Small Hive Beetle (Coleoptera: Nitidulidae) Attraction to a Blend of Fruit Volatiles

Author: Stuhl, Charles J.

Source: Florida Entomologist, 104(3) : 153-157

Published By: Florida Entomological Society

URL: https://doi.org/10.1653/024.104.0301

Research

Small hive beetle (Coleoptera: Nitidulidae) attraction to a blend of fruit volatiles

Charles J. Stuhl[1,*]

Abstract

The small hive beetle, *Aethina tumida* Murray (Coleoptera: Nitidulidae), belongs to a family of beetles known as sap beetles. As an agricultural pest they feed upon damaged, overripe fruits and vegetables, such as strawberries, corn, melons, tomatoes, and raspberries. The small hive beetle is a major parasite of honey bee hives worldwide. The beetle lives in the honey bee hive and feeds on honey, pollen, and honey bee brood. Fruit volatiles collected from overripe fruit provide for an effective attractant for both sexes of the small hive beetle. A laboratory trapping assay was performed using ripe fruit and a fruit-semiochemical attractant blend containing ethanol, ethyl butyrate, acetic acid, ethyl acetate, and acetaldehyde. Results indicated that the synthetic fruit blends captured beetles at the same rate as the cut fruit. The blend with the highest concentration had significantly more beetles captured. The key to an effective trapping system is a good attractant. The isolated fruit volatiles show promise as a possible attractant for control and monitoring of small hive beetle.

Key Words: *Apis mellifera*; sap beetles; *Aethina tumida*

Resumen

El pequeño escarabajo de la colmena, *Aethina tumida* Murray (Coleoptera: Nitidulidae), pertenece a una familia de escarabajos conocidos como escarabajos de la savia. Como plaga agrícola, se alimentan de frutas y verduras dañadas y demasiado maduras, como fresas, maíz, melones, tomates y frambuesas. El pequeño escarabajo de la colmena es un parásito importante de las colmenas de abejas en todo el mundo. El escarabajo vive en la colmena de abejas y se alimenta de miel, polen y las crías de abejas. Los volátiles de la fruta recolectados de la fruta demasiado madura proporcionan un atrayente eficaz para ambos sexos del pequeño escarabajo de la colmena. Se realizó un ensayo de trampeo de laboratorio utilizando fruta madura y una mezcla de atrayente semioquímico de fruta que contenía etanol, butirato de etilo, ácido acético, acetato de etilo y acetaldehído. Los resultados indicaron que las mezclas de fruta sintética capturaron los escarabajos al mismo razón que la fruta cortada. La mezcla con la concentración más alta capturó significativamente más escarabajos. La clave para un sistema de captura eficaz es un buen atrayente. Los volátiles aislados de la fruta se muestran prometedores como posible atrayente para el control y seguimiento del pequeño escarabajo de la colmena.

Palabras Clave: *Apis mellifera*; escarabajos de la savia; *Aethina tumida*

When fruits ripen, they begin to produce aromatic compounds that are released into the air, giving the mature fruit its pleasant odor. Fruits have evolved to be attractive for frugivores to perform seed-dispersal. In turn, the fruit provides a flesh rich in nutrients such as sugars, fats, proteins, vitamins, and minerals (ScienceDaily 2016). These low molecular weight olfactory signals are easily carried in the air and direct a frugivore to the fruit, whether it is a meal or a host site for reproduction (Reddy & Guerrero 2004.) Insect attraction to fruit is caused by fruit odors and by micro-organisms growing on and within the fruit (Becher et al. 2012).

Fruit volatiles and visual cues play an important role in many insect species, including the small hive beetle, *Aethena tumida* Murray (Coleoptera: Nitidulidae) (Parsons 1943; Hayashi 1978). Beetles of this family can be found feeding on tree sap, flowers, fresh and decaying fruits, and fungi (Parsons 1943; Hayashi 1978). There are only a few Nititulidae beetle species that are of agricultural importance. Sweet and field corn is the preferred host of the dusky sap beetle, *Carpophilus lugubris* Murray, and the corn sap beetle, *Carpophilus dimidiates* (F.) (Capinera 2001). Stored maize is infested by Freeman's sap beetle, *Carpophilus freemani* Dobson, and the confused sap beetle, *Carpophilus mutilatus* Erichson (all Coleoptera: Nitidulidae) (Arbogast & Throne 1997). Pineapples, strawberries, and an array of dried fruit have their own specialized nepticulid pests (Potter 1995). Small hive beetles can survive on fruit but prefer to feed and reproduce within honey bee hives. Additionally, small hive beetle adults are attracted to the honey bee colony by detecting hive odors (Torto et al. 2005).

Native to sub-Saharan Africa, this beetle has become a major pest of the Western and European honey bee (*Apis mellifera* L.; Hymenoptera: Apidae) worldwide. Except for Antarctica, small hive beetle is now present on all continents (Evans et al. 2018). The Africanized bee has an evolutionary history with the beetle and can maintain their hive with no deleterious effects from the beetle (Lundie 1940; Torto et al. 2005).

[1]USDA-ARS, Center for Medical, Agricultural and Veterinary Entomology, 1700 SW 23rd Drive, Gainesville, Florida 32608, USA; E-mail: charles.stuhl@usda.gov (C. J. S.)
*Corresponding author; E-mail: charles.stuhl@usda.gov

The Western honey bee is crucial in their role as pollinators for agricultural crops in the US. Approximately one-third of our foods rely on honey bees for pollination. Pollinators are critical to our nation's economy, food security, and environmental health. Honey bee pollination adds more than $15 billion in value to agricultural crops each yr and provides a foundation to ensure our diets are plentiful with fruits, nuts, and vegetables.

The widespread cultural practice for managing beetles is the use of apple cider vinegar and cooking oil placed in a Cutts Beetle Blaster trap (M0195, Dadant, Hamilton, Illinois, USA). Small hive beetle adults are attracted to the vinegar due to their evolutionary history with overripe fruit. Other attractants used are ripe banana peel and pollen patties inoculated with yeast (Hood & Miller 2003; Zawislak 2014). Small hive beetle has been observed feeding and reproducing on bananas, mango, grapes, strawberries, avocado, cantaloupe, pineapple, honeydew, and starfruit (Eischen 1999; Buchholz et al. 2008). Current trapping methods maintain the beetles at economic thresholds, but unfortunately none of these traps eliminate the beetles from the hive (Hood & Miller 2003). It has been demonstrated that small hive beetle can be attracted to a baited trap for control (Stuhl 2019). The beetle's attraction to fruit is dependent upon specific blends of volatile compounds and usually not a single compound. However, there are compounds within a blend that are essential to initiate a behavioral response (Light et al. 2001; Reddy & Guerrero 2004).

This research investigated the beetle's attraction to ripe cantaloupe (*Cucumis melo* L.; Cucurbitaceae), mango (*Mangifera indica* L.; Anacardiaceae), and peach (*Prunus persica* [L.] Batsch; Rosaceae). Fruits and a synthetic blend of compounds derived from fruit were presented to the beetles for attraction assays. This research reports an investigation comparing the effectiveness of natural and synthetic fruit volatile odors for attracting small hive beetle. It is hypothesized that the synthetic odors will be as effective in beetle attraction as their natural counterparts. The information from this research may lead to enhanced attraction when used in conjunction with current trapping methods.

Materials and Methods

SOURCE OF BEETLES

Laboratory colonies of small hive beetle were collected from wild populations in honey bee hives kept at the USDA-Agriculture Research Service, Center for Medical, Agricultural and Veterinary Entomology, Gainesville, Florida, USA, and were maintained for multiple generations. Beetles were reared on pollen dough (Global Patties, Butte, Montana, USA) inoculated with *Kalamata homer* L. (Oleaceae) yeast (Benda et al. 2008; Stuhl 2017). Beetles were sexed as per Neumann et al. (2013) and placed in separate containers. Insects were reared in a temperature-controlled chamber at 23 ± 5 °C, 60% RH, and photoperiod of 12:12 h (L:D).

VOLATILE COLLECTION

Volatiles were collected from ripe cantaloupe (*C. melo*), mango (*M. indica*), and peach (*P. persica*), all purchased from a local market. All fruit collections were performed at the USDA-ARS, Center for Medical, Agricultural and Veterinary Entomology, Gainesville, Florida, USA. Volatiles were collected using a head space collection technique as per Heath and Manukian (1992). Cut fruit was placed in a cylindrical glass volatile collection chamber that was 24 cm tall and 10 cm in diam. Dry charcoal filtered air was pushed into one end of the chamber and over the fruit, and passed through a volatile collection filter containing 50 mg of Tenex® Porous Polymer Adsorbent (Sigma-Aldrich, St. Louis, Missouri, USA) for 5 min via a vacuum system.

The volatile compounds collected from the fruit were analyzed by Gas Chromatography-Mass Spectroscopy (GC-MS) (Gas Chromatograph: Agilent 6890 with an HP-5MS capillary column of 30 m long, 0.25 mm inner diam, and 0.25 µm film thickness; Mass Spectroscope: Agilent 5973 mass selective detector, 70 eV, equipped with a thermal desorption cold trap injector [CP4010; Compacc, Bergen op Zoom, The Netherlands]). Headspace volatiles collected on Tenax® TA (Sigma-Aldrich, St. Louis, Missouri, USA) were released from the adsorbent by heating in the thermal desorption cold trap injector at 220 °C for 8 min within a flow of helium gas. The desorbed compounds were collected in the thermal desorption cold trap injector cold trap unit (SIL-5CB-coated fused silica capillary) at −130 °C. Flash heating of the cold trap unit injected the compounds into the capillary column of the gas chromatograph to which the cold trap unit was connected. The oven temperature of the gas chromatograph was programmed to rise from 40 °C (5 min hold) to 280 °C at 15 °C per min. The headspace volatiles were identified by comparing their mass spectra to those of the database (Wiley7N and Wiley275) and by comparing their retention times to those of authentic compounds. Volatiles were identified by comparison of mass spectra libraries (NIST 2014; Department of Chemical Ecology, Goteborg University, Goteborg, Sweden).

ELECTROPHYSIOLOGY RESPONSE TO FRUIT VOLATILES

The neurophysiological sensory response of male and female small hive beetle was measured to specific compounds isolated from fruit, ethanol, ethyl butyrate, acetic acid, ethyl acetate, and acetaldehyde. Individual compounds (Sigma-Aldrich, St. Louis, Missouri, USA) and blends (Table 1) were exposed to the beetle's antennae using an electroantennographic detector. A synthetic blend was created comprised of ethanol, ethyl butyrate, acetic acid, ethyl acetate, and acetaldehyde (Table 1). Extracts were analyzed with a gas chromatograph interfaced to both flame ionization and electroantennographic detectors. In this manner, antennal responses were matched with flame ionization detector signals for compounds eluting from the gas chromatograph. Volatile extracts were prepared in the method described above, and 1 µL aliquots were analyzed on a Hewlett-Packard (HP) 5890 Series II gas chromatograph equipped with an HP-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 by 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 is at 1 mL per min. The removal of the antenna was performed as described by Stuhl et al. (2011). *Aethina tumida* antennae were excised by grasping the scape at its base with a jeweler's forceps (No. 5, Integra Life Sciences, Plainsboro, New Jersey, USA). The extreme distal and proximal ends of the antennae were placed in conductivity gel (Parker labs, Fairfield, New Jersey, USA) between a forked electrode (Sentech, Buschbacher, Germany). The electroantennographic detector and flame ionization detector signals were recorded concurrently with a gas chromatography-electroantennographic detector program (Sentech, Eager, Germany), which analyzed the amplified signals on a personal computer.

Table 1. Concentrations of the synthetic fruit blends.

Blend	Ethanol	Ethyl acetate	Acetic acid	Acetaldehyde	Ethyl butyrate
1	2 mL	30 µL	30 µL	30 µL	1 µL
2	2 mL	15 µL	15 µL	15 µL	0.5 µL
3	2 mL	7.5 µL	7.5 µL	7.5 µL	0.25 µL

FLIGHT TUNNEL BIOASSAY

A flight tunnel bioassay was developed to determine the response of *A. tumida* to cut fruit and to 3 synthetic fruit blends (Table 1). Males and females (100 each) were combined and assayed in the flight tunnel. The concentration of the synthetic fruit blend was chosen based on the results obtained from the electrophysiological response to the fruit volatiles. The flight tunnel was constructed of clear acrylic sheets, measured 128 cm × 31.8 cm × 31.8 cm and was located inside a walk-in environmental chamber at the Center for Medical, Agricultural and Veterinary Entomology, Gainesville, Florida, USA. The room temperature ranged from 28.7 to 28.8 °C and relative humidity between 37.6 and 38.1%. 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 small hive beetle. 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 about 1,600 lux.

Two 3.8 L glass jars fitted with hose fittings contained the fruit and allowed air to pass over the odor source and the blank control and emerge separately in the flight tunnel. Air flow into the fruit containers was controlled by an adjustable flow meter (Aalborg Instruments, Monsey, New York, USA) set at about 0.5 L per min. Treated air emerged into 2 insect traps located at the upwind end of the tunnel placed midway between its ceiling and floor. Traps were constructed from 40-dram clear plastic snap cap vials (Thornton Plastics, Salt Lake City, Utah, USA). A 10-mm hole was placed in the center of the cap to allow insects to enter the chamber, and 200 beetles were placed in the flight tunnel. Insects could move freely within the flight tunnel for 2 h, after which the collection traps were inspected for a response. Insects were counted and recorded. The position of the treatment and control were alternated after each replication to prevent positional effects. There were 20 replicates performed for each fruit with combined males and females. This also was performed for the synthetic blends. Analysis of data was performed using ANOVA (SAS 2013).

OLFACTOMETER BIOASSAYS

A comparison of fruit odors was tested in a 4 choice olfactometer (Analytical Research Systems, Gainesville, Florida, USA) using the method of Vet et al. (1983). Four glass collection containers were attached to the arms of the olfactometer. Three fruit treatments, i.e., cantaloupe, mango, peach, and a blank control, were used in the bioassay. Charcoal filtered air at 1 mL per min was passed over a 30 g section of fruit that was placed in 475 mL glass jars fitted with hose fittings and allowed air to pass over the odor source and emerge into the olfactometer. A vacuum was set at 1.5 mL per min and attached to the bottom of the olfactometer central arena. The central arena and the collection arms of the olfactometer were covered with a black cloth to avoid light bias. Insects were introduced into the central chamber and allowed to make a choice. The insect response was recorded after 30 min. Before to the next replicate was done, the olfactometer was cleaned with mild soap and water and allowed to dry. A new section of fruit was used for each replicate. Airflow within the olfactometer was stabilized before beginning another replicate. The position of the 3 fruits and the blank were randomized in the 4 arms between replicates. The assay was performed in the same manner using the 3 blend concentrations (Table 1.) and a blank control. The blends were placed on a Whatman® 4.5 cm filter paper (W&H Balston Limited, St Albans, England) treated with 300 µL of the blend. Filter papers were placed in the 475 mL glass jar and presented in the same manner as the fruit, and were randomized after each replicate. Each assay consisted of 20 replicates of 20 adult beetles (10 each of males and females).

Results

IDENTIFICATION OF ATTRACTIVE FRUIT VOLATILES

Five key compounds were isolated from the over-ripe fruit. The most abundant compounds that were common in all fruit were ethanol, ethyl butyrate, acetic acid, ethyl acetate, and acetaldehyde.

ELECTROPHYSIOLOGY RESPONSE TO FRUIT VOLATILES

The antennae of male and female small hive beetle responded to the natural and synthetic fruit volatiles. The greatest response was to ethanol, ethyl butyrate, acetic acid, ethyl acetate, and acetaldehyde. There was no difference in response between sexes.

FLIGHT TUNNEL BIOASSAY

Fruit

The flight tunnel assay results indicated an attraction to the cut fruit. Males and females had the same responses to the odors from cut fruit ($F = 17.44$; df = 6; $P < 0.001$), and were more likely to be trapped by fruit than by their corresponding controls. Male ($F = 4799.58$; df = 2; $P < 0.0001$) and female ($F = 7831.75$; df = 2; $P < 0.0001$) beetles were influenced significantly by the mango fruit (Fig. 1). Additionally, a significant number of male ($F = 793.61$; df = 2; $P < 0.0001$) and female ($F = 3619.26$; df = 2; $P < 0.0001$) beetles were captured with cantaloupe. When presented the peach fruit, male ($F = 347.28$; df = 2; $P < 0.0001$) and female ($F = 964.46$; df = 2; $P < 0.0001$) beetles were attracted significantly to the peach fruit over the control (Fig. 1).

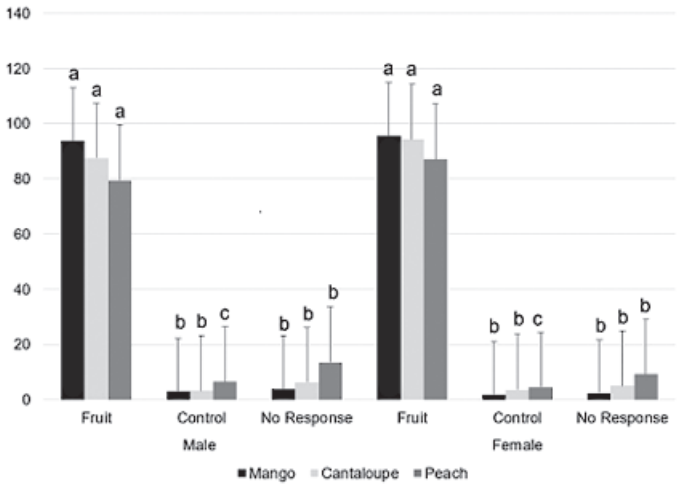

Fig. 1. Mean (SE) number of small hive beetle attraction to ripe cantaloupe, peach, and mango in a flight tunnel. Means with shared letters are not significantly different.

Blends

The results from the blend assays showed that males (F = 29102.79; df = 2; P < 0.0001) and females (F = 54347.80; df = 2; P < 0.0001) overwhelmingly selected Blend 1 over the control (Fig. 2). Blend 2 indicated that males (F = 2643.47; df = 2; P < 0.0001) and females (F = 4048.80; df = 2; P < 0.0001) responded to the blend over the control. Additionally, Blend 3 indicated that males (F = 836.59; df = 2; P < 0.0001) and females (F = 1745.11; df = 2; P < 0.0001) chose the blend over the control. For Blends 1 and 2, each sex chose the treatment, the blank control, and no response respectively.

OLFACTOMETER BIOASSAYS

The olfactometer assay results indicated a preference for a specific cut fruit. Males (F = 112.57; df = 3; P < 0.001) and females (F = 188.66; df = 3; P < 0.001) had a significant preference for mango (Fig. 3). Each sex chose cantaloupe, peach, and the blank control, respectively. For a specific blend, males (F = 302.09; df = 3; P < 0.0001) and females (F = 248.43; df = 3; P < 0.0001) more likely were found in the Blend 1 chamber. Blends 2 and 3 were equally attractive, followed by the blank control (Fig. 4).

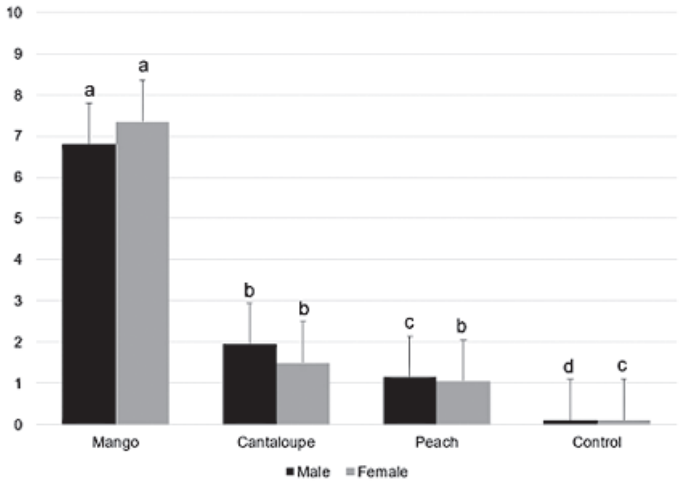

Fig. 3. Mean (SE) number of small hive beetle attraction to ripe cantaloupe, peach, and mango in an olfactometer. Means with shared letters are not significantly different.

Discussion

This study demonstrated that the small hive beetle, like other nitidulid beetles has an affinity for ripe fruit. It is believed that small hive beetle may have an evolutionary history with Kei apple, *Dovyalis caffra* Warb. (Salicaceae), which is a medium-sized tree native to southern Africa. It produces an edible acidic fruit that can be yellow or orange, about 2.5 to 4 cm diam. It has been demonstrated that the small hive beetle can feed and reproduce on this fruit (Stuhl, unpublished data). Beetles were influenced significantly by all fruits including mango (Fig. 1), whereas nitidulid beetle larvae have been collected from ripe mango (Williams et al. 1992a). This treatment captured the greatest number of beetles. This was demonstrated when the fruit was presented along with a blank control, as well as when it was presented alongside cantaloupe and peach in the olfactometer (Fig. 3). Additionally, a significant number of beetles were attracted and captured with cantaloupe. Cantaloupe was selected for its ability to become very odorous as it ripens. Traps baited with cantaloupe have been used to capture nitidulid beetles (Williams et al. 1992b; Price & Young 2006). *Glischrochilus* spp. Reitter (Coleoptera: Nitidulidae) commonly are found infesting damaged or overripe peaches (Hahn 1999). The yr-round availability of peaches from the market led to the use of this fruit as an attractant, which is very odorous as it ripens. This fruit captured a significant number of beetles compared to the control.

It was also demonstrated that 5 ripe fruit-derived compounds initiated a behavioral response in the small hive beetle. Gas chromatography-mass spectroscopy analyses identified the biologically active volatile compounds as ethanol, ethyl butyrate, acetic acid, ethyl acetate, and acetaldehyde. A synthetic blend of chemicals was formulated, and the blend was found to be attractive to the beetles. In gas chromatography-electroantennographic detector analyses, findings were confirmed further by presenting a synthetic blend to the insects which elicited a positive antennal neurophysiological response. The insects tested had an antennal response to individual compounds, but individual compounds did not initiate a behavioral response. It may be that the insects perceived the attractant as a blend rather than individual compounds.

The odor of ripe fruit alone may attract non-target insects such as honey bees. Therefore, the fruit odor may be more discriminatory

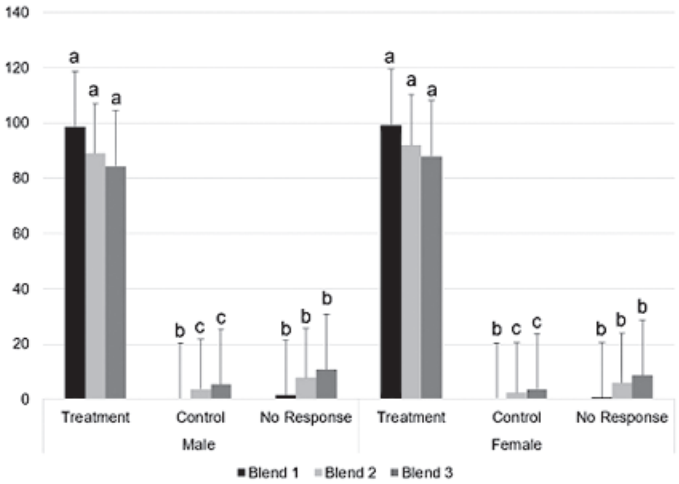

Fig 2. Mean (SE) number of small hive beetle attraction to 3 fruit volatile blend concentrations in a flight tunnel. Means with shared letters are not significantly different.

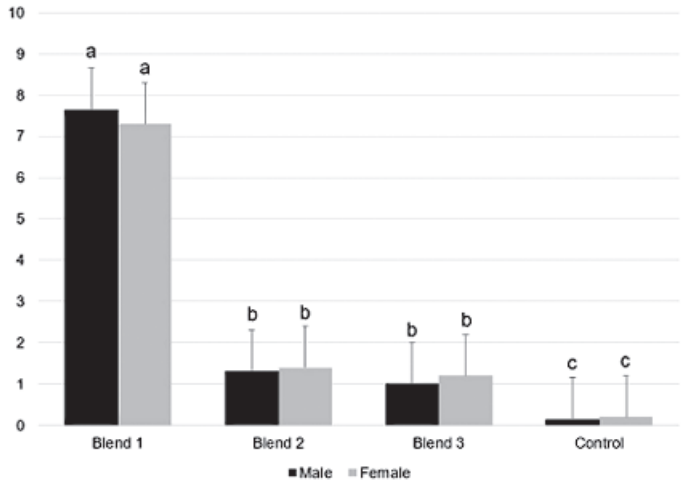

Fig. 4. Mean (SE) number of small hive beetle attraction to 3 fruit volatile blends in an olfactometer. Means with shared letters are not significantly different.

toward the small hive beetle if it is paired with a sex pheromone. This synergistic effect of using a host odor in conjunction with a sex pheromone has been observed in studies of other pests such as the maize weevil (Walgenbach et al. 1987). The blend concentrations showed an increase in insect capture as the blend concentration was increased. Blend 1 captured a greater number of beetles when compared to the other 2 blends; however, all the blends captured significantly more beetles over the control (Fig. 4).

Currently, the most widely used trap within the hive is a plastic container containing vegetable oil and apple cider vinegar. This method takes advantage of the beetle's preference for ripe and over-ripe fruit. In their search for feeding or oviposition sites, they fall into the trap and become trapped in the oil. In hives where the beetle population is high, large numbers of beetles can be found trapped in the oil. However, insects may be repelled by the odor of their decomposing conspecifics, making the trap unattractive over time (Chakraborty et al. 2019).

Control methods that are currently practiced do not offer complete management of this pest within a hive. The alternative would be a trap baited with an attractant that is placed within the apiary. Lin et al. (1992) has shown that this method can be effective for the monitoring and reduction of nitidulid beetle populations.

Results of this study suggest the use of a synthetic fruit volatile blend has the potential as an attractant for trapping and monitoring small hive beetle. Additional research will focus on placing the attractant in a trapping device within the hive. The fruit blend is a positive step in developing components that are attractive to the small hive beetle. Although the efficacy of these blends in flight tunnel assays was demonstrated, there is a need to advance to field studies in the future. This research has the potential to control and monitor this invasive species which is affecting honey bee survival worldwide.

References Cited

Arbogast RT, Throne JE. 1997. Insect infestation of farm-stored maize in South Carolina: towards characterization of a habitat. Journal of Stored Products Research 33: 187–198.

Becher PG, Flick G, Rozpędowska E, Schmidt A, Hagman A, Lebreton S, Larsson MC, Hansson BS, Piškur J, Witzgall P, Bengtsson M. 2012. Yeast, not fruit volatiles mediate *Drosophila melanogaster* attraction, oviposition, and development. Functional Ecology 4: 822–828.

Benda ND, Boucias D, Torto B, Teal P. 2008. Detection and characterization of *Kodamaea ohmeri* associated with small hive beetle *Aethina tumida* infesting honey bee hives. Journal of Apiculture Research 47: 194–201.

Buchholz S, Schäfer MO, Spiewok S, Pettis JS, Duncan M, Ritter W, Spooner-Hart R, Neumann P. 2008. Alternative food sources of *Aethina tumida* (Coleoptera: Nitidulidae). Journal of Apiculture Research 47: 202–209.

Capinera J. 2001. Handbook of vegetable pests, first edition. Academic Press, Elsevier, Amsterdam, The Netherlands.

Chakraborty TS, Gendron CM, Lyu Y, Munneke AS, DeMarco MN, Hoisington ZW, Pletcher SD. 2019. Sensory perception of dead conspecifics induces aversive cues and modulates lifespan through serotonin in Drosophila. Nature Communications 10: 2365. doi: 10.1038/s41467-019-10285-y

Eischen FA. 1999. Beetle watching. American Bee Journal 138: 452–453.

Evans JD, McKenna D, Scully E, Cook SC, Dainat B, Egekwu N, Grubbs N, Lopez D, Lorenzen MD, Reyna SM, Rinkevich FD. 2018. Genome of the small hive beetle (*Aethina tumida*, Coleoptera: Nitidulidae), a worldwide parasite of social bee colonies, provides insights into detoxification and herbivory. GigaScience 7: 138. doi: 10.1093/gigascience/giy138.

Hahn J. 1999. Sap beetles in home gardens. University of Minnesota Extension, St. Paul, Minnesota, USA.

Hayashi N. 1978. A contribution to the knowledge of the larvae of Nitidulidae occurring in Japan (Coleoptera: Cucujoidea). Insecta Matsumurana 14: 1–97.

Heath RR, Manukian A. 1992. Development and evaluation of systems to collect volatile semiochemicals from insects and plants using charcoal-infused medium for air purification. Journal of Chemical Ecology 14: 441–453.

Hood WM, Miller GA. 2003. Trapping small hive beetles (Coleoptera: Nitidulidae) inside colonies of honey bees (Hymenoptera: Apidae). American Bee Journal 143: 405–409.

Light DM, Knight AL, Henrick CA, Rajapaska D, Lingren B, Dickens JC, Reynolds KM, Buttery RG, Merrill G, Roitman J. 2001. A pear-derived kairomone with pheromonal potency that attracts male and female codling moth, *Cydia pomonella* (L.). Die Naturwissenschaften 88: 333–338.

Lin H, Phelan P, Bartelt R. 1992. Synergism between synthetic food odors and the aggregation pheromone for attracting *Carpophilus lugubris* in the field (Coleoptera: Nitidulidae). Environmental Entomology 21: 156–159.

Lundie AE. 1940. The small hive beetle, *Aethina tumida*. Science Bulletin. Department of Agriculture and Forestry, Union of South Africa, (220).

NIST – National Institute of Standards and Technology. 2014. NIST/EPA/NIH Mass Spectral Library with Search Program Data. Version NIST v17. Software Version 2.4. NIST, Gaithersburg, Maryland, USA.

Neumann P, Evans JD, Pettis JS, Pirk CW, Schäfer MO, Tanner G, Ellis JD. 2013. Standard methods for small hive beetle research. Journal of Apicultural Research 52: 1–32.

Parsons CT. 1943. A revision of Nearctic Nitidulidae (Coleoptera). Bulletin of Comparative Zoology 92: 121–248.

Potter MA. 1995. The Nitidulidae (Coleoptera) associated with strawberry in eastern Hillsborough County, Florida. MSc. Thesis, Entomology and Nematology Department, University of Florida, Gainesville, Florida, USA.

Price MB, Young DK. 2006 An annotated checklist of Wisconsin sap and short-winged flower beetles (Coleoptera: Nitidulidae, Kateretidae). Insecta Mundi 20: 69–84.

Reddy GVP, Guerrero A. 2004. Interactions of insect pheromones and plant semiochemicals. Trends in Plant Science 9: 253–261.

SAS. 2013. SAS system for Windows, version 9.4. SAS Institute, Cary, North Carolina, USA.

ScienceDaily. 2016. The ecological role of fruit aroma. www.sciencedaily.com/releases/2016/04/160412110508.htm (lasts accessed 10 Apr 2021).

Stuhl C, Sivinski J, Teal P, Paranhos B, Aluja M. 2011. A compound produced by fruigivorous tephritidae (Diptera) larvae promotes oviposition behavior by the biological control agent *Diachasmimorpha longicaudata* (Hymenoptera: Braconidae). Environmental Entomology 40: 727–736.

Stuhl C. 2017.Survival and reproduction of small hive beetle (Coleoptera: Nitidulidae) on commercial pollen substitutes. Florida Entomologist 100: 693–697.

Stuhl C. 2019. The development of an attract-and-kill bait for controlling the small hive beetle (Coleoptera: Nitidulidae). Apidologie 51: 428–435.

Torto B, Suazo A, Alborn H, Tumlinson JH, Teal PE. 2005. Response of the small hive beetle (*Aethina tumida*) to a blend of chemicals identified from honeybee (*Apis mellifera*) volatiles. Apidologie 36: 523–532.

Vet LE, Lenteren JV, Heymans M, Meelis E. 1983. An airflow olfactometer for measuring olfactory responses of hymenopterous parasitoids and other small insects. Physiological Entomology 8: 97–106.

Walgenbach CA, Burkholder WE, Curtis MJ, Khan ZA. 1987. Laboratory trapping studies with *Sitophilus zeamais* (Coleoptera: Curculionidae). Journal of Economic Entomology 80: 763–767.

Williams RN, Fickle DS, Galford JR. 1992a. Biological studies of *Brachyserphus abruptus* [Hym.: Proctotrupidae], a nitidulid parasite. Entomophaga 37: 91–98.

Williams RN, Blackmer JL, Richmond DS, Ellis MS. 1992b. Nitidulidae (Coleoptera) diversity in three natural preserves in Portage County, Ohio. Ohio Journal of Science 92: 82–87.

Zawislak J. 2014. Managing Small Hive Beetles. Division of Agriculture, University of Arkansas, Fayetteville, Arkansas, USA.