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**Original article**

# Response of the small hive beetle (*Aethina tumida*) to a blend of chemicals identified from honeybee (*Apis mellifera*) volatiles[1]

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**Abstract** – Coupled gas chromatographic-electroantennographic detection (GC-EAD) analyses of Super Q collected worker honey bee volatiles revealed several components that elicited antennal responses by the small hive beetle *Aethina tumida*. However, GC-MS analysis showed that eight of these EAD-active components dominated the volatile profile released into a wind tunnel by living adult worker honeybees and rubber septa impregnated with a Super Q extract of the volatiles of the bees in a 15-min bioassay. These components were identified as isopentyl acetate, 2-heptanone, octanal, hexyl acetate, nonanal, 2-nonanone, methyl benzoate and decanal. In dual-choice wind tunnel bioassays, the Super Q extract and a blend of the eight components elicited dose-dependent upwind responses from beetles relative to a solvent control. At 375-bee day equivalents, the Super Q extract and the 8-component blend elicited 76 and 74% upwind response, respectively, which compared with 84% response from approx. 150-200 living worker honey bees. In contrast, the Super Q extract and the 8-component blend lured only approx. 12 and 3% of beetles, respectively, into a trap compared to 48% by the odor from living adult worker bees.

***Aethina tumida* / *Apis mellifera* / volatile / alarm pheromone / wind tunnel**

## 1. INTRODUCTION

The small hive beetle (SHB, *Aethina tumida* Murray; Coleoptera: Nitidulidae) is a relatively minor parasitic pest of honeybee colonies in Africa. In the United States, where the beetle was recently detected in Florida in 1998, it has been reported to have attained pest status throughout the eastern and mid-western parts of the country (Sanford, 1998; Hood, 2000). The larval stage of the beetle is the most damaging to the honeybee colony, feeding on honey, pollen and brood (Lundie 1940; Schmolke, 1974; Eischen et al., 1998; Elzen et al., 2000). Larval excrement and fermentation of infested honey make it unfit for human consumption. Colonies weakened by beetle attack tend to collapse within two weeks, causing honeybees to abscond (Wenning, 2001). Currently, effective management tools are lacking to address the beetle problem. One promising approach to control the beetle is exploitation of its chemically-mediated host finding cues.

Elzen et al. (1999) trapped beetles in the field with traps baited with a combination of

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worker bees, pollen and honey. Olfactometric and wind tunnel studies have demonstrated the role of volatiles mediating attraction and confirmed the attractiveness to the beetle of volatiles from living adult worker bees, pollen, unripe honey and wax by-products ("slumgum") (Suazo et al., 2003). This study also showed that the response by females to these odors was significantly stronger than that of males. We have expanded on these findings, and in this study we report on the chemistry, electrophysiology and laboratory wind-tunnel responses of the small hive beetle to a blend of chemicals identified from honeybee volatiles.

## 2. MATERIALS AND METHODS

### 2.1. Insects

A colony of the SHB was started from beetles that were collected in the field in Umatilla, Florida during November, 1999. The beetles were reared in Plexiglas cages (25 × 25 × 25 cm) at room temperature (25 °C) with 14L: 10D photoperiod and provided with pollen-honey diet and water (Suazo et al., 2003). The female:male ratio in the colony was 2:1. Adult worker honeybees were obtained from colonies maintained at the USDA-ARS facilities in Gainesville.

### 2.2. Volatile collections

Volatiles were collected from adult worker honeybees by aeration and adsorption on dichloromethane-cleaned Super-Q traps (30 mg, Alltech, Nicholasville, KY) for two days at room temperature (Suazo et al., 2003). Briefly, 500–600 adult worker honeybees (represents approx. number of bees on a hive frame) were placed in a brass screen canister and provided with 50 g of sugar candy and water and then transferred into a cylindrical glass container (55-cm-long × 14-cm ID), fitted with a 12-port glass lid. Super Q filters were attached to two of the ports and volatiles were trapped on the filters by pulling charcoal-filtered and humidified air through the traps at 0.5 L/min for two days. Each filter was eluted with 200 µL of GC/GC-MS-grade dichloromethane (Burdick and Jackson, Muskegon, Michigan, USA), and the eluents pooled together and then stored at –70 °C prior to bioassays and analysis. This procedure was repeated with different populations of worker bees when more volatiles were needed for bioassays.

To identify the components released by test samples into the wind tunnel during bioassays which were run for 15 min, volatiles were collected from living adult worker honeybees (approx. 150–200) and rubber septa impregnated with the Super Q extract of volatiles from the bees. For comparison, volatiles were also collected from rubber septa impregnated with the synthetic blend of the identified components in the volatiles. The conditions for volatile collection were identical to those used for bioassays. Different doses (125–375 bee day equivalents) of the Super Q extract and synthetic blend, were loaded on rubber septa, air-dried for 3–4 h and then transferred into quick-fit glass containers (20-cm-long × 3-cm-OD) outside the wind-tunnel. Charcoal-purified air was passed over the test samples at 0.5 L/min and the odors collected on a Super Q filter connected to the outlet in the wind tunnel for 15 min. Each filter was eluted with 200 µL of GC/GC-MS-grade dichloromethane (Burdick and Jackson, Muskegon, Michigan, USA), and then stored at –70 °C prior to analysis by coupled gas chromatography-mass spectrometry.

### 2.3. Analyses of volatiles

Coupled gas chromatography-electroantennographic detection (GC-EAD) analysis was carried out on a Hewlett-Packard (HP) 5890 Series II gas chromatograph equipped with an HP-1 column (30 m × 0.32 mm ID × 0.25 µm, Agilent, Palo Alto, California, USA), with helium as the carrier gas. Extracts were analyzed in splitless mode at an injector temperature of 220 °C, and a split valve delay of 1 min. The oven temperature was held at 45 °C for 2 min, then programmed at 10 °C/min to 210 °C and held at this temperature for 10 min. The column effluent was split 1:1 for simultaneous detection by FID and EAD. For EAD detection, gold wires in drawn-out glass capillaries filled with Ephrussi-Beadle saline (Ephrussi-Beadle, 1936) served as reference and recording electrodes. The distal and proximal segments of the antenna of the beetle were placed in contact with the microelectrodes, and a humidified airstream (90–100% RH) was delivered at 1 mL/s over the antennal preparation. The microelectrodes were connected via an antennal holder to an AC/DC amplifier in DC mode (Syntech, Hilversum, The Netherlands). A GC-EAD program (Syntech GC-EAD 2000, Hilversum, The Netherlands) was used to simultaneously record and analyze the amplified EAD and FID signals on a PC. Five µL of the Super Q extract of honeybee volatiles were analyzed with either fresh male or female antenna for repeated sample analysis.

GC-MS analyses of the Super Q extracts of honeybee volatiles were carried out on an HP-6890 coupled to an HP5973 mass spectrometer (EI, 70 eV, Agilent, Palo Alto, California, USA) equipped with an HP-1 column (30 m × 0.25 mm ID × 0.25 µm,

Agilent, Palo Alto, California, USA), which employed the same temperature program used for the GC-EAD analysis. For the analysis, 100 ng of internal standard (butyl butyrate) were added to 40 μL of each volatile extract and 1 μL was analyzed. EAD-active compounds and volatiles trapped in the wind-tunnel were identified by comparing their mass spectral data with those in the library (NIST, 98K) of the mass spectrometer and by retention times and GC-EAD analysis with authentic commercial samples.

### 2.4. Chemicals

2-pentanone, 3-methyl-1-butanol, 2-methyl-1-butanol, sec-butyl acetate, isobutyl acetate, 2-hexanone, hexanal, butyl acetate, 1-hexanol, isopentyl acetate, 2-heptanone, heptanal, 2-heptanol, 6-methyl-5-hepten-2-one, α-pinene, β-pinene, 1-heptanol, octanal, hexyl acetate, 2-nonanone, methyl benzoate, nonanal, ethyl benzoate, benzyl acetate, decanal, citral, nerol and geraniol were purchased from Aldrich (Milwaukee, Wisconsin, USA). Purities ranged from 98% to 99.5%. 3-Methyl-2-buten-1-yl acetate was synthesized in the laboratory from acetyl chloride and 3-methyl-2-buten-1-ol (Aldrich, Milwaukee, Wisconsin, USA), while 3-methylbutan-1-yl propionate was synthesized from an acid catalyzed solution of propionic acid and 3-methyl-1-butanol. The purities of the two esters (> 99%) were checked by GC and their identities confirmed by comparison of their mass spectral data with library data (NIST, 98K) in the mass spectrometer. A similar analysis was also carried out on the volatiles captured on Super Q released from living honeybees, the Super Q extract and synthetic blend of identified compounds from the bee volatiles.

### 2.5. Wind-tunnel bioassays

Bioassays were carried out in a Plexiglas wind-tunnel (1.85 × 0.66 × 0.66 m) in a room maintained at 27 ± 1 °C and 50 ± 10% relative humidity (Suazo et al., 2003). Two 34-Watt fluorescent tubes (4 foot-long) placed 0.2 m above the wind tunnel provided illumination. Responding beetles released in the wind-tunnel were captured in two traps made out of plastic vials, (25 dram, BioQuip, Gardena, CA), fitted with a screen cone with a 5-mm opening, which allowed beetles to enter the vial but not exit. The traps were placed upwind, 0.3 m above the floor of the tunnel and were separated by 0.3 m from each other (Suazo et al., 2003). Wind speed inside the tunnel was set at 0.2 m/s. Bioassays were carried out between1900 and 2400 h, when beetle flight activity was at its peak, with 7–14 day old adults of mixed sex.

Living adult worker honeybees (approx. 150–200, obtained from frames in supers), Super Q collected volatile extracts of the worker honeybees and an 8-component synthetic blend comprising the major components identified in the volatiles of a representative sample of honeybee volatiles were used as odor sources. A 200 mg stock solution of the 8-component blend comprised isopentyl acetate, 2-heptanone, octanal, hexyl acetate, nonanal, 2-nonanone, methyl benzoate and decanal in the 44:100:17:4:5:41:16:13 ratio (based on GC-FID peak area) found in the natural volatile blend released by worker honey bees was formulated. Aliquots of the Super Q extracts and the synthetic blend in dichloromethane were loaded on red rubber septa (11 mm-OD, Wheaton, Millville, New Jersey, USA) (Heath et al., 1991). Doses were expressed as bee day equivalents (Suazo et al., 2003) (1 BDE = volatiles emitted by one bee in a day, which is approx. 65 ng of the 8-component blend). Four doses comprising approx. 50, 125, 250 and 375 bee day equivalents were tested in dual choice wind-tunnel bioassays. The control rubber septa used in each bioassay were treated with dichloromethane only. Prior to bioassays, the solvent was allowed to evaporate for 4 h. Treated and control rubber septa were placed in quick-fit glass containers (20-cm-long × 3-cm-OD) outside the wind-tunnel and a stream of purified air was passed through each container at a flow rate of 0.5 L/min (Suazo et al., 2003). One septum was used per replicate.

Twenty five beetles of mixed sex (7–14 days old), were released from plastic holding vials (25 dram, BioQuip, Gardena, CA), 1.5 m downwind from the odor source. For each dose, tests were replicated three times using one septum per replicate to ensure a relatively stable release rate per assay. For each replicate, the number of beetles responding was recorded for 15 min. The position of odor sources was switched between replicates to minimize positional bias. Beetles were deprived of food and water for one day prior to bioassays and were used only once. Bioassays were also conducted in which responses of beetles to dichloromethane impregnated rubber septa were compared to clean air.

The behavioral responses of beetles to the attractive sources during the 15 min bioassay period were scored as follows: Upwind response (UR), beetle took flight upwind following plume and clearly hovered within 5 cm of the odor source, but failed to enter the trap (responding beetles thereafter landed on the side or roof of the wind tunnel near the upwind end of the odor source); and trap capture (TC), beetle entered traps of the odor sources (Bruce and Cork, 2001; Downham et al., 2003).

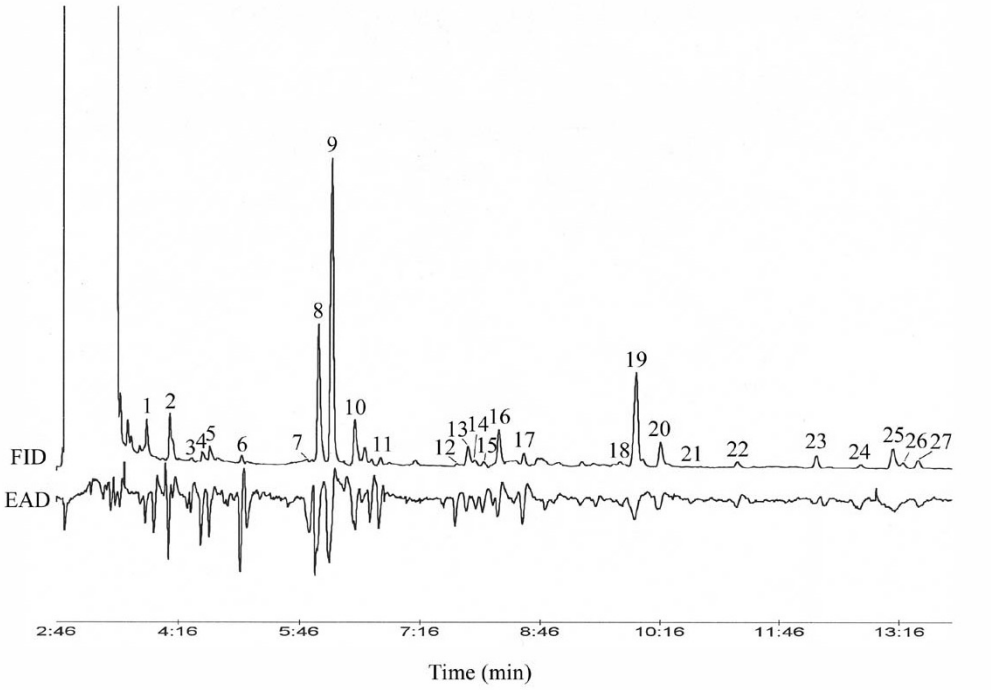


**Figure 1.** Representative GC-EAD profile using female antenna of *A. tumida*, responding to compounds in the Super Q extract of adult worker honeybee volatiles (See Tab. I for peak identities).

### 2.6. Data analyses

Percentages ($p$) of beetles performing upwind response (UR) and those captured in the traps (TC) were transformed by arcsin, $\sqrt{p}$ and were subjected to analysis of variance. Mean responses were compared and tested for significance by a LSD test ($P < 0.05$) (SAS Institute, 1999–2001, Version 8.2).

## 3. RESULTS

### 3.1. Analysis of volatiles

A representative GC-EAD profile of the volatiles collected from adult worker honeybees is shown in Figure 1. Forty one EAD-active peaks were detected in the volatiles, and twenty seven were identified by GC-MS. All of the EAD-active peaks were identified by their mass spectra and confirmed by GC retention time and GC-EAD comparison with authentic compounds (Tab. I). Among the EAD-active components, GC-MS identified isopentyl acetate, 2-heptanone, octanal, hexyl acetate, nonanal, 2-nonanone, methyl benzoate and decanal as the most abundant, eliciting strong antennal responses by the small hive beetle. These same eight components were identified by GC-MS analysis of volatiles released into the wind tunnel by living adult worker bees (Fig. 2) in our 15-min bioassay and from volatiles released from rubber septa impregnated with the Super Q extract of bee volatiles collected for two days (Fig. 3). The total ion chromatogram of this volatile blend was similar to that released by the 8-component blend of synthetic compounds into the wind tunnel in the 15-min bioassay (Fig. 3). Other EAD-active components identified in trace amounts in the volatiles released by the rubber septa impregnated with the Super Q extract included 3-methyl-1-butanol, butyl acetate, heptanal, α- and β-pinene and 6-methyl-5-hepten-2-one. However, GC-MS analysis showed that the volatiles released by different batches of adult worker honeybees

**Table I.** EAD-active components identified from Super Q trapped volatiles of honeybees.

| Peak | Compound | Honeybee |
|---|---|---|
| 1 | 2-pentanone | + |
| 2 | 3-methyl-1-butanol | + |
| 3 | sec-butyl acetate | + |
| 4 | isobutyl acetate | + |
| 5 | 2-hexanone | + |
| 6 | butyl acetate | + |
| 7 | 1-hexanol | + |
| 8 | isopentyl acetate | + |
| 9 | 2-heptanone | + |
| 10 | heptanal | (+) |
| 11 | 2-heptanol | + |
| 12 | 3-methyl-2-buten-1-yl acetate | + |
| 13 | 3-methylbutan-1-yl propionate | + |
| 14 | 6-methyl-5-hepten-2-one | + |
| 15 | β-pinene | (+) |
| 16 | octanal | + |
| 17 | hexyl acetate | + |
| 18 | 2-nonanone | + |
| 19 | methyl benzoate | + |
| 20 | nonanal | + |
| 21 | ethyl benzoate | + |
| 22 | benzyl acetate | + |
| 23 | decanal | + |
| 24 | Z-citral | + |
| 25 | E-citral | + |
| 26 | nerol | + |
| 27 | geraniol | + |

Key: + detected; (+) not always detected in three different samples analyzed by GC-MS.

differed quantitatively rather than qualitatively (Fig. 4).

### 3.2. Wind-tunnel bioassays

In dual-choice bioassays, upwind responses and trap captures elicited by the blank and air control from beetles compared to the different treatments was zero. Data was therefore analyzed as a no choice assay to allow for comparison between the different treatments containing honey bee volatiles. The Super Q extract and the 8-component blend elicited dose dependent upwind responses from beetles (Fig. 5). Upwind responses elicited by the Super Q extract and the 8-component blend at similar doses were not significantly different. At 375-bee day equivalents, upwind responses elicited by the Super Q extract, the 8-component blend and volatiles from approx. 150–200 worker honey bees were not significantly different. However, while volatiles from living worker bees lured 48% of beetles into the trap attached to the attractant source, the Super Q extract and the 8-component blend lured 12 and 3% of beetles, respectively, into the trap relative to control.

## 4. DISCUSSION

GC-MS analysis identified EAD-active components to consist primarily of alcohols, aldehydes, ketones and esters. Among these were the honeybee alarm pheromones, notably isopentyl acetate and 2-heptanone (Pettis et al., 1999), and the floral volatile components octanal, nonanal, decanal, hexyl acetate and methyl benzoate, which serve as attractants for various insects (Knudsen et al., 1993; Levin et al., 2001).

Bioassay results clearly showed that the Super Q extract and the 8-component blend elicited dose dependent upwind responses, in agreement with previous olfactometric and wind tunnel results which demonstrated that the small hive beetle was attracted to worker honeybee volatiles (Suazo et al., 2003). Although upwind response suggested that the small hive beetle detected the signal, the Super Q extract and the 8-component blend were not as effective as living bees in luring beetles into the trap attached to the attractant source in the wind tunnel. GC-MS identified the eight components in the synthetic blend and other components present in minor amounts in the volatiles released by living adult worker honeybees into the wind tunnel in our 15-min bioassay. This suggested that these minor components present in bee volatiles could play a role in small hive beetle attraction during the 15-min bioassay. This suggestion is further supported by GC-MS

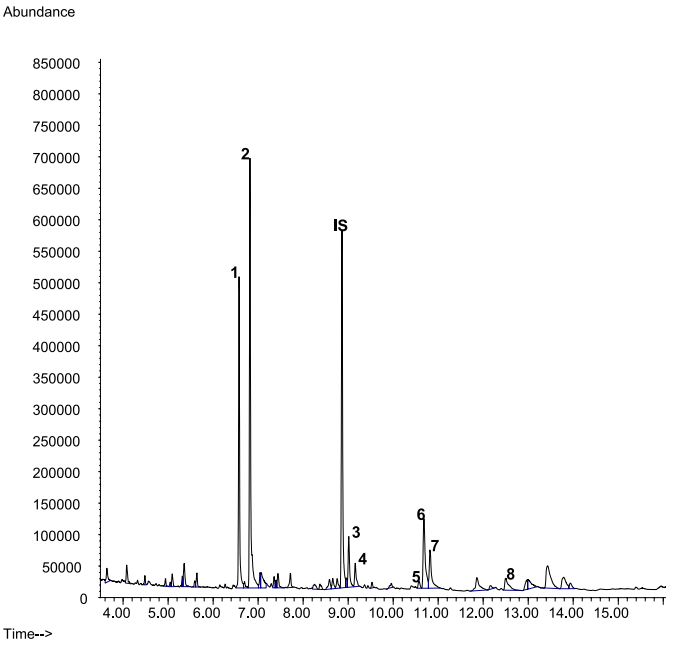


**Figure 2.** Representative total ion chromatogram of volatiles released by living adult worker honeybees (approx. 150–200) and captured on Super Q in a wind-tunnel during a 15-min bioassay. Peak number-compound: 1- isopentyl acetate; 2- 2-heptanone; 3- octanal, 4- hexyl acetate; 5- 2-nonanone; 6- methyl benzoate; 7- nonanal; 8- decanal; IS (internal standard)- butyl butyrate.

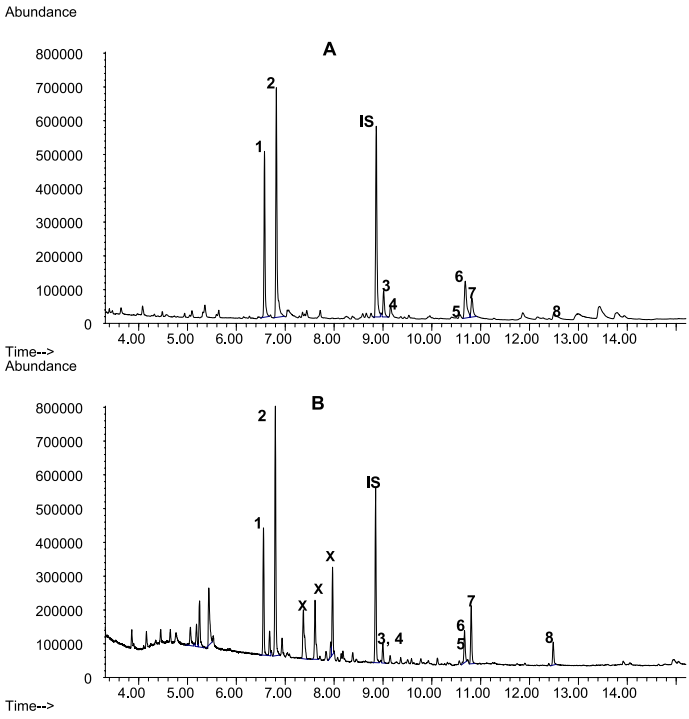


**Figure 3.** Representative total ion chromatograms of volatiles captured on Super Q in a wind-tunnel during a 15-min bioassay from rubber septa impregnated with (A) 250 BDE of Super Q volatile extract, and (B) 250 BDE of the 8-component synthetic blend identified in the volatiles of adult worker honeybees. Peak number-compound: 1- isopentyl acetate; 2- 2-heptanone; 3- octanal, 4- hexyl acetate; 5- 2-nonanone; 6- methyl benzoate; 7- nonanal; 8- decanal; IS (internal standard)- butyl butyrate; x- impurity.

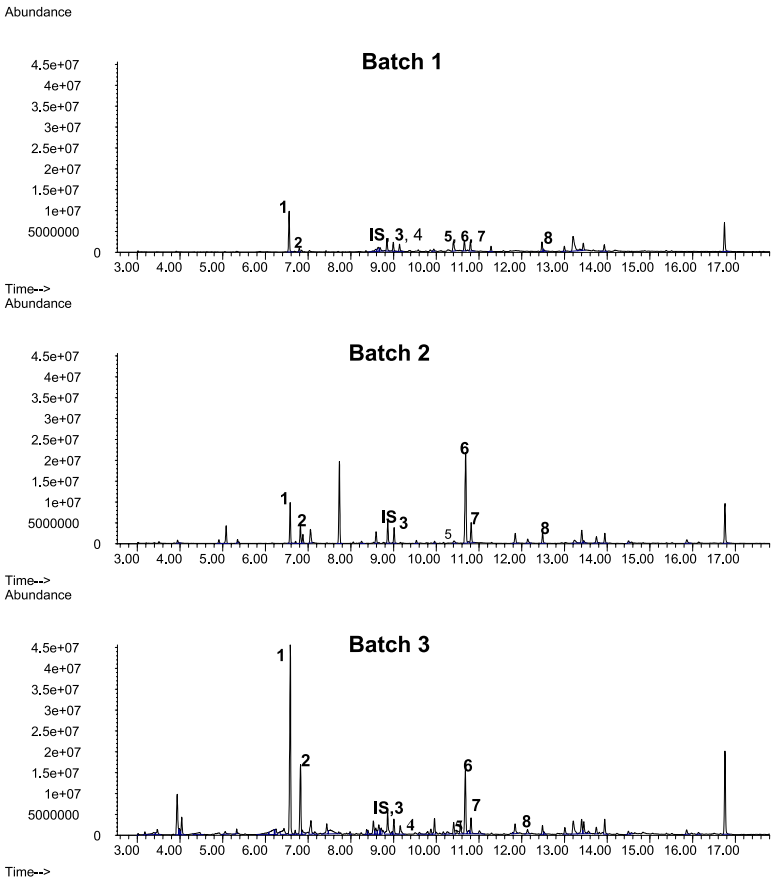


**Figure 4.** Representative total ion chromatograms of volatiles captured on Super Q for two days from different batches of adult worker honeybees. Peak number-compound: 1- isopentyl acetate; 2- 2-heptanone; 3- octanal, 4- hexyl acetate; 5- 2-nonanone; 6- methyl benzoate; 7- nonanal; 8- decanal; IS (internal standard)- butyl butyrate.

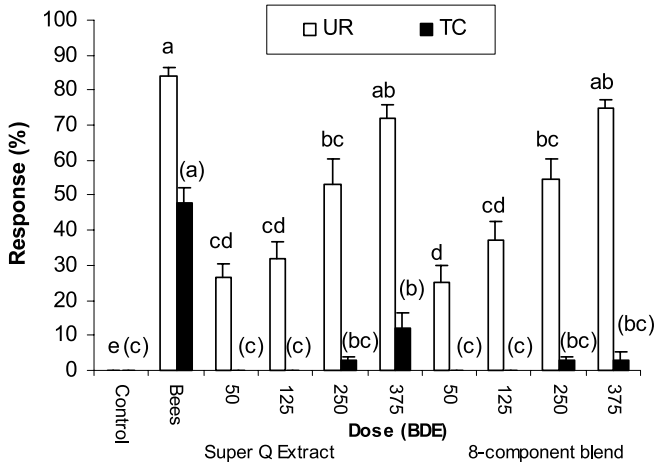


**Figure 5.** Wind-tunnel responses of *A. tumida* to blank control, 150–200 living adult worker honeybees, rubber septa impregnated with Super Q extract and an 8-component synthetic blend identified from the volatiles of the bees. Upwind responses, UR (open bars) and corresponding trap captures, TC (closed bars). For each test, there were three replicates. N = 25 beetles/replicate. Mean comparisons of upwind responses are indicated by letters; mean comparisons of trap captures are indicated by letters in parentheses. Means with the same letter were not significantly different ($P < 0.05$, LSD test).

analysis, which identified trace amounts of the EAD-active components 3-methyl-1-butanol, butyl acetate, heptanal, α- and β-pinene and 6-methyl-5-hepten-2-one in the volatiles released into the wind tunnel by the rubber septa impregnated with the Super Q extract which lured 4 times more beetles into the trap than the 8-component blend. But even this formulation of naturally-produced volatiles was less effective in attracting the small hive beetle than the volatiles released into the wind-tunnel by the bees. This suggests that volatiles released by the honeybees and not captured on the Super Q may, in fact, be crucial to small hive beetle attraction.

In olfactometer bioassays, seven-day old worker bees attracted six times more SHBs than three-day old bees (Suazo et al., 2003). In the present, as in the previous study carried out by Suazo et al. (2003), adult worker honeybees used for wind tunnel bioassays and the collection of volatiles were shaken off from frames in supers. Adult worker bees on these frames usually vary in age. Therefore, we cannot rule out the possibility that age differences in honeybees used in these studies might contribute to batch differences in volatile composition and beetle response. This possibility is supported by our GC-MS analysis of volatiles from different batches of honeybees collected for similar periods, which differed mainly quantitatively rather than qualitatively in some of the major components. GC-MS analysis of volatiles and bioassay results also suggest that the presence rather than the ratio of specific components in the volatiles may be important in the attractiveness of honeybee colonies to the small hive beetle.

Noteworthy in the present study is the composition of volatiles collected from worker honeybees. Volatiles were collected from bees in an aerated closed system, which did not simulate the natural conditions typical of a honeybee hive. Honeybees kept under these conditions are usually stressed, as indicated by the high levels of the alarm pheromone, isopentyl acetate, found in the volatiles of the different batches of worker bees used. Interestingly, odors released by these bees were very attractive to the small hive beetle. Wenning (2001), reports that honeybee hives weakened by stress either due to disease, pest, climatic changes or management practices are more attractive to the small hive beetle than healthy ones. Whether the alarm pheromone and other components identified in the volatiles in the present study contribute to the odor of a stressed honeybee hive will require further research. Future research will examine the contribution of other components in synergizing the attractiveness of the 8-component blend, and the development of this blend into an effective lure for trapping the small hive beetle.

## ACKNOWLEDGMENTS

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**Résumé – Réaction du Petit coléoptère des ruches (*Aethina tumida*) à un mélange de composés volatils identifiés chez l'Abeille domestique (*Apis mellifera*).** Le Petit coléoptère des ruches, *Aethina tumida* Murray (Coleoptera : Nitidulidae) est un nouveau ravageur invasif des colonies d'abeilles domestiques, *Apis mellifera* L., aux États-Unis. Nous avons étudié en tunnel de vol ses réactions à des composés chimiques volatils identifiés chez l'Abeille et qui avaient été auparavant caractérisés chimiquement et électrophysiologiquement. Les substances volatiles émises par les ouvrières d'abeilles ont été piégées sur un filtre Super Q, puis analysées par chromatographie en phase gazeuse couplée à la détection électroantennographique (GC-EAD) (Tab. I). Plusieurs composés déclenchaient des réponses antennaires de la part du Petit coléoptère des ruches. Ils ont été identifiés par chromatographie en phase gazeuse (GC) couplée à la spectrométrie de masse (MS). L'analyse en GC-MS a aussi porté sur les composés volatils émis dans le tunnel de vol par des ouvrières vivantes et par des bandes de caoutchouc imprégnées de l'extrait Super Q. On a pu ainsi identifier les composés émis dans le tunnel de vol durant des tests de 15 min. L'analyse a montré que le profil volatil était dominé par huit des composés actifs en EAD : l'acétate d'isopentyle (phéromone d'alarme des ouvrières d'abeilles), le 2-heptanone, l'octanal, l'acétate d'hexyle, le nonanal, le 2-nonanone, le benzoate de méthyle et le décanal. Les réactions du Petit coléoptère des ruches à l'extrait Super Q et au mélange de ces huit composés présentés dans un rapport naturel (44 : 100 : 17 : 4 : 5 : 41 : 16 : 13) ont été étudiées par un test de choix binaire en tunnel de vol. L'extrait Super Q et le mélange des huit composés ont déclenché de la part des coléoptères des réactions contre le vent dépendant de la dose par rapport au témoin (solvant). Pour un équivalent de 375 jours-abeilles (1 EJA = quantité émise/abeille/j ≈ 65 ng), l'extrait Super Q et le mélange des huit composés ont respectivement déclenché 76 et 74 % de réactions contre le vent.

C'est comparable aux 84 % de réactions à 150–200 ouvrières vivantes. Par contre l'attraction dans un piège par l'extrait Super Q et par le mélange des huit composés a été bien moindre que par l'odeur d'ouvrières vivantes : 12 et 3 % contre 48 % respectivement. A l'avenir la recherche doit porter sur l'amélioration de l'efficacité du leurre avec d'autres composés chimiques pour piéger le Petit coléoptère des ruches.

***Aethina tumida* / substance volatile / tunnel de vol / phéromone d'alarme / *Apis mellifera***

**Zusammenfassung – Reaktion des Kleinen Beutenkäfers (*Aethina tumida*) auf ein Gemisch von bei Honigbienen (*Apis mellifera)* nachgewiesenen flüchtigen Substanzen.** Der Kleine Beutenkäfer *Aethina tumida* Murray (Coleoptera: Nitidulidae) ist ein neuer eingeschleppter Schädling der europäischen Honigbienenvölker (*Apis mellifera*) in den Vereinigten Staaten von Amerika. Wir untersuchten die Reaktionen des Kleinen Beutenkäfers im Windkanal auf chemische Stoffe, die zuvor mit chemischen und elektrophysiologischen Methoden analysiert wurden. Die flüchtigen Stoffe der Arbeiterinnen der Honigbienen (*Apis mellifera)* wurden mit einem Super Q Filter aufgefangen und mit einer Kombination aus Gaschromatographie mit Elektroantennographie (GC-EAD) analysiert (Tab. I). Mehrere Komponenten dieser flüchtigen Stoffe erzeugten eine Reaktion des Kleinen Beutenkäfers. Diese Komponenten wurden mit einer Kombination von Gas- und Massenspektrometrie (GC-MS) analysiert. GC-MS Analysen wurden auch von den flüchtigen Stoffen gemacht, die von fliegenden Bienen und von Gummistreifen im Windtunnel abgegeben wurden, die mit dem Super Q Extrakt der flüchtigen Stoffe der Bienen imprägniert waren. Damit wurde die Identifikation von Komponenten ermöglicht, die im Windtunnel während der 15 minütigen Versuchsphase abgegeben wurde. Die Analyse zeigte, dass 8 der beim EAD aktiven Komponenten im Profil der flüchtigen Stoffe dominierten. Es bestand aus Isopentylacetat (Alarmpheromon der Honigbienen), 2-Heptanone, Octanal, Hexylacetat, Nonanal, 2-Nonanon, Methylbenzoat und Decanal. Die Reaktionen des Kleinen Beutenkäfers auf den Super Q Extrakt und auf ein Gemisch dieser 8 Komponenten in der nachgewiesenen natürlichen Zusammensetzung (44:100:17:4:5:41:16:13) wurden in einem (Alternativwahlversuch) Zweifachwahltest im Windtunnel untersucht. Der Super Q Extrakt und das Komponentengemisch riefen im Vergleich zum reinen Lösungsmittel eine dosisabhängige Gegenwindreaktion des Käfers hervor (Abb. 5). Bei Äquivalenten von 375-Bienentagen, lag die Gegenwindreaktion beim Super Q Extrakt und beim 8-Komponenten-Gemisch bei 76 % bzw. 74 %. Das ist vergleichbar mit der 84 % Reaktion auf etwa 150–200 lebende Arbeiterinnen. Im Gegensatz dazu war die Anlockung in eine Falle bei dem Super Q Extrakt und der 8-Komponentenmischung viel geringer als bei lebenden Bienen. Nur zwischen 12 % (Extrakt) und 3 % (Mischung) der Käfer wurden so gefangen, der Duft der lebenden Bienen lockte 48 % an. In Zukunft soll sich die Forschung auf die Steigerung der Effektivität der Attrappen durch andere Chemikalien zum Einfangen der Kleinen Beutenkäfer konzentrieren.

**Kleiner Beutenkäfer / *Aethina tumida* / Honigbienen / flüchtige Chemikalien / Alarmpheromon / Windkanal**

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