The importance of carcass volatiles as attractants for the hide beetle

*Dermestes maculatus* (De Geer)

C. von Hoermann a,*, J. Ruther b, S. Reibe c, B. Madea c, M. Ayasse a

a Institute of Experimental Ecology (Biology III), Ulm University, Albert-Einstein-Allee 11, 89069 Ulm, Germany
b Institute of Zoology, University of Regensburg, Universitätsstraße 31, 93053 Regensburg, Germany
c Institut für Rechtsmedizin, University of Bonn, Stiftsplatz 12, 53111 Bonn, Germany

**ARTICLE INFO**

**ABSTRACT**

A decaying cadaver emits volatile organic compounds that are used by necrophilous and necrophagous insects in order to find their brood substrate. Although volatile organic compounds (VOCs) that are released by carcasses have been identified, little is known about the specific compounds that are used by these insects while searching for a brood substrate. Therefore, we have investigated the chemical ecology involved in the attraction of the necrophagous hide beetle *Dermestes maculatus*, which feeds as an adult and larva upon decomposing carcasses. Our aims have been to identify the responsible compounds in the odours of the carcass that are important for the attraction of the beetles. Furthermore, we have studied sex- and age-related differences in beetle attraction and tested whether the hide beetle can distinguish between various stages of decomposition by means of the emitted odours. Headspace collection of volatiles released from piglet carcasses (bloated stage, post-bloating stage, advanced decay and dry remains), coupled gas chromatography-mass spectrometry (GC–MS), gas chromatography with electroantennographic detection (GC–EAD) and bioassays were conducted to identify the volatiles responsible for the attraction of the beetles. Freshly emerged male beetles were attracted by the odour of piglets in the post-bloating stage (9 days after death; Tmean = 27 °C) and the EAD-active compound benzyl butyrate. Statistical analysis revealed a higher relative proportion of benzyl butyrate in the odour bouquet of the post-bloating stage in comparison with the other stages. We therefore conclude that this compound plays an important role in the attraction of hide beetles to carcass odour. This underlines the potential use of *D. maculatus* for the estimation of the post mortem interval. The decomposition stage at which the female beetles are attracted to the odour of a cadaver remains unknown, as does the nature of this attraction. Pheromones (sexual or aggregation pheromones) might play an essential role correlated with their attraction to carrion and consequently with their attraction to the substrate for mating and ovipositioning.

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1. Introduction

Decaying cadavers are usually visited by species-rich insect communities [1]. The females of calliphorids (Diptera: Calliphoridae) visit cadavers and lay their eggs at ideal temperature and humidity conditions as early as a few hours after death [2], whereas dried-out carcasses are colonised at a later point of time in high abundance by species of the taxon Dermestidae (Coleoptera: Dermestidae) [3]. During the course of cadaver decomposition, the emitted volatile organic compounds (VOCs) are probably responsible for the attraction of cadaver-associated insects [4] and therefore have the potential to be used for answering forensic entomological questions, for instance regarding the post mortem interval. Because little is known about the attraction of necrophagous beetles to a carcass by means of olfactory cues, we have concentrated, in our study, on the hide beetle *Dermestes maculatus*, which feeds as an adult and larva on decomposing carcasses [5].

The females of this storage-pest insect can lay up to 800 eggs [6] and the hatched and characteristic bristled larvae feed preferably on the dried-out cadaver skin [7]. The developmental period of the adult insect fluctuates, depending on temperature, between approximately 90 days on average at 20 °C and approximately 36 days on average at 35 °C [8]; the arrival of adult beetles at the carcass has been shown to be predictable between 5 and 11 days post mortem in mesophytic and xerophytic habitats in Hawaii and other countries of the world [9].

Male and female beetles form mating and feeding aggregations on the cadaver [10]. Only the males possess a subepidermal exocrine pheromone gland and its development and subsequently the capability of pheromone emission is paralleled by the gonad
development which lasts about 2–3 weeks [11]. Fatty acid esters released by the males play a role in the communication system of D. maculatus. In particular, lower-boiling-point unsaturated esters elicit high olfactory receptor potentials and aggregation reactions in both sexes of the hide beetle [12,13]. However, whether these male specific aggregation pheromones also play a role in the attraction of female beetles to the cadaver remains unknown.

The aim of our study has been to investigate possible sex-related and age-related differences in the attraction of the hide beetle during cadaver decomposition. Furthermore, we have studied which volatile organic compounds of decaying piglet cadavers have a function as attractants for D. maculatus and how changes in the emitted odour bouquet during the decomposition process of a cadaver trigger attraction of the hide beetle.

2. Materials and methods

2.1. Test animals

2.1.1. Piglet carcasses

Seven piglet carcasses (Sus domesticus) were exposed on April 1–25, 2009 in plastic boxes (50 cm × 40 cm × 30 cm) at the Institut für Rechtsmedizin (University Bonn, Germany) and covered with gauze to prevent the access of blowflies. These carcasses were used for headspace volatile collection. Half-hour logging of the ambient temperature was performed with an ebro EBI-6 Data Logger (ebro Electronic GmbH, Germany). Two out of seven carcasses were in the dry-remains stage after having been exposed in an incubator at 25 °C from the end of January 2009 until the beginning of April. The carcasses were placed overnight beside a radiator in a closed room and, during the daytime, outdoors on the roof of the Institute for Legal Medicine.

2.1.2. Rearing of dermestid beetles

Rearing of the dermestid species D. maculatus for GC–EAD analysis and bioassays took place at the Institute of Experimental Ecology (University of Ulm, Germany). The breeding stock was kindly provided by Carsten Kopleck from the Zoological Museum Alexander Koenig (ZFMK, Bonn, Germany). The beetles were reared in a climate chamber in constant darkness under the following rearing conditions: rearing temperature: approximately 28 °C; humidity: approximately 80%; food: IAMS Kitten cat food (order from: www.1a-zoo.de) plus water supply (water-moistened sponge or Aqua-Gel (Bauer Handels GmbH, Adetswil, Switzerland)); rearing boxes: bowls made of plastic material (30 cm × 30 cm × 15 cm). The IAMS cat food served as the ground substrate and food supply of approximately 3 cm thickness. Pupae were removed from the rearing substrate and subsequently reared until eclosion in plastic boxes furnished with dry tissue paper.

For bioassays with freshly emerged hide beetles, freshly eclosed males and females were sex-segregated before mating by means of separate rearing boxes and tested 24 h after hatching. For bioassays with previously mated males and females, repeatedly mated males of 2–3 weeks of age with differentiated glands and gonads [11] and repeatedly mated females of 2–3 weeks of age were used.

2.2. Headspace volatile collection

Headspace volatile samples were collected between April 1–25, 2009 from seven piglet carcasses at four defined decomposition stages: bloated stage (days 2–5 after death); post-bloating stage (days 5–12 after death); advanced decay (days 17–28 after death) and dry remains (days 71–91 after death). Two different piglets were used for each decomposition stage (except for advanced decay). For volatile collection, the piglets were hermetically packed into commercial oven bags (Toppits®; 3 m × 31 cm extra broad). Only the bloated cadavers were packed in larger oven bags (LOOK® Ovens Bags, 45 cm × 55 cm, order from: www.cookability.biz) because of their increment in volume. Incoming air (100 ml/min) was sucked through a charcoal filter (30 mg, Supelco, Orbo 32 large) for cleaning purposes by using a membrane vacuum pump (DC12, FURGUT, Aichstetten, Germany) and subsequently passed through the oven bag with the cadaver inside. Outgoing air of the oven bag passed through an adsorbent tube for 4 h and carcass volatiles were collected in 5 mg Porapak® Q (Waters Division of Millipore, Milford, MA, USA) adsorbent material. Flow was controlled with a E29-C-150 MM2 sinter flowmeter (Air Products and Chemicals, Netherlands). As a control, we collected the volatiles of the empty experimental set-up under the same conditions. Adsorbed VOCs were eluted with 3 × 50 μl pentane/acetone (9:1) (Sigma–Aldrich, Munich, Germany, HPLC grade). The sample was stored until analysis in a sealed glass ampule at ~20 °C.

2.3. Bioassays

The attractiveness towards D. maculatus of volatiles emitted by the different decomposition stages (see above) of the piglet carcasses was tested in a double-Y-labyrinth olfactometer (Table 1). Furthermore, we tested the attractiveness of electrophysiologically active synthetic carcass volatiles at the same concentration as that in the attractive headspace sample of a post-bloated piglet cadaver (Fig. 2 and Table 1). The labyrinth consisted of a block of plexiglass (9 cm × 7 cm × 1 cm) with four outwardly oriented semicircular channels (0.5 cm deep) that were milled into the lower surface of the block in the shape of a double-Y (Fig. 1). This setup, which was originally designed for a study on ants, was used because it simulates perfectly the narrow and non-transparent feeding pathways through the cadaver [3]. In addition, the rough surface of a sheet of paper that was placed below the milled channels simulated the dried-out and rough cadaver tissue. This setup constrained the beetles to walk on the rough paper surface inside the predetermined channels. Preliminary tests with Y-tube olfactometers made of glass showed that the smooth and transparent walls were not accepted by the tested hide beetles (V. Hoermann, personal observation). Beetles had access to the labyrinth via a round entry arena. A sheet of paper was placed below the labyrinth.

Table 1

<table>
<thead>
<tr>
<th>Hide beetles tested</th>
<th>Dual-choice experiments</th>
<th>Synthetic copies of electrophysiologically active compounds against solvent controls (in 20 μl pentane test solvent) analogous to the attractive headspace sample of a post-bloated piglet cadaver</th>
</tr>
</thead>
<tbody>
<tr>
<td>Freshly emerged males</td>
<td>Bloated stagea against control Post-bloating stageb against control Advanced-decay stageb against control Dry remainsstageb against control</td>
<td>Ethyl butyrate (0.3446 μg/ml pentane) against control Isoamyl butyrate (2.0277 μg/ml pentane) against control Hexyl butyrate (0.1282 μg/ml pentane) against control 1-Octen-3-ol (0.3855 μg/ml pentane) against control Benzyl butyrate (0.025 μg/ml pentane) against control 2-Phenylethanol (0.901 μg/ml pentane) against control</td>
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<tr>
<td>Freshly emerged females</td>
<td>Bloated stagea against control Post-bloating stageb against control Advanced-decay stageb against control Dry remainsstageb against control</td>
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<tr>
<td>2–3 weeks old males</td>
<td>Bloated stagea against control Post-bloating stageb against control Advanced-decay stageb against control Dry remainsstageb against control</td>
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<td></td>
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</tbody>
</table>

a Day 9 post-mortem.
b Day 3 post-mortem.
c Day 17 post-mortem.
d Day 43 post-mortem.
HPLC-Analysentechnik, and flame-ionisation GC–EADs
labyrinth first effects. directed programme (as Fig. FSI-6478; G water). conditions 0.25 N/C2 Ringer with 0.25 ml/min). maculatus ml/min). the antenna was regularly changed to eliminate possible side-effects. Beetles were tested only once (N = 20 for each test). After every test, the labyrinth was cleaned with pure ethanol. The bioassays were performed in a climate chamber under red light at 28 °C and a relative humidity of 80%. These conditions were in accordance with the rearing conditions (Section 2.1.2).

2.4. Gas chromatography coupled with electroantennography (GC–EAD) Electrophysiological analyses were performed to identify those components of the various cadaver headspace samples that can be perceived by the male and female antennae of the hide beetles. The GC–EAD system comprised a HP 6890 Hewlett-Packard gas chromatograph (Agilent Technologies, Germany) with a flame-ionisation detector (FID) and an EAD setup (Syntexc, Hilversum, Netherlands). The antenna was dissected at the base by using micro-scissors and the tip of the terminal antennal segment was removed with a razor blade [14]. The antenna was subsequently mounted between two glass capillaries filled with insect Ringer solution (5 g NaCl, 0.42 g KCl, 0.19 g CaCl2 ad 1000 ml demineralised water). In order to record the FID and EAD responses simultaneously, the GC effluent was split (split ratio FID:EAD = 1:1) under a make-up gas supply (nitrogen, 30 ml/min). The effluent was humidified with a cleaned airflow of 100 ml/min and directed to the antennal preparation via a glass tube. Headspace samples were analysed on a polar GC capillary column (30 m = 0.25 mm, DB-Wax, film thickness 0.25 μm, Scientific, Folson, CA, USA). Hydrogen was used as a carrier gas (2 ml/min constant flow). One microlitre of the sample was injected in splitless mode (1 min) at an initial temperature of 40 °C. The oven temperature was increased at a rate of 10 °C/min to a final temperature of 230 °C (hold time: 26 min). In addition to the GC–EAD with headspace samples, we also measured the antennal responses to synthetic reference chemicals of the identified EAD-active compounds. The analysis of the synthetic chemicals was performed on a non-polar GC column (30 m = 0.25 mm, DB-5 ms, film thickness 0.25 μm, J&W). The temperature programme started at 50 °C and the oven was subsequently heated at 10 °C/min to a final temperature of 310 °C (hold time: 20 min). We performed 15 runs per headspace sample of each type of decomposition stage (4 × 15 runs) and a total of 77 runs with synthetic references of 7 identified EAD-active substances (ethyl butyrate, isobutyl butyrate, isobutyl hexanoate, hexyl butyrate, 1-octen-3-ol, benzyl butyrate and 2-phenylethanol). Compounds were termed "physiologically active" when they showed an EAD response in at least 4 of the runs at the same retention time.

2.5. Chemical analysis

2.5.1. Mass spectrometry (GC–MS) For structure elucidation, headspace samples were analysed by GC–MS on a Fisons 8060 GC (Fisons Instruments) equipped with a polar DB-Wax capillary column (30 m × 0.32 mm ID × 0.25 μm film thickness, J&W) operated in splitless mode (injector temperature: 240 °C) and coupled to a Fisons MD800 quadrupole MS running in the electron impact (EI) mode at 70 eV. Helium was used as the carrier gas at a head pressure of 5 kPa. The initial oven temperature was 40 °C; this was increased at 2 °C/min to 240 °C and held for 30 min. Identifications were performed by a comparison of the retention data and mass spectra with those of authentic reference compounds.

2.5.2. Gas chromatographic analysis (GC) For the quantification of carcass volatiles in the headspace samples, these were additionally analysed on a Thermo Trace GC 2000 (Thermo Finnigan, Germany) with hydrogen (2 ml/min) as the carrier gas. The GC was equipped with a polar DB-Wax column (analytical conditions as described above). To each sample, 1 µg tridecane was added as an internal standard. Quantification was performed by using Chrom-Card 2.2 chromatography software (Thermo Scientific, Massachusetts).

2.6. Statistical analysis

The Chi²-test was used for an evaluation of the results of choice experiments in the double-Y-labyrinth bioassays. For the comparison of odour bouquets from piglet carcasses at various stages, a principal component analysis (PCA) was performed by considering the relative amounts of 13 EAD-active compounds. The resulting five principal components (PCs) with Eigenvalues above one were used to perform a discriminant function analysis (DFA). The standardised discriminant function coefficients and the factor loadings after varimax rotation were used to assess the importance of individual compounds. A compound was considered to have a high factor loading when the loading was above 0.6. Finally, the relative proportions of the individual compounds were compared between the four tested decomposition stages by using a Mann–Whitney U-test with a p-value correction according to Benjamin and Hochberg [15]. All statistical analyses were performed with SPSS (Version 13.0, SPSS GmbH Software, Germany).

3. Results

3.1. Bioassays

In all the bioassays with both sexes and age groups of beetles (freshly emerged unmated males/virgin females and 2–3-week-old males/females) towards diluted headspace samples of the dry-reman stage, the bloated and the advanced decay stage, the samples were no more attractive to the hide beetles than the controls (chi-square tests; P > 0.05). However, if the choice experiments were conducted with freshly emerged males, the odour from the piglet carcasses in the post-bloating stage were significantly more attractive than the solvent control (chi-square test; Chi² = 22.500; FG = 1; n = 20; P < 0.001, Fig. 2). In a control experiment in which we tested pentane against pure air, no side preference indicated that our solvent did not influence the behaviour of the beetles (Fisher exact test; n = 9; P = 1.000, Fig. 2). In contrast to freshly emerged male beetles, freshly emerged females (chi-square test; Chi² = 0.1000; FG = 1; n = 20; P = 0.752, Fig. 2) and 2–3-week-old males (chi-square test; Chi² = 0.100; FG = 1; n = 20; P = 0.752) and 2–3-week-old female hide beetles (chi-square test; Chi² = 0.100; FG = 1; n = 20; P = 0.752, Fig. 2) showed no preference for the odour of piglet carcasses at the post-bloating stage.

In choice experiment performed with freshly emerged male hide beetles, a synthetic reference of the EAD-active compound benzyl butyrate was significantly more attractive than the control (chi-square test; Chi² = 4.900; FG = 1; n = 20; P = 0.027, Fig. 2).

Fig. 1. Schematic view of a double-Y-labyrinth. Only the first choice of beetles at the first Y-branching after the entrance (marked with a small circle) was recorded. (as described above) and one defined arm of the first Y after the entrance was impregnated with 20 μl of a test solution by using a micro syringe (100 μl, Gehrer HPLC-Analysentechnik, Chemnitz, Germany), whereas the remaining arm was treated with the solvent only and used as an empty control. Beetles were placed into the entry arena and the exit of the arena was opened after 1 min by rotation of an inserted plexiglass cylinder. We only recorded the first choice of the beetles at the first Y-branching after the entrance (Fig. 1). The side for the marked arm of the test-Y through the olfactometer was regularly changed to eliminate possible side-effects. Beetles were tested only once (N = 20 for each test). After every test, the labyrinth was cleaned with pure ethanol. The bioassays were performed in a climate chamber under red light at 28 °C and a relative humidity of 80%. These conditions were in accordance with the rearing conditions (Section 2.1.2).
3.2. Electrophysiology and chemical analysis

In the GC–EAD analysis of headspace samples of piglet carcasses at various decomposition stages, we registered 18 electrophysiologically active compounds. Thirteen substances could be identified by mass spectrometry. The identified compounds were ethyl butyrate, isoamyl butyrate, isobutyl hexanoate, hexyl butyrate, acetic acid, 1-octen-3-ol, propionic acid, isobutyric acid, butyric acid, hexanoic acid, benzyl butyrate, 2-phenylethanol and 2-phenylethyl butyrate (Fig. 3). All of these 13 substances could be found in headspace samples of a piglet carcass in the post-bloating stage, which was attractive for freshly emerged male hide beetles (Fig. 2).

However, during this study, only a synthetic copy of the EAD-active compound benzyl butyrate had a behaviour-modifying effect upon these freshly emerged hide beetles. In 15 GC–EAD runs with the odour of a post-bloated piglet cadaver using the antennae of hide beetles, benzyl butyrate was electrophysiologically active in 7 runs. There was no response in analyses using the other three decomposition stages, because of low concentrations (v. Hoermann, unpublished data). GC–EAD active synthetic copies of ethyl butyrate, isoamyl butyrate, hexyl butyrate, 1-octen-3-ol and 2-phenylethanol, which were additionally tested, were no more attractive than the control for freshly emerged male hide beetles in choice experiments. For the synthetic copy of isobutyl hexanoate, we

![Figure 2](image1.png)

**Fig. 2.** Response of *D. maculatus* in double-Y-labyrinth choice experiments to 1/100 pentane-diluted headspace samples of piglet carcasses in the post-bloating stage, the solvent pentane and a synthetic reference of the EAD-active compound benzyl butyrate (0.025 μg/ml pentane in 20 μl test solvent) (Chi² and fisher exact tests, *P* < 0.05, **P** < 0.001).

### Table 1

<table>
<thead>
<tr>
<th>Sample Description</th>
<th>EAD Response</th>
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</thead>
<tbody>
<tr>
<td>Freshly emerged male <em>D. maculatus</em> (n = 20)</td>
<td>Post-bloating</td>
</tr>
<tr>
<td>Freshly emerged male <em>D. maculatus</em> (n = 20)</td>
<td>Benzyl butyrate</td>
</tr>
<tr>
<td>Freshly emerged female <em>D. maculatus</em> (n = 9)</td>
<td>Pentane</td>
</tr>
<tr>
<td>2- to 3-week-old male <em>D. maculatus</em> (n = 20)</td>
<td>Post-bloating</td>
</tr>
<tr>
<td>2- to 3-week-old female <em>D. maculatus</em> (n = 20)</td>
<td>Post-bloating</td>
</tr>
</tbody>
</table>

![Figure 3](image2.png)

**Fig. 3.** GC–EAD active compounds in a headspace sample of a piglet carcass in the post-bloating stage (day 9 after death). EAD analysis was performed with the antenna of a male hide beetle. Only reproducible peaks were marked as EAD-active. In the present chromatogram, not all EAD-active compounds elicited an EAD-response. Acetic acid + 1-octen-3-ol, propionic acid, isobutyric acid, butyric acid, benzyl butyrate and an unknown substance elicited EAD-responses in the displayed chromatogram.
The dry-remains stage was significantly separated from the post-bloating stage (Fig. 4). DF2 significantly separated the advanced decay stage from the three remaining stages, whereas DF3 significantly separated the bloated stage from the three remaining stages (Fig. 4). The compounds that mainly contributed to the separation of the dry-remains stage and the post-bloating stage were benzyl butyrate, 2-phenylethanol, 2-phenylethyl butyrate, acetic acid + 1-octen-3-ol and three non-identified EAD-active compounds. Benzyl butyrate was found in the headspace samples of bloated piglet carcasses in significant lower relative amounts than in headspace samples of carcasses in the post-bloating stage (Mann–Whitney U-test, \( P < 0.01 \), Fig. 5). Again, the butyric acid ester was produced in smaller amounts in the advanced decay and dry-remains stages (Fig. 5).

4. Discussion

4.1. Which volatile organic compounds (VOCs) of decaying piglet cadavers have a function in the attraction of \( D. \) maculatus?

To date, numerous volatile organic compounds within the headspace of decomposing pig carcasses (\( S. \) domesticus L.) [16] and also of human corpses have been identified in several studies [4,17]. All of these investigations demonstrate the release of large amounts of VOCs during the decomposition process of vertebrate cadavers. The main components reported are acids, aromatic hydrocarbons, oxygenated compounds, sulphur and nitrogen compounds [16]. However, only a few compounds have been shown to be attractive to storage pest insects [5].

We have been able to identify 13 EAD-active compounds among the headspace volatiles of piglet carcasses at the post-bloating stage by GC–EAD and GC–MS analysis. Five out of 13 are butyrates and except for isovaleryl butyrate, all butyrates are solely detected in the post-bloating stage odour by the antennae of hide beetles. Thus, there is an indication that the antennal responses to butyrates are dose dependent. For instance, in the carrion beetles \( Nicrophorus \) vespillo and \( Nicrophorus \) vespilloides electroantennogram (EAG)
experiments with various concentrations of synthetic sulphur-containing volatile organic compounds (S-VOCs) were conducted [18]. The antennal responses were dose dependent. Therefore, in D. maculatus electrophysiological analyses (EAG) with different concentrations of butyrate should be performed to support our finding that low concentrations of certain of the compounds have been responsible for the missing EAD responses.

In the bioassay, freshly emerged male hide beetles are attracted exclusively by the synthetic benzyl butyrate. Therefore, we consider this compound to be an important component of the carcass odour bouquet contributing significantly to the attraction of hide beetles. This conclusion is supported by a forensic entomological case study from Argentina reporting the presence of Dermestes sp. and the red-legged ham beetle Necrobia rufipes on carcasses as indicators of the beginning of butyric acid fermentation [19]. Thereby, glucose is metabolised into butyric acid, acetic acid, hydrogen and carbon dioxide as final products of the fermentation process of the heterotrophic and anaerobic bacterium Clostridium butyricum [20].

Hence, our data suggest that D. maculatus functions as an indicator species for the beginning of butyric acid fermentation, especially for the release of benzyl butyrate. However, because only the synthetic reference of benzyl butyrate has a behaviour-modifying effect upon the hide beetle, we still have to perform further bioassays with synthetic references of the remaining (not tested in this study) EAD-active compounds, both individually and in combination with the proven attractant benzyl butyrate.

4.2. Are there sex-specific differences in the attraction of beetles?

Insect visitors of cadavers should use reliable chemical cues that help them to find its ephemeral, patchily distributed and rare food substrate. These cues additionally provide them with information about the decomposition process of a cadaver in order to avoid competition [1]. Niche capturing in various microhabitats might be different with regard to the diverse development stages and the two sexes.

In order to examine such a difference in sex and developmental stage regarding niche capturing, we performed bioassays with hide beetles of different sex and age. We could show that only freshly emerged male beetles responded in the bioassay. However, we could not clarify, in this study, the reason that neither freshly emerged nor 2–3-week-old females were attracted by the various carcass odour bouquets. A possible explanation might be that male-derived sex or aggregation pheromones might interact with carcass volatiles to attract female hide beetles to the oviposition substrate. This would imply that freshly emerged male beetles with immature gonads have to stay at least 2–3 weeks on a detected cadaver before they can attract receptive females using a combination of cadaver odour and pheromones. The latter are produced only by developed glands, 2–3 weeks after eclosion [11].

For instance, male beetles of the genus Nicrophorus (Silphidae: Nicrophorinae) are known to attract female beetles with a pheromone that they either release at a cadaver that is suitable for reproduction or independently of the presence of carrion [21,22]. Thus, the previously mentioned male isopropyl esters of the hide beetle are possibly sex pheromones used by males to attract females to an attractive oviposition site. Opportunistic males might also respond to the chemical signal of their competitors resulting in mixed sex aggregations. Such behaviour is known, for example, in the rove beetle Aleochara curtula (Coleoptera: Staphylinidae), in which male-pheromone-responding males probably exploit the emitting males’ signal for increased mating probabilities [23]. Further laboratory and field studies addressing the putative interaction between isopropyl esters and carcass odour are necessary to test this hypothesis. These results might be helpful in the development of a method for the calculation of post-mortem intervals (PMI) of previously skeletonised corpses, by using dermestid beetles as indicator organisms.

4.3. Does the composition of carcass volatile profiles depend on the time after death?

Statheropoulos et al. [4] have found changes of decomposition odour composition during the exposure time of human corpses. Moreover, the produced and released VOCs during the course of decomposition are known to attract many cadaver insects [4].

In order to assign this knowledge to the cadaver insect D. maculatus, we have performed a discriminant function analysis that enables a significant separation of the dry-remains stage from the post-bloating stage. The electrophysiologically active compound benzyl butyrate is significantly involved in this separation. Furthermore, the relative proportion of benzyl butyrate changes significantly during the course of cadaver decomposition (Fig. 5) and might be responsible for the observed attraction of freshly emerged hide beetles to carcasses in the post-bloating stage, because the emission of this compound is maximised at this stage (Figs. 2 and 5). The results clearly show that adult hide beetles appear considerably earlier at the cadaver as initially expected, possibly mediated by the increasing release of benzyl butyrate.

Such early cadaver colonisation by hide beetles has also been reported in an earlier study [7]. Braack explains this observation by the avoidance of intraspecific competition between adults and larvae of the hide beetle: this might be enabled by the earlier abundance of adult beetles and a food preference for moist muscle tissue or ligamentous tissue remains left by blowfly maggots. The larvae feed on the carcass skin at a later time and show their highest abundance when most of the adult beetles have left the carrion.

The maximal relative proportion of benzyl butyrate in an early decomposition period (day 5 till day 12 after death; $T_{\text{mean}} = 27^\circ$C) might thus serve as an indicator for the specific successional niche of the hide beetles at the carrion. We have been able to demonstrate this for freshly emerged male beetles in the bioassay. This observation coincides with the finding that dermestid beetles appear predictably between days 5 and 11 after death on a cadaver [8].

As a result, we have been able to show that decomposition is a dynamic process in time and that significant changes in the relative amounts of specific volatiles are responsible for the niche-specific attraction of carrion beetles, especially the male hide beetle. Because the composed bouquets of food odours are responsible for the attraction of many storage-pest insects [5], we suggest that benzyl butyrate is not alone responsible for the attraction of the hide beetle but acts in combination with specific relative amounts of other identified EAD-active substances. Currently, we are investigating this assumption on the basis of further bioassays and field experiments with the remaining identified EAD-active compounds of the odour bouquet of piglet carcasses in the post-bloating stage.

5. Conclusions

In the context of forensic chemoecological studies with the hide beetle D. maculatus, we have been able to show that freshly emerged male beetles are attracted by the odour of piglet carcasses during the post-bloating stage (day 9 after death; $T_{\text{mean}} = 27^\circ$C). Our investigations show furthermore that benzyl butyrate is a key component for beetle attraction. Benzyl butyrate is emitted in various amounts in the course of decay of the cadavers and reaches its maximum in the post-bloating stage. Our electrophysiological studies have shown that this substance can be detected by the beetles; a synthetic reference chemical is also behaviourally active in our bioassays.
Since female beetles are not attracted by the odour of piglet carcasses in various stages of decay, we hypothesize that pheromones of the males in addition to the cadaver odour play a role in female attraction.

The present study presents a first simplified step in finding out the mechanisms of hide beetle attraction to the food and breeding substrate by means of cadaveric odour. However, one should keep in mind that in this study we excluded blowflies that can constitute vectors for additional and new bacteria [24] or produce volatiles by themselves [25,26]. Both aspects can be involved in volatile production. Therefore, in future investigations blowfly maggots have to be included in the experimental setup and volatiles of different decomposition stages of piglet carcasses, which are already inhabited by blowfly maggots should be collected.

Acknowledgements

We thank the Glaxo SmithKline foundation, Munich, Germany, for financial support.

We are grateful to Carsten Kopleck (ZFMK - Zoological Research Museum Koenig, Bonn, Germany) for providing us with a strain of hide beetles and to Prof. Dr. K. Peschke for helpful discussions.

References


Please cite this article in press as: C. von Hoermann, et al., The importance of carcass volatiles as attractants for the hide beetle Dermestes maculatus (De Geer), Forensic Sci. Int. (2011), doi:10.1016/j.forsciint.2011.06.009