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ORIGINAL PAPER

Quantitative analysis of contents and volatile emissions from α-copaene and quercivorol lures, and longevity for attraction of *Euwallacea* nr. *fornicatus* in Florida

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Abstract

Ambrosia beetles in the cryptic species complex *Euwallacea* nr. *fornicatus* vector a fungal pathogen responsible for *Fusarium* dieback, a disease that impacts avocado (*Persea americana*), woody ornamentals, and numerous native trees in the USA (California, Florida), Israel, and other countries. Currently, these pests are detected with quercivorol lures (containing *p*-menth-2-en-1-ol isomers), but recent research identified an essential oil enriched in (-)-α-copaene as a new attractant. In this study, lure longevity and efficacy were assessed in three 12-week field tests conducted in Florida by deploying traps baited with quercivorol, α-copaene, and a combination of the two. A fourth test compared different formulations of quercivorol. Concurrent with field experiments, gas chromatographic analyses were conducted to quantify initial lure contents as well as volatile emissions from lures field-aged for 12 weeks. In all tests, the lure combination captured significantly more *E.* nr. *fornicatus* than the individual lures; and in two trials, synergistic attraction was observed. Field life of the combination lure was 12 weeks; longevity of single lures varied from 9 to 12 weeks. Twelve terpenoids were detected from the α-copaene-enriched oil, suggesting there may be additional attractants. Analysis of the quercivorol lure showed it contained 88% *trans*- and 9% *cis*-*p*-menth-2-en-1-ol. Results indicate that the combination of quercivorol and α-copaene provides a long-lasting, effective lure for early detection of *E.* nr. *fornicatus* in Florida. Further research is needed to determine which isomer of *p*-menth-2-en-1-ol is attractive to Florida *E.* nr. *fornicatus*, and if other members of the species complex are attracted to (-)-α-copaene.

Keywords Ambrosia beetle · Diastereomer · Enantiomer · *Fusarium* dieback · Kairomone · *p*-menth-2-en-1-ol

Key message

- Invasive ambrosia beetles in the *Euwallacea* nr. *fornicatus* complex vector a fungal pathogen that causes *Fusarium* dieback disease in avocado, landscape, and forest trees.
- Effective lures are critical for early pest detection and management.
- Quercivorol is the standard lure for *E.* nr. *fornicatus*, but α-copaene was recently identified as a new attractant.
- A two-component lure containing α-copaene and quercivorol provides more sensitive pest detection, with sustained low release of attractants, field longevity of 3 months, and minimal attraction of non-target beetles.

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Introduction

Ambrosia beetles (Coleoptera: Curculionidae: Scolytinae and Platypodinae) are among the most frequently intercepted non-native insects at ports of entry in the USA (Rabaglia et al. 2008), and several have become serious pests in forests, orchards, and nursery ornamentals (Hulcr and Dunn 2011). Native ambrosia beetles rarely cause injury to healthy trees, preferring to colonize stressed or dying trees that emit ethanol. As they bore into the xylem, females inoculate the gallery with conidia of their symbiotic fungi that are carried in mandibular or thoracic mycangial pits. The fungi growing in the galleries serve as the sole food source for both larvae and adults. In their native habitats, ambrosia beetles play an important role in maintaining overall forest health; they are one of the first insects to colonize dying trees, facilitating wood biodegradation.

Exotic ambrosia beetles sometimes interact with new naïve hosts in unpredictable ways. One particularly striking example is that of the redbay ambrosia beetle, *Xyleborus glabratus* Eichhoff, first detected in the USA in 2002 (Rabaglia et al. 2006). Ecologically, *X. glabratus* functions as a primary colonizer, attacking apparently healthy trees in the Lauraceae, an unusual host restriction and attack pattern for ambrosia beetles (Hanula et al. 2008; Kendra et al. 2014a). Its most prevalent symbiont, *Raffaelea lauricola* T. C. Harr., Fraedrich & Aghayeva (Ophiostomatales: Ophiostomataceae), is the causal agent of laurel wilt (Fraedrich et al. 2008). Systemic colonization of host xylem by *R. lauricola* triggers a defensive response which results in formation of tyloses, compromised water transport, and ultimately tree death (Inch et al. 2012; Ploetz et al. 2017a, b). Since 2002, millions of native bay trees (*Persea* spp.) have been killed in southeastern US forests (USDA-FS 2017), and 44,000 avocado trees (*Persea americana* Mill.) have succumbed in Florida (Florida Avocado Admin. Comm., Pybas D, personal comm.).

A second example, with greater global impact, is that of the invasive *Euwallacea* shot hole borers. In 2003 and 2004, ambrosia beetles morphologically indistinguishable from the Asian *Euwallacea fornicatus* Eichhoff were detected in California and Florida (Rabaglia et al. 2006), followed by detections in Israel (Mendel et al. 2012), Australia (Campbell and Geering 2011), and most recently Mexico (García-Avila et al. 2016). The primary symbionts of *Euwallacea* spp. are *Fusarium* spp. fungi (Hypocreales: Nectriaceae), some of which destroy functional xylem around the galleries, impairing water conduction and causing *Fusarium* dieback disease (Freeman et al. 2012). Molecular investigations of these beetles and their symbionts determined that populations from different geographic locations possess sufficient genetic diversity to constitute a cryptic species complex (collectively referred to as *E.* nr. *fornicatus*; Kasson et al. 2013; O'Donnell et al. 2015). The type species, the tea shot hole borer (TSHB), is a primary pest of tea, *Camellia sinensis* (L.) Kuntze (Ericales: Theaceae) (King 1940). Females prefer to bore into stems less than 20 mm in diameter (Hazarika et al. 2009), causing severe reduction in growth of tender foliage, the stage at which tea is harvested. In addition to tea, plant species from 35 families have been recorded as hosts of TSHB (Danthanarayana 1968).

Two distinct populations (species) of *E.* nr. *fornicatus* now exist in southern California, assigned the common names polyphagous shot hole borer (PSHB) and Kuroshio shot hole borer (KSHB) (Eskalen et al. 2013; Boland 2016). Like *X. glabratus*, PSHB and KSHB attack living hosts and vector pathogenic fungal symbionts, threatening native forests, urban landscapes, and the avocado industry. However, the California species have a more extensive host range, encompassing at least 60 phylogenetically diverse species (Eskalen 2017), including box elder (*Acer negundo* L., Sapindaceae), castorbean (*Ricinus communis* L., Euphorbaceae), California live oak (*Quercus agrifolia* Nee, Fagaceae), and red willow (*Salix laevigata* Bebb, Salicaceae); mass attacks can occur on the trunk and large branches of some hosts, resulting in tree mortality. With avocado, females tend to concentrate attacks near the base of secondary branches, causing localized lesions and branch death. Live, infested branches can also break under their own weight, particularly when in fruit, further reducing yields (Eskalen et al. 2013). Consequently, PSHB and KSHB pose a significant ecological threat to forest ecosystems in the western USA and Mexico, as well as an economic threat to avocado production [valued at 295.9 million USD in California (USDA-NASS 2017);1.1 billion USD in Mexico (SIAP 2015)].

A third distinct population, recently determined to be more closely aligned with TSHB (Stouthamer et al. 2017), is established in southern Florida. Although detected more than a decade ago, and reported from various hosts including ornamental royal poinciana [*Delonix regia* (Boj. ex Hook) Raf., Fabaceae], native species like swampbay [*Persea palustris* (Raf.) Sarg., Lauraceae] and wild tamarind [*Lysiloma latisiliquum* (L.) Benth., Fabaceae], and fruit crops such as avocado and mango (*Mangifera indica* L., Anacardiaceae), this species has been relatively uncommon and did not warrant pest status (Rabaglia et al. 2006; Carrillo et al. 2012, 2016; Owens et al. 2018). However, the situation changed in 2016 when increasing numbers of *E.* nr. *fornicatus* were found in commercial avocado groves of Miami-Dade County, FL (Carrillo et al. 2016; Kendra et al. 2017). The state's avocado industry (19.1 million USD; USDA-NASS 2017), already heavily impacted by laurel wilt, now faces a new threat with *Fusarium* dieback. Initial research indicates that topical insecticides are unlikely to provide effective management of *Euwallacea* spp. pests, as applications have limited efficacy and deterrence of new attacks is ephemeral (Jones et al. 2017).

Understanding the chemical ecology of dispersing *E.* nr. *fornicatus* females should facilitate development of effective detection systems, a key element for integrated pest management. Like other members within the tribe Xyleborini (subfamily Scolytinae), *E.* nr. *fornicatus* does not appear to utilize long-range attractive pheromones, as flightless males typically mate with their siblings (consanguineous polygyny) before females leave the brood gallery (Cooperband et al. 2016). However, in 2015, lures containing quercivorol [(1*S*, 4*R*)-*p*-menth-2-en-1-ol], the aggregation pheromone of *Platypus quercivorus* Murayama (Coleoptera: Curculionidae: Platypodinae) (Kashiwagi et al. 2006), were found to be attractive to *E.* nr. *fornicatus* in Florida (Carrillo et al. 2015), and subsequently in California (Dodge et al. 2017). Also in 2015, in a field test evaluating attractants for redbay ambrosia beetle (Kendra et al. 2015a), *E.* nr. *fornicatus* was intercepted in traps baited with an essential oil enriched in the sesquiterpene (-)-α-copaene (Kendra et al. 2016a). Subsequent research indicated that combining α-copaene with quercivorol increased captures of *E.* nr. *fornicatus* (Kendra et al. 2017). [Note on nomenclature: The common name 'quercivorol' was applied exclusively to the isomer (1*S*, 4*R*)-*p*-menth-2-en-1-ol (Kashiwagi et al. 2006), but commercial lures contain a mixture of four isomers. To be consistent with previous entomological literature (Carrillo et al. 2015; Byers et al. 2017; Dodge et al. 2017; Kendra et al. 2017), commercial lures for *E.* nr. *fornicatus* will be referred to as quercivorol lures in this report. Our discussion of the lure contents will address diastereomers of *p*-menth-2-en-1-ol].

The objectives of the current study were to evaluate quercivorol and α-copaene lures to (1) determine their detection efficacy and field longevity, deployed separately and in tandem, at sites that differed in population levels of *E.* nr. *fornicatus*, (2) quantify lure emissions over time and relate those data to field captures, and (3) analyze the lure constituents, potentially providing insight into additional attractive kairomones. While our initial tests were underway, the lure manufacturer modified the quercivorol formulation from a high-dose bubble lure to a low-dose mini-bubble. Therefore, we conducted additional tests to compare field performance and volatile emissions of these two formulations.

Materials and methods

Lures and traps

All field tests and laboratory analyses were conducted using plastic bubble lures (Synergy Semiochemicals Corp., Burnaby, BC, Canada). Treatments consisted of 50% α-copaene oil (2.0 mL loaded into a 2.9-cm-diameter bubble; product # 3302), high-dose quercivorol (290 mg in the standard 2.9-cm-diameter bubble; product # 3361), and low-dose quercivorol (97 mg in the new 1.2-cm-diameter 'mini'-bubble; product # 3402). Previous field tests, including those targeting *E.* nr. *fornicatus*, demonstrated that sticky panel traps were more effective than comparably baited Lindgren funnel traps for capture of in-flight ambrosia beetles (Kendra et al. 2012, 2017). Therefore, the current tests were conducted with bubble lures fastened above sticky traps constructed as described in Kendra et al. (2012). In brief, lures were attached to the bend of an S-shaped wire hanger and two sticky panels (Sentry wing trap bottoms, Great Lakes IPM Inc., Vestaburg, MI, USA) were hooked back-to-back from the bottom of the hanger. A control treatment consisted of an unbaited trap assembly. The wire hanger was inserted through the center of an inverted clear plastic plate (24 cm diameter) to serve as a rain shield that was taped in place just above the lures.

Field evaluations

Since populations of *E.* nr. *fornicatus* in Florida are more highly concentrated in avocado groves, all field trials were conducted in commercial groves in Miami-Dade County. Three replicate tests (at different sites) evaluated longevity of the field lures, presented alone and in combination; a fourth test directly compared efficacy of the two formulations of quercivorol. Field test 1 used the α-copaene lure, the high-dose quercivorol lure, a combination of these two lures, and an unbaited control. It was conducted from September 8 to December 1, 2016 (12 weeks) in a grove (25°30′22.17″N, 80°29′21.96″W) that was free of laurel wilt, but had a prior history of damaging populations of *E.* nr. *fornicatus* (Carrillo et al. 2016). The grove had a mixture of six different avocado cultivars, but beetles preferentially colonized cv. 'Donnie' (Kendra et al. 2017). Traps were hung only in rows containing 'Donnie' trees, spaced a minimum of 12 m apart within a replicate, and 15 m apart within replicate blocks (effective range of the standard quercivorol lures has been estimated to be < 3 m; Byers et al. 2017).

Field test 2 used the same four treatments as test 1, but was conducted in a grove (25°35′53.67″N, 80°27′50.68″W) that was heavily damaged by laurel wilt disease. There was minimal management at this grove; thus, the site had much higher numbers of bark and ambrosia beetles than site 1. This test was conducted for 12 weeks, from October 20, 2016 to January 12, 2017.

Field test 3 was conducted from February 17 to May 12, 2017 (12 weeks) in another grove (25°29′58.42″N, 80°29′19.80″W) affected by laurel wilt which had extremely high levels of *E.* nr. *fornicatus*. However, unlike at site two, the grove manager at this site practiced better sanitation, with prompt removal of trees symptomatic for laurel wilt. Treatments for this test consisted of the α-copaene lure, the new low-dose quercivorol lure, and a combination of the two (it did not include the α-copaene-only treatment).

Field test 4 was conducted at the same site as test 3, from May 16 to June 27, 2017 (6 weeks), and compared captures with six treatments: the α-copaene lure, the high- and low-dose quercivorol lures deployed alone and in combination with the α-copaene lure, and an unbaited control. For field tests 2–4, trap spacing was comparable to that described for field test 1. In each test, treatments were arranged in five linear replicates of a randomized complete block design. As in previous field trials with ambrosia beetles, traps were hung from branches well shaded by the canopy, at a height of ~ 1.5 m above ground (Kendra et al. 2011, 2014b, 2017; Brar et al. 2012; Owens et al. 2017), consistent with a mean flight height of 1.24 m reported for PSHB (Byers et al. 2017).

At weekly intervals, traps were rotated sequentially within each block and sticky panels collected/replaced such that by the end of the 12 week tests (tests 1, 2, 3), each lure had occupied the same position within a replicate block three times; with the 6 week test (test 4), each lure completed 2 full rotations through each position within a block. Panels were returned to the Subtropical Horticulture Research Station (SHRS; Miami, FL, USA) where beetles were removed and cleaned with a histological clearing agent (Histo-clear II, National Diagnostics, Atlanta, GA, USA), and stored in 70% ethanol. With the exception of *Hypothenemus* spp., all specimens within the Scolytinae and Platypodinae from field tests 1–3 were identified to species level according to Rabaglia et al. (2006) and Atkinson et al. (2013). For field test 4, only specimens of *E.* nr. *fornicatus* were tallied.

Chemical analysis

Lures of quercivorol (high-dose, lot # 160215) and α-copaene (lot # 160511), three replicates of each, were hung from trap assemblies (as described above) in a linear array within an avocado grove at SHRS and field-aged for 12 weeks from August 29 to November 21, 2016. A second set of quercivorol lures (high-dose, lot # 160726, three replicates) were placed in the grove from November 17, 2016 to February 8, 2017. A third set of quercivorol lures (low-dose, lot # 111816, three replicates) was deployed from February 7 to May 1, 2017. With all three batches, lures were removed from the field at regular intervals for analysis of volatile emissions following established procedures (Kendra et al. 2012, 2016b). Briefly, lures were placed in glass cylinders (10 cm diameter × 44 cm length), purified air was maintained at a flow rate of 1 L per min, and volatiles were collected in traps containing super-Q adsorbant (Analytical Research Systems, Gainesville, FL, USA). After 15 min, filters were removed and volatiles eluted with 200 µL methylene chloride (99.8% pure, Avantor Performance Materials, Inc., Center Valley, PA, USA). Hexadecane was added to each sample as an internal standard to make a final solution of 2.5 µg/µL sample. Upon completion of volatile extractions, lures were returned to the field and rotated sequentially among field positions, comparable to the procedure used for field tests. For each of the three sampling periods, environmental data (mean daily temperature, wind speed, and precipitation) were obtained from a weather station adjacent to SHRS (Kings Bay, Pinecrest, FL; https://www.wunderground.com/weather/us/fl/kings-bay).

To determine the chemical contents of lures, oil was extracted from fresh α-copaene and quercivorol lures (high-dose, lot # 160726), three replicates of each. Oil was diluted in methylene chloride in a 1:1000 ratio for analysis by gas chromatography–mass spectrometry (GC–MS) and gas chromatography–flame ionization detection (GC-FID). All samples (headspace volatiles and lure oil) were analyzed with a GC-FID (Thermoquest Trace GC 2000, Austin, TX, USA) equipped with a DB5-MS capillary column (25 m × 0.25 mm × 0.25 µm, Agilent Technologies, Santa Clara, CA, USA). GC method parameters for oil analysis are as follows: helium as carrier gas (1.3 mL min^{-1}), split less, injection temperature 225 °C, FID temperature 250 °C and oven temperature programmed 40–94 °C at 5 °C min^{-1}, then increasing at 2 °C min^{-1} to 180 °C, and lastly increasing at 20 °C min^{-1} to 240 °C. Oven parameters for headspace volatile analysis were 50–130 °C at a rate of 15 °C min^{-1}, then from 130 to 220 °C at 10° min^{-1}, and the final temperature was held for 4 min.

Lure contents were analyzed by GC–MS using an Agilent 5975B (Agilent Technologies) system equipped with a DB5-MS column. The carrier gas was helium, with a flow rate of 1.3 mL min^{-1}. GC oven temperature was kept at 45 °C for 1 min and programmed to 94 °C at a rate of 4 °C min^{-1}, and increased to 180 °C at a rate of 2 °C min^{-1}, and lastly increasing at 20 °C min^{-1} to 240 °C. The PTV injector temperature was 200 °C. Mass spectra were recorded at 70 eV. Mass range was *m/z* 35–450, ion source temperature was 230 °C, and the scan rate was 2.8 s^{-1}.

Enantio-GC analysis

Chiral analyses were carried out with an Rt-βDEXse 30 m, 0.32 mm × 0.25 µm column (Restek Corporation, Bellefonte, PA, USA) using a Trace GC Ultra (Thermo Scientific, Waltham, MA USA). Helium was used as a carrier gas at 1.2 mL min^{-1}. The samples were analyzed with a split ratio of 10:1. The injector and FID temperatures were 225 and 230 °C, respectively. Temperature program was as follows: for α-copaene, 40 °C, and programmed at a rate of 4 °C min^{-1} to 220 °C for 10 min; for quercivorol, 40 °C, and programmed at a rate of 2 °C min^{-1} to 220 °C for 10 min.

Solid-phase microextraction

In order to identify and confirm the enantiomeric composition of α-copaene, the ethanolic extract of *Cedrela odorata* L. (Meliaceae) was purchased (HawaiiPharm LLC, Honolulu, HI, USA); volatiles were collected by solid-phase microextraction (SPME) fiber coated with 100 µm polydimethylsiloxane (PDMS, Supelco Inc. Bellefonte, PA, USA). The extract was placed in a 15-mL amber vial. The vial was then sealed with plastic film and left at 40 °C for 10 min for equilibration of volatiles in the headspace. After equilibration, the film was pierced with the SPME needle and the fiber exposed to the sample headspace (HS-SPME) for 30 min at 40 °C. The fiber was then inserted into the injector port of the GC–MS for desorption 2 min. HS-SPME and subsequent analyses were performed in triplicate.

Identification of volatile compounds

Relative percentages of the separated compounds were calculated from integration of the peak areas in the GC-FID chromatograms. MassHunter software (B.07.02, Agilent Technologies, Santa Clara, CA, USA) was used for identifying the compounds. The identification of volatile compounds was based on the comparison of retention times with those of authenticated samples of α-copaene (Cas # 3856-25-5, Fluka Chemical Co., Buchs, SG, Switzerland), β-elemene (Cas # 515-13-9, LKT Laboratories Inc., Saint Paul, MN, USA), β-caryophyllene (Cas # 87-44-5, Sigma-Aldrich, St. Louis, MO, USA), α-humulene (Cas # 6753-98-6, Sigma-Aldrich, St. Louis, MO, USA), alloaromadendrene (Cas # 25246-27-9, Sigma-Aldrich, St. Louis, MO, USA), caryophyllene oxide (Cas # 1139-30-6, Sigma-Aldrich, St. Louis, MO, USA), α-phellandrene (Cas # 99-83-2, Sigma-Aldrich, St. Louis, MO, USA)] and computer matching against commercial [NIST (2017), Adams Library (Adams 2007), Flavors and Fragrances of Natural and Synthetic Compounds 3 (FFNSC#, 2015)] and our own library 'SHRS, DB5-MS Essential Oil Constituents' compiled from pure substances and components of known oils (Kendra et al. 2011; Carroll et al. 2011; Ali et al. 2013; Blythe et al. 2016; Kendra et al. 2017). Arithmetic retention indices (AI) were determined with the use of the retention times of a series of *n*-alkanes (C5–C25) that eluted over the whole span of the chromatogram and calculated according to the method of van den Dool and Kratz (1963).

Data analysis

Data from field tests were analyzed with analysis of variance (ANOVA); significant ANOVAs were then followed by mean separation with Tukey HSD test. When necessary, data were square root ($x + 0.5$)-transformed to stabilize variance prior to analysis. For field tests 1, 2, and 4, the individual trap captures with α-copaene and quercivorol lures were summed and compared to the captures obtained with the combination lure; analysis by *t* test was then used to assess for synergistic responses (defined as significantly higher captures with the combination lure as compared to summed captures with lures deployed separately). All analyses were conducted using Systat Software (2013). Unless noted otherwise, results are presented as mean ± SEM; probability was considered significant at a critical level of $\alpha = 0.05$.

Results

Field tests

In all field tests, the combination of quercivorol and α-copaene captured significantly more *E.* nr. *fornicatus* than other treatments (test 1: $F_{3,16} = 44.27$, $P < 0.001$, Fig. 1a; test 2: $F_{3,16} = 46.16$, $P < 0.001$, Fig. 1c; test 3: $F_{2,12} = 180.67$, $P < 0.001$, Fig. 1e; and test 4: $F_{5,24} = 31.79$, $P < 0.001$, Fig. 2). The single quercivorol and α-copaene treatments resulted in equivalent captures in field tests 1 and 2 (Fig. 1a, c); but in field test 4, the quercivorol mini-bubble captured more beetles than α-copaene (Fig. 2). Field test 4 also provided a direct comparison of the two quercivorol formulations, indicating there was no decrease in mean captures of *E.* nr. *fornicatus* with the reduced dose emitted by the mini-bubble; this was observed with quercivorol lures deployed individually or in combination with α-copaene (Fig. 2). In field test 1, the combination of quercivorol and α-copaene resulted in an additive effect on captures of *E.* nr. *fornicatus*, but no synergistic increase ($t = 2.090$, d$f = 8$, $P = 0.070$). However, a synergistic increase in captures was observed with the combination lure in field tests 2 and 4, including both formulations of quercivorol (test 2: $t = 2.915$, d$f = 8$, $P = 0.019$; test 4, high-dose: $t = 2.338$, d$f = 8$, $P = 0.010$; test 4, low-dose: $t = 2.349$, d$f = 8$, $P = 0.047$).

When summed captures were plotted by week, all three lures reflected similar population trends during field test 1. The combination lure and the α-copaene lure captured comparable numbers of beetles up through week 12; however, the quercivorol lure appeared to lose efficacy after week 9 (Fig. 1b). In field test 2, quercivorol and α-copaene-baited traps caught similar numbers of *E.* nr. *fornicatus* throughout the test, but captures were no different than those of the unbaited control during the final week of the experiment.

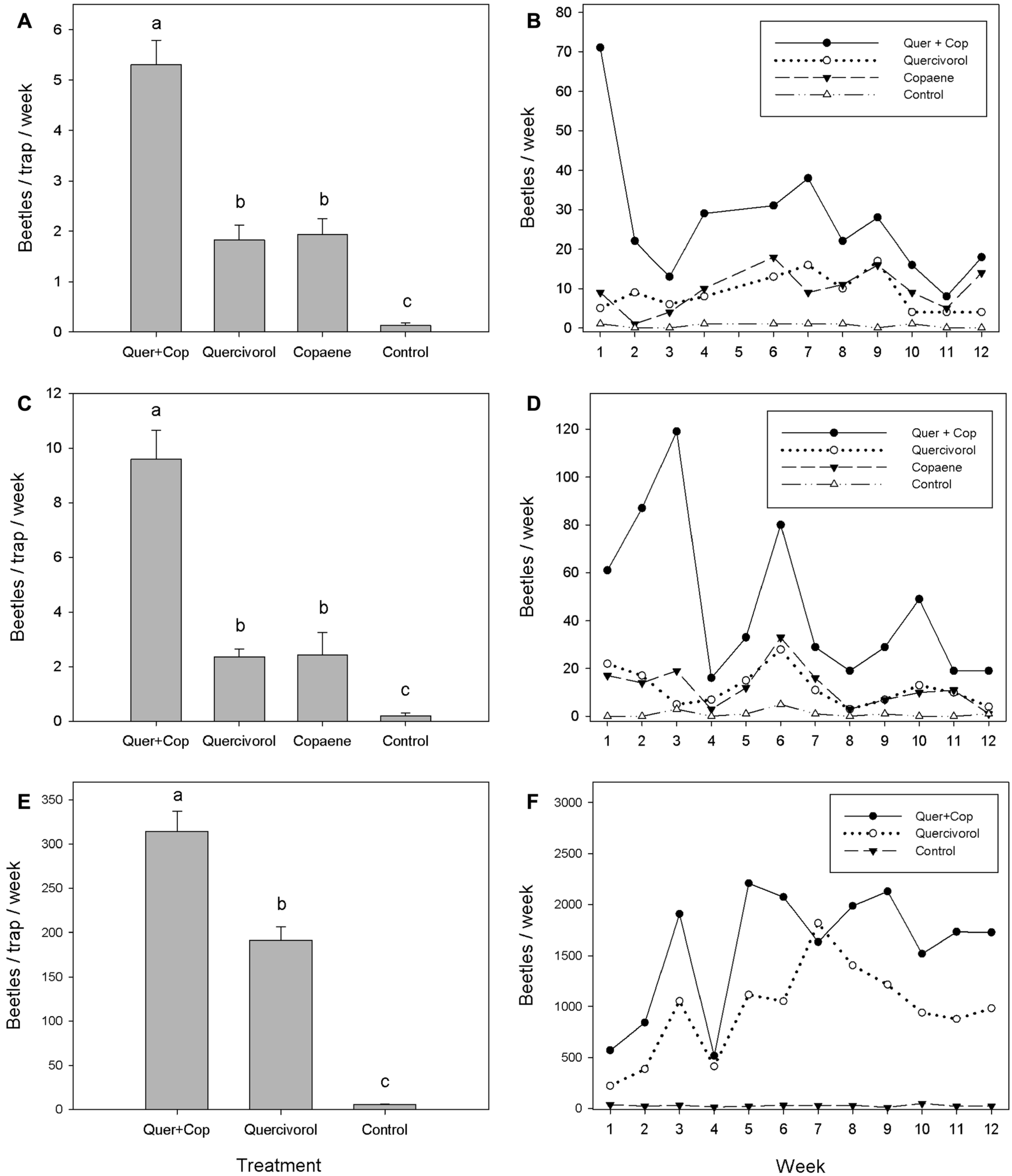


Fig. 1 Captures of female *Euwallacea* nr. *fornicatus* in three 12-week field tests conducted in Miami-Dade County, Florida, USA. Mean (± SEM) captures (**a**) and weekly captures (**b**) obtained in field test 1 (September 8–December 1, 2016). Mean (± SEM) captures (**c**) and weekly captures (**d**) obtained in field test 2 (October 20, 2016–January 12, 2017). Mean (± SEM) captures (**e**) and weekly captures (**f**) obtained in field test 3 (February 17–May 12, 2017). Lure treatments consisted of quercivorol, α-copaene, and a combination of the two. The control consisted of an unbaited trap. Bars topped with the same letter are not significantly different (Tukey's HSD mean separation, $P < 0.05$)

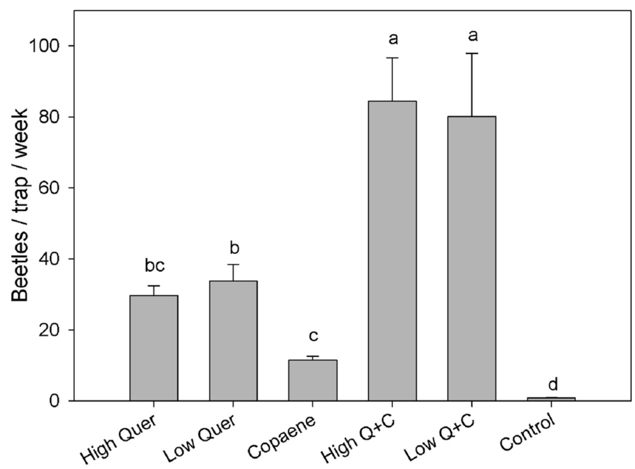


Fig. 2 Mean (± SEM) captures of female *Euwallacea* nr. *fornicatus* in a 6-week field test (test 4, May 16–June 27, 2017) conducted in Miami-Dade County, Florida, USA. Lure treatments consisted of high-dose and low-dose quercivorol, deployed separately and in combination with α-copaene, and α-copaene alone. The control consisted of an unbaited trap. Bars topped with the same letter are not significantly different (Tukey's HSD mean separation, $P < 0.05$)

The combination lure captured the highest number of beetles at every weekly sampling point, and indicated three flight peaks during the 12-week test (Fig. 1d). In field test 3, captures with the quercivorol mini-bubble were less than those of the combination, but the low-dose lure achieved sustained high captures of *E.* nr. *fornicatus* for the duration of the test (Fig. 1f). The *E.* nr. *fornicatus* populations observed in field tests 3 and 4 were much greater than any documented previously in Florida avocado groves (Carrillo et al. 2016; Kendra et al. 2017).

Non-target bark and ambrosia beetle captures were variable, depending on the field site (Table 1). In field test 1, conducted in a healthy well-maintained grove, non-target captures were relatively low, comprising 15.6% of the total captures. The predominant non-target scolytine was *Ambrosiodmus devexulus* (Wood), and it was captured more frequently in traps baited with α-copaene or the combination of α-copaene and quercivorol ($F_{3,16} = 15.230$, $P < 0.001$, Fig. 3a).

In field test 2, greater numbers of non-targets were captured due to the presence of numerous dead and laurel wilt-infected avocado trees adjacent to the study site. Although *E.* nr. *fornicatus* was the dominant species, 59.2% of the total captures were non-target beetles. Species caught in significant numbers included *A. devexulus*, *Xyleborinus saxesenii* (Ratzeburg), *Xyleborus affinis* Eichhoff, *Xyleborus bispinatus* Eichhoff, *Xyleborus volvulus* (Fabricius), and *Xylosandrus crassiusculus* (Motschulsky). However, only captures of *A. devexulus* varied among treatments, with the highest captures obtained in traps baited with α-copaene

Table 1 Scolytinae and Platypodinae (Coleoptera: Curculionidae) captured in field tests conducted in three avocado groves in Miami-Dade County, Florida, USA

Species	Test 1	Test 2	Test 3
Subfamily Scolytinae			
Tribe Xyleborini			
Ambrosiodmus devexulus (Wood)	129	96	79
Ambrosiodmus lecontei Hopkins	5	4	2
Euwallacea nr. *fornicatus* Eichhoff	1037	860	27,914
Premnobius cavipennis Eichhoff	2	0	10
Theoborus ricini (Eggers)	15	1	220
Xyleborinus andrewesi (Blandford)	0	5	8
Xyleborinus gracilis (Eichhoff)	8	4	17
Xyleborinus saxesenii (Ratzeburg)	1	199	35
Xyleborus affinis Eichhoff	1	102	21
Xyleborus bispinatus Eichhoff	6	138	11
Xyleborus ferrugineus (Fabricius)	0	0	3
Xyleborus glabratus Eichhoff	0	2	1
Xyleborus volvulus (Fabricius)	2	448	17
Xylosandrus compactus (Eichhoff)	0	1	5
Xylosandrus crassiusculus (Motschulsky)	1	118	6
Tribe Cryphalini			
Hypothenemus spp.	21	37	78
Subfamily Platypodinae			
Euplatypus parallelus (Fabricius)	0	95	0

($F_{3,16} = 7.347$, $P = 0.003$, Fig. 3b). Two *X. glabratus* were also captured in treatments containing α-copaene. In addition, this test included captures of the platypodine ambrosia beetle, *Euplatypus parallelus* (Fabricius), a species not encountered at the other two sites.

In field test 3, non-target scolytines comprised a very low percentage of the captures (only 1.8%), due in part to the extremely large numbers of *E.* nr. *fornicatus* present, and in part to the lack of dead and moribund trees which could serve as potential breeding substrates for secondary colonizers. As observed in previous field tests, more *A. devexulus* were captured with traps that included α-copaene ($F_{2,12} = 6.058$, $P = 0.015$, Fig. 3c), and this attractant also resulted in detection of a single *X. glabratus*. More *Theoborus ricini* (Eggers) were caught with the quercivorol and combination treatments than with the control treatment ($F_{2,12} = 20.467$, $P < 0.001$, Fig. 3d), and captures of this species were much greater than in previous tests.

Temporal analysis of lure emissions

Volatile emissions from the α-copaene lure decreased rapidly for the first 4 weeks after field exposure, but then stabilized at a low but constant release rate for the duration of

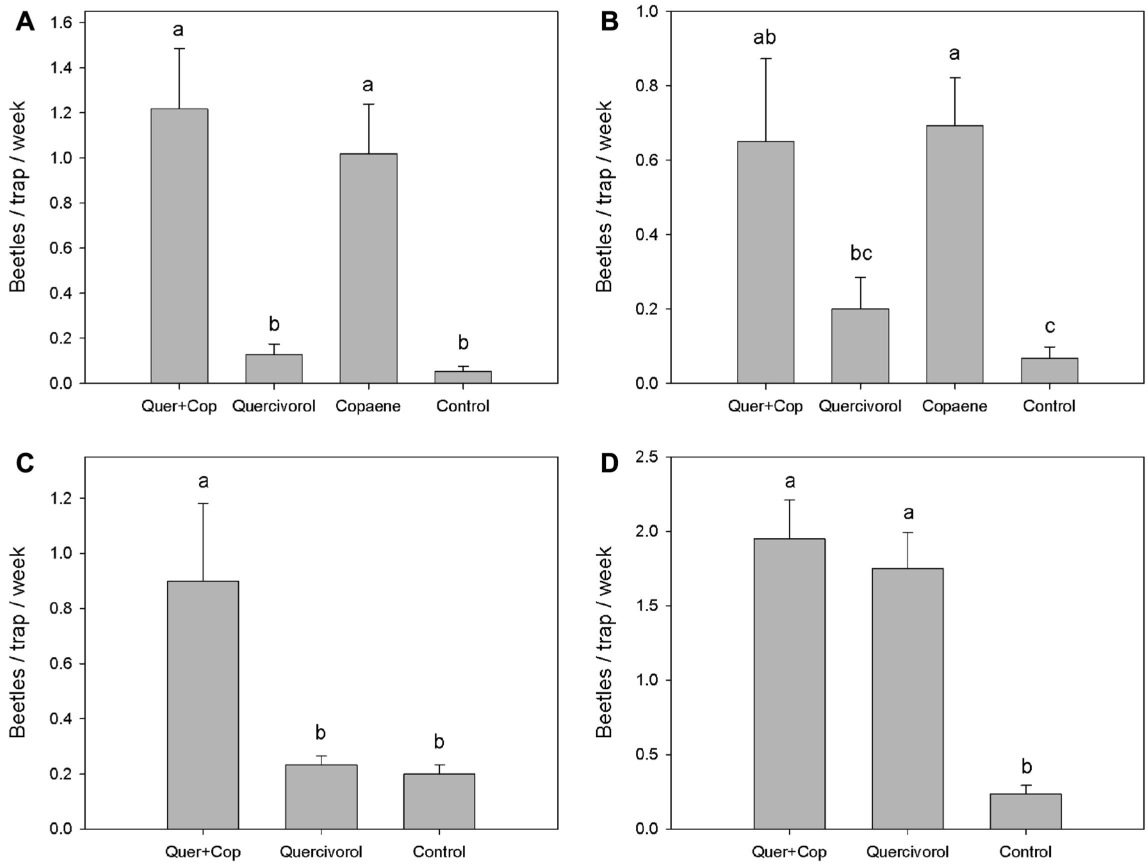


Fig. 3 Mean (± SEM) captures of female *Ambrosiodmus devexulus* in field test 1 (**a**), field test 2 (**b**), field test 3 (**c**), and of female *Theoborus ricini* in field test 3 (**d**). Lure treatments consisted of quercivorol, α-copaene, and a combination of the two. The control consisted of an unbaited trap. Bars topped with the same letter are not significantly different (Tukey's HSD mean separation, $P < 0.05$)

the 12 week period. α-Copaene emissions were best fit with an exponential decay regression model, $y = 47.70e^{(-0.08x)}$, $R^2 = 0.92$ (Fig. 4a). In contrast, emissions from the first set of quercivorol lures (high-dose, lot # 160215, used in field test 1) decreased steadily for 7 weeks after field exposure, and the dispensers appeared to be depleted of oil by the end of week 8, at which point quercivorol could no longer be detected with our sampling methods. Quercivorol emissions were best fit with the exponential decay model $y = 20.52e^{(-0.04x)}$, $R^2 = 0.89$ (Fig. 4b). Mean daily environmental conditions during this first sampling period were as follows—temperature (°C): mean = 25.67 ± 0.28, high = 30.16 ± 0.25, low = 21.59 ± 0.34; wind speed (m/s): mean = 3.05 ± 0.16, high = 7.16 ± 0.23; precipitation (cm): mean = 0.55 ± 0.14.

With the second set of quercivorol lures (high-dose, lot # 160511, used in field tests 2 and 4), emissions again dropped rapidly over the first 7–8 weeks, but could still be detected at very low levels during the final 3 weeks of analysis. Emissions from these lures followed the exponential decay model $y = 16.82e^{(-0.05x)}$, $R^2 = 0.89$ (Fig. 4c). Mean daily environmental conditions during the second sampling period were as follows—temperature (°C): mean = 22.21 ± 0.32, high = 26.89 ± 0.26, low = 17.42 ± 0.43; wind speed (m/s): mean = 3.20 ± 0.18, high = 7.10 ± 0.25; precipitation (cm): mean = 0.14 ± 0.04.

The change to a low-dose, small diameter bubble (used in field tests 3 and 4) resulted in sustained, very low-level emissions of quercivorol, again best described by an exponential decay model, $y = 0.60e^{(-0.02x)}$, $R^2 = 0.83$ (Fig. 4d). Emission rates quantified from the mini-bubble lures were comparable to those observed with the high-dose lures (second batch) during their last 4 weeks of field deployment. Mean daily environmental conditions during the third sampling period were as follows—temperature (°C): mean = 23.12 ± 0.26, high = 28.33 ± 0.22, low = 18.15 ± 0.40; wind speed (m/s): mean = 3.74 ± 0.20, high = 7.53 ± 0.20; precipitation (cm): mean = 0.29 ± 0.13.

Analysis of lure contents

Analysis by GC-FID and GC–MS indicated that the 50% α-copaene lure contained 12 sesquiterpenoids (Table 2). Although α-copaene was the primary sesquiterpene (mean

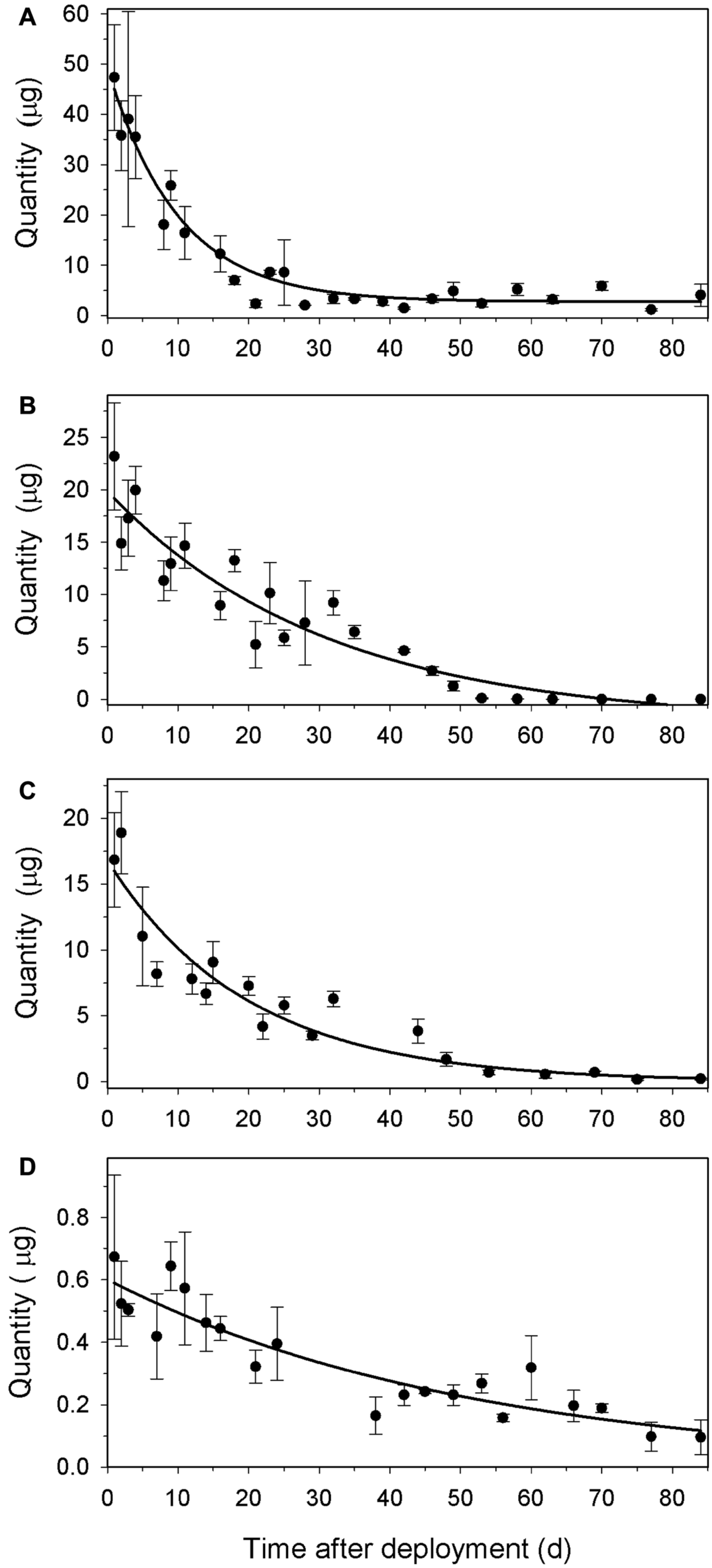


Fig. 4 Mean (± SEM) volatile emissions quantified from lures containing **a** 50% α-copaene oil, **b** high-dose quercivorol (used in field test 1), **c** high-dose quercivorol (field tests 2 and 4), and **d** low-dose quercivorol (field tests 3 and 4). For quercivorol lures, quantification is of *trans-p*-menth-2-en-1-ol, the dominant isomers. All lures were aged in the field for 12 weeks and periodically brought into the laboratory for volatile collections (super-Q adsorbant, 15 min) and analysis by GC–MS

Table 2 Mean (± SD) chemical composition of contents in the α-copaene-enriched lures ($n = 3$)

AI[a]	AI$_{\text{Lit}}$[b]	Compound	% Area$_{\text{FID}}$[c]	Identification method[d]
1324	1335	δ-Elemene	0.61 ± 0.01	MS
1340	1345	α-Cubebene	2.67 ± 0.25	MS
1379	1374	α-Copaene	51.26 ± 0.29	MS, RI, std
1384	1387	β-Cubebene	2.13 ± 0.60	MS
1399	1389	β-Elemene	1.45 ± 0.03	MS, RI, std
1417	1417	β-Caryophyllene	22.47 ± 0.22	MS, RI, std
1446	1452	α-Humulene	4.24 ± 0.03	MS, RI, std
1449	1458	Alloaromadendrene	1.42 ± 0.06	MS, RI, std
1466	1478	γ-Muurolene	1.58 ± 0.04	MS
1489	1500	α-Muurolene	1.05 ± 0.06	MS
1513	1522	δ-Cadinene	9.86 ± 0.04	MS
1566	1582	Caryophyllene oxide	1.25 ± 0.25	MS, RI, std

[a]Arithmetic retention indices calculated against *n*-alkanes

[b]Arithmetic retention indices from Adams Library (2007)

[c]Percent composition calculated from FID data

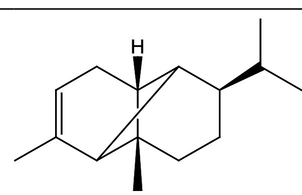
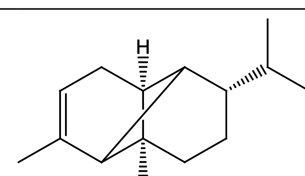
[d]MS = identification based on computer matching of the mass spectra with those of the NIST library (2017), FFNSC 3 (2015), Adams Library (2007), and comparison with literature data (Kendra et al. 2011, 2017; Carroll et al. 2011; Blythe et al. 2016); RI = identification by comparison with Retention Index of authentic compounds on DB-5MS column; std = identification by comparison of the retention time and mass spectrum

content 51.3%), β-caryophyllene was also abundant, constituting 22.5% of the oil. Other major sesquiterpene hydrocarbons included δ-cadinene, α-humulene, and α- and β-cubebene. Caryophyllene oxide was the only oxygenated sesquiterpene found in the lure. α-Copaene is a tricyclic sesquiterpene hydrocarbon and has two enantiomers (Table 3). To determine the correct peak assignment in chiral analyses, authenticated (−)-α-copaene (Fluka Chemical Co.) was used as a reference; retention times (R_t) of enantiomers were 27.73 min for (+)-α-copaene and 27.85 min for (−)-α-copaene, with a distribution of 5.72% (+)-α-copaene and 94.28% (−)- (Table 3). Additional confirmation was obtained by analysis of the essential oil from *Cedrela odorata*, previously reported to contain 8% (+)- and 92% (−)- α-copaene (Hardt et al. 1995). Our HS-SPME-GC enantio analysis of *C. odorata* volatiles indicated 3.62% (R_t: 27.76 min) for (+)-enantiomer and 96.38% (R_t: 27.87 min) for (−)-enantiomer. Once the correct peak assignment had been made, contents of the α-copaene lure were co-injected and comparison with reference chromatograms confirmed that the (−)-enantiomer was predominant (99.91%).

GC-FID and GC–MS analyses of the quercivorol lure indicated four peaks: two monoterpene hydrocarbons (α- and β-phellandrene) and two monoterpene alcohols (*cis*- and *trans*-*p*-menth-2-en-1-ol) (Table 4). The electron ionization mass spectra (EI MS) of the predominant peak of monoterpene alcohol (88%) showed a molecular ion at *m/z* 154 (M^+ for $C_{10}H_{18}O$, 8) with characteristic fragment peaks at *m/z* 43 (C_3H_7, 55), 55 (18), 69 (36), 71 (23), 77 (36), 79 (27), 81 (27), 83 (18), 84 (14), 91 (45), 93 ($M–C_3H_7–H_2O$, 100), 95 (9), 97 (5), 107 (6), 111 ($M–C_3H_7$, 45), 121 ($M–CH_3–H_2O$, 55), 136 ($M–H_2O$, 45), and 139 ($M–CH_3$, 73). The less abundant isomer (9%) possessed very similar MS with the following *m/z* peaks: 43 (55), 55 (9), 69 (27), 71 (36), 77 (36), 79 (45), 81 (23), 83 (18), 84 (18), 91 (45), 93 (100), 95 (36), 97 (9), 107 (6), 111 (36), 121 (29), 136 (32), 139 (55), and 154 (M^+ 5 for $C_{10}H_{18}O$, 8).

p-Menth-2-en-1-ol has stereoisomers at C1 and C4, resulting in four diastereoisomers [(−)-(1*S*, 4*R*)-4-isopropyl-1-methyl-2-cyclohexen-1-ol, (+)-(1*R*, 4*S*)-4-isopropyl-1-methyl-2-cyclohexen-1-ol, (−)-(1*S*, 4*S*)-4-isopropyl-1-methyl-2-cyclohexen-1-ol, and

Table 3 Mean (± SD) enantiomeric distribution of α-copaene in the α-copaene-enriched lures ($n = 3$) using an R_t-βDEXse column

Samples	 (+)-α-copaene R_t: 27.75 AI: 1427	 (−)-α-copaene R_t: 27.90 AI: 1431
α-Copaene lure	0.09 ± 0.01	99.91 ± 0.01
Reference samples		
(−)-α-copaene from Fluka	5.72 ± 0.29	94.28 ± 0.29
Cedrela odorata	3.62 ± 1.56	96.38 ± 1.56

R_t, retention time; AI, arithmetic retention index calculated against *n*-alkanes using R_t-βDEXse column

Table 4 Mean (± SD) chemical composition of contents in quercivorol lures ($n = 3$, lot #160726)

AI^a	AI^b_{Lit}	Compound	% $Area^c_{FID}$	Identification method[d]
1008	998	α-Phellandrene	1.42 ± 0.25	MS, RI, std
1029	1025	β-Phellandrene	1.57 ± 0.27	MS, RI
1121	1118	*cis*-*p*-menth-2-en-1-ol	8.93 ± 0.17	MS, RI
1142	1136	*trans*-*p*-menth-2-en-1-ol	88.09 ± 0.36	MS, RI

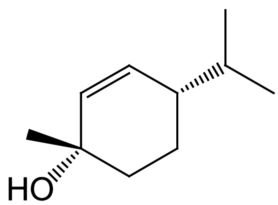
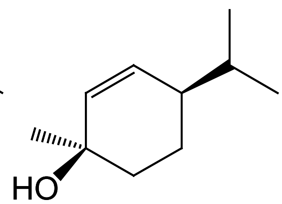
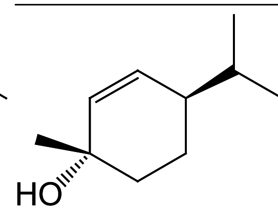
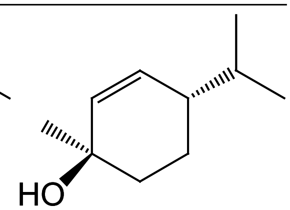
[a]Arithmetic retention indices calculated against *n*-alkanes

[b]Arithmetic retention indices from Adams Library (2007)

[c]Percent composition calculated from FID data

[d]MS = identification based on computer matching of the mass spectra with those of the NIST library (2017), FFNSC 3 (2015), Adams Library (2007); RI = identification by comparison of Retention Index with authentic compounds on DB-5MS column; std = identification by comparison of the retention time and mass spectrum of authentic standard

Table 5 Mean (± SD) enantiomeric distribution of *p*-menth-2-en-1-ol in quercivorol lures ($n = 3$) using an R_t-βDEXse column

Samples	*cis*- *p*-menth-2-en-1-ol		*trans*-*p*-menth-2-en-1-ol	
	 (−)-(1*S*, 4*S*)-4-isopropyl-1-methyl-2-cyclohexen-1-ol $R_{t:}$ 40.75 AI: 1344	 (+)-(1*R*, 4*R*)-4-isopropyl-1-methyl-2-cyclohexen-1-ol $R_{t:}$ 40.86 AI: 1346	 (−)-(1*S*, 4*R*)-4-isopropyl-1-methyl-2-cyclohexen-1-ol $R_{t:}$ 42.26 AI: 1367	 (+)-(1*R*, 4*S*)-4-isopropyl-1-methyl-2-cyclohexen-1-ol $R_{t:}$ 42.41 AI: 1369
Quercivorol lure	44.62 ± 1.74	55.38 ± 1.74	45.37 ± 1.63	54.63 ± 1.63
Reference oil (HTEO)	–	100 ± 0	–	100 ± 0

R_t retention time; AI arithmetic retention index calculated against *n*-alkanes using R_t-βDEXse column

(+)-(1*R*, 4*R*)-4-isopropyl-1-methyl-2-cyclohexen-1-ol] (Table 5). In this paper, *cis* (*Z*)- and *trans* (*E*)- configuration are defined by the priority of the functional groups attached to the double-bonded atoms, according to the sequence rules of Cahn et al. (1966); therefore, (1*S*, 4*R*)- and (1*R*, 4*S*)- are *trans*- as the OH is higher than CH_3 and isopropyl group higher than H, and (1*S*, 4*S*)-, (1*R*, 4*R*)- are *cis* isomers. With enantiomeric separation of the lure contents, achieved by GC with a chiral β-cyclodextrin capillary column (Rt-βDEXse), the predominant peak was observed to elute after the less abundant isomer. Since pure enantiomers are not available, the elution order of isomers was determined by using a natural source, *Haplophyllum tuberculatum* essential oil (HTEO), shown previously to contain *trans*-*p*-menth-2-en-1-ol (19.2%) and *cis*-*p*-menth-2-en-1-ol (13.2%) (Al-Rehaily et al. 2014). Based on the SciFinder database, HTEO consists of (1*R*, 4*R*)-*p*-menth-2-en-1-ol and (1*R*, 4*S*)-*p*-menth-2-en-1-ol enantiomers (Chemical Abstracts Service, https://scifinder.cas.org). With HTEO, retention times (R_t) of 40.86 min and 42.45 min were obtained for 100% (1*R*, 4*R*)-*p*-menth-2-en-1-ol and 100% (1*R*, 4*S*)-*p*-menth-2-en-1-ol, respectively. Analysis of the quercivorol lure under the same conditions yielded equivalent R_t values [40.86 and 42.42 min, assigned to (1*R*, 4*R*)- and (1*R*, 4*S*)-*p*-menth-2-en-1-ol, respectively], as well as R_t values of 40.75 and 42.26 min, assigned to (1*S*, 4*S*)-, and (1*S*, 4*R*)-*p*-menth-2-en-1-ol, respectively (Table 5). Based on results with the HTEO reference oil, we tentatively identified *p*-menth-2-en-1-ol isomers of the quercivorol lure, in order of elution, as (1*S*, 4*S*)-, (1*R*, 4*R*)-*cis*-*p*-menth-2-en-1-ol and (1*S*, 4*R*)-, (1*R*, 4*S*)-*trans*-*p*-menth-2-en-1-ol. Further support will require isolation of individual enantiomers to determine absolute configurations through a combination of nuclear magnetic resonance (NMR) techniques, circular dichroism (CD), and optical rotation.

Discussion

The combination of α-copaene and quercivorol lures is the most effective attractant identified to date for *E.* nr. *fornicatus* in Florida. In all four field trials, the combination captured significantly more beetles than the current standard, quercivorol alone, supporting results obtained previously in a series of shorter field tests (Kendra et al. 2017). Moreover, the present study indicated that the dual lure has a field life of at least 12 weeks under south Florida conditions, due to extended low release of volatiles from the bubble lure formulations. At the two sites with limited availability of dead trees, the combination lure also demonstrated good specificity, with *E.* nr. *fornicatus* comprising 86.1 and 98.2% of the total bark and ambrosia beetles captured (field tests 1 and 3, respectively). Sixteen non-target species were intercepted, but the majority were caught in comparable numbers with all treatments (including the unbaited control) and thus regarded as random, passive captures. Only *A. devexulus* was captured in higher numbers with α-copaene, and *T. ricini* demonstrated attraction to quercivorol.

The enhanced attraction of *E.* nr. *fornicatus* to the lure combination is likely the result of the two semiochemicals representing different critical resources for this species. We propose that, as with *X. glabratus* (Kendra et al. 2014a, 2016a; Owens et al. 2017), (−)-α-copaene is a primary kairomone that plays a dominant role in the host location process of dispersing female *E.* nr. *fornicatus.* This appears to be the case with avocado cv 'Donnie,' a preferred host, which has been found to contain high levels of α-copaene in GC–MS analyses of rasped wood (Owens et al. 2018). In addition, females may utilize the proximo-distal gradients in α-copaene (Niogret et al. 2013) to concentrate their attacks at the base of avocado branches. Although one isomer of *p*-menth-2-en-1-ol is an aggregation pheromone for *P. quercivorus*, and produced by that species (Kashiwagi et al. 2006; Tokoro et al. 2007), there is no evidence that any members of the *E.* nr. *fornicatus* complex synthesize this compound. It is proposed to be a fungal volatile emitted by *Fusarium* spp. symbionts (Cooperband et al. 2017). As such, *p*-menth-2-en-1-ol may function as a food attractant for female *E.* nr. *fornicatus*. A combination of (−)-α-copaene and *p*-menth-2-en-1-ol may provide a strong signal not only of an appropriate host species, but also of one at the proper stage to support growth of nutritional fungi. This is analogous to what has been reported for *X. glabratus*, where volatiles from the *Raffaelea* symbiont were synergistic with host-based odors to increase captures of in-flight females (Kuhns et al. 2014).

The commercial α-copaene lure contains an essential oil product enriched in this sesquiterpene through fractional distillation (Kendra et al. 2016a). Our analysis of the oil indicated > 50% α-copaene content, with 99.9% comprised of the (−)-enantiomer, but there are 11 other terpenoid constituents in this oil. After (−)-α-copaene, the most abundant compound is β-caryophyllene (22.5%), but there are appreciable quantities of other sesquiterpenes, including δ-cadinene (9.9%), α-humulene (4.2%), and α-cubebene (2.7%). All of these are hypothesized to be components of the host bouquet detected by female *X. glabratus* (Kendra et al. 2011, 2014a, 2015b, 2016a; Niogret et al. 2011). Given the similarities between *X. glabratus* and *E.* nr. *fornicatus* (Kendra et al. 2017), these compounds should be evaluated as potential attractants (alone or as synergists) for *E.* nr. *fornicatus.* It is also worth noting that the combination lure, with sustained α-copaene emissions, is effective for sensitive detection of *X. glabratus* (field tests 2 and 3), despite the rarity of this species in agro-ecosystems (Carrillo et al. 2012; Kendra et al. 2015a). Therefore, the two-component lure serves a dual purpose for detection of both disease vectors for at least 3 months in Florida.

Analysis of the quercivorol lure indicated that the predominant component is *trans*-*p*-menth-2-en-1-ol (88%). This determination, based on the sequence rules established by Cahn et al. (1966), is consistent with identification of the *trans*-isomer as the aggregation pheromone for *P. quercivorus* (Kashiwagi et al. 2006). [Note: There is a lack of consensus regarding nomenclature; other authors refer to (1*S*, 4*R*)-*p*-menth-2-en-1-ol as a *cis*-isomer (Mori 2006; Tokoro et al. 2007; Blair and Tuck 2009)]. However, our chiral analysis indicated that the *trans*-isomer component is comprised of 45% (1*S*, 4*R*)- and 55% (1*R*, 4*S*)-enantiomers, so it is not clear which enantiomer is attractive to *Euwallacea* spp.; it may not be the (1*S*, 4*R*)-enantiomer attractive to *P. quercivorus*. Although reformulated recently to a low-dose mini-bubble lure (in response to dose effects reported for the California species; Dodge et al. 2017), there was no decrease in efficacy for attraction of Florida *E.* nr. *fornicatus*, as documented by captures in field test 4. This suggests that female *E.* nr. *fornicatus* can detect *p*-menth-2-en-1-ol at very low levels, consistent with electroantennographic analyses of dose-dependent olfactory responses recorded by Kendra et al. (2017). Release rates are important in modulating insect behavior; host- or food-based attractants may become repellent if present at high concentrations (e.g., ammonia, a protein feeding cue; Bateman and Morton 1981; Kendra et al. 2005); this appears to apply to PSHB and KSHB with higher doses of *p*-menth-2-en-1-ol (Dodge et al. 2017). Although emissions of *p*-menth-2-en-1-ol were detected for 12 weeks with two batches of quercivorol lures, one batch of lures lost efficacy after 8–9 weeks. This may have been due to higher mean temperatures and precipitation during this sampling period (August–November 2016), which contributed to more rapid elution of volatiles. Despite a shorter field life of the quercivorol component, the lure combination captured *E.* nr. *fornicatus* for the duration of the 12 week test (field test 1).

Ethanol is another semiochemical that warrants further investigation for *E.* nr. *fornicatus* in Florida. Ethyl alcohol, a common attractant for secondary ambrosia beetles (Miller and Rabaglia 2009), was found to be repellent for species in California, decreasing captures when combined with quercivorol lures (Dodge et al. 2017). In contrast, low-dose ethanol was shown to be a weak attractant for Florida *E.* nr. *fornicatus* (Carrillo et al. 2015; Kendra et al. 2015a), and may potentially synergize attraction when added to the two-component lure. Ethanol is also reported to be an attractant for TSHB in Asia (Karunaratne et al. 2008), a species to which Florida *E.* nr. *fornicatus* is proposed to be more closely related than PSHB and KSHB (Stouthamer et al. 2017). However, incorporation of ethanol is likely to increase capture of non-target ambrosia beetles (Kendra et al. 2014b; Owens et al. 2017).

Although our tests were conducted in avocado groves, the combination of α-copaene and quercivorol lures will be highly effective for early detection of *E.* nr. *fornicatus* in other susceptible ecosystems, including forests and urban settings. For example, while surveying an avocado grove at the University of Florida, Homestead (Carrillo et al. 2016), large numbers of *E.* nr. *fornicatus* were caught with the two-component lure, but no infested avocado trees could be found in near proximity. Inspection of an adjacent woodland revealed infestations of *E.* nr. *fornicatus* in several trees, which led to the discovery of two new host species: *Lysiloma latisiliquum* and *Albizia lebbeck* (L.) Bentham (Fabaceae) (Owens et al. 2018). Area-wide surveys with this improved detection system are needed to determine the beetle's host range and to assess risk posed to both native and ornamental flora in Florida. With *X. glabratus*, the pathway of spread progressed from north to south via native trees (several *Persea* species), bringing laurel wilt to the avocado production area in southernmost Florida (Kendra et al. 2013; Ploetz et al. 2017b). With *E.* nr. *fornicatus*, the pathway is likely to proceed in the opposite direction. Large breeding populations in the avocado monocultures of Miami-Dade County may provide a source of dispersing females that can colonize nearby trees in natural and urban areas. Since avocado is a common backyard fruit tree throughout the state (Carrillo et al. 2012), this host may facilitate northward invasions. In addition, global commerce (anthropogenic transport of infested material) may provide another pathway for spread of adventive ambrosia beetles from Florida to neighboring regions. Based on our initial reports (Kendra et al. 2016a, 2017), SAGARPA (Secretaría de Agricultura, Ganadería, Desarrollo Rural, Pesca y Alimentación) in Mexico is now using the two-component lure in survey programs for *E.* nr. *fornicatus* and *X. glabratus* in high-risk areas (ports, international borders, and avocado production regions).

Since only females of *Euwallacea* spp. engage in flight, these pests are good candidates for population suppression through mass trapping, particularly in managed groves and urban environments. Feasibility of this strategy requires a highly attractive long-lasting lure, knowledge of the lure's effective attraction radius, and an inexpensive trap (Byers et al. 2017). Based on evaluations of the same quercivorol product used in our studies, Byers et al. (2017) concluded that this single bait, deployed in sticky traps, meets the criteria for mass trapping of PSHB, especially in the early spring prior to peak female dispersal. With *E.* nr. *fornicatus* in Florida, the two-component lure is more attractive than quercivorol alone, and therefore should be appropriate for mass trapping. Efficacy of this approach may potentially be increased by implementing a 'push–pull' system (Gillette et al. 2012)—combining a repellent (e.g., verbenone, Hughes et al. 2017) to 'push' beetles away from susceptible hosts along with a strong attractant to 'pull' beetles toward the traps—but this needs to be confirmed through field evaluations.

In conclusion, better detection of pest *E.* nr. *fornicatus* in Florida is accomplished using a combination of commercially available α-copaene and quercivorol lures. This two-component bait has field longevity of 12 weeks, attracts low numbers of non-target bark and ambrosia beetles, and has potential application for pest suppression by mass trapping. Further experimental research is needed to determine (1) which isomer(s) of *p*-menth-2-en-1-ol is attractive to Florida *E.* nr. *fornicatus*, (2) if ethanol can supplement the two-component lure to further improve pest detection, and (3) if (−)-α-copaene is attractive to other members of this cryptic species complex, in which case the two-component lure would constitute a significant improvement for detection of multiple invasive pests.

Author contribution statement

PK, DO, WM, NT, TN, and ES conceived, designed, and conducted the research; PK, NT, and DC contributed materials; PK, DO, NT, ES, and TN analyzed data; and DO, PK, and NT wrote the manuscript.

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Compliance with ethical standards

Conflict of interest All authors declare that they have no conflict of interest.

Ethical approval This article contains field studies that targeted insect pests and did not involve any protected or endangered species.

References

Adams RP (2007) Identification of essential oil components by gas chromatography/mass spectrometry, 4th edn. Allured Publishing Corp, Carol Stream

Ali A, Tabanca N, Demirci B, Baser KHC, Ellis J, Gray S, Lackey BR, Murphy C, Khan IA, Wedge DE (2013) Composition, mosquito larvicidal, biting deterrent and antifungal activity of essential oils of different plant parts of *Cupressus arizonica* var. *glabra* ('Carolina Sapphire'). Nat Prod Commun 8:257–260

Al-Rehaily AJ, Alqasoumi SI, Yusufoglu HS, Al-Yahya MA, Demirci B, Tabanca N, Wedge DE, Demirci F, Bernier UR, Becnel JJ, Temel HE, Baser KHC (2014) Chemical composition and biological activity of *Haplophyllum tuberculatum* Juss. essential oil. J Essent Oil Bear Plant 3:452–549

Atkinson TH, Carrillo D, Duncan RE, Peña JE (2013) Occurrence of *Xyleborus bispinatus* (Coleoptera: Curculionidae: Scolytinae) Eichhoff in southern Florida. Zootaxa 3669:096–100

Bateman MA, Morton TC (1981) The importance of ammonia in proteinaceous attractants for fruit flies (family: Tephritidae). Aust J Agric Res 32:883–903

Blair M, Tuck KL (2009) A new diastereoselective entry to the (1*S*, 4*R*)- and (1*S*, 4*S*)-isomers of 4-isopropyl-1-methyl-2-cyclohexen-1-ol, aggregation pheromones of the ambrosia beetle *Platypus quercivorus*. Tetrahedron Asymmetry 20:2149–2153

Blythe EK, Tabanca N, Demirci B, Tsikolia M, Bloomquist JR, Bernier UR (2016) *Lantana montevidensis* essential oil: chemical composition and mosquito repellent activity against *Aedes aegypti*. Nat Prod Commun 11:1713–1716

Boland JM (2016) The impact of an invasive ambrosia beetle on the riparian habitats of the Tijuana River Valley, California. PeerJ 4:e2141

Brar GS, Capinera JL, McLean S, Kendra PE, Ploetz RC, Peña JE (2012) Effect of trap size, trap height and age of lure on sampling *Xyleborus glabratus* (Coleoptera: Curculionidae: Scolytinae), and its flight periodicity and seasonality. Florida Entomol 95:1003–1011

Byers JA, Maoz Y, Levi-Zada A (2017) Attraction of the *Euwallacea* sp. near *fornicatus* (Coleoptera: Curculionidae) to quercivorol and to infestation in avocado. J Econ Entomol 110:1512–1517

Cahn RS, Ingold C, Prelog V (1966) Specification of molecular chirality. Angew Chem Int Ed Engl 5:385–415

Campbell P, Geering A (2011) Biosecurity capacity building for the Australian avocado industry—laurel wilt. In: Proceedings VII world avocado congress 2011 (Actas VII Congreso Mundial del Aguacate 2011). Cairns, Australia. 5–9 September 2011. http://www.avocadosource.com/WAC7/Section_03/CampbellPaul2011.pdf

Carrillo D, Duncan RE, Peña JE (2012) Ambrosia beetles (Coleoptera: Curculionidae: Scolytinae) that breed in avocado wood in Florida. Florida Entomol 95:573–579

Carrillo D, Narvaez T, Cossé AA, Stouthamer R, Cooperband M (2015) Attraction of *Euwallacea* nr. *fornicatus* (Coleoptera: Curculionidae: Scolytinae) to lures containing quercivorol. Florida Entomol 98:780–782

Carrillo D, Cruz LF, Kendra PE, Narvaez TI, Montgomery WS, Monterroso A, De Grave C, Cooperband MF (2016) Distribution, pest status, and fungal associates of *Euwallacea* nr. *fornicatus* in Florida avocado groves. Insects 7:55

Carroll JF, Tabanca N, Kramer M, Elejalde NM, Wedge DE, Bernier UR, Coy M, Becnel JJ, Demirci B, Baser KHC, Zhan J, Zhan S (2011) Essential oils of *Cupressus funebris*, *Juniperus communis*, and *J. chinensis* (Cupressaceae) as repellents against ticks (Acari: Ixodidae) and mosquitoes (Diptera: Culicidae) and as toxicants against mosquitoes. J Vector Ecol 36:258–268

Chemical Abstracts Service. https://scifinder.cas.org. Accessed on 26 Jan 2018

Cooperband MF, Stouthamer R, Carrillo D, Eskalen A, Thibault T, Cossé AA, Castrillo LA, Vandenberg JD, Rugman-Jones PF (2016) Biology of two members of the *Euwallacea fornicatus* species complex (Coleoptera: Curculionidae: Scolytinae), recently invasive in the USA, reared on an ambrosia beetle artificial diet. Agric For Entomol 18:223–237

Cooperband MF, Cossé AA, Jones TH, Carrillo D, Cleary K, Canlas I, Stouthamer R (2017) Pheromones of three ambrosia beetles in the *Euwallacea fornicatus* species complex: ratios and preferences. PeerJ 5:e3957

Danthanarayana W (1968) The distribution and host-range of the shot-hole borer (*Xyleborus fornicatus* Eichh.) of tea. Tea Q 39:61–69

Dodge C, Coolidge J, Cooperband M, Cossé A, Carrillo D, Stouthamer R (2017) Quercivorol as a lure for the polyphagous and Kuroshio shot hole borers, *Euwallacea* spp. nr. *fornicatus* (Coleoptera: Scolytinae), vectors of Fusarium dieback. PeerJ 5:e3656

Eskalen A, Stouthamer R, Lynch SC, Rugman-Jones PF, Twizeyimana M, Gonzalez A, Thibault T (2013) Host range of Fusarium dieback and its ambrosia beetle (Coleoptera: Scolytinae) vector in southern California. Plant Dis 97:938–951

Eskalen A (2017) Known suitable reproductive hosts of shot hole borer-Fusarium dieback in California. http://eskalenlab.ucr.edu/shotholeborerhosts.html. Accessed 28 Jan 2018

FFNSC 3 (2015) Flavors and fragrances of natural and synthetic compounds 3, mass spectral database. Wiley, Hoboken

Fraedrich SW, Harrington TC, Rabaglia RJ, Ulyshen MD, Mayfield AE III, Hanula JL, Eickwort JM, Miller DR (2008) A fungal symbiont of the redbay ambrosia beetle causes a lethal wilt in redbay and other Lauraceae in the southeastern USA. Plant Dis 92:215–224

Freeman S, Protasov A, Sharon M, Mohotti K, Eliyahu M, Okon-Levy N, Maymon M, Mendel Z (2012) Obligate feed requirement of *Fusarium* sp. Nov., an avocado wilting agent, by the ambrosia beetle *Euwallacea* aff. *fornicata*. Symbiosis 58:245–251

García-Avila CDJ, Trujillo-Arriaga FJ, López-Buenfil JA, González-Gómez R, Carrillo D, Cruz LF, Ruiz-Galván I, Quezada-Salinas A, Acevedo-Reyes N (2016) First report of *Euwallacea* nr. *fornicatus* (Coleoptera: Curculionidae) in Mexico. Florida Entomol 99:555–556

Gillette NE, Mehmel CJ, Mori SR, Webster JN, Wood DL, Erbilgin N, Owen DR (2012) The push-pull tactic for mitigation of mountain pine beetle (Coleoptera: Curculionidae) damage in lodgepole and whitebark pines. Environ Entomol 41:1575–1586

Hanula JL, Mayfield AE III, Fraedrich SW, Rabaglia RJ (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

Hardt IH, Rieck A, Fricke C, Koening WA (1995) Enantiomeric composition of sesquiterpene hydrocarbons of the essential oil of *Cedrela odorata* L. Flavour Fragr J 10:165–171

Hazarika LK, Bhuyan M, Hazarika BN (2009) Insect pests of tea and their management. Ann Rev Entomol 54:267–284

Hughes MA, Martini X, Kuhns E, Colee J, Mafra-Neto A, Stelinski LL, Smith JA (2017) Evaluation of repellents for the redbay ambrosia beetle, *Xyleborus glabratus*, vector of the laurel wilt pathogen. J Appl Entomol 141:653–664

Hulcr J, Dunn RR (2011) The sudden emergence of pathogenicity in insect-fungus symbioses threatens naïve forest ecosystems. Proc R Soc B Biol Sci 278:2866–2873

Inch SA, Ploetz RC, Blanchette R, Held B (2012) Histological and anatomical responses in avocado, *Persea americana*, induced by the vascular wilt pathogen, *Raffaelea lauricola*. Botany 90:627–635

Jones ME, Kabashima J, Eskalen A, Dimson M, Mayorquin JS, Carrillo JD, Hanlon CC, Paine TD (2017) Evaluations of insecticides and fungicides for reducing attack rates of a new invasive ambrosia beetle (*Euwallacea* sp., Coleoptera: Curculionidae: Scolytinae) in infested landscape trees in California. J Econ Entomol 110:1611–1618

Karunaratne WS, Kumar V, Pettersson J, Kumar NS (2008) Response of the shot-hole borer of tea, *Xyleborus fornicatus* (Coleoptera: Scolytidae) to conspecifics and plant semiochemicals. Acta Agric Scand Sect A—Anim Sci 58:345–351

Kashiwagi T, Nakashima T, Tebayashi S-I, Kim C-S (2006) Determination of the absolute configuration of quercivorol, (1*S*, 4*R*)-*p*-menth-2-en-1-ol, an aggregation pheromone of the ambrosia beetle *Platypus quercivorus* (Coleoptera: Platypodidae). Biosci Biotechnol Biochem 70:2544–2546

Kasson MT, O'Donnell K, Rooney AP, Sink S, Ploetz RC, Ploetz JN, Konkol JL, Carrillo D, Freeman S, Mendel Z, Smith JA, Black AW, Hulcr J, Bateman C, Stefkova K, Campbell PR, Geering ADW, Dann EK, Eskalen A, Mohotti K, Short DPG, Aoki T, Fenstermacher KA, Davis DD, Geiser DM (2013) An inordinate fondness for *Fusarium*: phylogenetic diversity of fusaria cultivated by ambrosia beetles in the genus *Euwallacea* on avocado and other plant hosts. Fungal Genet Biol 56:147–157

Kendra PE, Montgomery WS, Mateo DM, Puche H, Epsky ND, Heath RR (2005) Effect of age on EAG response and attraction of female *Anastrepha suspensa* (Diptera: Tephrididae) to ammonia and carbon dioxide. Environ Entomol 34:584–590

Kendra PE, Montgomery WS, Niogret J, Peña JE, Capinera JL, Brar G, Epsky ND, Heath RR (2011) Attraction of the redbay ambrosia beetle, *Xyleborus glabratus*, to avocado, lychee, and essential oil lures. J Chem Ecol 37:932–942

Kendra PE, Niogret J, Montgomery WS, Sanchez JS, Deyrup MA, Pruett GE, Ploetz RC, Epsky ND, Heath RR (2012) Temporal analysis of sesquiterpene emissions from manuka and phoebe oil lures and efficacy for attraction of *Xyleborus glabratus* (Coleoptera: Curculionidae: Scolytinae). J Econ Entomol 105:659–669

Kendra PE, Montgomery WS, Niogret J, Epsky ND (2013) An uncertain future for American Lauraceae: a lethal threat from redbay ambrosia beetle and laurel wilt disease (a review). Special issue: the future of forests. Am J Plant Sci 4:727–738

Kendra PE, Montgomery WS, Niogret J, Pruett GE, Mayfield AE III, MacKenzie M, Deyrup MA, Bauchan GR, Ploetz RC, Epsky ND (2014a) North American Lauraceae: Terpenoid emissions, relative attraction and boring preferences of redbay ambrosia beetle, *Xyleborus glabratus* (Coleoptera: Curculionidae: Scolytinae). PLoS ONE 9(7):e102086

Kendra PE, Montgomery WS, Niogret J, Schnell EQ, Deyrup MA, Epsky ND (2014b) Evaluation of seven essential oils identifies cubeb oil as most effective attractant for detection of *Xyleborus glabratus*. J Pest Sci 87:681–689

Kendra PE, Narvaez TI, Montgomery WS, Carrillo D (2015a) Ambrosia beetle communities in forest and agricultural ecosystems with laurel wilt disease (D3524). In: 53rd Annual Meeting of the Entomological Society of America, Minneapolis, MN, USA 15–18 November 2015. https://esa.confex.com/esa/2015/webprogram/Paper99702.html

Kendra PE, Niogret J, Montgomery WS, Deyrup MA, Epsky ND (2015b) Cubeb oil lures: Terpenoid emissions, trapping efficacy, and longevity for attraction of redbay ambrosia beetle (Coleoptera: Curculionidae: Scolytinae). J Econ Entomol 108:350–361

Kendra PE, Montgomery WS, Deyrup MA, Wakarchuk D (2016a) Improved lure for redbay ambrosia beetle developed by enrichment of α-copaene content. J Pest Sci 89:427–438

Kendra PE, Montgomery WS, Schnell EQ, Deyrup MA, Epsky ND (2016b) Efficacy of α-copaene, cubeb, and eucalyptol lures for detection of redbay ambrosia beetle (Coleoptera: Curculionidae: Scolytinae). J Econ Entomol 109:2428–2435

Kendra PE, Owens DR, Montgomery WS, Narvaez TI, Bauchan GR, Schnell EQ, Tabanca N, Carrillo D (2017) α-Copaene is an attractant, synergistic with quercivorol, for improved detection of *Euwallacea* nr. *fornicatus* (Coleoptera: Curculionidae: Scolytinae). PLoS ONE 12(6):e0179416

King CBR (1940) Notes on the shot-hole borer of tea *Xyleborus fornicatus* Eichhoff *fornicatior* Eggers. Tea Q 13:111–116

Kuhns EH, Tribuiani Y, Martini X, Meyer WL, Peña J, Hulcr J, Stelinski LL (2014) Volatiles from the symbiotic fungus *Raffaelea lauricola* are synergistic with manuka lures for increased capture of the redbay ambrosia beetle *Xyleborus glabratus*. Agric Forest Entomol 16:87–94

Mendel Z, Protasov A, Sharon M, Zveibil A, Ben Yehuda S, O'Donnell K, Rabaglia R, Wysoki M, Freeman S (2012) An Asian ambrosia beetle *Euwallacea fornicatus* and its novel symbiotic fungus *Fusarium* sp. pose a serious threat to the Israeli avocado industry. Phytoparasitica 40:235–238

Miller DR, Rabaglia RJ (2009) Ethanol and (−)-α-pinene: Attractant kairomones for bark and ambrosia beetles in the southeastern US. J Chem Ecol 35:435–448

Mori K (2006) Synthesis of (1*S*, 4*R*)-4-isopropyl-1-methyl-2-cyclohexen-1-ol, the aggregation pheromone of the ambrosia beetle *Platypus quercivorus*, its racemate, (1*R*, 4*R*)- and (1*S*,4*S*)-isomers. Tetrahedron Asymmetry 17:2133–2142

Niogret J, Kendra PE, Epsky ND, Heath RR (2011) Comparative analysis of terpenoid emissions from Florida host trees of the redbay ambrosia beetle, *Xyleborus glabratus* (Coleoptera: Curculionidae: Scolytinae). Florida Entomol 94:1010–1017

Niogret J, Epsky ND, Schnell RJ, Boza EJ, Kendra PE, Heath RR (2013) Terpenoid variations within and among half-sibling avocado trees, *Persea americana* Mill. (Lauraceae). PLoS ONE 8(9):e73601

NIST (2017) The NIST 17 Mass Spectrometer database, Scientific Instrument Services Inc., New Jersey. http://www.sisweb.com/software/ms/nist.htm. Accessed 23 Oct 2017

O'Donnell K, Sink S, Libeskind-Hadas R, Hulcr J, Kasson MT, Ploetz RC, Konkol JL, Ploetz JN, Carrillo D, Campbell A, Duncan RE, Liyanage PNH, Eskalen A, Na F, Geiser DM, Bateman C, Freeman S, Mendel Z, Sharon M, Aoki T, Cossé AA, Rooney AP (2015) Discordant phylogenies suggest repeated host shifts in the *Fusarium-Euwallacea* ambrosia beetle mutualism. Fungal Genet Biol 82:277–290

Owens D, Montgomery WS, Narvaez TI, Deyrup MA, Kendra PE (2017) Evaluation of lure combinations containing essential oils and volatile spiroketals for detection of host-seeking *Xyleborus glabratus* (Coleoptera: Curculionidae: Scolytinae). J Econ Entomol 110:1596–1602

Owens D, Cruz LF, Montgomery WS, Narvaez TI, Schnell EQ, Tabanca N, Duncan RE, Carrillo D, Kendra PE (2018) Host range expansion and increasing damage potential of *Euwallacea* nr. *fornicatus* (Coleoptera: Curculionidae) in Florida. Florida Entomol 101: in press. Accepted 9 Jan 2018

Ploetz RC, Hughes MA, Kendra PE, Fraedrich SW, Carrillo D, Stelinski LL, Hulcr J, Mayfield AE III, Dreaden TL, Crane JH, Evans EA, Schaffer BA, Rollins J (2017a) Recovery plan for laurel wilt of avocado, caused by *Raffaelea lauricola*. Plant Health Prog 18:51–77

Ploetz RC, Kendra PE, Choudhury RA, Rollins J, Campbell A, Garrett K, Hughes M, Dreaden T (2017b) Laurel wilt in natural and agricultural ecosystems: Understanding the drivers and scales of complex pathosystems. Special issue: Forest pathology and plant health. Forests 8:48

Rabaglia RJ, Dole SA, Cognato AI (2006) Review of American Xyleborina (Coleoptera: Curculionidae: Scolytinae) occurring north of Mexico, with an illustrated key. Ann Entomol Soc Amer 99:1034–1056

Rabaglia R, Duerr D, Acciavatti R, Ragenovich I (2008) Early detection and rapid response for non-native bark and ambrosia beetles. US Department of Agriculture, Forest Service, Forest Health Protection, Washington, DC. http://www.fs.fed.us/foresthealth/publications/EDRRProjectReport.pdf. Accessed 20 Oct 2017

(SIAP) Servicio de información agroalimentaria y pesquera (2015) Cierre de la producción agrícola por cultivo. http://infosiap.siap.gob.mx/aagricola_siap_gb/ientidad/index.jsp. Accessed 20 Oct 2017

Stouthamer R, Rugman-Jones P, Thu PQ, Eskalen A, Thibault T, Hulcr J, Wang L, Jordal BH, Chen C, Cooperband M, Lin C, Kamata N, Lu S, Masuya H, Mendel Z, Rabaglia R, Sanguansub S, Shih H, Sittichaya W, Zong S (2017) Tracing the origin of a cryptic invader: phylogeography of the *Euwallacea fornicatus* (Coleoptera: Curculionidae: Scolytinae) species complex. Agric For Entomol 19:366–375

Systat Software (2013) SigmaPlot® 13. User's guide. Systat Software, Inc., San Jose, CA, USA

Tokoro M, Kobayashi M, Saito S, Kinuura H, Nakashima T, Shoda-Kagaya E, Kashiwagi T, Tebayashi S-I, Kim C-S, Mori K (2007) Novel aggregation pheromone, (1*S*, 4*R*)-*p*-menth-2-en-1-ol, of the ambrosia beetle, *Platypus quercivorus* (Coleoptera: Platypodidae). Bull For For Prod Res Inst 6:49–57

(USDA-FS) United States Department of Agriculture, Forest Service (2017) Forest Health, Southern Regional Extension Forestry. Distribution of counties with laurel wilt as of 14 April 2017. http://southernforesthealth.net/fungi/laurel-wilt/distribution-map

(USDA-NASS) United States Department of Agriculture, National Agricultural Statistics Service (2017) Noncitrus fruits and nuts 2016 summary. USDA, Washington DC. http://usda.mannlib.cornell.edu/usda/current/NoncFruiNu/NoncFruiNu-06-27-2017.pdf

Van den Dool H, Kratz PD (1963) A generalization of the retention index system including linear temperature programmed gas-liquid partition chromatography. J Chromat A 11:463–471