A potential pheromone for the mass trapping of *Aethina tumida* (Coleoptera: Nitidulidae)

Charles J. Stuhl[1,*] *and Peter E. A. Teal*[1,†]

Abstract

Observations of wild and colony-reared small hive beetle populations indicate that the beetles form aggregations of many individuals of both sexes. Volatile collections performed on males and females have identified a potential male-produced pheromone comprised of 6-methyl-5-hepten-2-one, nonanal, and decanal. Flight tunnel and laboratory trapping assays were conducted using a synthetic pheromone blend (i.e., 6-methyl-5-hepten-2-one, nonanal, and decanal) and a blend of fruit volatiles (i.e., ethanol, ethyl butyrate, acetic acid, ethyl acetate, and acetaldehyde). Results showed that the synthetic pheromone blend along with a fruit-derived attractant captured significantly more beetles than the control. The identification of a potential pheromone is an important step in the search to provide effective control and monitoring of the small hive beetle.

Key Words: small hive beetle, *Apis mellifera*; chemical ecology; semiochemicals; aggregation

Resumen

Las observaciones de poblaciones de pequeños escarabajos de la colmena silvestre criados en colonias indican que los escarabajos forman agregaciones de muchos individuos de ambos sexos. Recolecciones volátiles realizadas sobre los machos y hembras han identificado una posible feromona producida por machos compuesta por 6-metil-5-hepten-2-ona, nonanal y decanal. Los ensayos de captura en laboratorio y en el túnel de vuelo se realizaron utilizando una mezcla de feromonas sintéticas (es decir, 6-metil-5-hepten-2-ona, nonanal y decanal) y de volátiles de frutas (etanol, butirato de etilo, ácido acético, acetato de etilo y acetaldehído). Los resultados mostraron que la mezcla de feromonas sintéticas junto con un atrayente derivado de frutas capturó significativamente más escarabajos que el control. La identificación de una feromona potencial es un paso importante en la búsqueda para proporcionar un control y seguimiento efectivos del pequeño escarabajo de la colmena.

Palabras Clave: escarabajo pequeño de la colmena; *Apis mellifera*; ecología química; semioquímicos; agregación

The western or European honey bee (*Apis mellifera* L.; Hymenoptera: Apidae) are susceptible to pests and diseases, some that are honey bee specific. These infestations and infections impact the health of a honey bee colony, ranging from minor stress to the death of the colony. The small hive beetle, *Aethina tumida* Murray (Coleoptera: Nitidulidae), is a European honey bee pest that is destructive to honey bee colonies. This nitidulid species originated in sub-Saharan Africa where it is considered a minor bee pest (Zawislak 2010). The small hive beetle has been present in the US since 1996 and on the Australian continent since 2000 (Neumann & Elzen 2004; Ellis & Hepburn 2006). The rapid spread of this pest throughout the world and its impact on honey bee populations has compelled the need for an effective trapping system to reduce its impact on honey production and honey bee survival that may lead to a reduction in pollination. Although it is the larvae of the small hive beetle that causes the damage inside the hive, there is no effective control measure for eliminating them; therefore, trapping must be targeted at the adult beetles (Hood 2004). There have been numerous attempts at developing control strategies for larvae and adults with the use of entomopathogenic fungi, nematodes, and use of pesticides to reduce populations of beetles within the hive (Cuthbertson et al. 2013). Honey bee and colony produced volatiles and pollen dough have been used in the past to attract adult small hive beetles (Suazo et al. 2003; Arbogast et al. 2007). Many of the commercially available pollen substitutes have the potential to be modified with an insecticide to bait and kill adult small hive beetles (Stuhl 2017). Previous studies have used corrugated cardboard and corrugated plastic sheets that had been treated with the active ingredient coumaphos. The small hive beetles enter the corrugated opening and encounter the insecticide. For larval control, GardStar® (Y-Tex Corp., Cody, Wyoming, USA), in an insecticidal soil drench, is applied outside of the hive (Neumann & Hoffmann 2008). This targets the small hive beetle larvae as they pupate in the soil and adults as they emerge. Unfortunately, these were just bioassays and none of these control measures are standard practice (Cuthbertson et al. 2013). Stuhl (2019) demonstrated that small hive beetle can be attracted to a trap containing an edible toxic bait for control using an attract and kill method. A bait treated with boric acid was placed in a trap that did not allow honey bee access. The small hive beetles ate the treated bait and died inside the trap within 24 h.

It has been shown that some species in the family Nitidulidae are found in ripe fruits and are considered pests of fruit and stored foods (Hood 2004, 2011). Small hive beetles have been shown to be attracted to fruit volatiles. Cantaloupe, *Cucumis melo* L. (Cucurbiataceae), a variety of muskmelon, has been found to be very attractive to small hive beetles and other nitidulids (Williams et al. 1984; Price & Young 2006).

Observations of wild small hive beetle populations and colony-reared beetles indicate that small hive beetles form aggregations of many individuals of both sexes (Mustafa et al. 2015). This observation led to the investigation into the possibility of a pheromone. The im-

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portance of pheromones has been well documented from the family Nitidulidae (Cossé & Bartelt 2000) and male produced pheromones have been identified in other Coleoptera such as *Gnathotrichus sulcatus* (LeConte) (Coleoptera: Curculionidae) (Byrne et al 1974). This study was conducted to determine if a pheromone could be identified from the small hive beetle, and additionally, could a synthetic pheromone blend be developed that initiated the same behavioral response in the adult small hive beetles. A trapping assay was developed using the pheromone and a fruit volatile blend isolated from ripe fruit that has shown to be attractive to small hive beetle by Stuhl (2021). The target strategy of the trap used was to direct attraction and capturing small hive beetle adults upon emergence from the soil before they enter the hive with the potential to develop an in-hive baited trap.

Materials and Methods

SOURCE OF SMALL HIVE BEETLES

Small hive beetles were collected from wild populations and then reared in laboratory colonies for 2 generations. Small hive beetles were collected with an aspirator from honey bee hives maintained at the US Department of Agriculture, Agriculture Research Service, Center for Medical, Agricultural, and Veterinary Entomology, Gainesville, Florida, USA. All small hive beetles were reared as per Stuhl (2022). Small hive beetles were sexed as pupae and placed in moistened soil in separate containers. Insects were reared in a temperature-controlled chamber at 23 ± 5 °C, 60% RH, and 12:12 (L:D) h photoperiod.

VOLATILE COLLECTIONS

Volatiles were collected separately from 100 colony-reared adult males 1 wk after emergence. All collections were performed at the US Department of Agriculture, Agriculture Research Service, Center for Medical, Agricultural, and Veterinary Entomology in Gainesville, Florida, USA. Volatiles were collected using a head space collection technique (Heath & Manukian 1992). The 100 small hive beetle males were placed in a glass volatile collection chamber (34 cm long × 4 cm outside diam) with a glass frit inlet, a glass joint outlet, and a single port collector base. The collection chamber was covered with a dark cloth and the male insects were allowed to aggregate for 1 h in the chamber before each collection. Dry charcoal filtered air was pushed into 1 end the chamber at 0.25 L per min and over the small hive beetles where it exited the chamber via a vacuum system. The air then passed through a volatile collection filter containing 50 mg of Tenax® porous polymer adsorbent (Sigma-Aldrich, St. Louis, Missouri, USA) for 5 min. There were 5 replicates performed for each sex. The filters were cleaned between collections by removing the gas chromatograph-mass spectrometer injection port liner and replacing it with the Tenax® filter. The gas chromatograph-mass spectrometer inlet temperature of 200 °C removed all traces of compounds. To quantify the amount of each compound in the sample, volatiles from 100 colony-reared adult males were collected using the previously stated head space collection technique. However, volatiles were collected onto Porapak Q adsorbent (Sigma-Aldrich, St. Louis, Missouri, USA) for 2 h.

IDENTIFICATION OF THE VOLATILE COMPOUNDS WITH GAS CHROMATOGRAPHY-MASS SPECTROMETRY

The volatile compounds collected onto the Tenax® filter were analyzed by gas chromatography-mass spectrometry. The gas chromatograph was an Agilent 6890 (Agilent Technologies, Inc., Santa Clara, California, USA) with a 30 m long HP-5MS capillary column with 0.25 mm inner diam and 0.25 µm film thickness. The mass spectrometer was an Agilent 5973 (Agilent Technologies, Inc., Santa Clara, California, USA) mass selective detector, 70 eV, equipped with an in-house designed thermal desorption cold trap injector (Alborn 2018). Headspace volatiles collected on the Tenax® filter were desorbed at 220 °C for 2 min by an increased flow of helium carrier gas. The desorbed compounds were trapped and focused by a thermal gradient on the first 5 cm of the column at −78 °C. The separation was initiated by turning off the coolant and allowing the trap to reach the oven temperature by convection heating, thus avoiding thermal degradation. The oven temperature of the gas chromatograph was programmed to rise from 30 °C (3-min hold) to 260 °C at 10 °C per min.

The small hive beetle pheromone collected onto the Porapac Q adsorbent were prepared by eluting the filter with 150 µL of dichloromethane containing 10 µg per mL hexacosane (Sigma-Aldrich, St. Louis, Missouri, USA) as an internal standard. Aliquots (2 µL) of the extracts were analyzed by gas chromatography-mass spectrometry as stated for the Tenax® filter without the use of the cold trap injector. The volatiles were identified by comparison of mass spectra with mass spectra libraries (NIST 2014) and with mass spectra and retention times of authentic standards.

SYNTHETIC PHEROMONE AND FRUIT BLEND PREPARATION

All chemicals were purchased from Sigma-Aldrich, St. Louis, Missouri, USA. The pheromone blend for the electrophysiological assays was prepared using dichloromethane (2 mL) in place of ethanol for use in the gas chromatograph. The pheromone blend consisted of 6 chemicals: 2 µL 6-methyl-5-hepten-2-one; 2 µL nonanal; 1 µL decanal; 1 µL acetic acid; 1 µL ethyl acetate; 1 µL octanal.

The blends for the flight tunnel and trapping assays were made using 99% ethanol. In our experience, ethanol is better tolerated by the insects during exposure assays than dichloromethane. The abundance of each compound was identified from the gas chromatograph-mass spectrometer and verified with known amounts of standards to determine the concentration to use in the assay. The pheromone blend consisted of 2 µL of 6-methyl-5-hepten-2-one, 2 µL nonanal, and 1 µL decanal in 10 mL of 99% ethanol, with the resulting pheromone blend having a concentration of 0.5 µL per mL of blend in ethanol. The fruit blend was created as outlined in Stuhl (2021) containing 1 µL of ethyl butyrate, 2 µL acetic acid, 2 µL ethyl acetate, and 1 µL acetaldehyde (all from Sigma-Aldrich, St. Louis, Missouri, USA) in 10 mL of 99% ethanol. The resulting fruit blend has a concentration of 0.6 µL per mL of fruit blend in 99% ethanol.

ELECTROPHYSIOLOGY RESPONSE TO THE VOLATILE COMPOUNDS

To determine if male and female small hive beetle had a sensory response to specific compounds isolated from small hive beetle adult males, both antennae of each beetle were exposed to the synthetic pheromone blend using a gas chromatograph-electroantennographic detector. The blend was injected into the gas chromatograph, split flow interfaced to both flame ionization and electroantennograph detectors. In this manner, antennal responses were matched with flame ionization signals for compounds eluting from the gas chromatograph. A 1 µL sample of the blend was injected into a Hewlett-Packard 5890 Series II gas chromatograph equipped with an Hewlett-Packard-5 column (30 m × 0.32 mm ID × 0.25 mm) (Agilent, Palo Alto, California, USA). The oven temperature was held at 40 °C for 5 min, then programmed to increase to 10°C per min to 220 °C and held at this temperature for 5

min. Helium was used as a carrier gas at a flow rate of 2.0 mL per min. A charcoal filtered humidified air stream was delivered over the antenna at 1 mL per min. Small hive beetle antennae were excised by grasping the scape at its base with jeweler's forceps (No. 5, Miltex GmbH, Rietheim-Weilheim, Germany). The extreme distal and proximal ends of the antennae were placed in conductivity gel (Parker Labs, Fairfield, New Jersey, USA) between a forked electrode (Syntech, Buschbacher, Germany). The gas chromatograph-electroantennal detector threshold was set to 2.5 millivolts per s. The electroantennal detector and flame ionization signals were concurrently recorded with a gas chromatograph-electroantennographic detector program (Syntech EAGPro, Eager, Germany), which analyzed the amplified signals. Both antennae of 20 male and 20 female small hive beetle were assayed. We calculated the percentage of small hive beetle males and females that had an antennal response using gas chromatograph-electroantennographic detector by compound.

FLIGHT TUNNEL BIOASSAY

A flight tunnel bioassay was developed to determine the response of small hive beetle to the synthetic pheromone blend along with a fruit blend as outlined in Stuhl (2021). Males, females, and both sexes combined were assayed. There were 10 replicates of each treatment (pheromone blend, fruit blend, pheromone+fruit blend). The flight tunnel, as stated in Stuhl et al. (2011), was constructed of clear acrylic sheets and measured 128.0 × 31.8 × 31.8 cm and was located inside a walk-in environmental chamber at the US Department of Agriculture, Agriculture Research Service, Center for Medical, Agricultural, and Veterinary Entomology in Gainesville, Florida, USA. Illumination was provided by fluorescent bulbs above the flight tunnel. The light source and the light emitted by the room lighting produced an illumination within the tunnel of 1,600 lux. The room temperature ranged from 28.7 to 28.8 °C and humidity was 37.6 to 38.1% RH. Air flow within the tunnel was produced by a shaded pole blower (Dayton, Niles, Illinois, USA), which pulled air into the tunnel through a charcoal filter and exhausted it outside the chamber. The exhaust end was screened to prevent insects from entering the tube. Airflow could be adjusted using a baffle inside a tube that connected the downwind end of the tunnel with the exhaust system of the hood. Air speed was maintained at 0.2 m per s. This flow was determined to be the speed that most stimulated flight in the small hive beetle (Stuhl 2017).

Two 3.8 L glass jars fitted with metal lids containing 2 brass hose fittings contained the treatments and allowed air to pass over the odor source in 1 jar and the blank control in the other and emerge separately in the flight tunnel. Air flow into the jars was controlled by an adjustable flow meter (Aalborg Instruments, Monsey, New York, USA) set at 0.5 L of air per min. The 2 flows of treated air emerged into 2 insect traps located at the upwind end of the tunnel placed midway between its ceiling and floor. The traps were constructed from 40-dram clear plastic snap cap vials (Thornton Plastics, Salt Lake City, Utah, USA). A 10 mm hole was made in the center of the cap to allow insects to enter the chamber. For combined sexes, 25 males and 25 females were placed in the flight tunnel and were checked every half h from 9:00 AM to 2:00 PM. When a single sex was assayed, 50 insects were placed in the flight tunnel and checked as stated for the combined sexes. A positive response was recorded when there was a small hive beetle inside the trap. The insect was removed from the trap and replaced with a naive insect from a stock cage, which was released into the flight tunnel. The position of the treatment and control were changed after each replication to prevent positional effects. The treatments assayed were the synthetic pheromone blend, the fruit blend, and ethanol as the control.

An aliquot (300 µL) of a blend was placed on a 4.5 cm diam filter paper (Whatman®, W&H Balston Limited, St. Albans, United Kingdom). Filter papers were placed in the glass jar and were randomized after each replicate. Statistical analyses were conducted by using SAS programming (SAS 2014). Analysis of variance (PROC ANOVA) followed by means separation through the Waller test was employed to compare the mean responses to various treatments.

TRAPPING BOIASSAY

Trapping assays were performed in a climate-controlled chamber at 23 ± 5 °C, 60% RH, and 12:12 (L:D) h photoperiod. An inverted Rescue Reusable Stink bug trap (Sterling International, Inc., Spokane, Washington, USA) was used in the assay. The trap was inverted to allow the entrance to face upright. Two traps, 1 containing the pheromone+fruit blend and a blank control, were suspended from the ceiling of a screen mesh cylindrical field cage (91.5 cm diam × 183.0 cm high). The blend contained 2 µL of 6-methyl-5-hepten-2-one, 2 µL nonanal, and 1 µL decanal, 1 µL ethyl butyrate, 2 µL acetic acid, 2 µL ethyl acetate, and 1 µL acetaldehyde in 10 mL of 99% ethanol. A 1 mL aliquot of the pheromone+fruit blend was delivered via an impregnated 3 cm cotton dental wick placed inside a 1 mL Eppendorf® tube (Sigma-Aldrich, St. Louis, Missouri, USA). The control of 99% ethanol was delivered in the same manner. The open tube was then attached to the inside chamber of the trap. The traps were positioned 1 m apart. A vial containing 200 male and 200 female newly emerged small hive beetle adults was opened inside the screen mesh cylindrical field cage. The assay was run for 24 h (9:00 AM–9:00 AM) after which the trapped insects were counted. The position of the treatment and control were changed after each replication. There were 10 replicates performed. Small hive beetles that did not respond were removed from the cage at the end of the assay and counted. A second cage of the same dimensions was used for the next repetition while the assayed cage vented for 24 h. A paired *t*-test procedure was performed to compare captures in the traps baited with pheromone+fruit blend and control (SAS 2014).

Results

IDENTIFICATION OF THE PHEROMONE

There were many peaks that were detected in the volatile extracts collected from the male small hive beetles, with 6 identified by gas chromatography-mass spectrometry: acetic acid, ethyl acetate, octanal, 6-methyl-5-hepten-2-one, nonanal, and decanal (Table 1). Three compounds, 6-methyl-5-hepten-2-one, nonanal, and decanal, were present in all of the volatile extracts. Many of the smaller peaks were from the plastics used in the collection of the volatiles (Fig. 1).

ELECTROPHYSIOLOGY RESPONSE TO THE SYNTHETIC PHEROMONE BLEND

Sensilla on the antennae of small hive beetle males and females responded to the synthetic pheromone blend comprised of acetic acid, ethyl acetate, octanal, 6-methyl-5-hepten-2-one, nonanal, and decanal. This procedure allowed for the evaluation and selection of the active compounds that initiated an electrophysiological response. The strongest responses were to 6-methyl-5-hepten-2-one, nonanal, and decanal (Fig. 2). The percentage of small hive beetle males and females that had an antennal response to the gas chromatograph-electroantennographic detector to the pheromone blend is shown in Table 1.

Table 1. Gas chromatograph-mass spectrometry components identified from male small hive beetles. Percentage of small hive beetle males and females that had an antennal response to the gas chromatography electroantennographic detector individual compounds.

Peak	Compound	Presence or absence	% Response	
			Male	Female
1	acetic acid	(+)	0.97	0.98
2	ethyl acetate	(+)	0.96	0.96
3	octanal	(+)	0.10	0.10
4	6-methyl-5-hepten-2-one	+	0.98	1.00
5	nonanal	+	0.90	0.94
6	decanal	+	0.98	0.98

Key: + detected; (+) not always detected in all samples analyzed by gas chromatograph-mass spectrometry.

FLIGHT TUNNEL BIOASSAY

The flight tunnel assays utilized a pheromone or fruit blend compared with a control. The final pheromone blend contained 6-methyl-5-hepten-2-one, nonanal, and decanal because it was present in all the small hive beetle volatile extracts and these 3 chemicals produced the strongest responses by gas chromatograph-electroantennal detector. The small hive beetles that selected the pheromone or fruit blend, control, and those that made no choice (no response) were counted. The pooled flight tunnel assay results indicated a slight attraction of small hive beetle to the pheromone blend (F = 138.63; df = 2; P < 0.0001). The capture rate for the pheromone blend was 39% and the blank control 1.5% (Fig. 3). However, the greatest response (60%) was from the small hive beetles that did not make a choice. There was no difference in attraction to the treatments amongst males and females (F = 1.28; df = 2; P = 0.1716). The greatest response was to the fruit blend (65%; F = 116.83; df = 2; P > 0.0001) (Fig. 4). This was followed by the small hive beetles that did not respond (32.6%) and those that selected the control (> 1%). There was no sexual bias in small hive beetle response amongst the treatments (F = 1.28; df = 2; P = 0.1716). There was a significant increase in trap capture when the pheromone blend was used in conjunction with the fruit blend (pheromone+fruit; F = 112682; df = 2; P > 0.0001) when compared to the control. The pheromone+fruit blend accounted for 98% of the small hive beetle capture, blank control > 1%, no response > 1% (Fig. 5). Males and females were equally attracted and showed no bias amongst the treatments (F = 1.45; df = 2; P = 0.2013).

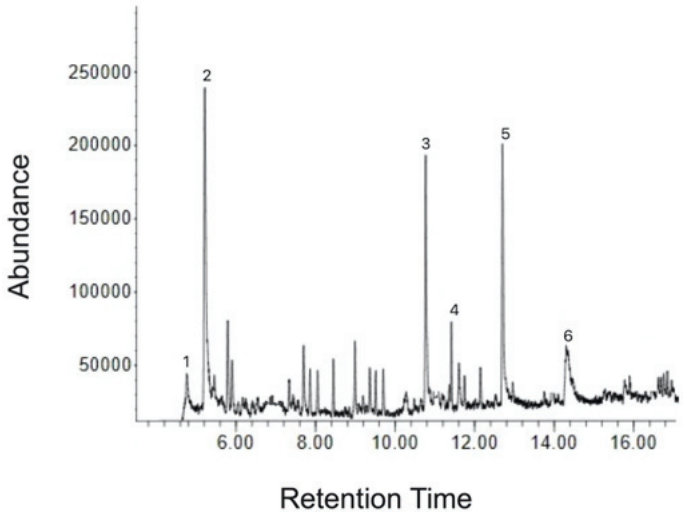

Fig. 1. Representative total ion chromatogram of volatiles released by male small hive beetles (n = 100) and captured on a Tenax® porous polymer adsorbent. Peak number compound: (1) acetic acid; (2) ethyl acetate; (3) octanal; (4) 6-methyl-5-hepten-2-one; (5) nonanal; (6) decanal.

TRAPPING BIOASSAY

The trapping bioassay confirmed the wind tunnel attraction to the pheromone+fruit blend compared to control. There was a significant increase in capture of small hive beetles to the pheromone+fruit blend (t = 1.53; df = 2; P > 0.0001). The trapping response for the pheromone+fruit volatile blend was 99%, blank control 1%, no response 1% (Fig. 6). There was no sexual bias in attraction to the treatments (t = −4.66; df = 2; P = 0.2136).

Discussion

The objective of this research was to investigate a potential pheromone associated with the small hive beetle that could be further developed to reduce beetle infestation in honeybee hives. There were multiple peaks that were detected in the volatiles collected from the male small hive beetles, with 6 identified by gas chromatograph-mass spectrometer. All the peaks were identified by their mass spectra and confirmed by gas chromatograph retention time and gas chromatography-electroantennographic detector comparison with authentic compounds that were presented to the small hive beetles in electroantennal detector assays. Many of the compounds seen in the collections were from the collection equipment and not produced by the small hive beetle. Gas chromatography-mass spectrometry identified 6-methyl-5-hepten-2-one, nonanal, and decanal as the most abundant and the 3 compounds elicited strong antennal responses by the small hive beetle using gas chromatograph-electroantennographic detector. Although the small hive beetles had an antennal response to individual compounds, only the blend initiated a strong behavioral response. This was further improved by the addition of the fruit blend that was previously shown to be attractive to the small hive beetles (Stuhl 2021). Flight tunnel bioassays indicated that the pheromone alone did not capture many small hive beetles and there were many beetles that did not respond to the treatment. However, observations during the assays indicated many clusters of small hive beetles throughout the assay chamber. This may be due to the presence of the pheromone, which alone causes the small hive beetles to aggregate and form these masses. A smaller, well ventilated assay chamber with individual small hive beetles may provide different results.

When the fruit odor alone was presented in the flight tunnel, there was a 65% capture of the released small hive beetles; however, the pheromone+fruit blend attracted 98% of the beetles. A similar syner-

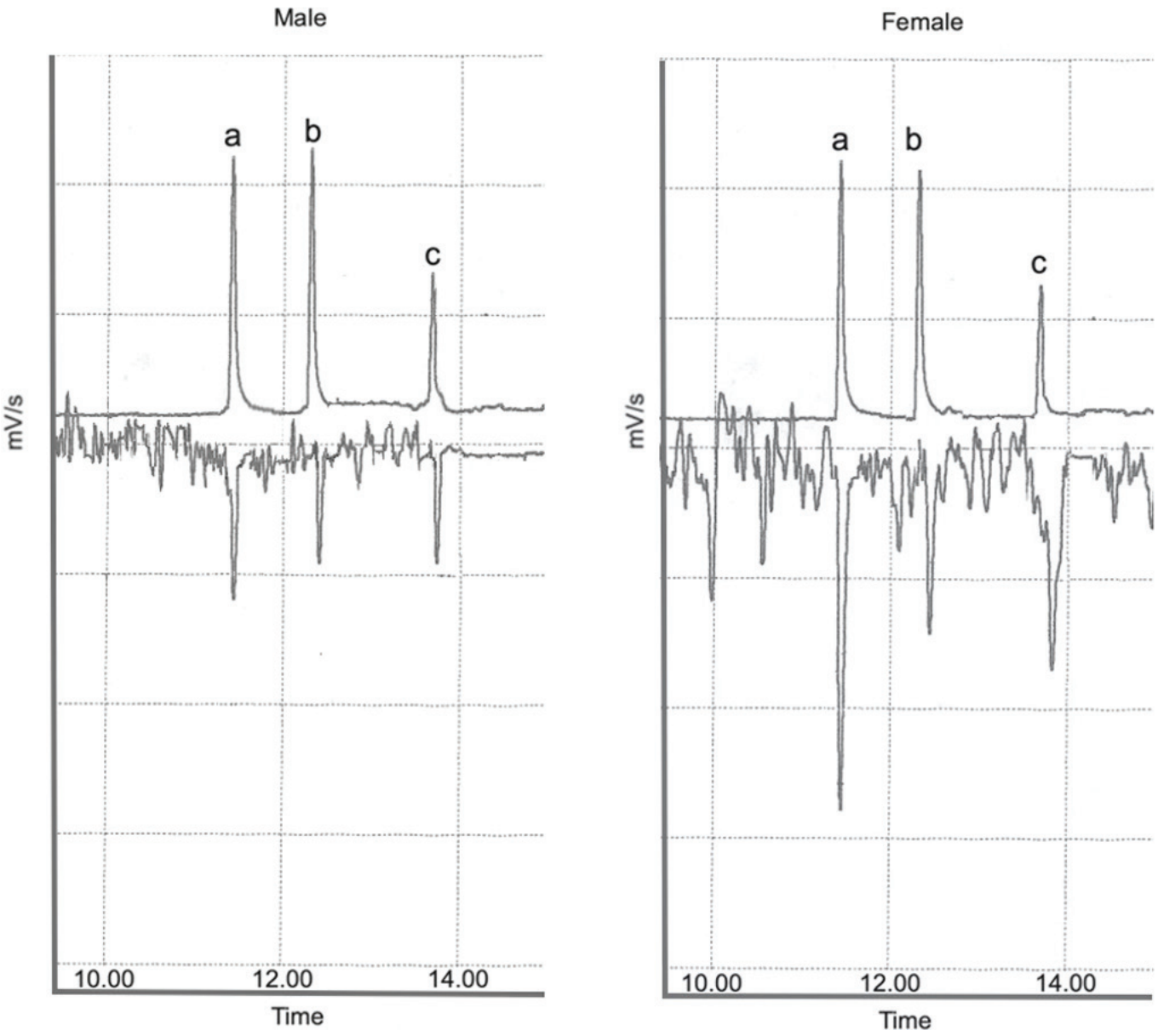

Fig 2. Small hive beetle male and female gas chromatography electroantennographic detector response to (a) 6-methyl-5-hepten-2-one; (b) 5-nonanal; (c) 6-decanal.

gistic effect when pairing a pheromone with a host odor has been seen for other Coleoptera species such as dusky sap beetle (Lin et al. 1992) and the maize weevil (Walgenbach et al. 1987). The association of volatiles from a food source or potential oviposition site with a pheromone greatly increases attraction (Bartelt & Dowd 1991).

We saw no sexual bias in attraction of small hive beetles to the pheromone blend. Mixed aggregations of male and female small hive beetles were present in the clusters of beetles in the assay chamber during exposure to the pheromone. Male and female jewel beetles are attracted to specific pheromones that are produced by the females. Studies have shown that these pheromones can elicit specific behaviors in males, such as antennal movements and wing-fanning, and that they play a critical role in the formation of mating pairs (Millar et al. 2018; Millar & Hanks 2021). Future studies could evaluate the effect of the small hive beetle pheromone on intricate mating behaviors.

The placement of baited traps in the large enclosure containing the pheromone+fruit blend resulted in a significantly large capture of the released small hive beetles. The control and those that did not respond were < 1%. Observations of the captured small hive beetles revealed aggregations of beetles within the traps. Small hive beetles within the trap formed aggregations and remained motionless. This behavior prevented the small hive beetles from escaping the trap. Further investigation of the high capture rate reported no sexual bias.

It is difficult to control this pest within a hive by means of an insecticide without harming the honey bee adults and brood (Kuan et al. 2018). A possible alternative would be a baited trap that is directed at the small hive beetle and restricts honey bee access. This method could be used to target this pest within and outside of the hive. Trapping has been successfully demonstrated for monitoring and the reduction of other nitidulid beetle populations (Peña et al. 1999). Historically, the most successful integrated pest management eradication of an agricultural pest was the Boll Weevil Eradication Program. The discovery of the male-produced pheromone of the cotton boll weevil, *Anthonomus grandis* Boheman (Coleoptera: Curculionidae), led to the development of an eradication strategy (Tumlinson et al. 1969). This strategy used pheromone in combination with a food odor for weevil

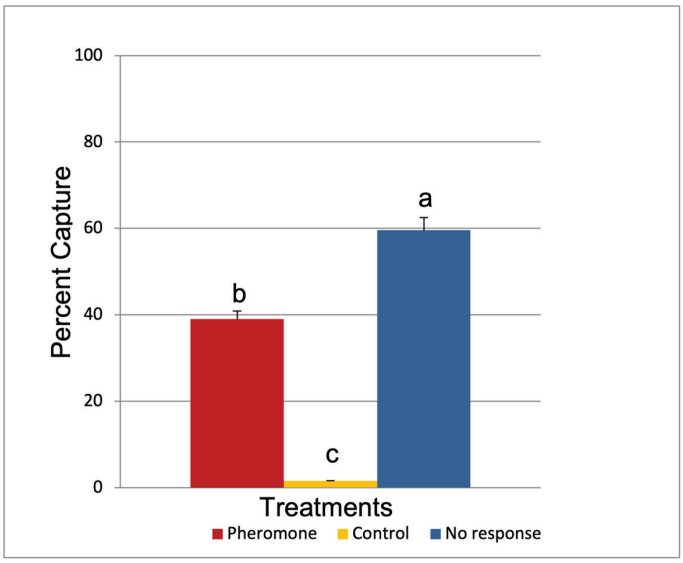

Fig 3. Attraction of combined male and female small hive beetle attraction to the pheromone blend in a flight tunnel. Means number of small hive beetles captured (± SE) with shared letters are not significantly different.

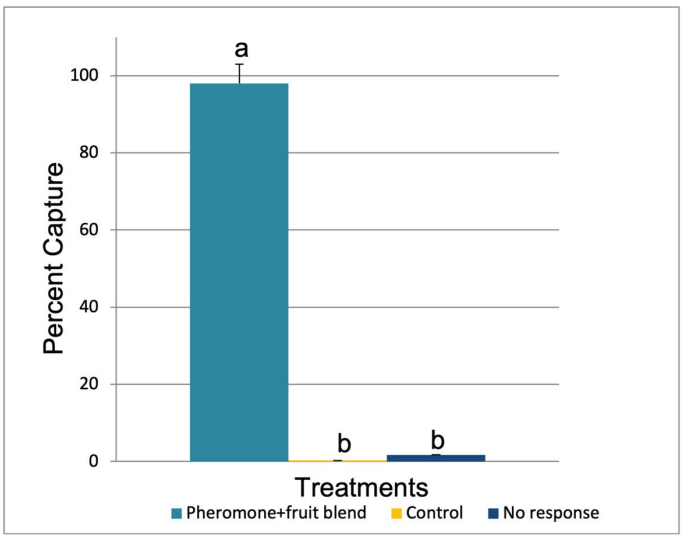

Fig 5. Attraction of combined male and female small hive beetles to pheromone+fruit blend in a flight tunnel. Mean number of small hive beetles captured (± SE) with shared letters are not significantly different.

detection, cultural practices by modifying the weevil's habitat to decrease its food supply, followed by chemical treatments that reduced the weevil populations. A similar integrated pest management strategy is needed for effective small hive beetle control. The further development of our pheromone+fruit blend may provide the key to sustainable management.

Future research will examine the role of other components in synergizing the attractiveness of the pheromone blend with the development into an effective attractant for small hive beetle control. Applications within the laboratory do not always transfer with the same results into a hive environment. Although no sex bias was observed in our laboratory assays, field studies need to confirm that all sexes will respond equally to the blend. Additionally, an investigation into the effectiveness of the blends over an extended period should be undertaken. This may be accomplished by assaying the blend in a variety of matrices.

The development of novel, safe, and sustainable alternatives to control honey bee pests that destroy the hive and have the potential to spread disease are needed urgently. Future research will examine the contribution of other components in synergizing the attractiveness of the pheromone blend with the development of this blend into an effective lure for trapping the small hive beetle.

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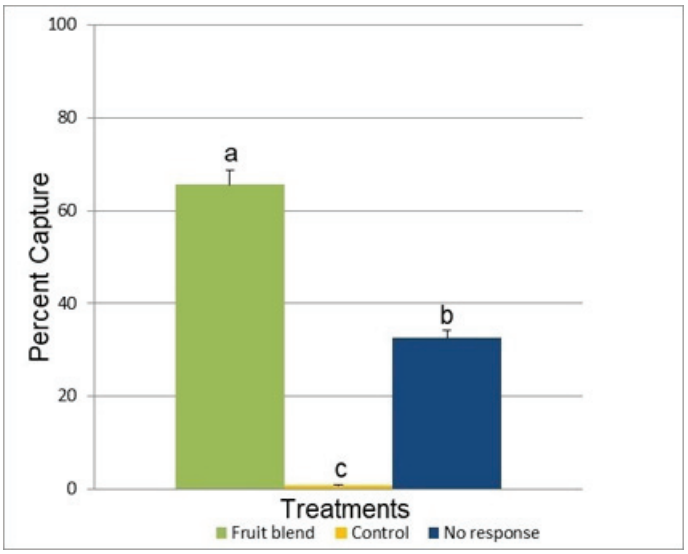

Fig 4. Attraction of combined male and female small hive beetles to the fruit blend in a flight tunnel. Means number of small hive beetles captured (± SE) with shared letters are not significantly different.

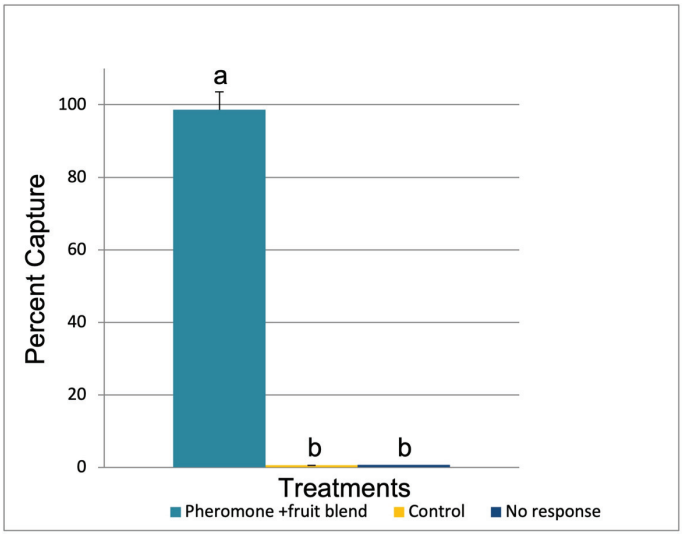

Fig 6. Attraction of small hive beetles to pheromone+fruit blend in a semi-field trapping assay. Mean number of small hive beetles captured (± SE) with shared letters are not significantly different.

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