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Three-dimensional flight tracking shows how a visual target alters tsetse fly responses to human breath in a wind tunnel

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Abstract. Tsetse flies *Glossina* spp. (Diptera; Glossinidae) are blood-feeding vectors of disease that are attracted to vertebrate hosts by odours and visual cues. Studies on how tsetse flies approach visual devices are of fundamental interest because they can help in the development of more efficient control tools. The responses of a forest tsetse fly species *Glossina brevipalpis* (Newstead) to human breath are tested in a wind tunnel in the presence or absence of a blue sphere as a visual target. The flight responses are video recorded with two motion-sensitive cameras and characterized in three dimensions. Although flies make meandering upwind flights predominantly in the horizontal plane in the plume of breath alone, upwind flights are highly directed at the visual target presented in the plume of breath. Flies responding to the visual target fly from take-off within stricter flight limits at lower ground speeds and with a significantly lower variance in flight trajectories in the horizontal plane. Once at the target, flies fly in loops principally in the horizontal plane within 40 cm of the blue sphere before descending in spirals beneath it. Successful field traps designed for *G. brevipalpis* take into account the strong horizontal component in local search behaviour by this species at objects. The results suggest that trapping devices should also take into account the propensity of *G. brevipalpis* to descend to the lower parts of visual targets.

Key words. Behaviour, *Glossina brevipalpis*, insect flight, olfaction, tsetse, vision.

Introduction

Tsetse flies, *Glossina* spp. (Diptera, Glossinidae) are currently confined to sub-Saharan Africa. They feed on vertebrate blood and can transmit trypanosomes that cause sleeping sickness in man and nagana in animals. The main strategy used by tsetse on searching for a host is to respond to wind direction when flying in an odour plume (Gibson & Brady, 1988; Gibson *et al*., 1991). Indeed, tsetse are capable of detecting hosts from up to 60–120 m downwind by olfactory cues (Vale, 1977). This permits the fly to come within the visual range of the host (Gibson *et al*., 1991), where it is then guided mainly by optical cues (Torr, 1989; Bursell, 1990; Green, 1993). A variety of devices consisting of two-dimensional (2D) targets (Vale, 1974) and three-dimensional (3D) traps (Challier & Laveissière, 1973) have been developed for tsetse based on their responses to visual and olfactory stimuli. *Glossina morsitans* is capable of detecting a shape 1 m in diameter from over 30 m (Gibson & Young, 1991) and *Glossina brevipalpis* (Newstead) can detect a biconical trap from a distance of 10–15 m (Dransfield, 1984). Objects coloured in phthalogen blue, white and black that contrast with background vegetation attract tsetse at short range (Green & Flint, 1986; Laveissière *et al*., 1987; Kappmeier & Nevill, 1999a). Olfactory stimuli derived from host odour, such as phenols (Bursell *et al*., 1988), acetone (Vale, 1980) and 1-octen-3-ol (Hall *et al*., 1984), are found to attract some species (Green, 1994). Although the

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attractiveness of visual devices is reliable, their efficiency in terms of inducing flies to land on them and/or enter traps remains quite low. To improve the efficacy of trapping devices, a better understanding of the role of both vision and olfaction in tsetse responses is necessary. For this reason, observations of the flight of the savannah tsetse species *Glossina pallidipes* and *Glossina morsitans morsitans* are made in the field. Tsetse overshoot an odour source in the absence of visual cues, leading to very sharp turns on the loss of the odour plume (Bursell, 1984), whereas their flight trajectory is altered with the addition of a visual stimulus, leading to circling flights around it (Gibson *et al*., 1991).

Because it is difficult to specify the precise location of an odour plume at a given time under field conditions as a result of the variability in air movements, some studies aim to investigate the behaviour of tsetse under more precisely controlled conditions in wind tunnels and such experiments allow the identification of host odour attractants for these flies (Warnes, 1990; Paynter & Brady, 1993; Harraca *et al*., 2009). Using 2D video recording, *G. m. morsitans* is shown to respond to a plume of CO_2 by using the visual flow field of their flight over the ground (Colvin *et al*., 1989) and flies also use wind speed to adapt their flight strategy by reducing their speed with increasing CO_2 concentration (Paynter & Brady, 1996). However, these conclusions are limited because of technical restrictions and such studies are unable to observe the details of the final approach to the stimulus source, which is crucial for understanding landing responses and trap entry. More recent sophisticated video-tracking systems are reported for the more precise analysis of insect flight where the dynamics of flight responses to stimuli can be recorded (El-Sayed *et al*., 2000; Rutkowski *et al*., 2009; Lacey & Cardé, 2011). In the present study, human breath is used to elicit oriented flight by the forest tsetse *G. brevipalpis* in a wind tunnel to human breath presented alone and to a blue sphere placed in a plume of breath. A 3D tracking system placed overhead allows precise characterization of the flight tracks and comparison of the flight behaviours between purely olfactory-guided and primarily visually-guided responses.

Materials and methods

Insects

Glossina brevipalpis pupae were obtained from a colony maintained for some 90 generations at the International Atomic Energy Agency (Siebersdorf Laboratories, Austria). Unmated males and females were separated in the morning of the first day after emergence and maintained separately in rectangular netting cotton cages (1-mm mesh; 25 × 15 × 15 cm) covered with a transparent plastic bag containing wet tissue to maintain 80% relative humidity (RH) in an environmental chamber at 26 ± 1 °C and 8 h of light, and 22 ± 1 °C and 10 h of dark, with 2-h light ramps at dawn and dusk. Flies were fed with manually defibrinated bovine blood through a silicone membrane (Langley & Maly, 1969) at intervals of 2–3 days from day 2 of emergence. Flies tested in the wind tunnel had received at least two blood meals to permit them develop their flight muscles (Dame *et al*., 1969). They were starved for 7–10 days before the experiments to elicit appetitive flight responses. The experiments took place during a 2-h period at dawn or dusk, corresponding to the daily activity peaks of *G. brevipalpis*, and each fly was tested only once.

Wind tunnel

The wind tunnel (working area: 250 × 100 × 100 cm) was made of nonreflecting glass (see Supporting information, Fig. S1). A centrifugal ventilator moved humid- and temperature-controlled air (85 ± 1% RH, 25 ± 0.1 °C) across its length at 40 cm s^{-1} in a closed loop through fin-tube heat exchangers and active charcoal cartridges placed at either end of the working area. A semi-laminar air flow was produced through a perforated steel screen (1 mm thick, 3 mm round holes; 51% air passage) covered with white mosquito netting (nylon; 1-mm mesh) placed upwind and a white nylon laminar flow screen (50-μm mesh) at the downwind end. Overhead illumination (36 W, >1 kHz; TL-D daylight tubes; Philips, The Netherlands) provided approximately 1000 lux on the floor, which corresponds to sunrise and sunset in African forests (Mihok *et al*., 1996). The floor was covered with medium density fibreboard (thickness 4 mm, light brown) and the sides with white cotton sheets backed by dark grey curtains forming approximately 30 vertical folds, 4 cm in width. This provided lateral visual cues for the flies to orientate.

Experimental set-up

Flies tested individually in the wind tunnel were transferred 3–10 min before being tested in a plastic release cage (transparent PVC cylinders; length 15 cm long, diameter 5 cm) with sliding ends covered with nylon netting (1-mm mesh). The release cage was placed horizontally on a stand at 40 cm from the floor at the downwind end of the tunnel and, after fly acclimatization, both doors were lifted slowly with a nylon line on a pulley. If the fly did not exit the cage during opening, it was successively exposed for 1 min to breath-free air (pre-test period) and then for 1 min to human breath (test period; see below). The same procedure was followed in the presence of the visual target (see below). Because we had established in preliminary experiments that human breath is a good attractant for *G. brevipalpis* in the wind tunnel, human breath produced by the same person was used as the air-borne stimulus with which to compare the flight responses of flies in the presence and absence of the visual target (see below). Human breath was introduced into the middle of the wind tunnel, 20 cm upwind of the perforated steel screen and at a height of 40 cm, via a tube bent upwind for 4 cm to provide a wide plume. In the situation without the visual target, breath was introduced by blowing (four blows each lasting approximately 15 s) directly into a silicone tube (inner diameter 5 mm). To achieve the same plume structure in the presence of the visual target, breath was introduced continuously by sucking air with a polytetrafluoroethylene diaphragm

pump (MPC 100-E; Saskia Hochvakuum- und Labortechnik, Germany) from a 25-L Tedlar gas-sampling bag (SKC; Eighty Four, North Strane, Pennsylvania) filled with human breath collected at least 30 min before the first experiment. The breath from the bag was released into the wind tunnel through a Teflon tube (inner diameter 4 mm) with its opening pointing upwind at the same position as above.

The structure of the plume of breath was visualized by generating a plume of ammonium acetate and by measuring the CO_2 concentration with an infrared CO_2 detector (LI-820; LI-COR Inc., Lincoln, Nebraska) at 18 different positions in the wind tunnel during breath delivery. Because of the semi-laminar air flow, the odour plume in the wind tunnel can be described as a truncated cone with a diameter of 40 cm at its apex entering the tunnel at a height of 40 cm to the centre of the plume, widening to a width of 80 cm downwind at a height of 40 cm to its centre at the position of the release cage (see representation in Fig. 2). Mean CO_2 increases were 303.3 ± 17.7 p.p.m. at the upwind end and 184.5 ± 23.8 p.p.m. at the release cage when breath was blown directly into the wind tunnel and 40.3 ± 3.3 p.p.m. upwind and 14.8 ± 1.9 p.p.m. at the release cage when breath was delivered from the gas-sampling bag. Despite these differences, experiments showed that flights to breath blown directly into the wind tunnel or pumped from the Tedlar bag were similar.

A polystyrene sphere (diameter 12 cm) covered with cotton dyed in phthalogen blue (IF3GM; Mount Kenya Textiles, Kenya) with a maximum spectral reflectance at 465 nm (Datacolor Check Spectrophotometer; Datacolor, Lawrenceville, New Jersey) was used as a visual stimulus in conjunction with human breath. This blue has been established as the optimum colour with which to attract tsetse to traps and targets in the field (Green, 1994). The sphere was placed on a transparent glass stem (diameter 8 mm) in the plume of breath at a height of 40 cm (30 cm from the upwind end and in line with the release cage). The presence of the blue sphere in the plume of breath did not affect the plume width, nor the overall CO_2 level at the release cage.

3D recording and track analysis

Two motion-sensitive video cameras (exA640–120 m; eXcite, Germany), equipped with wide-angle objectives (SV-M0614, C-mount, f = 6 mm; VS Technology, Japan), were placed 87 cm above the roof of the wind tunnel. A specially developed software using the HALCON software library, version 7.1 (MVTec, Germany) was used to determine the 3D position of a tsetse fly in the wind tunnel to within an accuracy of 4 mm over 1.8 m. The wind tunnel was thus transformed into a 3D space with the *x*-, *y*- and *z*-axes representing, respectively, the length, width and height of the wind tunnel.

Glossina brevipalpis performed different flight trajectories when exposed to human breath alone, such as successive upwind and downwind flights and local search flights at the ends of the wind tunnel. Upwind flights were extracted from the total track for further analysis. To ascribe overall characteristics to this type of flight according to context, the tracks were cut off at the apex of 180° loops made at either ends of the wind tunnel or after take-off. In addition, only upwind flights >1 m, starting 35 cm from the downwind end and occurring in a 60 cm wide zone in the middle of the wind tunnel (i.e. within the odour plume) were selected.

A distance based interpolation of the recorded tracks at 5-cm steps on the *x*-axis permitted resampling each flight path at defined positions and aligning them on this axis for the purpose of a comparison of flights between treatments. Different flight parameters such as total distance covered, instantaneous speed (i.e. ground velocity), straightness (defined as the distance between the first and the last position of an upwind flight divided by the distance covered by the insect), angular sum (defined as the sum of all the turns made by the fly) and instantaneous flight directions (defined as the 3D angle between the *x*-axis and each flight segment) were computed on the basis of Cartesian coordinates. The mean unit orientation vector of each track was characterized as the angles between the straight lines joining the first and last flight positions in the *xy* and *xz* planes and the *x*-axis, and vector length as an index of track straightness.

After pooling first and subsequent upwind flight responses to breath (not dissimilar at the 99% confidence level, Mann–Whitney *U*-test), the instantaneous *y*- and *z*-axis positions of flights to the different treatments, flight straightness indices, instantaneous flight directions and maximum ground velocities were compared using the Mann–Whitney *U*-test. The mean unit orientation vectors were compared using the *F*-test. The statistical software R, version 2.11.1 (http://www.r-project.org) was used for all of the statistical analyses. Because no differences were observed between the sexes, they were pooled for analysis.

Results

Characteristics of responses to olfactory and visual stimuli

Of the 55 flies tested with human breath alone, 80% took off during the test period, 49% flew upwind and 38% of these recordings were suitable for analysis ($n = 21$ flies). No flies took off during the pre-test period to air alone. The analyzed flights lasted between 4 and 141 s and covered distances between 6 and 153 m in the 2.5-m long wind tunnel. These flights were characterized by an upwind response in the plume of breath to the source, sometimes followed by local search behaviour at the upwind end of the wind tunnel. Afterwards, flies generally flew downwind toward the release cage where they undertook a second local search behaviour. This was followed, in 15 out of 16 recordings, by at least one subsequent upwind flight (Fig. 1A). When the plume of breath was lost, flies landed mainly at the downwind end or flew to the ceiling of the wind tunnel. Flights by *G. brevipalpis* stimulated by breath alone were characterized by 16 first (Fig. 1B) and 26 subsequent upwind flights.

With the blue target presented in the plume of breath, 32 flies were tested, 69% took off during the test period, 47% flew upwind and 34% were retained for analysis ($n = 11$ flies).

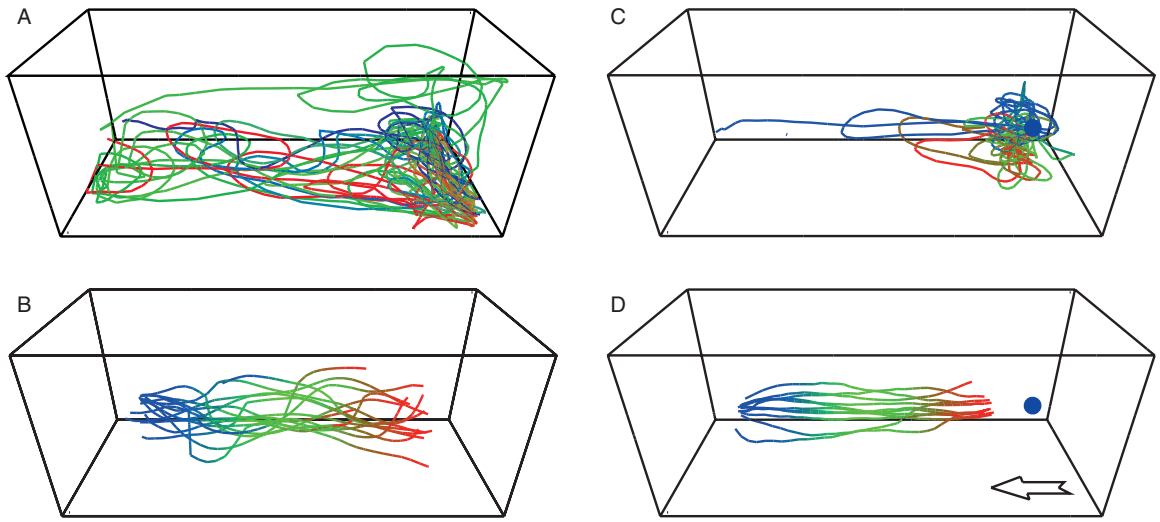

Fig. 1. Illustration of complete flights made by *Glossina brevipalpis* recorded in three dimensions in response to human breath in the absence (A) and presence (C) of an upwind visual target, and all first upwind flights of *G. brevipalpis* recorded in the absence (B; $n = 16$) and presence (D; $n = 11$) of a visual target. The outline of the wind tunnel ($2.5 \times 1 \times 1$ m) is viewed at 60° from above. Each flight starts downwind (left) in blue and finishes upwind (right) in red. The blue sphere at the upwind end (C, D) represents the visual target. The white arrow in (D) indicates the wind direction, common to all illustrations.

Flies did not take off when a visual target was presented on its own upwind but, on the delivery of human breath, flies flew for between 5 and 117 s and covered distances between 6 and 40 m. These flights were directed to the target (Fig. 1C), even when the visual target was placed outside the plume of breath (data not shown), followed by local search behaviour close to it. Of 11 upwind flights to the target (Fig. 1D), only one was followed by a downwind flight and no subsequent upwind flights were recorded when the visual target was present.

Trajectory of upwind flights: altitude, sinuosity and 3D straightness

On the vertical plane, *G. brevipalpis* flew in the lower part of our 1-m high wind tunnel in response to human breath. Indeed, in all upwind flights, most flies flew within 50 cm of the floor and none flew >70 cm. During the first upwind flights, *G. brevipalpis* stimulated by breath alone flew at a mean altitude of 37 ± 8 cm (Fig. 2A), which is significantly higher than during subsequent upwind flights made at a height of 19 ± 12 cm (Mann–Whitney U-test, W = 409 274, $P < 0.01$; see Supporting information, Fig. S2A). In the presence of the visual target, flies flew at a height of 39 ± 7 cm (Fig. 2B), which is close to the altitude of the first upwind flights to breath alone yet significantly higher. The variation of the mean angular trajectories in the vertical plane was much higher in the absence than in the presence of the visual cue, with variations of ±5.00° and ±2.06°, respectively (F-test, $F_{15,10} = 5.87$, $P < 0.01$; Fig. 3A). Moreover, a clear drop in altitude in the first part of the upwind flight was recorded only when no visual target was present (Fig. 2A).

On the horizontal plane, the upwind flights to the two treatments (i.e. to human breath with or without a visual target) represent two distinct flight patterns. Without the visual target, all upwind flights to breath alone ($n = 16$ first upwind flights plus 26 subsequent upwind flights) displayed a meandering pattern in the horizontal plane, covering a broad part of the wind tunnel width (Fig. 2C). By contrast, upwind flights to the visual target were significantly straighter and more confined to the middle of the wind tunnel, with a significantly lower variance in flight positions in the horizontal plane (F-test, $F_{354,1395} = 0.17$, $P < 0.01$; Fig. 2D; see Supporting information, Fig. S2B). Indeed, the variation in the mean angular trajectory in the horizontal plane was higher in the absence (±7.90°) than presence (±2.73°) of the visual target (F-test, $F_{15,10} = 8.34$, $P < 0.01$; Fig. 3B). The 3D straightness indices of 0.84 ± 0.10 generated for flights to breath alone and 0.97 ± 0.02 to breath in the presence of the visual target demonstrate a significant increase of aiming at the target by the flies in the plume of breath (Mann–Whitney U-test, W = 165, $P < 0.01$; Fig. 3; see Supporting information, Fig. S2C). In flights to the visual target, the flies turned approximately 2.5-fold less than in its absence (Mann–Whitney U-test, $W = 85\ 221$, $P < 0.05$), with a significant lower variance in their instantaneous directions (F-test, $F_{343,1353} = 0.3216$, $P < 0.01$; Fig. 4). In addition, these flies did not fly straight against the wind but progressed at a low angle (11.13 ± 4.06°) to the wind direction.

Ground velocity of upwind flights

Glossina brevipalpis reached a maximum speed of 4.64 m s^{-1} against the wind and no flies flew slower than 0.60 m s^{-1} during these experiments. Instantaneous ground velocity during upwind flights without a visual target increased gradually to reach a mean maximum velocity of 2.71 ± 0.75 m s^{-1} in the middle of the wind tunnel

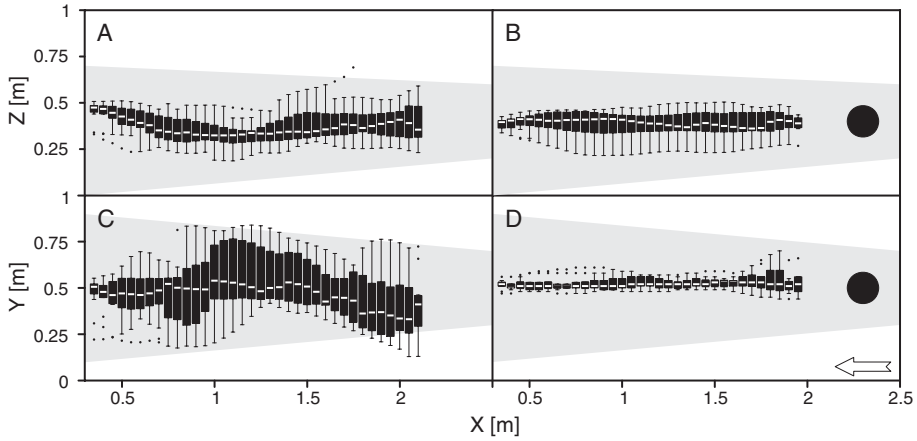

Fig. 2. Deviations of first upwind flights made by *Glossina brevipalpis* in the vertical (xz) and horizontal (xy) planes in response to human breath in the absence (A, C; $n = 16$) and presence (B, D; $n = 11$) of a visual target in the wind tunnel. Each box plot represents the position of the median, the 25th and 75th quartiles and the interquartile range; the dots represent outliers. The rectangle is the outline of the wind tunnel, the disc in (B) and (D) represents the visual target, the greyed-over area is an outline of the zone covered by the plume of breath and the white arrow indicates the wind direction, common to all illustrations.

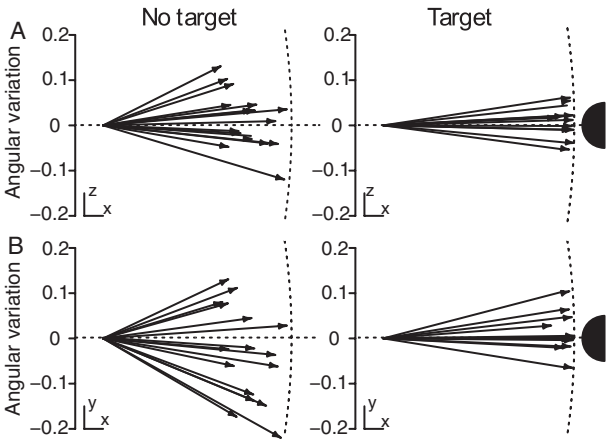

Fig. 3. Mean unit orientation vectors in the vertical (xz; A) and horizontal (xy; B) planes of upwind flights made by *Glossina brevipalpis* in response to human breath in the absence (left) and presence (right) of a visual target in the wind tunnel. Each arrow represents the orientation vector of one upwind flight by a fly, with the arrow's angle from horizontal corresponding to the mean angle of flight and the arrow length corresponding to the straightness of the flight track. The dashed arc represents the maximum straightness of 1. The visual target is visualized at right when present. The third two-dimensional plane (yz) is not represented because it did not provide any additional information.

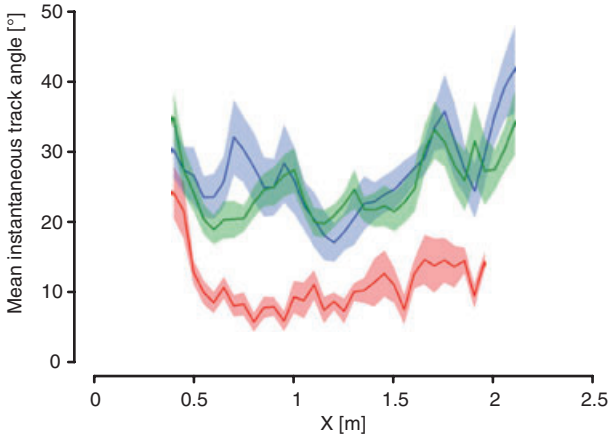

Fig. 4. Mean instantaneous track angles of flights along the wind tunnel (abscissa) in response to human breath in the absence (first upwind flights in blue, $n = 16$; subsequent upwind flights in green, $n = 26$) and presence (red, $n = 11$) of a visual target. Shaded areas represent the SEM.

(approximately 1 m from the release cage) and then decreased slightly to allow the flies to prepare for a sharp turn to avoid hitting into the upwind end of the wind tunnel (data not shown). In the presence of the visual target, the mean maximum ground velocity of upwind flights was significantly lower than without the visual target (2.13 ± 0.33 m s^{-1}; Mann–Whitney U-test, W = 134, $P < 0.01$; see Supporting information, Fig. S2D) and the variance in ground velocity was also lower than for all flights without the visual target (F-test, $F_{354,1395} = 0.63$, $P < 0.01$). During subsequent upwind flights without a target, the flies reached a mean maximum ground velocity of 2.59 ± 0.45 m s^{-1}, which is not different from the first upwind flights to the same treatment (Mann–Whitney U-test, $W = 197$, $P > 0.01$; F-test, $F = 2.66$, $P > 0.01$).

Local search behaviour linked to specific stimuli

On reaching the upwind end of the wind tunnel, *G. brevipalpis* undertook a local search behaviour. In the absence of the visual target, 67% of the flies flew in wide descending loops across the entire lower part of the upwind end (i.e. approximately 90 cm in width and within 40 cm of the floor) (Fig. 5A). Of these flies, 29% made the descending

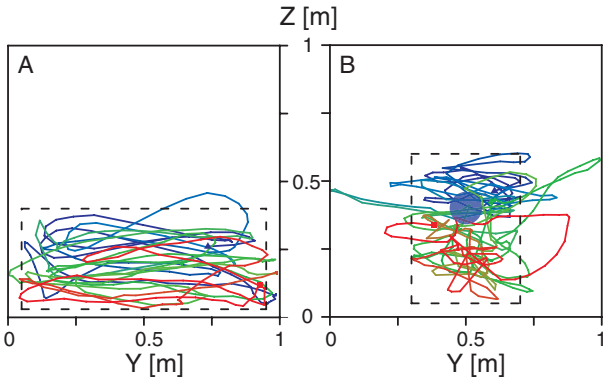

Fig. 5. Characteristic local search behaviours made at the upwind end of the wind tunnel by *Glossina brevipalpis* responding to breath alone (A) and by another fly responding to a visual target in a plume of breath (B). The *y*- and *z*-axes represent the width and height of the wind tunnel, respectively. Each flight track section begins in blue with a blue triangle and ends in red with a red square. The blue disc in (B) indicates the position of the visual target and the dashed rectangle indicates the zone where flight predominated in each case.

loops after the first upwind flight and 93% after a subsequent upwind flight. When the target was added to the plume of breath, 45% of the flights at the sphere were confined to its vicinity or immediately beneath it. These flies flew in loops within 40 cm of the sphere and then flew in a descending spiral within a column extending 60 cm from the floor (i.e. always near or below the target) (Fig. 5B).

Discussion

Response of G. brevipalpis *to the stimuli tested*

Glossina brevipalpis stay within the plume of breath, heading towards the source using wind-borne stimuli in the wind tunnel. The attraction of *G. brevipalpis* to human breath that is reported in the present study is somewhat at odds with the mild level of attraction recorded for *G. pallidipes* and *G. morsitans* to this stimulus in the field (Vale, 1979). However, as demonstrated by Colvin *et al*. (1989) and Paynter & Brady (1996), the CO_2 level present in breath induces, on its own, anemotactic responses from these two savannah species. Conversely, with *G. brevipalpis* in the present study, preliminary experiments with the wind tunnel show that, even though CO_2 activates the flies, only few of them initiate an upwind flight. Evidently, other host metabolites present in breath provide critical cues that lead to an upwind flight response (Harraca *et al*., 2009). The blue cloth used as visual target reflects light within the sensitivity spectrum of the tsetse fly eye (Green & Cosens, 1983) and is placed within the visual range of the fly (Gibson & Young, 1991). Despite this, resting flies do not respond to the stationary visual target presented upwind without stimulation from human breath. This confirms the field observations of Kappmeier & Nevill (1999b) suggesting that odour is more important than colour in the attraction of *G. brevipalpis*.

Flight characteristics of G. brevipalpis *to olfactory stimulation alone*

When no visual target is present, *G. brevipalpis* are shown to use horizontal zigzags by preference, rather than vertical shifts when progressing upwind, because they maintain the same altitude during their approach, although with wide deviations in the horizontal plane. This flight strategy allows the flies to sample a larger area of ground to minimize the risk of odour loss. Paynter & Brady (1996) report similar horizontal meandering flights for *G. m. morsitans* in response to CO_2 in the laboratory. Having failed to encounter an appropriate visual stimulus upwind at the odour source, *G. brevipalpis* fly downwind to undertake a second upwind attempt at finding the source, which is a behaviour that is rarely observed when the visual target is present. Although the first upwind flights take place at the altitude of take-off, the low upwind flights (~30 cm from the ground) that are recorded during subsequent upwind approaches are similar to the heights of displacements observed in the field for *G. pallidipes* and *G. m. morsitans* (Bursell, 1984; Gibson & Brady, 1988; Torr, 1988). In the present wind tunnel study, *G. brevipalpis* fly with a range of speeds between 0.6 and 4.6 m s^{-1}, faster than any previous wind tunnel reports for tsetse flies. The slight decrease in ground speed recorded at the upwind end of the wind tunnel is probably a result of the mechanical and aerodynamic needs of turning, as noted in the field for *G. pallidipes* and *G. m. morsitans* near visual targets (Gibson *et al*., 1991).

Flights by G. brevipalpis *to the blue target in a plume of breath*

The addition of the blue visual target to breath at the upwind end of the wind tunnel changes the flight behaviour of *G. brevipalpis* compared with the response to breath presented alone. Flights in the presence of the blue sphere are made at lower speeds and with lower horizontal deviations along the flight tracks, such that flies aim directly at the target, with finely controlled flight speeds and trajectories. This confirms the critical role of vision in the orientation behaviour of these diurnal insects towards resources. The straightness index of *G. brevipalpis* flights to breath alone is high (at 0.84 in the wind tunnel situation) but increases to 0.97 in the presence of the visual target. Coincidentally, using the same method to calculate straightness, Gibson *et al*. (1991) report a straightness index >0.9 for most of the upwind flights of *G. pallidipes* and *G. m. morsitans* to visual targets baited with odour in the field. Surprisingly, few flies landed on the visual target in the wind tunnel. This is probably a result of the absence of a black substrate that is known to induce a stronger landing response in *G. brevipalpis* than phthalogen blue (Kappmeier & Nevill, 1999a). Indeed, despite the fact that phthalogen blue cloth is almost twice as attractive as black for *G. brevipalpis*, the majority of flies that circle a 1-m^2 black or blue target in the field never land.

Local search behaviours

In the absence of the visual target, *G. brevipalpis* undertake a local search in the vicinity of the odour source at the upwind end of the wind tunnel, where flies describe descending wide horizontal castings, frequently leaving the plume of breath. This casting behaviour is undertaken predominantly after subsequent upwind flights to the source and can be interpreted as an effort by this highly visual fly species to find a route to move beyond the screen barrier to the odour source. By contrast, after the aimed flight to the target, the flies undertake a local search behaviour closely confined to a zone around and immediately beneath the blue sphere. This corresponds to what is recorded in the field, where up to 40% of *G. pallidipes* and *G. m. morsitans* respond to a black target by circling it (Gibson *et al*., 1991). The descending circling flights made beneath the target are probably indicative of a searching strategy by *G. brevipalpis* for a place to land, and can be related to the habit of this species (Kappmeier & Venter, 2007), as well as other forest tsetse flies, to alight on the lower part of objects (Vreysen *et al*., 1998). Such low flying behaviours are also observed in the field for *G. m. morsitans* and *G. pallidipes*, leading to the design of traps into which tsetse flies enter from below (Vale, 1982).

Implication for design of effective control devices for G. brevipalpis

The current wind tunnel observations on the flight responses of *G. brevipalpis* have a bearing on the design of effective control devices for this species. Indeed, the development of an effective 3D trap for *G. brevipalpis* has been hampered by the lack of a vertical flight response in this species that would allow it enter a cone leading to the cage above the attractive surfaces of traps, as in other tsetse fly-trapping devices (Kappmeier, 2000). To circumvent this, the H trap with side cones emerging at ground level was developed, which captures *G. brevipalpis* that enter and fly horizontally within the trap. However, the best design of this H trap is no more than 50% efficient, leading Kappmeier (2000) to conclude that this is a result of flies leaving the device. These observations can now be supported by the present recordings of *G. brevipalpis* flight responses, where a horizontal component in local search behaviour is accompanied by an important descent to fly at lower levels from the ground. Departure of flies from the bottom of the H trap, and not just via the entrances as suggested by Kappmeier (2000), could in this case contribute further to its poor efficiency. Retaining flies in the trap through the addition of a base has already been recognized by Ndegwa & Mihok (1999) in the development of the S3 trap for *Glossina swynnertoni*, a species that also shows a predilection to fly low around and within this trapping device.

Conclusions

The wind tunnel findings reported in the present study demonstrate how *G. brevipalpis* can use both olfactory and visual information to navigate towards resources. The role of olfaction has already been documented in the field for this species (Kappmeier & Nevill, 1999a), and the current 3D video tracking demonstrates how a visual target modifies significantly the flight response. In addition, the present recordings provide information on local search behaviours in this species, which may lead to an improvement of trapping devices.

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Supporting Information

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Figure S1. Photo of the wind tunnel set up showing the underneath container with the fin-tube heat exchangers, connected to the upwind container with, in turn, the active charcoal filter, volatile stimulus introduction zone and laminar flow screen covered with a mosquito netting (visible). The two motion-sensitive cameras are integrated in the overhead lighting (dotted circles). The blue visual target is shown and wind direction is indicated. For more details see text.

Figure S2. Parameters of *G. brevipalpis* flight responses to human breath in presence and absence of a visual target: flight altitude (A), variance in flight track positions in the horizontal plane (B), straightness (C) and maximum ground velocity (D). Each box plot represents the position of the median, the 25th & 75th quartiles and the interquartile range; dots represent outliers.

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References

Bursell, E. (1984) Observations on the orientation of tsetse flies (*Glossina pallidipes*) to wind-borne odors. *Physiological Entomology*, **9**, 133–137.

Bursell, E. (1990) The effect of host odor on the landing responses of tsetse flies (*Glossina morsitans morsitans*) in a wind-tunnel with and without visual targets. *Physiological Entomology*, **15**, 369–376.

Bursell, E., Gough, A.J.E., Beevor, P.S. *et al*. (1988) Identification of components of cattle urine attractive to tsetse flies, *Glossina* spp (Diptera, Glossinidae). *Bulletin of Entomological Research*, **78**, 281–291.

Challier, A. & Laveissière, C. (1973) Un nouveau piège pour la capture des glossines (*Glossina*: Diptera, Muscidae) description et essais sur le terrain. *Cahiers ORSTOM Serie Entomologie Medicale et Parasitologie*, **11**, 251–262.

Colvin, J., Brady, J. & Gibson, G. (1989) Visually-guided, upwind turning behaviour of free-flying tsetse flies in odor-laden wind: a wind tunnel study. *Physiological Entomology*, **14**, 31–39.

Dame, D.A., Birkenmeyer, D.R. & Bursell, E. (1969) Development of the thoracic muscle and flight behaviour of *Glossina morsitans orientalis* Vanderplank. *Bulletin of Entomological Research*, **59**, 345–350.

Dransfield, R.D. (1984) The range of attraction of the biconical trap for *Glossina pallidipes* and *Glossina brevipalpis*. *Insect Science and its Application*, **5**, 363–368.

El-Sayed, A.M., Godde, J. & Arn, H. (2000) A computer-controlled video system for real-time recording of insect flight in three dimensions. *Journal of Insect Behavior*, **13**, 881–900.

Gibson, G. & Brady, J. (1988) Flight behavior of tsetse flies in host odor plumes: the initial response to leaving or entering odor. *Physiological Entomology*, **13**, 29–42.

Gibson, G. & Young, S. (1991) The optics of tsetse fly eyes in relation to their behavior and ecology. *Physiological Entomology*, **16**, 273–282.

Gibson, G., Packer, M.J., Steullet, P. & Brady, J. (1991) Orientation of tsetse-flies to wind, within and outside host odor plumes in the field. *Physiological Entomology*, **16**, 47–56.

Green, C.H. (1993) The effects of odors and target color on landing responses of *Glossina morsitans morsitans* and *Glossina pallidipes* (Diptera, Glossinidae). *Bulletin of Entomological Research*, **83**, 553–562.

Green, C.H. (1994) Bait methods for tsetse fly control. *Advances in Parasitology*, **34**, 229–291.

Green, C.H. & Cosens, D. (1983) Spectral responses of the tsetse fly, *Glossina morsitans morsitans*. *Journal of Insect Physiology*, **29**, 795–800.

Green, C.H. & Flint, S. (1986) An analysis of color effects in the performance of the F2 trap against *Glossina pallidipes* (Austen) and *G. morsitans morsitans* (Westwood) (Diptera, Glossinidae). *Bulletin of Entomological Research*, **76**, 409–418.

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

Harraca, V., Syed, Z. & Guerin, P.M. (2009) Olfactory and behavioural responses of tsetse flies, *Glossina* spp., to rumen metabolites. *Journal of Comparative Physiology A: Neuroethology Sensory Neural and Behavioral Physiology*, **195**, 815–824.

Kappmeier, K. (2000) A newly developed odour-baited 'H trap' for the live collection of *Glossina brevipalpis* and *Glossina austeni* (Diptera: Glossinidae) in South Africa. *Onderstepoort Journal of Veterinary Research*, **67**, 15–26.

Kappmeier, K. & Nevill, E.M. (1999a) Evaluation of coloured targets for the attraction of *Glossina brevipalpis* and *Glossina austeni* (Diptera: Glossinidae) in South Africa. *Onderstepoort Journal of Veterinary Research*, **66**, 291–305.

Kappmeier, K. & Nevill, E.M. (1999b) Evaluation of a proposed odour-baited target to control the tsetse flies *Glossina brevipalpis* and *Glossina austeni* (Diptera: Glossinidae) in South Africa. *Onderstepoort Journal of Veterinary Research*, **66**, 327–332.

Kappmerier, K. & Venter, G.J. (2007) Evaluation and improvement of sticky traps as monitoring tools for *Glossina austeni* and *G. brevipalpis* (Diptera: Glossinidae) in north-eastern KwaZulu- Natal, South Africa. *Bulletin of Entomological Research*, **97**, 545–553.

Lacey, E.S. & Cardé, R.T. (2011) Activation, orientation and landing of female *Culex quinquefasciatus* in response to carbon dioxide and odour from human feet: 3-D flight analysis in a wind tunnel. *Medical and Veterinary Entomology*, **25**, 94–103.

Langley, P.A. & Maly, H. (1969) Membrane feeding technique for tsetse flies (*Glossina* spp). *Nature*, **221**, 855–856.

Laveissière, C., Couret, D. & Grébaut, P. (1987) Recherches sur les écrans pour la lutte contre les glossines en région forestière de Côte d'Ivoire. Mise au point d'un nouvel écran. *Cahiers ORSTOM Serie Entomologie Medicale et Parasitologie*, **25**, 145–164.

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

Ndegwa, P.N. & Mihok, S. (1999) Development of odour-baited traps for *Glossina swynnertoni* (Diptera: Glossinidae). *Bulletin of Entomological Research*, **89**, 255–261.

Paynter, Q. & Brady, J. (1993) Flight responses of tsetse-flies (*Glossina*) to octenol and acetone vapor in a wind-tunnel. *Physiological Entomology*, **18**, 102–108.

Paynter, Q. & Brady, J. (1996) The effect of wind speed on the flight responses of tsetse flies to CO_2: a wind-tunnel study. *Physiological Entomology*, **21**, 309–312.

Rutkowski, A.J., Quinn, R.D. & Willis, M.A. (2009) Three-dimensional characterization of the wind-borne pheromone tracking behavior of male hawkmoths, *Manduca sexta*. *Journal of Comparative Physiology A: Neuroethology Sensory Neural and Behavioral Physiology*, **195**, 39–54.

Torr, S.J. (1988) Behavior of tsetse flies (*Glossina*) in host odor plumes in the field. *Physiological Entomology*, **13**, 467–478.

Torr, S.J. (1989) The host-orientated behavior of tsetse flies (*Glossina*): the interaction of visual and olfactory stimuli. *Physiological Entomology*, **14**, 325–340.

Vale, G.A. (1974) Responses of tsetse flies (Diptera, Glossinidae) to mobile and stationary baits. *Bulletin of Entomological Research*, **64**, 545–588.

Vale, G.A. (1977) Flight of tsetse flies (Diptera-Glossinidae) to and from a stationary ox. *Bulletin of Entomological Research*, **67**, 297–303.

Vale, G.A. (1979) Field responses of tsetse flies (Diptera, Glossinidae) to odors of men, lactic acid and carbon dioxide. *Bulletin of Entomological Research*, **69**, 459–467.

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

Vale, G.A. (1982) The trap orientated behavior of tsetse flies (Glossinidae) and other Diptera. *Bulletin of Entomological Research*, **72**, 71–93.

Vreysen, M.J.B., Zhu, Z.R. & Saleh, K.M. (1998) Field responses of *Glossina austeni* to sticky panels on Unguja Island, Zanzibar. *Medical and Veterinary Entomology*, **12**, 407–416.

Warnes, M.L. (1990) The effect of host odor and carbon dioxide on the flight of tsetse flies (*Glossina* spp) in the laboratory. *Journal of Insect Physiology*, **36**, 607–611.

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