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Original article

Composition of volatiles from fermenting pollen dough and attractiveness to the small hive beetle *Aethina tumida*, a parasite of the honeybee *Apis mellifera**

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Abstract – The response of the small hive beetle (SHB), *Aethina tumida* Murray, to volatiles from a pollen-based diet conditioned by the feeding of 100 adult virgin female or male SHBs (4–8 weeks old) for 1, 3, 7 or 14 days is described and compared to that of the same diet inoculated with the yeast *Kodamaea ohmeri* (NRRL Y-30722), isolated from the beetle. In a wind tunnel, volatiles from pollen dough conditioned by beetles of either sex for 3 or 7 days lured significantly more beetles into traps than volatiles from unconditioned dough. In contrast, trap captures with volatiles from dough conditioned for 1 and 14 days were weakly attractive. In cage bioassays, when naïve, unfed, virgin, SHBs (3–4 days old) were given a choice between yeast-inoculated pollen dough and non-inoculated dough, the responses were similar to those obtained in the wind tunnel with dough conditioned by SHBs for 3 and 7 days. Chemical analysis revealed high levels of fermentation-related products in volatiles that attracted the beetle.

***Aethina tumida* / *Apis mellifera* / pollen dough / volatile / electrophysiology**

1. INTRODUCTION

The small hive beetle (SHB), *Aethina tumida* Murray (Coleoptera: Nitidulidae), is a pest of European honeybees (*Apis mellifera* L.) in the United States and Australia. Surveys in the US during 2004 indicated that the beetle had spread to 30 states (Hood, 2004). The beetle is weakly attracted to bucket traps baited with a combination of honey, pollen and adult bees, but not to traps baited with honey and pollen, or brood alone (Elzen et al., 1999). In wind tunnel studies, adult SHBs responded to a variety of chemical cues, including worker bee volatiles, pollen, unripe honey and honeybee colony by-products ("slum gum") (Suazo et al., 2003). Further studies on worker bee volatiles led to identification of a blend of eight chemicals (isopentyl acetate, 2-heptanone, octanal, hexyl acetate, nonanal, 2-nonanone, methyl benzoate and decanal) that weakly attracted the SHB in wind tunnel assays (Torto et al., 2005).

Small hive beetles show a stronger response to the volatiles of freshly-collected bee pollen (Suazo et al., 2003) than to those of commercially packaged bee pollen, alone or in combination with honey, as observed by Elzen et al. (1999) in a trapping study. Whether SHBs will respond to a pollen-honey mixture made with commercially packaged bee pollen and

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conditioned by feeding beetles is unknown. In this study, we examined the potential of a pollen-honey mixture (pollen dough) as a lure for the SHB. The specific objectives were: (a) to compare the beetle's response to volatiles from pollen dough conditioned by the feeding of adult beetles to volatiles from unconditioned pollen dough, (b) to compare the beetle's response to volatiles from pollen dough inoculated with the yeast *Kodamaea ohmeri* (NRRL Y-30722) isolated from the beetle (Teal. et al., 2006) and (c) to identify the electrophysiologically-active compounds.

2. METHODS AND MATERIALS

2.1. Insects

Adult small hive beetles (SHBs) were collected from managed honeybee colonies in Lake City, Florida to start a laboratory colony. To ensure colony vigor, colonies were maintained for only six months and then restarted with freshly collected field beetles. Also, to ensure that the SHBs were virgins for use in assays, we collected pupae from our rearing soil and sexed them based on pupal secondary sexual characters described previously for the nitulid beetle, *Meligethes aeneus* Fabricius (Charpentier and Weibull, 1994). The beetles were reared on a pollen-honey diet (pollen dough) using methods similar to those described by Suazo et al. (2003). The pollen dough was prepared from commercially packaged bee pollen (Y.S. Organic Bee Farms, Sheridan, Illinois, USA), commercial pollen substitute (Bee Pro®, Mann Lake Ltd., Hackensack, MN, USA) and warm honey (34 °C) (1:12:18).

2.2. Odor sources

Two types of odor sources were tested: one consisted of pollen dough that had been fed upon (conditioned) by 100 adult SHBs, either males or virgin females, for 1, 3, 7 or 14 days; the other consisted of pollen dough that had been inoculated with a yeast (NRRL Y-30722) isolated from the beetle (Teal et al., 2006). For conditioning, 10 g of freshly prepared pollen dough (see Sect. 2.1 Insects) and a water-filled vial (15 mL, Millipore-pure™, 18 MΩ) were placed in a quickfit aeration chamber (33 cm long × 3.5 cm OD). A dental wick (Richmond Dental, Richmond, NC, USA), inserted through a hole in the snap cap of the vial and into the water, gradually moistened the dough. For each conditioning period, 100 beetles were placed in each of four chambers – males in two and females in two – and held at 26 °C. For bioassays and volatile collection, the chambers were emptied, except for the pollen dough.

Inoculated pollen dough was prepared by mixing the yeast with Millipore-pure™ water and pollen dough, 1:100:1000 by wt and allowing the resulting dough to ferment for 5 days.

2.3. Wind-tunnel bioassays

The response of adult beetles (virgin males or females) to volatiles released by conditioned pollen dough was tested by dual choice bioassays in a wind tunnel (1.85 × 0.66 × 0.66 m) (Suazo et al., 2003; Torto et al., 2005). The beetles were deprived of food and water for 1 day prior to the bioassay. A stream of purified air was passed through each of two glass tubes (33 cm long × 3.5 cm OD) at rate of 0.5 L/min and into the wind tunnel. One tube contained 10 g of unconditioned pollen dough (control) and the other 10 g of pollen dough that had been conditioned for 1, 3, 7 or 14 days. Twenty-five virgin males or females (4–8 week old) were released 1.5 m downwind from the two odor sources and the numbers captured in traps attached to the sources were recorded for 10 min. Each comparison was replicated three times with a different batch of beetles, and the positions of the odor sources were reversed between replicates to minimize positional bias. A fresh sample of conditioned and unconditioned pollen dough was used for each sex.

2.4. Laboratory cage bioassays

The response of naïve, unfed, virgin adult males and females (3–4 days old) to yeast-inoculated pollen dough was tested by dual choice bioassays using a trap in a screen mesh cage (72 × 40 × 40 cm). The trap consisted of a flat plywood panel with two holes (each 2 cm in diam. and 12 cm apart) that led to separate 500 mL plastic containers (Rubbermaid, Huntersville, NC, USA) attached beneath it. Each hole opened directly into a triangular enclosure formed by three strips of wood (0.8 × 0.8 × 6.0 cm) attached to the underside of the plywood panel and covered with 7-mesh screen. Openings (0.5 cm wide) at the apices of each triangle allowed beetles to enter the container below.

A Petri dish (6 cm-diam × 2 cm-high) for holding the odor source was glued to the bottom of one container. The Petri dish was covered with a tight-fitting lid perforated by about 60 pin-holes that allowed the release of odors into the screen cage.

The odor sources included 10 g of pollen dough inoculated with the yeast *Kodamaea ohmeri* (NRRL Y-30722) vs. 10 g non-inoculated pollen dough. The positions of the odor sources were switched between assays to eliminate positional bias. Twenty-five male or female beetles were released from a vial at the center of the wooden panel midway between the two odor sources, and the numbers captured in the two containers were recorded after 10 min. Each comparison was replicated three times with a different batch of beetles in each replicate. One sample of the inoculated and non-inoculated pollen dough was used for each sex of the beetle tested in the three replicates.

2.5. Collection and analysis of volatiles

Volatiles were collected from conditioned and unconditioned pollen dough (three replicates each) by passing charcoal-filtered and humidified air at 0.5 L/min over the pollen dough and through a Super-Q adsorbent trap (30 mg, Alltech, Nicholasville, KY) for 1 h at room temperature. Each filter was eluted with 150 μL of GC/GC-MS-grade dichloromethane (Burdick and Jackson, Muskegon, Michigan, USA), and stored at -70 °C prior to analysis by coupled gas chromatography-electroantennogram detection (GC-EAD) and coupled gas chromatography-mass spectrometry (GC-MS) analysis.

Aliquots of 5 μL of the volatile extracts from unconditioned and conditioned pollen dough were analyzed by GC-EAD (Syntech GC-EAD 2000, Hilversum, The Netherlands) on a Hewlett-Packard (HP) 5890 Series II gas chromatograph equipped with an EC-1 column (30 m × 0.25 mm ID × 0.25 μm) (Alltech, Houston, Texas, USA), as previously described (Torto et al., 2005). The oven temperature was held at 35 °C for 5 min, then programmed to increase at 10 °C/min to 220 °C and held at this temperature for 5 min. For EAD, excised antennae of either male or female beetles were inserted into the openings of glass capillaries containing gold wires and filled with Ephrussi-Beadle saline solution (Ephrussi and Beadle, 1936). GC-EAD was carried out similarly on the volatile extract of the yeast inoculated pollen dough on a HP-5 column (30 m × 0.32 mm ID × 0.25 μm) (Agilent, Palo, Alto, California, USA). The oven conditions were: 30 °C for 5 min, rate at 10 °C/min to 220 °C and held at this temperature for 5 min.

GC-MS analyses of the volatile extracts 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 temperature program used for the GC-EAD analysis with the EC-1 column. For the analysis, 500 ng of internal standard (butyl butyrate) were added to 40 μL of each volatile extract and 1 μL was analyzed. The components of the volatiles were identified by comparing their mass spectral data with those in the library (U. S. Dept. of Commerce, 2005) of the mass spectrometer and by retention time analysis and mass spectra with authentic commercial samples. To identify components released by conditioned pollen dough during the bioassays, we used Super Q (for 10 min) to collect volatiles directly from the wind tunnel under conditions identical to those used in the bioassays. Also, to identify volatiles not captured on the Super Q filter, air from the headspace of the conditioned pollen dough was drawn into a 100 μL syringe and analyzed directly by GC-MS. This sample was analyzed on a Varian 3400 GC equipped with a cool on-column injector coupled to a Finnigan Magnum mass spectrometer. The GC was fitted with a DB-5 column (30 m × 0.25 mm ID × 0.25 μm) (J & W Scientific, Folsom, California, USA). The injector temperature was held at 20 °C for 0.5 min before increasing to 220 °C. The oven temperature was held at 20 °C for 5 min, increasing at 10 °C/min to 220 °C and held at this temperature for 5 min.

2.6. Chemicals

Acetaldehyde, ethanol, 2-methyl-1-propanol, ethyl acetate, 3-methyl-1-butanol, 2-methyl-1-butanol, hexanal, isopentyl acetate, 2-heptanone, 6-methyl-5-hepten-2-one, α-pinene, β-pinene, 3-octanone, octanal, 2-nonanone, nonanal, decanal, ethyl propionate, ethyl 2-methylpropionate, 2-methylpropanyl acetate, ethyl butyrate, ethyl 2-hydroxypropionate, ethyl 2-methylbutyrate, ethyl 3-methylbutyrate 2-methylbutanyl acetate, ethyl hexanoate, ethyl octanoate, ethyl nonanoate, ethyl decanoate, benzyl alcohol, 2-phenylethanol and 2-phenylethyl acetate were purchased from Aldrich

Table I. Two-way analysis of variance: percentage of adult *Aethina tumida* responding to male- or female-conditioned pollen dough in wind tunnel.

Source of variation	df	SS	MS	F	P
Male-conditioned pollen					
Sex	1	24.00	24.00	0.33	0.573
Conditioning period	3	6533	2177	29.97	< 0.001
Sex x Conditioning period	3	45.33	15.11	0.21	0.889
Residual	16	1163	72.67		
Total	23	7765	337.60		
Female-conditioned pollen					
Sex	1	192.70	192.70	2.04	0.173
Conditioning period	3	4215	1405	14.85	< 0.001
Sex x Conditioning period	3	103.3	34.44	0.364	0.780
Residual	16	1515	94.67		
Total	23	6026	262.00		

(Milwaukee, Wisconsin, USA). Purities ranged from 98% to 99.5%.

2.7. Data analysis

In both the wind tunnel and cage bioassays, the data were expressed as percentages (of the total number of beetles introduced) that responded to each of the two choices. Statistical analysis was done with SigmaStat 3.1 (Systat Software, Port Richmond, California), and in all cases the data passed tests for normality and equal variance ($P < 0.05$) without transformation. Percentages of beetles captured by treated and control traps in the wind tunnel were compared for each of the eight conditioning treatments (male- or female-conditioned for 1, 3, 7, or 14 days) separately, and the significance of conditioning and sex of the responding beetles were tested by two-way ANOVA. Two-way ANOVA was also used to examine the effect of sex and duration of conditioning on the response to either male- or female-conditioned pollen dough in the wind tunnel and to examine the significance of yeast-inoculation and sex of the responding beetles in the cage bioassays. The Holm-Sidak method was used, following all ANOVA, for pairwise multiple comparisons of means.

3. RESULTS AND DISCUSSION

3.1. Wind tunnel bioassays

The results showed that in general the responses of male and female SHBs to volatiles from conditioned and unconditioned pollen dough were similar, although the response varied with the duration of conditioning. The volatiles from pollen dough conditioned for 1, 3, or 7 days by either sex of the SHB lured significantly more beetles into the traps in the wind tunnel than unconditioned pollen dough (Fig. 1). In contrast, the number of beetles lured into traps by volatiles from dough conditioned for 14 days by either males or females did not differ significantly from the controls (Fig. 1). Two-way ANOVA confirmed that the response of the beetles to the volatiles was significantly affected by the duration of conditioning in both male- and female-conditioned pollen dough, but not by the sex of the responding beetles (Tab. I). Pairwise multiple comparisons (Holm-Sidak) showed that the response (of both sexes combined) to male-conditioned pollen dough differed significantly between any two conditioning periods with the exception of 1 and 14 days. The response to female-conditioned pollen differed significantly between any two conditioning periods with the exception of 3 and 7 days. This, together with the pattern of variation displayed in Figure 1, suggests an increase in release of volatiles over time that peaks between 3 and 7 days, and then declines.

3.2. Laboratory cage bioassays

Both sexes of the SHB were more strongly attracted to yeast-inoculated pollen dough

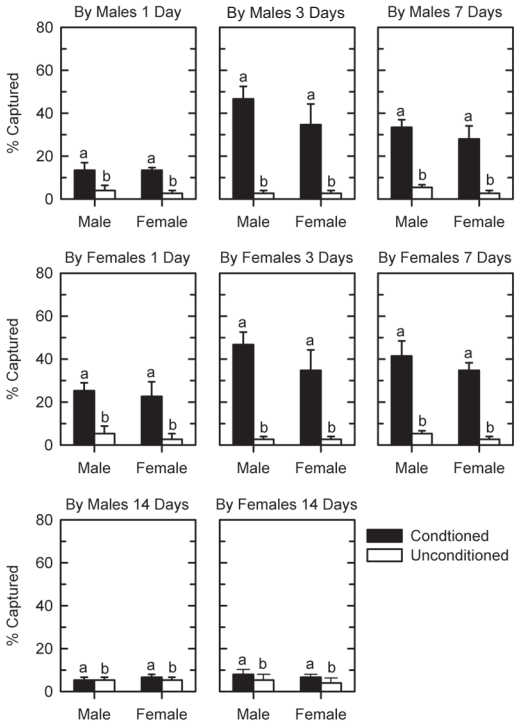

Figure 1. Wind tunnel responses of adult male and female *A. tumida* (4–8 week old) to volatiles released by unconditioned pollen dough and by pollen dough conditioned for 1, 3, 7, or 14 days by feeding of adult male and female beetles. Mean of 3 replicates (percentage captured out of 25). Error bars represent standard error. Bars with the same letter are not significantly different (two-way ANOVA, pairwise multiple comparisons by the Holm-Sidak method, $P < 0.05$). Conditioned 1 day by: males, $F_{(1,8)} = 18.6, P < 0.01$; females, $F_{(1,8)} = 22.5, P < 0.01$. Conditioned 3 days by: males, $F_{(1,8)} = 61.7, P < 0.01$; females, $F_{(1,8)} = 44.5, P < 0.01$. Conditioned 7 days by: males, $F_{(1,8)} = 53.3, P < 0.01$; females, $F_{(1,8)} = 70.3, P < 0.01$). Conditioned 14 days by: males, $F_{(1,8)} = 0.25, P = 0.63$; females, $F_{(1,8)} = 1.46, P = 0.26$.

(treatment) than to non-inoculated pollen dough (control) ($F_{(1,8)} = 137.6, P < 0.01$) (Fig. 2). There was a significant interaction between sex and treatment ($F_{(1,8)} = 8.0, P = 0.02$). Males were significantly more responsive to the yeast-inoculated pollen dough than females ($P = 0.02$), but there was no difference in the responses of males and females to non-inoculated pollen dough ($P = 0.29$) (Holm-Sidak) (Fig. 2).

3.3. Analysis of volatiles

The GC-EAD profiles of pollen dough conditioned by either sex varied with the length of the conditioning period, but no sex specific components were identified. As shown by representative profiles (Figs. 3, 4), antennae of both males and females consistently detected 10 components in volatiles from unconditioned pollen dough, and at least 20 in volatiles from pollen dough conditioned for 3 or 7 days and in the volatiles of the pollen dough inoculated with the yeast (NRRL Y-30722) (Fig. 5).

GC-MS analysis resulted in identification of several EAD-active components, mainly alcohols, aldehydes and ethyl esters, by comparison of mass spectral data with the library data of the mass spectrometer (U. S. Dept.

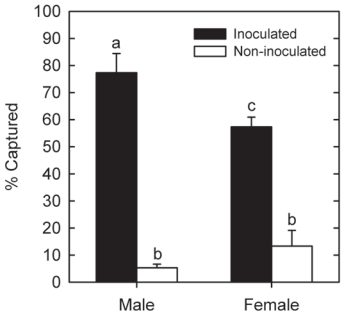

Figure 2. Cage responses of adult male and female *A. tumida* (3–4 days old) to volatiles released by pollen dough 7 days after inoculation with yeast (NRRL Y-30722) (solid bars) and by unconditioned pollen dough (open bars). Mean of 3 replicates (percentage captured out of 25). Error bars represent standard error. Bars with the same letter are not significantly different (two-way ANOVA, pairwise multiple comparisons by the Holm-Sidak method, $P < 0.05$).

of Commerce, 2005). Identification of these components was confirmed through comparison with authentic compounds by GC-EAD and GC-MS (Figs. 2, 3). Table II compares the variation in composition of some of the EAD-active compounds between the volatiles of conditioned and unconditioned pollen dough. GC-MS determined that the combined level of 3-methyl-1-butanol and 2-methyl-1-butanol in the volatiles of unconditioned pollen dough was 8%, with benzaldehyde plus α-pinene accounting for 18% of the total volatiles. When the pollen dough was conditioned for 3 days by the beetles, the combined level of 3-methyl-1-butanol and 2-methyl-1-butanol in the volatiles increased eight-fold (65%), with benzaldehyde and α-pinene disappearing in the volatiles. In addition, several ethyl esters of short chain fatty acids and acetates of some of the alcohols including 3-methyl-1-butanol and 2-methyl-1-butanol appeared as new components in the volatiles. These ethyl esters plus acetates accounted for the remaining 35% of the total volatiles. Similar results were obtained for the composition of these compounds in the volatiles of the pollen dough conditioned by either sex of the beetle for 7 days and inoculated with the yeast isolated from the beetle. Four additional EAD-active components, including acetaldehyde, ethanol, ethyl acetate and 2-methyl-1-propanol, were identified in air samples taken from the headspace of pollen dough conditioned for 3 and 7 days and inoculated with the yeast, suggesting fermentation of the dough and enzymatic oxidation and esterification of acetaldehyde, ethanol and other alcohols in the volatiles. Each of these four fermentation components elicited a response from male and female antennae when 1 μL of synthetic samples, prepared in dichloromethane at concentrations of 100 ng/μL, were analyzed by GC-EAD. These components eluted with the solvent peak, except for acetaldehyde, which eluted before the peak. Most of these EAD-active components were not detected in volatiles from dough conditioned for 14 days, although benzyl alcohol, 2-phenylethanol and 2-phenylethyl acetate and fatty acid derivatives of the alcohols were identified in the volatiles. Among these components, 2-phenylethanol (which was EAD-active) accounted for ~45% of the total. The fatty acid derivatives were not EAD-active.

Results of the present study indicate that odors from a pollen-honey based diet unconditioned by beetles is only weakly attractive to SHBs. These results are consistent with those of Elzen et al. (1999) who found in field experiments that bucket traps baited with a combination of pollen and honey trapped no beetles in 48 h. On the other hand, our results also indicate that pollen dough conditioned for 3–7 days by SHB or inoculated with the yeast, releases common fermentation products that act as an effective lure for the beetle. Our ongoing work in Florida and Pennsylvania apiaries has already demonstrated the effectiveness of traps baited with either conditioned or yeast-inoculated pollen dough in detecting and monitoring the beetle (Torto et al., unpubl. data; Arbogast et al., unpubl. data).

Our results also suggest that the presence of acetaldehyde, ethanol, ethyl acetate and ethyl esters of short chain fatty acids in the conditioned and yeast inoculated pollen dough may be necessary for attraction of SHBs. This suggestion is supported by bioassays. Volatiles from pollen dough conditioned for 3 to 7 days, which include these components, attracted

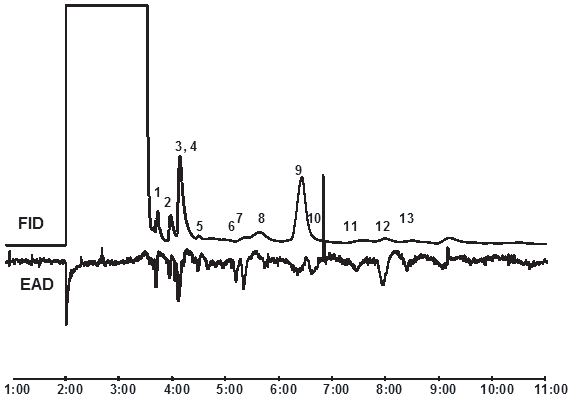

Figure 3. Representative GC-EAD profile using male or female antennae of *A. tumida* responding to compounds in the Super Q extract of unconditioned pollen dough. 1: unidentified, 2: ethyl propionate, 3: 3-methyl-1-butanol, 4: 2-methyl-1-butanol, 5: hexanal, 6: ethyl butyrate, 7: butyrolactone*, 8: 2-heptanone, 9: benzaldehyde + α-pinene, 10: 6-methyl-5-hepten-2-one, 11: 3,5-octadien-2-one*, 12: 3,5-octadien-2-one isomer*, 13: nonanal. * Identified by comparison of mass spectral data with library data only.

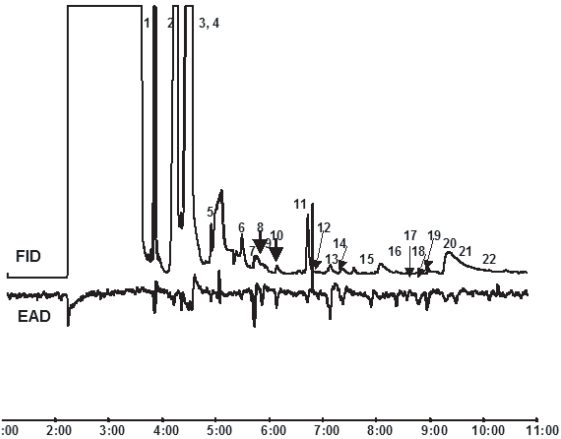

Figure 4. Representative GC-EAD profile using male or female antennae of *A. tumida* responding to compounds in the Super Q extract of conditioned pollen dough for 3 days by adult males (above) and females (below). 1: unidentified, 2: ethyl propionate, 3: 3-methyl-1-butanol, 4: 2-methyl-1-butanol, 5: ethyl 2-methylpropionate, 6: ethyl butyrate, 7: unidentified, 8: ethyl 2-methylbutyrate, 9: ethyl 3-methylbutyrate, 10: unidentified, 11: isopentyl actetate, 12: ethyl pentanoate, 13: 3-octanone, 14: ethyl hexanoate, 15: 2-nonanone, 16: nonanal, 17: ethyl octanoate, 18: decanal, 19: ethyl nonanoate, 20: 2-phenylethanol, 21: unidentified, 22: ethyl decanoate.

significantly more beetles than volatiles from pollen dough conditioned for 14 days, which did not contain these low molecular weight compounds. Thus the lower attractiveness of the pollen dough conditioned for 14 days is suggestive of the disappearance of attractant and the appearance of repellent compounds in the volatiles, and possibly an antagonistic interaction between the yeast associated with the SHB and a different microorganism. Interestingly, acetaldehyde, ethanol, ethyl acetate and several of these ethyl esters have been identified in volatiles released by microbial cultures, and these volatiles either act alone as kairomones or synergize the activity of pheromones in various nitidulid species

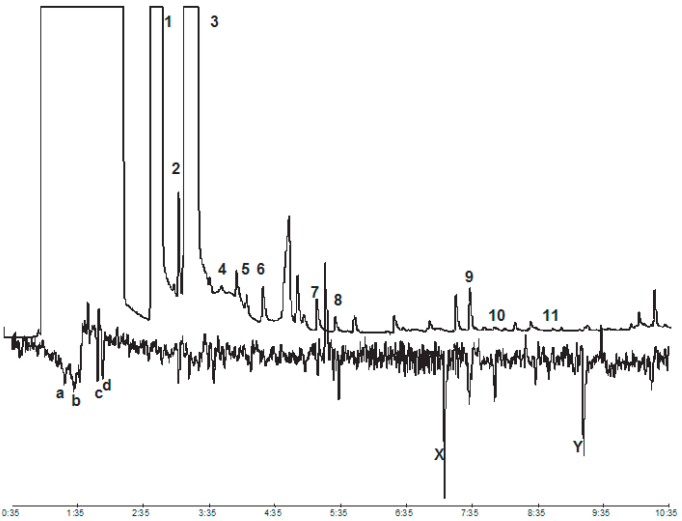

Figure 5. Representative GC-EAD profile using male antennae of *A. tumida* responding to compounds in the Super Q extract of pollen dough inoculated with the yeast (NRRL Y-30722) for 7 days. 1: unidentified, 2: ethyl propionate, 3: 3-methyl-1-butanol, 4: 2-methyl-1-butanol, 5: ethyl 2-methylpropionate, 6: ethyl butyrate, 7: ethyl 2-methylbutyrate, 8: ethyl 3-methylbutyrate, 9: isopentyl actetate, 10: ethyl pentanoate, 11: ethyl hexanoate. Components eluting with solvent identified as a:- ethanol, b: unidentified, c: ethyl acetate, d: 2-methyl-1-propanol. X and Y are electrical spikes.

Table II. Composition of some EAD-active components in the volatiles of conditioned pollen dough for 1, 3, 7 and 14 days[a] by adult *Aethina tumida*.

		Conditioned (days)							
Peak #	Compound	1 male	1 fem.	3 male	3 fem.	7 male	7 fem.	14 male	14 fem.
1	Unidentified	15.2	16.2	20.9	26.4	14.9	15.1	5.2	1.8
3 + 4	3-Methyl-1-butanol + 2-Methyl-1-butanol	25.9	39.3	64.9	65.4	68.5	68.1	0.9	0.7
6	Ethyl butyrate	1.1	0.6	1.7	1.0	2.2	4.1		
11	Isopentyl acetate			1.4	0.3	1.9	0.8		
13	3-Octanone			0.6	0.6	0.3	0.8		
14	Ethyl hexanoate			0.4	0.4	0.4	0.9		
20	2-Phenylethanol							53.8	36.9

[a] In order of increasing retention times.

(Smilanick et al., 1978; Lin and Phelan, 1991; Phelan and Lin, 1991; Bartelt et al., 1993; James et al., 1996; Nout and Bartelt, 1998; Bartelt and Zilkowski, 1998; Zilkowski et al., 1999; Teal et al., 2006).

Strikingly, 2-phenylethanol, also a fermentation product (Zilkowski et al., 1999, Zhu et al., 2003), is the major component of volatiles released by pollen dough conditioned for 14 days. In preliminary studies using solid phase microextraction (SPME) techniques, we traced the source of 2-phenylethanol to the frass of the beetles. This suggests that the relatively large proportion of 2-phenylethanol in volatiles released by pollen dough conditioned for 14 days is due to frass buildup, resulting in a lower attractiveness of the pollen dough. The residual pollen dough enriched with frass volatiles might explain why in the field hives that are badly infested with SHBs, particularly larvae of the beetle, are often abandoned by both bees and adults of the beetles.

In summary, this study indicates that when SHBs, regardless of age, feed on a mixture

of pollen and honey, volatiles that attract hive beetles are released, and that the release of these volatiles are due to fermentation by microorganisms including the yeast *Kodamea ohmeri*, previously isolated from the beetle acting on pollen (Teal et al., 2006). Research into the identities of behaviorally-active components in the volatiles could provide methods for developing synthetic lures as an alternative to the use of the yeast that can be exploited for management of the beetle.

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La composition des substances volatiles issues de la pâte de pollen fermentée et son attractivité pour le Petit coléoptère des ruches, *Aethina tumida*, parasite de l'Abeille domestique, *Apis mellifera*.

***Aethina tumida* / *Apis mellifera* / substance volatile / produit de nourrissement / mélange pollen miel / attractivité / électrophysiologie**

Zusammenfassung – Die Zusammensetzung von Duftstoffen aus fermentiertem Pollenteig und deren Attraktivität auf den Kleinen Beutenkäfer, *Aethina tumida*, einem Parasiten der Honigbiene *Apis mellifera*. Der Kleine Beutenkäfer, *Aethina tumida* Murray (Coleoptera: Nitidulidae), ein Parasit der europäischen Honigbiene (*Apis mellifera* L.) in den USA, reagiert auf verschiedene chemische Substanzen aus dem Bienenvolk wie z.B. auf flüchtige Komponenten der Bienen, Pollen, unreifen Honig und Nebenprodukte wie Bienenwachs-Trester. Vor kurzem konnte in Windtunneltests gezeigt werden, dass ein Gemisch von 8 volatilen Komponenten der Arbeitsbiene, bestehend aus Isopentylacetat, 2-Heptanon, Octanal, Hexylacetat, Nonanal, 2-Nonanon, Methylbenzoat und Decanal, eine schwach attraktive Wirkung auf Käfer hat. In Fallentests reagieren die Käfer stärker auf Duftstoffe von frisch gesammelten Pollen als auf kommerziell vertriebenen Pollen für Paketbienen. Es ist aber unbekannt, ob der Kleine Beutenkäfer auf ein Futtergemisch reagiert, dass aus Pollen, Pollenersatzstoffen und Honig besteht ("Pollenteig") und dessen Zusammensetzung zusätzlich durch fressende Käfer verändert wird. In dieser Arbeit untersuchten wir, ob ein solcher Pollenteig als Köder für den Kleinen Beutenkäfer verwendet werden kann.

Die Reaktionen der Käfer auf die Duftstoffe von unverändertem Pollenteig sollten verglichen werden mit (a) Duftstoffen aus Pollenteig, der durch fressende Käfer verändert wurde und (b) Duftstoffen aus Pollenteig, der mit Hefen, die von Käfern isoliert wurden, beimpft wurde (NRRL Y-30722). Darüber hinaus sollten die elektrophysiologisch aktiven Substanzen identifiziert werden (c).

Duftstoffe von Pollenteig, an dem 3 bzw. 7 Tage lang 100 adulte unverpaarte Käfer beiderlei Geschlechts (4–6 Wochen alt) gefressen hatten, zeigten im Windtunnelversuch die höchste Attraktivität und lockte signifikant mehr Käfer in die Fallen als Duftstoffe von unverändertem Pollenteig. Dagegen waren Fallen mit Duftstoffen von Pollenteig, an dem die Käfer nur für einen Tag gefressen hatten, nur wenig attraktiv. Pollenteig, an dem die Käfer für 14 Tage gefressen hatten, war am wenigsten attraktiv und unterschied sich nicht von dem unbehandelten Kontrollteig. Es gab keine signifikanten Unterschiede in der Reaktion zwischen Männchen und Weibchen.

In Käfigtests konnten 3–4 Tage alte unverpaarte und nicht gefütterte Käfer wählen zwischen unbehandeltem Pollenteig und Pollenteig, der mit Hefe beimpft wurde. Der „kontaminierte" Pollenteig war im Käfigtest ähnlich attraktiv wie der Futterteig im Windtunnelversuch, an dem zuvor 3 bzw. 7 Tage lang andere Käfer gefressen hatten. Allerdings wurden im Käfigtest signifikant mehr Männchen als Weibchen in die Fallen gelockt.

Die chemischen Analysen ergaben höhere Anteile an Fermentationsprodukten (kurzkettige gesättigte Alkohole und entsprechende Ethylester) in den Duftstoffen der attraktiven Pollenteige im Vergleich zu den im Biotest unattraktiven Pollenteigen.

***Aethina tumida* / *Apis mellifera* / Pollenteig / Duftstoffe / Elektrophysiologie**

REFERENCES

Bartelt R.J., Seaton K.L., Dowd P.F. (1993) Aggregation pheromone of *Carpophilus antiquus* (Coleoptera: Nitidulidae) and kairomonal use of *C. lugubris* pheromone by *C. antiquus*, J. Chem. Ecol. 10, 2203–2216.

Bartelt R.J., Zilkowski B.W. (1998) A versatile and quantitative volatile-delivery system for laboratory bioassays and examples of use with host-related attractants for *Carpophilus humeralis*, J. Chem. Ecol. 24, 535–558.

Charpentier R., Weibull J. (1994) Secondary sexual differences of pollen beetle *Meligethes aeneus* Fabr. (Coleoptera:Nitidulidae) pupae, J. Appl. Entomol. 118, 100–102.

Elzen P.J., Baxter J.R., Westervelt D., Randall C., Delaplane K.S., Cutts L., Wilson W.T. (1999)

Field control and biology studies of a new pest species, *Aethina tumida* Murray (Coleoptera, Nitidulidae), attacking European honeybees in the western hemisphere, Apidologie 31, 361–366.

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

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

James D.G., Bartelt R.J., Moore C.J. (1996) Trap design effect on capture of *Carpophilus* spp. (Coleoptera: Nitidulidae) using synthetic aggregation pheromone and a coattractant, Ecol. Behav. 89, 648–653.

Lin H., Phelan L. (1991) Identification of food volatiles attractive to dusky sap beetle, *Carpophilus lugubris* (Coleoptera: Nitidulidae), J. Chem. Ecol. 17, 1273–1286.

Nout M.R., Bartelt R.J. (1998) Attraction of a flying nitidulid (*Carpophilus humeralis*) to volatiles produced by yeasts grown on sweet corn and a corn-based medium, J. Chem. Ecol. 24, 1217–1239.

Phelan L., Lin H. (1991) Chemical characterization of fruit and fungal volatiles attractive to dried-fruit beetle, *Carpophilus hemiptera* (Coleoptera: Nitidulidae), J. Chem. Ecol. 17, 1253–1272.

Smilanick J.M., Ehler L.E., Birch M.C. (1978) Attraction of *Carpophilus* spp. (Coleoptera: Nitidulidae) to volatile compounds present in figs, J. Chem. Ecol. 4, 701–707.

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.

Teal P.E.A, Torto B., Boucias D., Tumlinson J.H. (2006) In-hive trap and attractant composition for control of the small hive beetle, *Aethina tumida*, US Patent: 20060141904.

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

U. S. Department of Commerce (2005) NIST/EPA/NIH mass spectral library (NIST05), J. Wiley, New York, [online] http://www.nist.gov/srd/nist1a.htm (accessed on 3 May 2007).

Zhu J., Park K., Baker T.C. (2003) Identification of odors from overripe mango that attract vinegar flies, *Drosophila melanogaster*, J. Chem. Ecol. 29, 899–909.

Zilkowski B.W., Bartelt R.J., Blumberg D., James D.G., Weaver D.K. (1999) Identification of host-related volatiles attractive to pineapple beetle *Carpophilus humeralis*, J. Chem. Ecol. 25, 229–252.