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Host range expansion and increasing damage potential of *Euwallacea* nr. *fornicatus* (Coleoptera: Curculionidae) in Florida

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Abstract

Ambrosia beetles in the *Euwallacea* nr. *fornicatus* complex (Coleoptera: Curculionidae) vector *Fusarium* spp. fungi pathogenic to susceptible hosts, including avocado, *Persea americana* Mill., (Lauraceae). Previous survey traps in Florida avocado groves indicated significant beetle populations in several groves with minimal observed beetle activity, suggesting an external beetle source. A natural area near one such grove revealed *E.* nr. *fornicatus* colonization of wild tamarind, *Lysiloma latisiliquum* (L.) Bentham (Fabaceae). A survey of the natural area was conducted to understand the role that natural areas might play in *E*. nr. *fornicatus* ecology in southern Florida. Headspace volatiles from rasped avocado and *L. latisiliquum* bark were analyzed by gas chromatography-mass spectroscopy (GC-MS) to identify potential attractants. Genetic analysis confirmed that these beetles and their symbiotic fungi are of the same complex that attacks Florida avocado. Gas chromatography-mass spectroscopy analysis indicated that avocado is high in α-copaene (a known attractant of *E*. nr. *fornicatus*), but this kairomone is lacking in *L. latisiliquum*. Host diam and ht were examined for potential influence on colonization behavior. *Albizia lebbeck* (L.) Bentham (Fabaceae) and an unknown shrub also were observed to be suitable hosts. Concurrent with this study, a nearby grove of soursop, *Annona muricata* L. (Annonaceae), was found to have infestations of *E.* nr. *fornicatus*. *Euwallacea* nr. *fornicatus* populations are increasing in Florida and other cultivated and native trees are potentially at risk. Further research is warranted to better understand the ecology of this emerging pest and the chemical cues used for host location.

Key Words: ambrosia beetle; avocado; *Fusarium* dieback; headspace volatiles; *Lysiloma latisiliquum*

Resumen

Los escarabajos ambrosiales en el complejo *Euwallacea* cr. *fornicatus* (Coleoptera: Curculionidae) transmiten hongos patógenos de *Fusarium* spp. a hospederos susceptibles, incluyendo el aguacate, *Persea americana* Mill., (Lauraceae). Trampas usadas anteriormente para monitorear los huertos de aguacate en la Florida indicaron poblaciones significativas de escarabajos en varios huertos con una actividad mínima observada sobre los arboles de aguacate lo que sugiere una fuente externa de escarabajos. Un área natural cerca de uno de estos huertos reveló la colonización de *E.* cr. *fornicatus* sobre el tamarindo silvestre, *Lysiloma latisiliquum* (L.) Bentham (Fabaceae). Se realizó un sondeo del área natural para comprender el papel que las áreas naturales podrían jugar en la ecología de *E.* cr. *fornicatus* en el sur de la Florida. Se analizaron los volátiles "headspace" del aguacate raspado y la corteza de *L. latisiliquum* por medio del cromatografía de gases y espectrometría de masas (CG-SM) para identificar atrayentes potenciales. El análisis genético confirmó que estos escarabajos y sus hongos simbióticos son del mismo complejo que ataca al aguacate de Florida. El análisis de cromatografía de gases y espectrometría de masas indicó que el aguacate tiene un alto contenido de α-copaeno (un atrayente conocido de *E.* cr. *fornicatus*), pero hace falta esta kairomona en *L. latisiliquum*. Se examinaron el diámetro y la altura del hospedero para determinar su posible influencia sobre el comportamiento de colonización. También, se observaron *Albizia lebbeck* (L.) Bentham (Fabaceae) y un arbusto desconocido como hospederos adecuados. Simultáneamente con este estudio, se encontró que un huerto de guanábana (*Annona muricata* L.: Annonaceae) cercano, que tenía infestaciones de *E*. cr. *fornicatus*. Las poblaciones de *Euwallacea* cr. *fornicatus* están incrementando en la Florida y otros árboles cultivados y nativos están potencialmente en riesgo. Se necesita más investigación para comprender mejor la ecología de esta plaga emergente y las señales químicas que utiliza para localizar los hospederos.

Palabras Clave: escarabajos ambrosiales; aguacate; muerte decendente *Fusarium*; volátiles headspace; *Lysiloma latisiliquum*

The introduction of exotic ambrosia beetles (Coleoptera: Curculionidae: Scolytinae) poses a serious threat to U.S. forest health and several agricultural tree fruits. Ambrosia beetles culture symbiotic fungi inside galleries that they excavate in host trees as their sole food source (Ploetz et al. 2013; Hulcr & Stelinski 2017). Although most ambrosia beetles are benign, some introduced species attack and even cause the death of apparently healthy trees (Ploetz et al. 2013). In 2002, the redbay ambrosia beetle, *Xyleborus glabratus* Eichhoff (Coleoptera: Curculionidae), was discovered in Georgia (Rabaglia et al. 2008). Its primary nutritional symbiont, *Raffaelea lauricola* T.C. Harr.,

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Fraedrich & Aghayeva (Ophiostomataceae) is a devastating, systemic pathogen of North American Lauraceae, including avocado, *Persea americana* Mill. (Lauraceae) (Mayfield et al. 2008; Kendra et al. 2014a; Ploetz et al. 2017). Unlike many ambrosia beetles, *X. glabratus* is not attracted to standard ethanol lures and is, in North America, a specialist on the Lauraceae family. Extensive research examining host preferences among the Lauraceae and their volatile kairomones was instrumental in developing sensitive lures for detection programs (Hanula & Sullivan 2008; Kendra et al. 2011, 2014a, b, 2016a, b).

In 2002 and 2003, exotic ambrosia beetles identified as the tea shot hole borer, *Euwallacea fornicatus* Eichhoff (Coleoptera: Curculionidae), were discovered in Florida and California (CABI 2017). Subsequent molecular analysis of these beetle populations indicated the presence of multiple cryptic species in the United States, and all species groups in North America are referred to as *Euwallacea* near *fornicatus* (O'Donnell et al. 2015; Stouthamer et al. 2017). The primary nutritional symbionts are members of the Ambrosia Fusarium Clade, some of which destroy functional xylem around the galleries, resulting in *Fusarium* dieback disease (Freeman et al. 2012; Eskalen et al. 2013; Kasson et al. 2013; O'Donnell et al. 2015). In its native southeast Asia range, *E. fornicatus* attacks dozens of hosts in 35 plant families, including economically important fruit trees (Danthanarayana 1968). The cryptic species in California also are extremely polyphagous, attacking hundreds of host species (Eskalen et al. 2013). Members of the complex attack avocado in the US and Israel (Carrillo et al. 2012, 2016; Eskalen et al. 2012, 2017; Mendel et al. 2012; Kendra et al. 2017).

Since its discovery in Florida avocado in 2010, *E.* nr. *fornicatus* has spread throughout the commercial avocado production region centered around Homestead, Florida, USA (Carrillo et al. 2012, 2015). In early 2016, the beetle first caused extensive damage to avocado (cv. 'Donnie,' planted in 2004) in a single grove, killing branches up to 12.7 cm diam. This outbreak prompted a survey for *E.* nr. *fornicatus* and revealed abundant beetle populations in several groves that did not exhibit signs of significant beetle injury (sawdust sticks, sugary exudate, dead twigs and branches), suggesting that beetles were originating from either visually inaccessible locations in the groves or migrating from non-avocado hosts (Carrillo et al. 2016). One such grove with large beetle numbers captured in traps was located near a natural area. Very few hosts from Florida have been recorded, despite the growing populations and potential for extreme polyphagy. Previous host records include mango (*Mangifera indica* L., Anacardiaceae), swamp bay (*Persea palustris* [Raf.] Sarg. [Lauraceae]), and royal poinciana (*Delonix regia* [Boj. ex Hook] Raf [Fabaceae]) (Rabaglia et al. 2006; Carrillo et al. 2012, 2016). We hypothesized that tree species in the natural area may serve as a source of beetles flying to the trap located in the avocado grove. Furthermore, if additional, acceptable hosts were discovered, analysis of their volatile profiles might help guide further bioassays to develop improved lures, as was the case with *X. glabratus*.

Materials and Methods

HOST SURVEY

The diam of all trees greater than 2.5 cm (measured at a ht of 1.5 m) within the 0.37 hectare natural area (located at the UF/IFAS Tropical Research and Education Center, 25.5073°N, 80.5038°W) were measured and examined for signs of ambrosia beetle activity during Dec 2016. When beetle damage was observed, galleries were examined for the presence of *E.* nr. *fornicatus*. In May 2017, *E.* nr. *fornicatus* damage was identified by the owner of a soursop grove [*Annona muricata* L. (Annonaceae)]. The grove was visited to assess damage and symptoms.

BEETLE IDENTIFICATION AND COMMUNITY COMPOSITION

A total of 5 beetles and 5 samples of xylem tissue around the galleries were collected from 2 *Lysiloma latisiliquum* (L.) Bentham (Fabaceae) trees. Fungal isolation followed methods by Carrillo et al. (2016). Beetles were surface-disinfected with 70% ethanol, and xylem tissue was disinfected with 10% bleach and 70% ethanol prior to *Fusarium* isolation. The beetle head macerate and sapwood tissue were plated on potato dextrose agar supplemented with 0.1g per L streptomycin (PDA+). *Fusarium*-like colonies were counted on the agar plates for the beetle head isolates, and distinct morphologies were individually cultured on PDA+ for both wood tissue and beetle isolates. Pure isolates were obtained by monosporic cultures.

Total genomic DNA was extracted from the beetle bodies and from the mycelia of fungal isolates following a modified cetyl trimethylammonium bromide (CTAB) protocol (Doyle and Doyle 1987). For *Fusarium* spp. identification, portions of the EF-1α and DNA-directed RNA polymerase II largest and second largest subunits RBP1 and RBP2 were amplified using primers EF1 and EF2 (O'Donnell et al. 1998), R8/R8 (O'Donnell et al. 2010) and 5f2 / 7cr and 7cf / 11ar (Liu et al. 1999). For beetle identification, portions of the cytochrome oxidase subunit (COI), elongation factor-1α (EF-1α), CAD protein, 16s mtDNA, and 28s rDNA were amplified by PCR using primers sets LCO1490/ HCO2198, ets149/efa754, apCADforB2/ apCADrevlmod, forB2/rev and B2 LR-J-12961/LR-N-13398 and D2F1/D3R2; 3665/4048 according to Dole et al. (2010). PCR products were purified using ExoSAP-IT (Affymetrix, Inc., Santa Clara, California, USA) and sequenced in both directions. Nucleotide alignments were performed in the NCBI Basic Local Alignment Search Tool (BLAST) for sequence identification.

The ambrosia beetle species composition was estimated from 4 dead *L. latisiliquum* trees and from 4 avocado branches for comparison. Polypropylene micro-centrifuge tubes (1.7 mL; Thomas Scientific, Swedesboro, New Jersey, USA) were used to trap ambrosia beetles emerging from their natal galleries. The ends of centrifuge tubes were cut and covered with screening material. Tanglefoot® trap (The Scotts Co., Marysville, Ohio, USA) coated the inside of the centrifuge tube and tubes were glued onto the bark of infested trees to enclose gallery entrance holes. Galleries were selected according to their size; galleries that were obviously too small for *E.* nr. *fornicatus* were not sampled. Very few small gallery entrances were present. Sampled galleries were located 0.3 to 6 m above-ground. Thirty tubes were deployed per *L. latisiliquum* tree. One-hundred-four vials were attached to avocado branches. Branches were removed from trees and placed in plastic emergence containers (167 liter Brute® containers, Rubbermaid Commercial Products, Winchester, Virginia, USA) prior to trap tube attachment. Intercepted scolytines were identified to species according to Rabaglia et al. (2006) and Atkinson et al. (2013).

DENSITY OF ENTRANCE HOLES ON *LYSILOMA LATISILIQUUM*

Gallery density of 4 dead *L. latisiliquum* with active beetle infestation was estimated every 1.5 m above the ground on the main trunk and at several points on secondary branches until section diam was less than 2.5 cm. At each location, galleries were counted from sections measuring either 929 cm^2 (basal trunk locations), 465 cm^2 (upper trunk locations), or 232 cm^2 (small branches).

CHEMICAL ANALYSIS

Headspace volatiles were collected and analyzed from 5 samples of rasped outer bark of apparently healthy, non-infested *L. latisiliquum* and 1 sample of avocado (c.v. 'Donnie') using methods similar to Niogret et al. (2011). Ten grams of the outer bark was rasped with a micro-

plane. Bark shavings were transferred to a glass cylinder (10 cm diam × 44 cm length) for headspace collection. A purified air stream flowed over the wood shavings at a rate of 1 L per min and was pulled through a super-Q filter by vacuum for 1 h. Volatiles then were eluted from the filter with 200 µl of methylene chloride (99.8% pure, Avantor Performance Materials, Inc., Center Valley, Pennsylvania, USA). For quantification, a hexadecane (CAS Registry No. 544-76-3, Sigma-Aldrich, St. Louis, Missouri, USA) internal standard was added to each sample to make a solution of 2.5 µg hexadecane per µl of sample.

Samples were injected into a gas chromatograph-flame ionization detector (GC-FID, Trace GC2000, Thermoquest Corporation, Austin, Texas, USA) and a gas chromatograph-mass spectrometer (Agilent 5975B, Agilent Technologies, Santa Clara, California, USA). The column of both instruments was a DB5-MS capillary column (25 m × 0.25 mm × 0.25 µm, Agilent Technologies). Oven temperature was programmed at 45 °C for 1 min, then to 94 °C at a rate of 4 °C per min, then to 180 °C at a rate of 2 °C per min, and then to 240 °C at a rate of 20 °C per min. Gas chromatograph-mass spectrometer chromatograms were analyzed with MassHunter software (B.07.02, Agilent Technologies) and compounds were identified by correlating mass spectra to the NIST (2011) database, the Adams Library (Adams 2007), and our own "SHRS Essential Oil Constituents" library, authentic samples, and the components of known essential oils (Kendra et al. 2011; Ali et al. 2014; Blythe et al. 2016; Kendra et al. 2017). The authentic compounds [cyclosativene (CAS Registry No. 22469-52-9); β-elemene (CAS Registry No. 515-13-9); β-caryophyllene (CAS Registry No. 87-44-5); alloaromadendrene (CAS Registry No. 25246-27-9); (*Z*)-3-hexen-1-ol (CAS Registry No. 928-96-1); eucalyptol (CAS Registry No. 470-82-6)] were purchased from Sigma-Aldrich, St. Louis, Missouri, USA, and α-copaene (CAS Registry No. 3856-25-5) was from Fluka Chemical Co. (Buchs, SG, Switzerland). Retention indices (RI) were calculated according to the method of van Den Dool & Kratz (1963) using a homogenous series of *n*-alkanes (C_8-C_{40}). Chemical quantity was calculated by comparing gas chromatograph-flame ionization detector peak area to the area of the hexadecane standard (2.5 µg per µl).

DATA ANALYSIS

The number of *E.* nr. *fornicatus* galleries in the 4 measured trees was estimated by multiplying the counted gallery entrances in a sample by the area of a cylinder for the corresponding trunk section. This estimate then was multiplied by the tree's proportion of *E.* nr. *fornicatus*-identified galleries from the trap samples. The effect of section diam and ht of the tree on the number of galleries was analyzed with analysis of variance and Tukey's Test for mean separation (Systat Software 2010).

Results

HOST SURVEY

Of the 536 trees measured in the natural area, 68 could not be reliably identified. *Lysiloma latisiliquum* was the dominant species present, comprising 55.4% of the stand (Table 1). Thirty-nine dead or dying *L. latisiliquum* trees exhibited ambrosia beetle attack symptoms consistent with trees infested by *E.* nr. *fornicatus*. In living branches and on small dead trees and branches, galleries often were concentrated at nodes. On large dead trees, high densities of beetle galleries were present on the main trunk and branches (Fig. 1). Dried sap flow was observed on the trunks of several live or recently killed trees infested with *L. latisiliquum*. The nearest *L. latisiliquum* to a survey trap in the adjacent avocado orchard was 15 m.

A single *Albizia lebbeck* (L.) Benth. (Fabaceae) was damaged by *E.* nr. *fornicatus*, and another dead *A. lebbeck* had *E.* nr. *fornicatus* galleries. One shrubby plant that could not be identified had several stems with *E.* nr. *fornicatus* galleries. Only 2 leaf clusters remained on a single stem when the plant was discovered (Table 1).

There were 2 dead branches on a single gumbo limbo tree, *Bursera simaruba* (L.) Sargent (Burseraceae) with ambrosia beetle galleries. Although *E.* nr. *fornicatus* was not recovered from the galleries, 2 specimens were removed from sap flowing from galleries located at the base of the branch. Ambrosia beetle injury, if caused by *E.* nr. *fornicatus*, was similar to avocado damage, where beetles concentrate on secondary branches without attacking the main trunk.

The soursop grove infested by *E.* nr. *fornicatus* was located 2.0 km southwest from the infested natural area. Damage was light, and limited to the lower, deeply shaded branches. Trees had a dense canopy and were planted about 2 m apart. *Euwallacea* nr. *fornicatus* was observed in several small branches that began dying. Gallery entrances mostly were located at nodes. The grove owner practiced regular sanitation, pruning infested branches and destroying them.

BEETLE AND FUNGI IDENTIFICATION

All 5 beetles removed from galleries for genotyping matched previous sequences for *E.* nr. *fornicatus* sp. 2, also identified as the type species with the common name tea shot hole borer (for nomenclature discussion, see O'Donnell et al. 2015 and Stouthamer et al. 2017; Table 2). *Fusarium* sp. AF-8 was isolated from 4 beetles with a mean of 680 ± 143 (SE) colony forming units per beetle and from galleries in both sampled trees. *Fusarium* sp. AF-9 was recovered from a single *E.* nr. *fornicatus* gallery in 1 tree, but was not recovered from *E.* nr. *fornicatus*.

The percentage of *E.* nr. *fornicatus* galleries sampled with the modified centrifuge tubes ranged from 28.6 to 83.9% on the 4 dead *L. latisiliquum* trees. Ambrosia beetles were not recovered from 15 of the 120 vials, and 4 vials had fallen from the tree and were not recovered. *Theoborus ricini* Wood & Bright (Coleoptera: Curculionidae) accounted for between 12.9 to 60.7% of the galleries. Single *Xyleborus ferrugineus* Fab. and *Xyleborus bispinatus* Eichhoff (Coleoptera: Curculionidae: Scolytinae) galleries were identified from a single dead tree. In avocado, the percentage of confirmed *E.* nr. *fornicatus* galleries ranged from 41.7 to 75.6% (mean 60.6%). No emergence or beetle activity was observed from 16 galleries, and ambrosia beetle activity was detected in 17 vials (heavy sawdust deposition), but beetles either escaped or failed to be captured. Only 8 vials captured *T. ricini*.

ATTACK DENSITY

Galleries were counted at 36 sites (diam of trunk or branches > 2.5 cm) combined from the 4 trees. Trunk diam ranged between 5.3 and 25.1 cm. Average gallery density was 0.11 galleries per cm^2. Extrapolating over the entire tree, suitable *L. latisiliquum* can support a significant *E.* nr. *fornicatus* population (Table 3). Gallery density was influenced by tree section diam, with the smallest diam having fewer attacks ($F = 4.88$; df = 6, 29; $P = 0.001$; Fig. 2a). Gallery density was not influenced by ht ($F = 0.27$; df = 5, 30; $P = 0.926$; Fig. 2b).

HEADSPACE ANALYSIS

The avocado sample contained numerous terpenoids, 12 of which were identified (Table 4). The most abundant was α-copaene. Four chemicals were identified from *L. latisiliquum* (Table 4). The green leaf volatiles (Z)-3-hexen-1-ol and 1-hexanol were emitted in large quantity.

Table 1. Plant species present in the Florida natural area in which *Euwallacea* nr. *fornicatus* was discovered in *Lysiloma latisiliquum*. Hosts infested with *Euwallacea* nr. *fornicatus* are marked with an asterisk.

Species	No. (% of total)	Mean diameter (cm)	No. dead trees with *Euwallacea* nr. *fornicatus*
Fabaceae			
*Lysiloma latisiliquum**	297 (55.4%)	11.7	36
*Albizia lebbeck**	12 (2.2%)	8.1	2
Sapotaceae			
Chrysophyllum oliviforme	83 (15.3)	7.4	
Sideroxylon salicifolium	7 (1.3)	13.3	
Primulaceae			
Ardisia escallonioides	3 (0.4)	5.7	
Casuarinaceae			
Casuarina spp.	2 (0.4)	37.3	
Anacardiaceae			
Schinus terebinthifolius	11 (2.1)	6.8	
Metopium toxiferum	1 (0.2)	14.6	
Combretaceae			
Conocarpus erectus	3 (0.6)	16.9	
Myrtaceae			
Eugenia spp.	1 (0.2)	2.7	
Unidentified	2 (0.4)	11.0	
Ficus aurea	8 (5.2)	24.9	
Fagaceae			
Quercus virginiana	2 (0.4)	7.2	
Pittosporaceae			
Pittosporum ferrugineum	9 (1.5)	5.8	
Burseraceae			
Bursera simaruba	21 (3.9)	20.7	
Simaroubaceae			
Simarouba glauca	1 (0.2)	18.5	
Rutaceae			
Zanthoxylum fagara	1 (0.2)	7.4	
Meliaceae			
Swietenia mahahoni	2 (0.4)	15.3	
Rhamnaceae			
Colubrina arborescens	1 (0.2)	12.2	
Rosaceae			
Prunus myrtifolia	1 (0.2)		
Unidentified*	1 (12.7)	8.8	
Unidentified non-host	67 (12.5)	3.7	

Anisol also was detected in 4 of the 5 samples. Eucalyptol, a monoterpene ether, was the only terpenoid detected from *L. latisiliquum*, and in low quantity.

Discussion

The discovery of infested native trees indicates that natural areas in Florida may function as reservoirs for pest *E.* nr. *fornicatus* populations capable of dispersing to avocado and other cultivated fruit trees. This is the first time that the beetle has been reported from *L. latisiliquum* and *Annona muricata*. *Lysiloma latisiliquum* is widely distributed throughout Central America, the Caribbean, and southern Florida. It is a pioneer species, capable of growing on calcareous soils common in Miami-Dade County (Brown & Cooprider 2013). *Euwallacea* nr. *fornicatus* may become an increasingly serious threat to native forest stands, as *E.* nr. *fornicatus* populations have in California (Eskalen et al. 2013; Boland 2016). At the time of sampling, 13% of the *L. latisiliquum* trees in the natural area were infested by *E.* nr. *fornicatus*, and gallery counts indicated that infested *L. latisiliquum* can support significant *E.* nr. *fornicatus* populations. It is highly likely that beetles from this natural area were captured by the nearby survey trap in the avocado grove.

Other *Albizia* spp. and members of the Burseraceae family have been recorded as hosts for the tea shot hole borer in Asia (Danthanarayana 1968). The polyphagous shot hole borer (*Euwallacea* sp.) in California also attacks *Bursera* spp. (Eskalen et al. 2013). Although *E.* nr. *fornicatus* was not recovered from galleries in *B. simaruba*, beetles were removed from sap flow at the branch collar. This suggests that either *B. simaruba* is a host in Florida, it contains attractive kairomones, or *E.* nr. *fornicatus* was responding to fungal volatiles produced by galleries of previous ambrosia beetles colonizing the wood (*X. glabratus* is attracted to symbiont fungal volatiles [Kuhns et al. 2014a]). A third species in the natural area that was colonized by *E.* nr. *fornicatus* could not be identified, and it is very likely that other native plant species are hosts for *E.* nr. *fornicatus*. *Euwallacea* nr. *fornicatus* has been recovered only from a few plant species in Florida, while in California and Asia, hundreds of species are attacked by the *E.* nr. *fornicatus* complex (Danthanarayana 1968; Amarasinghe & Devy 2003; Eskalen et al. 2013). It is interesting to note also that *Fusarium* species AF-9 previously has been identified only from beetles in Costa Rica (Kasson et al. 2013). It

Fig. 1. Ambrosia beetle gallery entrances in the trunk of a *Lysiloma latisiliquum*. *Euwallacea* nr. *fornicatus* and *Theoborus ricini* were the two most abundant species of ambrosia beetle recovered from *L. latisiliquum*.

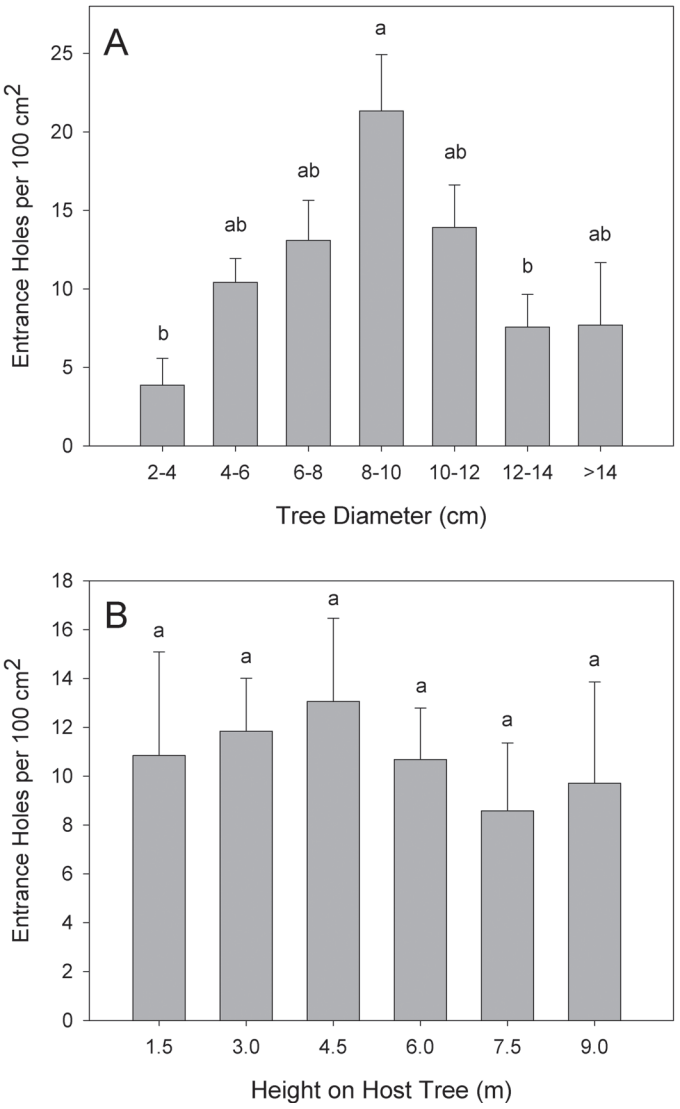

Fig. 2. Relationships among host tree diameter, height above ground, and site of attack by *Euwallacea* nr. *fornicatus.* Density of beetle entrance holes versus the trunk or branch diameter (A) and the trunk or branch height (B) of host *Lysiloma latisiliquum* from 4 trees. Mean values topped by the same letter are not significantly different (Tukey's test, $P = 0.05$).

is not known how different *Fusarium* species affect host selection and colonization by *E.* nr. *fornicatus*.

In avocado, beetle attack usually is confined to the secondary branches and activity often does not extend past the branch collar, but in *L. latisiliquum*, beetles readily colonized the main trunk. *Euwallacea* nr. *fornicatus* galleries were present only on soursop branches, not the trunk, and galleries were concentrated at nodes. On *L. latisiliquum*, there were differences in beetle colonization at locations of differing diam but not ht. Differences in colonization behavior within a host and among host species could be due to either visual or semiochemical cues. *Xyleborus glabratus* prefers larger diam hosts (Mayfield & Brownie 2013; Kendra et al. 2013). At the same time, Niogret et al. (2013)

Table 2. Genotyping results of *Euwallacea* nr. *fornicatus* and associated *Fusarium* collected from *Lysiloma latisiliquum* in southern Florida.

Species	Locus	% similarity	Gene Bank Accession Number
Euwallacea fornicatus sp. 2	COI	100	KM406728
(TSHB)	EF-1α	100	KM406747
	CAD	99	KM406713
	16s mtDNA	98	KM406663
	28s rDNA	99	KM406679
Fusarium AF-8	EF	100	KC691549
	RBP1	100	KC691607
	RBP2	100	KC691638
Fusarium AF-9	EF	99	KM406625
	RBP1	100	KC691613
	RBP2	100	KM406646

Table 3. Proportion and estimated number of *Euwallacea* nr. *fornicatus* gallery entrances on four infested, dead *Lysiloma latisiliquum* trees.

Tree	Diameter (1.5 m)	No. of sections	Proportion of *Euwallacea* nr. *fornicatus* galleries	Proportion of *Theoborus ricini* galleries	Estimated no. of *Euwallacea* nr. *fornicatus* galleries per tree
1	10.4 cm	4	0.286	0.607	817
2	25.1 cm	14	0.367	0.5	2,372
3	5.3 cm	5	0.321	0.357	374
4	13.2 cm	6	0.838	0.129	3,885

discovered terpenoid gradients among avocado trunks, branches, leaf petioles, and leaves, with some being produced in greater quantity in the trunk and others by smaller branches and leaves. The lack of terpenoid emissions from *L. latisiliquum* indicates that *E.* nr. *fornicatus* is responding to other volatiles. Eucalyptol was detected in low quantity from rasped bark samples. This chemical has been identified previously as an attractant for another primary ambrosia beetle colonizer, *X. glabratus* (Kuhns et al. 2014b). Subsequent studies found eucalyptol to be less attractive than essential oil lures high in sesquiterpenes (Kendra et al. 2016b). The most abundant volatile from avocado was α-copaene, which was consistent with previous reports. This chemical is also a known attractant for both *E.* nr. *fornicatus* and *X. glabratus* (Kendra et al. 2016a, 2017). Both β-caryophyllene and germacrene-D, identified from avocado, are potential attractants for *E.* nr. *fornicatus* (James 2007). However, lures containing pure β-caryophyllene, presented alone or in combination with essential oil lures (Kendra et al. 2016a), were not attractive to *X. glabratus*.

It is not known what physiological condition the *L. latisiliquum* trees were in at the time of initial *E.* nr. *fornicatus* colonization, although Nov and Dec 2016 weather was unusually hot and dry. Trees under severe drought stress produce ethanol (Kimmerer & Kozlowski 1982), and ethanol in combination with other host volatiles is attractive to *E. fornicatus* in Asia (Karunaratne et al. 2008). However, there are indications that the presence of ethanol decreases trap captures of the *E.* nr. *fornicatus* species present in California (Dodge et al. 2017). Analytical chemistry techniques sensitive to low-molecular weight compounds, such as solid phase microextraction, may further aid in identification of attractive kairomones.

To date, the only commercial attractants for *E*. nr. *fornicatus* are quercivorol (Carrillo et al. 2015) and a proprietary essential oil enriched in α-copaene (Kendra et al. 2017). The combination of both lures results in additive or synergistic increase in beetle capture, depending on population levels (Kendra et al. 2017). Identification of additional hosts is an important step toward the identification of other attractive terpenoids that could improve attractiveness of field lures for *E*. nr. *fornicatus*. With *X. glabratus*, it has been hypothesized that optimal host location is achieved by detection of a complex terpenoid mixture, a 'signature bouquet' of the Lauraceae (Kendra et al. 2014a). In Sri Lanka, 7 plant species are reported to be more attractive to *E. nr. fornicatus* than tea (Amarasinghe & Devy 2003). One of these species, *Jacaranda mimosifolia* Don (Bignoniaceae), is widely planted in southern Florida. Further studies comparing the volatile emissions from reported and observed hosts, including avocado and *Annona* spp., may identify unique attractants that potentially could improve detection of *E.* nr. *fornicatus* in Florida.

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Table 4. Headspace volatile identification from 10 g rasped outer bark volatiles collected onto super-Q for one hour.

RI[a]	RI_{Lit}[b]	Identification	Quantity per µL
avocado			
1326	1335	δ-elemene	0.88
1337	1345	α-cubebene	1.29
1356	1369	cyclosativene	3.37
1365	1374	α-copaene	10.03
1376	1387	β-cubebene	0.77
1379	1389	β-elemene	1.98
1430	1417	β-caryophyllene	2.39
1445	1458	alloaromadendrene	4.4
1462	1479	γ-muurolene	1.00
1466	1484	germacrene-D	3.54
1506	1522	δ-cadinene	2.01
1540	1559	germacrene-B	0.40
Lysiloma latisiliquum			
856	850	(Z)-3-hexen-1-ol	10.72 ± 2.69
869	863	1-hexanol	2.70 ± 0.68
915	913	anisol	0.91 ± 0.28
984		unidentified[c]	0.26 ± 0.04
1028	1026	eucalyptol	0.61 ± 0.46

[a]RI: Relative retention indices calculated for *n*-alkanes;
[b]RRI_{Lit}: Relative retention indices from Adams Library (Adams, 2007)
[c]Mass spectra of unidentified compound [*m/z* (% int.)]: 49 (100), 43 (76), 84 (65), 57 (63), 86 (49), 41 (37), 51 (33), 55 (24), 71 (22), 72 (22), 99 (18), 108 (10), 128 (2)

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