Sampling and Biostatistics

Comparison of Trap Designs for Detection of Euwallacea nr. fornicatus and Other Scolytinae (Coleoptera: Curculionidae) That Vector Fungal Pathogens of Avocado Trees in Florida

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Abstract

Laurel wilt and Fusarium dieback are vascular diseases caused by fungal symbionts of invasive ambrosia beetles (Coleoptera: Curculionidae: Scolytinae). Both diseases threaten avocado trees in Florida. Redbay ambrosia beetle, Xyleborus glabratus, is the primary vector of the laurel wilt pathogen, Raffaelea lauricola, but in recent years this symbiont has been transferred laterally to at least nine other species of ambrosia beetle, which now comprise a community of secondary vectors. Dieback disease, caused by Fusarium spp. fungi, is spread by shot hole borers in the Euwallacea fornicatus species complex. In this study, we conducted field tests in Florida avocado groves to compare efficacy of four trap designs for detection of Scolytinae. Treatments included an 8-funnel Lindgren trap, black 3-vane flight interception trap, green 3-vane interception trap, white sticky panel trap, and an unbaited sticky panel (control). In two tests targeting E. nr. fornicatus and X. glabratus, traps were baited with a two-component lure (α-copaene and quercivorol). In a test targeting other species, traps were baited with a low-release ethanol lure. For E. nr. fornicatus, sticky panels and black interception traps captured significantly more beetles than Lindgren traps; captures with green traps were intermediate. With ethanol-baited traps, 20 species of bark/ambrosia beetle were detected. Trap efficacy varied by species, but in general, sticky traps captured the highest number of beetles. Results indicate that sticky panel traps are more effective for monitoring ambrosia beetles than Lindgren funnel traps, the current standard, and may provide an economical alternative for pest detection in avocado groves.

Key words: ambrosia beetle, Fusarium dieback, laurel wilt, Persea americana, Xyleborus glabratus

Production of avocado (Persea americana Mill. [Laurales: Lauraceae]) in Florida is currently threatened by two vascular diseases—laurel wilt and Fusarium dieback—both caused by fungal symbionts of invasive ambrosia beetles (Coleoptera: Curculionidae: Scolytinae: Xyleborini) endemic to Southeast Asia, but now established in the United States. The primary vector of laurel wilt is redbay ambrosia beetle, Xyleborus glabratus Eichhoff, first detected near Savannah, Georgia in 2002, and now found in 11 southeastern states (USDA-FS 2019). The predominant symbiont of X. glabratus is Raffaelea lauricola T. C. Harr., Fredrich & Aghayeva (Ophiostomatales: Ophiostomataceae) (Harrington et al. 2008), a fungus that triggers a host defensive response that includes extensive tylose formation in xylem vessels, ultimately leading to compromised water transport, systemic wilt, and death of infected trees (Ploetz et al. 2012). Initially regarded as an epidemic affecting native Lauraceae, primarily redbay [Persea borbonia (L.) Spreng. [Laurales: Lauraceae]] and swampbay [P. palustris ( Raf.) Sarg. [Laurales: Lauraceae]], laurel wilt quickly spread southward into Florida where it became apparent that avocado was also susceptible (reviewed by Kendra et al. 2013). Contrary to predictions, populations of X. glabratus did not increase rapidly within commercial groves, a monoculture of apparently suitable host trees. Although avocado wood is highly attractive to females due to emissions of appropriate kairomones (Kendra et al. 2011a, 2012b, 2014a), and the species can develop successfully within cut avocado logs under laboratory conditions (Brar et al. 2013), avocado trees in situ appear to be poor reproductive hosts (Carrillo et al. 2012). Despite very low numbers of X. glabratus in Florida groves (Kendra et al. 2017, Owens et al. 2019a), laurel wilt is still prevalent and continues to spread throughout the avocado production area of Miami-Dade County (Ploetz et al. 2017a). This is the result of a community of ambrosia beetles attracted to (Kendra et al. 2011b) and breeding in (Carrillo et al. 2012) avocado wood, as well as an unexpected,
unprecedented lateral transfer of R. lauricola to at least nine additional species which now function as potential secondary vectors of the pathogen (Carrillo et al. 2014, Ploetz et al. 2017b).

Fusarium dieback is a more recent wilt disease that affects avocado, woody ornamentals, and native trees in California (Eskalen et al. 2012, 2013), Florida (Carrillo et al. 2016; Owens et al. 2018), and elsewhere (Mendel et al. 2012, García-Avila et al. 2016, Paap et al. 2018). It is caused by a complex of fungal symbionts (primarily in the genera Fusarium, Graphium, and Acremonium) vectored by shot hole borers in the Euwallacea fornicatus cryptic species complex (Freeman et al. 2013, Carrillo et al. 2016, Lynch et al. 2016). There are seven described species within this complex (Smith et al. 2019), with two established in California and another in South Florida. The Florida species was recently reported as Euwallacea perbreviss (Schedl) (Smith et al. 2019), but due to a lack of consensus regarding nomenclature (O’Donnell et al. 2015, Stouhamer et al. 2017, Gomez et al. 2018), the conservative, collective term E. near fornicatus will be retained in this report. Unlike R. lauricola that spreads systemically throughout a host tree, the Fusarium pathogen introduced by E. nr. fornicatus remains localized near the beetle entrance holes and galleries, typically at the base of a branch, which results in wilt and dieback of individual branches.

Field lures have been developed for detection and monitoring of both X. glabratus and E. nr. fornicatus. The chemical ecology of host-seeking female X. glabratus has been the subject of research for over a decade, and a series of essential oils have been utilized as kairomone attractants, culminating in a distilled essential oil product highly enriched in (-)-α-copaene, which is the current standard lure (Kendra et al. 2016a, 2016b, 2018). In a field test targeting X. glabratus in avocado (Kendra et al. 2015), it was discovered that females of E. nr. fornicatus are also attracted to α-copaene. Subsequent research demonstrated that α-copaene is equal in attraction to quercivorol, a food-based attractant and the standard lure for E. nr. fornicatus (Carrillo et al. 2015); moreover, a combination of α-copaene and quercivorol lures result in synergistic attraction and improved pest detection (Kendra et al. 2017). This two-component lure has field longevity of 3 mo (Owens et al. 2019a) and a sampling range of 30–35 m (Owens et al. 2019b).

The objective of the current study was to evaluate efficacy of four trap designs, baited with the best available attractants, for detection of pest ambrosia beetles in Florida avocado groves. The two-component lure was used to target X. glabratus and E. nr. fornicatus in two field tests. A third field test was conducted using low-release ethanol lures, a general attractant for many ambrosia beetles (Miller and Rabaglia 2009), but not for X. glabratus (Kendra et al. 2014b) or E. nr. fornicatus (Carrillo et al. 2015). The latter test was intended to evaluate the effect of trap design on detection of the community of ambrosia beetles in this agroecosystem, particularly species known to be secondary vectors of the Laurel wilt pathogen (Carrillo et al. 2014, Ploetz et al. 2017b).

The two-component lure has been used previously in Florida grove surveys (Carrillo et al. 2016) and has been adopted by SAGARPA (Secretaría de Agricultura, Ganadería, Desarrollo Rural, Pesca y Alimentación) in Mexico in surveillance programs for both pests, yet it has not been determined whether quercivorol functions as an attractant or repellent for X. glabratus. Therefore, we also conducted laboratory bioassays with newly emerged female X. glabratus to assess behavioral responses to α-copaene and quercivorol, presented alone and in tandem, to test for a potential negative response to quercivorol in the combination lure.

Materials and Methods
Experimental Insects
Xyleborus glabratus used in behavioral bioassays were reared from infested swampywood wood collected in Broward County, FL. Logs (8–12 cm diameter) were placed flat on moist paper towels in a shallow plastic emergence chamber (122 cm long × 61 cm wide × 18 cm high; Sterilite Corp., Townsend, MA) containing a tight-fitting lid, which had two screened ventilation holes (12 cm diameter) at each end. The chamber was maintained at 24°C under natural light and checked daily. Newly emerged females were collected by hand with a camel hair brush and held in a small plastic storage box with moist tissue until needed for testing.

Laboratory Bioassays
Test substrates consisted of two plastic bubble lures obtained from Synergy Semiochemicals Corp. (Delta, BC, Canada). The first lure contained 50% α-copaene oil (2.0 ml loaded into a 2.9-cm-diameter bubble; product #3302), with a release rate of 10 mg/d reported by the manufacturer. The second lure contained quercivorol (97 mg in a 1.2-cm-diameter bubble; product #3402), with a release rate of 0.7 mg/d. An empty plastic bubble dispenser served as a negative control (blank treatment). [Note: the lure commonly referred to as ‘quercivorol’ contains a mixture of four isomers of p-menth-2-en-1-ol; only the 1S,4R-enantiomer has been termed quercivorol (Kashiwagi et al. 2006), and it is not the major component in the lure (Owens et al. 2019a).] Bioassays were conducted in rectangular plastic bins (identical to the one described above for beetle rearing), using a modified protocol from that reported previously (Kendra et al. 2016a). A paper lining was placed on the bin floor to facilitate walking. Test lures were hung at opposite ends of the bin using wire hooks (paper clips) taped to the bin wall, and a piece of sticky card (4 × 8 cm) was placed directly beneath each lure to retain attracted beetles. Tests were initiated in the early afternoon, prior to onset of the female flight window (~16:00 h EDST; Kendra et al. 2012a). For each replicate run, 10 females (≤48 h postemergence) were introduced at the center of the arena and immediately covered with an inverted glass funnel (6 cm diameter). After 5 min acclimation, the funnel was lifted gently to release the beetles and start the bioassay. The bin was then covered and left undisturbed (18 h) until the next morning, at which point the test was concluded and beetles counted on each card.

Five replicated binary-choice tests were conducted (copaene vs blank, quercivorol vs blank, combination lure vs blank, copaene vs quercivorol, and combination lure vs copaene). Each choice test was replicated six times, using a new cohort of beetles and paper liner, and reversing the position of test substrates between replicate runs.

Traps
Four trap designs were compared in this study. Three are available commercially and specifically intended for monitoring wood-boring beetles: a black 8-unit Lindgren funnel trap (BioQuip Products, Inc., Rancho Domínguez, CA; product #2853; Fig. 1A), a black 3-vane flight interception trap (Synergy Semiochemicals Corp.; product #4057; Fig. 1B), and a green 3-vane flight interception trap (Synergy Semiochemicals Corp.; product #4058; Fig. 1C). With these ‘wet trap’ designs, the collection cups were filled with 300 ml of an aqueous solution of 10% propylene glycol (Low-Tox antifreeze; Prestone, Danbury, CT) to retain and preserve captures. The fourth design consisted of a sticky panel trap (Fig. 1D) used previously in our field evaluations of scolytine lures (Kendra et al. 2012c, 2014b) and host preferences (Kendra et al. 2011a, 2014a). This latter trap
was assembled from two white sticky panels (23 × 28 cm Sentry wing trap bottoms; Great Lakes IPM, Vestaburg, MI), oriented back-to-back, suspended from a wire hanger, and topped with an inverted plastic plate (24 cm diameter) to provide a protective covering comparable to that found on the three commercial trap designs.

**Field Tests**

In two field trials designed for *E. nr. fornicatus* and *X. glabratus*, the four trap designs were baited with a combination of α-copaene and quercivorol lures (described above). In addition, an unbaited white sticky trap was used as a negative control to assess passive baseline captures. Field test 1 was conducted from 10 October to 19 December 2017 (10 wk) in an avocado grove affected by laurel wilt (25° 29′ 58.42″ N, 80° 29′ 19.80″ W); however, the grove was well managed with prompt removal of symptomatic trees. Field test 2 was conducted from 21 December 2017 to 1 March 2018 (10 wk) in a grove free of laurel wilt (25° 30′ 20.19″ N, 80° 29′ 21.96″ W).

A third 10-wk field trial was designed to sample the overall community of bark and ambrosia beetles. This test utilized low-release ethanol lures (Synergy Semiochemicals Corp.; product #3344; release rate of 15 mg/d) to bait the four trap treatments and also included an unbaited sticky trap control. Field test 3 was conducted from 27 March to 5 June 2018 at another avocado grove affected by laurel wilt (25° 27′ 73.00″ N, 80° 32′ 47.60″ W). Unlike the site used for test 1, this grove had an ample number of stressed and dying trees available as breeding substrates for beetles.

All three tests followed a randomized complete block design with five replicate blocks; each block consisted of a row of traps hung ~1.5 m above ground (Brar et al. 2012) in well-shaded locations in trees asymptomatic for laurel wilt or *Fusarium* dieback. There was a minimum spacing of 12 m between adjacent traps within a row, and 15 m spacing between rows. At weekly intervals, traps were serviced to collect beetles and replace reten- tion fluid/sticky cards. In addition, trap treatments were rotated sequentially within each row. By the end of a 10-wk test, each trap had completed two full rotations through each field position within a row. All samples were processed in the laboratory (USDA-ARS, Miami, FL) under a stereo microscope (Discovery.V12; Carl Zeiss Microscopy, LLC, White Plains, NY). Specimens from sticky panels first were cleared by briefly soaking them in Histo-Clear II (National Diagnostics, Atlanta, GA). With the exception of *Hypothenemus* spp., all scolytine and platypodine beetles were identified to species level according to Rabaglia et al. (2006) and Atkinson et al. (2013).

**Statistical Analysis**

Data were analyzed using Systat Software (2017). Paired t-tests were used to analyze results from the binary-choice bioassays. Field captures were expressed as beetles/trap/week (captures pooled over time for each treatment within a replicate block) and results 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. Results are presented as mean ± SEM; probability was considered significant at a critical level of α = 0.05.

**Results**

**Laboratory Bioassays**

Five replicated binary-choice tests were conducted to assess behavioral responses of female *X. glabratus* to α-copaene and quercivorol lures, presented alone and in combination (Table 1). Overall, the response rate was very high, with an average of 91.68 (± 1.58)% of the beetles responding to one of the two choices. When given a choice between lures and a blank control, females were attracted to α-copaene alone and the combination of α-copaene plus quercivorol, but not to quercivorol alone. When presented with α-copaene versus quercivorol, females were more attracted to α-copaene. When presented with the combination of α-copaene and quercivorol versus α-copaene alone, females were equally distributed among the two choices (the choice between α-copaene and quercivorol displayed no attraction in all previous tests). These results provide no evidence that quercivorol functions as an attractant or a repellent for *X. glabratus*. Use of the two-component lure for dual detection of *E. nr. fornicatus* and *X. glabratus* is valid.
Table 1. Response (mean ± SEM) of female Xyleborus glabratus to α-copaene (Cop) and quercivorol (Quer) lures presented in binary-choice bioassays

<table>
<thead>
<tr>
<th>Choice 1/Choice 2</th>
<th>% Respondinga</th>
<th>% at Choice 1b</th>
<th>% at Choice 2b</th>
<th>t</th>
<th>df</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cop/blank</td>
<td>95.0</td>
<td>89.5 ± 3.9</td>
<td>10.5 ± 3.9</td>
<td>14.43</td>
<td>10</td>
<td>&lt;0.001*</td>
</tr>
<tr>
<td>Quer/blank</td>
<td>91.7</td>
<td>51.1 ± 3.8</td>
<td>48.9 ± 3.8</td>
<td>0.415</td>
<td>10</td>
<td>0.687</td>
</tr>
<tr>
<td>Cop+Quer/blank</td>
<td>95.0</td>
<td>85.9 ± 4.3</td>
<td>14.1 ± 4.3</td>
<td>11.85</td>
<td>10</td>
<td>&lt;0.001*</td>
</tr>
<tr>
<td>Cop/Quer</td>
<td>86.7</td>
<td>73.8 ± 4.7</td>
<td>26.2 ± 4.7</td>
<td>7.173</td>
<td>10</td>
<td>&lt;0.001*</td>
</tr>
<tr>
<td>Cop+Quer/Cop</td>
<td>90.0</td>
<td>52.6 ± 2.3</td>
<td>47.4 ± 2.3</td>
<td>1.625</td>
<td>10</td>
<td>0.135</td>
</tr>
</tbody>
</table>

*aPercentage of beetles responding to either choice.

bPercentage of responding beetles attracted by each choice.

*cResponse to choice 1 significantly higher.

Fig. 2. Mean (± SEM) captures of female Euwallacea nr. fornicatus. (A) Field test 1: 10-wk trial in an avocado grove with laurel wilt. (B) Field test 2: 10-wk trial in an avocado grove with laurel wilt. Treatments consist of a white sticky panel trap (Sticky), black 3-vane flight interception trap (Black), green 3-vane flight interception trap (Green), black 8-unit Lindgren funnel trap (Lindgren), and an unbaited sticky trap (Control). All traps except control baited with a combination of α-copaene and quercivorol lures. Bars topped with the same letter are not significantly different (Tukey HSD mean separation, P < 0.05). Please note differences in scale for the y-axis.

Field Tests

In field test 1 (Fig. 2A), there were significant differences in mean capture of female E. nr. fornicatus among the five treatments (F = 31.434; df = 4, 20; P < 0.001). Highest numbers of beetles were caught with the sticky panel trap, followed by intermediate numbers with the black and green 3-vane traps, low numbers with the Lindgren funnel trap, and no captures with the unbaited trap. Captures with both the sticky trap and the black 3-vane trap were significantly higher than those obtained with the standard Lindgren trap. Captures with the green 3-vane trap were equivalent to those obtained with the standard Lindgren trap but significantly higher than those of the unbaited control. Captures with the Lindgren trap were statistically no different from those of the unbaited control.

In field test 2 (Fig. 2B), there were similar differences in mean capture of E. nr. fornicatus among treatments (F = 21.488; df = 4, 20; P < 0.001), but the population level was approximately four times higher at this site. The sticky panel trap and black 3-vane trap caught equally high numbers of beetles, followed by intermediate captures with the green 3-vane and Lindgren traps, and low captures with the unbaited control. As observed in field test 1, both the sticky trap and black 3-vane trap caught significantly more E. nr. fornicatus than the standard Lindgren trap. At this higher beetle density, captures with the Lindgren funnel trap were greater than those of the control trap.

The combination of α-copaene and quercivorol lures resulted in good specificity for attraction of female E. nr. fornicatus, which comprised 74.3 and 75.9% of the trap captures in field tests 1 and 2, respectively (Table 2). Species with the next highest captures in test 1 were Ambrosiodmus devexulus (Wood), representing 9.3% of the total, and Hypothenemus spp., representing 6.9%. A single specimen of X. glabratus was caught in a baited sticky trap in test 1. In test 2, Hypothenemus spp. comprised 10.2% and Theoborus ricini (Eggers) 6.1% of the overall trap captures.

In field test 3 (Fig. 3), there were significant differences in mean capture of bark and ambrosia beetles (all species combined) among the trap designs baited with ethanol lures (F = 30.031; df = 4, 20; P < 0.001). Highest numbers of beetles were caught with the sticky panel trap, followed by intermediate numbers with the black 3-vane trap and Lindgren funnel trap, and lowest captures with the green 3-vane trap, which were statistically no different from those obtained with the unbaited control trap.

Diversity of species and total numbers captured were greater in field test 3 than in the previous two tests (Table 2). Species of Hypothenemus made up the largest percentage of captures in ethanol-baited traps, comprising 57.9% of the total, followed by Xyleborus saxesenii (Ratzeburg) at 17.3%, Xyleborus bispinatus Eichhoff at 9.4%, Xyleborus affinis Eichhoff at 4.9%, Xyleborus volvulus (Fabricius) at 4.6%, and Premnomius cavipennis Eichhoff at 1.3%. Euwallacea nr. fornicatus represented less than 1% of the captures, and three specimens of X. glabratus were trapped (two on baited sticky traps and one on an unbaited sticky trap). All 10 species known to harbor R. lauricola were detected at this site (indicated by an asterisk in Table 2).

With the six most abundant species from test 3, numbers were high enough to permit analysis of trap efficacy (Fig. 4). Sticky panel traps consistently caught the highest number of beetles, but performance of other trap designs varied depending on species. For
Table 2. Bark and ambrosia beetles captured in three 10-wk field tests conducted in commercial avocado groves in Miami-Dade County, FL

<table>
<thead>
<tr>
<th>Species</th>
<th>Test 1*</th>
<th>Test 2*</th>
<th>Test 3*</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Subfamily Scolytinae</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Tribe Dryococini</td>
<td></td>
<td></td>
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<tr>
<td>Coccytaspis carphagus (Hornung)</td>
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<td>18</td>
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<tr>
<td><strong>Tribe Xyleborini</strong></td>
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<td></td>
</tr>
<tr>
<td>Ambrosiadia devexus (Wood)</td>
<td>120</td>
<td>75</td>
<td>5</td>
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<tr>
<td>Ambrosiadia lecontei Hopkins*</td>
<td>7</td>
<td>8</td>
<td>22</td>
</tr>
<tr>
<td>Euscelidius nr. formicatus Eichhoff</td>
<td>960</td>
<td>3623</td>
<td>141</td>
</tr>
<tr>
<td>Premniobius cavipennis Eichhoff</td>
<td>0</td>
<td>8</td>
<td>181</td>
</tr>
<tr>
<td>Theocharis ricini (Eggers)</td>
<td>31</td>
<td>293</td>
<td>26</td>
</tr>
<tr>
<td>Xyleborinus andrewesi (Blandford)*</td>
<td>0</td>
<td>13</td>
<td>89</td>
</tr>
<tr>
<td>Xyleborinus gracilis (Eichhoff)*</td>
<td>0</td>
<td>22</td>
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<td>2499</td>
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<td>Xyleborus affinis Eichhoff*</td>
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<td>693</td>
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<td>83</td>
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<td>75</td>
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<tr>
<td>Xyleborus ferrugineus (Fabricius)*</td>
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<td>75</td>
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<td>Xyleborus voluculus (Fabricius)*</td>
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<td>665</td>
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<td>Xylosandrus compactus (Eichhoff)</td>
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<td>0</td>
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<td>Xylosandrus crassiusculus (Motschulsky)*</td>
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<td>97</td>
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<td><strong>Tribe Cryptobius</strong></td>
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<td>Cryptocarenus hexae (Hagedorn)</td>
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<tr>
<td>Hypothenemus spp.</td>
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<td><strong>Tribe Oryctes</strong></td>
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<td>Cortylopus pupulus Eichhoff</td>
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<td></td>
<td></td>
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<tr>
<td>Euplatypus parallellus (Fabricius)</td>
<td>7</td>
<td>0</td>
<td>52</td>
</tr>
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</table>

*Traps baited with a combination of α-copaene and quercivorol lures.
*Traps baited with low-release ethanol lures.
*Species from which Raffaeela lauricola, etiological agent of laurel wilt, has been isolated (Ploetz et al. 2017b).

Discussion

Development of improved semiochemical-based detection systems for ambrosia beetles will rely on a better understanding of the chemical ecology and flight behavior of the adult female during that brief stage when she leaves the natal tree and searches for a new host. For X. glabratus, the process has been viewed as a multistep process involving sequential input of pertinent environmental information, including long-range olfactory cues, mid-range visual cues, and final integration of close-range/contact cues that result in a behavioral switch from ‘host-seeking’ to ‘host-acceptance’ mode (Kendra et al. 2014a). Optimization of pest detection requires discovery of effective attractants as well as detection of appropriate trap designs for your target species. Since effective attractants are known, the current study examined the latter requirement for ambrosia beetles that vector phytopathogenic fungi in Florida.

The multiple funnel trap developed by Lindgren (1983) has long been the standard used by action agencies for detection and population monitoring of bark and ambrosia beetles. However, our results indicate that the trap design does not always provide adequate detection of critical ambrosia beetle species in Florida. In field tests 1 and 2, E. nr. formicatus was trapped at significantly higher numbers with the sticky panel trap and the black 3-vane trap. The four X. glabratus captured (tests 1 and 3) were all intercepted with sticky traps, results consistent with a previous comparison between Lindgren and sticky traps conduct at a north Florida site with higher numbers of X. glabratus (Kendra et al. 2012c). In field test 3, mean cumulative captures of all beetle species were again significantly higher with the sticky panel trap than with the Lindgren trap, including captures of X. saxenesii and X. volvulus, potential carriers of the laurel wilt pathogen (Carrillo et al. 2014, Ploetz et al. 2017b).

Brar et al. (2012) evaluated the effect of Lindgren trap size (4, 8, 12, and 16 funnels) on captures of X. glabratus. An increase from 4 to 8 funnels resulted in a significant increase in captures; however, further increment in funnels did not improve the numbers captured. Based on these results, our choice of the 8-unit funnel trap should have represented the optimal Lindgren design for field comparison.

In comparing the black and green versions of the 3-vane flight interception trap, captures of all species were less with the green trap. Since construction was identical with these two traps, the difference in captures indicates a strong influence of visual cues. As with the Lindgren funnel trap, the black color of the 3-vane trap, in combination with its elongated cylindrical shape, is likely representative of a tree trunk or branch silhouette, providing an additional signal consistent with host location, as demonstrated for...
X. glabratus (Mayfield and Brownie 2013). It should be noted that, in the latter study, the visual cue was not sufficient on its own, but increased female attraction only when presented in combination with an appropriate chemical cue (a host-based kairomone).

In all field tests, the white sticky panel trap consistently captured the highest number of beetles. This suggests that the lures utilized were sufficiently attractive to bring females close enough to make contact with the adhesive, after which there was no opportunity for escape. With the other three trap designs, it is possible for small beetles to land on the trap, fail to detect appropriate short-range/contact cues, and then fly off. Authors (P.E.K. and W.S.M.) have observed females of X. glabratus to hover near a lure but not land (during live collections reported in Kendra et al. 2012b) or land on the upper rim of a Lindgren funnel, but not fall into the trap (during field tests reported in Kendra et al. 2012c). Even with the sticky panel design, trapping efficacy has been estimated to be only 44.0 ± 2.3% for X. glabratus (field cage release-recapture tests with α-copaene lure; Kendra et al. 2016b) and 31.2 ± 3.7% for E. nr. fornicatus (open field release-recapture tests with two-component lure; Owens et al. 2019b). Therefore, trap captures are only indicative of relative

**Fig. 4.** Mean (± SEM) captures of the six most abundant species in field test 3: 10-wk trial in an avocado grove with laurel wilt. Treatments consist of a white sticky panel trap (Sticky), black 3-vane flight interception trap (Black), green 3-vane flight interception trap (Green), black 8-unit Lindgren funnel trap (Lindgren), and an unbaited sticky trap (Control). All traps except control baited with a low-release ethanol lure. Bars topped with the same letter are not significantly different (Tukey HSD mean separation, *P* < 0.05). Please note differences in scale for the *y*-axis.
species abundance at an experimental site. The trapping period, including length of study and time of year, may also affect the species abundance and composition detected, since population levels fluctuate seasonally (Kendra et al. 2012c, 2016a; Johnson et al. 2016). Additional factors influencing trapping efficacy include vertical distribution and temporal flight periodicity of host-seeking beetles (Kendra et al. 2012a, Menocal et al. 2018). The current field tests were conducted with traps hung at a height of 1.5 m above ground, based on previous studies with X. glabratus (Brar et al. 2012) and E. nr. fornicatus (Byers et al. 2017); however, a recent study with unbaited sticky cards indicated that many species fly at much greater heights (Menocal et al. 2018). In addition, diurnal species like X. glabratus, E. nr. fornicatus, Xyleborinus andreweesi (Blandford), X. saxesenii, and Hypothenemus sp. (Kendra et al. 2012a, 2012b, 2017; Johnson et al. 2016; Menocal et al. 2018) are more likely to utilize visual cues incorporated into the trap design when compared with species that engage in flight near sunset and after dark, like X. bispinatus and Xylosandrus crassiusculus (Motschulsky) (Kendra et al. 2012b, Menocal et al. 2018).

In conclusion, the results of this study indicate that 1) quercivilor does not function as a repellent for X. glabratus and therefore the two-component lure (α-copaene and quercivilor) is appropriate for dual detection of E. nr. fornicatus and X. glabratus; and 2) of the four trap designs tested, the sticky panel trap provides the best overall detection and monitoring of ambrosia beetles in Florida avocado groves. Efficacy of other traps was variable and depended upon the target species. For E. nr. fornicatus, both the sticky panel trap and the black 3-vane trap provided more sensitive detection than the standard Lindgren funnel trap. Previous field comparisons indicated that sticky traps also improve detection of X. glabratus, and the current study suggests that sticky traps far exceed other designs for detection of X. saxesenii and X. volvulus. In terms of costs associated with the products evaluated, the current posted price of sticky panels is US$0.98 each, 8-unit funnel traps US$64.39 each, and 3-vane traps US$30.12 each. Sticky panel traps may provide a more effective and economical alternative for growers and regulatory agencies that monitor for pest ambrosia beetles. If a wet trap design is highly preferred, use of the black 3-vane trap would result in a reduction of program costs over the currently used Lindgren trap.

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