THE INITIAL FLIGHT DIRECTION OF TSETSE (DIPTERA: GLOSSINIDAE) EXPOSED TO NATURAL AND SYNTHETIC OX ODOUR

C.A. Groenendijk[1,2], N.T. Griffiths[3] & W. Takken[2]

[1]Tsetse and Trypanosomiasis Control Branch P.O.-Box CY 52 Harare Zimbabwe
[2]Department of Entomology Wageningen Agricultural University, P.O.-Box 8031 EH Wageningen The Netherlands
[3]Imperial College Silwood Park Ascot

Key Words: artificial odour, initial flight direction, *Glossina pallidipes*, Tsetse-fly.

Summary

In the field in Zimbabwe, the behaviour of tsetse (Diptera: Glossinidae) released 10 m downwind of an odour source was studied with a video camera and with electric nets. Video studies showed that in the absence of odour, 46% of the released *Glossina pallidipes* Austen turn downwind and 32 % turned upwind. Tsetse left the box at a constant rate. When an artificial odour mixture containing carbon dioxide, acetone, octenol and phenols was used significantly fewer tsetse, 35 %, turned downwind and more tsetse, 37 %, turned upwind. In the presence of odour, tsetse left the TRB later and not at a constant rate. When the TRB was placed in a complete ring of electric nets, the release of natural ox odour changed the distribution of tsetse to the downwind electric nets compared to the no odour treatment. Artificial odour, with and without carbon dioxide, had no effect on the distribution of tsetse over the electric nets. The difference between the video study and the electric net study is attributed to the 50% efficiency of electric nets. We infer from the results that 10% of the tsetse departing from the TRB reacts to the presence of odour immediately.

INTRODUCTION

In recent studies (Griffiths *et al.*, 1995, Groenendijk *et al.*, in prep), marked *Glossina pallidipes* Austen (Diptera: Glossinidae) were released from a tsetse release box (TRB) placed at various distances downwind of an odour source. In both studies, the recapture percentage of marked tsetse at the source declined with increasing distance between odour source and release site in the presence of odour, whereas the recapture percentage remained constant in the absence of odour.

These differences in recapture percentage might be caused by the initial flight direction tsetse take when they leave the TRB. At short distances from the source, an odour plume is only a few metres wide (Brady & Griffiths, 1993). Tsetse might leave the TRB in an escape response, during which they presumably do not respond to odour. An escape response of one second at the preferred groundspeed of 5 m/s (Brady, 1991) would then be enough to take tsetse out of the odour plume. Groenendijk *et al* (in prep) suggest that this was one of the reasons why they recaptured fewer tsetse than Griffiths *et al* (1995).

Here we report the results of studies on the behaviour of tsetse just after they left the TRB. Tsetse leaving the TRB were either recorded on video or captured with electric nets placed at less than two metres from the TRB.

MATERIAL & METHODS

Experiments were conducted near Rekomitjie Research Station, Zambezi Valley, Zimbabwe, where *G. pallidipes* is abundant. Experiments with electric nets were conducted from October 1993 until September 1994 and tsetse were videoed during five consecutive days in August 1994. The electric nets were removed during the video experiment.

Tsetse were caught with epsilon traps (Hargrove & Langley, 1990) during the three hours preceding the release. The traps were baited with acetone released from a small bottle (0.5 g/h), and 1-octen-3-ol (0.4 mg/h), 4-methylphenol (0.8 mg/h) and 3-n-propylphenol (0.1 mg/h) released from a polythene sachet (Laveissière *et al.*, 1990). Traps were fitted with a non-return netting cage of 20 x 10 x 10 cm. After removal, the cages containing tsetse were kept in a cool box with a damp cloth until the tsetse were marked with a small spot of artist's oil paint which indicated the day of release. An earlier study showed no effect of a similar paint or colour on the survival of marked tsetse (Vale *et al.*, 1976).

Marked tsetse were put singly in plastic tubes in the release box (Fig. 1). The tubes were 10 cm long and 5 cm in diameter. The lower ends of the tubes were covered with netting in which a small slit was made to allow the insertion of a tsetse. The top ends of the tubes were covered with a black cloth which, when pulled away, simultaneously exposed all tubes. The release boxes were placed downwind of the source, underneath a hessian shade. The black cloth was pulled away by a string from a distance of 30 m across the prevailing wind direction, 10 minutes after odour release was started. There were not always enough flies to fill all release tubes, the number released varied from 35 to 125.

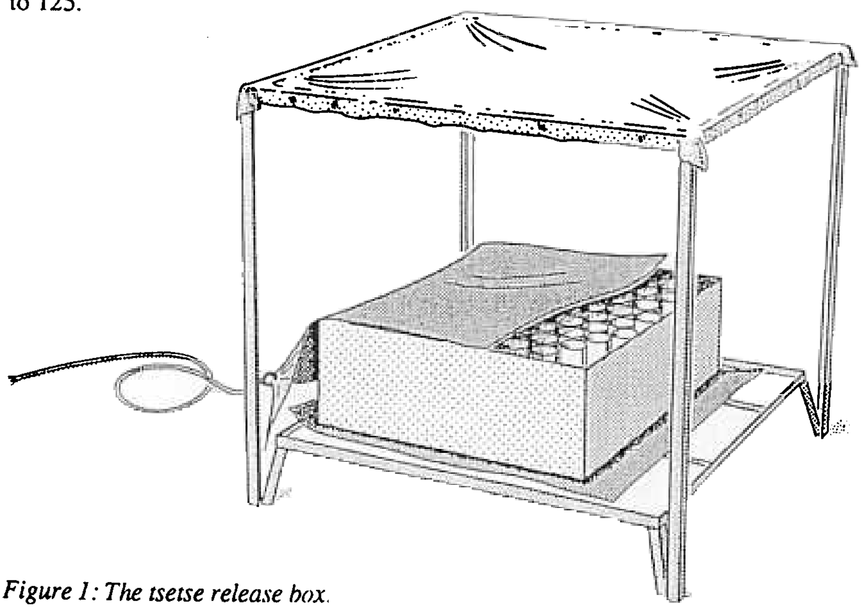

Figure 1: The tsetse release box.

Natural ox odour (OX) and two mixtures of known kairomones were tested. The Artificial Odour (AO) consisted of acetone (0.2 g/h), 1-octen-3-ol (0.16 mg/h), 4-methylphenol (0.32 mg/h) and 3-n-propylphenol (0.04 mg/h), released from a polythene sachet. Complete Artificial Odour (CAO) consisted of AO supplemented with carbon dioxide (480l/h). As a control, we used no odour (NO). Because of time constraints, only NO and the CAO mixture were tested when video recordings were made.

The TRB was placed 10 m downwind of an odour source, in the centre of a complete ring of eight electric nets (Vale, 1974). Each net was 1.5 m square and placed 1.8 m from the TRB. Electrocuted tsetse fell straight down (Vale, 1974) on corrugated iron sheets where they were retained with polybutene. The corrugated iron sheets were divided in sixteen sectors, two per net each 22.5° wide. The experiment was conducted during the hour before sunset.

With electric nets we could separate males from females. However, about 50% of the tsetse colliding with an electric net are killed or stunned, the others escape apparently unscathed (Packer & Brady, 1990, Griffiths & Brady, 1994). In this experiment the latter group could get killed on the next contact, with another electric net, which could seriously alter the distribution of recapture of tsetse over the 16 sectors.

The behaviour of tsetse after opening of the TRB was recorded on video (Gibson & Brady, 1985) to eliminate the effect of net efficiency. This meant that we could not separate the sexes from each other. The TRB was placed 10 m downwind of the odour source, on a floor of black velvet. The field of view of the camera was ± 2 x 3m, i.e. up to 1 or 1.5 m from the centre of the TRB. The edge of the field of view was closer to the centre of the box than the electric nets. The video equipment recorded with a time base. On the first day, recordings were made for 45 minutes. Analysis showed that virtually all tsetse had left the box after 20 minutes and on subsequent days recordings were made for 22 minutes.

Chi-square tests were used to test for significant differences in distribution of recaptured tsetse over the net sectors. A circle, divided in 16 sectors of 22.5 °, was drawn on an acetate sheet, which was placed in front of the monitor when analysing the video tapes. For each tsetse fly, the time at which it appeared from under the shade (appearing time), the sector in which it appeared, (initial direction), and the sector in which it left the field of view of the camera (leaving direction) were recorded. If a tsetse fly left in another sector than it appeared, this was classified as a turn, directed either upwind or downwind and measured in sectors. We used run tests to see whether tsetse followed each other. If a tsetse fly appeared within 0.5 s of the previous tsetse and left the field of view within 45° (two sectors) of the previous tsetse, it was considered a 'follower'. To test for differences between cumulative curves of tsetse leaving the TRB, and for goodness of fit of the observed curves, the Kolmogorov-Smirnov test was used.

RESULTS

The sectors 1 and 16, 2 and 15 etc., were pooled (i.e. added and the resulting percentage halved) because they had the same orientation relative to the mean wind direction. This resulted in eight sectors, from sector one, pointing upwind, to sector eight, pointing downwind. There was no difference in distribution over the nets or recapture percentages between males and females, the data of the sexes were therefore pooled.

Electric nets. Each treatment was repeated at least 6 times and at least 800 tsetse were released per treatment. The recapture rate varied, but not significantly, from 39 % with ox odour to 51 % with CAO. The remaining tsetse probably flew over the nets. The artificial mixtures and the control all caused a similar distribution of catches over the nets. The release of OX odour caused a significant shift of the catch to the downwind net, compared to the other odours (χ^2, $P<0.025$). AO caused a shift towards the crosswind oriented nets, compared to CAO (χ^2, $P<0.025$) (Fig. 2).

Video recordings. In the presence of odour 309 tsetse were observed and in the absence of odour 203. Most tsetse appeared in view within the first minute after opening and 95-100% had left the TRB after 20 minutes. A few tsetse did not leave the field of view immediately, but returned under the shade or settled on it. These tsetse later followed another tsetse leaving the box. We ignored these flies in the analysis of odour effects on departure directions.

Tsetse released in the presence of odour, appeared later than those released in the absence of odour (Kolmogorov-Smirnov, DN= 0.122, P<0.05). In the absence of odour, tsetse appeared at a constant rate (Kolmogorov-Smirnov: DN 0.043, P>0.05). In the presence of odour, the cumulative departure curve of tsetse appearing in view differed significantly from the best-fit single exponential (Kolmogorov Smirnov: DN=0.112, P<0.05).

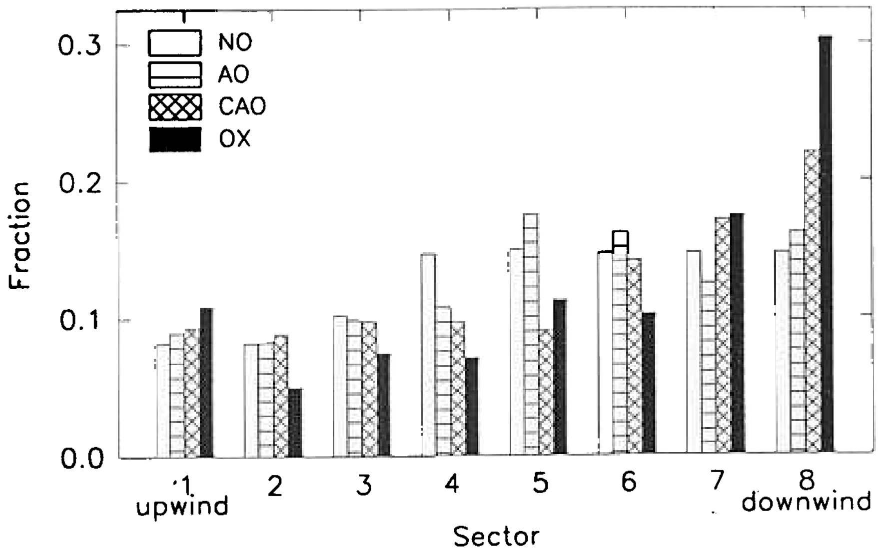

Figure 2: *The distribution of tsetse over the sectors of electric nets in the presence of different odours.*

When CAO was present, there were no more or fewer runs than expected, and tsetse were thus not influencing each others flight direction. When odour was absent, there were significantly more runs than expected (P<0.05). This suggested that tsetse avoided each other.

Video studies showed no significant effect of CAO on the sectors in which tsetse appeared into view or left the field of view. But there was a difference in turn size and direction (P<0.05). When odour was present, fewer tsetse (35 % vs 46 %) turned downwind, whereas more tsetse did not turn (28 % vs 22 %) or turned upwind (37 % vs 32 %). Thus, a fraction of the tsetse responded immediately, to the presence of odour.

Comparison of methods. There was no significant difference in distribution of tsetse over the sectors between video recordings and electric net catches of tsetse in the absence of odour (c^2, P>0.05). In the presence of CAO, 22 % of the tsetse were caught on the downwind nets, significantly more than the 10 % that left the field of view of the video camera in a downwind direction (χ^2, P<0.005).

DISCUSSION

In the video studies, tsetse behaviour could only be observed while they flew from the edge of the hessian shade to the edge of the videoed area, a distance of 1 to 1.5 m, which tsetse can cross in 0.2 s (Brady, 1991). In this limited field of view, we observed an effect of CAO: more tsetse turned upwind, or flew straight ahead than in the absence of odour. However, we could not detect an effect of CAO on the flight directions in which the tsetse appeared or left the field of view.

The delay in appearing time when odour was present was short: during the first minute, tsetse appear on average 4 s later. Due to the hessian shade, we could not see what happened during this delay. Tsetse might have perched on the edge of the release tube or the shade, or might have flown in a tight circle before appearing in view. The shortness of the delay makes it unlikely that it is associated with the landing behaviour near the source, which generally lasts about a minute (Bursell, 1984, 1987, Torr, 1988).

The results of the CAO treatment were similar to those of Griffiths *et al.* (1995), who placed the TRB with the tubes facing upwind. The differences in recapture between the experiments of Groenendijk *et al.* (in prep) and Griffiths *et al.* (1995) are probably due to the difference in odour release rate.

On about ten occasions, tsetse appearing from under the hessian shade, turned and perched on the edge of the shade. They later followed other tsetse leaving the box. Perhaps these were males responding to prospective mates, as has been observed for *G. morsitans morsitans* (Brady, 1991).

Electric net studies showed a downwind bias, whereas video studies showed no bias in take-off directions. The former effect could be an artefact. Only 50% of the tsetse colliding with the electric net are killed, the others 'bounce away', apparently unscathed (Packer & Brady, 1990, Griffiths & Brady, 1994). Tsetse that survived their first contact with an electric net, could either hit another electric net, again with a 50% chance to get killed, or escape by flying over the top of the nets. Ultimately, about 50% escaped. Tsetse that were killed on the second or third time they struck the net, could change the apparent distribution of take-off directions.

Fewer tsetse avoid nets when they approach face on than when they approach obliquely (Packer & Brady, 1990). For a tsetse taking off from the box, the electric net straight ahead is obviously approached face-on, and the other nets are all approached more or less obliquely. This may have suppressed turning behaviour, because that would have taken a tsetse towards a more visible net. About 27 % of the tsetse approaching an electric net, avoid it if it is standing in the sun and about 40 % avoid an electric net standing in the shade (Griffiths & Brady, 1994). The wind was blowing from East to West and the 'downwind' nets would thus always be between the release box and the sun and this might have made these nets less visible than the upwind nets. That could explain the apparent preference for the downwind flight direction in the electric nets experiment.

These experiments showed that some tsetse react almost immediately after they left the TRB to natural host odour and to an artificial host odour mixture of carbon dioxide, acetone, octenol and phenols. The percentage of tsetse that reacted was smaller than the percentage recaptured at an odour baited target (Griffiths *et al.*, 1995), and some tsetse probably reacted to the odour in a later stadium.

Acknowledgements

We thank the Assistant Director, Tsetse and Trypanosomiasis Control Branch, Department of Veterinary Services, Zimbabwe for his hospitality, Messrs. F. Vakayi, A. Ndoro, C. Mashongwa and S. Mutanga for technical assistance and Dr. J.C. van Lenteren for valuable comments on earlier drafts of the paper. CAG was funded by The Netherlands Foundation for the Advancement of research in the Tropics (WOTRO) (W84-314) and NG was funded by the Overseas Development Administration of the Foreign and Commonwealth Office, London (X0138).

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