Aethina tumida (Coleoptera: Nitidulidae) attraction to volatiles produced by Apis mellifera (Hymenoptera: Apidae) and Bombus impatiens (Hymenoptera: Apidae) colonies

Jason Graham, James Ellis, Mark Carroll, Peter Teal

Apidologie (2011) 42:326–336

DOI: 10.1007/s13592-011-0017-9

Original article

Aethina tumida (Coleoptera: Nitidulidae) attraction to volatiles produced by *Apis mellifera* (Hymenoptera: Apidae) and *Bombus impatiens* (Hymenoptera: Apidae) colonies

Jason R. GRAHAM[1], James D. ELLIS[1], Mark J. CARROLL[2], Peter E. A. TEAL[2]

[1]Entomology Nematology Department, Honey Bee Research and Extension Laboratory, University of Florida, P.O. Box 110620, Bldg. 970 Natural Area Dr., Gainesville, FL 32611, USA
[2]Center for Medical, Agricultural, and Veterinary Entomology, Agricultural Research Service, Chemistry Research Unit, U.S. Department of Agriculture, 1600 Southwest 23rd Drive, Gainesville, FL 32608, USA

Received 16 December 2009 – Revised 30 April 2010 – Accepted 7 June 2010

Abstract – In this study, small hive beetle (SHB) attraction to whole honey bee and bumble bee colony volatiles as well as volatiles from individual colony components was investigated using four-way olfactometer choice tests. This was done to determine the role olfactory cues play in SHB host location and differentiation. Results from the bumble bee bioassays suggest that SHBs are attracted to adult bumble bees, stored pollen, brood, wax, and whole colony volatiles though not to honey volatiles. The honey bee bioassay results suggest that SHBs are attracted to adult honey bees, brood, honey, stored pollen, wax, and whole colony volatiles. SHBs did not exhibit a preference for honey bee or bumble bee component volatiles in the honey bee vs. bumble bee bioassays. Collectively, the data suggest that SHB attraction to bumble and honey bee colony volatiles is mediated chemically.

Aethina tumida **/** ***Bombus impatiens*** **/** ***Apis mellifera*** **/ choice test / olfactometer**

1. INTRODUCTION

The small hive beetle (Coleoptera: Nitidulidae, *Aethina tumida* Murray, hereafter referred to as SHB) is a pest of Western honey bees (Hymenoptera: Apidae, *Apis mellifera* L.) that is found in colonies of African subspecies of honey bee (its natural host) and more recently in colonies of European subspecies of honey bees. The occurrence of SHBs in European honey bee colonies is due to the recent accidental introduction of the SHB into the USA and Australia from its native range of sub-Saharan Africa (Elzen et al. 1999; Hood 2000, 2004; Neumann and Elzen 2004; Ellis and Hepburn 2006; Neumann and Ellis 2008). Moreover, investigators have shown that commercial bumble bee (Hymenoptera: Apidae, *Bombus* spp.) colonies in the beetle's introduced range can serve as alternative hosts for the SHB, a relationship that may be detrimental to wild and managed populations of bumble bees (Ambrose et al. 2000; Stanghellini et al. 2000; Spiewok and Neumann 2006; Hoffmann et al. 2008).

Several studies have confirmed that SHBs can survive in and cause significant damage to bumble bee colonies. Both Ambrose et al. (2000) and Stanghellini et al. (2000) found that commercial colonies of *B. impatiens* Cresson artificially

Corresponding author: J.R. Graham, jgraham@ufl.edu
Manuscript editor: Yves Le Conte

infested with SHBs had fewer live bees, more dead bees, and more comb damage than non-infested colonies. Further, SHBs were able to complete their lifecycle within three *B. impatiens* colonies infested with 20 SHB each. The authors concluded that if SHBs are able to locate and invade commercial and wild bumble bee colonies, then bumble bees and the ecological communities they support may be at considerable risk (Ambrose et al. 2000; Stanghellini et al. 2000).

Studies by Spiewok and Neumann (2006) indicate that SHB adults invade bumble bee colonies even when honey bee colonies are present. The authors found that commercial *B. impatiens* colonies maintained within close proximity (~100 or ~500 m) to infested honey bee colonies became infested naturally with SHBs and successful SHB reproduction occurred within these colonies. Hoffmann et al. (2008) placed four commercial *B. impatiens* colonies and four honey bee colonies in a closed greenhouse and released 1,000 SHBs to study possible SHB preference for honey bee or bumble bee colonies. The colonies of both bee types were invaded by SHBs that readily oviposited in the colonies and showed no apparent preference to honey bee over bumble bee colonies. The investigators also observed defensive behaviors exhibited by adult bumble bees attempting to defend their colonies from SHB invasion (Hoffmann et al. 2008).

Regarding SHB attraction to host colonies, Spiewok and Neumann (2006) found that in four-square choice tests, SHBs were attracted to both adult bumble bee workers and stored pollen (also called "bee bread") from bumble bee colonies. As a result of their work, Spiewok and Neumann (2006) concluded that SHBs are expected to infest not only wild and commercial *B. impatiens* nests but also nests of other bumble bee species.

While it is clear that SHBs are capable of invading *B. impatiens* colonies (Ambrose et al. 2000; Stanghellini et al. 2000; Spiewok and Neumann 2006; Hoffmann et al. 2008), it remains unclear what mediates the invasion. Hypothesizing that SHB attraction to bumble bee colonies is mediated chemically, as is their attraction to honey bee colonies (Suazo et al. 2003; Torto et al. 2005, 2007a, b), we tested SHB attraction to airborne volatiles from whole honey bee and bumble bee colonies as well as volatiles from individual colony components (adult bees, brood, honey, stored pollen, and wax). We further hypothesized that volatiles from bumble bee and honey bee colonies, particularly those from adult bees and pollen, would be equally attractive to SHB.

2. MATERIALS AND METHODS

2.1. Bumble bees and honey bees

In January 2009, four commercial *B. impatiens* colonies (collectively termed a quad) were purchased from Koppert Biological Systems, Inc. (Romulus, MI, USA) and placed at the University of Florida's Bee Biology Unit in Gainesville, FL (N 29° 37.629″ W 82° 21.405″). Each colony contained a corn syrup/water solution, provided by Koppert Biological Systems, Inc., for periods of low nectar flow. All colonies had a reproductive queen, 200–250 workers, brood, and nesting material made of cotton and plastic. The quad was placed on a wooden pallet and the colony entrances opened, permitting the bees to forage.

Four honey bee colonies were also maintained at the University of Florida's Bee Biology Unit in Gainesville, FL. All colonies were housed in full-sized, Langstroth-style hives, headed by a single queen, and had been used as production colonies during the previous year. The colonies were provided a corn syrup/water solution during periods of low nectar flow. The bumble bee and honey bee colonies were maintained in the same location within 35 m of one another in order to expose the bees to identical environmental conditions and forage availability.

2.2. SHBs

The SHBs used in the bioassays were mass-reared at the Honey Bee Research and Extension Laboratory (HBREL, University of Florida, Gainesville) using modified rearing methods originally outlined by Mürrle and Neumann (2004). All SHBs used in the various bioassays were 1–2-week-old adults of mixed

sex. After emergence, the SHBs were fed only sugar water for the 3 days immediately prior to their use in the bioassays. The bioassays were conducted between 18:00 and 2:30 of the following morning when SHBs seem most active (Schmolke 1974; Elzen et al. 1999; Ellis et al. 2003).

2.3. Olfactometer

A four-way olfactometer was used to determine SHB attraction to honey bee and bumble bee colony component and whole colony volatiles. The olfactometer (3.66 m×3.66 m×30.5 cm) was a four-port/four-choice arena olfactometer with a removable lid (ARS, Inc., Gainesville, FL, USA) (Figures 1, 2, and 3). The main body and ports were composed of solid UHMW polyethylene with a 0.95-cm-thick clear Plexiglas removable lid. At the four odor source ports, glass Internal Odor Source Adapters/Insect Isolation Traps (IOS/IIT) were attached to collect SHBs responding to the odor source and to prevent them from returning to the arena (Figures 1 and 2). Colony materials that served as an odor source were housed in a glass Odor Source Container (OSC). Odors were relayed from the OSC to the IOS/IIT by corrugated FEP tubing [1.15 cm inside diameter (ID)×1.27 cm outside diameter (OD); Cole-Parmer, Vernon Hills, IL, USA]. Positive air flow through the system was provided by two portable filtered-air pumps and was regulated by carboloy air flow regulators (Aalborg Instruments and Controls, Inc., Orangeburg, NY, USA) set at 0.5 l/min (Figures 1 and 2).

The original insect inlet port was modified to form a SHB release port constructed of 0.32 cm industrial tubing (Cole-Parmer), nylon mesh, and 0.64 cm industrial tubing (Cole-Parmer; Figure 3). Air flow from all four odor sources was pulled from the arena through the release port by a house vacuum attached to the release port by Master flex® Tygon® tubing (Cole-Parmer) (Figures 1, 2, and 3). Vacuum pullout of the olfactometer was maintained at 2 l/min by a carboloy air flow regulator (Aalborg Instruments and Controls).

The bioassays were conducted in a walk-in incubator at the USDA/ARS (CMAVE, Gainesville, FL, USA) maintained at approximately 31°C and 65% RH with four banks of Ecolux® fluorescent lighting (General Electric Company, Fairfield, CT, USA).

2.4. Colony component odors

2.4.1. Bumble bee whole hive

Whole bumble bee colony odors were collected from colonies enclosed in a large container. Two bumble bee colonies were removed from a quad and placed into a steel pan with 1.25-cm holes (for ports) drilled into either end and enclosed with a glass lid. The two colonies were placed side by side within the container, and the lid was secured with duct tape to prohibit air from escaping. The two bumble bee colonies were considered equivalent in strength to one five-frame nucleus colony of

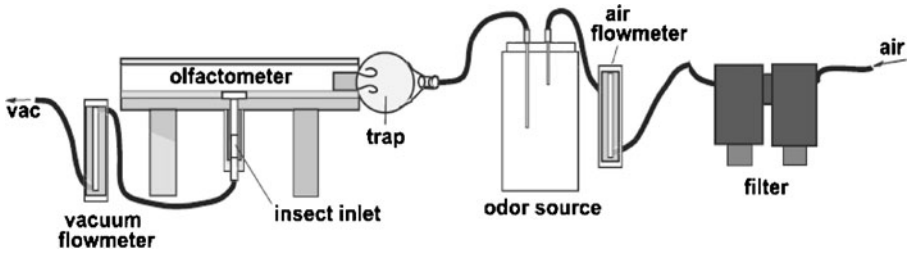

Figure 1. Lateral view of the four-way olfactometer (modified from Carroll et al., in preparation). The olfactometer is connected to an insect inlet port attached to the vacuum, drawing air from the odor source over the insect as it is released into the olfactometer. The vacuum is attached to a flowmeter (2 l/min) drawing four times the air delivered by each of the four ports (0.5 l/min), each port delivering air through air flowmeters, odor sources, and glass traps.

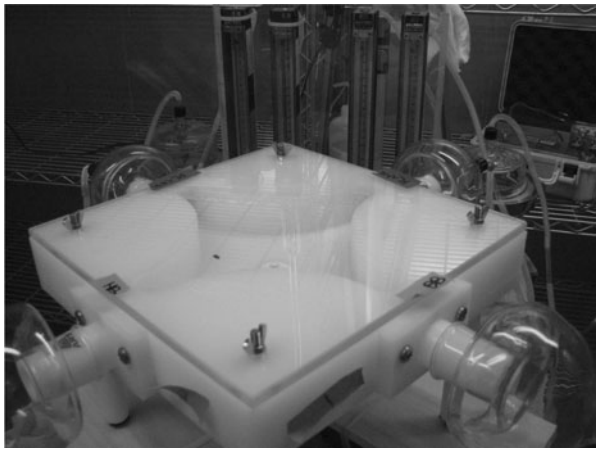

Figure 2. The four-way olfactometer used for SHB choice tests at the Center for Medical, Agricultural, and Veterinary Entomology (CMAVE, USDA–ARS, Gainesville, FL, USA). Filtered air is passed over bee constituents being tested and released through ports connecting the glass insect traps to the choice arena. *Photo*: Jason R. Graham.

honey bees. We did this to avoid potential quantitative differences in volatiles produced by honey bee and bumble bee colonies of different sizes.

2.4.2. Honey bee whole hive

To collect odors from a whole honey bee colony, a 1.25-cm port hole was drilled into the front and back

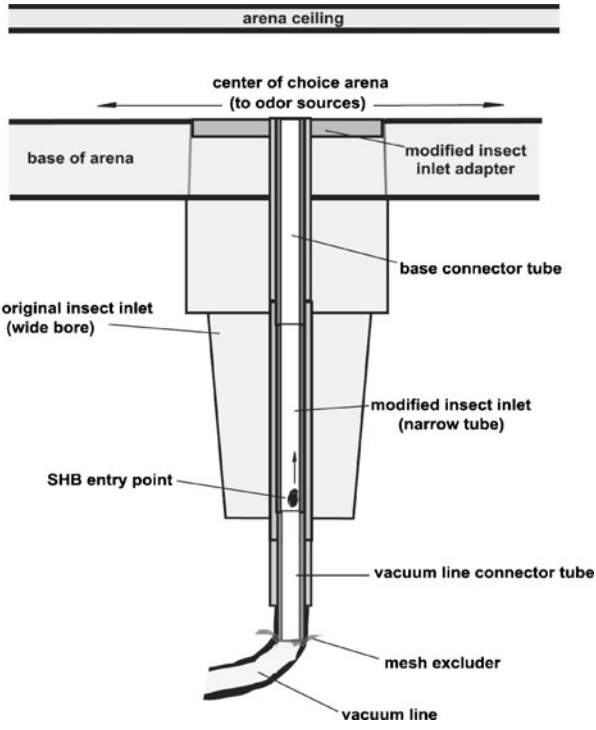

Figure 3. Lateral view of the four-way olfactometer showing insect inlet (Carroll et al., in preparation). The vacuum line connector tube is detached from the vacuum line and a SHB is loaded into the vacuum connector tube. A mesh excluder prevents the SHB from entering the vacuum line while the SHB travels past the SHB entry point and up the modified insect inlet.

of a healthy, five-frame nucleus colony. The colony contained five (23.2 cm×47.9 cm) frames with 21.27 cm Plasticell® foundation (Dadant and Sons, Inc., Hamilton, IL, USA), a queen, brood, honey, pollen, wax, and adult honey bees. A telescoping lid was placed on the top and bottom of the nuc body and any cracks on the periphery where air could escape were sealed with duct tape.

2.4.3. Adult bees

All adult bumble bees (~32 g per colony) were collected from four bumble bee colonies and placed by colony into separate glass OSCs (3.8 l each). The collection chambers were maintained at 33°C, 65% humidity, and 12 h of daylight. The adults were provisioned with a cotton wick saturated with a 50% sugar water solution. The adult honey bees (~32 g per colony) also were collected from four colonies and handled identically. All bee OSCs were connected to the olfactometer within 0.5 h of bee capture and the bioassay began approximately 1 h after capture.

2.4.4. Brood

Odors from honey bee brood of all ages (egg to pupae) were collected by taking one brood frame from each of two colonies and using tin snips to cut out and remove a square of brood weighing ~32 g from both. The brood squares were pushed back into the frame immediately and returned to the honey bee colony. This gave the bees time to remove the macerated brood and repair the wax located on the edge of the newly cut squares. After 2 days, the brood squares were removed and placed into OSCs ~30 min prior to the bioassay. One square was used to compare the attractiveness of brood odors against an air blank, while the other square was used to compare the relative attractiveness of honey bee and bumble bee brood odors.

Two groups (~32 g each) of live larvae/pupae were collected from the commercial bumble bee colonies and placed into separate OSCs. One group was used to compare the attractiveness of brood odors against an air blank, while the other group was used to compare the relative attractiveness of bumble bee and honey bee brood odors. The majority of the bumble bee brood collected was enclosed in wax cells which differ in color and texture from wax cells containing honey or pollen. To avoid disturbing or injuring the brood within, and to keep the bioassay as natural as possible, any closed brood cells were left unopened during the bioassays.

2.4.5. Stored pollen, honey, and wax

Stored pollen (~32 g), honey (~32 g), and wax (~32 g) were collected separately from each of the honey bee and bumble bee colonies. One collection of stored pollen was made from two honey bee colonies using a small autoclaved spatula. The bumble bee pollen was collected from two bumble bee colonies by removing and opening pollen cells and extracting the stored pollen with an autoclaved spatula. All stored pollen portions were weighed on filter paper and then transferred directly into the OSC.

Honey bee honey was collected from two frames, one each from two separate colonies. The honey collection was made by scraping honey out of the wax cells using an autoclaved spoon. The honey then was transferred into a glass Petri dish and weighed. Bumble bee honey was collected by squeezing the wax honey cells to force the honey into a glass Petri dish and was then weighed. The components were transferred into their respective OSCs and used in studies within 2 days of collection.

To collect honey bee wax, a frame containing honey and pollen was placed within 10 m of managed honey bee colonies. The bees from these colonies "robbed" the combs, leaving only the wax behind. Once the wax was empty, it was scraped from the frame using an autoclaved hive tool. The wax was put into an OSC approximately 0.5 h prior to the bioassay. Wax cells containing no stored pollen, honey, or brood were collected from two bumble bee colonies, weighed, and then put into an OSC.

2.5. Choice bioassays

The choice bioassays were designed to test SHB attraction to (1) bumble bee colony component volatiles in the absence of other odorants (bumble bee, single odor source bioassay), (2) honey bee colony component volatiles in the absence of other odorants (honey bee, single odor source bioassay),

and (3) honey bee and bumble bee colony component volatiles (honey bee and bumble bee, dual odor source bioassay). The single odor bioassays were composed of the whole colony or individual component in an OSC connected to one of the four ports of the olfactometer. The other three ports were connected to a supply of clean, filtered air. For the dual odor source bioassays, the four ports were connected to OSCs housing bumble bee whole colony or individual components at one port, the honey bee equivalent at a second port, and clean, filtered air in the other two ports. Each port delivered air at 0.5 l/min while the vacuum port pulled air through the system at a rate of 2 l/min.

The SHBs were held in a 1 l container with a cotton wick saturated with sugar water (50:50 solution) while the olfactometer was being set up. The sex of the SHBs was not determined but left instead to chance; therefore, gender-specific behaviors were not evaluated. Individual SHBs were never used twice. A hit (=choice) was scored when the SHBs crossed the threshold from the olfactometer into a glass trap (see Figures 1 and 2). If a test SHB was unresponsive after 10 min, it was removed from the arena and replaced with another SHB**.** The positions of the odor source and control ports were chosen randomly and changed after every five SHBs individually tested. For each trial, 40 responding SHBs were used and the trials typically took ~5 h to complete. Each group of five individual SHBs released was considered a replicate and the ports were repositioned randomly between replicates.

2.6. Statistical analysis

For all analyses, the data were compared between SHB responses to main effects (whole colonies and colony components) and controls, which were adjusted for the number of ports emitting odor. In the honey bee vs. bumble bee bioassay, the main effects included SHB responses to honey bee and bumble bee components separately (first analysis) and averaged together (second analysis). All analyses were done using a one-way ANOVA with means compared using Tukey tests (JMP v. 7, 2008; SAS Institute, Inc., Cary, NC, USA).

3. RESULTS

The SHBs tested in the bumble bee component bioassays were attracted to ports emitting volatiles from adult bumble bees, stored pollen, brood, wax, and the whole colony, though not honey, significantly more than they were attracted to the control ports emitting filtered air (Table I). Component volatiles from honey bee adults, brood, honey, pollen, wax, and whole colonies were more attractive to SHBs than clean air emitted by control ports in the honey bee component bioassays (Table II).

The results from the bumble bee vs. honey bee component bioassays are reported in Table III. SHBs were attracted significantly to component volatiles produced by honey bee and bumble bee brood, stored pollen, and wax though not to

Table I. Attraction of SHBs to bumble bee colony components.

Bumble bee	Colony component	Control	ANOVA
Adults	2.4±0.5a	0.8±0.2b	(F=8.6; df=1,15; P=0.01)
Brood	3.9±0.4a	0.4±0.1b	(F=57.8; df=1,15; P<0.01)
Colony	3.0±0.2a	0.6±0.1b	(F=141.9; df=1,15; P<0.01)
Honey	1.6±0.4a	1.1±0.1a	(F=1.8; df=1,15; P=0.2)
Stored pollen	3.0±0.3a	0.6±0.1b	(F=70.3; df=1,15; P<0.01)
Wax	3.8±0.3a	0.4±0.1b	(F=167.2; df=1,15; P<0.01)

Data are the number of beetles (mean ± SE) attracted to odor source ports in a four-way olfactometer. Each replicate represents five successive releases of individual beetles into the olfactometer eight different times. “Colony component” includes average SHB responses to a single port that emitted air passed over one of the bumble bee colony components. “Control” represents the average SHB response to three ports that emitted filtered air (air blank control). Row means followed by the same letter are not different at $\alpha \leq 0.05$ (Tukey test).

Table II. Attraction of SHBs to honey bee colony components.

Honey bee	Colony component	Control	ANOVA
Adults	2.8±0.4a	0.7±0.1b	(F=27.7; df=1,15; P<0.01)
Brood	2.9±0.2a	0.7±0.1b	(F=84.3; df=1,15; P<0.01)
Colony	2.6±0.3a	0.8±0.1b	(F=44.2; df=1,15; P<0.01)
Honey	3.1±0.4a	0.6±0.1b	(F=36.1; df=1,15; P<0.01)
Stored pollen	2.0±0.5a	1.0±0.2b	(F=4.5; df=1,15; P=0.05)
Wax	3.9±0.3a	0.3±0.1b	(F=131.9; df=1,15; P<0.01)

Data are the number of beetles (mean ± SE) attracted to odor source ports in a four-way olfactometer. Each replicate represents five successive releases of individual beetles into the olfactometer eight different times. "Colony component" includes average SHB responses to a single port that emitted air passed over one of the bumble bee colony components. "Control" represents the average SHB response to three ports that emitted filtered air (air blank control). Row means followed by the same letter are not different at $\alpha \leq 0.05$ (Tukey test).

those produced by adults, whole colonies, or honey (Table III). When SHB responses to component volatiles produced by both bees were pooled and averaged, we found SHBs more attracted to ports emitting component volatiles produced by all bee components than to control ports with the exception of honey (Table IV).

4. DISCUSSION

In general, the choice bioassays provide an understanding of what olfactory stimuli attract SHBs to bumble and honey bee colonies (Tables I, II, III, and IV). The bumble bee bioassay results suggest that SHBs are attracted to volatiles from bumble bee adults, brood, stored pollen, wax, and whole bumble bee colonies, though not to honey (Table I). This supports findings by Spiewok and Neumann (2006) who showed that SHBs were attracted to bumble bee adults and stored pollen from bumble bee colonies more than to the three control squares in four square choice tests. SHB attraction to wax, which may absorb volatiles of components stored within it, is not surprising as SHB were attracted to stored pollen and brood (Table I), both found in wax. Why SHBs were not attracted to bumble bee honey is unclear,

Table III. Attraction of SHBs to honey bee or bumble bee colony components.

Odorant	Honey bee component	Bumble bee component	Control	ANOVA
Adults	1.5±0.2a	1.5±0.2a	1.0±0.1a	(F=3.1; df=2,23; P=0.07)
Brood	1.5±0.2a	1.6±0.2a	0.9±0.1b	(F=5.5; df=2,23; P=0.01)
Colony	1.4±0.3a	1.5±0.2a	1.1±0.1a	(F=1.0; df=2,23; P=0.39)
Honey	1.8±0.3a	1.0±0.2b	1.1±0.2ab	(F=3.9; df=2,23; P=0.04)
Stored pollen	1.8±0.3a	1.6±0.3a	0.8±0.1b	(F=5.2; df=2,23; P=0.01)
Wax	1.6±0.2a	1.6±0.3a	0.9±0.1b	(F=5.1; df=2,23; P=0.02)

Data are the number of beetles (mean ± SE) attracted to odor source ports in a four-way olfactometer. Each replicate represents five successive releases of individual beetles into the olfactometer eight different times. "Honey bee component" and "Bumble bee component" include SHB responses to a port that emitted air passed over one of the bumble bee colony components and to a port that emitted air passed over one of the honey bee colony components, respectively. "Control" represents the average SHB response to two ports that emitted filtered air (air blank control). Row means followed by the same letter are not different at $\alpha \leq 0.05$ (Tukey test).

Table IV. Attraction of SHBs to bee colony components.

Odorant	Bee component	Control	ANOVA
Adults	1.5±0.1a	1.0±0.1b	(F=14.0; df=1,15; P<0.01)
Brood	1.6±0.1a	1.0±0.1b	(F=50.0; df=1,15; P<0.01)
Colony	1.4±0.1a	1.1±0.1b	(F=5.5; df=1,15; P=0.03)
Honey	1.4±0.2a	1.1±0.2a	(F=1.3; df=1,15; P=0.3)
Stored pollen	1.7±0.1a	0.8±0.1b	(F=22.1; df=1,15; P<0.01)
Wax	1.6±0.1a	0.9±0.1b	(F=42.0; df=1,15; P<0.01)

Data are the number of beetles (mean ± SE) attracted to odor source ports in a four-way olfactometer. Each replicate represents five successive releases of individual beetles into the olfactometer eight different times. "Bee component" includes the average SHB response to two ports, one that emitted air passed over one of the bumble bee colony components and the other that emitted air passed over one of the honey bee colony components. "Control" represents the average SHB response to two ports that emitted filtered air (air blank control). Row means followed by the same letter are not different at $\alpha \leq 0.05$ (Tukey test).

especially since they were attracted to honey bee honey. This needs to be investigated further.

The data on SHB attraction to component volatiles from bumble bee colonies (Table I) support that from previous investigations (Spiewok and Neumann 2006; Hoffmann et al. 2008). While Spiewok and Neumann (2006) conducted their investigations using field studies and Hoffmann et al. (2008) used a greenhouse, we present data from choice tests conducted with an olfactometer. This allows for the study of SHB decision-making in the absence of visual cues. Collectively, the data suggest that SHBs use airborne volatiles to locate host colonies. This seems intuitive as SHBs typically search for their host colonies in the evening and at night (Schmolke 1974; Elzen et al. 1999) and once they find them, live in host colonies where light is restricted.

SHBs also were attracted to volatiles from honey bee adults, brood, honey, stored pollen, wax, and whole colonies (Table II). This is consistent with some of the findings of Suazo et al. (2003) who showed in olfactometric and wind tunnel choice tests that SHB were attracted to volatiles from adult worker honey bees, volatiles from a mixture of honey/propolis/pollen/wax from honey bee colonies, and to volatiles from freshly collected pollen, but not to brood, beeswax, or commercially available pollen volatiles. SHB attraction to a mixture of honey/propolis/pollen/wax was not tested in this study but rather their attraction to honey, stored pollen, and wax separately.

Suazo et al. (2003) found that volatiles from honey bee brood (10 g pupae removed from the comb) were not attractive to SHB, whereas our results suggest that volatiles from honey bee brood (32 g eggs, larvae, pre-pupae, and pupae within the comb) were attractive. There are three possible reasons for this difference. First, we tested SHB attractiveness to all brood stages simultaneously, whereas Suazo et al. (2003) tested attractiveness only to pupae. Secondly, SHB attraction to brood may be dose dependent and positively correlated. We tested SHB attraction to three times more brood per replicate than did Suazo et al. (2003). Finally, the chemical signals produced by the brood may have been different in both studies due to our respective methods of handling the brood prior to analysis. Immature brood removed from individual cells, as in Suazo et al. (2003), may have emitted a different volatile profile from that of brood left in the comb (reported here).

In this study, SHBs did not exhibit a preference for component volatiles produced by either honey bees or bumble bees over those produced by the other. These findings support those of Hoffmann et al. (2008) who in greenhouse studies found that SHB went to honey bee colonies and

bumble bee colonies equally and preferred "bee colonies" of either type to empty nest boxes. Hoffmann et al. (2008) also noted general defense mechanisms used by the bumble bee against the SHB, but suggested that these mechanisms are not enough to avoid SHB infestation indefinitely. The data we present here and that of Hoffmann et al. (2008) suggest that wild and commercial bumble bee colonies are vulnerable to SHB invasion.

SHBs have also been found in other social bee colonies such as stingless bee colonies of *Dactylurina staudingerii* in Africa (Mutsaers 2006) and *Trigona carbonaria* in Australia (Greco et al. 2009). Beekeepers have witnessed the devastation that SHBs can exact on honey bee colonies. If other bee colonies face the same threat, the results could be disastrous to commercial and wild bee populations as well as the ecological communities they support. If, for example, bumble bee colonies were found to be damaged by SHB invasion, their value to agriculture through the pollination services they provide may decrease due to the general loss of infested colonies. This loss would be detrimental as bumble bees are important pollinators (they have long tongues, can be used in greenhouses, fly and forage in unfavorable conditions, and buzz pollinate plants—Goulson 2003; Heinrich 2004; Mader et al. 2010).

SHB spillover from commercial bumble bee colonies to wild colonies has not been documented, but wild colonies contain the same components as commercial ones and the volatiles of these components are now known to attract SHB, making SHB attraction to wild colonies likely. Future investigations need to be conducted to determine if SHBs have begun to infest wild bumble bee colonies or if they pose a real threat to commercial bumble bee colonies managed for pollination purposes.

ACKNOWLEDGMENTS

The authors wish to thank Nicole Benda, Tricia Toth, Anthony Vaudo, Eddie Atkinson, Hannah O'Malley, and Meredith Cenzer who provided technical assistance during the study.

Attraction d' *Aethina tumida* (Coleoptera: Nitulidae) par des substances volatiles émises par les colonies d' *Apis mellifera* (Hymenoptera: Apidae) et de *Bombus impatiens* (Hymenoptera: Apidae).

***Aethina tumida* / *Bombus impatiens* / *Apis mellifera* / olfactomètre / test de choix / communication chimique**

Zusammenfassung – Die Anlockung von *Aethina tumida* (Coleoptera: Nitidulidae) durch flüchtige Substanzen aus Völkern von *Apis mellifera* (Hymenoptera: Apidae) und *Bombus impatiens* (Hymenoptera: Apidae). Der kleine Beutenkäfer (*Aethina tumida* Murray, Coleoptera: Nitidulidae; SHB), ein Schädling der westlichen Honigbiene (*Apis mellifera* L.; Hymenoptera: Apidae), wurde in den Vereinigten Staaten auch in kommerziell genutzten Hummelvölkern (*Bombus impatiens* Cresson, Hymenoptera: Apidae) nachgewiesen. Ein Befall mit SHB kann sowohl für kommerziell genutzte als auch für wilde Hummelnester verheerende Folgen haben und wegen des wertvollen Beitrags der Hummeln zur Bestäubung ein ernstes Problem darstellen. Weiterhin könnten Hummelvölker dadurch eine unkontrollierte Quelle für die Vermehrung von SHB darstellen. Aus diesen Gründen ist es wichtig zu verstehen, welche Faktoren die Attraktivität von Hummelnestern für SHB beeinflussen.

Ausgehend von der Hypothese, dass die Anziehung der SHB zu den Hummeln wie bei Honigbienen auf chemischem Weg erfolgt, untersuchten wir die Attraktivität von durch die Luft übertragenen flüchtigen Stoffen auf SHB. Diese waren entweder aus vollständigen Völkern, oder aus einzelnen Komponenten (erwachsene Bienen, Brut, Honig, Pollenvorrat und Wachs) von Bienen oder Hummeln gewonnen worden.

Die Wahlversuche wurden so durchgeführt, dass die Attraktivität von (1) Komponenten aus Hummelnestern in Abwesenheit anderer Duftstoffe getestet wurde (Hummel gegen Kontrolle), (2) Komponenten aus Honigbienenvölkern in Abwesenheit anderer Duftstoffe (Honigbiene gegen Kontrolle), und (3) Komponenten der beiden Bienenarten gegeneinander (Hummel gegen Honigbiene). Bei allen Analysen wurden die Daten für die Anzahl der Ausgänge des Olfaktometers korrigiert und mittels einer 1-way ANOVA und eines Tukey-Tests ausgewertet.

Die Ergebnisse des Biotests zu der Attraktivität von Hummeln (Table I) deuten an, dass SHB von erwachsenen Hummeln, Pollenvorrat, Brut und

Wachs, sowie von ganzen Hummelnestern angelockt werden, jedoch nicht von Honig. Erwachsene Honigbienen, Brut, Honig, Pollenvorrat, Wachs sowie aus dem gesamten Bienenvolk gewonnene Duftstoffe waren für SHB attraktiver als saubere, gefilterte Luft die durch die Kontrollausgänge strömte (Table II). Die Käfer zeigten im Biotest keine Präferenz für Honigbienen oder Hummeln, aber sie wurden, mit der Ausnahme von Honig, von den Duftstoffen beider Bienenarten stärker angelockt als von der gefilterten Kontroll-Luft (Table IV).

Die Wahlversuche lieferten daher eine Erklärung, durch welche Stimulanzien die Beutenkäfer an Hummelnester und Bienenvölker angelockt wurden. Insgesamt scheinen die Daten anzuzeigen, dass die Käfer von Hummelnestern genauso stark angezogen werden wie von Bienenvölkern, und dass diese Attraktivität auf chemischem Weg erfolgt. Imker sind bereits Zeugen der Verwüstungen geworden, die SHB in Bienenvölkern auslösen können. Wenn Hummelnester auf die gleiche Art und Weise bedroht werden, könnte das Ergebnis sowohl für die kommerziell genutzte als auch für die wilde Hummelpopulation und die von ihnen abhängigen ökologischen Gemeinschaften verheerend sein.

***Aethina tumida* / *Bombus impatiens* / *Apis mellifera* / Wahlversuch / Olfaktometer**

REFERENCES

Ambrose, J.T., Stanghellini, M.S., Hopkins, D.I. (2000) A scientific note on the threat of small hive beetles (*Aethina tumida* Murray) to bumble bee (*Bombus* spp.) colonies in the United States. Apidologie **31**, 455–456

Ellis, J.D., Hepburn, H.R. (2006) An ecological digest of the small hive beetle (*Aethina tumida*), a symbiont in honey bee colonies (*Apis mellifera*). Insect. Soc. **53**, 8–19

Ellis, J.D., Hepburn, H.R., Ellis, A.M., Elzen, P.J. (2003) Social encapsulation of the small hive beetle (*Aethina tumida* Murray) by European honeybees (*Apis mellifera* L.). Insect. Soc. **50**, 286–291

Elzen, P.J., Baxter, J.R., Westervelt, D., Randall, C., Cutts, L., Wilson, W.T., Eischen, F.A., Delaplane, K. S., Hopkins, D.I. (1999) Status of the small hive beetle in the U.S. Bee Cult. **127**, 28–29

Goulson, D. (2003) Bumblebees: behaviour and ecology. Oxford University Press, Oxford

Greco, M., Hoffmann, D., Dollin, A., Duncan, M., Spooner-Hart, R., Neumann, P. (2009) The modified pharaoh approach: stingless bees mummify beetle parasites alive. Available at Nature Proceedings http://dx.doi.org/10.1038/npre.2009.2591.2

Heinrich, B. (2004) Bumblebee economics. Harvard University Press, Cambridge (reprint 1979)

Hoffmann, D., Pettis, J.S., Neumann, P. (2008) Potential host shift of the small hive beetle (*Aethina tumida*) to bumblebee colonies (*Bombus impatiens*). Insect. Soc. **55**, 153–162

Hood, W.M.M. (2000) Overview of the small hive beetle, *Aethina tumida*, in North America. Bee World **81**, 129–137

Hood, M.W. (2004) The small hive beetle, *Aethina tumida*: a review. Bee World **85**, 51–59

Mader, E., Spivak, M., Evans, E. (2010) Managing alternative pollinators: a handbook for beekeepers, growers and conservationists. NRAES, Ithaca

Mürrle, T., Neumann, P. (2004) Mass production of small hive beetles (*Aethina tumida*, Coleoptera: Nitidulidae). J. Apic. Res. **43**, 144–145

Mutsaers, M. (2006) Beekeepers' observations on the small hive beetle (*Aethina tumida*) and other pests in bee colonies in West and East Africa. In: Proceedings of the 2nd EurBee conference, Prague, Czech Republic, p. 44

Neumann, P., Ellis, J.D. (2008) The small hive beetle (*Aethina tumida* Murray, Coleoptera: Nitidulidae): distribution, biology and control of an invasive species. J. Apic. Res. **47**, 181–183

Neumann, P., Elzen, P.J. (2004) The biology of the small hive beetle (*Aethina tumida*, Coleoptera: Nitidulidae): gaps in our knowledge of an invasive species. Apidologie **35**, 229–247

Schmolke MD (1974) A study of *Aethina tumida*: the small hive-beetle, University of Rhodesia (Zimbabwe), Salisbury (Harare)

Spiewok, S., Neumann, P. (2006) Infestation of commercial bumblebee (*Bombus impatiens*) field colonies by small hive beetles (*Aethina tumida*). Ecol. Entomol. **31**, 623–628

Stanghellini, M.S., Ambrose, J.T., Hopkins, D.I. (2000) Bumble bee colonies as potential alternative hosts for the small hive beetle (*Aethina tumida* Murray). Am. Bee. J. **140**, 71–75

Suazo, A., Torto, B., Teal, P.E.A., Tumlinson, J.H. (2003) Response of the small hive beetle (*Aethina tumida*) to honey bee (*Apis mellifera*) and beehive-produced volatiles. Apidologie **34**, 525–533

Torto, B., Suazo, A., Alborn, H., Tumlinson, J.H., Teal, P.E.A. (2005) Response of the small hive beetle (*Aethina tumida*) to a blend of chemicals identified

from honeybee (*Apis mellifera*) volatiles. Apidologie **36**, 523–532

Torto, B., Boucias, D.G., Arbogast, R.T., Tumlinson, J. H., Teal, P.E.A. (2007a) Multitrophic interaction facilitates parasite–host relationship between an invasive beetle and the honey bee. Proc. Natl. Acad. Sci. USA **104**, 8374–8378

Torto, B., Arbogast, R.T., Alborn, H., Suazo, A., van Englesdorp, D., Boucias, D., Tumlinson, J.H., Teal, P.E.A. (2007b) Composition of volatiles from fermenting pollen dough and attractiveness to the small hive beetle, *Aethina tumida*, a parasite of the honey bee, *Apis mellifera*. Apidologie **38**, 380–389