

# Upwind flight responses of tsetse flies (*Glossina* spp.) (Diptera: Glossinidae) to acetone, octenol and phenols in nature: a video study

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## Abstract

Video observations were made of tsetse flies (mainly *Glossina pallidipes* Austen) as they approached, responded to and left a vertical (1 m square) black target in odour plumes of acetone, or a 4:1:8 mixture of octenol:propylphenol:methylphenol, or acetone plus this "4:1:8" mixture, or in no odour. No differences in mean flight speed or turn size in any of these situations were detected. With the odour source 5 m upwind of the target, the flight tracks of tsetse arriving at and leaving the target were significantly biased towards upwind, highly so when 4:1:8 was present, marginally so in acetone alone. With the source 10 m upwind, the same biases were still present but weaker. Circling flights around the black target were more frequent in acetone plus 4:1:8 than in no odour (26% vs 15%), but in either odour alone were only just significantly more than in no odour. Upwind turning at the target was more frequent (25% vs 17%) in acetone alone than in no odour (though not in 4:1:8 alone). It is concluded that 4:1:8 elicits an upwind anemotactic response comparable in strength to that in CO<sub>2</sub>, and that acetone elicits a similar response more weakly, but may be more involved in potentiating visual responses.

## Introduction

Hitherto it has seemed that no odours other than carbon dioxide, or mixtures that include CO<sub>2</sub>, stimulate upwind flight by tsetse flies. Thus, even though acetone or octenol used as odour baits substantially increase trap catches (Vale, 1980; Vale & Hall, 1985a, 1985b), when Torr (1988) released either of these odours alone upwind of a 'primary' plume that included CO<sub>2</sub>, neither of them recruited tsetse upwind from the primary plume. Similarly, when he used an open ring of electrocuting nets, neither acetone nor octenol increased the catch at the upwind net over the odourless situation (Torr, 1990). Likewise, Bursell found no upwind bias in take-off directions evoked by acetone and octenol without CO<sub>2</sub> (Bursell, 1987), and he tested phenols only with CO<sub>2</sub> (Bursell *et al.*, 1988).

However, it has now been found that both acetone and octenol elicit upwind flight in a laboratory wind-tunnel (Paynter & Brady, 1993). It is therefore important to

know whether they do in fact have this effect in the field, but that it has simply not been detected. We examined this question by recording the flight tracks of tsetse with video in Zimbabwe, using acetone and the combination of octenol and phenols now used commonly to bait targets and traps in fly control programmes. We report here on the analysis of these tracks.

## Methods

The main observations were made at Rekomitjie Research Station, in the Zambezi Valley (Zimbabwe), in a site closely similar to those used by Vale and Torr. Two species of tsetse were present: *Glossina pallidipes* Austen and *G. morsitans morsitans* Westwood, but as these were in a ratio of about 20:1 (see Torr, 1988; Vale *et al.*, 1988), the observations refer, in effect, mainly to the former.

The set-up consisted of a video-camera raised c.2.5 m above the ground and looking down at a vertical, 1 m square black target aligned with the mean wind direction; the target sat on a 2 × 3 m black velvet groundsheet to improve resolution of the tsetse flies (see Gibson *et al.*, 1991, for details); the camera 'saw' an area of about 1.5 × 2.5 m at ground level.

The following odours were tested. 1. A bottle releasing acetone at *c.*500 mg/h. 2. A 5 × 5 cm polythene satchel releasing *c.*400, 100 and 800 µg/h of, respectively, 1-octen-3-ol, 3-*n*-propylphenol, and 4-methylphenol (hereafter called "4:1:8"). 3. The acetone and 4:1:8 sources combined (which is the 'synthetic ox' odour currently most widely used in baited target campaigns – Vale *et al.*, 1988). 4. No odour as the control.

To ensure that the target was almost continuously within the odour plume, three point sources (each releasing at these rates) were placed in an upwind arc with the angle between the outer sources subtending 60° to the target, and 30° between adjacent sources. Trials with smoke showed that in wind of > *c.*1 m/s the plume expanded at *c.*10°, and in slower winds expanded more broadly. Thus, with the sources at either 5 or 10 m distant up the mean wind line, the wind could normally swing by at least ±45° without carrying the plume clear of the videoed site.

Several days of recording were done, at various distances between the odour source and the target, ranging from 5 to 25 m. Data from only the 5 m and 10 m distances are analysed here, because the response was already waning by 10 m (see below), and in the 15 m observations most of the wind was < 0.5 m/s so that it would have taken over 30 s for the odour to reach the target, making the presence of odour highly uncertain.

All the analysed tracks therefore relate to two days of recording, with all four odour tests carried out on the same day for each odour distance. The recordings were made between *c.*16.00 and 17.45 h local time, on 25 September (5 m) and 4 October 1991 (10 m), when the maximum temperature was *c.*36° and 37°, respectively,

and fell only *c.*1° between the start and finish of recording. The different odours were tested in different orders on the two days.

Flight tracks were transcribed onto acetate sheets direct from the monitor screen and, taking 180° as due upwind, were analysed for: arrival angle (1st 3 video frames), leaving angle (last 3 frames), net total turn angle (with clockwise parts of a track cancelling out anticlockwise), and flight speed (= ground speed). The tracks were also classified into five types: net turn upwind, net turn downwind, 'straight' (= net turn < 20°), 'circling' (= net turn > 100° and concentric upon the target), and 'bimodal' (track including left and right-hand turns). Contemporary wind speed and direction were recorded *c.*2 m downwind of the site with a solid-state ultrasonic anemometer (1012-S, Gill Instruments).

One 22 min video tape was recorded of each odour situation. Starting at least 3 min into each tape (to remove all human disturbance effects), all flight tracks identifiable as almost certainly of tsetse (see Gibson & Brady, 1988) were transcribed, until at least 120 had been accumulated. Then the wind record was examined and, initially, only those tracks that coincided with wind of at least 0.2 m/s that blew within 45° of the target were used, until *c.*100 tracks were accumulated. When subsequent analysis revealed that the 45° wind specification made no apparent improvement to the upwind bias of the tracks, all the transcribed tracks were analysed.

Two earlier experiments sampled upwind flight tendencies with electric nets, i.e. independently of video. In the first (over 8 days in March 1990 at Rekomitjie), a 1 × 2 m, double-sided electric net (see Vale, 1980) was odour-baited with acetone and 4:1:8 as above. The centre 1 m<sup>2</sup>

Table 1. Mean directions of flight tracks of tsetse flies relative to the wind direction in different odour situations (see text); expressed in bold as the mean vector length (*r* of the Raleigh test), and beneath this as the angular difference between the mean flight vector and due upwind.

	Acetone	4:1:8	Acetone + 4:1:8	No odour
<b>A. Odour 5 m distant</b>				
No. of flight tracks ( <i>n</i> )	157	145	140	120
Arriving tracks	<b>0.112 n.s.<sup>§</sup></b> 115°	<b>0.306***</b> 6°	<b>0.251***</b> 70°	<b>0.016 n.s.</b> 200°
Leaving tracks	<b>0.156*</b> 38°	<b>0.260***</b> 14°	<b>0.233***</b> 47°	<b>0.071 n.s.</b> 3°
Leaving tracks of crosswind arrivers#	<b>0.314**</b> 21°	<b>0.283*</b> 4°	<b>0.465***</b> 32°	<b>0.145 n.s.</b> 40°
<b>B. Odour 10 m distant</b>				
No. of flight tracks ( <i>n</i> )	109	121	107	104
Arriving tracks	<b>0.099 n.s.</b> 45°	<b>0.188*</b> 44°	<b>0.242**</b> 47°	<b>0.145 n.s.</b> 9°
Leaving tracks	<b>0.119 n.s.(*)</b> 79°	<b>0.202**</b> 27°	<b>0.142 n.s.</b> 55°	<b>0.130 n.s.</b> 12°

Significance values are based on the Raleigh test for the flight tracks being concentrated about the mean vector (Batschelet, 1981); note that non-significant mean vectors can be closer to upwind than significant ones (e.g. the no-odour leaving tracks); it is the overall concentration of data around the mean vector that directs the Raleigh test. \**P* < 0.05, \*\**P* < 0.01, \*\*\**P* < 0.001. For analysis restricted to flights when the wind came from within ±45° of the odour source: (\*)*P* < 0.05, §*P* < 0.07. #ns for crosswind leavers = 35–47.

Table 2. Characteristics of flight tracks of tsetse flies in different odours (see text); number of tracks analysed (*n*) as in Table 1.

	Acetone	4:1:8	Acetone + 4:1:8	No odour
<b>A. Mean flight track characteristics at 5 m from odour source</b>				
Mean flight speed $\pm$ SE (m/s)	3.54 $\pm$ 0.18	4.62 $\pm$ 0.21	4.21 $\pm$ 0.16	4.13 $\pm$ 0.26
Mean turn size $\pm$ SE ( $^{\circ}$ )	101 $\pm$ 9	104 $\pm$ 11	101 $\pm$ 10	90 $\pm$ 9
<b>B. Percentages of different categories of track (5 m and 10 m data combined)</b>				
Circling	22(*) <sup>§</sup>	22(*) <sup>§</sup>	26**	15
Straight (<20 $^{\circ}$ )	27*	30 n.s. <sup>§</sup>	29 n.s. <sup>§</sup>	37
Upwind turning	25*	23 n.s.	22 n.s.	17
Downwind turning	18 n.s.	16 n.s.	14 n.s.	19
Bimodal turning	8 n.s.	10 n.s.	9 n.s.	13

In section A, no means differ significantly. In section B, \* $P < 0.05$ , (\*\*) $P = 0.06-0.07$ , \*\* $P < 0.01$  for significance of difference from control (no odour) percentage ( $\chi^2$ ). <sup>§</sup>When combined these pairs of figures have  $P < 0.05$ .

was 'baited' visually between the electric nets with black cloth, flanked each side by  $\frac{1}{2}$  m<sup>2</sup> mosquito-netting screens. In the second (over 8 days in September 1991 at Nguruman, south-west Kenya), a 1 m<sup>2</sup>, double-sided electric net (the two sides separated by mosquito netting only) was baited with a horizontal c.50  $\times$  30 cm diameter black drum centred c.50 cm upwind of the net, and with acetone (as above) plus a bottle of fermenting ox urine (which mainly releases various phenols, Dransfield *et al.*, 1986). The close similarity of the Zimbabwe and Kenya results (table 3) suggests that the visual target used in Kenya did not significantly obstruct the arrival of tsetse at the upwind-facing electric net. Both electric nets were set across the mean wind line, with sticky trays below to catch the stunned flies. They were visited every 10 min (with the power switched off) to collect and count the caught flies, with the upwind- and downwind-facing catches separated.

### Results

The site was set up so that the highly consistent wind blew mainly from the odour source to the black target; during the observations its mean vector was never more than 30 $^{\circ}$  away from 180 $^{\circ}$  (= direction between target and centre odour source) with angular deviations (SD) of only 36–47 $^{\circ}$ . The mean windspeed in the different odour situations ranged from 0.5 to 1.2 m/s (SDs c.  $\pm$  0.4), but had no detectable effect on the behaviour. Mean flight speeds

ranged from 3.4 to 4.6 m/s, with no significant differences between the four odour situations (table 2A). Similarly, mean turn sizes did not differ significantly between the four odour situations (table 2A).

The basic flight direction details are given in table 1. At 5 m downwind of the odour source, the directions of arrival of the flies at the target were highly significantly biased upwind when 4:1:8 was in the odour, whereas flights in the absence of odour showed no such bias. The effect of acetone on upwind arrival was much less marked, although it approached significance if analysis was restricted to times when the wind blew the odour plume directly over the target. At 10 m range, the upwind bias of arrivals in 4:1:8 was still evident, but rather less significant, and the distribution of flights in acetone was statistically indistinguishable from that in the absence of odour. There was also no evidence that acetone augmented the upwind bias in 4:1:8 when it was used in the combined odour.

The flight directions of tsetse *leaving* the field of view (and target) were similarly biased upwind in 4:1:8, but now also reached significance in acetone. However, as the great majority of flights were relatively straight (70% turning < 100 $^{\circ}$ ), this is perhaps hardly surprising; most flies arriving upwind flight would simply continue on in that direction. For this reason we looked more closely at those flies that approached the target in crosswind flight (by arriving through the two 90 $^{\circ}$  sectors that were orthogonal to the wind line).

Of these, those that made moderate turns of 20–100 $^{\circ}$  and were thus neither 'straight' fliers nor 'circlers' – see Methods) commonly responded in the odour by turning upwind and leaving with a distinct upwind bias (table 1A row 4). And now the acetone plume did have a significant effect. Indeed, it even enhanced the response to 4:1:8, since the Raleigh statistic for these crosswind fliers in 4:1:8 was almost doubled when acetone was added ( $r$  increasing from 0.28 to 0.47 – cf. 0.26 vs 0.23 for the overall leaving tracks in row 3 of table 1A). The equivalent analysis of crosswind arrivers in the situation at 10 m revealed no significant responses, however.

Although log-survivorship analysis of all 562 tracks at 5 m provided no evidence for the distribution of turn sizes being anything other than monotonic (cf. Gibson *et al.*, 1991), inspection suggested the existence of qualita-

Table 3. Percentage of tsetse flies caught in 10-min samples on the downwind-facing side of two-sided, odour-baited electric nets (see text).

Catch type	Sample no. ( <i>n</i> )	% flies caught in upwind flight ( $\pm$ SE)
Electrified 1 m <sup>2</sup> black target (Zimbabwe)	37	62.7 $\pm$ 2.0
Black 30 $\times$ 50 cm target with 1 m <sup>2</sup> electric net (Kenya)	26	62.0 $\pm$ 3.9
Upwind arrivers (90–270 $^{\circ}$ ) when 4:1:8 present (from Table 1A)	285 (flies)	62.8 $\pm$ 2.9

tively different kinds of flight. All the tracks were therefore classified into five types (Methods). The results (table 2B) show small, but significant differences between the odour and control situations, with circling being more frequent in the presence of the combined odour, and upwind turning more frequent (and straight flights less frequent) in acetone.

Finally, we had previously done two other tests which can be used to see whether the behaviour observed with video manifests itself as a differentiable upwind:downwind ratio in flies caught on standard electric nets baited with odour and visual targets (Methods). It is apparent from table 3 that it does: even in the absence of CO<sub>2</sub>, flies showed a distinct (c.62%) bias for getting caught on the downwind-facing side of such devices, and this bias is very similar to the proportion of flies arriving in upwind flight that we observed with video.

### Discussion

There are difficulties with interpreting these observations because the tsetse detected by the video were presumably not all responding to the experimental stimuli. For instance, even when no odour was present about 15% of approaching flies circled the visual target, so only about 10% more did so when it was in a plume of acetone and 4:1:8 (table 2B). Conversely, nearly a third of all flies in odour made no obvious response at all, travelling straight through the set-up, turning less than 20° as they passed the target, so most of the crosswind and downwind fliers among these were presumably responding to stimuli other than those being provided experimentally.

It is therefore not surprising that the upwind biases observed are less than dramatic: at best only about 50% of the tsetse arrived through the 120° upwind-pointing sector, where random distribution would predict 33% doing so anyway. Nevertheless, the video system demonstrated that tsetse do respond to acetone, octenol and phenols in the field by the same kind of upwind anemotaxis they show to CO<sub>2</sub> or whole ox odour (Gibson & Brady, 1988; Torr, 1988, 1990).

In fact, the 4:1:8 combination odour could not separate responses to octenol from those to phenols, so it is not clear which component(s) were the effective anemotactic stimulants. However, octenol on its own evokes upwind flight in a wind-tunnel (Paynter & Brady, 1993), and the fermented cow urine (which contains no octenol, D.R. Hall, pers. comm.) used in the Kenya electric net experiment, evoked upwind approaches to the target (table 3), so it seems likely that each of the four odour components, acetone, octenol, propylphenol, and/or methylphenol, elicit upwind anemotaxis in tsetse.

The upwind bias in the video observations was particularly clear in the 4:1:8 combination; although it was also detectable in acetone on its own, it was so only at 5 m from the source. However, it is interesting that even though these overall arriving and leaving directions were barely significantly biased in acetone, when analysis was restricted to tsetse approaching in crosswind flight – and therefore to flies which were probably experiencing the acetone odour afresh – upwind turning and departure

were highly significant. Moreover, this acetone response seems to have augmented the otherwise stronger upwind response to 4:1:8.

The percentage of these crosswind tsetse that left through the upwind 120° sector was close to 50% in each of the odours, which is, surprisingly, virtually the same as the 47% level reported for acetone plus CO<sub>2</sub> by Gibson *et al.* (1991). Moreover, in 4:1:8 the upwind arriving tsetse (whose view of the target will mainly have been edge on) formed 51% of the total, and this also is the same as the 47% upwind leaving bias in CO<sub>2</sub> plus acetone in the visually similar situation studied by Gibson *et al.* (1991) when no target was present. On the other hand, among the crosswind arriving tsetse (for whom the target will have been most obvious) the level of target circling in acetone and 4:1:8 was less than half that observed in acetone plus CO<sub>2</sub> (i.e. 25% *vs* the 56% in Gibson *et al.*, 1991), even though the proportion of circlers that left upwind was scarcely less than in CO<sub>2</sub> plus acetone (36% *vs* the 40% in Gibson *et al.*, 1991).

It is difficult to make precise comparisons between these observations and those in the controlled conditions of a wind-tunnel, because of the difficulty of comparing like with like in terms of odour concentration in the two situations. However, in just above ambient CO<sub>2</sub> (0.05%), c.67% of *G. m. morsitans* flew upwind in the wind-tunnel (Colvin *et al.*, 1989), compared with c.64% doing so in c.1 ng/l octenol ( $\approx 10 \times$  threshold conc.), and c.67% in c.1 ng/l acetone ( $\approx$  threshold conc.) (Paynter & Brady, 1993). Even here, therefore, the difference from CO<sub>2</sub> is slight.

These similarities with and without CO<sub>2</sub> raise the question of why upwind anemotaxis in the field has frequently been reported for odours that include CO<sub>2</sub>, but not for acetone or octenol without it (Bursell, 1987; Torr, 1988, 1990 – and see Introduction). The explanation proposed by Paynter & Brady (1993, who discuss this more fully, is that in Torr's 1988 double-plume experiment, the flies turned back on losing contact with the high CO<sub>2</sub> concentration near the source of the downward primary plume, because at that concentration CO<sub>2</sub> is a close-range attractant only. They also argue that in Torr's 1990 experiment, the ring-of-nets sampling system was too insensitive to detect the 'signal' of the small upwind bias in acetone or octenol above the high random 'noise' due to the fact that electric nets kill only about 50% of tsetse flies on first strike.

In view of the similar levels of upwind bias we now find with *and* without CO<sub>2</sub>, we are no longer convinced by the latter explanation, though we have no alternative to offer. The fact that Torr did not use phenols might explain the lack of upwind flight he found in octenol, because our 4:1:8 mixture elicited the strongest upwind biases, but it cannot explain his acetone results, nor the difference between his octenol findings and ours in the wind-tunnel. Bursell *et al.* (1988) report upwind take-offs to some phenols in a wind-tunnel, but all their test odours included CO<sub>2</sub> at about 10 times ambient concentration.

We conclude that the 4:1:8 octenol:phenol combination is an effective stimulus for upwind orientation in tsetse comparable in effect to CO<sub>2</sub>, but that acetone on its own is rather less so, although it may be more involved than octenol and phenols in potentiating visual responses to host-like targets.

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