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PAPER

PATHOLOGY/BIOLOGY

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Responses of *Lucilia sericata* Meigen (Diptera: Calliphoridae) to Cadaveric Volatile Organic Compounds*

ABSTRACT: Flies of the Calliphoridae Family are the most forensically important insects because of their abundance on the decedent during the first minutes following death. Necrophagous insects are attracted at a distance by a decomposing body, through the use of volatile chemical cues. We tested the possible attractive role of some volatile organic chemicals (VOCs) released by decaying cadavers, on male and female of *Lucilia sericata* Meigen (Diptera: Calliphoridae). Two complementary approaches were used. Electroantennography (EAG) allowed identifying the semiochemicals that are detected by the olfactory system of *L. sericata*. Dose–response tests with EAG showed that dimethyl disulfide (DMDS) and butan-1-ol elicited the highest responses. Behavioral assays showed that, among the VOCs tested, DMDS and butan-1-ol are attractive for *L. sericata*, while the other VOCs are repulsive or do not cause any behavior. Our results may have potential implications in a better understanding of attractiveness of blowflies toward a corpse.

KEYWORDS: forensic science, forensic entomology, electroantennography, olfactometer, behavior, chemical communication, volatile organic compounds

Forensic entomology is a branch of the forensic sciences, which studies insects and other arthropods (e.g., mites) in a medico-legal context (1–5). Insects are predominantly used in this discipline to determine the time of colonization which might be interpreted as the death of an organism, otherwise known as the postmortem interval. Postmortem interval is the period of time between death and corpse discovery and is based on the age of the insect present on a corpse (3,5–7). Insects can also reveal information in cases of abuse or neglect of children or elderly (5,8,9), providing information on the causes of death (5,10–14).

After death, the body is quickly visited and colonized by many invertebrates with the majority being necrophagous insects (3,5,7). Diptera and coleoptera have particular relationships with decomposing remains that constitute a rich ephemeral resource (15–17). These insects are attracted and colonize a cadaver in a relative predictable sequence called the entomofaunal succession (9,18–22). This succession takes place as different stages of decay offer different composition of nutrients that are used by specialized insect species.

The blowfly (Diptera: Calliphoridae) such as *Calliphora vomitoria* L. and *Lucilia sericata* Meigen are generally the most forensically important insects in Europe. They are indeed the most numerous insects on dead bodies and are usually the first to colonize it (3,5,7,23–25). These first cadaver colonizers are attracted by the early decomposition odor, even from long distances (several kilometers) (3,23,26–31). At such distances, visual cues probably play a limited role in attracting the insects to the body. Their attraction is therefore thought to be reliant on volatile chemical cues (25,32,33). It is speculated that the volatile organic chemicals (VOCs) released during the decompositional process attract a wide range of insects (27,34–37). Forensic entomologists often raise the question, Do the cadaveric VOCs regulate the necrophagous insects' behavior (38)? However, the relationship that may exist between cadaveric VOCs and necrophagous insects is poorly studied (39). Because of the sensitivity of their olfactory system, it is possible that insects also might be used to develop a novel method for detecting and locating chemicals associated with decomposition (40,41).

The goal of this study was to identify the semiochemicals mediating the corpse attractiveness to *L. sericata*. To undertake this study, we conducted both electrophysiological and laboratory-based behavioral experiments.

Materials and Methods

Rearing of Insects

L. sericata was selected because of its relative abundance on decaying corpses in Europe and because it is one of the first species to arrive on a dead body in this region (31). The flies were kept on a 16-h light:8-h dark photoperiod and at ±23°C. Males and females were maintained together in a rearing cage (55 cm × 60 cm × 48 cm) supplied with sucrose, dried milk, and water. Defrosted pork chop was supplied to provide a protein source. The experiments were conducted with insects aged 5–15 days.

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Chemicals

Previous studies on cadaveric VOCs have been conducted at the Department of Functional and Evolutionary Entomology (Gembloux Agro-Bio Tech, University of Liege, Liege, Belgium) where researchers identified a hundred cadaveric VOCs that are specifically released during the pig decompositional process (38). To conduct this study, we have selected the five most abundant compounds we found in our previous work (38) and that were also commonly reported as being part of a corpse's decaying odors (37,38,42–44). We also selected two other compounds reported to be important in the decay process: putrescine and cadaverine (43,44).

The chemicals tested in this study were therefore putrescine (IUPAC name: butane-1,4-diamine, Fluka (Buchs, Switzerland) 32790, purity of >99%), cadaverine (IUPAC name: pentane-1,5-diamine, Fluka 33211, purity of >97%), butan-1-ol (Sigma (St. Louis, MO) 24124, purity of >99%), butanoic acid (Fluka 19210, purity of >99.5%), indole (Fluka 57190, purity of >98.5%), dimethyl disulfide (DMDS) (IUPAC name: methyldisulfanylmethane, Fluka 40221, purity of >98%), and phenol (Fluka 77612, purity of >99%).

Electroantennography

Whole insects were covered and immobilized using plasticine, but leaving the antennae free to move. One of the antennae was mounted in Ag-AgCl glass capillary electrodes filled with saline solution (NaCl, 7.5 g/L; $CaCl_2$, 0.21 g/L; KCl, 0.35 g/L; and $NaHCO_3$, 0.2 g/L) and in contact with a silver wire. Half of the last distal antennal segment was immersed into the saline solution of the recording electrode. The second antenna was excised from the head, and the reference electrode was inserted into the head of the insect. This setup was shown to produce elegant results on house fly antennae (45).

The direct current potential was recorded on a computer (AutoSpike v. 3.2; Syntech, Hilversum, the Netherlands) using an amplifier (IDAC-4; Syntech) with 100-fold amplification. This amplification is necessary to have a sufficiently high level to drive a recording device. A 0.5-cm^2 piece of filter paper, which was impregnated with 10 μL of the tested chemical, was placed in a Pasteur pipette and used to puff an air sample in a constant flow (1.5 L/min) during 0.5 sec. The air was charcoal-filtered and humidified continuously. As a control, the working antenna was first stimulated with semiochemical-free filter paper (mechanical stimulus). Following the control stimulation, each insect was stimulated with increasing doses (0.001, 0.01, 0.1, 1, and 10 mg) of the seven chemical compounds to be tested. To determine whether previous exposure affected the response of the insect to additional treatments, an electroantennography (EAG) experience with four insects and one compound (DMDS) has been made. The orders of doses were presented in a random sequence. Finally, to compare the average EAG responses between increasing doses (0.001, 0.01, 0.1, 1, and 10 mg) and random sequence, a one-way analysis of variance (ANOVA) was conducted.

For each doses, Table 1 shows that there was no interaction between the current treatment and the previous treatment and so, prior exposure did not affect subsequent responses.

TABLE 1—*Comparisons of the average electroantennography responses between increasing doses and random sequence.*

Doses (mg)	$F_{1,22}$	p
10	0.01	0.940
1	0.17	0.686
0.1	0.47	0.498
0.01	0.03	0.871
0.001	1.34	0.259

A preliminary study using different solvents was made to choose the most adequate solvent for our EAG tests. We tested six solvents randomly: n-hexane (Merck (Darmstadt, Germany), 96%), dichloromethane (Fluka, >99.8%), diethyl ether (IUPAC name: ethoxyethane; VWR, 99.7%), n-pentane (Merck, 95%), paraffin oil, and distilled water. The solvent used for dilution was tested alone and must elicit minimal EAG responses, indistinguishable from responses to air (mechanical stimulus). This preliminary step highlighted the best performance of distilled water as solvent, which is also used by Kelling et al. (46).

Because phenol was insoluble in distilled water at the dose of 10 mg, this compound was not tested in EAG at this dose. Moreover, because indole was not soluble in distilled water at the doses of 10, 1, and 0.1 mg, it was tested only in EAG with the doses of 0.01 and 0.001 mg. In olfactometer assays, indole was tested only with a dose of 0.05 μg.

Thirty seconds separated each puff. This time is necessary to repolarize the antenna. Each insect was stimulated with all dilutions of the seven cadaveric VOCs, and the EAGs were recorded from 10 insects of both sexes.

Y-Tube Olfactometer Assays

Y-tube olfactometer (also called a two-arm olfactometer) was used to investigate the behavioral responses of *L. sericata* exposed to olfactive stimuli. The main arm and the two arms were made of Teflon® (Bohlender, Grünsfedl, Germany). The main arm was 8.5 cm long and 1.5 cm wide. The two arms of the olfactometer were 20 cm long and 1.5 cm wide. The chambers were made of glass and have a basal diameter of 16 cm and a height of 16 cm. A pump was used to pull air through the glass chambers and the Y-tube. Airflow through each of the olfactometer arm was maintained at constant rate flow (800 mL/min). White light placed above the olfactometer provided uniform lighting (1362 Lux).

Each chemical was tested alone, at the doses of 100 and 0.05 μg. The semiochemicals were applied on a 2-cm^2 piece of filter paper and randomly placed in one of the two glass chambers, while a control filter paper was placed in the other. Fifty starved insects of each sex were tested for each compound. Each individual insect was introduced into the Y-tube at the entrance of the main branch and thus had a choice between the test chemical and the control. New filter papers were used for each insect. The Y-tube was washed after each test with warm water and dichloromethane. Each insect was allowed to spend a maximum of 15 min in the olfactometer. The test was stopped when an insect made a choice. An insect was considered to have made a choice when it moved into one of the glass chambers. The measured response in each test was calculated as the number of *L. sericata* attracted by the tested compound.

Statistical Analysis

Statistical tests were performed using the statistical software Minitab® v15.0 (State College, PA) for Windows® (Windows Corporation, Redmond, WA).

The EAG responses were analyzed by a three-way ANOVA. Three analyses of variance (with factors being sex, VOCs, and doses) were conducted because phenol and indole were not tested

at all doses. When a significant difference in EAG response based on the VOCs tested was observed, a multiple comparison of the means by the method of Newman and Keuls was made. Also, a multiple comparison of the means by the method of orthogonal polynomials was made when there was a significant difference in EAG response based on the doses of the VOCs tested. These analyses sought to answer three questions: (i) is there a significant difference in perception between males and females? (ii) which products? and (iii) which concentrations are better perceived by *L. sericata*?

A chi-squared goodness-of-fit test was used to determine the significance of the differences between the numbers of *L. sericata* choosing the test compound or control arm of the olfactometer.

Results

Electroantennography

All tested cadaveric chemicals were perceived by the olfactory system of *L. sericata*. The three-way ANOVA (with factors being sex, VOCs, and doses) indicated that for each dose, the tested VOCs were perceived differently, that is, at the doses of 10, 1, 0.1, 0.01, and 0.001 mg ($F_{4,450} = 98.23$, $p < 0.001$; $F_{5,432} = 89.01$, $p < 0.001$; $F_{5,432} = 35.78$, $p < 0.001$; $F_{6,252} = 24.64$, $p < 0.001$; and $F_{6,252} = 3.41$, $p = 0.003$, respectively). The multiple mean comparisons by the Newman and Keuls test are shown in Table 2. This comparison showed that DMDS and butan-1-ol induced higher electrical responses when compared with the other compounds, at the doses of 10 and 1 mg. For the other tested doses, the tested insects were more sensitive to DMDS than to the other chemicals. Females were also more sensitive to the cadaveric volatile chemicals than males. Significant differences between males and females were observed at the doses of 10 mg ($F_{1,450} = 9.47$, $p = 0.002$), 1 mg ($F_{1,432} = 10.65$, $p = 0.001$), and 0.1 mg ($F_{1,432} = 6.69$, $p = 0.010$). There was no difference in perception between males and females at the doses of 0.01 mg ($F_{1,252} = 3.17$, $p = 0.076$) and 0.001 mg ($F_{1,252} = 0.01$, $p = 0.7920$).

Females' and males' antennal responses were dose dependent for cadaverine ($F_{4,450} = 9.66$, $p < 0.001$), DMDS ($F_{4,450} = 106.39$, $p < 0.001$), butan-1-ol ($F_{4,450} = 57.66$, $p < 0.001$), butanoic acid ($F_{4,450} = 2.83$, $p = 0.024$), putrescine ($F_{4,450} = 4.80$, $p = 0.001$), and phenol ($F_{3,432} = 3.56$, $p = 0.014$); that is, an increase in stimulus vapor concentration typically caused an increase in the action potential rate (47). The multiple comparisons of the means by the method of orthogonal polynomials showed that antennal responses induced by cadaverine, DMDS, and butan-1-ol significantly increased in a quadratic manner with the logarithm of the doses (Fig. 1). Moreover, for DMDS, a difference in perception according to the doses between males and females was observed ($F_{3,432} = 3.97$, $p < 0.001$) when antennal perceptions were compared at the doses of 1, 0.1, 0.01, and 0.001 mg. The antennal responses of butanoic acid and putrescine significantly increased in a linear manner with the logarithm of the doses. For phenol, the antennal responses did not follow any relationship to the logarithm of the doses.

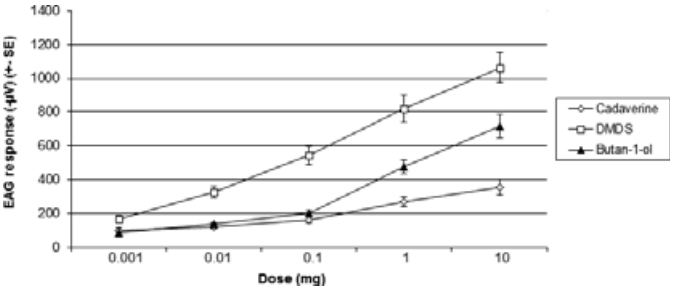

FIG. 1—*Effect of doses of cadaverine, dimethyl disulfide (DMDS), and butan-1-ol on antennal responses (±SE) of Lucilia sericata.*

Y-Tube Olfactometer

In the behavioral assay, 1,4-diaminobutane (putrescine) was repulsive for female flies at the dose of 0.1 mg ($\chi^2 = 7.37$, $p = 0.007$) (Table 3). At this dose, the other compounds did not elicit any observable behavior in both males and females. DMDS attracted females at the dose of 0.05 µg ($\chi^2 = 6.48$, $p = 0.011$), while butan-1-ol attracted both males and females at 0.05 µg ($\chi^2 = 3.92$, $p = 0.048$ and $\chi^2 = 9.68$, $p = 0.002$, respectively). The other compounds were not attractive or repulsive to *L. sericata* at the two tested doses.

Discussion

DMDS and butan-1-ol were reported as major chemicals to be released from decaying pig carcasses (38,44). These two chemicals were the most perceived by the fly antennae in our EAG experiments. We observed that the largest depolarizations were elicited by DMDS. In our bioassay, both DMDS and butan-1-ol were attractive for female flies at the dose of 0.05 µg (and not at a higher dose, i.e., 100 µg). Sulfurous compounds like DMDS strongly attract carrion flies as *L. sericata* (29,48,49) or *Musca domestica* (50–53). Also, the behavioral assays at the dose of 100 µg showed that DMDS and butan-1-ol did not show any significant attractiveness. This may be due to the difference in concentrations of these two compounds in the field (39), and this dose may be too high to attract *L. sericata*.

Cadaverine and putrescine are compounds usually associated with the decaying processes (43,54). However, these two diamines were not detected from decaying corpses in several studies

TABLE 2—*The multiple comparisons of the average electroantennography (EAG) responses with Newman and Keuls test.*

10 mg			Butanoic acid*	Putrescine*	Cadaverine*	Butan-1-ol	DMDS
			237 ± 16	303 ± 31.9	356 ± 44.2	718 ± 68.7	1062 ± 89.7
1 mg		Phenol*	Putrescine*	Butanoic acid*	Cadaverine*	Butan-1-ol	DMDS
		208 ± 21.3	208 ± 25.3	218 ± 19.7	270 ± 29.6	476 ± 39.4	819 ± 79.5
0.1 mg		Phenol*	Butanoic acid*	Cadaverine*	Putrescine*	Butan-1-ol*	DMDS
		144 ± 10.5	150 ± 13.3	160 ± 15.2	174 ± 14.6	202 ± 17.2	542 ± 52.3
0.01 mg	Phenol*	Butanoic acid*	Cadaverine*	Butan-1-ol*	Putrescine*	Indole*	DMDS
	103 ± 10.7	117 ± 9.7	118 ± 12.2	136 ± 7.7	158 ± 11.1	172 ± 19.5	327 ± 33.1
0.001 mg	Butan-1-ol*	Putrescine*	Cadaverine*	Butanoic acid*	Phenol*	Indole*	DMDS
	86 ± 12.8	93 ± 11.5	99 ± 13.6	106 ± 14.4	106 ± 9.4	139 ± 13.1	166 ± 22.2

Average EAG responses ± SE. For each dose, chemicals sharing the * induce similar EAG responses as a result of Newman and Keuls test. $\alpha = 0.05$. DMDS, dimethyl disulfide.

TABLE 3—*Behavioral responses of* Lucilia sericata *to seven VOCs.*

Compounds	Sex	Doses (μg)	χ^2	*p*
1,4-Diaminobutane	Females	100	7.37	0.007**
		0.05	1.28	0.258
	Males	100	1.65	0.199
		0.05	1.28	0.258
1,5-Diaminopentane	Females	100	3.00	0.083
		0.05	0.00	1.000
	Males	100	2.08	0.149
		0.05	0.08	0.777
Butan-1-ol	Females	100	1.28	0.258
		0.05	9.68	0.002**
	Males	100	0.18	0.668
		0.05	3.92	0.048*
Butanoic acid	Females	100	2.88	0.090
		0.05	1.28	0.258
	Males	100	0.72	0.396
		0.05	0.08	0.777
Indole	Females	100	–	–
		0.05	0.32	0.572
	Males	100	–	–
		0.05	2.00	0.157
DMDS	Females	100	2.47	0.116
		0.05	6.48	0.011*
	Males	100	1.65	0.199
		0.05	2.88	0.090
Phenol	Females	100	2.47	0.116
		0.05	0.00	1.000
	Males	100	0.19	0.662
		0.05	0.09	0.090

* and **, respectively, indicate the differences between control and VOC at $p < 0.05$ and $p < 0.01$. $\alpha = 0.05$. DMDS, dimethyl disulfide.

(38,42, 44,54). In agreement with Easton and Feir (55), there was no response to cadaverine in EAG and in our behavioral assays. However, behavioral assay with putrescine showed that this VOC was clearly repulsive at the dose of 100 μg, which is also in agreement with Easton and Feir (55).

Although indole and phenol had been suggested as putative attractants and stimulants of oviposition for blowflies, including *L. sericata* (50–53,56–58), our work showed that phenol and indole did not induce any behavioral response. Our results are also in accordance with those of Easton and Feir (55) who found that indole was unattractive to *L. sericata*.

Park and Cork (49) reported that butanoic acid inhibited the spontaneous activity of all antennal neurons studied in female *Lucilia cuprina* Wiedemann. Moreover, other studies showed that butanoic acid did not always inhibit antennal olfactory receptor neurons but stimulated the olfactory neurons in several insect species like *Stomoxys calcitrans* L. (59) or *M. domestica* L. (50–53). In this study, butanoic acid was not perceived by *L. sericata* in EAG test and in bioassays. Additional studies based on the activation or the inhibition of the antennal neurons of *L. sericata* by butanoic acid must be realized for a better understanding of this olfactory process.

The behavioral assays showed that the behavior of the blowflies is influenced by the tested dose. Moreover, it is necessary to keep in mind that there are huge differences in volatilities among the VOCs tested in our experiments (39,60).

Our electrophysiological results also showed that *L. sericata* females were more sensitive to decomposing odors than males. In our olfactometer, females and males showed different behavior for all tested chemicals except for butan-1-ol. Females only responded to DMDS and putrescine. In insect species where males and females have the same food preferences, sexual dimorphism of the olfactory system has also been demonstrated (61). Sukontason et al. (61), based on the observations of *Parasarcophaga dux* and *L. cuprina*, showed that the number of sensory pits in female flies is greater than in males of the same species. This information supports the hypothesis that the abundant sensory pits would help female flies to be more sensitive in olfactory reception. However, in some tsetse species, the electrophysiological responses of females to host odors were higher than those of males, whereas in other tsetse species, males were more sensitive than females (46,62).

In conclusion, our results may have potential implications in a better understanding of attractiveness of blowflies toward a corpse. The comprehension of the role of odors in the behavior of necrophagous insects would make it possible to consider new developments in forensic sciences and the research of the bodies with the use of insects that may act as cadaveric "biodetectors." In general, there has been very little study performed in the area of the cadaveric volatile compounds that influence the behavior of necrophagous insects. Further researches on the behavior of necrophagous insects are currently conducted at the Department of Functional and Evolutionary Entomology.

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