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The effect of wind speed on the flight responses of tsetse flies to CO_2: a wind-tunnel study

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Abstract. Female *Glossina morsitans morsitans* Westwood were video-recorded in a wind-tunnel as they entered, in cross-wind flight, a broad plume of CO_2 (a component of host odour). At a wind speed that corresponds with peak catches in the field (*c.* 0.6 m s^{-1}) odour produced both significant upwind turning responses (in-flight anemotaxis) and kinetic responses (reduced flight speed and increased sinuosity (°m^{-1})). At a wind speed of *c.* 0.2 m s^{-1} flies displayed anemotactic, but not kinetic, responses to odour. At very low wind speeds (0.1 m s^{-1}) neither upwind turning responses nor kinetic responses to odour were detected. The results are discussed with regard to current theory of host-location by tsetse.

Key words. *Glossina*, tsetse fly, anemotaxis, klinokinesis, edge detection, chemotaxis, flight, orientation, host-finding, odour plume behaviour.

Introduction

Host odour baits greatly increase the number of tsetse caught at visual traps (e.g. Vale, 1980; Vale & Hall, 1985a, b) and many studies show that upwind anemotaxis in response to odour is important for host-location: in response to host odour tsetse take off in an upwind direction (Bursell, 1987; Torr, 1988) and exhibit optomotor-steered upwind anemotaxis in flight (see Gibson *et al.*, 1991, for references). Successful location of an invisible odour source by this method requires that the odour plume extrapolates reliably back upwind towards the source (see David, 1986). In vegetation this depends on the wind speed because the straightness of airflow increases with increasing wind speed, up to *c.* 1 m s^{-1} (Brady *et al.*, 1989). It has recently been shown that there is often an inverted 'U'-shaped relationship between the wind speed and the catch of tsetse flies at odour-baits (Brady *et al.*, 1995), suggesting that tsetse are more successful at locating odour sources as the wind speed increases to *c.* 0.5–1 m s^{-1}. At higher wind speeds the catch declines, perhaps because the 'active space' of the plume is reduced by turbulence and increased dilution of the odour.

Wind speeds experienced by flies in the field are commonly rather low; for example, Brady *et al.* (1989) recorded the modal wind speed in typical tsetse habitat in Zimbabwe to be 0.25 m s^{-1}. Nevertheless, large numbers of flies may still be caught when the wind is virtually zero, since Brady *et al.* (1995, Experiment 8) record 25% of catches in such conditions, suggesting that odour source-location is not always dependent on the presence of wind. Source location under these circumstances is presumably based on kinetic responses (see Warnes, 1990).

Williams (1994), however, argued that kinetic responses alone are rather inefficient unless the turning responses have some bias towards the odour source (which would require an anemotactic element). This paper describes the responses of tsetse flies to CO_2 in a wind-tunnel at a wind speed associated with peak catches in the field (Brady *et al.*, 1995), and at lighter wind speeds where the problems of odour-based anemotaxis will be greater.

Materials and Methods

Insects. Only mature, virgin, female *Glossina morsitans morsitans* Westwood were used. Pupae (supplied by the Tsetse Research Laboratory, Bristol) and adults were kept at 27 ± 1°C, 65 ± 5% r.h. and LD 12:12 h. On emergence, virgin females were separated from males, before mating. They were offered a blood-meal on a rabbit's ear 2 days after emergence and every third day thereafter. Between feeds they were kept together in a 50-cm-cubed cage, to encourage flight and thus flight-muscle development (Bursell & Kuwenga, 1972). Individuals that took off spontaneously and probed the cage netting after 3 days without feeding were selected for experiment. Flies that were recovered undamaged after testing were given a further blood-meal and re-used after a further 3 days of starvation.

The wind-tunnel. The wind-tunnel was the same as that used by Paynter & Brady (1993). Video-recording of the flies' flight

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tracks was carried out with a camera situated 1 m above the glass ceiling of the working section, the whole of which was shrouded in black cloth to isolate it from external stimuli. The tunnel floor was yellow with large red spots to provide optomotor cues. Reductions in wind speed were achieved by partially shutting the intake to the electric fan and were measured by timing puffs of smoke over a known distance within the wind-tunnel.

Host odour. CO_2, as a surrogate for host odour, was delivered from a cylinder via a 'gap' flowmeter (Flowbits Ltd) through twelve plastic pipes of equal length and diameter whose ends were arranged in a 25 × 60 cm grid of three rows of four at the upwind end of the wind-tunnel and upwind of a turbulence-generating baffle (*loc. cit.*). The odour plume so produced, when visualized by blowing smoke through the system, was lightly turbulent and evenly filled the whole of the tunnel apart from a 25 cm corridor of clean air next to the fly-release battery. The boundary between clean and odour-free air was discrete and did not vary by more than ±5 cm.

At the highest wind speed, CO_2 was released at a rate of 2 l min^{-1}, which is equivalent to that released by one cow (Vale, 1980). The concentration in the wind-tunnel would have been *c.* 0.013% above ambient (which is *c.* 0.03%; Gillies, 1980). This concentration should be representative of the concentrations encountered a few metres downwind of a cow in nature. Wind speeds tested were 0.58, 0.18 and 0.10 m s^{-1}. To ensure that the concentration of CO_2 remained unchanged for each wind speed, the flow rate of CO_2 was reduced in proportion to the reduction of the volume of air flowing through the wind-tunnel.

Flux versus concentration. In this experiment the *flux* of odour molecules (i.e. molecules encountered per unit time) experienced by a tsetse fly will have been related to the wind speed as well as the odour concentration (Elkinton & Cardé, 1984). Therefore it could be argued that by keeping the concentration of odour constant in this experiment, the flux of molecules passing the flies' antennae will have been approximately 6 times higher for the 0.58 m s^{-1} wind speed than for the 0.10 m s^{-1}.

However, because any flying insect is not stationary relative to the wind, the flux of molecules it encounters will be directly related to its *airspeed* times the odour concentration. Hence, because tsetse flight speeds are typically considerably faster than the wind speed (up to 10× in nature; Brady *et al.*, 1995), the flux they encounter will be relatively unaffected by the wind speed. If one considers a tsetse fly entering the tunnel, in crosswind flight, the airspeed it encounters will be the hypotenuse of the vector triangle formed by its ground speed and the wind speed. For a fly crossing a 0.58 ms^{-1} wind at a ground speed of 2.5 ms^{-1} (Paynter & Brady, 1993) its airspeed will be just 3% greater than if it had crossed a 0.1 m s^{-1} wind at the same ground speed. Even for a fly flying directly up or down wind the difference will be less than 20%. Thus, if instead of holding the odour concentration constant we had adjusted it to keep the flux of molecules passing a stationary point constant, the actual flux encountered by the tsetse in flight in the tunnel would have varied by several fold between the treatments. Therefore, we decided to keep the odour concentration constant, rather than to adjust it to keep the flux constant relative to the wind speed.

Experimental protocol. Experiments were carried out in the first and last 2 h of the photophase when the flies are most active (Brady, 1988). Flies were presented to the tunnel in a battery of ten clear plastic specimen tubes (55 mm long × 35 mm diam.; one fly per tube), having been acclimatized in the tube for at least 8 h before each experiment (as in Paynter & Brady, 1993). The battery was fitted to the side of the tunnel with the tubes' open ends against an aperture in the tunnel wall. The back and sides of the battery were opaque and the tubes' open ends were closed by a transparent plastic shutter that was slid gently open at the start of each experiment. After an initial 5 min to allow the flies in the tubes to resettle, the video was switched on, a stopwatch started and the shutter opened. Recording continued for a further 3 min.

Analysis. Analysis was carried out by digitizing the *x*/*y* co-ordinates of each fly's position every 60 ms of its video-recorded flight track, from when it initially entered the tunnel until it left the field of view, with a BBC microcomputer reading the position of a cursor displayed on the monitor screen. Any subsequent flight tracks by the same fly were ignored. The output from the digitizer was analysed to reveal for each track: *entry angle*, the direction of the track as the fly entered the tunnel (first 60 ms), where a track across the tunnel = 0° and an entry angle to the left was positive (= up-tunnel) and to the right, negative (= down-tunnel); *exit angle*, the direction the fly left the field of view (last 60 ms), measured as above; and *net total turn angle*, the algebraic sum of left-hand and right-hand turns (i.e. with pluses cancelling minuses), a positive number therefore denoting a net left-hand (upwind) turn, and a negative a net right-hand (down-wind) turn. Flies that failed to reach the odour were ignored.

To remove potential biases from the plume edge or tunnel walls, analysis of the kinetic responses was restricted to a central area drawn onto the monitor screen (representing 0.5 × 1.0 m at the wind-tunnel floor; see Paynter & Brady, 1993). Flight tracks of flies entering this rectangular space were digitized as before, but also included tracks made by flies that were ignored from the previous analysis because their initial flight failed to reach odour, provided their subsequent flight crossed the centre of the tunnel. The tracks were analysed to reveal: *mean ground speed*, the horizontal distance travelled divided by the time taken; *mean air speed*, calculated by summing the vectors produced by the ground speed and the wind speed for each fly as it exited the rectangular flight space; *total turn angle*, the sum of left-hand turns and right-hand turns, ignoring their sign; *sinuosity*, the number of degrees turned per metre; and *angular velocity*, the rate of change of angular deviation of a fly measured in $°s^{-1}$. Distortions were minimized (*loc. cit.*) so that the plotted positions were accurate to within ±12%.

Results

When flies took off across the initially odourless wind in the tunnel they either flew with no initial bias to the wind direction or significantly downwind (Table 1). The control flies, which flew throughout in clean air, continued onwards more or less as they entered, so that they exited the tunnel either with no relationship with the wind direction, or significantly downwind (at 0.58 m s^{-1}).

Once flies entered CO_2 in winds of 0.58 and 0.18 m s^{-1}, they left the field of view significantly more frequently upwind than the 50% random expectation; they also turned highly

Table 1. The percentage of entry angles, exit angles and net total turns that were upwind in clean air (controls) and in CO_2 (experiments) in the three wind speeds. n = number of flies.

Treatment	Wind speed ($m\,s^{-1}$)		% Respouse		
			Enter up	Exit up	Turn up
Controls					
C.1	0.58	48	38	29**	44
C.2	0.18	57	51	58	58
C.3	0.10	53	36*	57	58
CO_2					
Ex.1	0.58	66	36*	67*	73**
Ex.2	0.18	50	40	68**	72**
Ex.3	0.10	50	48	60	54

*$P < 0.05$, **$P < 0.01$, ***$P < 0.001$ for differences from 50% (χ^2

significantly upwind. When the wind speed was further reduced, to $0.10\,m\,s^{-1}$, no significant upwind orientations were detected, however.

Analysis of the kinetic responses to odour revealed significant differences for mean ground and air speeds, and also for track sinuosities, between flies in clean air and CO_2 for the $0.58\,m\,s^{-1}$ wind speed only (Table 2). The mean ground and air speeds in CO_2 were reduced by about 17% and 15%, respectively, while the sinuosity was increased by about 70%.

The kinetic flight characteristics in odour-free air scarcely varied among the different wind speeds, though the ground and air speeds were about 10% greater in the $0.58\,m\,s^{-1}$ wind. In CO_2, however, the ground and air speeds were significantly faster and straighter in the lowest wind speed.

Discussion

We found significant kinetic responses to CO_2 only in our fastest wind speed ($0.58\,m\,s^{-1}$). Warnes (1990), however, reports flight speed reductions related to the odour concentration for both *G.morsitans* and *G.pallidipes*, at a wind speed of *c.* $0.1\,m\,s^{-1}$ (the lowest we tested). This may be because, although the concentrations of CO_2 that he introduced into his tunnel were initially lower than ours, his tunnel lacked a turbulence generating baffle and introduced odour in only a single row of four pipes (cf. our grid of pipes) so that the greater degree of mixing with clean air in our experiment will have produced more evenly low concentrations of CO_2 within the tunnel. It is also likely that his ground speed extrapolations were more precise than ours because his flies were more constrained in the vertical plane than ours, reducing errors due to camera distortion. Nevertheless, what is clear from the results is that not only are the anemotactic responses sensitive to wind (Table 1), but so also are the kinetic responses (Table 2).

Williams (1994) considers three methods of olfactory host location: anemotaxis, klinokinesis (by which he means kineses in general) and 'edge detection'. The last will, in effect, be anemo*taxis* if the flies turn upwind on entering odour (see Gibson *et al.*, 1991) or an internally steered ('idiothetic') schemakinesis (Kennedy, 1986) if the flies turn back when they leave odour.

Table 2. Mean (± SE), speeds, sinuosities, and angular velocities of the flight tracks of flies in clean air (controls) and odour (CO_2) in the three wind speeds.

Treatment	Wind speed (ms^{-1})		Ground speed ($m\,s^{-1}$)	Air speed ($m\,s^{-1}$)	Sinuosity ($°m^{-1}$)	Angular velocity ($°s^{-1}$)
Controls						
C.1	0.58	70	3.11 (0.12)a*	3.14 (0.13)a*	29.5 (3.4)a*	117.5 (10.8)a
C.2	0.18	100	2.73 (0.09)b	2.70 (0.09)b	35.5 (3.3)a	120.2 (8.3)a
C.3	0.10	89	2.84 (0.09)b	2.83 (0.09)b	30.2 (3.5)a	104.7 (9.7)a
CO_2						
Ex.1	0.58	50	2.53 (0.09)a*	2.58 (0.08)a*	50.1 (8.1)a*	151.4* (17.5)a
Ex.2	0.18	97	2.64 (0.10)a	2.62 (0.09)a	37.2 (4.3)ab	114.8 (7.9)b
Ex.3	0.10	80	3.03 (0.11)b	3.03 (0.10)b	24.0 (3.3)b	95.5 (8.8)b

n = the number of flies. The values for sinuosity and angular velocity were $\log_{10}$ transformed to normalize them for analysis; the detransformed values (±SEM) are shown. Values within a half column not followed by the same letter indicate a significant difference between the means for the controls or for the CO_2 means respectively. Values in a column followed by an asterisk indicate a significant difference between the control value and the respective CO_2 value at the same wind speed ($P < 0.05$; t test).

The absence of detectable anemotactic upwind turning in the 0.1 m s^{-1} wind is perhaps to be expected; the lateral drift of the flies (David, 1986) may be too slight for them to detect – at least in the short time they had available for a response in the wind-tunnel (<300 ms). Less expected is the lack of evidence for either ortho- or klinokinetic responses to CO_2 in both the 0.18 and the 0.10 m s^{-1} winds. If this result is translatable to the field situation, it leaves schemakinetic edge detection as the only means of biasing a random walk towards an odour source for tsetse flies in the very low wind speeds in which we have caught them at odour-baited traps (Brady *et al.*, 1995, Experiment 8).

The maximum tunnel wind speed we tested (~0.6 m s^{-1}) is close to tsetses' optimum wind speed for odour-source location in nature. This seems to be the cross-over point between the flies benefitting from the odour plume straightening out as the wind speed increases (Brady *et al.*, 1989) and being disadvantaged by the plume's reduced active space due to increased turbulence in higher winds (Brady *et al.*, 1995).

Williams's (1994) models of host location predict that, because tsetse are capable of just a few minutes of flight each day (Brady, 1988), they should sample the wind frequently in flight and adjust their direction to upwind when the wind speed is relatively fast and is therefore a relatively good indicator of a host's direction (because the odour plume straightens out as the wind speed increases; Brady *et al.*, 1989). In this situation, the slowing down and turning more often which we observed may allow the fly to sample the wind direction and odour concentration more frequently per unit displacement towards the host, and thus allow more frequent turns in odour. That should improve the fly's chances of locating the host, unless the increased turning extends the flight time beyond a minute or two.

At lower wind speeds, on the other hand (because of the odour plume's much worse extrapolation back towards its source), orientation by this combination of kineses and anemotaxis could well lead a fly to take such a tortuous route towards the host that it would exhaust its immediate fuel reserves before it managed to locate the host. Williams suggests that a less careful 'aim into the wind and shoot' response may be the best compromise under these circumstances. These effects could explain why the flies responded both anemotactically and klinokinetically to odour in the 0.58 m s^{-1} wind speed, but only anemotactically in a 0.18 m s^{-1} wind.

Williams also argues that if the wind is perfectly straight (because it is blowing at >1 m s^{-1}; Brady *et al.*, 1989), then flies should fly straight upwind without slowing down or turning more, because any such kinetic responses would simply delay the fly without increasing its chances of finding the host. We could not test such winds in our tunnel, but in the field tsetse appear to be less successful at finding odour sources when the wind rises above about 0.75 m s^{-1} (Brady *et al.*, 1995).

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