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Research

A Potential Repellent Against the Coffee Berry Borer (Coleoptera: Curculionidae: Scolytinae)

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Abstract

The coffee berry borer, *Hypothenemus hampei* (Ferrari), continues to pose a formidable challenge to coffee growers worldwide. Because of the cryptic life habit of the insect inside coffee berries, effective pest management strategies have been difficult to develop. A sesquiterpene, (*E,E*)-α-farnesene, produced by infested coffee berries has been identified as a potential repellent against the coffee berry borer both in laboratory bioassays and a field experiment in Hawaii. Various laboratory bioassays revealed significantly lower levels of infestation in berries treated with different concentrations of the (*E,E*)-α-farnesene. A field experiment in Hawaii resulted in up to 80% decreased coffee berry borer captures in traps containing a standard 3:1 methanol:ethanol attractant and a bubble cap formulation of (*E,E*)-α-farnesene compared to traps with just the attractant. (*E,E*)-α-farnesene was still active 19 wk after installation in the coffee plantation, based on 59% lower insect captures in traps containing the attractant + (*E,E*)-α-farnesene (1,737 insects) compared to traps containing the attractant (4,253 insects). The easy to install bubble caps are a welcome contrast with other pest management strategies that require spraying. The placement of (*E,E*)-α-farnesene in bubble caps in coffee plantations when coffee berries first become susceptible to infestations (ca. 90 d post-flowering) might result in lower infestation levels throughout the season, and consequently, increased yields and profits.

Key words: bark beetles, broca del café, (*E,E*)-α-farnesene, coffee berry borer management, push-pull

The most economically important insect pest affecting coffee production (*Coffea arabica* and *Coffee canephora*) worldwide is the coffee berry borer, *Hypothenemus hampei* (Ferrari) (Coleoptera: Curculionidae: Scolytinae) (Vega et al. 2015). Adult females bore a hole in the disc of the coffee berry (i.e., the circular area at the lower end of the berry, opposite the pedicel) and lay their eggs in galleries built throughout the endosperm, with larvae and adults feeding on the coffee seed. Most of the insects' life cycle is spent inside coffee berries, thus making the insect quite difficult to control both by chemical and nonchemical methods. Annual yearly losses caused by the coffee berry borer in Brazil have been estimated at $215–$358 million per year (Oliveira et al. 2013), suggesting that yearly losses caused by the insect on a worldwide basis must easily surpass $500 million.

Even though at least nearly two dozen studies have examined cues used by the coffee berry borer to localize the berry (Prates 1969; Esquinca Avilés, 1986; Gutiérrez-Martínez et al. 1990; Mathieu et al. 1991; Mendoza-Mora 1991; Giordanengo et al. 1993; Gutiérrez-Martínez and Virgen Sánchez 1995; Gutiérrez-Martínez and Ondarza 1996; Mathieu et al. 1996; Brun and Mathieu 1997; Velasco Pascual et al. 1997a, b; Mathieu et al. 1998, 1999; Borbón Martínez et al. 2000; Cárdenas 2000; Mathieu et al. 2001; Saravanan and Chozhan 2003; Ortiz et al. 2004; Rojas 2005; Dufour and Frérot 2008; Mendesil et al. 2009; Jaramillo et al. 2013; Njihia et al. 2014), research involving coffee berry borer repellents remains vastly underexplored, with just a handful of studies. For example, Borbón Martínez et al. (2000) reported on various green leaf volatiles that reduced coffee berry borer trap captures when compared to a 3:1 mixture of methanol:ethanol, used as an attractant. In a laboratory study, Mathieu et al. (2001) found that virgin and mated nulliparous females were repelled by unidentified volatiles from red coffee berries. Góngora et al. (2012) identified isoprene as a possible coffee berry borer repellent. Finally, Njihia et al.

(2014) identified brocain (1,6-dioxaspiro[4,5]decane) as an attractant at low doses and as a repellent at high doses (> 160 ng/µl); frontalin (1,5-dimethyl-6,8-dioxabicyclo[3.2.1]octane) was also found to have repellent effects (> 40 ng/µl).

Vega et al. (2011) reported that an increase in coffee berry borer female density in artificial diet (i.e., under unlimited food resources) resulted in a reduction in fecundity and hypothesized that a possible mechanism for this reduction in fecundity could be the production of volatiles that act as host-marking pheromones, antiaggregation pheromones, or oviposition deterrence chemicals, all of which could serve as repellents. Such volatiles would reduce intraspecific competition, as has been reported for many insects (Vega et al. 2011). Based on these results, several experiments were conducted to elucidate whether coffee berries infested with the coffee berry borer were producing repellent compounds, resulting in the identification of a sesquiterpene, (*E,E*)-α-farnesene, as a potential repellent in laboratory and field trials.

Materials and Methods

Coffee Berries and Coffee Berry Borers

Coffee berry borer infested and uninfested coffee berries (*C. arabica*) were collected in coffee plantations in Kona, Hawaii and sent to the ARS laboratories in Peoria (Illinois) and Beltsville (Maryland). Adult female coffee berry borers reared in artificial diet (Vega et al. 2011) were used in laboratory bioassays. Age of the adult females was not controlled throughout the experiments.

Volatile Collection and Identification

The first identification of volatiles of interest was based on placing a single green field infested coffee berry in a capped glass vial. The headspace volatiles were collected by placing a solid-phase microextraction (SPME, 100-µm polydimethylsiloxane fiber) needle (Supelco, Inc., Bellefonte, Pennsylvania) 1–2 mm above the hole bored by the insect to enter the berry. A needle was also placed in close proximity to an artificially bored uninfested berry in a glass-capped vial. After 1 h of collection, the needle was retracted and analyzed by gas chromatography–mass spectrometry (GC-MS). The experiment had five replicates.

In a subsequent experiment, 10 infested berries and 10 uninfested green coffee berries were placed in 100-ml glass volatiles collector flasks (five replicates each). The collection flasks were connected to glass thermometer adapters (male 24/40 joint, Ace Glass, Vineland, NJ). The adapters had Teflon fittings at each end to hold a volatile trap filter (6 × 0.4 cm ID) containing 100 mg of HayeSep-Q (Restek, Bellefonte, PA) and through which air was drawn (50 ml/min) by vacuum. The inlet filter cleaned incoming air and the second filter trapped the volatiles emitted within the flasks. Collection duration was continuous for 4 d, and collected volatiles were recovered by rinsing the outlet HayeSep-Q filter with 400 µl of petroleum ether into a vial. Collection flasks were kept in an incubator at 27°C with a relative humidity of about 50%. Light was provided by eight 40 W fluorescent tubes set about 0.5 m above and behind the collection flasks, and the daily light cycle was a 17:7 L:D h photoperiod.

Instrumentation

Volatile collections were analyzed by GC with flame-ionization detection (FID) and coupled GC-MS. Samples were injected in splitless mode using a Hewlett Packard 6890 GC, interfaced to a Hewlett Packard 5973 mass-selective detector (electron impact, 70 eV). For most analyses, a 30-m DB-5 capillary column (0.25 mm ID, 0.25-µm film thickness, J&W Scientific, Folsom, CA) was used. The temperature program was 50°C for 1 min, then rising to 280°C at 10°C per min, and holding for 5 min at 280°C. The temperature of the inlet was 250°C, and the transfer line temperature was 280°C. The Wiley MS library (Wiley 2005) was installed on the data system. All the GC analyses used He as carrier gas at constant pressure (41.4 KPa).

Chemicals

(*E,E*)-α-farnesene was purified (90% pure) from a mixture of farnesene isomers (Bedoukian Research, Danbury, CT; product code 808) by preparative high-performance liquid chromatography (HPLC). The system consisted of a Waters M6000 pump, Waters R401 differential refractometer detector, and $AgNO_3$-treated column. The $AgNO_3$ column was prepared from a 25-cm × 4.6-mm-ID silica column (Alltech, Adsorbosphere, 5 µm) by the method of Heath and Sonnet (1980). The elution solvent was 5% ether in hexane. The identification of (*E,E*)-α-farnesene was verified by an authenticated sample of natural (*E,E*)-α-farnesene (70% pure, Contech Enterprises Inc., Victoria, British Columbia, Canada). The Contech sample contained approximately 18% of an additional unidentified farnesene isomer. The first three bioassays experiments used the HPLC-purified material. A sample of (5R,7S)-conophthorin, used as a positive GC-EAD control (Jaramillo et al. 2013), was obtained from Synergy Semiochemicals Corp. (Burnaby, Canada). (*E,E*)-α-farnesene (70% pure) for the bubble cab field formulations was obtained from Bedoukian Research (Danbury, CT).

Electrophysiology

Coupled GC-electroantennographic detections (GC-EAD) were made by methods and equipment generally described by Cossé and Bartelt (2000). GC-EAD connections were made by inserting a glass-pipette silver-grounding electrode (WPI Inc., Sarasota, FL) into the back of an excised female beetle head. A second glass-pipette silver-grounding probe pierced the tip of one of the clubbed antennae. Both electrodes were filled with a Beadle-Ephrussi saline solution (Ephrussi and Beadle 1936). Electrodes were placed into position using battery-powered piezo-drive micromanipulators (Sensapex, Oulu, Finland). The recording electrode was connected to a battery-powered preamplifier (Iso-Dam, WPI Inc., Sarasota, FL), which connected to an AC/DC UN-06 amplifier (Syntech, Hilversum, the Netherlands). Acquisition and analysis of responses were performed by a computer equipped with an analog to digital conversion board (IDAC-2, Syntech) running GC-EAD software (Syntech). Approximately, 25 ng/compound was blown over the antennal preparation by injecting 100-ng samples (solution in petroleum ether) of a mixture of standard compounds containing (5R,7S) conophthorin and (*E,E*)-α-farnesene (70% pure, Contech, 1:1, splitless) with approximately 50% blown over the antennal preparations (1:1 FID:EAD split). GC-EAD was conducted using a 15-m DB-5 capillary column (0.25 mm ID, 1.0-µm film thickness, J&W Scientific, Folsom, CA). The thinner film and shorter column length compared to the GC-MS column allowed for faster elution times, which accommodated the sometimes short life span of the antennal preparation. The temperature program was 50°C for 1 min, then rising to 280°C at 25°C per min, and holding for 5 min at 280°C. The temperature of the inlet was 250°C, and the transfer line temperature was 280°C.

Laboratory Bioassays

Bioassays were conducted with (*E,E*)-α-farnesene at four concentrations: 0.4, 4, 40, and 80 µg/µl. For each one of these concentrations,

we applied 2.5 µl to both ends of a green coffee berry (i.e., the pedicel and the disc) to obtain a 1, 10, 100, and 200 µg range at each end of the coffee berry. For the controls, we used petroleum ether, which was the solvent used to prepare the four concentrations of (*E*,*E*)-α-farnesene. All experiments were conducted in scintillation vials containing a small layer of plaster of Paris to absorb excess moisture. A Percival Scientific E-36L growth chamber (Percival Scientific Inc., Perry, IA) kept dark and set at 25°C was used in all bioassays.

Two preliminary bioassays were conducted to assess the possible repellent effect. In the first one, we compared 40 µg/µl (*E*,*E*)-α-farnesene to the control. One berry was placed in each scintillation vial immediately after applying the treatments, and one adult female coffee berry borer was introduced in the vial. There were 20 replicates in total. Percent bored berries were determined 1 and 17 h post-application. In the second preliminary bioassay, we compared 0.4, 4, and 40 µg/µl (*E*,*E*)-α-farnesene to the control using the method described above. There were 20 replicates (berries) for each (*E*,*E*)-α-farnesene concentration and 19 for the control. Percent berries bored by the insect were determined 1 and 24 h post-application.

In a third bioassay, percent bored berries were determined using 4 and 40 µg/µl (*E*,*E*)-α-farnesene and compared to the control. Run at different times over four dates, there were a total of 50 berries (i.e., replicates) for the control, 87 for the low (*E*,*E*)-α-farnesene concentration, and 15 for the high concentration. One berry was placed in each scintillation vial immediately after applying the treatments, and one adult female coffee berry borer was introduced in the vial. Numbers of berries that had been bored by the insect were assessed at 1 and 24 h post-application.

Subsequent bioassays were conducted using the (*E*,*E*)-α-farnesene purchased from Contech. We determined percentage coffee berries bored by the insect after applying 40 or 80 µg/µl (*E*,*E*)-α-farnesene compared to the control. The higher concentration was included to determine insect responses to twice the previous highest dose tested. We ran three bioassays per day, each consisting of three repetitions of 10 replicates each for the three treatments (control, 40, 80 µg/µl) for a total of 30 insects per treatment per day. The bioassays were repeated on three different dates for a grand total of 90 insects per treatment. One berry was placed in each scintillation vial immediately after applying the treatments, and one adult female coffee berry borer was introduced in the vial. We determined the percent berries that had been bored by the insect 1 and 24 h post-application.

Choice bioassays were conducted to test the effects of the higher dosage (80 µg/µl) using three scintillation vials joined by glass tubes (Fig. 1). One female coffee berry borer adult was released in the central arena and had a choice of going to a berry treated with 80 µg/µl (*E*,*E*)-α-farnesene or to an untreated berry. Once the insects were released, the vials were capped. We repeated the experiments six times, with four repetitions consisting of 10 individual choice bioassays tests, one repetition consisting of 20, and one repetition consisting of nine (n = 69). Percent berries that had been bored by the insect were assessed 1 and 24 h post-application.

Field Experiment

A field experiment was conducted at a *C. arabica* (cultivar typica) plantation growing at full sun in Pahala, Hawaii (Cloud Rest plantation; 19°11′54.02″ N; 155°31′17.53″ W; 1,720 m above sea level; Fig. 2), with ca. 65% coffee berry borer infestation. Samples (2 ml) of (*E*,*E*)-α-farnesene (Bedoukian Research, Danbury, CT) were formulated (Synergy Semiochemicals Corp., Burnaby, Canada) into 29-mm diameter polyethylene bubble caps, 10-mm deep. Elution studies conducted by Synergy Semiochemicals Corp. in an aging chamber at a constant 25°C indicated (*E*,*E*)-α-farnesene elutes at 5–25 mg per day over a 90-d period.

Fig. 1. Choice bioassay setup using three scintillation vials joined by glass tubes. Holes were made in the scintillation vials with a DeWalt 6.4-mm diamond drill bit. One adult female was released in the central arena and had a choice of going to the control berry or to a berry treated with 80 µg/µl (*E*,*E*)-α-farnesene.

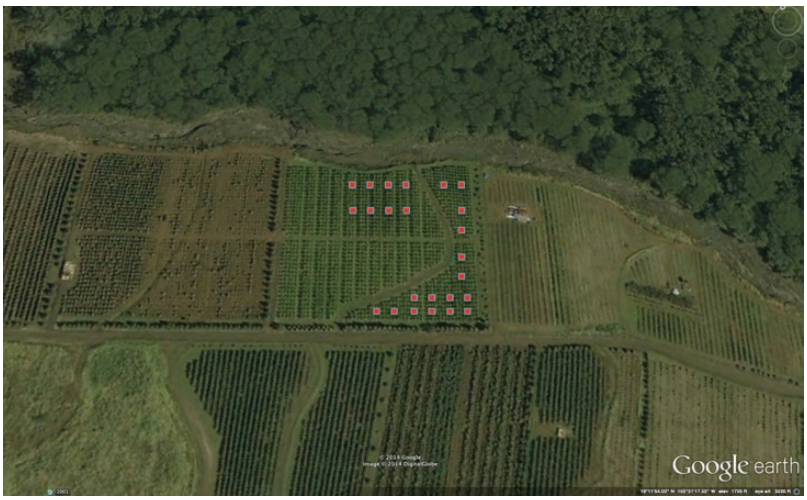

Fig. 2. Google Maps view of Cloud Rest coffee plantation in Pahala, Hawaii, where the field experiment was conducted. Each red square indicates location of traps (n=12/trt).

Commercially available coffee berry borer traps (BROCAP®, Agroindustrias Unidas de México, México; Fig. 3) were hung ca. 1.2 m (4′) high from a coffee branch, with 12 replicates for each of two treatments: (1) traps containing the standard methanol:ethanol attractant at a 3:1 ratio (Mendoza Mora 1991, Vega et al. 2015); and (2) traps containing both the attractant and the (*E*,*E*)-α-farnesene bubble cap formulation. The experimental design was a paired *t*-test. Figure 3A shows the trap design with the attractant dispenser immediately above the funnel and the bubble cap in the upper left side (white plastic). Figure 3B shows a closeup of the bubble cap. Trapped beetles were collected in soapy water. The methanol:ethanol dispenser and trapping solution were refilled as needed. Traps were spaced 20 m apart from each other (Fig. 2). The number of insects captured in each trap was determined every week for 19 wk.

Statistical Analysis

Results were analyzed using Student *t*-test in Excel (Microsoft, Inc, Bellevue, WA) to determine the significance of the difference of the means. In all tests, a two-tailed distribution was assumed. In all but the last experiment (the field study) equal variance was assumed. For the field study, which was designed as a paired *t*-test (a design commonly used for these types of field studies), a paired *t*-test analysis was employed. This analysis was also appropriate because the number of beetles captured in a single week varied by as much as an order of magnitude. Results for the *t*-test are displayed as the probability that the means of the two populations were equal. Thus, a probability of 0.05 indicates that the means are unequal at the 95% confidence level.

Results

Volatile Collection and Identification

GC-MS comparisons of the SPME volatile profiles showed consistently four compounds in single coffee berry borer infested berries but not in the control berries (indicated with an asterisk; Fig. 4). These compounds (MW 204) were tentatively identified as sesquiterpenes by the mass spectral library. One of the sesquiterpenes was tentatively identified as (*E*,*E*)-α-farnesene. The GC retention time (14.84 min) and mass spectrum of this sesquiterpene match those of the HPLC-purified (*E*,*E*)-α-farnesene and the (*E*,*E*)-α-farnesene obtained from Contech. The compound ratios of these four sesquiterpenes were variable, but (*E*,*E*)-α-farnesene was the major component in all five replicates. Besides quantitative differences, there were very few qualitative differences in the HayeSep-Q collected volatiles between 10 infested and 10 uninfested berries, but hints of the earlier SPME compounds (Fig. 4) of interest were detectable in the infested sample. Different from the SPME collections, these dynamic HaySep-Q collected volatiles were collected from the whole berry and thus showed a relative abundance of compounds compared to the static and focused SPME collections. These additional compounds interfered with purification attempts (HPLC and LC) of the sesquiterpenes of interest, and because of the very small amounts (picograms) of sesquiterpenes present with SPME samples of infested berries samples and the very similar mass spectra, no further attempts were made to identify the remaining three sesquiterpenes of interest.

Electrophysiology

The GC-EAD analysis for a mixture of standard compounds is shown in Fig. 5. The responses were variable due to differences in antennal preparations; however, female coffee berry borer antennae gave positive EAD responses to (*E*,*E*)-α-farnesene (**b**) and an unidentified farnesene isomer (**c**) as well as to the positive (5R,7S)-conophthorin (**a**) control. Because of the very small amounts of volatiles collected, no successful GC-EAD responses were recorded with the SPME collected volatiles and only standard samples were used to determine antennal activity.

The three additional unidentified sesquiterpenes might potentially be behaviorally active compounds; however, those compounds would also be present in the control traps because the whole of the coffee orchard was infested with the beetles. The detected *E*,*E*-farnesene seems to be localized with the bored hole of the infested berry and less with the infested berry as a whole, even though this was not fully examined in this study. In more natural circumstances, *E*,*E*-farnesene might deter additional beetles boring into an already infested berry at *E*,*E*-farnesene concentrations that are much lower than used in this study. However, in this study, the concentrations of *E*,*E*-farnesene used in bioassays and field experiments still deterred the beetles by delayed berry boring in the bioassays and lower trap catches in the field indicating similar deterrent effects at higher concentrations. The unidentified EAD-active *E*,*E*-farnesene isomer in the Contech samples was not present in the *E*,*E*-farnesene supplied by Bedoukian for the field bubble cap fomulations and thus would not be part of the observed field behavioral activity.

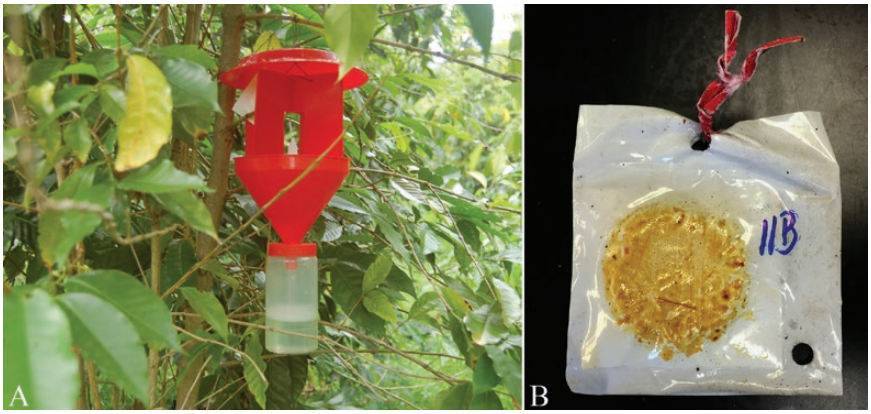

Fig. 3. (A) BROCAP® trap containing the standard methanol:ethanol attractant at a 3:1 ratio and the (*E*,*E*)-α-farnesene bubble cap. The attractant dispenser is immediately above the funnel, and the bubble cap can be seen in the upper left side (white plastic). (B) Closeup of a bubble cap.

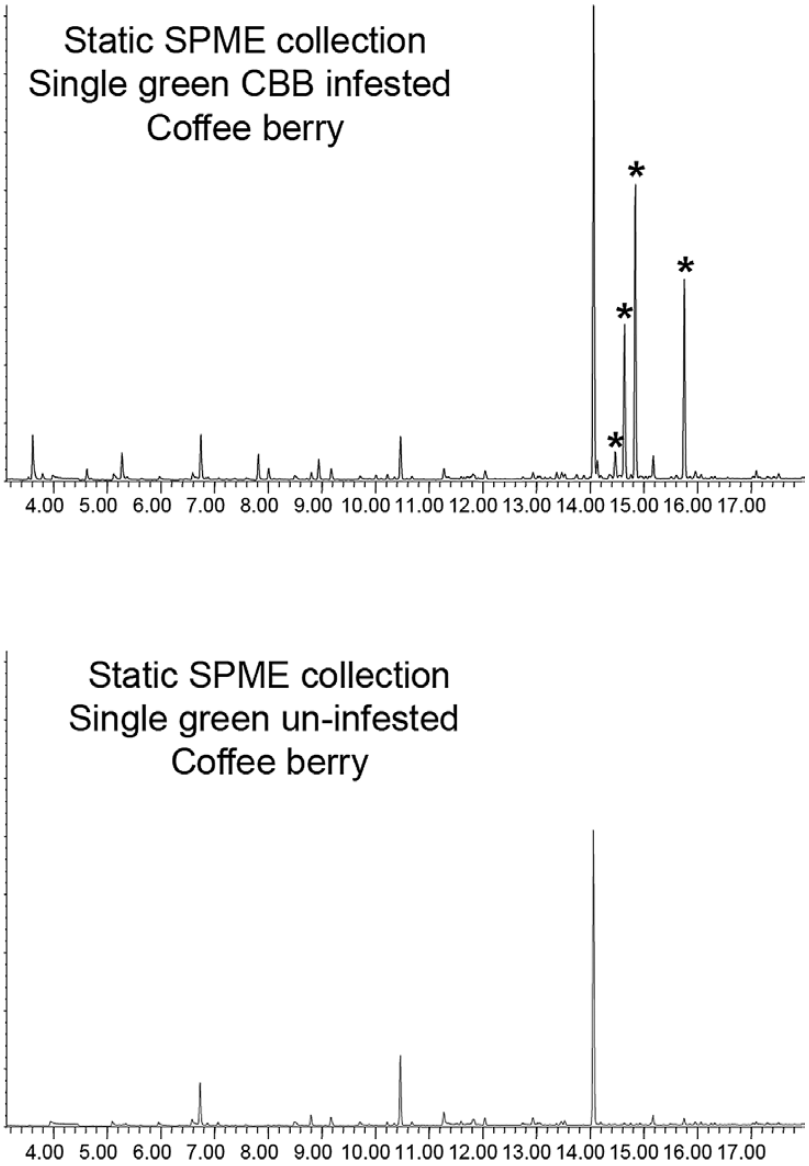

Fig. 4. Static solid-phase microextraction (SPME) headspace volatiles collection of single coffee berry borer-infested berry and uninfested coffee berry. (*E*,*E*)-α-farnesene eluted at 14.84 min.

Laboratory Bioassays

The two preliminary bioassays showed a lower percentage of coffee berries being bored after application. One hour after applying 40 µg/µl (*E*,*E*)-α-farnesene to both ends of the coffee berries, 55% of the berries in the control had been bored by insects in contrast to 10% in the treated berries (*n*=20/trt). At 17 h post-initiation, 65% of the berries in the control had been bored versus 10% in treated berries. In the second preliminary bioassay, application of (*E*,*E*)-α-farnesene at 0.4, 4, and 40 µg/µl resulted in 70%, 30%, and 5% bored berries 1 h post application, respectively, compared to 89.5% in the control, and in 75%, 30%, 25% bored berries 24 h post-application, respectively, compared to 95% in the control.

In the third bioassay, percent bored berries were determined using 4 and 40 µg/µl (*E*,*E*)-α-farnesene and compared to the control. Means at 1 and 24 h post-application for the control (63% and 94%, respectively) and for 4 µg/µl (*E*,*E*)-α-farnesene (16% and 89%, respectively) were significantly different at the 95% confidence level (*P*=0.007, *P*=<0.001, respectively), indicating that as time increased from 1 h to 24 h, the percentage of bored berries increased significantly. At 1 h post-application, there were also significant differences at the 95% confidence level between the control and 4 µg/µl (63% vs 16%, respectively; *P*=<0.001), indicating an initial strong repellent effect. This effect disappears at 24 h, when mean comparisons between the control and 4 µg/µl reveal no significant differences (94% vs 89%, respectively; *P*=0.264). There were not enough data points collected at 40 µg/µl to permit a valid statistical analysis.

Results of the three separate bioassays per day, repeated over 3 d using the Contech Enterprises (*E*,*E*)-α-farnesene, revealed that means at 1 versus 24 h post-application for the control (82% vs 91%), 40 µg/µl (52% vs 79%), and 80 µg/µl (26% vs 43%) were significantly different at the 95% confidence level (*P*=0.048, *P*=0.004, *P*=0.014, respectively), indicating that as time increased from 1 h to 24 h, the percentage bored berries increased significantly. At 1-h post-application, there were also significant differences at the 95% confidence level between the control and 40 µg/µl (82% vs 52%, respectively; *P*=<0.001) and between the control and 80 µg/µl (82% vs 26%,

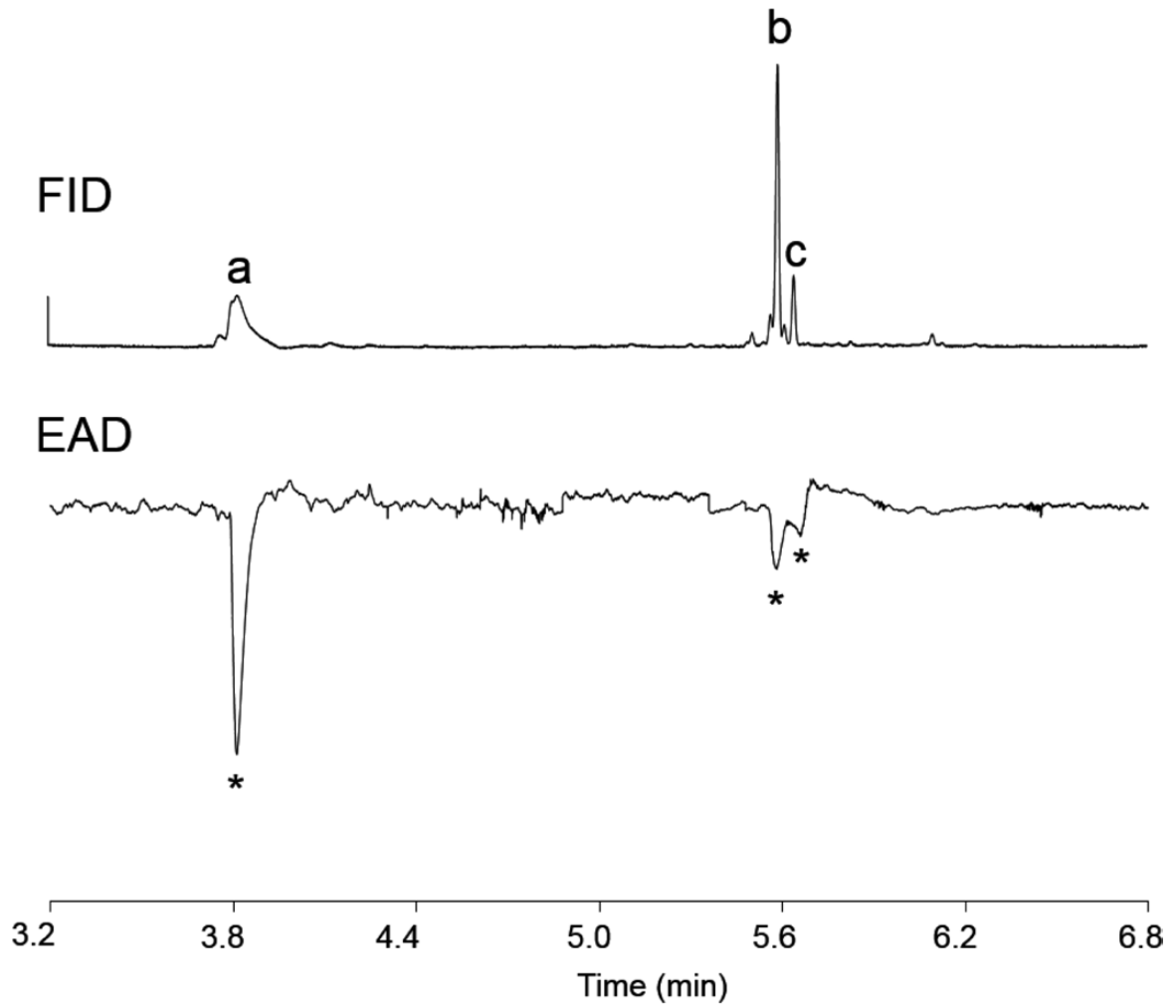

Fig. 5. Simultaneously recorded gas chromatogram (flame ionization detector [FID]) and electroantennogram (EAD) from a female coffee berry borer antenna responding (*) to standard samples of (5R,7S)-conophthorin (**a**), (*E,E*)-α-farnesene (**b**), and an unidentified farnesene isomer (**c**).

respectively; P=<0.001). At 24 h post-application, there were significant differences at the 90% confidence level between the control and 40 µg/µl (91% vs 79%, respectively; P=0.093) and significant differences at the 95% confidence level between the control and 80 µg/µl (91% vs 43%, respectively; P=<0.001).

Results of the choice bioassays experiments (Table 1) revealed that 1 h post application, there were no significant differences between (a) the number of insects in the control compared to the center arena (P=0.539), the center arena versus 80 µg/µl (*E*,*E*)-α-farnesene (P=0.255), or the control versus 80 µg/µl (P=0.390). In contrast, 24 h post-application, there were significant differences in (a) the number of insects in the control compared to the center arena (P=0.008) and the control versus 80 µg/µl (P=0.02; paired *t*-tests), while there were no significant differences in the center arena versus 80 µg/µl (P=0.967). One-hour post-application, there were significant differences (P = 0.028) in the percentage of bored berries in the control (16%) versus the treated berries (0%), and 24-h post-application, the difference was highly significant (P = <0.001), with 45% of the control berries being bored in contrast to 9% in the treated berries (Table 1).

Field Experiment

A total of 58,018 coffee berry borers were captured over 19 wk (Table 2). The mean ± SD for weekly captures was 2,208 ± 1,556 in traps with the attractant in contrast to 846 ± 791 in traps containing both the attractant and (*E*,*E*)-α-farnesene. These differences were highly significant (P = <0.001; paired *t*-tests). In two instances, captures were 80 and 81% lower (Table 2) in traps with attractant and (*E*,*E*)-α-farnesene. Over the 19-wk sampling period, traps with the attractant and (*E*,*E*)-α-farnesene had 62% lower captures (16,073 insects) than traps containing just the attractant (41,945 insects). The bubble caps installed on August 18, 2014 were never replaced, thus remaining effective for at least 19 wk (Table 2).

Discussion

Several diverse roles for (*E*,*E*)-α-farnesene have been discovered in nature. For example, (*E*,*E*)-α-farnesene has been identified in the natural waxy coating of apples (Huelin and Murray 1966); as an attractant and oviposition stimulant for *Cydia pomonella* (Lepidoptera: Tortricidae; Wearing and Hutchins 1973, Sutherland et al. 1977); as an alarm pheromone for *Prorhinotermes canalifrons* (Isoptera: Rhinotermitidae; Šobotník et al. 2008); as a possible repellent against *Pityophthorus pubescens* (Coleoptera: Curculionidae: Scolytinae; López et al. 2013); and as a component of the *Anoplophora glabripennis* pheromone (Coleoptera: Cerambycidae; Crook et al. 2014), among others.

Table 1. Results of choice bioassay using three scintillation vials joined by glass tubes (Fig. 1). Female adults were released in a central arena (center) and had a choice of going to the control berry or to a berry treated with 80 µg/µl (*E,E*)-α-farnesene. Percent berries that had been bored by the insect were assessed 1 and 24 h post-application

Rep	1 h (berries bored in parenthesis)			24 h (berries bored in parenthesis)			*n*
	Control	Center	80 µg/µl	Control	Center	80 µg/µl	
1	3(0)	4	3(0)	3(3)	4	3(2)	10
2	2(0)	8	0(0)	7(6)	3	0(0)	10
3	3(2)	2	4(0)	4(3)	1	4(2)	9
4	4(3)	5	1(0)	6(5)	2	2(0)	10
5	3(2)	2	5(0)	4(4)	2	4(1)	10
6	10(5)	6	4(0)	11(11)	6	3(0)	20
Total	25(12)	27	17(0)	35(32)	18	16(5)	69
% insects in each arena	34%	40%	26%	50%	25%	25%	-
P		0.539 (control vs center)			0.008 (control vs center)		-
		0.255 (center vs 80 µg/µl)			0.967 (center vs 80 µg/µl)		-
		0.390 (control vs 80 µg/µl)			0.020 (control vs 80 µg/µl)		-
% bored berries	16%	-	0%	45%	-	9%	-
P		0.028 (control vs 80 µg/µl)			<0.000 (control vs 80 µg/µl)		-

Table 2. Weekly coffee berry borer captures in experiment conducted at an abandoned coffee plantation growing at full sun in Pahala, Hawaii (Fig. 2). Insects were captured in BROCAP® traps with attractant and in traps combining the attractant and (*E,E*)-α-farnesene (Fig. 3)

Week no.	Sampling date (2014)	Attractant	Attractant + (*E,E*)-α-farnesene	Total	% Reduction
1	25 Aug	3,408	690	4,098	80%
2	1 Sept	2,802	750	3,552	73%
3	8 Sept	1,297	467	1,764	64%
4	15 Sept	786	191	977	76%
5	22 Sept	1,323	376	1,699	72%
6	29 Sept	856	289	1,145	66%
7	6 Oct	646	295	941	54%
8	13 Oct	906	343	1,249	62%
9	20 Oct	961	405	1,366	58%
10	27 Oct	1,233	749	1,982	40%
11	3 Nov	1,024	559	1,583	45%
12	10 Nov	3,688	1,817	5,505	51%
13	17 Nov	2,827	1,049	3,876	63%
14	24 Nov	2,238	428	2,666	81%
15	1 Dec	3,888	1,017	4,905	74%
16	8 Dec	1,154	547	1,701	53%
17	15 Dec	6,404	3,529	9,933	45%
18	22 Dec	2,251	835	3,086	63%
19	29 Dec	4,253	1,737	5,990	59%
	TOTALS	**41,945**	**16,073**	-	**62%**
	Mean ± SD	2,208 ± 1,556	846 ± 791	3,054 ± 2,294	-
	***P*=**		**<0.001**	-	-

(*E,E*)-α-farnesene is a volatile sesquiterpene with two conjugated double bonds. These reactive molecules are susceptible to inactivation by photochemical and air oxidation (Anet 1969, Spicer et al. 1993). For sustained longevity in the field, it must be protected from degradation. In most coffee-growing areas, there are frequent rains and periods of intense sunlight, which will preclude using (*E,E*)-α-farnesene as a direct foliar spray. In addition, farnesene could also cause phytoxicity (Ju and Curry 2000; Araniti et al. 2013, 2016), another reason for its unsuitability as a foliar spray. Passive volatile delivery systems are regulated by Fick's Law of diffusion (Fick 1855, Philibert 2005), and very small reservoirs such as microcapsules have a limited capacity to sustain delivery of volatiles over long periods of time. Microencapsulation is also expensive and seldom used for semiochemical delivery systems. On the other hand, inexpensive polyethylene blister packages known as bubble caps, have much larger reservoirs and offer a more favorable surface area to volume ratio. Bubble caps deliver many semiochemicals reliably and have made repellant semiochemical usage practical and affordable. For example, hundreds of thousands of 3-methyl-2-cyclohexenone bubble caps are used annually in North America as an antiaggregation pheromone to protect Douglas-fir trees from Douglas-fir beetle attack (*Dendroctonus pseudotsugae* Hopkins; Coleoptera: Curculionidae: Scolytinae) (Wakarchuk, unpublished data). Bubble caps can be tailored for sustained field use, selecting from a variety

of membranes that can also aid in screening out ultraviolet radiation and augmenting stabilizers added to the semiochemical reservoir. In the present work, the bubble caps were still active 19 wk after installation, which is similar to the other sesquiterpene bubble cap lures deployed in Florida (Kendra et al. 2016). Shelf life of the bubble caps under freezer conditions is stable; often a year or more storage is not detrimental to the product (Wakarchuk, unpublished data). These properties make a bubble cap system very appealing for economical delivery of the potential coffee berry borer repellent. The field deployment of an effective coffee berry borer repellent could become a pest management strategy that would not require combination with other tactics in order for it to be effective in reducing infestation levels and consequently, increasing yields.

Simultaneous use of coffee berry borer attractants and repellents alone or in combination as a push-pull strategy (Hassanali et al. 2008, Khan et al. 2011, López et al. 2013, Njihia et al. 2014) merits detailed study in coffee plantations. In a 3-wk field experiment in a Kenyan coffee plantation, the *Dendroctonus* antiaggregation pheromone frontalin was shown by Njihia et al. (2014) to act 'both as a repellent and an inhibitor of *H. hampei* attractants.' Traps containing the methanol:ethanol (1:1) attractant and frontalin had 77% lower trap captures than when the attractant was used by itself (4.44 ± 1.32 vs 19.00 ± 4.29, respectively). In contrast to the present study, in which (*E,E*)-α-farnesene was still active 19 wk post placement in the field, all the chemicals used in the Kenya field study were replaced on a weekly basis (Njihia et al. 2014).

Even though the use of coffee berry borer repellent plants in the field remains mostly unexplored, work by Pohlan (2005) and Pohlan et al. (2008) suggests that *Canavalia ensiformis* (Leguminosae) might have coffee berry borer repellent effects in coffee plantations in Mexico. The possible use of repellent plants in coffee plantations needs further study.

In conclusion, use of bubble caps formulations of (*E,E*)-α-farnesene with a long field life and that growers can easily install on coffee branches could become a reliable and easy-to-implement pest management strategy against the coffee berry borer.

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We dedicate this paper to the memory of Thomas "Bull" Kailiawa III (1963–2017), a preeminent Hawaiian coffee grower that allowed us to conduct fieldwork in Kailiawa Coffee Cloud Rest plantation, in Pahala, HI.

References Cited

Anet E. F. L. J. 1969. Autoxidation of α-farnesene. Aust. J. Chem. 22: 2403–2410.

Araniti, F., E. Graña, M. J. Reigosa, A. M. Sánchez-Moreiras, and M. R. Abenavoli. 2013. Individual and joint activity of terpenoids, isolated from *Calamintha nepeta* extract, on *Arabidopsis thaliana*. Nat. Prod. Res. 27: 2297–2303.

Araniti, F., E. Graña, U. Krasuska, R. Bogatek, M. J. Reigosa, M. R. Abenavoli, and A. M. Sánchez-Moreiras. 2016. Loss of gravitropism in farnesene-treated arabidopsis is due to microtubule malformations related to hormonal and ROS unbalance. PLoS ONE. 11: e0160202.

Borbón-Martínez, O., O. Mora Alfaro, A. Cam Oehlschlager, and L. M. González. 2000. Proyecto de trampas, atrayentes y repelentes para el control de la broca del fruto de cafeto, *Hypothenemus hampei* L. (Coleoptera: Scolytidae). Memorias del XIX Simposio Latinoamericano de Caficultura, San José, Costa Rica, pp. 331–348.

Brun, L. O., and F. Mathieu. 1997. Utilisation d'un piège à attractif kairomonal pour le suivi des populations du scolyte du café en champ. 17th International Scientific Colloquium on Coffee, Nairobi, Kenya, pp. 714–717.

Cárdenas, M. R. 2000. Trampas y atrayentes para monitoreo de poblaciones de broca del café *Hypothenemus hampei* (Ferrari) (Col., Scolytidae). Memorias del XIX Simposio Latinoamericano de Caficultura, San José, Costa Rica, pp. 369–379.

Cossé, A. A., and R. J. Bartelt. 2000. Male-produced aggregation pheromone of *Colopterus truncatus*: structure, electrophysiological, and behavioral activity. J. Chem. Ecol. 26: 1735–1748.

Crook, D. J., D. R. Lance, and V. C. Mastro. 2014. Identification of a potential third component of the male-produced pheromone of *Anoplophora glabripennis* and its effect on behavior. J. Chem. Ecol. 40: 1241–1250.

Dufour, B. P., and B. Frérot. 2008. Optimization of coffee berry borer, *Hypothenemus hampei* Ferrari (Col., Scolytidae), mass trapping with an attractant mixture. J. Appl. Entomol. 132: 591–600.

Ephrussi, B., and G. W. Beadle. 1936. A technique of transplantation for *Drosophila*. Am. Nat. 70: 218–225.

Esquinca Avilés, H. A. 1986. Búsqueda de semioquímicos en la interacción cafeto *Coffea* spp. – broca del café *Hypothenemus hampei* (Ferrari) mediante bioensayos olfatométricos. B.S. thesis, Universidad Autónoma de Chiapas, México.

Fick, A. 1855. Ueber diffusion. Ann. Phys. 170: 59–86.

Giordanengo, P., L. O. Brun, and B. Frerot. 1993. Evidence for allelochemical attraction of the coffee berry borer, *Hypothenemus hampei*, by coffee berries. J. Chem. Ecol. 19: 763–769.

Góngora, C., E. Macea, A. M. Castro, A. M., S. Idarraga, M. A. Cristancho, P. Benavides, D. Galbraith, C. Vanier, and A. L. Gaitán. 2012. Interaction between coffee plants and the insect coffee berry borer, *Hypothenemus hampei*. 24th International Scientific Colloquium on Coffee, San José, Costa Rica, pp. 533–541.

Gutiérrez-Martínez, A., and R. N. Ondarza. 1996. Kairomone effect of extracts from *Coffea canephora* over *Hypothenemus hampei* (Coleoptera: Scolytidae). Environ. Entomol. 25: 96–100.

Gutiérrez-Martínez, A., and A. Virgen Sánchez. 1995. Guía Práctica: Preparación de extractos crudos para obtener los semioquímicos volátiles para el manejo de la broca del café (*Hypothenemus hampei*). El Colegio de la Frontera Sur, Tapachula, Chiapas, México.

Gutiérrez-Martínez, A., R. Haro-González, and S. Hernández Rivas. 1990. Kairomona responsable de la atracción de la broca del café *Hypothenemus hampei* Ferrari (Coleoptera: Scolytidae) al grano de café. Congreso Nacional MIP, Managua, Nicaragua, pp. 28–36.

Hassanali, A., H. Herren, Z. R. Khan, J. A. Pickett, and C. M. Woodcock. 2008. Integrated pest management: the push–pull approach for controlling insect pests and weeds of cereals, and its potential for other agricultural systems including animal husbandry. Phil. Trans. R. Soc. B. 363: 611–621.

Heath, R. R., and P. E. Sonnet. 1980. Technique for *in situ* coating of Ag+ onto silica gel in HPLC columns for the separation of geometrical isomers. J. Liq. Chromatogr. 3: 1129–1135.

Huelin, F. E., and K. E. Murray. 1966. (E,E)-α-farnesene in the natural coating of apples. Nature. 210: 1260–1261.

Jaramillo, J., B. Torto, D. Mwenda, A. Troeger, C. Borgemeister, H. M. Poehling, and W. Francke. 2013. Coffee berry borer joins bark beetles in coffee klatch. PLoS ONE. 8: e74277.

Ju, Z., and E. A. Curry. 2000. Lovastatin inhibits α-farnesene biosynthesis and scald development in 'Delicious' and 'Granny Smith' apples and 'd'Anjou' pears. J. Amer. Soc. Hort. Sci. 125: 626–629.

Kendra, P. E., W. S. Montgomery, M. A. Deyrup, and D. Wakarchuk. 2016. Improved lure for redbay ambrosia beetle developed by enrichment of α-copaene content. J. Pest Sci. 89: 427–438.

Khan, Z., C. Midega, J. Pittchar, J. Pickett, and T. Bruce. 2011. Push–pull technology: a conservation agriculture approach for integrated management of insect pests, weeds and soil health in Africa. Int. J. Agric. Sustainability. 9: 162–170.

López, S., C. Quero, J. C. Iturrondobeitia, A. Guerrero, and A. Goldarazena. 2013. Electrophysiological and behavioural responses of *Pityophthorus pubescens* (Coleoptera: Scolytinae) to (E,E)-α-farnesene, ®-(+)-limonene and (S)-(-)-verbenone in *Pinus radiata* (Pinaceae) stands in northern Spain. Pest Manag. Sci. 69: 40–47.

Mathieu, F., L. Brun, and B. Frérot. 1991. Preliminary results on comparative GC analyses of volatiles produced by the coffee berries. 14th International Scientific Colloquium on Coffee, San Francisco, California, pp. 687–689.

Mathieu, F., C. Malosse, A.-H. Cain, and B. Frérot. 1996. Comparative headspace analysis of fresh red coffee berries from different cultivated varieties of coffee trees. J. High Resol. Chromatogr. 5: 298–300.

Mathieu, F., C. Malosse, and B. Frérot. 1998. Identification of the volatile components released by fresh coffee berries at different stages of ripeness. J. Agric. Food Chem. 46: 1106–1110.

Mathieu, F., L. O. Brun, B. Frérot, D. M. Suckling, and C. Frampton. 1999. Progression in field infestation is linked with trapping of coffee berry borer, *Hypothenemus hampei* (Col., Scolytidae). J. Appl. Entomol. 123: 535–540.

Mathieu, F., V. Gaudichon, L. O. Brun, and B. Frérot. 2001. Effect of physiological status on olfactory and visual responses of female *Hypothenemus hampei* during host plant colonization. Physiol. Entomol. 26: 189–193.

Mendesil, E., T. J. Bruce, C. M. Woodcock, J. C. Caulfield, E. Seyoum, and J. A. Pickett. 2009. Semiochemicals used in host location by the coffee berry borer, *Hypothenemus hampei*. J. Chem. Ecol. 35: 944–950.

Mendoza Mora, J. R. 1991. Resposta da broca-do-café, *Hypothenemus hampei*, a estímulos visuais e semioquímicos. M.S. thesis, Universidade Federal de Viçosa, Brasil.

Njihia, T. N., J. Jaramillo, L. Murungi, D. Mwenda, B. Orindi, H. M. Poehling, and B. Torto. 2014. Spiroacetals in the colonization behaviour of the coffee berry borer: a 'push-pull' system. PLoS ONE. 9: e111316.

Oliveira, C. M., A. M. Auad, S. M. Mendes, and M. R. Frizzas. 2013. Economic impact of exotic insect pests in Brazilian agriculture. J. Appl. Entomol. 137: 1–15.

Ortiz, A., A. Ortiz, F. E. Vega, and F. Posada. 2004. Volatile composition of coffee berries at different stages of ripeness and their possible attraction to the coffee berry borer *Hypothenemus hampei* (Coleoptera: Curculionidae). J. Agric. Food Chem. 52: 5914–5918.

Philibert, J. 2005. One and a half century of diffusion: Fick, Einstein, before and beyond. Diffus. Fundamen. 2: 1.1–1.10.

Pohlan, H. A. J. 2005. Manejo de la cenosis en cafetales y sus impactos sobre insectos, con especial énfasis en la broca del café, pp. 22–30. In J. F. Barrera (ed.), Simposio sobre la situación actual y perspectivas de la investigación y manejo de la broca del café en Cuba, Costa Rica, Guatemala y México. Sociedad Mexicana de Entomología, Tapachula, Chiapas, México.

Pohlan, H. A. J., M. J. J. Janssens, B. Giesemann Eversbusch. 2008. Impact of *Canavalia* cover crop management in *Coffea arabica* L. on plant- invertebrate associations. Open Agric. J. 2: 84–89.

Prates, H. S. 1969. Observações preliminares da atração da broca do café, *Hypothenemus hampei* (Ferrari, 1867), a extratos de frutos do cafeeiro (cereja e verde). Solo (Brazil) 61: 13–14.

Rojas, J. 2005. Ecología química de la broca del café y sus parasitoides, pp. 12–21. In J. F. Barrera (ed.), Simposio sobre la situación actual y perspectivas de la investigación y manejo de la broca del café en Cuba, Costa Rica, Guatemala y México. Sociedad Mexicana de Entomología, Tapachula, Chiapas, México.

Saravanan, P. A., and K. Chozhan. 2003. Monitoring and management of coffee berry borer, *Hypothenemus hampei* Ferrari (Scolytidae: Coleoptera). Crop Res. 26: 154–158.

Šobotník, J., R. Hanus, B. Kalinová, R. Piskorski, J. Cvačka, T. Bourguignon, Y. Roisin. 2008. (E,E)-α-farnesene, an alarm pheromone of the termite *Prorhinotermes canalifrons*. J. Chem. Ecol. 34: 478–486.

Spicer, J. A., M. A. Brimble, and D. D. Rowan. 1993. Oxidation of α-farnesene. Aust. J. Chem. 46: 1929–1939.

Sutherland, O. R. W., C. H. Wearing, and R. F. N. Hutchins. 1977. Production of (E,E)-α-farnesene, an attractant and oviposition stimulant for codling moth, by developing fruit of ten varieties of apple. J. Chem. Ecol. 3: 625–631.

Vega, F. E., F. Infante, and A. J. Johnson. 2015. The genus *Hypothenemus*, with emphasis on *H. hampei*, the coffee berry borer, pp. 427–494. In F. E. Vega and R. W. Hofstetter (eds.), Bark beetles: biology and ecology of native and invasive species. Academic Press, San Diego, CA.

Vega, F. E., M. Kramer, and J. Jaramillo. 2011. Increasing coffee berry borer (*Hypothenemus hampei*; Coleoptera: Curculionidae: Scolytinae) female density in artificial diet decreases fecundity. J. Econ. Entomol. 104: 87–93.

Velasco Pascual, H., B. Beristain Ruiz, S. Díaz Cárdenas, J. M. Llavén Gómez, and A. F. Velázquez Velázquez. 1997a. Respuesta de la broca del fruto *Hypothenemus hampei* Ferr. a extractos de cerezas de café utilizados como atrayentes en Tepatlaxco, Veracruz, México. Informe de proyecto. Sistema de Investigación del Golfo de México, México.

Velasco Pascual, H., J. M. Llavén Gómez, and A. F. Velázquez Velázquez. 1997b. Respuesta a extractos de cerezas de café utilizados como atrayente para hembras intercosecha de la broca del fruto *Hypothenemus hampei* Ferr. Memorias XVIII Simposio Latinoamericano de Caficultura, San José, Costa Rica, pp. 349–352.

Wearing, C. H., and R. F. N. Hutchins. 1973. (E,E)-α-farnesene, a naturally occurring oviposition stimulant for the codling moth *Laspeyresia pomonella*. J. Insect Physiol. 19: 1251–1256.

Wiley. 2005. Wiley. Spectral Library, [CD-ROM]. 7th ed. Wiley, New York.