House Flies and Pig Manure Volatiles: Wind Tunnel Behavioral Studies and Electrophysiological Evaluations[1]

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ABSTRACT Nine compounds from the headspace of fresh pig manure were identified as electrophysiologically active on virgin female house fly (*Musca domestica* L.) antennae, and mixtures of these compounds attracted female house flies in wind-tunnel behavioral assays. Identification was accomplished by using coupled gas chromatographic-electroantennographic (GC-EAG) recordings, coupled gas chromatographic-mass spectrometric (GC-MS) analysis, electroantennographic (EAG) assays of standards, and wind tunnel behavioral studies. The pig manure volatiles eliciting responses from female antennae were butanoic acid, 3-methylbutanoic acid, dimethyldisulfide, dimethyltrisulfide, dimethyltetrasulfide, phenol, benzeneethanol, indole, and 3-methylindole. In EAG dose-response tests butanoic acid, 3-methylbutanoic acid, indole, and 3-methylindole elicited the highest responses on female house fly antennae, and dimethyltrisulfide, phenol, and benzeneethanol elicited more moderate responses. In wind-tunnel behavioral studies, female house flies flew upwind in the plume and landed on the source in response to pig manure volatiles. The ability of individual EAG-active compounds to attract female house flies was not significantly greater than that of the control. However, two mixtures, one comprised of seven EAG-active compounds and a second made up of only three compounds, were capable of attracting female flies in a similar manner as found with pig manure. The behavioral significance of these compounds as potential attractants is discussed.

KEY WORDS Diptera, Muscidae, *Musca domestica*, house fly, pig manure volatiles, gas chromatographic-electroantennographic recordings, mass spectrometry, attractants, wind tunnel, behavior

The house fly (*Musca domestica* L.) enjoys a cosmopolitan distribution. The enormous potential that this species possesses for pathogen transmission is well documented (Greenberg 1973, West & Peters 1973). In addition to transmitting disease the house fly is a major pest in or around livestock, swine, and poultry

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facilities (West 1951). Heavy fly populations not only can cause actual economic losses in production to various classes of livestock but also can cause economic losses due to lawsuits against livestock production units due to the flies, odor, and dust being cited as nuisances (Campbell 1993). Insecticides have been used as the primary method for fly control for the last 100 yr and are still widely employed today. In spite of the large numbers of chemical products available, heavy fly populations are still present at livestock installations and they do disperse into urban areas (Thomas & Skoda 1993). Many house fly control failures have been attributed to insecticide resistance (Meyer et al. 1987, Scott & Georghiou 1985). One of the alternative control methods for house flies is attractants, which can be deployed in the domestic and feedlot environment. However, existing commercial house fly attractant baits have shown mixed results (Browne 1990). Studies on attractants for the house fly are not new and have focused on evaluating the attractancy of single compounds or complex odorant mixtures in olfactometers, small rooms, and the field (West & Peters 1973 and references therein). These studies have demonstrated that mixtures are more attractive than single chemicals (Brown et al. 1961, Mulla et al. 1977, Künast & Günzrodt 1981). The most effective attractants for house flies are natural products, and especially effective are the products of putrefaction and fermentation, the sources of which can serve as oviposition sites and food sources. Dairy products and sugar-containing substances also are considered attractive (Künast & Günzrodt 1981).

Manure and spilled feed are the principal breeding media for the house fly (Skoda & Thomas 1993). Larsen et al. (1966) showed that odor taken from air that has passed through a mixture of water and pig manure was attractive to house flies in an olfactometer, and that of eight different types of manure tested, pig manure was the most favorable site for oviposition. Considerable effort has been expended to identify the compounds emanating from livestock manure. In pig manure, at least 140 different volatile compounds and gases have been identified (Yasuhara & Fuwa 1979, Spoelstra 1980, Yasuhara et al. 1984, O'Neill & Phillips 1992, Chen et al. 1994). There is, however, a paucity of literature regarding the actual volatile compounds in pig manure that are responsible for attracting house flies.

In the present study we report the use of a novel approach to identify possible attractants for the house fly. Pig manure volatiles were analyzed by coupled gas chromatographic-electroantennographic (GC-EAG) and coupled GC-mass spectrometry (GC-MS) assays to isolate and identify those compounds in the headspace of pig manure that are biologically active. We then tested synthetic samples of these compounds, alone and as mixtures, in wind-tunnel behavioral bioassays.

Materials and Methods

Insects. *Musca domestica* were obtained as pupae from a colony maintained at the Department of Entomology, Iowa State University, and kept at 25°C under a 14:10 (L:D) h photoperiod regime until they emerged. Adults were chilled (ca. 90 s at -20°C) and the sexes separated within 12 h after they emerged. Males and females were placed in separate cages and given access to

water and a mixture of dry whole milk and powdered sugar (1:1). Flies used in experiments were between 5 and 16 d old.

Collection of pig manure volatiles. Pig manure, comprised of pig urine and feces, was gathered immediately after excretion. The manure was divided into 2.5-g samples that were placed in 2-dram (7.4-ml) vials filled with 2.5 g of HPLC-grade water and capped with Teflon-coated septa (Supelco, Inc., Bellefonte, Pennsylvania). Fifty-gram samples also were prepared and placed in 100-ml polypropylene bottles filled with 50 g of HPLC-grade water. All samples were stored at -20°C.

Headspace collections of volatiles from the pig manure were obtained by two different methods. The first method, which does not require the use of solvents, collected pig manure volatiles directly from 2-dram sample vials by solid phase microextraction (SPME) (Supelco, Inc., Bellefonte, Pennsylvania). The SPME unit consisted of a fiber coated with 100 μm of polydimethylsiloxane contained within a syringe. The fiber was extruded from the syringe and inserted into the headspace of a 2-dram vial containing pig manure for 10 min at room temperature (ca. 25°–28°C), after which it was retracted and immediately inserted into the injector port of a GC (HP 5890 gas chromatograph, Hewlett-Packard Co., Palo Alto, California) where it was thermally desorbed. For the second method, a 50-g pig manure sample was placed in a two-necked 250-ml flask. Charcoal-filtered air was blown over magnetically stirred manure at a rate of 3 ml/s for 16 h at room temperature. Volatiles were collected in a glass tube (10 cm long × 4 mm ID) containing 50 mg of pre-cleaned Tenax TA (Alltech Associates, Inc., Deerfield, Illinois) placed between two glass wool plugs. The Tenax TA trap was desorbed with 1 ml of redistilled HPLC-grade hexane. Each sample was concentrated to ca. 200 μl under nitrogen and immediately analyzed by GC-EAG and GC-MS.

Chemical analyses. One-microliter aliquots of the Tenax TA-collected pig manure volatiles were injected in splitless mode onto 30 m × 0.25 mm ID fused silica capillary gas chromatographic columns, coated either with DB-1 or DB-225 (J & W Scientific, Folsom, California) for analysis by GC-EAG and GC-MS. Column conditions were as follows: He carrier gas flow of 1.5 ml/min, injector temperature 250°C, 1 min delay on inlet purge, 4 min at 35°C then 25°C/min to 320°C (DB-1) or 15°C/min to 220°C (DB-225). The SPME samples were analyzed by using the above mentioned conditions with a 4-min delay on inlet purge.

GC-MS analyses were performed by using the HP 5890 gas GC with a direct interface to a Hewlett-Packard 5970 mass selective detector (electron impact, 70 eV).

Electroantennogram responses. Simultaneous GC-EAG and EAG analyses were performed according to standard methods (e.g., Cossé et al. 1995, Baker et al. 1991) by using a HP 5890 GC. EAG recordings were made by inserting a glass pipette Ag/AgCl saline electrode (World Precision Instruments, Sarasota, Florida) in the back of an excised house fly head. A second saline recording electrode was placed in contact with the distal end of the funiculus of one of the antenna. Both pipettes were filled with Beadle-Ephrussi (Ephrussi & Beadle 1936) saline. To examine antennal sensitivity to the identified pig manure volatiles, EAGs that were not coupled to the GC were

recorded in response to a dose-response series of commercial compounds. Serial dilutions of the tested compounds were made in redistilled HPLC-grade methylene chloride such that the tested compounds were applied to filter-paper-strips (0.5 cm × 3.0 cm, Whatman No. 1) in 10 µl of solvent. The filter-paper-strips were placed inside glass Pasteur pipettes (15 cm long). The antenna was continuously flushed with a charcoal-filtered and moistened airstream. The air, flowing at a rate of 20 ml/s, was delivered through a glass tube (8 mm ID) ending 10 mm in front of the preparation. Two milliliters of the atmosphere of the stimulus pipette was puffed into the constant airstream by a mechanical puffing device (Syntech, Hilversum, The Netherlands), delivering 0.1-s puffs. The stimulus was injected into the airstream 15 cm upstream from the antenna. Control puffs (filter paper plus solvent) were presented to each EAG preparation before and after the test compounds. To compensate for possible deterioration of the preparation and differences in quality of the electrical connection, EAG amplitudes were normalized by dividing the amplitude of the EAG generated from a test compound by the mean of the control response. Each value thus yielded an estimate of relative EAG amplitude. Within a particular series of test compounds, presentation of the test compounds was randomized. EAG data were subjected to ANOVA and mean responses were compared by using the LSD method (Sokal & Rohlf 1981).

Chemicals. Butanoic acid, 3-methylbutanoic acid, 3-methylindole, indole, 3-methylphenol, 4-methylphenol, and benzeneethanol were obtained from Sigma Chemical Co. (St. Louis, Missouri) and were >99% pure (label information and GC analyses). Phenol, δ-3-carene, (±)-α-pinene, (1S)-(-)-β-pinene, and 4-ethylphenol were purchased from Aldrich Chemical Co. (Milwaukee, Wisconsin) and were >95% pure. Dimethyltrisulfide was obtained from Narchem Corp. (Chicago, Illinois) and was >99% pure. The dimethyltetrasulfide, purchased from Oxford Organics, Inc. (Elizabeth, New Jersey) was a mixture of dimethyltetrasulfide (33%) and dimethyltrisulfide (50%) based on GC-MS comparisons with the commercial sample of dimethyltrisulfide.

Wind tunnel. Behavioral assays were conducted in a modified 2.4 m × 1.0 m × 1.0 m wind tunnel described previously by Miller & Roelofs (1978). The wind speed was adjustable (0 to 100 cm/s) and was set at 50 cm/s. The temperature in the wind tunnel was 23 ± 2°C. White paper covered the outside of the tunnel to eliminate potential distractions to flies and diffused the light coming from four pairs of white fluorescent lamps running the length of the tunnel top and positioned 1 m above the ceiling of the tunnel. A visual pattern consisting of 6-cm-diam red vinyl dots was placed underneath the transparent tunnel floor. Small flaps covering peep holes in the white-paper paneling were opened from time to time to allow for observations of the flies during experiments. The flight tracks of the flies, together with the audio observations of the experimenter, were recorded onto cassette tape by using a Toshiba KV 6300A recorder and a Sony M374 black-and-white video camera located ca. 50 cm above the ceiling of the tunnel. The field of view for all recordings was 1.2 m long extending from 55 cm to 175 cm downwind from the upwind end of the tunnel and extending the width of the tunnel. Recordings of fly behaviors were played back in slow motion through a 47.5-cm black-and-white Panasonic WV 5470 television monitor.

Wind-tunnel behavioral bioassays. Virgin females were placed in groups of five or six in screened release cages (6 cm × 10 cm ID), covered with Petri dish lids, 3 h before being tested, and then were released between 5–8 h into the photophase. The release cage was placed on a platform (15 cm × 15 cm) 60 cm away from the downwind screen and 25 cm above the tunnel floor. The Petri dish lid was attached to a wire that was pulled up from the tunnel top ca. 15 s after the introduction of the odor source. The clear Petri dish lid together with its friction-free rapid removal prevented the evocation of an escape response in the flies (Holmqvist & Srinivasan 1991). The odor source, formulated in 10 ml of water, was introduced in a Petri dish (1.5 cm × 10 cm ID) placed on a platform 60 cm away from the upwind screen and 25 cm above the tunnel floor. The odor source was presented as a turbulent plume (as visualized by $TiCl_4$ smoke prior to the bioassays) that moved over and through the release cage. The distance between the release cage and odor source was 1 m. In addition to video recording, the behavior of flies was recorded by the experimenter from the side, who observed whether or not the flies were flying at the height of the odor plume and also gave additional information about the 3-dimensional aspects of the flight tracks. Tracks were recorded for 4 min after the removal of the Petri dish cover. Upon analysis during replay those tracks in which flies exhibited plume-oriented upwind flight behavior were scored for the upwind flight distance attained by the flies (either 60 cm or 95–100 cm from the release point) and also for whether or not the flies landed on the source. The upwind progress score was calculated per fly unit, with a fly unit expressed as the total number of plume-oriented upwind flights per group divided by the number of individual flies per group. Odor sources were chosen at random, each source was tested on one single day, and the experiments were performed for 12 consecutive days. The data were subjected to ANOVA and means were compared by using the T-method (Sokal & Rolhf 1981).

Flight track analyses. Individually released females had their upwind flight tracks video-recorded from ca. 50 cm above the tunnel ceiling by using two Sony M374 black-and-white video cameras connected to two Toshiba KV 6300A video recorders. Measuring the total field of view of the partial overlapping camera views at the level of a $TiCl_4$ smoke plume (25 cm above the wind tunnel floor) yielded a 1.0 m (1.4 m rectangular area, which extended from 40 cm to 180 cm downwind from the upwind end of the tunnel. Only flight tracks of females that flew upwind in the plume and contacted the pig manure (2.5 g in 10 ml of H_2O) odor source were analyzed. The recordings of the female flight tracks were played back frame-by-frame through a 47.5-cm black-and-white Panasonic WV 5470 television monitor. Every other frame (each 1/30th s) together with three points of reference on the wind tunnel floor and the fly's location were captured by using a computer video frame-grabber board (Odavision4, Hammerkop Co., Massachusetts). The X and Y coordinates of the fly location in a two-dimensional plane were obtained by using digitizing Mantid computer software (Hammerkop Co., Massachusetts) and were analyzed for net upwind flight speed. The release of the individual females was similar to that described for the wind-tunnel bioassays with the exception that the release cage and odor source were placed 50 cm away from the tunnel ends, creating a distance of 1.2 m between release cage and odor source.

Results

Analyses of pig manure volatiles. Twenty-two GC-EAG analyses of the volatiles collected from pig manure were obtained by using 22 different female house fly antennae. Only those GC peaks that consistently revealed simultaneous EAG activity were targeted for further analysis. GC-EAG analyses consistently revealed eight GC peaks on both DB-1 and DB-225 columns with corresponding EAG activity on female house fly antennae (Fig. 1 and Table 1, peak no. 3, 4, 7, 9, 15, 17, 18, and 19). The retention times on both columns and GC-MS spectra of peak no. 3, 4, 7, 9, 15, 17, 18, and 19 corresponded precisely to those of eight compounds known to be present in the headspace of pig manure: butanoic acid, 3-methylbutanoic acid, dimethyltrisulfide, phenol, benzeneethanol, dimethyltetrasulfide, indole, and 3-methylindole, respectively. Moreover, combined GC-EAG recordings on both DB-1 and DB-225 columns demonstrated that butanoic acid, 3-methylbutanoic acid, dimethyltrisulfide, phenol, benzeneethanol, dimethyltetrasulfide, indole, and 3-methylindole had retention times identical to peak no. 3, 4, 7, 9, 15, 17, 18, and 19, respectively, from the collected pig manure volatiles, and that these compounds were EAG-active (Table 1). Seven of our 22 GC-EAG analyses showed an additional (ninth) EAG-active peak, and a subsequent GC-MS library search tentatively identified this peak as dimethyldisulfide (Table 1). Comparison between SPME-collected volatiles vs. Tenax TA-collected materials revealed no prominent differences in the number of compounds detected. However, there were differences in the ratios of the compounds collected via each method, and compounds eluting from the capillary columns during the first 3 min of the runs where hidden by the solvent peak in the Tenax collection method. In both methods, 4-methylphenol was always the major compound collected.

Electroantennogram responses. The EAG responses of female house fly antennae to puffs of seven of the GC-EAG-active compounds were recorded at five dosages (Fig. 2). EAG amplitudes increased to puffs from cartridges containing from 0.1 µg up to 100 µg for all of the compounds tested. All responses were significantly higher than those from the control (solvent) cartridges. Even at the lowest dosage tested, the responses to all of the compounds were at least 1.5-times greater than to those of the control. Analysis of variance of the antennal responses indicated that there were significant differences not only between the responses of the flies to the various doses tested (F= 227.34, df= 4, 315; $P<<$ 0.001) and between the different chemicals (F= 73.76, df= 6, 315; $P<<$ 0.001) but also that the interaction between doses and chemicals was significant (F= 21.96, df= 24, 315; $P<<$ 0.001). Furthermore, the data show that, of the compounds tested, butanoic acid elicited the highest EAG responses at all dosages except 0.1 µg. Conversely, benzeneethanol elicited the lowest responses at all dosages except 0.1 µg (Fig. 2). The EAG responses elicited by 3-methylindole and indole were not significantly different from each other at all dosages tested. The dose-response curves also indicate that maximum responses had been reached at the 100-µg dosage with butanoic acid, 3-methylbutanoic acid, 3-methylindole, and dimethyltrisulfide, whereas responses to the 1,000-µg dosage of indole, phenol, and benzeneethanol had still not peaked (Fig. 2).

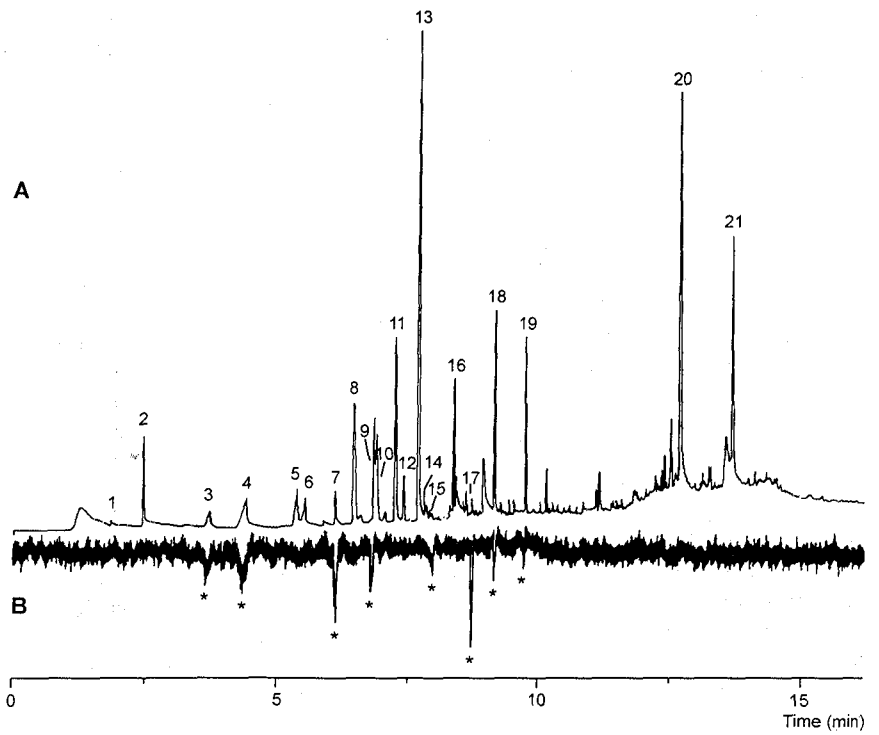

Fig. 1. Simultaneously recorded gas chromatogram of pig manure volatiles collected by solid phase microextraction (A) and electroantennogram of a female house fly antenna (B). Electrophysiologically active compounds are labeled with an asterisk. Number labels indicate identified materials (see Table 1).

Wind-tunnel behavioral assays. Virgin female house flies readily flew upwind to volatiles emitted from pig manure: 44 landings on the source were recorded using four groups of flies (totaling 22 flies released) (Fig. 3). Female flies also flew upwind and landed on the source in response to volatiles emitted from mixtures of chemicals identified from the headspace of pig manure. In instances where extended observations of individual flies were possible, flies made several upwind-oriented flights in the plume of varying distances and made several successful landings on the source during the 4-min response time. Typically, after removal of the Petri dish cover, the quiescent female would remain inactive for several seconds before flying upwind in a zigzag pattern

Table 1. Analytical results of pig manure volatiles.

Peak no.[a]	Compound	GC-EAG active[b]	Identification[c]
1	dimethyldisulfide	+	MS
2	acetic acid	-	MS
3	butanoic acid	+	GC & MS
4	3-methylbutanoic acid	+	GC & MS
5	pentanoic acid	-	MS
6	hexanoic acid	-	MS
7	dimethyltrisulfide	+	GC & MS
8	α-pinene	-	GC & MS
9	phenol	+	GC & MS
10	β-pinene	-	GC & MS
11	δ-3-carene	-	GC & MS
12	limonene	-	MS
13	4-methylphenol	-	GC & MS
14	3-methylphenol	-	GC & MS
15	benzeneethanol	+	GC & MS
16	4-ethylphenol	-	GC & MS
17	dimethyltetrasulfide	+	GC & MS
18	indole	+	GC & MS
19	3-methylindole	+	GC & MS
20	hexadecanoic acid	-	MS
21	octadecanoic acid	-	MS

[a]Numbers correspond to labeled peaks in Fig. 1.
[b]Gas chromatographic-coupled-electroantennogram (GC-EAG) (see also Fig. 1); +, active; -, not active.
[c]Identification is based on a mass spectrum library search (MS) and/or comparison of mass spectra of the natural material with that of the identified synthetic compound (GC & MS).

toward the pig manure odor source. Females that upon release flew upwind in the plume and landed directly on the source exhibited a mean net upwind flight speed of 40.4 cm/s (±14.4 SD, n= 4) (Fig. 4). Of the females released in groups of five or six, only a few flew directly to the manure, the majority flying toward the source in the plume and aborting their upwind progress at various distances downwind of the source. Those females that landed on the source were observed to feed. Analysis of variance of the behavioral responses showed that there were significantly more upwind flights and landings on the source with pig manure than with the water control (Table 2). The attractancy of individual EAG-active compounds was not significantly different than that of the control. However, two mixtures, one comprised of seven EAG-active compounds and a second made up of equal amounts of butanoic acid, 3-methylindole, and dimethyltrisulfide, elicited upwind flights at a rate not significantly different than that to pig manure. However, the rate of landing on the source was lower than that evoked by pig manure. The frequencies of

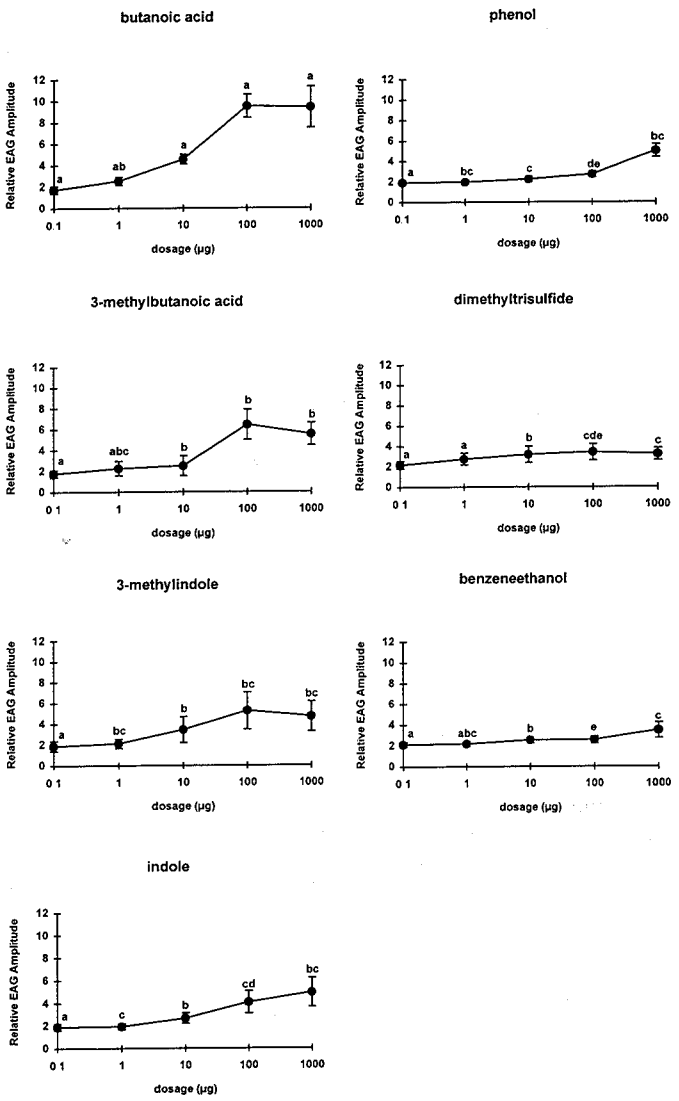

Fig. 2. Electroantennogram (EAG) dose responses of female house fly antennae to selected known volatile compounds released by pig manure. Stimuli were synthetic standards. EAG amplitudes were normalized by dividing the amplitude of the EAG generated from the test compounds by the mean of the control responses. Standard deviations of mean responses are presented as error bars. EAG responses for each dosage were compared by ANOVA. Dosage means between the different stimuli with the same letter are not significantly different by the LSD method (Sokal & Rohlf 1981) at $P < 0.05$.

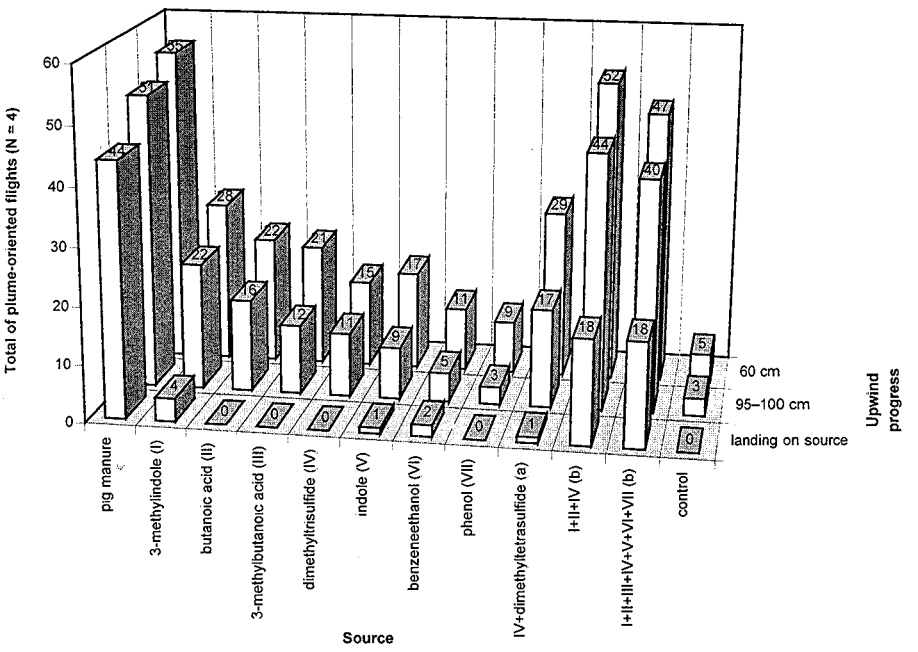

Fig. 3. Wind-tunnel behavioral responses of female house flies to pig manure, synthetic pig manure volatiles, and mixtures of synthetic pig manure volatiles. Odor sources were formulated in 10 ml of water at dosages of 20 μg except for pig manure (2.5 g). (a) ratio of 1.5 : 1, 20 μg total amount and (b) ratios of 1 : 1, 20 μg of each compound. Females were released in four groups, each containing five or six flies, totalling 22 flies for each treatment.

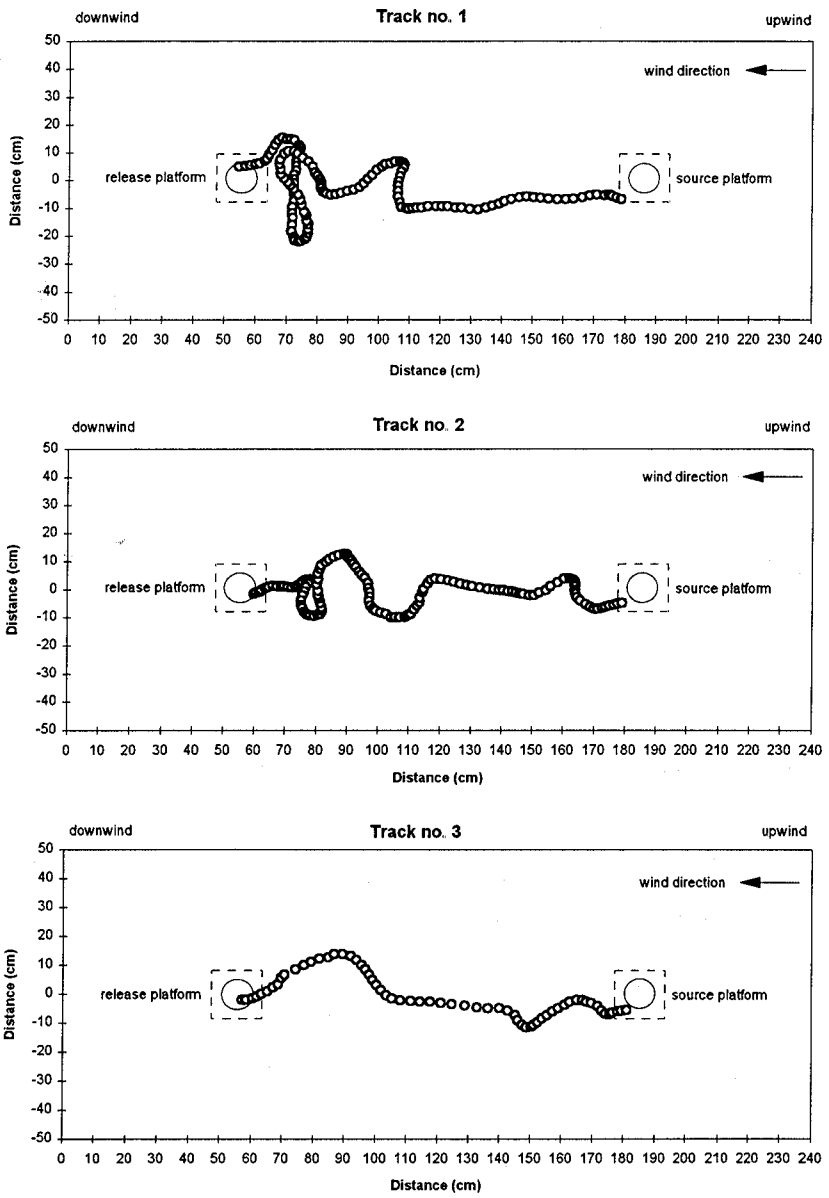

Fig. 4. Three flight tracks of flying *M. domestica* females in response to pig manure odor (2.5 g in 10 ml of H_2O). Large rectangular boxes represent the wind-tunnel floor area. Wind speed was set at 50 cm/s. Dots are 1/30th s apart.

Table 2. Wind tunnel behavioral responses of house flies to pig manure, individual pig manure volatiles, and mixtures of pig manure volatiles.

Source[b]	Groups[c]	Plume-oriented upwind progress/fly unit[a]		
		60 cm	95-100 cm	landing on source
		mean ± SD[d]	mean ± SD[d]	mean ± SD[d]
pig manure[e]	4	2.50 ± 0.69a	2.33 ± 0.48a	2.01 ± 0.55a
3-methylindole (I)	4	1.23 ± 0.91ab	0.98 ± 0.62bcd	0.18 ± 0.14c
butanoic acid (II)	4	1.00 ± 0.32ab	0.74 ± 0.29bcd	0c
3-methylbutanoic acid (III)	4	0.88 ± 0.94ab	0.51 ± 0.48bcd	0c
dimethyltrisulfide (IV)	4	0.68 ± 0.34ab	0.48 ± 0.23bcd	0c
indole (V)	4	0.76 ± 0.38ab	0.41 ± 0.26cd	0.04 ± 0.08c
benzeneethanol (VI)	4	0.53 ± 0.32b	0.24 ± 0.25d	0.09 ± 0.11c
phenol (VII)	4	0.44 ± 0.53b	0.15 ± 0.30d	0c
IV+dimethyltetrasulfide (1.5:1)[f]	4	1.38 ± 0.90ab	0.80 ± 0.38bcd	0.04 ± 0.08c
I+II+IV (1:1:1)	4	2.34 ± 1.40a	1.97 ± 1.14ab	0.80 ± 0.42b
I+II+III+IV+V+VI+VII (1:1:1:1:1:1:1)	4	2.12 ± 1.42ab	1.78 ± 1.27abc	0.81 ± 0.55b
control	4	0.24 ± 0.25b	0.15 ± 0.19d	0c

[a] Fly unit is expressed as the total number of plume-oriented flights per group divided by the number of individual flies per group.
[b] 20 µg suspended in 10 ml of H_2O.
[c] Four groups of 5 or 6 females each, totalling 22 females.
[d] Means with the same letter are not significantly different by the T-method (Sokal & Rohlf 1981) at $P < 0.05$.
[e] 2.5 g suspended in H_2O.
[f] 20 µg in total.

upwind flight and landing on the source in response to these synthetic mixtures were, however, significantly higher than that of the control.

Discussion

House fly attractants derived from mammalian excretory products have been the subject of many studies (West & Peters 1973 and references therein). However, these attractant studies were performed with mixtures of complex substances or empirical compositions, without any systematic approach to an understanding of the actual chemical stimuli inducing responses in the flies. Our results are based on, for the house fly, novel approaches that allowed us to identify electrophysiologically active compounds in the headspace of pig manure and to test their efficacy in attracting house flies in a wind-tunnel behavioral assay.

The GC-EAG analyses demonstrated that female house fly antennae selectively responded to only nine of the numerous compounds that were present in the headspace of pig manure. We identified these compounds as butanoic acid, 3-methylbutanoic acid, dimethyldi-, tri-, and tetrasulfide, phenol, indole, 3-methylindole, and benzeneethanol. All nine electrophysiologically active compounds have been previously identified in pig manure (Yasuhara & Fuwa 1979, Spoelstra 1980, Yasuhara et al. 1984, O'Neill & Phillips 1992, Chen et al. 1994), and with the exception of benzeneethanol, are key components of the objectionable or malodorous nature of swine waste (Spoelstra 1980, O'Neill & Phillips 1992, Chen & Liao 1994, Chen et al. 1994). Our wind-tunnel behavioral assays showed that two mixtures, one comprised of seven EAG-active compounds and a second containing butanoic acid, 3-methylindole, and dimethyltrisulfide, were capable of attracting female flies at a rate similar to that of pig manure. However, the attractancy of individual EAG-active compounds was not significantly greater than that of the control.

The solid phase microextraction (SPME) headspace sampling with GC separation provided a useful method for analyzing volatiles emitted by pig manure. The use of this technique overcomes many of the drawbacks that can occur with more standard methods, and all of the GC-EAG spectra we obtained exhibited a high degree of uniformity. However, GC-EAG analyses serve only as a first step in any attempt to identify behaviorally active compounds present in the pig manure. In this study we did not attempt to quantify or qualify all volatile substances emitted by pig manure, nor did we attempt to evaluate the efficiency of the SPME sampling method. Therefore, some electrophysiologically active compounds may have gone undetected due to the relatively low amounts eluting from the GC column and/or due to their inability to be adsorbed onto the SPME polymer needle.

Several of the identified EAG-active compounds also have been found to evoke antennal responses in other dipterans. Tsetse flies (*Glossina* spp.) are attracted to buffalo and ox urine, and both EAG and single-cell recordings have shown that attractiveness of urine can be attributed to the presence of phenol, 3- and 4-methylphenol, 3- and 4-ethylphenol, as well as 4-*n*-propylphenol (Den Otter 1991, Den Otter et al. 1993, Saini & Hassanali 1992). Similar EAG work done on the stable fly, *Stomoxys calcitrans* (L.), demonstrated the

electrophysiological activity of 3-methylphenol (Schofield et al. 1995), but responses to butanoic acid as well as to the earlier published EAG-active acetic acid (Warnes & Finlayson 1986) were not significant. All of these phenolic compounds, with the exception of 4-*n*-propylphenol, have been identified in swine waste (Spoelstra 1980, O'Neill & Phillips 1992), and at least four of these compounds (phenol, 3- and 4-methylphenol, and 4-ethylphenol) have been positively identified in our SPME samples of pig manure. However, 4-methylphenol, one of the most important components in an attractant blend for *Glossina* species did not elicit any EAG response with female house fly antennae, even though it is the most abundant component in the pig manure (Fig. 1). Likewise, no significant EAG responses occurred when 3-methylphenol or 4-ethylphenol eluted from the GC column. The only phenolic compound that did elicit a significant response with female house fly antennae was phenol (Table 1). In dose-response series, EAGs from female house flies only started to increase at the 1 mg dosage level, suggesting that there are fewer receptors for phenolic compounds on the antennae of house flies than on stable fly and tsetse fly antennae.

Cork (1994) identified several EAG-active compounds from larval wound fluid that elicited responses from screwworm, *Cochliomyia hominivorax* (Coquerel), antennae. The array of compounds identified from wounds was similar to that found in the headspace of our pig manure samples. EAGs from *C. hominivorax* in response to a homologous series of aliphatic carboxylic acids showed that acids with C_4- and C_5-carbon chain lengths elicited the strongest responses. In addition, Cork (1994) showed that relatively strong EAG responses were evoked with 1-octen-3-ol, 3-methylphenol, indole, phenol, dimethyldisulfide, and 3-methylindole, even though the latter was not present in larval wound fluid. Benzeneethanol present in the larval wound fluid was mentioned to be EAG active. Several of the volatile fatty acids tested with *C. hominivorax* also were present in the pig manure samples (Table 2), but only butanoic acid and 3-methylbutanoic acid elicited a response from female house fly antennae. Additional EAG dose-response tests will have to be performed to confirm that house fly antennae predominately respond to aliphatic carboxylic acids with C_4-carbon chain lengths.

Our wind-tunnel behavioral assay demonstrated that female house flies fly upwind to and land on a source of a mixture of seven of the nine electrophysiologically active pig manure volatiles as well as to a three-component mixture. The observed flight behaviors of the flies to pig manure and to the synthetic mixtures exhibited striking similarities to those of male moths flying upwind towards their conspecific sex pheromones. That is, quiescent female flies would, at the introduction of the odor source, walk around the release cage for several seconds, after which they flew upwind in the odor plume in an oscillating zigzag pattern in the direction of the odor source (Fig. 4). Whether house flies are indeed using flight mechanisms similar to those of moths (Baker 1989, Vickers & Baker 1994) to progress upwind towards fecal odor sources might be addressed in a future study.

Most studies on house fly attractants have shown mixtures to be better attractants for house flies than single compounds. However, house flies can be attracted to single odor sources of indole, 3-methylindole, or butanoic acid in

the field (Brown et al. 1961, Frishman & Matthysee 1966, Mulla et al. 1977). Our wind-tunnel behavioral assays did not show any significant attractancy for the individually tested compounds. This might be due to the differences in concentration between prior studies employing field trapping (at least 25- to 250-fold higher) and our wind-tunnel assays.

An indication that our identified compounds have potential as an effective house fly bait comes from work done on the chemical attractant for screwworms, swormlure-4 (Jones et al. 1976, Coppedge et al. 1977, Mackley & Brown 1984). This attractant contains four compounds that have been identified in our study; butanoic acid, indole, phenol, and dimethyldisulfide. Research performed on nontarget insects attracted to an older formulation of swormlure-4, swormlure-2 (Coppedge et al. 1977), showed that of the 168 collected insect species (totaling 4,640 insects), 4% were in the family Muscidae (Peterson et al. 1981). In addition, preliminary trapping tests in a domestic environment comparing commercially available house fly traps baited with either a mixture of sugar and house fly pheromone or with a mixture containing butanoic acid, 3-methylindole, and dimethyltrisulfide showed that house flies are more attracted to the synthetic mixture than to the sugar-pheromone baited trap (A. A. Cossé & T. C. Baker, unpubl. data). However, further research will be needed to determine whether the identified compounds can be formulated into an effective house fly bait.

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