Behavioural and electrophysiological responses of females of two species of tabanid to volatiles in urine of different mammals

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Abstract. Urine volatiles from different ungulates (cows, horses and sheep) were tested as bait for tabanids in southeastern France using Nzi traps during the early summer of 2011. Tabanus bromius Linnaeus, 1758 and Atylotus quadrifarius (Loew, 1874) (both: Diptera: Tabanidae) were the most captured species, respectively representing 57% and 41% of all tabanids collected (all of which were female). Horse urine significantly increased catches of T. bromius (1.6-fold) and A. quadrifarius (3.5-fold), and sheep urine significantly increased catches of A. quadrifarius (2.5-fold). In parallel, an electroantennogram (EAG) study was conducted for the first time on these two species, in which EAGs were recorded using 1-octen-3-ol and extracts of the same urine samples used in the field. For T. bromius, the EAG response to 1-octen-3-ol increased quasi-sigmoidally with dose, with a maximum response at ≥100 μg on filter paper. For both species of tabanid, cow and horse urine elicited larger EAGs than did sheep urine. The behavioural implications in host-seeking and feeding habits are discussed.

Key words. Tabanidae, baits, electroantennography, host odours, Nzi trap, octenol, olfaction, urine.

Introduction

In their quest for important resources such as mates, nutrients and oviposition and resting sites, insects rely on olfaction to a large extent. Although olfaction has been studied in many insect species, comprehensive data come mainly from a few groups, such as cockroaches, moths, vinegar flies, honeybees and mosquitoes (de Bruyne & Baker, 2008). In hematophagous Diptera, odours are among the primary external cues affecting host-seeking and feeding behaviour. Olfactory mechanisms have been well characterized in mosquitoes, which are important vectors of human diseases such as yellow fever, dengue and malaria (Zwiebel & Takken, 2004), but other biting insects, such as tabanids, which are vectors of principally veterinary importance, have been less studied.
Canary Islands (Gutierrez et al., 2010). Tabanids and stable flies are likely to have transmitted the parasite among the animals, showing the potential risk for the transmission of exotic pathogens by biting flies in Europe.

Like other haematophagous Diptera, female tabanids can find a host by heat, odour or visual cues (Allan et al., 1987). Tabanids are mainly diurnal and visual stimuli seem to assist their final orientation in host location, whereas olfactory stimuli seem to be used for long- and short-range orientation (Gibson & Torr, 1999). Recently, tabanids were shown to use polarized light reflected from an animal’s coat as a signal in finding a host (Horvath et al., 2010). Most studies that have examined the odour-oriented responses of these insects have been conducted in Africa, where Tabanidae are sympatric with Glossina spp., and in North America. Experiments have mainly consisted of screening known tsetse attractants in outdoor conditions using electric nets or various trapping systems (Gibson & Torr, 1999). Results have shown that aged cow urine, octenol (1-octen-3-ol) and phenols (3- and 4-methylphenol)—but not ketones (including acetone)—are effective attractants for species of Tabanidae, although there are differences in efficacy among groups and species (Gibson & Torr, 1999; Mihok et al., 2007; Mihok & Mulye, 2010). Synergism between ammonia and phenols, a combination that frequently occurs in aged urine, has also been demonstrated for Hybomitra species (Diptera: Tabanidae) in Ontario (Mihok & Lange, 2011).

To characterize the activity of these mammalian kairomones on biting flies, electroantennography (EAG) has previously been used to record chemo-reception on the antennae of Glossina spp. (Diptera: Glossinidae) (Bursell et al., 1988; Den Otter, 1991; Gikonyo et al., 2002) and Stomoxys calcitrans (Linnaeus, 1758) (Diptera: Muscidae) (Schofield et al., 1995; Birkett et al., 2004; Jeanbourquin & Guerin, 2007). It was found that 1-octenol-3-ol, 3-methylphenol and 4-methylphenol were the most electrophysiologically active chemicals for these species. However, prior to the present study, no EAG experiments had previously been conducted in tabanids.

In Europe, the only previous studies of attractants of tabanids were carried out in Croatia, where the efficacy of synthetic and natural substances in catching tabanids was investigated (Krcmar et al., 2006, 2010; Krcmar, 2007). Aged animal urine, 4-methylphenol, and a mixture of octenol, acetone and ammonia appeared to be effective attractants.

To better understand the involvement of odour stimuli in host location and host choice of temperate tabanids, the present study coupled, for the first time, a field experiment with EAG tests. We chose to compare the aged urine of different ungulates as natural attractants because aged urine is a blend of active compounds, is host-specific (Madubunyi et al., 1996) and has a composition that varies with host physiology (Torr et al., 2006). The aim of the study was to evaluate the attractiveness of urine from different mammal species in a Mediterranean steppe environment that has been exclusively grazed by sheep for more than 20 centuries, and to record the electrophysiological responses of tabanids to the extracts of the urine samples used in the field.

Providing the first EAG recordings of tabanids has allowed us to investigate the correspondence between their behavioural and physiological responses to host odours. This study has also allowed us to look at the host-seeking and feeding behaviours of tabanids from a new angle.

Materials and methods

Field experiment

Study site. The field experiment was conducted in the Coussouls de Crau Nature Reserve in southeastern France. La Crau is a unique French landscape of semi-arid pastures. The selected site for the study was the Peau de Meau sheepfold (43°33'N, 04°50'E; elevation 10 m a.s.l., area 160 ha), which has dry steppe vegetation with 50% stone cover (see Google Earth). This region is characterized by a Mediterranean climate with high interannual variability, low rainfall (400–500 mm/year) with maximum precipitation in spring and autumn, long hot summers and mild winters (mean annual temperature 14 °C). On average, the sun shines 3000 h per year and a very strong predominant wind (the mistral) blows from the northwest 334 days per year, thus increasing evapotranspiration (Blight et al., 2011). In the plain, flocks of Merinos d’Arles sheep are rotated from hay fields (fourth cutting) in autumn to the steppe in spring and transhumance to the Alps for the summer is carried out by truck (Buisson & Dutoit, 2006). During the study, no sheep were present in the Peau de Meau sheepfold, but flocks were present in other sheepfolds that were <1 km distant.

La Crau was a choice study site. Firstly, sheep grazing has been the traditional form of land use in this region for many centuries and thus sheep are the quasi-exclusive host for haematophagous Diptera here. Secondly, the area is wide open and has only herbaceous plants and thus there are no high woody plants to disturb either the visual cues of Nzi traps from a distance (Mihok, 2002) or the movement of air; this supports the reliability of directional cues available to tabanids orienting towards an odour source (Gibson & Torr, 1999).

Meteorological data. Investigations into meteorological effects on the daily activity of tabanids have shown that different species respond differently to weather variables. The meteorological variables that influence the activity of tabanids are wind velocity, temperature, relative humidity (RH) or evaporation, atmospheric pressure, and sky radiation or cloud cover (Burnett & Hays, 1974; Dale & Axtell, 1975; Alverson & Noblet, 1977; Van Hennekeler et al., 2011). In the present study, daily climate data were obtained from the meteorological station at Istres, which is located 20 km from the study site. The weather changed during the experiment, with alternating sunny and cloudy days. Maximum daily temperatures ranged from 25.9 °C to 30.7 °C, mean wind chill temperatures ranged from 24.2 °C to 29.9 °C and mean RH ranged from 37% to 64%. Wind chill considers the effect of wind on heat loss from exposed skin (Bluestein et al., 2002). There was no precipitation except on 22 June 2011 (0.2 mm) and 23 June 2011 (0.4 mm). The maximum wind speeds were in
Trapping and experimental design. New Nzi traps were used as standard traps (Fig. 1). The Nzi trap is a multipurpose trap for biting flies designed by Mihok (2002). The blue and black components of the traps were made from the same fabric (SuperMaine 300 g cotton/polyester 65/35%; TDV Industries, Laval, France). The cone and the back of the trap were made from polyester mosquito netting.

The experiment consisted of setting up one 4 × 4 Latin square maintained in place during 14–17 June 2011 and a second identical Latin square maintained in place during 20–23 June 2011. Four Nzi traps were set up in the open facing west at 200-m intervals along a line running northeast to southwest, perpendicular to the predominant wind and parallel to the closest canal. This alignment was chosen firstly because flies approach a host from downwind (Phelps & Vale, 1976) and secondly because the Nzi trap has a single entrance and thus is dependent on its orientation to the sun. The highest catches of tabanids have been found to occur in traps facing west (Mihok et al., 2006).

Three traps were baited with urine and one trap was left unbaited as a control. On the first day, 50 mL of aged urine was placed in a plastic vial with a 2.5-cm aperture and the vial was placed in the back corner of each trap. Baits and controls were randomly moved every day.

The bait urine was collected directly from the bladders of several adult animals in a slaughterhouse (sheep urine sample was a mixture pooled from 10 ewes; cow urine was pooled from 30 cows and one ox; horse urine was pooled from 10 mares and one gelding). The urine was collected from adults because young animals have lower levels of phenols in their urine (Torr et al., 2006). Each sample of urine was kept for 3 weeks at room temperature (20–22 °C) in a 1.5-L plastic bottle and was then stored at −20 °C in 250-mL bottles.

Responses of tabanids to urine volatiles

The trap’s collectors containing the tabanids (which were all female) were removed daily and transported to the laboratory. All live tabanids were identified individually and placed in mesh cages (55 × 80 × 40 cm) with access to a 10% sucrose solution provided on thin sponges to prolong their survival (Auroi, 1985). Electroantennogram recordings were conducted on tabanids in good condition for 1–7 days following capture. The ovarian age and the meal status of the females tested were unknown. Electroantennography was not performed in males because only females were collected during this study.

Laboratory experiment

Odour stimuli. Each sample of aged urine was maintained with 50 mL of dichloromethane (1 : 1) in an Erlenmeyer flask for 4 h. These extracts were then aliquoted and stored at −20 °C. 1-octen-3-ol, a well-known attractant for many haematophagous Diptera (Hall et al., 1984; Gibson & Torr, 1999), was used to validate the experimental system. Aliquot dilutions of pure octenol (1 mg/μL) were prepared using dichloromethane (0.1, 0.01, 0.001 mg/μL). Octenol at 0.1 mg/μL was used as a positive control, and dichloromethane was used as a negative control.

Electroantennography. Antennal electrical responses to volatile compounds were recorded with an EAG device (Stimulus controller CS-55 and signal acquisition controller IDAC-2; Syntech, Hilversum, the Netherlands). Recordings were made using electrolyte-filled (0.1 m KCl) glass capillary electrodes, with Ag/AgCl wire making contact with the recording apparatus. The tabanids were first immobilized by cold temperatures (2 min at −20 °C). Electroantennograms were recorded using a technique similar to those previously used in biting flies (Den Otter & Saini, 1985; Den Otter et al., 1988; Schofield et al., 1995; Jeanbourquin & Guerin, 2007). The antenna was excised from the tabanid’s head as the EAG signal has been found to be better without the head (F. Baldacchino and J. Cadier, unpublished data, 2011). The indifferent electrode was inserted into the pedicel of the antenna and the recording electrode was brought into contact with the distal end of the flagellum. The majority of olfactory sensilla (described in females of four species of tabanid) are present on the first segment of the flagellum (Parashar et al., 1994). Furthermore, previous electrophysiological experiments have shown the presence of gustatory chemosensilla at the tip of the tabanid antennae (Stoffolano et al., 1990).

The antenna was maintained in a humidified charcoal-filtered air stream delivered at 13.5 mL/s through a metal tube. Each stimulus was prepared by depositing 10 μL of the tested solution on a strip of filter paper (40 × 5 mm) placed in a glass Pasteur pipette. The solvent was allowed to evaporate for 15 min before the first use and the same pipettes were used for four antennae from different individuals (corresponding to 1 h of the experiment). The tip of the pipette was connected to the metal tube, and the stimulus was delivered to the antenna using an air pulse (20 mL/s for 0.8 s). Stimuli were released successively in random order at 60-s intervals to avoid receptor saturation.

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Table 1. Female tabanids collected by Nzi traps in the Peau de Meau sheepfold, June 2011.

<table>
<thead>
<tr>
<th>Species</th>
<th>Total, n</th>
</tr>
</thead>
<tbody>
<tr>
<td>Tabanus bromius Linnaeus, 1758</td>
<td>3999</td>
</tr>
<tr>
<td>Atylotus quadrifarius (Loew, 1874)</td>
<td>2920</td>
</tr>
<tr>
<td>Atylotus flavoguttatus (Szilady, 1915)</td>
<td>62</td>
</tr>
<tr>
<td>Tabanus regularis Jaenicke, 1866</td>
<td>38</td>
</tr>
<tr>
<td>Tabanus recurus Loew, 1858</td>
<td>11</td>
</tr>
<tr>
<td>Hybomitra ciureai (Seguy, 1937)</td>
<td>4</td>
</tr>
<tr>
<td>Hybomitra expollicata (Pandelle, 1883)</td>
<td>1</td>
</tr>
<tr>
<td>Tabanus cordiger Meigen, 1820</td>
<td>1</td>
</tr>
<tr>
<td>Tabanus eggeri Schiner, 1868</td>
<td>1</td>
</tr>
<tr>
<td>Tabanus sudeticus Zeller, 1842</td>
<td>1</td>
</tr>
</tbody>
</table>

Statistical analyses

We compared catches from different trap positions, different trapping days and different baits using the non-parametric Kruskal–Wallis test. Differences in catches between baited and unbaited traps were evaluated using the non-parametric Wilcoxon signed-rank test. The effects of meteorological conditions on catches were analysed using the Spearman’s rank correlation test. Differences in EAG responses and odour stimuli were evaluated using the non-parametric Wilcoxon signed-rank test. All analyses were performed using PAST Version 2.12 (Hammer et al., 2001).

Results

Field experiment

A total of 7038 tabanids (all females) belonging to 10 species in the genera Tabanus, Atylotus and Hybomitra were collected (Table 1). All the species are common in southern France (Rageau & Mouchet, 1967; Chvala et al., 1972; Raymond, 1978). Catches were dominated by two species: Tabanus bromius (57%) and Atylotus quadrifarius (41%). Daily catches of T. bromius were positively correlated with maximum temperature and mean wind chill temperature (R = 0.80, P = 0.02 and R = 0.95, P = 0.0003, respectively). No significant correlation with maximum temperature or mean wind chill temperature emerged for A. quadrifarius (R = 0.34, P = 0.40 and R = 0.52, P = 0.19, respectively).

Only the two dominant species T. bromius and A. quadrifarius were sufficiently abundant to be studied. Comparisons of the catches at different trap positions and on different trapping days for both species showed a significant difference only among trapping days for T. bromius (Kruskal-Wallis; H = 22.5, P = 0.002).

Our results showed that:

- Nzi traps baited with aged cow urine seemed to increase catches of T. bromius (2.0-fold) and A. quadrifarius (1.7-fold) in comparison with unbaited Nzi traps (Table 2), but the increases were not statistically significant.
- Nzi traps baited with aged horse urine significantly enhanced catches of T. bromius (1.6-fold) and A. quadrifarius (3.5-fold) in comparison with unbaited traps.
- Nzi traps baited with aged sheep urine collected more T. bromius (1.7-fold) and significantly more A. quadrifarius (2.5-fold) in comparison with unbaited traps.
- No significant differences emerged between baits for either T. bromius (H = 0.34, P = 0.84) or A. quadrifarius (H = 2.05, P = 0.35).

Laboratory experiment

For T. bromius, EAG peak amplitude increased in a dose-dependent fashion with an increased octenol dose, and the mean EAG amplitude elicited by each of the doses (oct 1 mg/μL: 1.25 ± 0.06 mV; oct 0.1 mg/μL: 1.38 ± 0.08 mV; oct 0.01 mg/μL: 1.23 ± 0.08 mV; oct 0.001 mg/μL: 0.66 ± 0.06 mV) was greater than that elicited by the control (dichloromethane: 0.26 ± 0.03 mV) (Fig. 2). The mean EAG amplitude elicited by octenol at 0.001 mg/μL (10 μg) was significantly lower than that for higher doses.

Table 2. Catches of Tabanus bromius and Atylotus quadrifarius with Nzi traps baited with urine from different types of animals.

<table>
<thead>
<tr>
<th>Species</th>
<th>Total catches</th>
<th>Catch index*</th>
<th>Median value†</th>
<th>Percentiles‡</th>
<th>W/P-value§</th>
</tr>
</thead>
<tbody>
<tr>
<td>Tabanus bromius</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Unbaited traps</td>
<td>620</td>
<td>Control</td>
<td>46.5</td>
<td>27.75/107.75</td>
<td>–</td>
</tr>
<tr>
<td>Aged cow urine</td>
<td>1289</td>
<td>2.08</td>
<td>89</td>
<td>33/195.75</td>
<td>23/0.548</td>
</tr>
<tr>
<td>Aged horse urine</td>
<td>996</td>
<td>1.60</td>
<td>112</td>
<td>71/202.25</td>
<td>330.043</td>
</tr>
<tr>
<td>Aged sheep urine</td>
<td>1094</td>
<td>1.76</td>
<td>87</td>
<td>50.5/160.5</td>
<td>29/0.151</td>
</tr>
<tr>
<td>Atylotus quadrifarius</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Unbaited traps</td>
<td>334</td>
<td>Control</td>
<td>35</td>
<td>17.75/67</td>
<td>–</td>
</tr>
<tr>
<td>Aged cow urine</td>
<td>569</td>
<td>1.70</td>
<td>83</td>
<td>43/100</td>
<td>29/0.152</td>
</tr>
<tr>
<td>Aged horse urine</td>
<td>1179</td>
<td>3.53</td>
<td>101</td>
<td>68.5/200</td>
<td>36/0.011</td>
</tr>
<tr>
<td>Aged sheep urine</td>
<td>838</td>
<td>2.51</td>
<td>70.5</td>
<td>62.25/171.25</td>
<td>35/0.019</td>
</tr>
</tbody>
</table>

*Ratio of the total catches in the baited traps/total catches in the unbaited traps.
†Median value for catch/day/trap.
‡25th percentile/75th percentile for catch/day/trap.
§Results of non-parametric Wilcoxon signed-rank test (baited vs. unbaited traps), significant at P < 0.05.
Significant results are in bold.
Responses of tabanids to urine volatiles

The dose–response relationship was sigmoidal, with EAG amplitudes reaching a maximum at a dose of 0.01 mg/μL octenol. For A. quadrifarius, the mean EAG amplitude elicited by each dose of octenol (oct 1 mg/μL: 0.77 ± 0.04 mV; oct 0.1 mg/μL: 0.79 ± 0.03 mV; oct 0.01 mg/μL: 0.76 ± 0.06 mV; oct 0.001 mg/μL: 0.65 ± 0.04 mV) was greater than that of the control (dichloromethane: 0.43 ± 0.04 mV), but EAGs were much lower than those of T. bromius.

For T. bromius, mean EAG amplitudes elicited by the three types of urine (cow: 1.34 ± 0.12 mV; horse: 1.28 ± 0.11 mV; sheep: 1.15 ± 0.06 mV) were greater than those of the control, but there was no significant difference between urine and octenol at 0.1 mg/μL (Fig. 3). For A. quadrifarius, mean EAG amplitudes elicited by the three types of urine (cow: 1.65 ± 0.13 mV; horse: 1.64 ± 0.12 mV; sheep: 1.23 ± 0.05 mV) were greater than those elicited by the control and octenol. For both species of tabanid, horse urine and cow urine elicited larger EAGs than sheep urine.

Discussion

Behaviour

Baited and unbaited Nzi traps captured a large number of female tabanids in the plain of La Crau during the study period. On average, we collected 220 tabanids per day per trap. Although two main species of tabanid coexist in this region, a variety of species were collected. The efficacy of Nzi traps has been evaluated in Africa, North America and Australia (Mihok, 2002; Mihok et al., 2006; Van Hennekeler et al., 2008), but no study has previously reported the use of these traps in Europe. Although we did not compare different trap designs, Nzi traps seemed to be very effective for catching temperate tabanid species (A. quadrifarius, T. bromius spp.). For T. bromius, catches differed significantly among trapping days. Maximum and wind chill temperatures were factors with positive correlation to daily catches of T. bromius; catches were lower on cloudy days. These results confirm the effects of meteorological variables, particularly temperature and cloud cover, on the daily activity of tabanids (Van Hennekeler et al., 2011).

Tabanus bromius and A. quadrifarius seemed to be attracted by aged cow urine. These results were not statistically significant because of the limited number of traps and the large differences in daily catches, but the catch index was >1.5 for both species. Aged cow urine has also been shown to increase catches of Hybomitra spp. in Canada by 1.5–1.9 times using Nzi traps (Mihok & Mulye, 2010) and of tabanids [Tabanus spp., Hybomitra bimaculata (Macquart, 1826) and Haematopota pluvialis (Linnaeus, 1758)] in Croatia by 51 times using canopy and malaise traps (Krcmar et al., 2006). Cow urine plus acetone and octenol also increased catches of...
tabanids (Ancala spp. and Tabanus spp.) in Mali by 2.4 times using pyramidal traps, and by 5.1 times using Vavoua traps (Djitéye et al., 1998).

Horse urine significantly enhanced catches of both the main tabanid species (1.6–3.5 times), and sheep urine significantly enhanced catches of A. quadrifarius (2.5 times). The Croatian study also showed that catches of tabanids were increased by horse urine (36 times) and sheep urine (30 times) (Krcmar et al., 2006). However, these results cannot be usefully compared with our findings as canopy and malaise traps collected very few tabanids without bait, whereas unbaited Nzi traps were very effective (Mihok & Mulye, 2010). In the present study, a comparison of total catches by each bait found no significant differences. Therefore, the three unguulate urines could be considered as effective attractants for tabanids.

Physiological activity

For T. bromius, the highest EAGs were elicited by octenol. This confirms results for other biting flies such as stable flies (Warner & Finlayson, 1986; Schofield et al., 1995) and tsetse flies (Hall et al., 1984; Den Otter et al., 1988). We also found a dose–response relationship, as has been shown by Schofield et al. (1995) and Den Otter et al. (1988). In our study, EAGs peaked at a dose of 100 μg. This is compatible with the highest EAGs recorded for stable flies and tsetse flies at similar doses of 200 μg and 41.5 μg, respectively (Den Otter et al., 1988; Schofield et al., 1995). At high doses of octenol, EAG amplitude decreased between 10^2 μg and 10^6 μg because the antenna reached saturation.

Electroantennography studies must use physiologically meaningful concentrations to prevent potentially misleading results (Bruce et al., 2005). Dose–response curves usually show a regular increase in depolarization with an increase in compound quantity (Vittecoq et al., 2011). However, several examples have shown that similar antenna depolarization can be obtained from very different stimulus concentrations, including amounts of compounds in much higher concentrations than would occur naturally (Sasso et al., 2009; Suchet et al., 2011).

For A. quadrifarius, EAGs elicited by octenol were very low in comparison with EAGs elicited by urine, and relative EAG amplitudes for octenol never exceeded twice the relative EAG amplitude for dichloromethane. These results contrasted markedly with those obtained for T. bromius, S. calcitrans and Glossina spp. Octenol was less electrophysiologically active for A. quadrifarius than for other biting flies. However, there is considerable evidence of the attractiveness of octenol to tabanids. For example, in the Croatian study, octenol increased catches of tabanids by 7.7 times (Tabanus spp. and H. pluvialis) in canopy traps (Krcmar et al., 2005), and in the Canadian study, it increased catches of tabanids up to 3.6 times (Tabanus spp. and Chrysops spp.) on sticky enclosures surrounding Nzi traps (Mihok et al., 2007). Yet in Croatia, catches of Atylotus loewianus (Villeneuve, 1920) collected from canopy traps baited with octenol and aged horse urine did not differ significantly (Krcmar et al., 2005), and aged donkey urine collected significantly more A. loewianus than did traps baited with a mixture of octenol, acetone and ammonia (5 : 3 : 2) (Krcmar et al., 2010). The low electrophysiological activity of octenol on antennae of A. quadrifarius may explain why not all biting flies respond to octenol alone or in combination with other attractants.

For T. bromius and A. quadrifarius, urine elicited large EAG amplitudes, and horse urine and cow urine elicited larger EAGs than sheep urine. Differences in EAGs may be explained by differences in the volatile chemicals emitted by aged urines. These results are consistent with the biology of tabanids because most species, particularly those of the Tabaninae subfamily, feed predominantly on large mammals such as cattle, horses and deer (Mullen & Durden, 2002). In the field, the attractiveness of the three types of urine was quite similar despite some differences in the catch index. Aged urine gradually accumulates phenolic attractants as a result of microbial activity (Okech & Hassanali, 1990) and probably many other active chemicals that may have indifferent or repellent effects. Mammal urines and their phenol constituents (3-methylphenol, 4-methylphenol, 3-n-propylphenol) have been tested in outdoor experiments in tabanids with contrasting results (Gibson & Torr, 1999; Mihok & Mulye, 2010). Synergism between ammonia and phenols is likely to explain these observations (Mihok & Lange, 2011). Moreover, Torr et al. underlined the effects of host physiology (weight and/or age) on the presence and level of kairomones in cattle odour, including urine (Torr et al., 2006). Therefore, it would be useful to compare the composition of volatile odorants in different mammal urines using gas chromatography and to conduct EAG tests in tabanids to determine the physiological activities of phenols and other urinary compounds such as ammonia, as has been done with tsetse flies (Bursell et al., 1988) and stable flies (Jeanbourquin & Guerin, 2007).

Host-seeking and feeding behaviour

In the field, we observed a significant attraction of A. quadrifarius to sheep odours, although the species was only moderately sensitive to the extract of aged sheep urine. In this area, sheep are the quasi-exclusive hosts of these flies. During the study, two sheep flocks and a few horses were present <1 km from the trapping zone. Associative learning experiments previously conducted on haematophagous Diptera have shown that these insects are able to recognize an odour associated with a bloodmeal. For example, Culex quinquefasciatus (Diptera: Culicidae) adults can learn to associate a conditioned odour (vanilla or strawberry) with an unconditioned stimulus such as sugar or a blood source (Tomberlin et al., 2006), and tsetse flies were found to prefer to feed on a host encountered for a first meal 2 days previously (Bouyer et al., 2007). Although the attraction of aged sheep urine for A. quadrifarius contrasts with the trophic preferences of Tabaninae, it may be that individuals that have previously encountered a sheep are then attracted by sheep odours.

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Studies using serological tests to identify the sources of bloodmeals have shown that tabanids have opportunistic feeding habits (Magnarelli & Anderson, 1980; Muzari et al., 2010). In Louisiana in the U.S.A., domesticated animals, especially cattle and horses, are the main hosts of tabanids, but, in localities from which cattle are absent, deer are of major importance (Wilson & Richards, 1969). In Australia, Muzari et al. (2010) found that all tabanids fed on at least three of the four hosts tested (horse, cow, macropod and pig) suggesting a level of opportunistic feeding (despite a preference for macropods).

A comparison using ungulate urine in a location in which cattle or horses are the predominant hosts may be useful to follow up on the physiological and ecological explanations of the present results.

**Control strategy**

Tabanid control is difficult to achieve. Typical host contact is only about 4 min per fly during blood feeding, which may occur only once every 3–4 days (Mullen & Durden, 2002). As horse flies are partly independent of livestock, integrated control strategies are required to reduce their impact (Foil & Hogsette, 1994). The use of traps provides a potential control technique, but the effective reduction of the number of tabanids attacking cattle requires the development of better attractants. Several studies in Africa, North America and Europe have tested the use of different attractants, including synthetic compounds, alone or in mixtures, or excretory products of host animals, such as urine (Gibson & Torr, 1999; Mihok & Mulye, 2010). Contrasting results have emerged from these studies, which suggests that it will be very difficult to find a universal attractant for Tabanidae, a family of over 4000 species and at least 137 genera (Foil & Hogsette, 1994). The present study’s first EAG recordings in two different tabanid species showed their physiological activities to be dissimilar. Moreover, it is likely that host availability will influence the behavioural responses of horse flies to host odours. The results of the present study may be useful for matching the bait used in trapping devices to species of Tabanidae and the livestock present, and thus improving the control of horse flies in a push–pull strategy.

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