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Chemostimuli implicated in selection of oviposition substrates by the stable fly *Stomoxys calcitrans*

P. JEANBOURQUIN and P. M. GUERIN
Laboratory of Animal Physiology, Institute of Biology, University of Neuchâtel, 2009 Neuchâtel, Switzerland

Abstract. Horse and cow dung were tested as substrates for oviposition by the stable fly *Stomoxys calcitrans* (L) (Diptera: Muscidae) in laboratory cages. Odour alone from either horse or cow dung was sufficient to attract flies for oviposition. This was confirmed in wind tunnel experiments, where both horse and cow dung were shown to attract gravid stable flies. However, when *S. calcitrans* was offered a choice between these two oviposition substrates, flies always chose horse dung over cow dung, both when allowed to contact the substrates and when relying on dung odour alone. Analyses of volatile compounds emanating from horse and cow dung by gas chromatography linked antennogram recordings from *S. calcitrans* antennae revealed no differences in the chemostimuli released from the two substrates. The predominant chemostimulant compounds in both substrates were carboxylic acids (butanoic acid), alcohols (oct-1-en-3-ol), aldehydes (decanal), ketones (octan-3-one), phenols (*p*-cresol), indoles (skatole), terpenes (β-caryophyllene) and sulphides (dimethyl trisulphide). Higher levels (20–40 p.p.m.) of carbon dioxide were recorded over horse dung compared with cow dung, a factor that may contribute to the preference exhibited by *S. calcitrans* for this substrate for oviposition.

Key words. Antennogram, olfaction, oviposition, sensory ecology, stable fly.

Introduction

Stomoxys calcitrans (L) is a worldwide livestock pest and important economic losses are encountered when stable flies are present in large numbers (Campbell *et al.*, 1977). Cattle are particularly sensitive to the annoyance caused by stable flies, which results in reduced weight gains, weight loss and sometimes dramatically reduced milk production (Stork, 1979). Traditional control measures for reducing fly infestations mostly involve the removal or dispersal of any substrate that can serve as a breeding site (rotting straw, manure) so that, in the case of dispersal, it dries out before the maggots can complete their development (Zumpt, 1973). Many efforts are made to render these substrates unsuitable for larval development (Axtell, 1986), but little interest has been shown in understanding how flies locate them.

Numerous observations in the field have noted the flexibility stable flies show in their choice of oviposition substrates. It seems, indeed, that all kinds of decomposing organic matter of plant origin, such as silage, rotting hay, grass clippings, garden compost and even sea grass deposits, may form appropriate breeding media (Zumpt, 1973; Axtell, 1986). Animal faeces are also commonly visited by gravid females; Hafez & Gamal-Eddin (1959) reported preferences by *S. calcitrans* for equine (donkey and horse) manure, especially when mixed with straw and wetted with urine. They also observed that cattle dung was only slightly attractive to ovipositing females, and that sheep and goat droppings were the least favoured oviposition substrates. Animal faeces were much less attractive when not mixed with fermenting plant material (Hafez & Gamal-Eddin, 1959).

Given the apparent variety of potential substrates and the role olfaction might play in guiding ovipositing stable flies, we can hypothesize that flies rely on a set of chemostimuli shared by all these substrates for their location. In addition, volatile compounds arise from the degradation of carbohydrates, lipids and proteins through microbial activity. Some of these, such as indoles, are known to attract ovipositing female mosquitoes to

Correspondence: Dr Patrick M. Guerin, Institute of Biology, Faculty of Science, University of Neuchâtel, Rue Emile-Argand 11, Case Postale 158, 2009 Neuchâtel, Switzerland. Tel.: + 41 32 718 30 66 (direct); Fax: + 41 32 718 30 01; E-mail: patrick.guerin@unine.ch

fermented grass infusions (Beehler *et al.*, 1994; Millar *et al.*, 1994; Allan & Kline, 1995). Furthermore, decomposing organic matter releases carbon dioxide (CO_2), which, as it attracts stable flies (Vale, 1980; Mihok *et al.*, 1996), may also be implicated in their oviposition behaviour.

In this study, we assessed the role of olfaction in guiding *S. calcitrans* to suitable substrates for oviposition, hypothesizing that flies primarily locate a suitable oviposition site at a distance using olfactory cues and depend on visual cues and information from contact chemoreceptors to determine their acceptance or rejection of the substrate. To test this, we observed *S. calcitrans* behaviours in a wind tunnel on exposure to horse and cow dung odours and conducted oviposition assays with horse and cow dung – two potential oviposition substrates commonly encountered in temperate latitudes. We then analysed the volatile composition of these odours using gas chromatography linked antennogram recordings to identify the compounds that elicit responses from the antennal receptor cells of stable flies.

Materials and methods

Insects and oviposition substrates

Pupae of *S. calcitrans* were supplied by Novartis Animal Health S.A. (St-Aubin, Switzerland) and kept in an environmental chamber until emergence under a LD 12 : 12 h cycle at 26–28°C, 65% RH in light and 22°C, 90% RH in dark, with light increasing and decreasing for 2 h at dawn and dusk. Newly emerged flies were sexed daily and 15 males and 25 females were held in cotton mesh (1 mm) cages (15 × 25 × 15 cm). Flies were fed heparinized bovine blood (Veterinary School, University of Bern, Bern, Switzerland) on cotton treated daily with 5 mL fresh blood. Four days after emergence, 10 gravid females used for the oviposition experiments (below) were placed in a bigger cage (30 × 30 × 30 cm) made of white nylon netting (2 mm) with access to a 10% sucrose solution on cottonwool. The 15 remaining females were separated from the males and used in wind tunnel experiments (below). Fresh horse and cow dung was collected from hay- and grass-fed animals on a farm at Fleurier (Neuchâtel, Switzerland) and stored at 4°C until needed.

Oviposition assays

The cage containing 10 gravid females was placed on black cardboard (0.2 mm thick) pierced at opposite corners (centres 25 cm apart) with two 8.5-cm diameter holes that were covered with black nylon netting (0.25 mm mesh). A plastic dish (10 cm in diameter, 5 cm deep) containing 25 g of either cow or horse dung was placed under one hole in the cardboard; a dish of water-soaked cottonwool was placed under the opposite opening as a control. Oviposition tests were also made in the environmental cabinet (conditions as above) where flies laid eggs during the photophase. The numbers of eggs laid per cm^2 over the dung and control dishes and elsewhere on the floor of the cage were counted and compared using the Kruskal–Wallis test. When significant, subsequent pairwise comparisons were made using the Mann–Whitney test. Four replicates were carried out for each treatment. The same procedure was used for choice experiments where the attractiveness of 25 g horse dung was compared with that of 25 g cow dung in dishes under the two respective openings. The cage was carefully washed and the position of the holes under the cage rotated between replicates to avoid any bias. Moreover, an experiment with empty plastic dishes was made to check for contamination of the black netting covering the holes. The concentration of CO_2 above both waste substrates was also recorded for 24 h with a Li-820 gas analyser (Li-Cor®, ± 5 p.p.m. resolution; LI-COR Biosciences, Lincoln, NE, U.S.A.) linked to a portable computer.

Oviposition experiments where the flies had access to the dung for oviposition were conducted in cotton netting (IVF Technology AG, Neuhausen, Switzerland) cages (15 × 25 × 15 cm) on a tray. A plastic dish (9 cm in diameter, 1.5 cm deep) containing 20 g of either cow or horse dung was placed in the cage. Eggs laid in the dung and elsewhere in the cage were counted and the respective proportions for cow and horse dung were compared using the Mann–Whitney test. Three replicates were made for each treatment.

Wind tunnel experiments

The wind tunnel (170 × 60 × 60 cm, wind speed 30 cm/s) was constructed of non-reflecting glass as described in Syed & Guerin (2004), with the following modifications. The downwind end (fly release point) of the nylon net flight cylinder which stretched the length of the tunnel (1.5 m long, 20 cm diameter, 1 mm mesh) was closed by a metal grid (3 mm mesh) with a centred 3 × 3 cm opening in order to prevent released flies from escaping downwind. The wind tunnel was completely wrapped in white paper. Flies tested in the wind tunnel were transferred individually into plastic release cages (transparent PVC cylinders, 13 cm long, 4.5 cm diameter), of which the downwind ends were covered with nylon netting (1 mm mesh) and the upwind ends were fitted with a flat sliding nylon mesh (1 mm) opening. The release cage was placed horizontally at the downwind end of the nylon net flight cylinder with the cage's upwind opening aligned against the metal grid with the 3 × 3 cm opening at the downwind end of the flight cylinder. Cages with flies were kept in the environmental cabinet (28°C, 65% RH) with the wind tunnel for at least 1 h before tests.

To test the odour of natural substrates, 50 g dung (test) and 50 mL distilled water (control) were placed in 1-L glass gas-wash bottles equipped with a glass T-connector. One end of the T-connector was linked to a stimulus delivery device blowing charcoal-filtered air at 400 mL/min through Teflon® tubing into the bottle. The other end was linked via Teflon® tubing to a vertical aluminium tube (4 mm internal diameter) that served to guide the headspace vapour from the gas-wash bottle through an aluminium funnel (4 cm diameter) facing downwind at a height of 35 cm at the upwind end of the wind tunnel. The 4-cm diameter funnel opening was perforated with 16 holes (0.4 mm diameter). The odour plume structure was checked visually by

generating a plume of ammonium acetate. The plume covered the flight cylinder uniformly from 15 cm downwind of the aluminium release funnel to its exit.

The responses of individual gravid female *S. calcitrans* to odours emanating from cow and horse dung were tested during the photophase. After 2 min of acclimatization in the wind tunnel, the nylon-mesh door of the fly release cage was lifted slowly and flies were successively exposed for 2 min to odour-free air (control) and then for 2 min to the test odour from a second funnel standing beside the first. Effects of test stimuli were estimated by the percentage of flies activated on exposure to dung volatiles and the percentage of flies attracted to the odour source. As flies were naturally active, we considered a fly to be activated when it took off more than three times in the flight cylinder and that it was attracted when it flew more than 50 cm of the 130 cm distance from the release cage towards the source. Flies that were activated or that flew upwind during the control period were discarded. OBSERVER software (Noldus Technologies, Wageningen, the Netherlands) was used for recording fly behaviours. Comparisons between test odours were performed on counts recorded using chi-square tests, but results are presented as the percentages of flies activated and attracted for clarity. Variations in CO_2 levels during stimulation with cow and horse dung were measured at the funnel exit and at the fly release point.

Volatiles collection

A sample of 50 g of either cow or horse dung was introduced into a 1-L gas-wash flask and left for 1 h at room temperature. Charcoal-filtered air was pulled for 20 min at 50 mL/min successively through the bottle via a glass T-connector and then through a commercial Tenax™ GR (30% graphite) cartridge (6 mm outer diameter; Gerstel® GmbH & Co., Muelheim an der Ruhr, Germany) connected to the outlet end of the connector.

Gas chromatography linked electroantennographic detection

The antenna of a gravid female *S. calcitrans* used as a biological detector (Arn *et al.*, 1975) in gas chromatography (GC) analysis of dung volatiles was mounted (Guerin & Visser, 1980) between two glass electrodes (2 mm outer diameter) filled with 0.1 M KCl. Electroantennographic detector (EAD) responses were recorded in parallel with the flame ionization detector (FID) response of the gas-chromatograph (GC 5300; Carlo Erba Instruments, Milan, Italy) on a computer through a 16-bit analogue/digital Intelligent Data Acquisition Controller (IDAC) box (Syntech, Hilversum, the Netherlands). The GC was equipped with a thermal desorption unit (TDS 2; Gerstel®) and a cooled injection system (CIS 3; Gerstel®) installed on the head of a polar high-resolution capillary column (Free Fatty Acids Phase (FFAP), length 30 m, internal diameter 0.25 mm, film thickness 0.25 μm; BGB Analytik, Boeckten, Switzerland).

Dung volatiles trapped on the Tenax™ GR cartridge were thermally desorbed using the TDS 2 programmed to increase from 30°C to 220°C at 30°C/min. A heated (240°C) transfer line evacuated the volatiles to the CIS 3 cooled to – 80°C with liquid nitrogen, where volatiles were cryotrapped. The CIS was then heated (12°C/s) to reach 240°C to transfer (splitless mode) the volatiles onto the high-resolution capillary column. Hydrogen at 50 cm/s was used as the carrier gas. The temperature programme was set at 40°C for 5 min, rising by 5°C/min to 230°C. The column effluent was split equally with a metal splitter (Gerstel®) between the FID (set at 250°C) and a heated (250°C) transfer line that conveyed the column effluent to a glass water-jacketed tube (7 mm internal diameter), where a charcoal-filtered humidified (90–100% RH) air stream at 1 m/s carried the volatiles to the fly antenna (Steullet & Guerin, 1994).

An averaged GC-EAD trace for each oviposition substrate was redrawn from the three most representative GC-EAD analyses of the cow and horse dung extracts. Only antennal responses recorded in at least two traces to a given constituent of the extract were used.

Gas chromatography coupled mass spectrometry

Biologically active constituents of either cow or horse dung volatiles that evoked electroantennogram (EAG) responses from *S. calcitrans* antennae were identified by gas chromatography linked mass spectrometry (GC-MS) using an HP 5890 Series II GC equipped with a TDS 2 and CIS 3, and with a 30-m FFAP high-resolution capillary column (see above) linked to a mass selective detector (HP 5971 A) with helium at 30 cm/s as the carrier gas (Steullet & Guerin, 1994). The TDS 2, CIS 3 and GC oven conditions were the same as for the GC-EAD recordings.

Kovat's retention indices (RI) were used to locate compounds that caused EAD responses in dung odour extracts and the identities of these chemostimuli were determined by comparing the mass spectra with those in mass spectrum libraries (Wiley or Nist98). Identified compounds were then compared with the corresponding injected synthetic analogues (when available) or with RI values from the literature (www.flavornet.org).

Results

Oviposition assays

When horse or cow dung was presented beneath the nylon mesh, flies laid significantly more eggs over dung than over water or elsewhere in the cage (Fig. 1). Means of 3.45 ± 1.13 and 1.96 ± 0.50 eggs/cm^2 were laid on horse and cow dung, respectively, whereas the number deposited on the control and elsewhere in the cage did not exceed means of 0.08 ± 0.03 and 0.39 ± 0.01 eggs/cm^2, respectively. However, when provided with a choice between horse and cow dung in the same cage, flies laid significantly more eggs over horse dung (5.82 ± 1.57 eggs/cm^2) than over cow dung (0.04 ± 0.01 eggs/cm^2) (Fig. 2). When no substrate was presented, flies deposited their eggs randomly, showing no preference for any oviposition site inside the cage (data not shown).

The concentrations of CO_2 over horse and cow dung during the 24 h of continuous recording were quite different (Fig. 3).

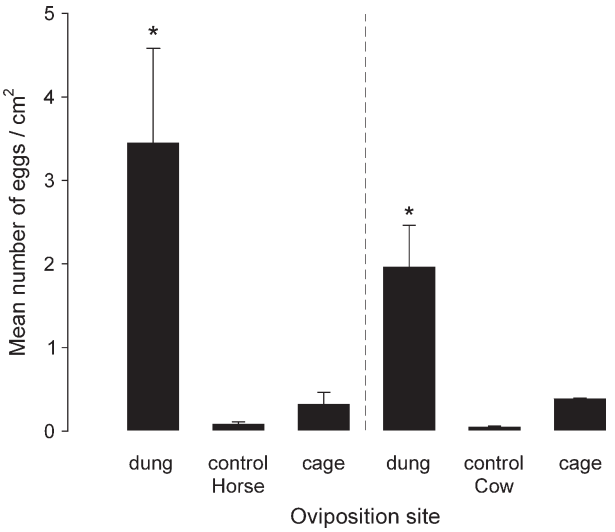

Fig. 1. Mean number of eggs/cm^2 laid by *Stomoxys calcitrans* ($n = 10$) over 24 h in response to horse or cow dung volatiles presented in separate cages compared with eggs laid over the control (wet cottonwool) and elsewhere in the cage. *$P < 0.05$, Mann–Whitney test ($P < 0.01$, Kruskal–Wallis test). Vertical lines indicate the standard error of the mean.

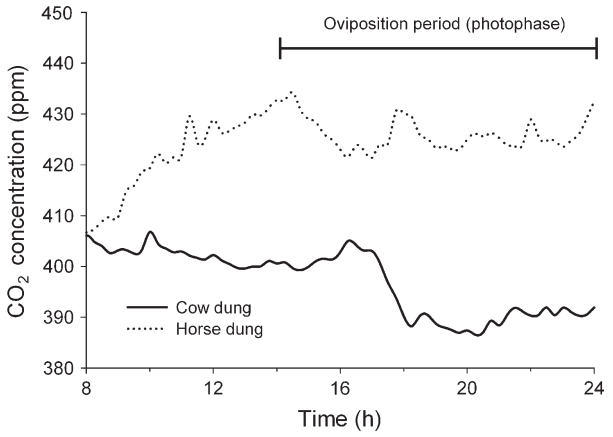

Fig. 3. Carbon dioxide levels (p.p.m.) recorded over 25 g samples of cow and horse dung, respectively, over a 24-h period (first 8 h not shown) as recorded with a Li-Cor® gas analyser. The *Stomoxys* oviposition period ran from 14 h onwards (i.e. start of the photophase).

For the first 8 h, the CO_2 level over both horse and cow dung was similar, at around 400 p.p.m., only marginally above ambient levels (*c*. 350 p.p.m.). From this point, however, CO_2 levels over the two substrates began to diverge. Whereas the level over cow dung remained approximately constant at 390–400 p.p.m. for the next 16 h, the level of CO_2 over horse dung increased to around 420–430 p.p.m., giving rise to a difference of some 20–40 p.p.m. between horse and cow dung from 12 h onwards (i.e. 2 h before the start of the photophase).

In oviposition experiments where the flies had access to the dung, a significantly higher number of eggs were deposited by the 10 *S. calcitrans* females on horse dung (547 ± 218 eggs) compared with elsewhere in the cage (38 ± 33 eggs) (Fig. 4). By contrast, the flies avoided laying eggs directly on cow dung (5 ± 3 eggs), instead depositing significantly more eggs within 3 cm of the cow dung (506 ± 148 eggs).

Wind tunnel experiments

Exposure to both horse and cow dung volatiles activated and attracted flies exposed in the wind tunnel (Table 1). Although the

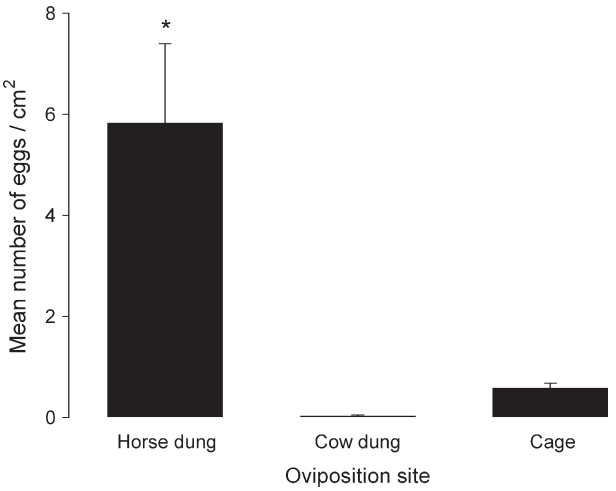

Fig. 2. Mean number of eggs/cm^2 laid by *Stomoxys calcitrans* ($n = 10$) over 24 h in choice tests between horse and cow dung volatiles in the same cage. *$P < 0.05$, Mann–Whitney test ($P < 0.01$, Kruskal–Wallis test). Vertical lines indicate the standard error of the mean.

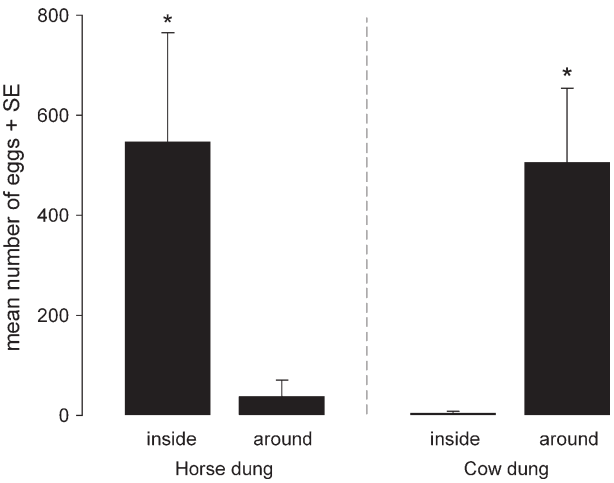

Fig. 4. Mean number of eggs laid over 24 h by *Stomoxys calcitrans* ($n = 10$) with access to either horse or cow dung in separate cages. *$P < 0.05$, Mann–Whitney test. Vertical lines indicate the standard error of the mean.

Table 1. Behavioural responses of *Stomoxys calcitrans* gravid females following exposure to dung odours in a wind tunnel. The percentages of flies activated and attracted to the odour source were recorded. A fly was considered activated when it took off more than three times in the flight cylinder and attracted when it flew more than 50 cm from the release cage towards the source. Flies that were activated or attracted during the control period were discarded. Chi-square tests were performed on the raw counts for statistical comparison of activation and attraction between treatments.

Treatment	n*	n†	% activated	% attracted
Cow dung	32	22	68.2	63.6
Horse dung	32	22	77.3	68.2

n* = number of flies tested.
n† = number of flies that responded during stimulus delivery only.

difference was not significant, a marginally higher percentage of flies was activated and attracted when stimulated with horse dung volatiles. High spontaneous activity resulted in activation of about 30% of flies during the 2-min control periods.

Increased CO_2 concentrations were noted at the odour delivery point in the wind tunnel for horse and cow dung, at 43 ± 15 p.p.m. and 162 ± 49 p.p.m., respectively. These variations at the upwind end of the wind tunnel resulted in fluctuations at the fly release point of 1 ± 0.5 p.p.m. for horse and 2 ± 1 p.p.m. for cow dung.

GC linked EAD analyses of dung odour extracts

Despite some differences in the GC profiles recorded for the cow and horse dung volatile collections on the porous polymer, *S. calcitrans* showed similar patterns of antennal responses to the odour constituents of the two substrates (Fig. 5). The volatiles that elicited electrophysiological responses belong to different chemical classes, including carboxylic acids, short chain alcohols and aldehydes, ketones, indoles, phenols, sulphides and terpenes. Of 27 compounds eliciting EAG response from the two substrates, stable flies consistently responded to 25 compounds occurring in both, with oct-1-en-3-ol and dimethyl trisulphide evoking the strongest antennogram responses relative to the amount of each of the two products in the odour extracts. Responses to butanoic acid, α-humulene, acetophenone, isovaleric acid and borneol, clearly visible in the averaged EAD trace for horse dung volatiles, were also visible in the GC-EAD analyses of cow dung volatiles, but were much weaker because of the lower levels of these products in the latter. Likewise, heptan-1-ol was only detected by *S. calcitrans* in cow dung odour and β-caryophyllene in horse dung odour, whereas GC-MS analyses revealed that these chemostimuli may occasionally occur in both substrates. Electroantennographic detector responses 11 and 12 were elicited by co-eluting products (peak 11 acetic acid plus decanal and peak 12 propanoic acid plus octan-1-ol) such that these products may have contributed in a combined manner to the amplitudes of these EAD responses.

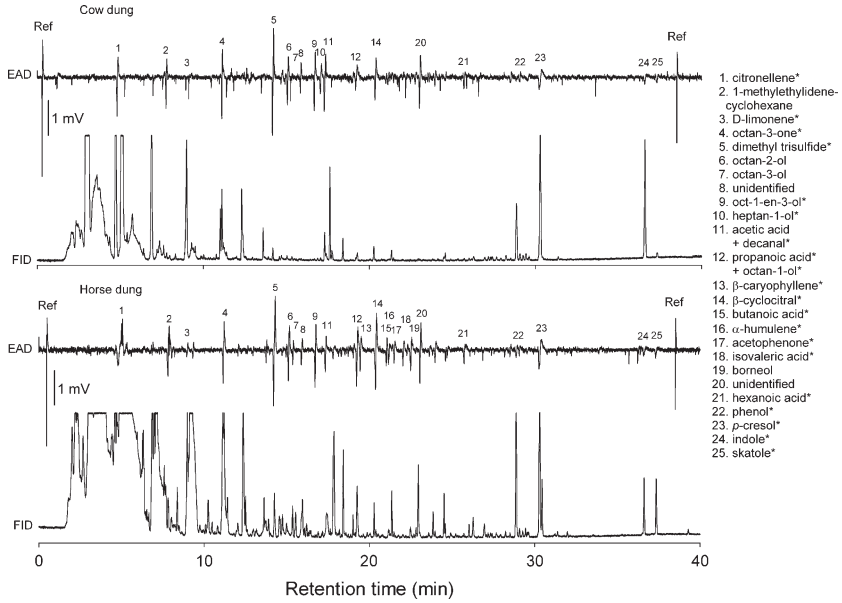

Fig. 5. Antennal responses of *S. calcitrans* to cow and horse dung volatiles thermally desorbed onto a high-resolution gas chromatographic column from a porous polymer. The lower traces in each analysis represent the signal from the flame ionization detector (FID); the upper traces represent the averaged electroantennographic detector (EAD) responses of three female stable flies to substrate volatiles. Synthetic analogues of compounds marked with * showed electrophysiological activity in GC-EAD or electroantennogram recordings. Reference responses (Ref) were recorded at the start and end of each analysis using an odour puff of oct-1-en-3-ol from 1 μg in a stimulus syringe. 1 = citronellene*; 2 = 1-methylethylidene-cyclohexane; 3 = D-limonene*; 4 = octan-3-one*; 5 = dimethyl trisulphide*; 6 = octan-2-ol; 7 = octan-3-ol; 8 = unidentified; 9 = oct-1-en-3-ol*; 10 = heptan-1-ol*; 11 = acetic acid + decanal*; 12 = propanoic acid* + octan-1-ol*; 13 = β-caryophyllene*; 14 = β-cyclocitral*; 15 = butanoic acid*; 16 = α-humulene*; 17 = acetophenone*; 18 = isovaleric acid*; 19 = borneol; 20 = unidentified; 21 = hexanoic acid*; 22 = phenol*; 23 = *p*-cresol*; 24 = indole*; 25 = skatole*.

Discussion

This study has shown that stable flies can use olfactory cues from a distance to locate a suitable substrate on which to oviposit. Although the importance of olfaction for oviposition in some haematophagous arthropods such as mosquitoes is well documented (Bentley & Day, 1989; Beehler *et al.*, 1994; Allan & Kline, 1995), information about the role of olfaction in *S. calcitrans* oviposition is lacking. Despite the tendency of stable flies to oviposit anywhere in the current laboratory experiments, the oviposition assay used in this research demonstrates the importance of olfactory cues in stable fly ovipositioning. In the absence of contact with the substrate, *S. calcitrans* is able to locate either horse or cow dung by relying only on odour cues. This is supported by the observations in the wind tunnel, which showed that stable flies were attracted to both substrates. When provided with a choice, *S. calcitrans* clearly shows an oviposition preference for horse over cow dung, confirming earlier field observations (Hafez & Gamal-Eddin, 1959). In order to explain this olfactory-mediated preference, the antennal responses of *S. calcitrans* to the odours of horse and cow dung were recorded, but the antennal receptor cell response profiles recorded do not show major differences in the pattern of chemostimuli emanating from the two substrates. In fact, most of the chemostimuli occur consistently in the odours of both substrates. However, higher levels of CO_2 were recorded over horse dung at the time when flies started to oviposit, which may have influenced the preference. The oviposition substrates selected by many blood-feeding dipteran females (stable flies, horn flies, mosquitoes) mainly comprise decomposing vegetal matter in which bacterial degradation of the medium results in the production of volatile compounds, such as carboxylic acids, short chain aliphatic alcohols, phenols, indoles, sulphides, terpenes and CO_2. Romero *et al.* (2006) have shown that female stable flies are capable of selecting an oviposition site based on microbially derived stimuli.

The new chemostimulant identified here for *S. calcitrans*, dimethyl trisulphide, is widespread in potential oviposition substrates, originating from methionine degradation (Mackie *et al.*, 1998). This product is also released from fermented infusions of Bermuda grass and serves as an oviposition stimulant for *Culex* mosquitoes (Du & Millar, 1999). Dimethyl trisulphide has been detected in pig manure and was shown to attract houseflies when combined with butanoic acid and skatole (Cossé & Baker, 1996). The GC-EAD recordings from antennal receptor cells in this study confirmed dimethyl trisulphide as a chemostimulant in both horse and cow dung. Furthermore, a sensory threshold in the picogram range was recorded here and this compound attracts stable flies in the wind tunnel (Jeanbourquin & Guerin, 2007), suggesting a role for dimethyl trisulphide in the sensory ecology of these flies.

Other chemostimuli that occurred in our dung extracts were carboxylic acids, which have been shown to play a role in host location in mosquitoes (Knols *et al.*, 1997; Bosch *et al.*, 2000; Costantini *et al.*, 2001) and ticks (Donzé *et al.*, 2004). Broce & Haas (1999) observed a clear preference on the part of gravid stable flies for aged cow manure, a substrate that produces twice the concentration of volatile carboxylic acids as fresh manure (Miller & Varel, 2001). Aliphatic C_7–C_8 alcohols found in our dung extracts were previously shown to elicit EAG responses from *Stomoxys* antennae (Schofield *et al.*, 1995) and oct-1-en-3-ol was also detected in trace amounts in our GC-EAD analyses of cow and horse dung. Whereas the electrophysiological and behavioural effects of oct-1-en-3-ol on various haematophagous insects are well documented (Hall *et al.*, 1984; French & Kline, 1989; Kline *et al.*, 1990; Gibson & Torr, 1999), and although the lowest antennogram threshold in stable flies was recorded with this chemostimulant (Jeanbourquin & Guerin, 2007), little is known about the possible influence of aliphatic alcohols on oviposition in *S. calcitrans*.

The GC-EAD analyses in the current study also revealed the occurrence of phenol, *p*-cresol, indole, skatole and a range of terpenes as chemostimuli from both horse and cow dung. Numerous studies highlight the release of phenols and indoles from substrates that are known to attract different mosquito spp. for oviposition (Du & Millar, 1999; Mboera *et al.*, 2000). The terpenes identified in the current GC-EAD analyses of dung as volatile chemostimulants for stable fly antennal receptor cells were citronellene, D-limonene, β-caryophyllene, β-cyclocitral and α-humulene. Stable flies are known to take nectar and pollen from plants as energy sources for flight (Zumpt, 1973; Jones *et al.*, 1985, 1992). Although terpenes may be suspected as being primarily of use to *S. calcitrans* for locating flowers, the preference shown by stable flies for oviposition substrates containing plant material (Hafez & Gamal-Eddin, 1959; Zumpt, 1973) suggests these chemostimulants also play a role in the location of suitable oviposition substrates.

Interestingly, some plants, mainly belonging to Araceae, are known to mimic dung odour to attract pollinators, including many Diptera associated with the ecological recycling of dung and carrion (Kite, 1995; Skubatz *et al.*, 1996). Most of the odour compounds produced by these plants were also present in our odour extracts of cow and horse dung as chemostimulants for stable flies. This suggests that a complex blend of terpenes, carboxylic acids, aliphatic alcohols and aldehydes, ketones, phenols, indoles and sulphur-containing compounds may serve to lure *Stomoxys* spp. searching for dung or dung-like substrates on which to oviposit.

Higher CO_2 levels were recorded over horse dung, the preferred substrate for ovipositing females in this study. CO_2 is known to be a strong attractant for haematophagous insects (Cork, 1996; Gibson & Torr, 1999). Its attractiveness to stable flies has been documented (Vale, 1980; Cilek, 1999), as has a concentration threshold of close to 60 p.p.m. for behavioural response (Schofield & Brady, 1997). In the same manner that gravid *Bactrocera tryoni* fruit flies rely on CO_2 for oviposition (Stange, 1999), the higher level of CO_2 over horse dung may have been sufficient to account for the preference of females for horse dung in our tests. In addition, CO_2 is probably released from grass infusions that attract mosquitoes for oviposition (Millar *et al.*, 1992; Allan & Kline, 1995; Isoe & Millar, 1995).

In experiments where ovipositing *S. calcitrans* were able to make contact with the horse and cow dung substrates, the females demonstrated a clear preference for horse dung for oviposition. Here the flies hid their eggs in the horse dung but avoided the cow dung, preferring to oviposit in the vicinity of the latter through the net of the cage. We suggest that flies unable to hide their eggs

in cow dung oviposit around it, providing larvae with a nearby food resource. The more fibrous structure of horse dung allows flies to lay their eggs in crevices of the substrate protected from desiccation and predation. This is in agreement with findings by Hafez & Gamal-Eddin (1959), who reported that oviposition substrates chosen by gravid *Stomoxys* sp. females must be loose and porous, further underlining the importance of both chemoreception and proprioreception in the acceptance of a substrate for oviposition in flies (Städler *et al.*, 1995).

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References

Allan, S.A. & Kline, D.L. (1995) Evaluation of organic infusions and synthetic compounds mediating oviposition in *Aedes albopictus* and *Aedes aegypti* (Diptera: Culicidae). *Journal of Chemical Ecology*, **21**, 1847–1860.

Arn, H., Städler, E. & Rauscher, S. (1975) The electroantennographic detector – a selective and sensitive tool in gas chromatographic analysis of insect pheromones. *Zeitschrift für Naturforschung*, **30**, 722–725.

Axtell, R.C. (1986) *Fly Control in Confined Livestock and Poultry Production*. Technical Monograph, pp. 1–59. Ciba-Geigy Corporation, Greensboro, NC.

Beehler, J.W., Millar, J.G. & Mulla, M.S. (1994) Field evaluation of synthetic compounds mediating oviposition in *Culex* mosquitoes (Diptera: Culicidae). *Journal of Chemical Ecology*, **20**, 281–291.

Bentley, M.D. & Day, J.F. (1989) Chemical ecology and behavioural aspects of mosquito oviposition. *Annual Review of Entomology*, **34**, 401–421.

Bosch, O.J., Geier, M. & Boeckh, J. (2000) Contribution of fatty acids to olfactory host finding of female *Aedes aegypti*. *Chemical Senses*, **25**, 323–330.

Broce, A.B. & Haas, M.S. (1999) Relation of cattle manure age to colonization by stable fly and house fly (Diptera: Muscidae). *Journal of the Kansas Entomological Society*, **72**, 60–72.

Campbell, J.B., White, R.G., Wright, J.E. *et al*. (1977) Effects of stable flies on weight gains and feed efficiency of calves on growing or finishing rations. *Journal of Economic Entomology*, **70**, 592–594.

Cilek, J.E. (1999) Evaluation of various substances to increase adult *Stomoxys calcitrans* (Diptera: Muscidae) collections on Alsynite cylinder traps in north Florida. *Journal of Medical Entomology*, **36**, 605–609.

Cork, A. (1996) Olfactory basis of host location by mosquitoes and other haematophagous Diptera. *Olfaction in Mosquito–Host Interactions, Ciba Foundation Symposium* (ed. by G.R. Bock & G. Cardew), **200**, pp. 71–88. John Wiley & Sons, Chichester.

Cossé, A.A. & Baker, T.C. (1996) House flies and pig manure volatiles: wind tunnel behavioural studies and electrophysiological evaluations. *Journal of Agricultural Entomology*, **13**, 301–317.

Costantini, C., Birkett, M.A., Gibson, G. *et al*. (2001) Electroantennogram and behavioural responses of the malaria vector *Anopheles gambiae* to human-specific sweat components. *Medical and Veterinary Entomology*, **15**, 259–266.

Donzé, G., McMahon, C. & Guerin, P.M. (2004) Rumen metabolites serve ticks to exploit large mammals. *Journal of Experimental Biology*, **207**, 4283–4289.

Du, Y.S. & Millar, J.G. (1999) Electroantennogram and oviposition bioassay responses to *Culex quinquefasciatus* and *Culex tarsalis* (Diptera: Culicidae) to chemicals in odours from Bermuda grass infusions. *Journal of Medical Entomology*, **36**, 158–166.

French, F.E. & Kline, D.L. (1989) 1-octen-3-ol, an effective attractant for Tabanidae (Diptera). *Journal of Medical Entomology*, **26**, 459–461.

Gibson, G. & Torr, S.J. (1999) Visual and olfactory responses of haematophagous Diptera to host stimuli. *Medical and Veterinary Entomology*, **13**, 2–23.

Guerin, P.M. & Visser, J.H. (1980) Electroantennogram responses of the carrot fly, *Psila rosae*, to volatile plant components. *Physiological Entomology*, **5**, 111–119.

Hafez, M. & Gamal-Eddin, F.M. (1959) Ecological studies on *Stomoxys calcitrans* L. and *Sitiens* Rond, in Egypt, with suggestions on their control. *Bulletin de la Société Entomologique d'Egypte*, **XLIII**, 245–283.

Hall, D.R., Beevor, P.S., Cork, A. *et al*. (1984) 1-Octen-3-ol: a potent olfactory stimulant and attractant for tsetse isolated from cattle odours. *Insect Science and its Applications*, **5**, 335–339.

Isoe, J. & Millar, J.G. (1995) Characterization of factors mediating oviposition site choice by *Culex tarsalis*. *Journal of the American Mosquito Control Association*, **11**, 21–28.

Jeanbourquin, P. & Guerin, P.M. (2007) Sensory and behavioural responses of *Stomoxys calcitrans* to rumen volatiles. *Medical and Veterinary Entomology*, **21**, 217–224.

Jones, C.J., Hogsette, J.A., Patterson, R.S. & Milne, D.E. (1985) Effects of natural saccharide and pollen extract feeding on stable fly (Diptera: Muscidae) longevity. *Environmental Entomology*, **14**, 223–227.

Jones, C.J., Milne, D.E., Patterson, R.S. *et al*. (1992) Nectar feeding by *Stomoxys calcitrans* (Diptera: Muscidae) – effects on reproduction and survival. *Environmental Entomology*, **21**, 141–147.

Kite, G.C. (1995) The floral odour of *Arum maculatum*. *Biochemical Systematics and Ecology*, **23**, 343–354.

Kline, D.L., Takken, W., Wood, J.R. & Carlson, D.A. (1990) Field studies on the potential of butanone, carbon dioxide, honey extract, 1-octen-3-ol, 1-lactic acid and phenols as attractants for mosquitoes. *Medical and Veterinary Entomology*, **4**, 383–391.

Knols, B.G.J., Van Loon, J.J.A., Cork, A. *et al*. (1997) Behavioural and electrophysiological responses of the female malaria mosquito *Anopheles gambiae* (Diptera: Culicidae) to Limburger cheese volatiles. *Bulletin of Entomological Research*, **87**, 151–159.

Mackie, R.I., Stroot, P.G. & Varel, P.H. (1998) Biochemical identification and biological origin of key odour components in livestock waste. *Journal of Animal Science*, **76**, 1331–1342.

Mboera, L.E.G., Takken, W., Mdira, K.Y. *et al*. (2000) Oviposition and behavioural responses of *Culex quinquefasciatus* to skatole and synthetic oviposition pheromone in Tanzania. *Journal of Chemical Ecology*, **26**, 1193–1203.

Mihok, S., Maramba, O., Munyoki, E. & Saleh, K. (1996) Phenology of Stomoxyinae in a Kenyan forest. *Medical and Veterinary Entomology*, **10**, 305–316.

Millar, J.G., Chaney, J.D., Beehler, J.W. & Mulla, M.S. (1994) Interaction of the *Culex quinquefasciatus* egg raft pheromone with a natural chemical associated with oviposition sites. *Journal of the American Mosquito Control Association*, **10**, 374–379.

Millar, J.G., Chaney, J.D. & Mulla, M.S. (1992) Identification of oviposition attractants for *Culex quinquefasciatus* from fermented Bermuda grass infusions. *Journal of the American Mosquito Control Association*, **8**, 11–17.

Miller, D.N. & Varel, V.H. (2001) *In vitro* study of the biochemical origin and production limits of odorous compounds in cattle feedlots. *Journal of Animal Science*, **79**, 2949–2956.

Romero, A., Broce, A. & Zurek, L. (2006) Role of bacteria in the oviposition behaviour and larval development of stable flies. *Medical and Veterinary Entomology*, **20**, 115–121.

Schofield, S. & Brady, J. (1997) Effects of carbon dioxide, acetone and 1-octen-3-ol on the flight responses of the stable fly, *Stomoxys calcitrans*, in a wind tunnel. *Physiological Entomology*, **22**, 380–386.

Schofield, S., Cork, A. & Brady, J. (1995) Electroantennogram responses of the stable fly, *Stomoxys calcitrans*, to components of host odour. *Physiological Entomology*, **20**, 273–280.

Skubatz, H., Kunkel, D.D., Howald, W.N. *et al*. (1996) The *Sauromatum guttatum* appendix as an osmophore: excretory pathways, composition of volatiles and attractiveness to insects. *New Phytologist*, **134**, 631–640.

Städler, E., Renwick, J.A.A., Radke, C.D. & Sachdev-Gupta, K. (1995) Tarsal contact chemoreceptor response to glucosinolates and cardenolides mediating oviposition in *Pieris rapae*. *Physiological Entomology*, **20**, 175–187.

Stange, G. (1999) Carbon dioxide is a close-range oviposition attractant in the Queensland fruit fly *Bactrocera tryoni*. *Naturwissenschaften*, **86**, 190–192.

Steullet, P. & Guerin, P.M. (1994) Identification of vertebrate volatiles stimulating olfactory receptors on tarsus I of the tick *Amblyomma variegatum* Fabricius (Ixodidae) I. Receptors within the Haller's organ capsule. *Journal of Comparative Physiology, A: Sensory, Neural, and Behavioral Physiology*, **174**, 27–38.

Stork, M.G. (1979) The epidemiological and economic importance of fly infestation of meat and milk producing animals in Europe. *Veterinary Record*, **105**, 341–343.

Syed, Z. & Guerin, P.M. (2004) Tsetse flies are attracted to the invasive plant *Lantana camara*. *Journal of Insect Physiology*, **50**, 43–50.

Vale, G.A. (1980) Field studies of the responses of tsetse flies (Glossinidae) and other Diptera to carbon dioxide, acetone and other chemicals. *Bulletin of Entomological Research*, **70**, 563–570.

Zumpt, F. (1973) *The Stomoxyine Biting Flies of the World*, pp. 175. Gustav Fisher Verlag, Stuttgart.

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