The behaviour of tsetse flies in an odour plume

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This thesis contains results of a research project of the Wageningen Agricultural University, Department of Entomology and the Tsetse and Trypanosomiasis Control Branch of the Department of Veterinary Services, Zimbabwe ministry of Agriculture. The project was supported by the Netherlands foundation for the advancement of tropical research (WOTRO), (Project nr W84-314), which is subsidized by the Netherlands Organization for Scientific Research and the Ministry of Development coorporation.

Stellingen

1 Als kooldioxide wordt toegevoegd aan een artificieel geurmengsel, neemt het percentage tseetseevliegen dat reageert toe, maar de aard van de reactie van de tseetseevliegen verandert niet.
-dit proefschrift

2 In tegenstelling tot wat eerder werd gesteld (Randolph *et al.*), neemt de tseetseevlieg *Glossina pallidipes* bloedmaaltijden kort na elkaar als de mogelijkheid zich voordoet.
-dit proefschrift
-Randolph, S.E., Rogers, D.J. & Kiilu, J. 1991. The feeding behaviour, activity and trappability of wild female *Glossina pallidipes* in relation to their pregnancy cycle. Medical and Veterinary Entomology 5: 335-350.

3 De vangsten van vallen zijn vaak ten onrechte gebruikt om uitspraken te doen over het gedrag van insekten.
-Vale, G.A., 1993. Development of baits for tsetse flies (Diptera: Glossinidae) in Zimbabwe. Journal of Medical Entomology 30: 831-842.
-dit proefschrift

4 De conclusie van Madubunyi dat een biconische val geen gastheer-zoekende tseetseevliegen aantrekt, wordt niet gestaafd door zijn gegevens.
-Madubunyi, L.C., 1995. Bloodmeal evacuation from the midgut of *Glossina pallidipes* in relation to tsetse foraging behaviour and trappability. Ecological Entomology 20: 146-152.

5 In windtunnels en op loopbollen zijn de structuur van de wind en de geurpluim te weinig gevarieerd om het zoekgedrag van insekten goed te kunnen bestuderen.

6 Ondanks enkele elegante studies die de bruikbaarheid van het Gaussiaanse model van een geurpluim voor insektenstudies ernstig aantasten, blijft dit model hardnekkig opduiken.
-Gillies, M.T., 1988. Anopheline mosquitoes: vector behaviour and bionomics. In: Malaria, principles and practice of Malariology (W.H. Wernsdorfer & I. McGregor, Eds.). Churchill Livingstone, Edinburgh. pp. 453-483.
-Knols, B.G.J., 1996. Odour-mediated host-seeking behaviour of the Afro-tropical malaria vector *Anopheles gambiae* Giles. Ph.D.-thesis Wageningen Agricultural University.

7 Genetische manipulatie levert slechts een snellere en bredere toepassing van veredeling. Het gebruik van deze techniek om organismen resistent te maken tegen ziekten en plagen levert alleen tijdwinst, maar geen fundamentele oplossing.
-Collins, F.H. & Besonski, N.J., 1994. Vector Biology and the control of malaria in Africa. Science 264: 1874-1875.

8 In Zimbabwe wordt niet alleen aanzienlijk meer natuur beschermd dan in Nederland, maar die natuur wordt ook beter beschermd. Enige bescheidenheid bij de beoordeling van de Zimbabwaanse natuurbeschermingsmaatregelen zou de Nederlandse natuurbeschermers dan ook niet misstaan.

9 Dat de natuur in Nederland niet veel voorstelt, werd al in 1943 opgemerkt, maar niet door een bioloog.
- Bloem, J.C., 1943. De Dapperstraat.

10 De in de herijkingsnota verkondigde politiek van verlicht eigenbelang is slechts een sanctionering van de reeds bestaande praktijk.

11 Er gaan stemmen op om het budget voor ontwikkelingssamenwerking te verlagen omdat ontwikkelingssamenwerking weinig effectief is. Als dat een goede reden is, dient ook het budget van o.a. de ministeries van Justitie en LNV verlaagd te worden.

12 Omdat een mens geboren in een 'ontwikkeld' land bijna 80 keer zoveel milieugebruiksruimte neemt als een mens geboren in een 'onderontwikkeld' land, dient de bevolkingsgroei overal ter wereld even voortvarend te worden aangepakt.

13 De door King en Ormerod voorgestelde stop op het financieren van respectievelijk gezondheidszorg, malariabestrijding en tseetseebestrijding, hebben tot gevolg dat de armsten de hoogste prijs moeten betalen voor de "verduurzaming" van de wereldeconomie.
-King, M., 1990. Health is a sustainable state. The Lancet 336: 664-667.
-King, M., 1991. Malaria control and the demographic trap. The Lancet 338:124.
-Ormerod, W.E., 1976. Ecological effect of Control of African Trypanosomiasis. Science 191: 815-821.

14 Het gebruik van politiek en ambtelijk jargon en het clichématige taalgebruik in soaps, reality tv en reclame, vormen een grotere bedreiging voor het Nederlands dan de adoptie van woorden uit een andere taal.

15 Harry Mulisch schrijft te slecht om de Nobelprijs te krijgen.

16 Dat de anti-conceptie pil in een kinderveilige verpakking zit, geeft reden tot ongerustheid.

Stellingen behorende bij het proefschrift 'The behaviour of tsetse flies in an odour plume'.

Wageningen, 26 april 1996

C.A. Groenendijk

Aan: Loes, Wanny en Dick

Foreword

Many people have helped me during the nearly five years it took to conduct the experiments, analyze the results and write the thesis.

Willem Takken initiated the project and gave me the opportunity to do this research, for which I am grateful. I thank him and Joop van Lenteren for providing me with advice when I was in Zimbabwe, but also for leaving me the freedom to conduct experiments as I saw fit. During my stay in Wageningen they gave valuable comments on my writings, sometimes within a day.

I am especially indebted to Glyn Vale and John Hargrove. Glyn showed me how to perform field experiments of high quality. His criticisms on my experiments and papers were sometimes severe, but always constructive and to the point. I have greatly benefitted from his knowledge of tsetse fly behaviour and his almost unlimited creativity in the design and realization of field experiments. He also instilled in me a healthy suspicion of trap catches. John helped me with the analysis of physiological data and helped me straightening out my thinking on that field. He also provided constructive comments on my papers.

All experiments were conducted at Rekomitjie Research Station of the Tsetse and Trypanosomiasis Control Branch, Department of Veterinary services of Zimbabwe. I am grateful to Mr. Vitalis Chadenga, the Assistant Director of the branch, who organized administrative support to obtain the necessary permits and logistic support for the project. For additional logistic and administrative support I thank mr. Chirinda, the manager of Rekomitjie Research Station, messrs. Saunders and Mandizvidza of the Regional Tsetse and Trypanosomiasis Control Programme and mrs. Buunk and mrs. Klunder of the Department of Entomology in Wageningen.

The field experiments would not have succeeded without the help of the "Gang of four": Frederick Vakayi, Sunwell Mutanga, C. Mashongwa and Aleck Ndoro. They were always cheerful, even at five in the morning, and we worked together in a relaxed atmosphere. The Gang marked over 40,000 tsetse flies and counted the catches, in total well over half a million insects. They also protected the experiments and Mukoni, the helpful ox, against elephants, buffaloes, lions and puff adders.

Furthermore I thank, Steve Torr, Martin Warnes, Clement Mangwiro, Odwell Muzare, 'Fido' Phelps, Nigel Griffiths, John Brady, Karen Voskamp and

many other 'regulars' at Rekomitjie for their constructive comments on experiments and papers and for their pleasurable company during the evening hours in Rekomitjie. My time in Wageningen was a pleasant one due to my collegues at the Department of Entomology; the informal Friday evening meetings were always enjoyable.

I thank A. Otten for help with the statistical analysis of the data in Chapter 5, 6, 7 and 8 and Piet Kostense for drawing some of the figures. The cover was designed by Loes Roos.

I thank Loes and some close friends for regularly drawing my attention away from tsetse flies.

Contents

1. Introduction

1.1 General

Both male and female tsetse flies (Diptera: Glossinidae) are obligate blood feeding insects. They feed only on the blood of vertebrates, mainly mammals (Weitz, 1970; Robertson, 1983; Staak *et al.*, 1986), but they do not live in close association with their host. Tsetse live 30 (♂) to 120 (♀) days (Hargrove, 1990) and need to obtain a blood meal approximately every three days, so they regularly face the task to find a relatively small, scarce and mobile food source. To find their hosts, tsetse use smell and vision (Vale, 1974a).

Tsetse are by far the most important vector of the trypanosome species (Kinetoplastida: Trypanosomatidae) that cause nagana and sleeping sickness. These are potentially lethal diseases in man and cattle. Species of the morsitans group of tsetse, to which *Glossina pallidipes* Austen and *G. morsitans morsitans* Westw. belong, are the most widespread and common disease vectors (Jordan, 1986). These tsetse species occur mainly in Eastern Africa, from Ethiopia to Mozambique, in savanna woodland (Jordan, 1986).

1.2 Foraging for hosts

Pianka (1966) described two general foraging strategies of animals: Wide Foraging (WF), in which the searcher is moving continuously, and Sit and Wait (S&W), in which the searcher remains stationary. The S&W foraging strategy is best suited for highly mobile, densely distributed prey, whereas the WF strategy seems optimal for sparsely distributed sedentary prey (Pianka, 1966). These two strategies are now considered the extremes of saltatory search (O'Brien *et al.*, 1990). The hosts of tsetse are relatively scarce but mobile and therefore the foraging strategy used by tsetse could lie anywhere between the two extremes. Males fly approximately 30 - 50 minutes, and females about 5 minutes per day (Bursell & Taylor, 1980; Randolph & Rogers, 1978, 1981) and tsetse seem to be on the S&W end of the saltatory search continuum. The

relative importance of S&W and WF may differ between age groups because young tsetse do not have fully developed flight muscles (Hargrove, 1975, 1991).

Tsetse are probably responsive to host cues while sitting and while flying (Brady, 1972; Vale, 1980; Gibson & Brady, 1988; Torr, 1988a; Gibson *et al.*, 1991; Hargrove, 1991; Warnes, 1992). They also seem to use vision while they are sitting and waiting, and smell when they are foraging widely (Vale, 1974b). Tsetse therefore do not fit very well in the saltatory search continuum.

The majority of *G.m. morsitans* and *G. pallidipes* rests on the underside of branches, not more than 3 m above the ground (Pilson & Leggate, 1962; Pilson & Pilson, 1966). A good indication of the wind direction can be obtained at these sites because the airflow is laminar (Brady *et al.*, 1989). These sites also give a good view on their surroundings. When tsetse search for cues in flight, they fly downwind (Gibson *et al.*, 1991). That is the optimal search direction in an environment with variable winds (Sabelis & Schippers, 1984; Dusenbery, 1989), such as that of tsetse (Brady *et al.*, 1990).

Tsetse use flight as a host foraging strategy during a limited period of the day. The daily spontaneous activity of *G. m. morsitans* and *G. pallidipes* shows a U-shape: peak responsiveness to baits occurs in the early morning and in the late afternoon (Pilson & Pilson, 1967; Dean *et al.*, 1969; Brady, 1972; Van Etten, 1982; Hargrove & Brady, 1992). The pattern of male *G. pallidipes* catches at baits differs slightly from the laboratory pattern; this difference is attributed to the effect of temperature (Van Etten, 1982). The daily rhythm of activity is mainly influenced by an endogenous clock (80 %) and by the ambient temperature (20 %) (Brady & Crump, 1978). The daily activity observed in the field does, however, depend on the sampling method (Rawlings *et al.*, 1994). In the laboratory, the mean daily spontaneous activity of *G. m. morsitans* males increases linearly with time passed since the last blood meal, whereas the spontaneous activity of females increases exponentially (Brady, 1972, 1975). The threshold for spontaneous activity is probably influenced by body weight, abdominal volume or a variable closely correlated to one of these (Brady, 1975). This increased activity is governed by the endogenous clock: when tsetse become more active, they do not extend the period of activity, but increase the activity during the set activity period.

Whether the time spent on WF, which is probably what the spontaneous activity in the laboratory represents, also increases with progressing starvation in the field, or starts at a set time since feeding, is hotly debated (Rogers, 1977; Randolph & Rogers 1978; Langley & Wall, 1990; Randolph *et al*, 1991; Hargrove & Packer, 1993). All this work refers to male tsetse, where fat reserves and blood meal size are not influenced by the pregnancy cycle. Randolph *et al.* (1991) suggest that tsetse will only feed at a set time since their last meal, and that the probability that tsetse obtain a blood meal within one day after initiating their search is almost one. Hargrove & Packer (1993) argue that tsetse will feed whenever the opportunity arises, and that the time spent on wide foraging increases with time since the last feed.

For females, the same reasoning can be applied, but the problem is more complicated because of the reproduction. Only one egg ovulates at a time. The egg stays in the uterus, a specialized part of the common oviduct. After about three days, the egg hatches. The larva staysin the uterus for 6 days and feeds from the secretions of 'milk glands' (for details, see Buxton, 1955).

To save as much energy as possible for reproduction, the best strategy for the female would be to fly as little as possible and to try to obtain a meal from every host that passes by. However, with the increasing size of the larva, the amount of blood that can be ingested decreases. Taking a meal is risky, so to optimize the chance of survival, feeding as infrequently as possible would be the best strategy. (Rogers, 1977; Randolph & Rogers 1978; Randolph *et al*, 1991). Only Randolph and coworkers (1991) have investigated the feeding cycle in females, also taking the pregnancy into account. They found that female *G. pallidipes* caught in traps had in general fed some 72 hours earlier and that feeding can occur on any day of the pregnancy cycle.

1.3. Host location

The range of attraction for tsetse can be divided in long range and short range. Long range is the area in which host kairomones are above threshold concentration (Willemse & Takken, 1994), i.e. the active space. Short range is

the area in which the host is visible. First, I will discus the structure of the odour plume, which is important to host-location strategies by means of odour (e.g. Mafra-Neto & Cardé, 1994). Then orientation at long range and the influence of kairomones on orientation at long range will be discussed. Finally, orientation at short range and the effect of kairomones thereon will be reviewed.

1.3.1 Odour plume structure

The first descriptions of the shape of an odour plume used a Gaussian model developed by Sutton (1953). This model gives the time-averaged concentration of the odour at a given point downwind of the source (Wright, 1958; Bossert & Wilson, 1963). There is a large difference between the time-averaged model and the instantaneous structure of an odour plume (Fig. 1). The Gaussian models of odour dispersion were evaluated for male moths by Elkinton *et al.* (1984). These moths respond instantaneously to odour and models that produce time-averaged concentrations were consequently judged inadequate to predict the active space of an odour source. Tsetse also respond instantaneously to odour and, therefore, models of the instantaneous structure of an odour plume are necessary to understand host-location behaviour by tsetse.

At a stationary sampler, an odour plume appears as a series of bursts, which vary in strength and duration (Murlis & Jones, 1981). Instantaneous measurements, such as burst length, the time between bursts and the concentration in one burst, vary little with distance from the source and do therefore not give reliable information about the distance between the receiver and the source. Intermittency increases with increasing distance (Murlis *et al.*, 1990), but is also influenced by the turbulence of the air flow, by the source size (Fackrell & Robins, 1982, cited in Murlis *et al.*, 1992) and by changes in wind direction. Intermittency is therefore not a good clue for host location.

However, odour pockets move downwind and an insect that is orientating towards the source, is flying upwind. Therefore the concentration

of odour in the odour pocket has less impact than the flux of odour (Elkinton & Cardé, 1984). Mean flux (amount of odour that passes the scent organ per unit of time), dose (total amount of odour), peak value of flux and the maximum peak recorded in short time samples (to exclude variation due to large-scale meandering of the plume) all decreased systematically with increasing distance from the source (Murlis *et al.*, 1990). Measurements of these plume characteristics could provide an insect with reliable information about the distance to the odour source.

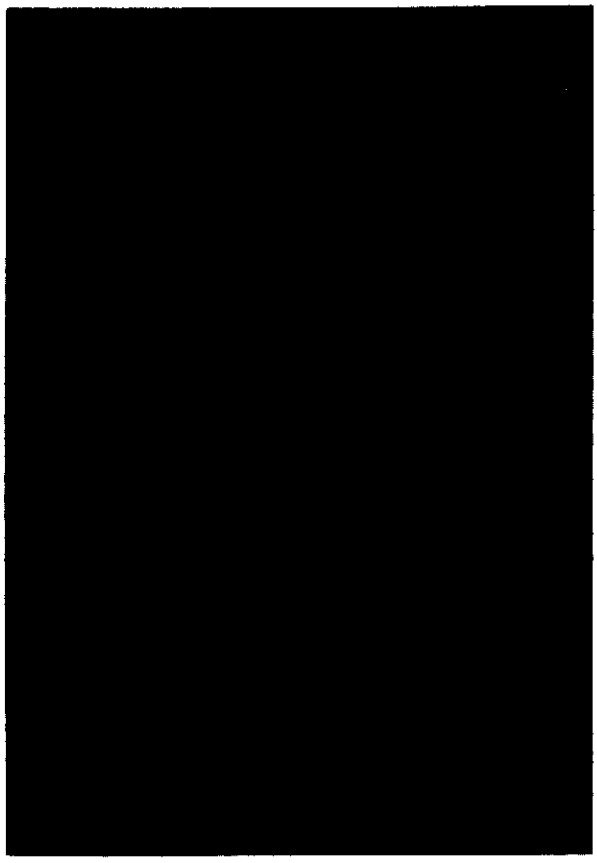

Figure 1: A photo of a smoke plume, which is a good model for an odour plume.

The large scale meandering of a plume is caused by changes in the wind direction (David *et al.*, 1982, 1983). The correlation between the initial direction of a parcel of air and its subsequent direction decreases with decreasing wind speed (Elkinton *et al.*, 1987; Brady *et al.*, 1990). Over distances of 10-20 m in grassland, each parcel of air travels in an almost straight line and at a nearly constant speed. In forest without understorey and in woodland with a thick understorey, the wind speed is lower than in open grassland and the rate of change of wind direction is much higher (Brady *et al.*, 1989, 1990). In tsetse habitat, the wind direction may change up to 20° per second.

The instantaneous concentration of a plume varies little with distance, while its time-averaged concentration decreases with distance from the source. Consequently, the probability that an insect encounters a detectable odour pocket, decreases with increasing distance from source. The precise pattern of the decrease in each direction depends on wind parameters like the change in wind direction per unit of time, the prevailing wind direction, and the wind speed.

Simple upwind flight therefore does not necessarily bring the tsetse to the odour source. Tsetse probably use another or an additional turning behaviour when losing contact with odour to remain in the vicinity of the source.

1.3.2 Long range orientation

How do tsetse, which are fast flying insects, cope with the frequent changes in wind direction and the low wind speed? Experiments show that tsetse can detect wind direction while in flight. About 60 % of the tsetse entering an odour plume, make a turn. Nearly two thirds of these tsetse turn upwind, but do not make large turns (Gibson & Brady, 1988; Colvin *et al.*, 1989; Torr, 1989). As a result, tsetse that respond to odour are not very precisely orientated towards the wind direction (Torr, 1988c). In an odour plume, tsetse turn frequently and fly slower (Warnes, 1990a; Gibson *et al.*, 1991). This latter mechanism and their tendency to turn more often while in odour - especially

when the odour release rate is high (Warnes, 1990a; Paynter & Brady, 1993) - may serve to keep the tsetse in the vicinity of the plume and its source (Warnes, 1990a). When tsetse lose contact with odour while in crosswind flight, they turn either downwind or upwind, those turning upwind making sharper turns than those turning downwind. Tsetse that lose contact with odour while in upwind flight, overshoot the odour source by about 2 m (Bursell, 1984, Torr, 1988c) and then turn downwind (Gibson & Brady, 1985, 1988). Given their flight speed of 5 m/s (Brady, 1991), tsetse apparently react as if they have lost contact with the plume when they do not encounter host odour for 0.4 s. During their flight in a generally upwind direction, tsetse may divert from their flight course to investigate host-like objects (Torr, 1989).

From the above, it appears that tsetse might be using a biassed random movement to locate their host (Brady *et al.*, 1990; Williams, 1994; Griffiths *et al.*, 1995), with the bias caused by the wind direction. They combine this with turns of more than 150° when they lose contact with odour.

In tsetse habitat at 15 m downwind of the source, the probability that the wind blows from the source when odour is detected, may be as low as 30 % (Brady *et al.* 1989). The bias probably decreases exponentially with increasing distance. The chance of finding the source with biassed random movement depends on the extent of the bias. Numerical solutions of this problem, assuming a circular active space around the source, indicate that the chance of success is close to unity if the bias exceeds 20 % (Fisher & Lauffenburger, 1987). And indeed, at least 80 % of the tsetse that detect ox odour in a clearing, find the source (Vale, 1980).

1.3.3 The effect of odour on long range orientation

Whether tsetse react to individual components of host odour with a specific behaviour, and if so, which behaviour is caused by which odour, remains largely unknown (Willemse & Takken, 1994).

Carbon dioxide elicits upwind flight in tsetse (Torr, 1990) and is thought to be a close range attractant only (Paynter & Brady, 1993). Acetone and 1-

octen-3-ol (henceforth termed octenol) may increase the visual responses of tsetse (Torr, 1990; Brady & Griffiths, 1993) and elicit upwind turning responses (Paynter & Brady, 1993). The combination of 3-n-propylphenol and 4-methylphenol increases trap catches more than target catches (Vale *et al.*, 1988), indicating that these components affect trap entering responses. These components also elicit upwind turning behaviour in tsetse, when used in combination with octenol (Brady & Griffiths, 1993).

The kairomones for tsetse might be divided in three different groups: 1) carbon dioxide and acetone, which are exhaled by host animals and these components are synergists; 2) octenol, emanated by hosts, but also by non-host organisms (Buttery & Kamm, 1980) and 3) the synergistically acting compounds 3-n-propylphenol and 4-methylphenol, which are found in the urine of hosts. Kairomones emanated by hosts indicate that the host is nearby and their biological role therefore seems clear. The kairomones from urine do not necessarily indicate that the host is close by, because urine poured onto the soil was attractive for one day (Vale *et al.*, 1986). These compounds may serve to bring tsetse to places were animals have been recently, and which might be frequented regularly by host animals.

The synergisms between carbon dioxide and acetone (Torr, 1990) and between 3-n-propyl phenol and 4-methyl phenol (Owaga *et al.*, 1988; Vale *et al.*, 1988), indicate that the composition of the odour is also important. Targets baited with imitations of ox odour catch fewer tsetse than targets baited with natural ox odour (Torr *et al.*, 1995); the more complete the artificial mixture is, the higher the catch (Willemse & Takken, 1994). The threshold at which tsetse respond to host odour might be lower when more attractive components are present in the mixture, thus enlarging the active space of the odour plume. This has been shown for some mosquito species (Gillies & Wilkes, 1969, 1972) and for fruit moths (Linn *et al.*, 1986, 1991). Natural ox odour elicits host-location behaviour at approximately 90 m downwind of the source (Vale, 1977a), while tsetse could not locate a source of acetone and carbon dioxide from more than 45 m downwind (Vale, 1984). However, these two odours differed in release rate and in composition and it is unknown to which extent the two factors influenced the result.

The catch at the odour source increases with odour release rate (Hargrove & Vale, 1978; Filledier *et al.*, 1988; Hargrove *et al.*, 1995). The release rate of a single-component plume increased the catch until a release rate equivalent to 10 oxen was reached. When a multi-component plume was used, the catch increased even when the release rate was increased to a level higher than that of 10 oxen (Torr, 1990). Since 80-100% of the tsetse encountering a host odour plume of one ox react to it and find the source (Vale, 1980), a higher dose probably attracts tsetse from further away (Hargrove & Vale, 1978; Torr, 1990, Hargrove *et al.*, 1995). However, only about 25 % of the tsetse flying downwind of an odour plume, detect the odour and it is also possible that the percentage of the tsetse passing downwind of the source that detects the odour increases with dose.

1.3.4 Short range orientation

Tsetse that have contacted odour, divert to host-like objects (Vale, 1974b; Torr, 1989). Many of the early traps relied only on visual attraction of tsetse to the trap (for a review see Buxton, 1955). Tsetse eyes are inferred to be capable of discrimination of cryptic hosts at high light intensities (Gibson & Young, 1991). Tsetse are sensitive to a spectral range from 300 to 700 nm, the near u.v. and most of the range visible to the human eye. Peak sensitivity occurs at 350-365 nm and a second peak is present at 450-520 nm (Davis & Gooding, 1983; Green & Cosens, 1983). Targets reflecting in these wavelengths are more attractive than targets reflecting in the green and yellow wavelengths (Green, 1986; Torr 1989). Landing responses of *G. pallidipes* and *G.m. morsitans* are strongest on black targets (Vale, 1982; Green, 1986).

The propensity of tsetse to divert to an object also depends on its shape and size and on the composition of the plume (Vale, 1979; Warnes, 1990b; Packer & Warnes, 1991). A larger proportion of the attracted tsetse alight on large targets than on small targets (Vale, 1974b; Hargrove, 1980; Vale, 1993). Simple shapes like a circle or a square, in colours contrasting with the surroundings, are most attractive (Torr, 1989). Near the source, and in the

presence of carbon dioxide, colour is not important (Green, 1986) and tsetse alight on host-like objects.

Uniformly coloured targets are more attractive than striped targets and vertically striped targets are more attractive than horizontally striped targets (Barrass, 1960; Brady & Shereni, 1988; Gibson, 1992). Horizontal stripes are in fact repellent, very few tsetse land on, or circle, a horizontally striped target (Gibson, 1992).

1.4. Host selection

Tsetse have catholic feeding habits (Weitz, 1970; Robertson, 1983; Staak *et al.*, 1986). Apparent host preference is mainly the result of host density (Vale & Cummings, 1976; Robertson, 1983), host body mass (Hargrove & Vale, 1978; Hargrove *et al.*, 1995) and host complacency (Vale, 1977b; Boyt *et al.*, 1978; Pilson *et al.*, 1978). Tsetse seem unable to separate complacent and non complacent hosts from a distance (Vale, 1977b). However, some host species seem to be more repellent (humans) or attractive (pigs) than their weight predicts (Hargrove, 1976; Vale 1977b; Mérot *et al.*, 1986).

1.5. Structure of the thesis

An extensive review of the effects of odour on host location by tsetse appeared recently (Willemse & Takken, 1994). It reveals that a lot of work has been done to elucidate the behaviour of tsetse and to maximize the catch of tsetse at a bait. Recent behavioural studies were done with video cameras, which could not distinguish the species and sex of the observed tsetse. Nor could the observed tsetse be analyzed with respect to fat reserves and pregnancy stage. It was also unknown from how far away those tsetse came and how long they had been flying. The effect of physiological state of the fly on its foraging strategy also remains unknown. The sensitivity of tsetse odour receptors is influenced by the physiological state of the tsetse (Den Otter *et al.*, 1991) and

might therefore influence foraging behaviour of tsetse. Furthermore, no studies have been made to compare tsetse behaviour in odour plumes of different composition and dose.

First, I studied the effect of the physiological state of tsetse on their foraging strategy. **Chapter 2** describes the diurnal rhythm in the activity and the host-location success of tsetse. In **chapter 3**, I present data on the fat reserves, which give an indication of the physiological state and pregnancy stage of tsetse that engage in either wide foraging or sit and wait. The foraging strategy of females is only known from studies with traps, but it has been suggested that, for males, studies with traps give an indication of the maximum, rather than the average, time that tsetse do not feed (Langley & Wall, 1990; Hargrove & Packer, 1993).

In **chapter 4**, I describe studies on a release method for marked tsetse. This method will be used in later chapters.

In **chapter 5 and 6**, I studied the effect of odour composition and odour release rate on host-location efficiency of tsetse and on the size of the active space of the odour. As stated earlier, several authors have suggested that an increase in release rate or an increase in the number of components increases the active space of an odour source. These data will help to evaluate the importance of several host odour components in host location. This may lead to improvements of the current bait technology.

The effect of odour and distance from the source on flight direction of tsetse is studied in **chapter 7**. The results I obtained will help to evaluate current hypotheses of the host-location strategy used by tsetse.

Little was known about the effect of fat and haematin content and pregnancy on the host-location strategy of female tsetse flies. It was also unknown whether different odours would attract different parts of the tsetse population. In **chapter 8** I describe experiments in which the physiological state of tsetse that were marked and tsetse that were recaptured or caught at targets baited with different odours was analyzed.

The thesis is concluded with a general discussion of the results in **chapter 9**.

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Part 1: Foraging Behaviour

2. Diurnal patterns in activity and response to host cues in tsetse (Glossinidae), Tabanidae and Stomoxynae

Abstract

In Zimbabwe, from before sunrise until after sunset, hourly catches of *Glossina morsitans morsitans* Westw. and *G. pallidipes* Austen were made from a stationary unbaited electric net, an ox fly-round, an electrified target, an epsilon trap and a biconical trap. The latter three were baited with artificial host odour. Catches of tsetse were low from dawn to early afternoon, peaking just before sunset. Despite the broad similarity in diurnal patterns, there were some consistent differences between the sampling methods. The fraction of the daily catch caught in the afternoon with the target or the traps was larger for males than for females. The catch at the unbaited electric net probably gave the best estimate of the diurnal rhythm of flight activity of tsetse. Compared to the net catch, trap catches of tsetse were relatively high during the middle of the day and low in the early morning and late afternoon. The differences are attributed to sampling biases of traps. The pattern of the target catches was not significantly different from the pattern of unbaited net catches. The catch pattern at the mobile bait was significantly different from that of the net. This is attributed to the response of tsetse sitting on vegetation, which were not sampled by the unbaited net, to the ox fly-round. It seems that target catches can be used to monitor diurnal rhythms in tsetse activity. The pattern of Stomoxynae-catches on targets resembled that of tsetse, however, no peak was evident in the electric net catches. Catches of the tabanid *Philoliche (Stenophara) zonata* Walker showed a sharp peak in the early afternoon.

Introduction

In most field studies of the diurnal activity of tsetse (*Glossinidae*), hosts or artificial host cues have been used, combined with hand net catching (e.g: Pilson & Pilson, 1967; Dean *et al.*, 1969; Van Etten, 1982) or with electric nets (Hargrove & Brady, 1992). However, these catches result from a long chain of distinctive responses and the catches are governed by the probability that each response occurs (Vale, 1993a). Trap catches for instance, depend on the probabilities of (1) tsetse encountering the stimuli from a distant trap; (2)

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navigation to the trap; (3) entry into the trap; (4) movement from the entrance of the trap to the cage. Each of these groups of responses covers several separate stages of behavioural steps, some of which may be alternatives. When responding to natural hosts, the steps 3 and 4 there are landing and searching for a feeding site. Sampling methods relying on different behavioural responses of tsetse, show different diurnal catch patterns (Rawlings *et al.*, 1994), because alternative steps may be influenced by external factors to a different extent.

The diurnal activity of tsetse in the absence of host cues has been studied in the laboratory (Brady, 1972; Van Etten, 1982). Whether the activity pattern found in the laboratory is equivalent to activities in relation to host location in the field, is not known. Several authors (Vale 1974b, 1980; Hargrove, 1991; Groenendijk, 1996) have suggested that flight in the absence of external stimuli, here termed spontaneous flight, is an important strategy of tsetse to encounter host cues. Males of *G. pallidipes* Austen from two different localities in Kenya, Nguruman in the South and Mwalewa Forest in the Southeast, kept under identical conditions in the laboratory, exhibit different patterns of activity (Van Etten, 1982). Both populations show differences between activity pattern in the laboratory and the catch pattern at the site where the population originated. The differences were attributed to ambient temperature, which is thought to influence the activity patterns of the two populations in different ways (Van Etten, 1982), but it cannot be excluded that the two populations respond differently to the bait. Females were not tested in the laboratory. If the diurnal activity patterns are the same, as they are in *G. morsitans morsitans* Westw. (Brady, 1972), the difference in catch pattern between the sexes is due to different responses to bait or temperature.

In this chapter, the diurnal catch pattern of apparently spontaneous flight of *G. pallidipes* and *G. m. morsitans* during the rainy season is compared with catch patterns obtained with different sampling methods, to determine the effect of sampling method on the diurnal pattern obtained.

Study area & Methods

The experiment was conducted during the rainy season, January to April 1993, for 28 days at Rekomitjie Research Station, Zambezi Valley, Zimbabwe, where *G. pallidipes* is abundant and *G.m. morsitans* also occurs. Five sampling devices were operated from 05.00 h (before sunrise) to 19.00 h (after sunset) in woodland dominated by *Colophospermum mopane* Kirk ex Benth..

Stationary baits. These consisted of a biconical trap (Challier *et al.*, 1977), an epsilon trap (Hargrove & Langley, 1990) and an electrified standard target (Vale, 1993b). The latter was a 1 x 1 m black cloth with a 1 x 0.5 m sheet of fine netting on each flank: flies contacting the standard target were killed or stunned by a grid of fine wires and fell to be held in a sticky tray on the ground. The stationary baits were baited with acetone (500 mg/h), 1-octen-3-ol (0.4 mg/h), 4-methyl phenol (0.8 mg/h) and 3-n-propyl phenol (0.1 mg/h).

Ox fly-round. Two men led a black ox of approximately 400 kg along a 3 km path, stopping for 1 minute at intervals of 100 m. At the end of the path, reached after about 3 h, the party travelled back along the same path. Blood-feeding flies were caught continuously as they landed on the ox.

Unbaited electric net. This consisted of a 1.5 x 1.5 m electric net (Vale, 1974a). No visual or odour bait was present. Tsetse are not attracted to the stimuli provided by the electric net (Vale, 1974a), but video studies show that some 40% of the tsetse approaching a net are not caught because they either avoid the net or are not killed when contacting it (Packer & Brady, 1990; Griffiths & Brady, 1994).

The temperature was measured hourly at a weather station, approximately 2.5 km from the experimental site.

Experimental Design. Eight sites, at 200 m distance from each other, were cleared. The sites were divided in two groups (A & B) of four sites (1-4). Two randomized block designs were nested to allow for site and time effects. The stationary baits and the unbaited electric net were randomly placed on sites 1-4 in both groups for the duration of four days. The four-day period was divided in two sub-periods of two days. Within each sub-period, the groups were randomly assigned to be operated during either the even or the odd

hours. Thus, if group A was operating during the first hour of the day, it was switched off and made inconspicuous at the end of the first hour: odour baits were removed and the traps and target laid flat on the ground. During the second hour, Group B was switched on and catches from the devices in group A were counted and removed. This procedure was then repeated at the end of each hour. After four days, the treatments were randomly placed at another site in the same group. The ox fly-round party followed a path stretching along two sides of a rectangle enclosing the sites where the stationary baits were placed, at a distance of 500 m.

Hourly catches were summed over all days and expressed as fraction of the total catch. Differences in diurnal distribution of catches between catching methods were tested with the χ^2-test. All treatments were operated every day and heterogeneity between days was therefore ignored.

An indication of where distributions differed was obtained by calculating the fraction caught at each hour of the day with the different methods. To compare different methods, the fraction of one method was divided by the corresponding fraction of the other method. Confidence intervals of this ratio were calculated according to Noether (1957). When the confidence interval did not embrace 1, the ratio was judged to be significantly different from unity.

Results

Tsetse. Few tsetse were caught during the morning and the early part of the afternoon, until 15.00 h. To obtain enough tsetse of each species and sex per category in a χ^2-test, the catches were pooled in a morning catch (05.00-12.00) and an early (12.00-15.00), middle (15.00-17.00) and late (17.00-19.00) afternoon catch.

G. pallidipes catches from all catching methods showed an afternoon peak (Fig.1) as did the *G. m. morsitans* catches from the standard target, the epsilon trap and the ox fly-round (Fig. 2). The *G. m. morsitans* catches from the unbaited net were small and the pattern could not be compared with patterns in the catches of other methods (Fig. 2). For *G. pallidipes*, the diurnal catch

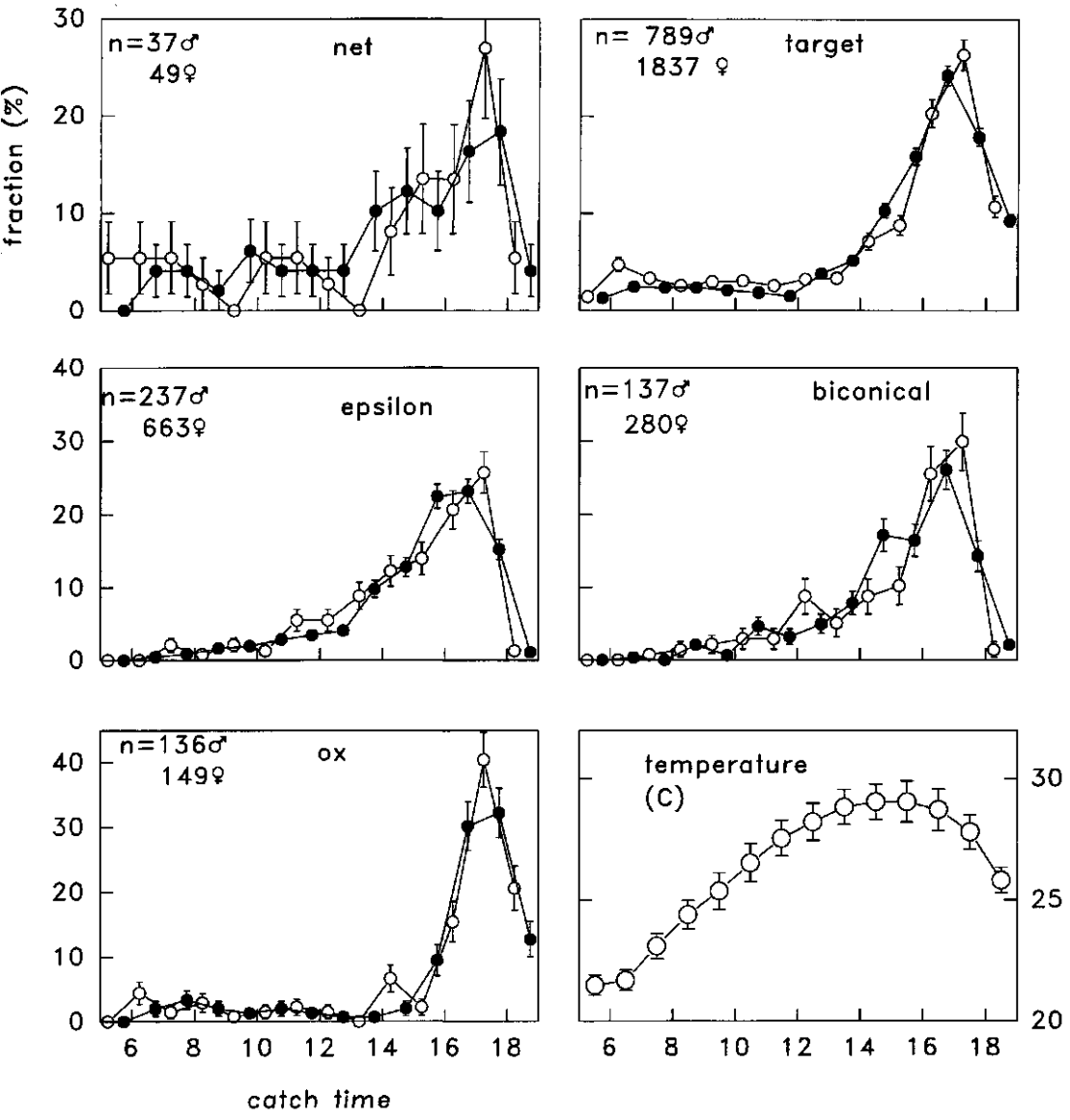

Fig. 1: The distribution of catches of female (dots) and male (circles) *G. pallidipes* over the day. Catches expressed as percentage of the total catch (± 95% confidence interval) in all graphs (note different range of y-axes in different graphs). The average temperature (°C ± 95 % confidence interval) is shown in the lower right graph.

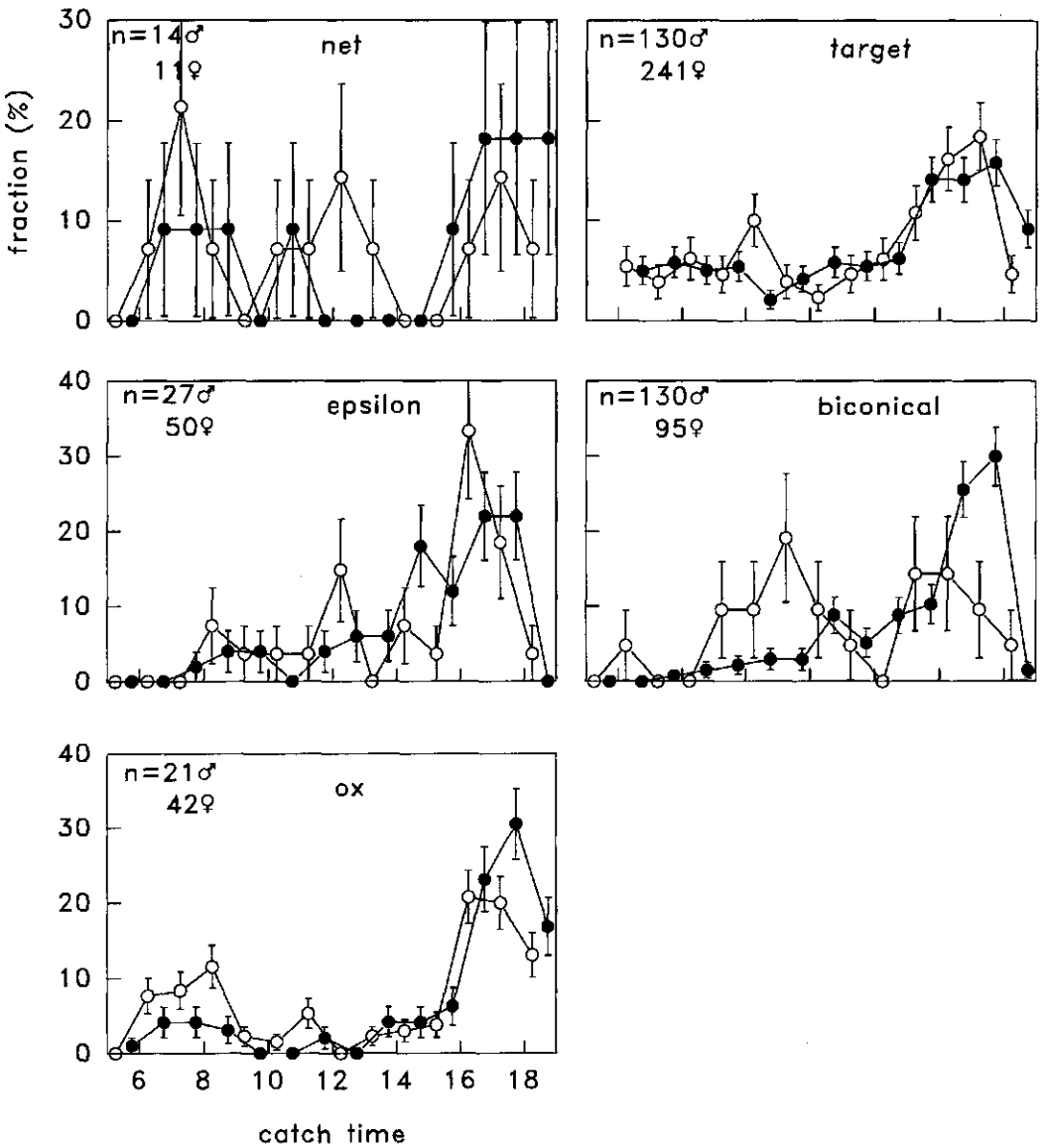

Fig. 2: The distribution of catches of female (dots) and male (circles) *G. m. morsitans* over the day. Catches expressed as percentage of the total catch (± 95% confidence interval) in all graphs (note different range of y-axes in different graphs).

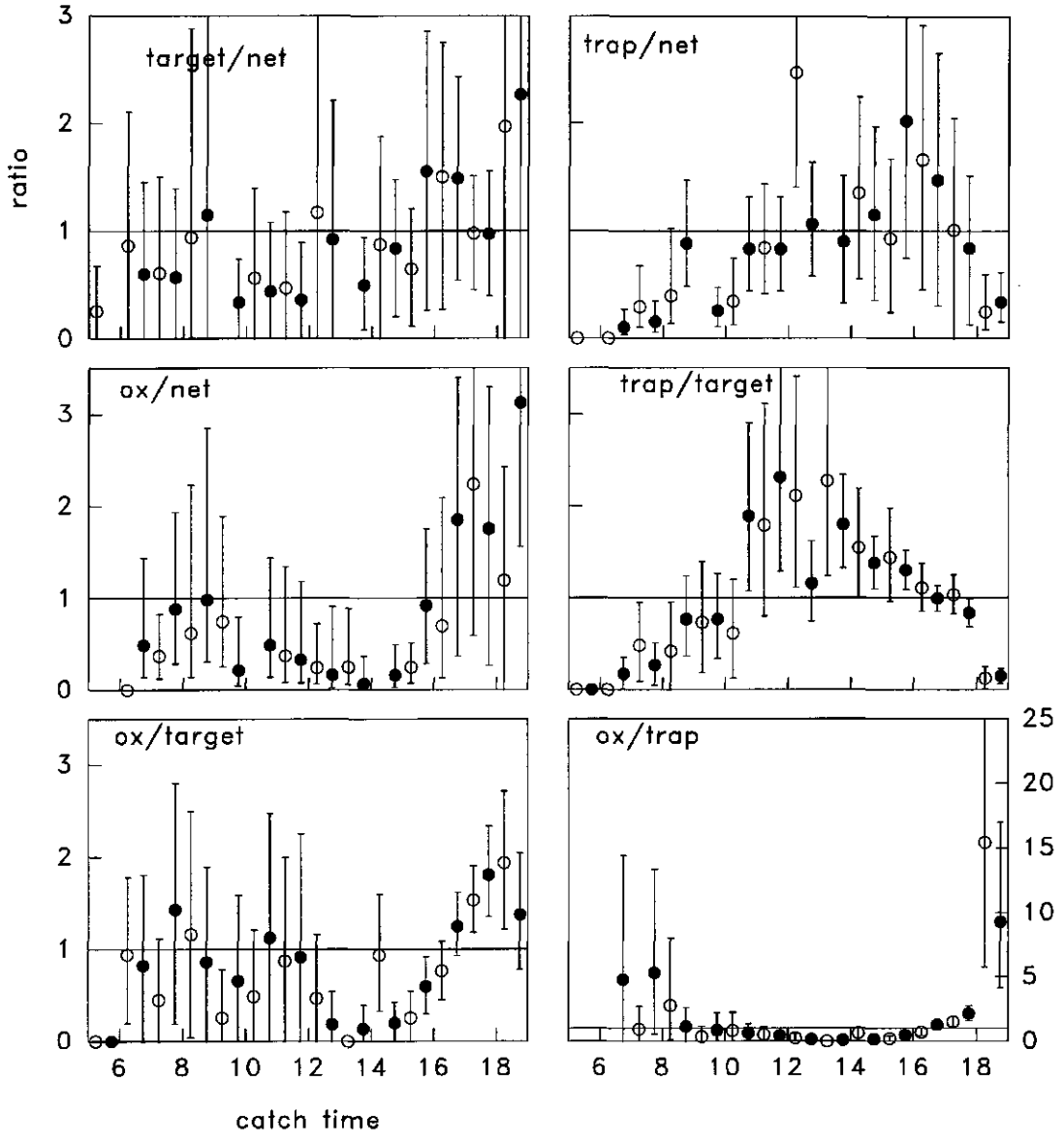

Fig. 3: The ratio of relative fractions (± 95% confidence interval) of different sampling methods of female (dots) and male (circles) *G. pallidipes* over the day. Where data are missing, the denominator was 0.

pattern of the unbaited net and the standard target resembled each other. The diurnal catch patterns of the epsilon and the biconical trap also resembled each other, but differed from the catch patterns of the other sampling devices (Table 1 and 2).

The diurnal pattern in catches of *G. pallidipes* males and females at the unbaited net and the ox fly-round were not significantly different. With other sampling methods, the catches of males peaked one hour later in the afternoon than catches of females (χ^2, P<0.01, df=3). Patterns of *G. m. morsitans* male and female catches only differed significantly in the ox fly-round (Fig. 1). Males were more responsive during the morning, while females were more responsive during the evening.

The diurnal patterns of the two species caught with the target were significantly different (χ^2, P<0.005, df=3), as were the diurnal patterns of the males caught with the ox fly-round (χ^2, P<0.005, df=3).

Table 1: Results of χ^2-square tests. The null hypothesis was that distributions of hourly catches of male and female *G. pallidipes* did not differ between methods. The hours 5-12, 12-15, 15-17 and 17-19 were lumped. *: P< 0.05, **: P<0.025, ***: P<0.01, ****: P<0.005, ns= null hypothesis not rejected.

	net	target	epsilon	biconical	ox fly-round
males					
net	-	ns	***	*	****
target		-	****	****	****
epsilon			-	ns	****
biconical				-	****
females					
net	-	ns	***	**	****
target		-	****	****	****
epsilon			-	ns	****
biconical				-	****

For the *G. pallidipes* catches, the fraction of the catch caught during the middle of the day with the traps is significantly higher than the fraction caught with the unbaited net or the standard target (Fig. 3).

Tabanidae and Stomoxynae. Many other Diptera were caught as well. Catches were sorted in Stomoxynae spp. and several species of Tabanidae. The non-biting Muscidae were pooled. The catch of Stomoxynae at the unbaited net showed no peak, but the response to the target showed a peak in late afternoon (Fig. 3). For Stomoxynae spp. there was no significant difference between net and target catch (χ^2, 0.10>P>0.05, df=3) but for other Muscidae, the difference was significant (χ^2, P<0.005, df=3). Muscidae were continuously present in large numbers around the ox fly-round party. Since the party could not catch every muscid at the moment it landed, these flies were ignored. For the tabanid *Philoliche (Stenophara) zonata* Walker, the catch from the unbaited net, the target and the ox fly-round showed a peak around midday (Fig. 4).

Table 2: Results of χ^2-square tests. The null hypothesis was that distributions of hourly catches of male and female *G. m. morsitans* did not differ between methods. The hours 5-12, 12-15, 15-17 and 17-19 were lumped. The diurnal pattern of male and female catches of the net, standard target and traps did not differ, and for these treatments the sexes were therefore pooled. *: P<0.05, **: P<0.025, ***: P<0.01, ****: P<0.005, ns= null hypothesis not rejected, #= insufficient data.

	net	target	epsilon	biconical
net	-	ns	***	*
target		-	***	*
epsilon			-	ns
biconical				-
ox fly-round				
females	#	**** ****	****	
males	#	ns	*	*

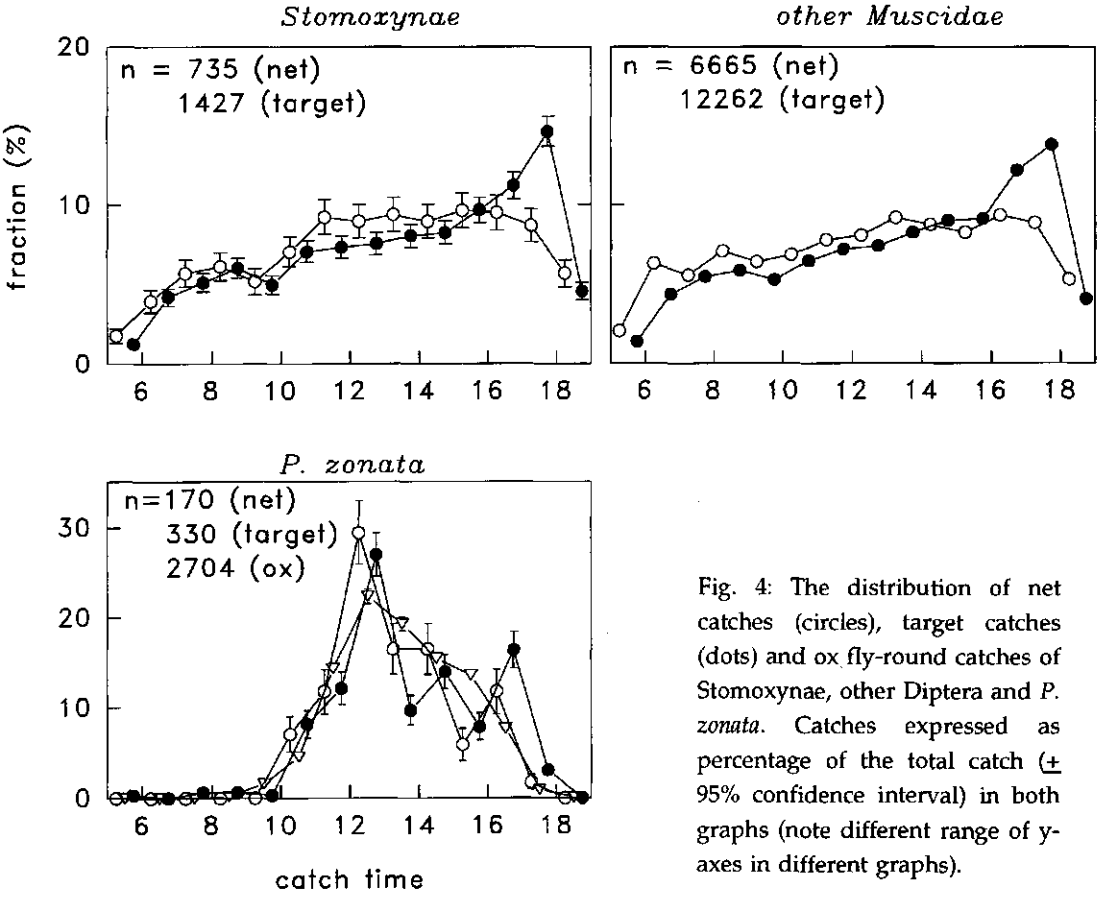

Fig. 4: The distribution of net catches (circles), target catches (dots) and ox fly-round catches of Stomoxynae, other Diptera and *P. zonata*. Catches expressed as percentage of the total catch (± 95% confidence interval) in both graphs (note different range of y-axes in different graphs).

The diurnal pattern of unbaited net- and target catches were not significantly different, but the catch with the ox fly-round differed from both the net catch pattern (χ^2, $P<0.025$, df=3), and the target catch pattern (χ^2, $P<0.005$, df=3). Trap catches of other Diptera were too low for statistical analysis.

Discussion

The unbaited electrified net is not attractive to tsetse and therefore probably gave the best estimate of the spontaneous flight activity in the field. The catch pattern of male *G. pallidipes* at the unbaited electrified net did not differ much from the pattern of spontaneous activity of *G. pallidipes* males from Nguruman in the laboratory (Van Etten, 1982). This difference might have been caused by the temperature (Brady and Crump, 1978). The difference with the laboratory activity of males from Mwalewa, Kenya on the other hand, was large. This difference between the spontaneous activity pattern of the two populations may be genetically determined (Van Etten, 1982).

Van Etten did not test females in the laboratory, but pregnant female *G.m. morsitans* show essentially the same pattern as males (Brady, 1972). There was no difference between the spontaneous flight activity of male and female *G. pallidipes*, as measured by the unbaited electric net catch. It thus seems that later capture of males by odour-baited stationary traps and targets is caused by a behavioural response of the males, not by a different activity rhythm. However, the sample sizes were small, which may have made the test too unsensitive.

The diurnal pattern of tsetse catches at the target in these experiments was similar to that found previously in Zimbabwe (Brady & Crump, 1978; Hargrove & Brady, 1992) when temperatures were in the same range. The diurnal pattern of the unbaited net catch was similar to the diurnal pattern of catches at the standard target. The similarity between the catch of active tsetse and of tsetse responding to the target supports earlier suggestions that the catch at a stationary target consists mainly of tsetse that were already in flight when they responded to the bait (Vale 1974b and 1980; Hargrove, 1991; Groenendijk, 1996).

Other sampling devices showed differences in diurnal catch patterns. The diurnal patterns of responses of *G. pallidipes* and *G. m. morsitans* to traps differed significantly from the diurnal pattern of spontaneous activity as indicated by the catch pattern of the unbaited net. For *G. pallidipes*, ratios of the relative frequency of net and trap catches and of target and trap catches were

significantly below unity in the early morning and in the late afternoon (Fig. 2). This difference may have been due to the trap entry response, which increases with light intensity in the trap (Brightwell *et al.*, 1991).

A comparison between the pattern obtained from the ox fly-round and patterns from traps and the target is more complicated. The ox fly-round presented a different set of stimuli: mobile, larger and of a different colour than the target and traps used and with a different odour. Odours have no effect on the catch size at a moving bait (Vale, 1974b) and presumably also not on the diurnal pattern of the catch. The target catches were similar to those of Hargrove and Brady (1991), who used a smaller target, and the size of the target and its colour also seem to have little effect. Possible diurnal variations in the human catching efficiency and in escape responses of tsetse may also have played a role in the diurnal pattern obtained. However, tsetse can probably discern an object like a handnet against the sky at low light intensities (Gibson & Young, 1991), and escape reactions were therefore probably not hindered by low light intensities. The catch efficiency of humans was not assessed, but conceivably declined with light intensity and during the course of the day due to fatigue. Since catches were highest at the end of the afternoon, when human catch efficiency was at its lowest, the peak afternoon catches may have been underestimated.

Therefore, mobility apparently is the most important factor causing the difference in catch-patterns between the ox fly-round and other methods. Many of the tsetse responding to a mobile bait are sitting on vegetation (Vale, 1974b). This implies that *G. pallidipes* resting on vegetation was only in a responsive state in the late afternoon, whereas *G. m. morsitans* was responsive in the early morning and the late afternoon. The other catching devices only caught tsetse that were active when they sensed the bait (Vale, 1980; Hargrove, 1991; Groenendijk, 1996).

Work done on *G. morsitans submorsitans* in The Gambia shows the same difference in patterns between trap and ox fly-round catches during the rainy season (Rawlings *et al.*, 1994) as were seen in *G. m. morsitans* in Zimbabwe. Ox fly-round catches show a bimodal pattern, but trap catches show a single peak in the afternoon. However, the trap catches of *G. m. morsitans* and *G. pallidipes*

showed a greater decline during the last hour of daylight than catches of *G. m. submorsitans* in The Gambia.

Both Stomoxynae and other Muscidae show activity, as measured by the unbaited net, throughout the day. Activity oriented to the target is concentrated in the late afternoon. For Stomoxynae the difference between the two sampling methods was nearly significant, for other Muscidae it was significant. The impression from the ox fly-round team was that Muscidae were present throughout the day in large numbers. Thus the peak in target catches in the afternoon might reflect either a behavioural response from the muscids, or a change in attractiveness of the target due to environmental factors.

The unbaited net catch of *P. zonata* showed the same pattern as the catch at the stationary target. But the patterns of the net catch and the target catch are different from that of the ox fly-round catch. It is likely that with the ox fly-round another, or larger, part of the population of *P. zonata* is sampled. This may be due to the different odour composition of the ox fly-round, or to the movement. Seeing the large difference in numbers caught by the ox fly-round (2704) and the target (330), it seems likely that *P. zonata* is strongly attracted to one of these factors.

Odour baited targets are the best option for the study of the diurnal rhythm in activity of *G. pallidipes* and probably *G. m. morsitans*, yielding high catches and a pattern similar to that from an unbaited net. Traps are biased with respect to the net and target catches, underestimating activity of tsetse in the early morning and the late afternoon.

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3. The response of tsetse flies to artificial baits in relation to age, nutritional and reproductive state

Abstract

In various vegetation types in Zimbabwe, the catches of *Glossina pallidipes* Austen and *G. morsitans morsitans* Westw. (Diptera: *Glossinidae*) at a target baited with odour (acetone, 1-octen-3-ol and two phenols) were positively correlated with catches of the same species at an unbaited net. No correlation existed between target catches and hand net catches of tsetse flies sitting on the vegetation. *G. pallidipes* females caught at a target and at an unbaited net were older than those caught from vegetation. Of the female *G. pallidipes* caught at the target, 46% were in the first 3 days of pregnancy. Of those caught at the unbaited net, significantly fewer, 21%, were in this stage. *G. pallidipes* males caught from vegetation contained more fat (3.07 $\pm$ 0.333 mg) than those caught at the unbaited net (2.06 $\pm$ 0.339 mg) or at the target (2.19 $\pm$ 0.218 mg). It is inferred that target catches consisted predominantly of tsetse which were already in flight when they sensed the stimuli from the target, and that target catches were biased towards female *G. pallidipes* in the first 3 days of pregnancy.

Introduction

Blood-feeding arthropods may use two different strategies to locate a host: 'sit and wait' or 'active search'. The latter requires more energy, but may lead to more frequent encounters with host cues. There is evidence that tsetse (Diptera: Glossinidae) can employ both strategies since both sitting tsetse and those in apparently spontaneous flight are responsive to host stimuli (Brady, 1972; Torr, 1988; Vale, 1980). The importance of 'sit and wait' or 'active search' strategies for host location may differ between *Glossina* species (Warnes, 1992).

The large catches of tsetse at targets (Vale, 1993) and traps (Hargrove, 1991), compared with a relatively low tsetse density (Vale *et al.*, 1988) and the seemingly short range of visual and olfactory perception, have been put forward in support of the strategy of active search for host location (Hargrove,

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1991). However, estimations of the range of attraction to a stationary target are imprecise and vary from 90 m for an ox (Vale, 1977) to 40 m for a mixture of acetone and carbon dioxide (Vale, 1984). A small increase in the range leads to a large increase in the area where sitting tsetse could be stimulated. There is also evidence that older tsetse may rely more on active search than younger tsetse (Hargrove, 1991). The role and importance of active search as a strategy for tsetse has not been well determined to date.

In the present study, simultaneous measurements were made of the numbers and physiological condition of (1) tsetse which had arrived at a baited target and thus were supposed to have responded to olfactory and visual host stimuli, (2) tsetse which were in flight in the apparent absence of olfactory and visual host stimuli and (3) tsetse sitting on vegetation. Correlations between numbers and physiological state of tsetse of these three behavioural groups were then used to infer the relative importance of 'sit and wait' and 'active search' as host finding strategies.

Materials & Methods

Experiments were carried out near Rekomitjie Research Station in the Zambezi Valley of Zimbabwe. Two species of tsetse, *G. pallidipes* Austen and *G. morsitans morsitans* Westw. occur there with the former being the most abundant (Hargrove, 1991). Experiments were conducted between August 1991 and July 1993 during the 3 h preceding sunset, when most tsetse are active (Chapter 1) and caught (Hargrove & Brady 1992; Chapter 1).

Tsetse engaged in different types of behaviour, were sampled by three methods: (1) An electrified standard target (Vale, 1993), consisting of a 1 x 1 m black cloth as a visual target, with a 1 m high x 0.5 m wide sheet of fine netting on each flank. An electrified grid of fine wires covered both sides of target and netting. Flies contacting the grid were killed or stunned and fell to be held in a sticky tray on the ground. The standard target was baited with acetone (0.5 g/h), 3-n-propyl phenol (0.1 mg/h), 1-octen-3-ol (0.4 mg/h) and 4-methyl phenol (0.8 mg/h), released on the downwind side, at ground level,

from the centre of the target. This blend is a reasonable imitation of ox odour (Torr, 1990). Tsetse caught at the target were considered to have responded to the attractants and were termed 'at target'. (2) An unbaited electric net of 1.5 x 1.5 m (Vale, 1974) without visual target, intercepted tsetse flying in the apparent absence of host cues; these tsetse were termed 'in flight'. (3) Two persons searched the preferred resting sites of tsetse (Pilson & Leggate, 1962) and caught tsetse from vegetation using a hand net. The searched area was a triangle, approximately 5000 m^2, extending 100 m downwind along the main wind direction and 100 m crosswind at its base on the downwind side. This triangle covered the whole area where tsetse using the 'sit and wait' strategy would have been stimulated by odour from a source at the upwind apex of the triangle. Captured tsetse were termed 'on vegetation'.

Two seasons were distinguished: dry (May to November) and wet (December to April). To obtain a reasonable variation in the number of tsetse caught on vegetation, three vegetation types were chosen: riverine, with many suitable sites for sit and wait, grassland, with a few of such sites, and woodland, with an intermediate number of such sites. In each vegetation type three experimental sites were selected, situated 200 m apart across the main wind direction. Treatments were incorporated in a Latin square design of treatments x sites x days. Each Latin square was conducted at least once each season and in each vegetation type.

The maximum temperature was read daily from a Stevenson's screen at Rekomitjie Research Station.

The daily catches were transformed to $\log_{10}$ (n+1) to stabilize the variance and the mean transformed catch of each treatment in a Latin square was used in a linear regression of tsetse caught 'at target' on tsetse caught 'in flight' or 'on vegetation'. The exact relationship could not be determined because both axes contained errors of measurement, while the regression method assumes that all errors occur in the values plotted on the y-axis. Since the regression of y on x assumes that all the errors were in the y-values and the regression of x on y assumes that all errors were in the x-values, the true relationship lies between these two lines (Kendall & Stuart, 1973). Both

regression coefficients were therefore calculated and the one of x on y is given as its reciprocal.

The reproductive and nutritional state of the *G. pallidipes* catch were analyzed during a 10-day-period in August 1992. The legs and wings of each fly were excised and discarded. Females were dissected to determine the ovarian age category (Challier, 1965) and the pregnancy stage (Hargrove, 1994). The fat content of the carcass was determined as described by Potts (Mulligan, 1970). Males with a residual dry weight of less than 7 mg were considered to be immature (Hargrove & Packer, 1993).

Tsetse were caught with epsilon traps (Hargrove & Langley, 1990) in an adjacent area during the same period. Data on the pregnancy stage of the females captured with these traps were also used.

Results

For both species and both sexes, linear regression analysis of all catches during the 2-year period showed a significant positive correlation between the log-transformed numbers of tsetse caught 'in flight' and 'at target', with up to 75% of the variation explained (Table 1 & Fig. 1). No correlation was found between the log-transformed numbers of tsetse caught 'at target' and 'on vegetation' (Table 1 & Fig. 1). The regression coefficient did not vary significantly with regard to sex or season and the data of the seasons and the sexes were therefore pooled. The number of tsetse caught 'at target', 'in flight' or 'on vegetation' was not correlated with the maximum temperature.
Since the analyzed samples of *G. pallidipes* females caught 'in flight' and 'at rest' were small, comparisons between the complete age distributions or pregnancy cycle distributions could not be made. Instead, females were pooled in two age groups and three pregnancy stage groups.

Table 1: Regression analyses of tsetse caught 'at target' on tsetse caught 'in flight' or 'on vegetation': log(tsetse 'at target'+1)= a + b x log(tsetse 'in flight' or 'on vegetation' +1) and its reciprocal. ***: P<0.01; ns: not significant (P>0.05).

species	a	b	r^2
G.m. morsitans			
'at target vs 'in flight'	0.731	1.800	0.57 ***
'in flight' vs 'at target'	-0.158	3.019	
'at target' vs 'on vegetation'	0.974	1.091	0.29 ns
'on vegetation' vs 'at target'	-0.125	3.049	
G.pallidipes			
'at target' vs 'in flight'	1.199	1.533	0.75 ***
'in flight' vs 'at target'	-0.483	2.011	
'at target' vs 'on vegetation'	1.795	0.546	0.03 ns
'on vegetation' vs 'at target'	-0.015	4.167	

Females were classified as 'young' (ovarian age-classes 0 to 3) and 'old' (ovarian age-classes 4 + 4n to 7 + 4n). A significant difference in distribution of females over these categories was found between females caught 'at target' and 'on vegetation' (χ^2, P<0.025, df=1). Fifty percent of the females caught 'on vegetation' were young, whereas this was the case with only 21 % and 26 % of females caught 'at target' and 'in flight' respectively. No age difference was found between females caught 'at target' and 'in flight' (χ^2, P>0.25, df=1) or between females caught 'on vegetation' and 'in flight' (χ^2, 0.1<P<0.25, df=1). The last result may be due to the low power of the test, caused by the small sample size.

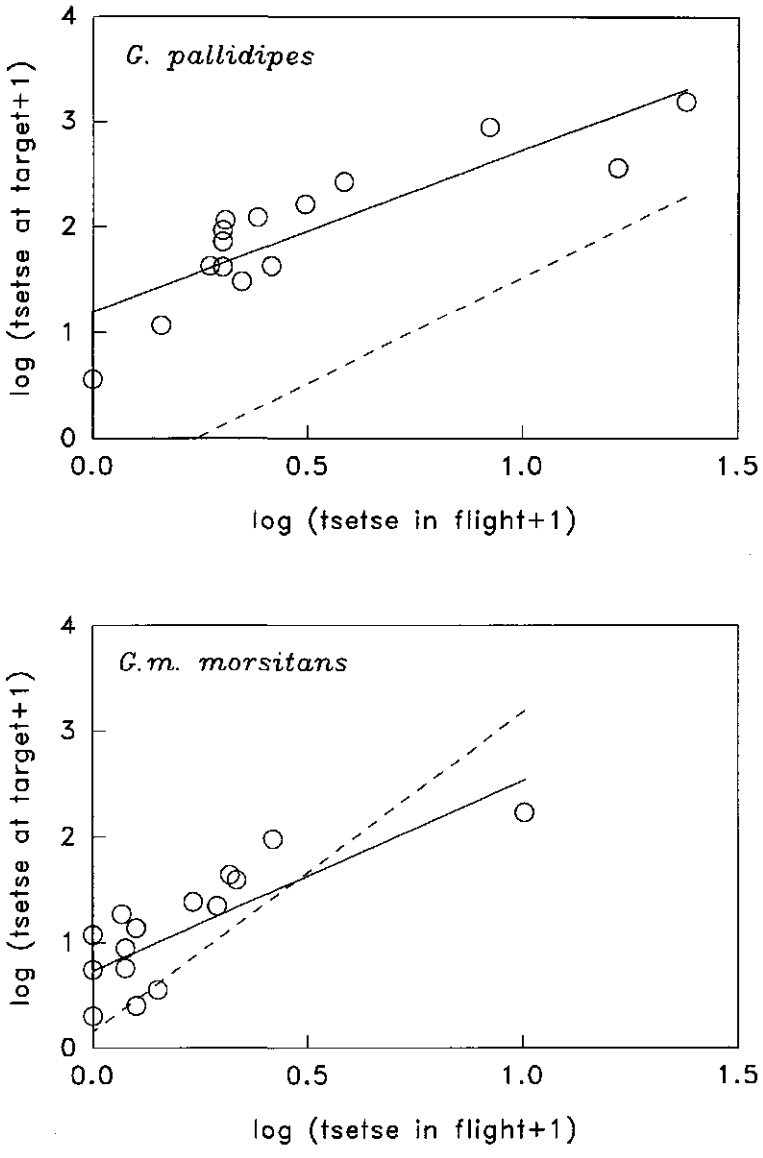

Fig. 1: Correlation between catches of tsetse 'in flight' and 'at target'. Continuous line: regression of tsetse 'at target' on tsetse 'in flight' Dotted line: reciprocal of the regression of tsetse 'in flight' on tsetse 'at target'.

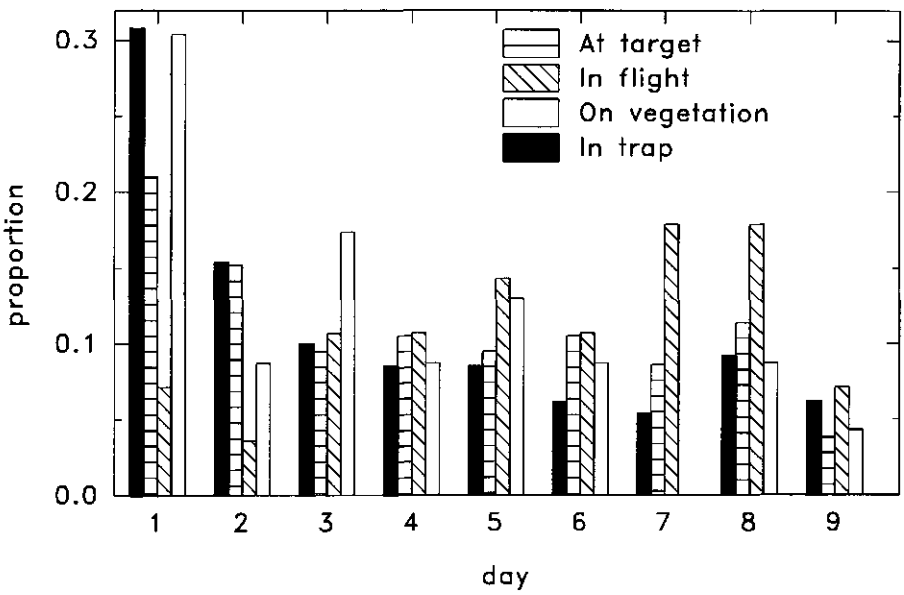

Fig. 2: The distribution of the catch of *G. pallidipes* females over a 9-day pregnancy cycle. Sample sizes were 28, 102, and 21 respectively for tsetse caught 'in flight', 'at the target' and 'on vegetation'. Trap catches are shown as well.

Pregnancies were classified as 'early' (day 1-3), 'middle' (day 4-6), and 'late' (day 7-9). Females caught 'in flight' were in a later stage of their pregnancy than females caught 'at target' (χ^2, $P<0.05$, df=2) and females caught 'on vegetation' (χ^2, $P<0.025$, df=2) (Fig 2). No difference was found between females caught 'at target' and 'on vegetation' (χ^2, $P>0.25$, df=2).

Females caught with different devices did not differ in fat content. The mean fat level ($\pm$ 95 % confidence interval) of male *G. pallidipes* caught 'on vegetation' was 3.07 ± 0.333 mg, significantly higher than that of males caught 'at target', which was 2.06 ± 0.339 mg, or 'in flight', which was 2.19 ± 0.218 mg ($P<0.005$, F-test).

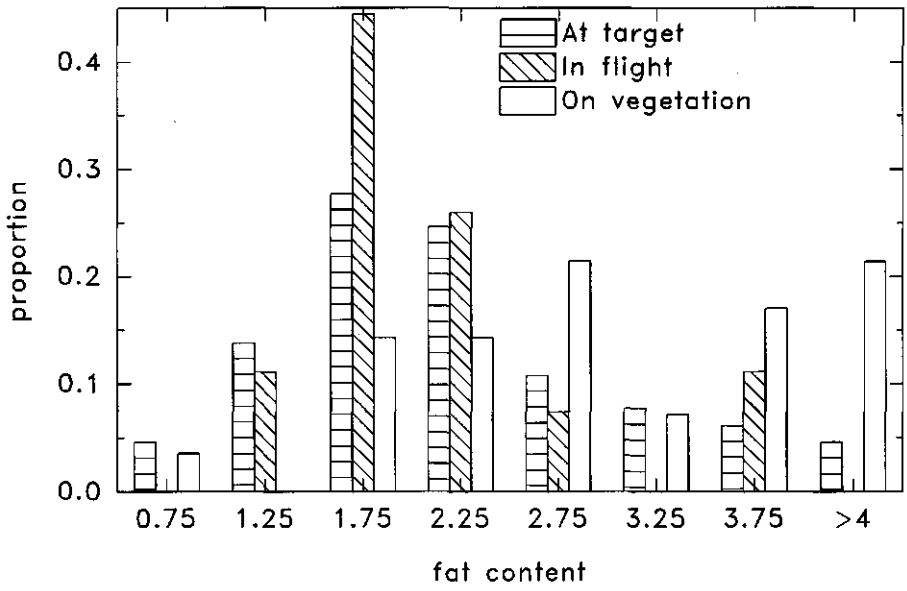

Fig. 3: Chloroform extractable fat levels in male *G. pallidipes*. Sample sizes were 65, 27 and 28 respectively for tsetse caught 'at target', 'in flight' and 'on vegetation'.

The distribution of males caught 'at target' over different fat categories shows that the majority have a fat content of 1.5 to 2.5 mg, but with a large variation (Fig. 3). One immature male was caught 'at target' and it contained no fat. Immature flies use the blood meal to build up flight muscles, not fat reserves (Langley, 1970). This fly was therefore not used in the analysis and comparison between different samples.

Discussion

The positive correlation between log transformed numbers of tsetse 'in flight' and 'at target' during the whole year, is consistent with the hypothesis that the

majority of the tsetse caught at an odour baited stationary target were 'in flight' when they first sensed the cues. This was further supported by the similarity in fat content in male tsetse caught 'in flight' and 'at target' and the similar age of female *G. pallidipes* caught 'in flight' and 'at target'. The similarity in diurnal patterns of activity and responsiveness (Chapter 1) also supports the above mentioned hypothesis.

However, females caught 'in flight' were in a later stage of the pregnancy than females caught 'at target'. This may have been due to the stimuli used with the standard target. The odour was a reasonable imitation, but did not contain all attractants present in ox odour (Torr, 1990). It is possible that females in the early stage of pregnancy have a greater chance of being captured because they run a higher risk of starvation than females in the middle stage of pregnancy. Females in the early stage of their pregnancy generally have low energy reserves. They are unlikely to have fed during the last stage of their previous pregnancy, while the larva they then carried grew quickly (Randolph *et al.*, 1991). Consequently, females in the early stage of pregnancy are more likely to respond to an incomplete range of host stimuli. However, because the sample size of tsetse caught 'in flight' was small, this relationship must be treated cautiously.

In order to be caught, a tsetse fly must enter a trap. This requires a series of behavioural steps through which the tsetse fly must persist. Consequently, the trap catch should show a bias towards females in the early stage of pregnancy. Traps were not part of the experimental design, but the catches of traps in the vicinity of the experiment indeed showed a bias towards females in the early stages of pregnancy (Fig. 2).

About 20 - 40 % of the tsetse avoid the unbaited electric nets (Packer & Brady, 1990; Griffiths & Brady, 1994) and this lowered the number of tsetse captured 'in flight'. However, there is no reason to assume that the physiological state of the tsetse flies influenced avoiding behaviour.

The samples of *G. pallidipes* caught 'at target' and 'in flight' contained fewer young females than the sample of *G. pallidipes* caught 'on vegetation'. This supports the suggestion of Hargrove (1991) that the bias towards older tsetse in trap and target catches is caused by an increased flight activity in

older females and not by a greater capability in older tsetse to find the odour source.

The distribution of female *G. pallidipes* caught 'on vegetation' or 'at target' over the pregnancy cycle was similar. If the tsetse caught on vegetation were resting, one would expect females in the early stages of pregnancy to be slightly under-represented and those in the late stage of pregnancy to be over-represented in the sample. However, the opposite was the case. It seems that the tsetse caught with a hand-net from vegetation, although in most aspects different from tsetse caught 'in flight' or 'at target', were only part of the population of tsetse sitting on vegetation. It might be possible that females in the earlier stages of pregnancy chose more exposed perching sites than those in the other stages. Alternatively, females in the early stage of pregnancy were initially resting and responded to the humans taking the sample, while females in the later stages of pregnancy did not respond.

Female *G. pallidipes* caught with different devices did not differ in fat content. However, the measured fat content contained fat from the female and from the egg or larva she carried. The fat content of the larva increases with progressing pregnancy (Randolph *et al.*, 1991). The measured fat content is thus strongly influenced by the pregnancy stage (Rogers & Randolph, 1978), but part of it, the larval fat, is not available to the adult female. This may have obscured a possible effect of female fat reserves on availability to the different catch methods.

Male *G. pallidipes* caught 'at target' or 'in flight' had a lower fat content than the 2.8 mg estimated by Hargrove & Packer (1993). However, the activity of male *G. pallidipes*, and consequently the chance that they are captured 'in flight' or 'at target', probably increases with time since feeding. This implies that 24 hours and onwards since feeding, the activity of males increases with decreasing fat content (Hargrove & Packer, 1993). It then follows that males caught 'in flight' and 'at target' have an average fat content below the estimated 2.8 mg. But the variation in fat content is large because some males are caught during their first bout of activity following their last blood meal. That is indeed what was observed here (Fig. 3).

It is inferred that *G. pallidipes* and *G. m. morsitans* caught on a stationary odour baited target were already in flight at the time they first sensed the stimuli. Furthermore, female *G. pallidipes* in the early stage of pregnancy were more readily caught at the target than those in later stages of pregnancy.

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Part 2: Host-location Behaviour

4. The initial flight direction of tsetse (Diptera: Glossinidae) exposed to natural and synthetic ox odour

Abstract

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. When an artificial odour mixture containing carbon dioxide, acetone, octenol and phenols was used, 35% turn downwind; significantly less, but still a large percentage. The difference in upwind turns was smaller: in the absence of odour 32% turned upwind, in the presence of odour 37% turned upwind. In the absence of odour, tsetse left the box at a constant rate and appeared to avoid each other. In the presence of odour, this avoidance disappeared and tsetse left the TRB later and not at a constant rate. When the TRB was placed in a complete ring of electrified nets, only the release of natural ox odour changed the distribution of tsetse over the electric nets compared to the no odour treatment. The artificial odour mixture, with and without carbon dioxide, had no effect on the distribution of tsetse over the electric nets. Most tsetse were caught while flying in a downwind direction. 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 released or departing from the TRB reacts to the presence of odour immediately.

Introduction

In recent studies (Griffiths *et al.*, 1995), marked *Glossina pallidipes* Austen (Diptera: Glossinidae) were released from a release box placed at various distances downwind of an odour source. 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.

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This trend in recapture percentage might be caused by the initial flight direction tsetse take when they leave the release box. At short distances from the source, an odour plume is only a few metres wide (Brady & Griffiths, 1993). 11Tsetse might leave the release box 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.

I want to use this release technique to compare the behaviour of tsetse in odour plumes of different composition and release rate. First, I evaluated the behaviour of tsetse leaving a release box which had the release tubes pointing upward, to avoid any bias in the flight direction of tsetse. The results of these studies are reported here. Tsetse leaving the release box were recorded on video or caught with electric nets placed at 1.8 m from the release box.

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.

Capture and Release. 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.

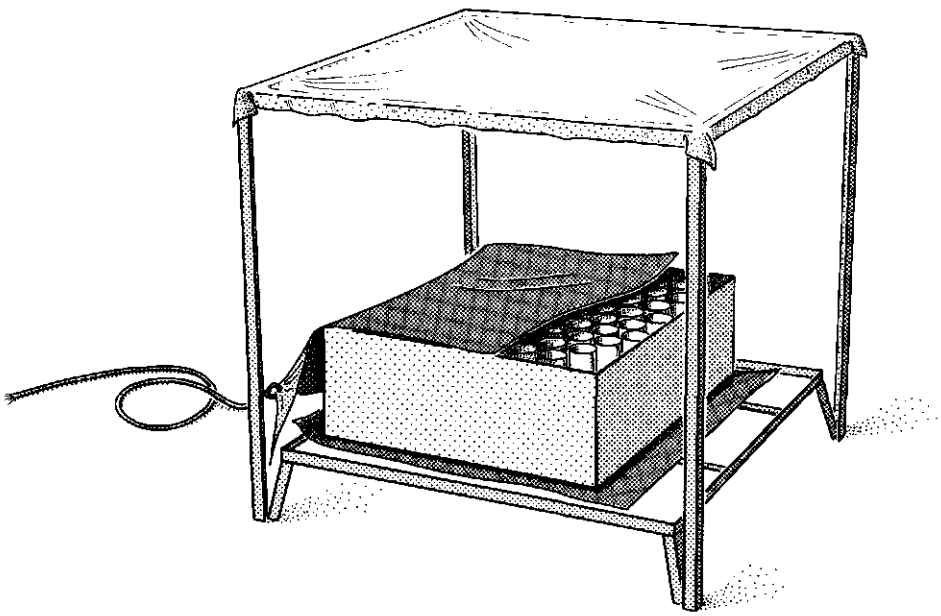

Figure 1: The tsetse release box.

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 box was 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.

Odour. 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 (480 l/h). As a control, no odour (NO) was dispensed. Because there was relatively little time to use the video equipment, only the control and the CAO mixture were tested when video recordings were made.

Recapture. The release box was placed in the centre of a complete ring of eight electric nets (Vale, 1974), each 1.5 m square. The distance from the centre of the release box to an electric net was 1.8 m. 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.

Video. The behaviour of tsetse after opening of the release box 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 release box was placed 10 m downwind of the odour source, on a floor of black velvet. The field of view of the camera was $\pm$ 2 x 3 m, i.e. up to 1 or 1.5 m from the centre of the release box. 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.

Analysis. 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 release box, 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 (Fig. 2). 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 5 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 must have flown 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, $P<0.025$, $df=7$). AO caused a shift towards the crosswind oriented nets, compared to CAO (χ^2, $P<0.025$, $df=7$) (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 (Fig. 3) and 95 to 100 % had left the release box after 20

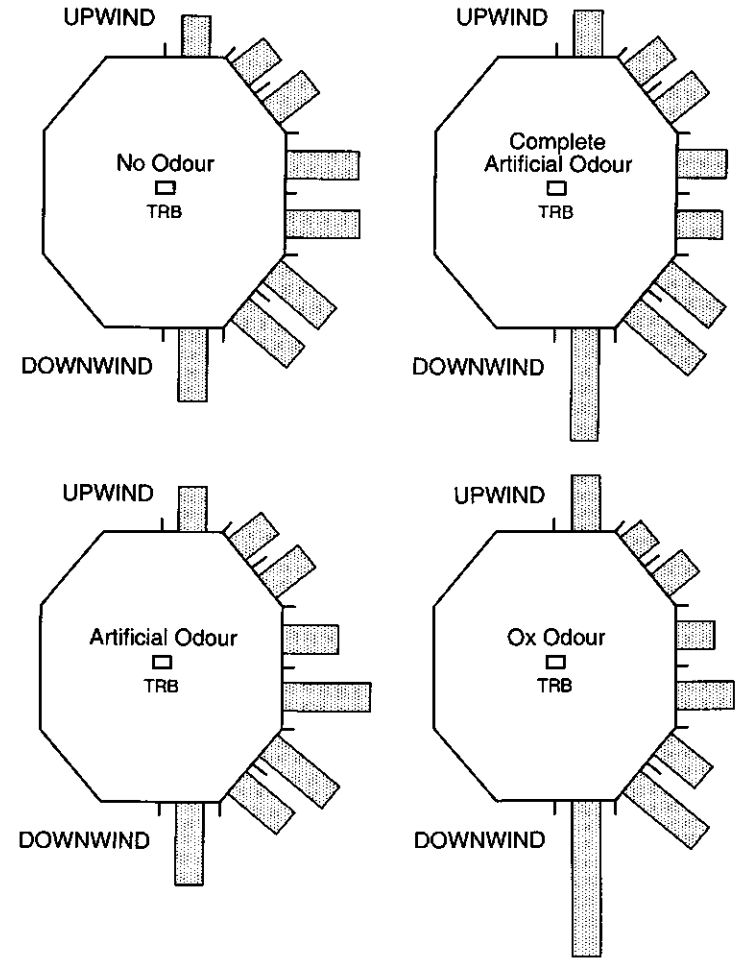

Figure 2: The distribution of tsetse over the sectors of electric nets in the presence of different odours.

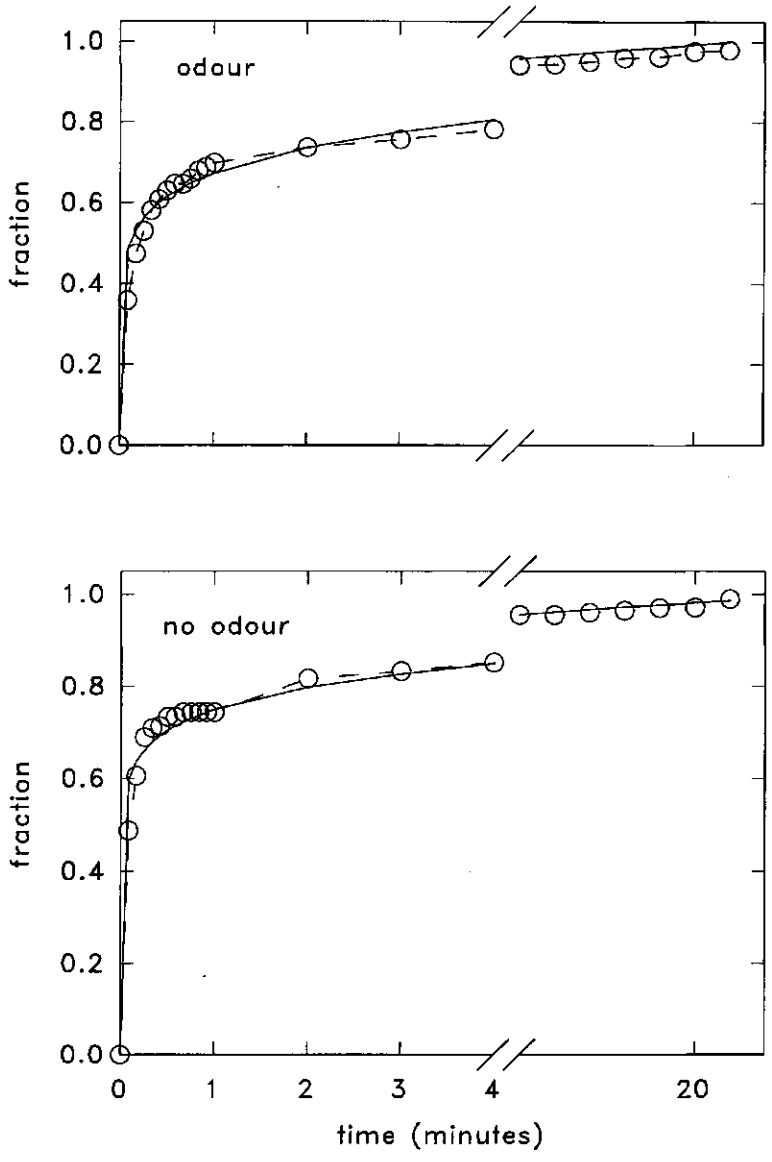

Figure 3: Cumulative distribution of time of appearance of tsetse. A: in the presence of odour; B: in the absence of odour. solid line: best fit single exponential.

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).

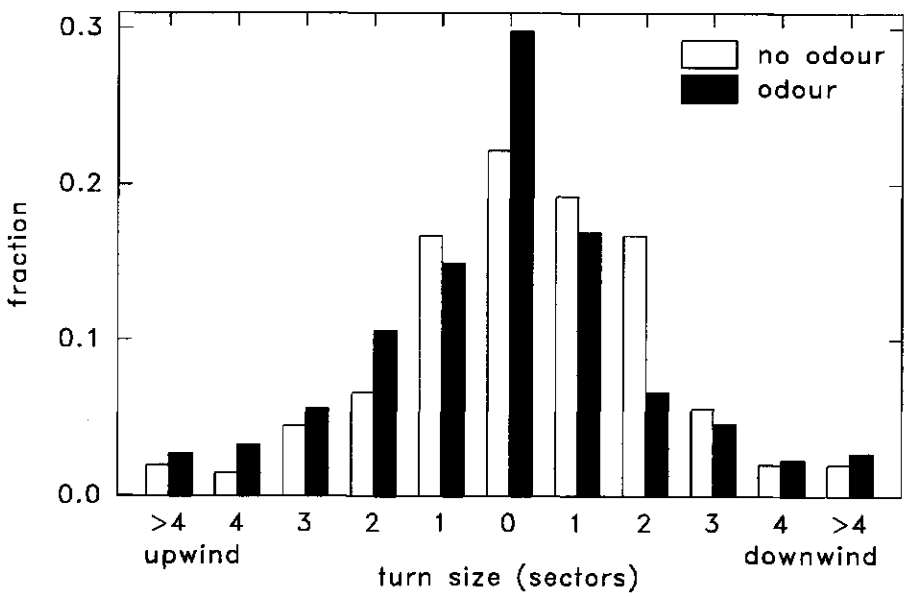

Figure 4: The turn size and direction made by tsetse in the presence and absence of CAO.

When CAO was present, there were no more or less 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 (χ^2, $P<0.05$, df=9). 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 (χ^2, $P>0.05$, df=7). 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, $P<0.005$, df=7).

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 release box with the tubes facing upwind. The differences in recapture between the experiments described in chapter 5 and 6 and those of 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).

The results of the study with electric nets appear to contradict the results of the video study: video studies showed no bias in take-off directions, while electric net studies showed a downwind bias. The latter 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 release box 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.

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5. Host odour composition affects host location by tsetse (Diptera, Glossinidae)

Abstract

Marked *Glossina pallidipes* Austen were released downwind of an odour source in the field in Zimbabwe and the percentage recaptured at the source on the same day was measured. In the absence of odour, 1.3 % of the marked tsetse released from a box or refuge were recaptured, independent of the distance between release point and odour source. When natural ox odour or a blend of carbon dioxide, acetone, octenol and phenols was dispensed, the untransformed recapture percentage of box-released tsetse released at 10 m was 18 %. The recapture percentage decreased to 2 % for tsetse released at 100 m. Recapture percentages were significantly higher than in the absence of odour at all release distances for ox odour and for release distances up to 75 m downwind for the artificial odour. When a combination of acetone, octenol and phenols or carbon dioxide on its own was dispensed, recapture percentages decreased from 6 % for tsetse released at 10 m to 0 % for tsetse released at 100 m. With these odours, recapture percentages were higher than in the absence of odour when tsetse were released at 20 m from the source, but were lower than recaptures in the presence of ox odour or the artificial mixture with carbon dioxide. Recapture percentages of tsetse spontaneously leaving refuges were higher than those of box-released tsetse. Proximity of source had no effect on the recapture percentage of refuge-leaving tsetse. The results are discussed in relation to host-location efficiency of tsetse, which seems close to 100 % when host odour is detected at less than 30 m downwind of the host.

Introduction

Host odours are important cues used by *Glossina pallidipes* Austen (Diptera: Glossinidae) in host location at long distance (see Willemse & Takken, 1994). Targets baited with the best imitation of ox odour catch fewer tsetse than targets baited with natural ox odour (Torr *et al.*, 1995). Composition of the host odour thus plays an important role in host location, but how odour composition affects the numbers of tsetse arriving at the source is unknown.

This chapter has been submitted in slightly different form as: Groenendijk, C.A. & W. Takken, Host odour composition affects host location by tsetse (Diptera: Glossinidae), to *Physiological Entomology*.

Two mechanisms are proposed to explain the role of odour composition: (1) the active space of the odour plume increases, resulting in more tsetse detecting the plume (Hargrove *et al.*, 1995); (2) tsetse navigate more successfully in a plume with more attractants.

When tsetse detect ox odour in a large clearing, stretching 120 m downwind of the source, they find the source with 80 to 100 % probability (Vale, 1980). But only 25 to 30 % of the tsetse entering a clearing from elsewhere detect the odour. In a recent mark-release-recapture study, a good imitation of ox odour, but at a dose much higher than from one ox, gave results comparable to Vale's 1980 data (Griffiths *et al.*, 1995). A comparison between these two experiments is not straightforward: the dose rates and odour composition differed between the experiments and both will affect the catch (Willemse & Takken, 1994; Hargrove *et al.*, 1995). Moreover, Vale conducted his experiment in a large clearing where directional information from the wind is more reliable than in the 'mopane' woodland where Griffiths *et al.* worked (Brady *et al.*, 1989).

Here I describe three experiments carried out to investigate the effect of odour composition on host-location efficiency by tsetse. Marked tsetse were released from 'release boxes', modified from Griffiths *et al.* (1995). I assessed the effect of distance between release point and odour source and the effect of odour composition on the numbers of marked tsetse recaptured at the odour source. This experiment was repeated with tsetse leaving an artificial refuge (Vale, 1971). Lastly, I used a 'booster' odour source to attract large numbers of tsetse and determined how many of these tsetse transferred to a secondary source placed at various distances upwind of the booster odour.

Material and Methods

Experiments were conducted from May 1993 to October 1994 near Rekomitjie Research Station, Zambezi Valley, Zimbabwe. Study sites were at the edge of the riverine vegetation (described by Vale, 1971) and in 'mopane' woodland

(described by Vale, 1974a). The following three experiments were carried out to study odour source location by *G. pallidipes*.

The 'Box Release' Experiment

Source of tsetse. Tsetse were caught with epsilon traps (Hargrove & Langley, 1990) from 10.00 to 15.00 h. The traps were baited with acetone (0.5 g/h), released from a small bottle, 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) and fitted with a non-return cage of netting, 20 x 10 x 10 cm. After removal, the cages containing tsetse were kept in a cool box with a damp cloth until they were marked. The mark consisted of two small spots of artist's oil paint which indicated the day and the distance of release. An earlier study showed no effect of a similar paint or colour on the survival of marked tsetse (Vale *et al.*, 1976).

Release. Marked tsetse were put singly in one of the plastic tubes in the release box (Chapter 4, p.53). 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. Flies were released at distances of 10, 20, 30, 40, 50, 75 and 100 m downwind of the odour source. There were not always enough flies to fill all release tubes, the number released per box or refuge varied from 35 to 125. Each combination of distance and odour was replicated at least four times, and a minimum of 275 tsetse were released.

Recapture. An electrified standard target (Vale, 1993) was placed at the source of the odour (Fig. 1). The target consisted of a 1 x 1 m black cloth with a 1 x 0.5 m sheet of fine netting on each flank. Cloth and netting were covered by an electrified grid of fine copper wires; flies contacting the grid were killed or stunned, fell down and were held in a sticky tray on the ground. The visual target was used because tsetse attracted by odour only, show an imprecise orientation to the odour source (Vale, 1974b). Catches and odour release started 1.5 - 2 hours before sunset and stopped at sunset. The target placed at the odour source will henceforward be termed the 'Main target'.

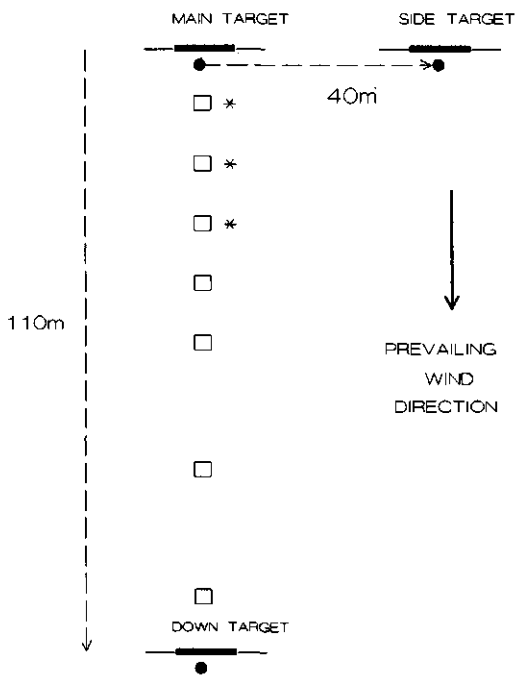

Figure 1: Overview of the experimental set-up. Open square= tsetse release site; dot= odour source. Stars: positions of refuges.

Two more electrified standard targets were used. One was placed at 110 m downwind of the source and will henceforth be termed 'Down target'. The other was placed 40 m from the Main target, in a direction across the prevailing wind direction (Fig. 1) and will henceforth be termed 'Side target'. Catches at these targets indicated the number of tsetse that were responsive but 'missed' the Main target.

The numbers of unmarked tsetse caught at the targets were also recorded, to allow a comparison between trends in recaptures and catches.

Odour. Four odours were tested: (1) natural ox odour (OX), dispensed via the fan-powered ventilation shaft of a roofed pit (Vale, 1974b) which contained a live ox of 550 kg; (2) Complete Artificial Odour (CAO), which consisted of carbon dioxide (480 l/h), released from a pressurized cylinder, and

acetone (0.2 g/h), 1-octen-3-ol (0.16 mg/h), 4-methyl phenol (0.32 mg/h) and 3-n-propyl phenol (0.04 mg/h), released from a polythene sachet; (3) Artificial Odour, (AO), which contained no carbon dioxide, but was otherwise identical to CAO; (4) Carbon dioxide only, (C), at 480 l/h. As a control, no odour (NO) was dispensed. The dose rate of CAO used here generally results in catches that are about twice as large as catches with the odour of one ox (Vale & Hall, 1985).

The Down and Side targets were always baited with the same odour, Standard Artificial Odour (SAO). SAO was identical to AO but dispensed at 2.5 times the dose rate of AO. This large dose was used in an attempt to attract tsetse that were released but did not react to the odour dispensed from the Main target.

The 'Refuge Leaving' experiment

Tsetse. During the hot season, September to October, tsetse were collected from artificial refuges (Vale, 1971) during the hottest time of the day. These tsetse were then handled like the tsetse used in the box release experiment, but placed in a release refuge (fig. 2A).

Departure. The front opening of the release refuge faced North, i.e., crosswind. This orientation ensured that (1) tsetse could not see the visual target while they were in the refuge; (2) the sun would not shine into the refuge during the time of the experiment; (3) those tsetse that flew straight out of the refuge and did not detect the odour released at the Main target, had a reasonable chance of encountering the odour dispensed at the Side target, which was stationed 40 m to the North of the Main target. The refuge was closed with a cover of netting during marking (Fig. 2B). Tsetse were put in the refuge through a small hole in the back. When all tsetse were inserted, this hole was closed and the tsetse were left to settle in the refuge. Tsetse that still tried to escape 10 minutes after marking, were trapped as they tried to escape and were removed from the system (Fig. 2C). The release of odour was then started and the netting cover was removed from the opening of the release refuge without disturbing the tsetse resting inside.

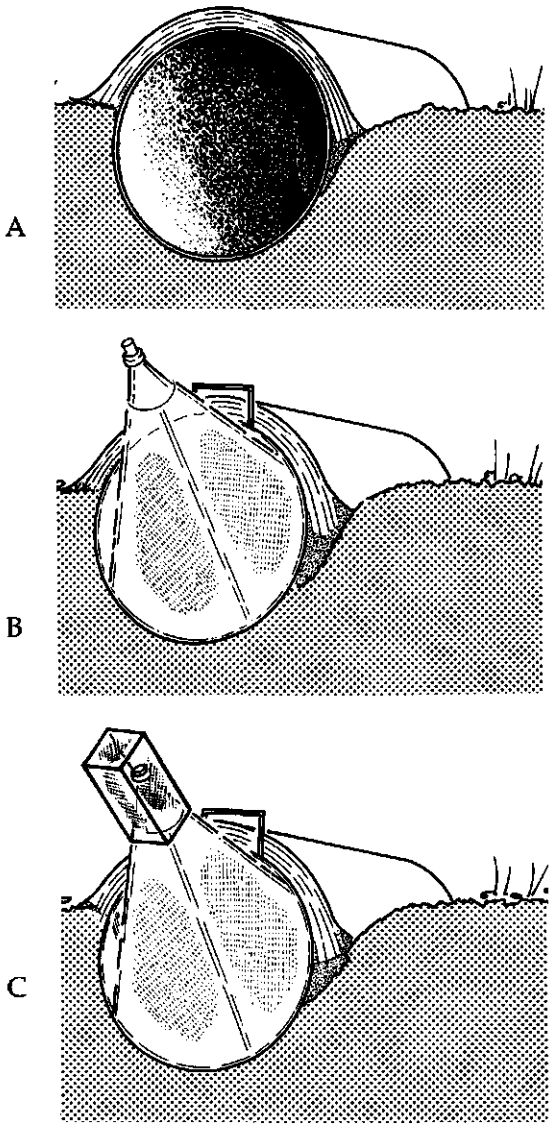

Figure 2: The release refuge. A: with main entrance/exit open; B: with netting cover which prevented recently marked flies from escaping; C: with non-return cage to remove tsetse that would not settle from the system.

Recapture and odour. Targets were placed as in the box release experiment, but with the Down target 50 m downwind of the odour source. Catches were made from around 15.00 h, when odour release started, until sunset (approx. 18.00h). Fly numbers were relatively low during these months, and therefore only three distances, 10, 20 and 30 m, and two odours, CAO and AO, were tested.

The 'booster' experiment

This experiment was used as a control for the box-release experiment. The behaviour of the tsetse in the box-release experiment was perhaps affected by handling and releasing from the boxes. Furthermore, the trapped tsetse may have comprised a biased sample of host seeking tsetse. Griffiths *et al.* (1995) found no difference between males caught with a trap or with an electrified target, but a small difference in fat content between females caught with the two methods. Trap (Randolph *et al.*, 1991) and target (see Chapter 3) catches contain more females in the early stage of pregnancy than expected from a random sample.

A large dose of odour (CO_2 (600 l/h), acetone (4 g/h), 1-octen-3-ol (3.2 mg/h), 4-methylphenol (6.4 mg/h) and 3-n-propylphenol (0.8 mg/h), termed booster odour, without a visual stimulus near the source, attracted many tsetse. If the active space of the test odour encompassed the source of the booster odour, tsetse attracted to the booster may transfer to the test odour (Torr, 1988b). This greatly increased the catch at the source of the test odour. Since tsetse do not transfer from a source containing carbon dioxide to one without it (Torr, 1988b), only CAO was tested. CAO was released at 10, 20, 30 and 40 m and at infinite distance (booster switched off) upwind of the booster odour.

Wind parameters and temperature

During all the experiments, wind direction was measured once every minute in 10° sectors at a site 40 m South of the Main target. The average wind direction, the variation in wind direction and the departure of the average wind direction from the prevailing wind direction, were calculated. The

maximum temperature was measured at a weather station at about 2 km distance.

Statistical design

A nested randomized block design was used, with groups of days as blocks. Odour treatments were assigned to days within a block and release distances were assigned to each odour treatment. The variance in the data was stabilized with an arcsine transformation of percentages of recaptured tsetse and a $\log_{10}$ (catch + 1) transformation of the numbers of captured unmarked tsetse. The effects of treatments were assessed with an analysis of variance. Where more than two means were compared, a Least Significant Difference test was used.

Effects of treatment and weather on recapture data were assessed with a logistic regression (Hosmer & Lemeshow, 1989) using the Egret statistical package. To normalize the distribution, recapture data were transformed to log {P/(1-P)}, with P = the chance of recapture. A log-transformation of the distances resulted in a linear correlation between recapture data and distance for each odour. This facilitated a comparison of the regression models of different odour treatments. The slope of each odour treatment was estimated independently, while for the other variables (e.g. distance) one coefficient was estimated for all odour treatments. The general equation is:

$$\text{Recapture} = C + aX + bY + dZ + \begin{matrix} f1 \\ f2 \\ f3 \\ f4 \\ f5 \end{matrix} * \text{odour} \qquad (1)$$

in which a,b,d = coefficients of the variates X,Y and Z
C = constant
f1 to f5 = coefficients of odour treatment 1 to 5

To assess the goodness of fit, an r^2 -type measure was calculated (Hosmer & Lemeshow, 1989, p. 148).

Results

No significant differences in behaviour between males and females were found, and the sexes were therefore pooled in the results reported below. In total 10,589 marked tsetse were placed in release boxes and 4,133 marked tsetse were placed in release refuges.

The 'Box Release' experiment

On average, 95 % of the marked tsetse took off during the experiment. This percentage was not correlated with treatments and flies that did not leave were therefore not used in the analysis. There was also no significant difference between recapture percentages at different experimental sites and hence the results of the sites were pooled.

In the absence of odour, the recapture percentage of tsetse at the Main target was 1.3 % and independent of the distance between release point and Main target. This is significantly lower (χ^2, $P<0.01$, df=1) than would be expected on the grounds of random flight directions from the release site, followed by visual homing-in to the target from about 5 m distance (Griffiths *et al.*, 1995), which predicts recapture percentages from 15.9 % to 1.6 % for releases at 10 to 100 m downwind of the source.

When flies were released in the presence of OX or CAO, recapture percentages were higher than in the absence of odour, for OX at all distances tested and for CAO at all distances except 100 m (Table 1). In the presence of CAO, the number of recaptured tsetse was either similar to the expectation from random flight direction (at 10, 40 and 100 m), or higher (χ^2, $P<0.01$, df=1). Compared to expectations based on random radial dispersion, the numbers of tsetse recaught when OX was dispensed were lower than expected at 10 m, higher at 20, 30 and 50 m (χ^2, $P<0.01$, df=1) and similar at 40 and 75 m. In the presence of AO and C, only recapture percentages of tsetse released at 20 m were higher than recapture percentages in the absence of odour.

Table 1: Detransformed mean percentages of *G. pallidipes* recaptured at the Main target after release from the box. Means followed by different letters in the same row indicate significant differences (Least Significant Difference Test).

Release distance		Odour					P
(m)	N	No odour	Complete artificial	Artificial	CO_2 only	Ox odour	
10	8	0.4^{a}	11.8^{c}	3.3^{ab}	2.3^{ab}	8.0^{b}	<0.001
20	15	0.9^{a}	12.3^{c}	3.2^{b}	3.5^{b}	12.0^{c}	<0.001
30	7	0.7^{a}	7.0^{b}	1.9^{a}	1.0^{a}	10.6^{b}	<0.001
40	7	0.1^{a}	4.0^{c}	0.7^{ab}	1.1^{abc}	5.6^{c}	<0.01
50	5	1.2^{a}	6.4^{b}	1.7^{a}	1.0^{a}	6.3^{b}	<0.05
75	4	0.0^{a}	3.8^{b}	--	0.7^{ab}	2.2^{b}	<0.05
100	4	0.0^{a}	2.1^{ab}	--	0.1^{ab}	1.9^{b}	<0.05

N = number of replicates

Table 2: Detransformed mean catches of unmarked *G. pallidipes* at the targets during box release experiments. Means in the same column followed by different letters are significantly different (Least Significant Difference Test).

Odour	Main	Side	Down
No odour	16^{a}	55^{b}	45^{ab}
Complete artificial	84^{c}	42^{b}	37^{a}
artificial	32^{b}	42^{ab}	39^{ab}
CO_2 only	53^{bc}	51^{b}	64^{b}
Ox odour	76^{c}	28^{a}	27^{a}
P	<0.001	<0.05	<0.025

For tsetse released at less than 40 m, the numbers recaptured were lower then expected from random radial dispersion (χ^2, $P<0.01$). At greater distances, the recapture percentages were similar to those of the NO treatment. The catch of unmarked tsetse at the Main target showed a trend similar to that of the recapture percentages: the release of odour increased the catch and OX and CAO attracted more tsetse than AO or C (Table 2).

Recapture percentages at the Main target were correlated with the distance between release site and source, the odour composition and the maximum temperature. The odour treatment and the release distance both decreased the deviance of the model by a larger percentage than temperature (Table 3). The regression coefficients of the odour treatments were all different from each other. CAO had the highest regression coefficient (Table 3; Fig. 3).

Table 3: Results of the logistic regression of box-released *G. pallidipes* recaptures at the Main target. $P<0.001$ in each case. The slopes of the odour treatments differed significantly from each other ($P<0.001$)

Term	Coefficient	Decrease in deviance
Constant	-2.10	
Odour		36 %
Complete artificial	2.10	
Artificial	0.88	
CO_2	0.60	
Ox odour	2.02	
distance	-0.02	29 %
temperature	-0.05	3 %
estimate of r^2	0.56	

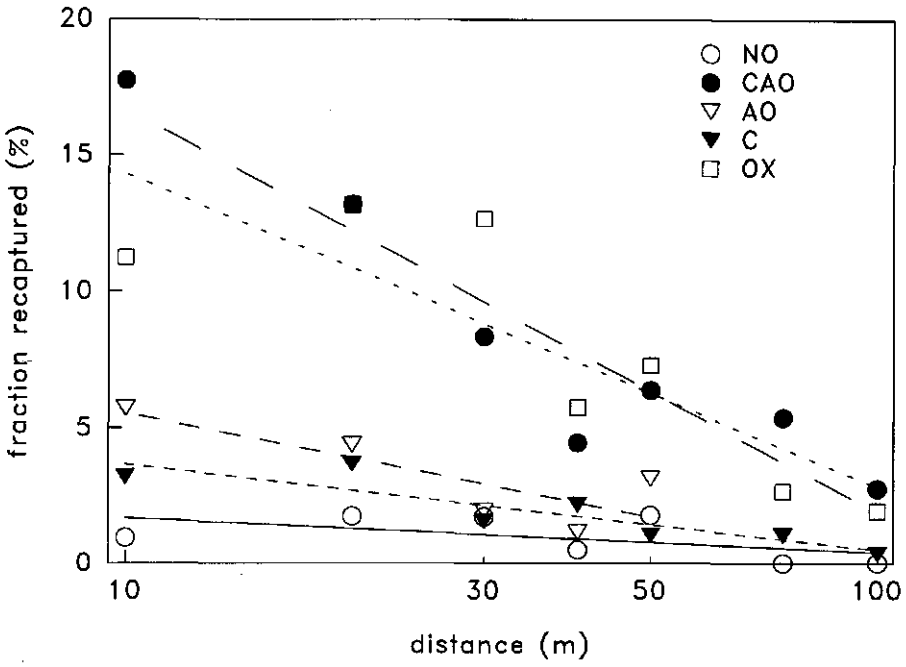

Figure 3: Transformed recapture percentages of box-released tsetse at the main target plotted against release distance (log-scale). Regression coefficients are given in Table 4.

When odour was released at the Main target, recapture percentages of flies at the Side target were significantly lower than in the control ($P<0.01$) but the composition of the odour did not change the recapture percentage. Furthermore, the recapture percentage was negatively correlated with the square of the distance between release box and Side target and was positively correlated with the variation in wind direction and with the departure of the average wind direction from the prevailing wind direction. However, these variables decreased the deviance of the model by 5 % at most. The complete model explained about 10 % of the variation. Use of OX at the Main target decreased the catch of unmarked tsetse at the Side target.

At the Down target, recapture percentages of tsetse were not correlated with the odour dispensed at the Main target. The negative correlation between recapture percentages and the distance between Down target and the release point explained 29 % of the variation. A positive correlation with the variability in wind direction explained a further 7 % of the variation and the complete model explained about 34 %. Release of C increased the catch of unmarked tsetse at the Down target.

The 'Refuge Leaving' experiment

Five percent of the marked tsetse did not settle in the refuge and were discounted (see Methods). No tsetse remained in the refuge at the end of the experiment. Recapture percentages were higher than in the release box experiment. During September 1994 box-release and refuge-leaving experiments were conducted at the same site, but the choice of release method depended on the temperature. Table 4 shows that temperature had an effect on the recapture percentage, which makes statistical testing of the difference in recapture percentage between methods impossible.

Recapture percentages at the Main target were affected by the type of odour used, but not by the distance between release point and Main target (Fig. 4). Recapture percentages at the Down target were negatively correlated with increasing distance from the release site. Recapture percentages at the Side target were negatively correlated with temperature, but correlations decreased the deviance of the model by only 6 %.

Booster experiment

Catches of *G. pallidipes* declined with increasing distance between the booster odour and the test source (Fig. 4). When the test odour was dispensed at less than 20 m upwind of the booster odour, more tsetse were caught than when the test source was placed at infinite distance upwind of the booster ($P<0.05$; LSD-test).

Table 4: Results of the logistic regression of refuge-leaving *G. pallidipes* at the Main target. P< 0.005 in each case. The slopes of the odour treatments differed significantly from each other (P<0.01)

Term	Coefficient	Decrease in deviance
Constant	-2.85	
Odour		46 %
Complete artificial	1.558	
Artificial	1	
estimate of r^2	0.46	

Discussion

Effects of release method

Box-released tsetse were collected from traps, which sample tsetse that are in flight and react to odour (Hargrove, 1991), whereas refuge-leaving tsetse were collected from refuges which sample tsetse that respond negatively to light at high temperatures and were not host-seeking at the time of collection (Vale, 1971). One might therefore expect the recapture of box-released tsetse to be higher than that of refuge-leaving tsetse, but the opposite was the case. Video recordings (Chapter 4) showed that 80 % of the flies left in the first minute after the release box was opened. Griffiths *et al.* (1995) suggest that this is a response to opening of the box and may be considered an unnatural departure. But there was no relation between the rapidity of take-off and probability of recapture of tsetse (Griffiths *et al.*, 1995). Furthermore, I found that different odours had the same effect on marked and unmarked tsetse in the box-release experiment.

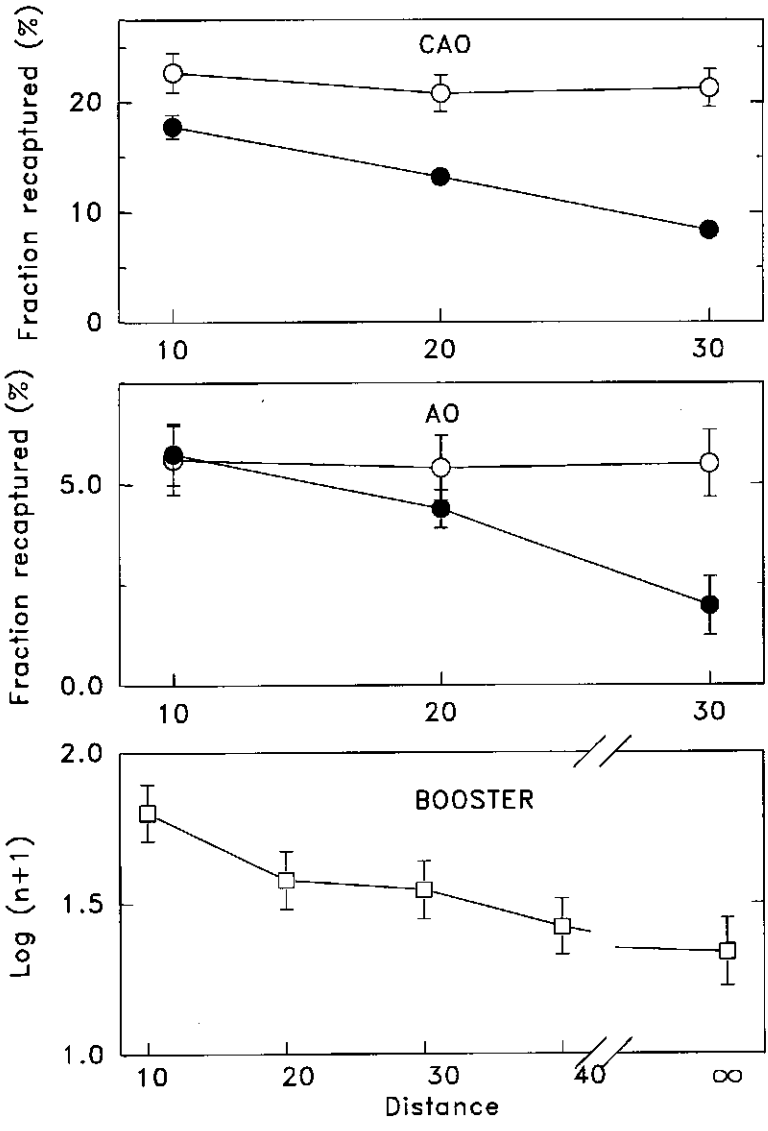

Figure 4: The effect of release distance on recapture percentages (± SEM) of box-released (dots) and refuge-leaving (circles) tsetse at the Main target when CAO or AO was dispensed, and the effect of distance between test odour source and booster source on the catch (± SEM) *G. pallidipes* at the test source.

From these data I infer that the tsetse behaved naturally after they had left the box and that a difference in recapture between box-released and refuge-leaving tsetse was not caused by behavioural changes in the former.

The difference in recapture might be explained by the structure of the odour plume. The chance that odour is present when the majority of the tsetse leave the release box, obviously decreases with distance from the odour source (Murlis & Jones, 1981) and flies that do not encounter odour fly downwind (Gibson *et al.*, 1991). The decline in recaptures of box-released tsetse was probably largely caused by the declining chance that odour was present near the box when it was opened. The same explanation may apply to the declining catches at the test odour of the booster experiment. The chance that odour from the test source is present at the booster, which is necessary in order to guide a fly further upwind, declines with increasing distance between the booster and the test source.

Unlike in the box-release experiment, tsetse did not leave the refuge all at once when it was opened. Since there was no effect of release distance on the recapture percentage, there appeared to be no decrease in the probability that tsetse encounter odour after leaving the refuge. It is possible that, due to wind turbulence at the entrance, odour entered the refuge and that responsive tsetse only left the refuge when they detected host odour. This is supported by the higher recapture percentage of refuge-leaving tsetse. When taking the 50 % efficiency of electric nets in account (Packer & Brady, 1990; Griffiths & Brady, 1994), our recapture percentage of refuge-leaving tsetse was 43 %.

Effects of odour composition on recapture percentage and range of detection

Tsetse flies fly in a downwind direction in the absence of odour (Gibson *et al.*, 1991). Video data (Chapter 4) showed that tsetse left the release box in all directions. However, within the field of view (2 x 3 m) 63 % of the tsetse that left in upwind direction, almost immediately turned downwind in the absence of odour. Few tsetse that left in a downwind direction, turned upwind. Also, the recapture percentages in the absence of odour were significantly lower than expected from random radial dispersion (Griffiths *et al.*, 1995). Therefore, I assume that when the recapture percentage from a given release

point was significantly higher in the presence of odour than in the absence of odour, tsetse could detect the odour at that release point.

Air turbulence disperses plumes of different composition in the same way (Murlis *et al.*, 1992). Tsetse released at the same distance of the source thus had the same chance of encountering the plume, irrespective of the composition of the plume. Consequently, differences in recapture percentage were due to differences in plume composition, because the release rate of other components were unchanged.

More tsetse were recaptured when released at 20 m downwind of a source of CAO than when released 20 m downwind of a source of C or AO. Tsetse thus had a greater chance to reach the source when the odour contained more components. CAO still elicited a detectable response in tsetse released at 50 m downwind, but C and AO did not. Thus, both C and AO can be detected at 50 m downwind, but only the combination of these odours elicited a response that brought the flies in the vicinity of the odour source. This was probably due to the synergistic effect between acetone and carbon dioxide (Torr, 1990). OX still elicited a detectable response in tsetse released at 100 m downwind. This finding is similar to the estimate of Vale (1977) and is the first direct confirmation of that estimate. Tsetse respond to a combination of acetone and carbon dioxide from about 45 m downwind (Vale, 1984). This range falls between the ranges I found for CAO and either C or AO. This may be due to the synergism between carbon dioxide and acetone (Torr, 1990), but Vale used a high dose of acetone. This complicates the comparison with our data because dose also affects the recapture rates (Willemse & Takken, 1994; Hargrove *et al.*, 1995).

I detected a response to CAO up to 75 m downwind from the source with the box release experiment, but with the booster experiment I did not detect a response at more than 30 m downwind from the source. This difference was probably due to the variability of the catches in the latter experiment. In general, a doubling in the catch is necessary to show a clear and significant difference between two treatments (Torr *et al.*, 1995). Alternatively, fewer flies may have transferred from the booster odour to the test odour because of the lower concentration of the latter.

The difference between recapture percentages of box-released tsetse at the Main target with OX and CAO was small but significant, whereas the components that both mixtures had in common were released at twice the rate in CAO. This, and the fact that only OX odour depressed the catch of unmarked tsetse at the Side target, suggests that there is at least one more attractive component in ox odour beside the ones present in CAO. This agrees with recent findings of Torr *et al.* (1995) who caught more tsetse at a target baited with ox odour than at a target baited with the known components of ox odour. However, the percentage of refuge-leaving tsetse I recaptured with CAO, was similar to the percentage Torr (1988a) observed to react to ox odour.

Efficiency of host location

Vale (1980) estimated that in his experiment approximately 25 % of the tsetse that entered his test arena were caught at the source of ox odour marked with a visual bait. When OX was dispensed and the release box placed at not more than 30 m from the source, the recapture percentages were similar to Vale's results, taking the 50 % efficiency of the electric nets into account. The percentage of recaptured tsetse leaving the refuge was similar to the percentage that responds to ox odour when in a refuge (Torr, 1988a). This suggests a host-location efficiency close to 100 % at distances from 10 to 30 m downwind of the source, similar to the estimate of efficiency by Vale (1980). Fewer tsetse were recaptured when released at greater distances, possibly because of the weaker directional information of the wind within our 'mopane' vegetation versus Vale's clearing (Brady *et al.*, 1989).

CAO had the same composition as the odour used by Griffiths *et al.* (1995), but released at a lower rate. The 20 % recapture percentage at 30 m of Griffiths *et al.* (1995) was 2.5 times higher than the 8 % (untransformed) obtained in this study. The recapture percentages of tsetse released at 50 and 75 m were similar in the two studies. The different orientation of the release box in the two studies had no effect (see Chapter 4) and it is thus likely that the difference was caused by the difference in odour release rate.

Effects of wind parameters

The recapture percentage at the Main target was not correlated with the wind parameters used (average wind direction, variance of the wind direction and departure from the prevailing wind direction). This is no surprise because these parameters were averages, whereas previous work showed that tsetse react to the instantaneous wind direction (e.g. Gibson & Brady, 1988; Gibson *et al.*, 1991; Brady & Griffiths, 1993).

The recapture percentages at the Side target were positively correlated with the variance and the departure of the wind direction from the prevailing wind direction. However, these variates decreased the deviance of the model only marginally. These variates probably only indicated an increased chance that the odour from the Side target was present near the release box at the time of release. Recapture percentages at the Down target were correlated with the variance in wind direction. This catch might have consisted mainly of tsetse that first flew downwind and then encountered, and responded to, the odour released at the Down target.

Orientation mechanisms

A more complete odour source was found with greater probability and from a greater distance. This was probably caused at least partly by the synergism between components, which might lower the concentration at which a tsetse responds to an individual odour pocket. An odour plume appears to a sampler, in this case a tsetse fly, as a series of bursts that vary in strength and duration (Murlis & Jones, 1981). In a more complete odour, a tsetse might also detect bursts of lower concentration ('strength') due to synergism and consequently encounter more odour pockets per unit of time. It seems likely that tsetse use a form of biassed random walk to locate an odour source they have detected (Brady *et al.*, 1990; Williams, 1994; Griffiths *et al.*, 1995). The bias is then provided by a combination of upwind take-off in odour and in-flight corrections of direction when contact with odour is made or lost. If tsetse react to wind direction more frequently in a more complete plume, this should result in a higher probability of (re)gaining contact with odour, which, over time, should result in a higher bias of flight direction towards the source.

According to theoretical studies (Fisher & Lauffenburger, 1987; Williams, 1994) a higher bias results in a higher chance that the source is found.

The relation between the bias in the biassed random walk and the probability of success is not linear (Fisher & Lauffenburger, 1987). When the bias is below 20 %, the chance of success increases quickly with increasing bias, but above 20 %, the chance of success increases more slowly to an asymptote. These theoretical calculations assume a circular active space. However, the size and shape of the active space of an odour source varies with variability in wind speed and wind direction (Brady *et al.*, 1995; Griffiths & Brady, 1995). The probability of finding the source with random biassed walk would then depend on wind parameters. In mopane woodland, odour arrived from within 10° of the direction of an odour source at 15 m distance for about 30 % of the time (Brady *et al.*, 1989).

Tsetse also change their flight direction when they no longer detect odour for some time (Bursell 1984; Gibson & Brady, 1988; Torr, 1988b), at least at less than 15 m downwind of the source. Presumably these turns are made to relocate the odour plume. This would increase their chance to locate the host. From the results of the refuge experiment, it appears that between 10 and 30 m from the source, a decrease in bias of wind direction with respect to the source has little effect on host-location efficiency.

When the bias in wind direction to the source is low and the source is far away, a lower 'sampling frequency' is an advantage, given the limited flight time of tsetse (Williams, 1994). The intermittency of a plume increases with distance from the source (Murlis *et al.*, 1992). If tsetse react to wind direction only when they detect odour, their responses would optimize their chance to find a source. The two ways in which odour composition could affect host location which were proposed in the introduction, are then two effects of the same mechanism.

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6. Odour release rate affects the host-location efficiency of tsetse

Abstract

Marked tsetse flies, *Glossina pallidipes* Austen, were released downwind of an odour source in the field in Zimbabwe and the percentage recaptured at the source on the same day was measured. An odour mixture consisting of carbon dioxide, acetone, 1-octen-3-ol and phenols was released at three different rates. With the medium odour release rate, marked tsetse were recaptured from up to 75 m distance. Of the tsetse released at 10 m distance, 15 % was recaptured. This percentage declined with distance to 5% for tsetse released from 75 m. The low odour release rate, a quarter of the medium rate, only attracted tsetse from 20 m and less; the recapture percentage declined from 5% at 10 m to 2 % at 30 m. The decrease in release rate thus caused a decline in plume length and a decline in host-location efficiency or recruitment of tsetse to the plume. When the medium release rate was increased fourfold, the resulting high odour release rate did not increase the recapture percentages from the same distances, nor did the distance from which tsetse were recaptured increase, compared to the medium rate.

Introduction

The rate at which host odour is released, has a strong effect on the number of tsetse flies (Diptera: Glossinidae) caught at the source (Hargrove & Vale, 1978; Torr, 1990; Hargrove *et al.*, 1995). The positive correlation between odour release rate and catch appears to be a simple power law which might reflect the relationship between the magnitude of the odour flux or dose (sensu Murlis *et al.*, 1992) and the distance from the source (Hargrove *et al.*, 1995). In this paper, the word 'rate' will refer to known release rates at the source. The word 'dose' will refer to the number of odour molecules in an odour pocket (Murlis et al., 1992). Odour release rate might increase the catch at the source in two ways: (1) the active space increases or (2) tsetse navigate more successfully. Simple catch methods cannot distinguish between these effects.

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An odour plume consists of odour pockets that are separated by clean air (Murlis & Jones, 1981). Tsetse probably navigate towards the source by means of a biassed random walk (Brady *et al.*, 1990; Williams, 1994). The bias towards the source is the result of upwind take-off in odour (Bursell, 1987) and upwind turns when odour is encountered in flight (Gibson & Brady, 1988; Brady & Griffiths, 1993). In chapter 5 I argued that a more complete odour plume causes a larger bias in flight directions towards the source because it produces more detectable odour pockets.

If more detectable odour pockets are the clue to the higher efficiency, then a higher odour release rate should increase the catch of marked tsetse. Here I report on field experiments that were designed to test this hypothesis. Marked *Glossina pallidipes* Austen were released at 10 to 100 m downwind of an odour source. A difference was observed in the numbers of tsetse recaptured when three different odour release rates were used.

Materials and Methods

Experiments were conducted during the rainy season, from December 1994 to March 1995, near Rekomitjie Research Station, Zambezi Valley, Zimbabwe. The rainfall was 50 % below average and tsetse were not as abundant as in previous years.

Tsetse were collected in an epsilon trap (Hargrove & Langley, 1990), marked with artist's oil paint and were released from a release box (Chapter 4) in 'open' woodland. Recaptures were made at the odour source with an electrified standard target (Vale, 1993). The target consisted of a 1 x 1 m black cloth with a 1 x 0.5 m sheet of fine netting on each flank. Cloth and netting were covered by an electrified grid of fine copper wires; flies contacting the grid were killed or stunned, fell and were then trapped in a sticky tray on the ground. The visual target was used because tsetse attracted by odour only, show an imprecise orientation to the odour source (Vale, 1974b). The target

placed at the source of the test odour will henceforward be termed the 'Main target'. Two more electrified standard targets were used, as in Chapter 5. One was placed at 110 m downwind of the source and will henceforth be termed 'Down target'. The other was placed 40 m to the side of the Main target, in a direction across the prevailing wind direction and will henceforth be termed 'Side target'. Recaptures at these targets indicated the number of tsetse that were responsive but 'missed' the Main target. The numbers of unmarked tsetse caught at the targets were also recorded, to compare trends in recaptures and catches.

Experiments started 1.5 - 2 h before sunset and stopped at sunset (approx. 18.00 h). Tsetse were released 10 minutes after the odour dispensers and the electrified targets were turned on. A full account of the procedure is given in chapter 5.

Odour

Three release rates of the same odour were tested: (1) a low rate, which consisted of carbon dioxide (120 l/h), released from a pressurized cylinder, and acetone (0.05 g/h), 1-octen-3-ol (0.04 mg/h), 4-methylphenol (0.08 mg/h) and 3-n-propylphenol (0.01 mg/h), released from a polythene sachet (Laveissière *et al.*, 1990); (2) a medium rate, which was four times higher than the low rate; and (3) a high rate, which was 16 times higher than the low rate.

Wind parameters and temperature

During all the experiments wind direction was measured once every minute in bins of 10° at a site 40 m South of the Main target. The average wind direction, the variation in wind direction and the departure of the average wind direction from the prevailing wind direction were calculated. The maximum temperature was measured at a weather station at about 2 km distance.

Statistical design

A nested randomized block design was used, with groups of days as blocks. Odour treatments were assigned to days within a block and release distances

were assigned to each odour treatment. The variance in the data was stabilized with an arcsine transformation of percentages of recaptured tsetse and a $\log_{10}$ (catch + 1) transformation of the numbers of unmarked tsetse caught. The effects of treatments were assessed with an analysis of variance. Where more than two means were compared, a Least Significant Difference test was used.

Effects of treatment and weather on recapture data were assessed with a logistic regression (Hosmer & Lemeshow, 1989) using the Egret statistical package. To normalize the distribution, recapture data were transformed to log {P/(1-P)}, with P = the chance of recapture. A log-transformation of the distances resulted in a linear correlation between recapture data and distance for each odour. This facilitated a comparison of the regression models of different odour treatments. The slope of each odour treatment was estimated independently, while for the other variables (e.g. distance) one coefficient was estimated for all odour treatments. The general equation is:

$$\text{Recapture} = C + aX + bY + dZ + \begin{matrix} f1 \\ f2 \\ f3 \end{matrix} * \text{odour} \qquad (1)$$

in which

a,b,d	=	coefficients of the variates X,Y and Z
C	=	constant
f1 to f3	=	coefficients of odour treatment 1 to 3

To assess the goodness of fit, an r^2-type measure was calculated (Hosmer & Lemeshow, 1989, p. 148).

Results

About 5 % of the tsetse did not leave the release box, irrespective of treatment. These tsetse were ignored in the analysis. Females and males reacted in a similar way and the results of the sexes were pooled.

When tsetse were released in the presence of the low rate and as far as 50 m downwind of the source, the percentage recaptured at the Main target was about half that of tsetse released in the presence of the medium or the high rate (Table 1).

There were no clear differences in the percentages of marked tsetse recaptured at the Main target in the presence of the high or the medium rate. When tsetse were released at more than 50 m downwind of the source, the odour release rate did not affect the recapture percentage. The catch of unmarked tsetse was highest when the medium rate was used (Table 2). Logistic regression showed that the best model explained 46 % of the variation in recapture percentages at the Main target. The most important factors were odour release rate and distance of release; wind parameters only had a minor influence (Table 3 & Fig. 1).

Table 1: Mean percentage and average numbers of *G. pallidipes* recaptured at the Main target after release from a box. N= number of replicates; P= probability. Different letters in the same row indicate significant differences (Least Significant Difference Test).

Release distance (m)	N	Release rate			P
		Small	Medium	Large	
10	8	5.1^{a}	15.2^{b}	8.3^{ab}	<0.05
20	15	4.4^{a}	11.1^{b}	9.5^{b}	<0.001
30	7	1.7^{a}	4.0^{ab}	4.2^{b}	<0.05
40	7	2.4^{a}	4.3^{a}	5.0^{a}	ns
50	5	1.9^{a}	5.7^{b}	3.4^{ab}	<0.025
75	4	1.2^{a}	5.0^{a}	5.3^{a}	ns
100	4	1.4^{a}	1.1^{a}	1.4^{a}	ns

Table 2: Detransformed mean catches of unmarked *G. pallidipes* at the targets during box release experiments. Different letters in the same column indicate significant differences (Least Significant Difference Test).

Release rate	Main	Side	Down
Small	16^a	8^a	15^b
Medium	38^b	13^a	19^b
Large	18^a	7^a	8^a
P	<0.005	ns	<0.05

Table 3: Results of the logistic regression of *G. pallidipes* recaptures at the Main target after release from a box. In all cases P<0.005

Term	coefficient	decrease in deviance
Constant	-1.42	
Release rate		42.9%
Small[a]	0.25	
Medium[b]	0.95	
Large[c]	0.56	
distance	-0.05	42.7 %
distance squared	0.002	3.4 %
Wind parameters		
average direction	-0.06	6.0 %
Departure from axis	-0.08	5.0 %
estimate of r^2	0.46	

Odour release rate had no effect on the recapture percentage at the Side and Down target. Only 7 % of the variance in recapture percentages at the Side target was explained by the best model. The recapture percentage was negatively correlated with the square of the distance between Side target and release box and with the variation in wind direction. The recapture percentage was positively correlated with the distance between the Side target and the release point.

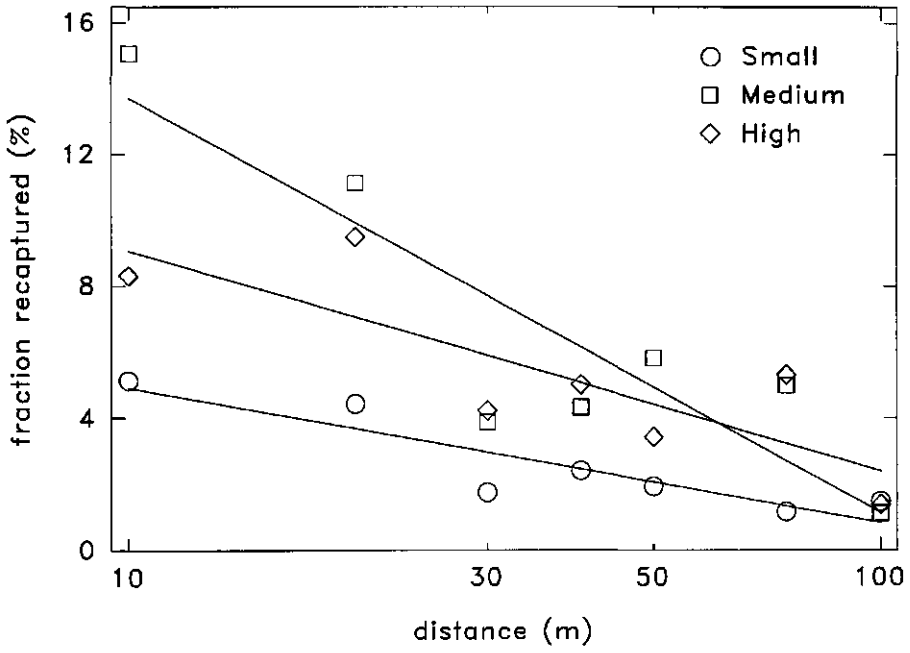

Figure 1: Transformed recapture percentages of box-released tsetse at the main target plotted against release distance (log-scale). Regression coefficients are given in Table 3.

About 21 % of the variation in recapture percentage at the Down target was explained by the logistic model. The recapture percentage was negatively correlated with the distance between release point and Down target squared. This factor explained 17 % of the variation. Negative correlations between wind direction and variation in wind direction and the recapture percentage accounted for the remaining 4 %. The high rate decreased the catch of unmarked tsetse at the Down target significantly.

Discussion

The fourfold increase in odour release rate from a low to a medium rate more than doubled the recapture percentages up to 75 m from the source. The apparent length of the plume increased from 20 m to 50 m. This is the first direct confirmation that an increase in release rate at the source increases the plume length (Hargrove & Vale, 1978; Hargrove *et al.*, 1995). The results also confirm that the release rate of the odour increases the efficiency of host-location or the number of tsetse that detects the odour. The increase in the active space and the increase in the host-location efficiency both contributed about 50 % of the increase in the total recapture percentage at the Main target.

The fourfold increase in odour release rate from medium to high did not increase the recapture percentages. The medium rate reaches 75 m downwind and up to that distance, the high rate did not increase the host-location efficiency. When I used the medium or the high release rate, I did not recapture more tsetse released at 100 m than could be expected in the absence of odour. The high release rate apparently did not increase the active space either. When the dose is increased much more, the catch at the source increases too (Hargrove *et al.*, 1995). It is thus possible that the dose used in our experiment was not large enough to have a measurable effect at 100 m downwind.

It is unlikely that repellency played a role. The catch of unmarked tsetse at the Down target declined significantly when odour was released at the high rate, but so did the catch at the Main target. Hargrove *et al.* (1995) used a

larger release rate of artificial odour than we used and found no repellency. The decrease with the high release rate probably only indicated that the population of *G. pallidipes* was decreasing during the experiment.

Host-location efficiency of tsetse from these distance might thus be optimal when odour is released at the medium rate. However, I recaptured fewer tsetse than Griffiths *et al.* (1995), even though I released odour at about 1.5 times the rate they did. The release methods, which differed in detail, do not affect the behaviour immediately after release (Chapter 4).

It is possible that the variation in wind direction played a role. The experiment was conducted in the rainy season, when the wind direction is more variable than in the dry season (Groenendijk, unpublished results). Tsetse probably use a 'biassed random walk' (Williams, 1994; Griffiths *et al.*, 1995, Chapter 5) in which the bias is achieved by taking off in upwind direction and turning upwind when odour is detected. The probability that the wind direction indicates the true direction to the source decreases with increasing variability in wind direction. The bias in the random biassed walk then decreases and the probability that the source is found decreases as well.

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7. Flight orientation of tsetse flies (Diptera: Glossinidae) at various distances downwind of an odour source in open and dense vegetation

Abstract

Marked *Glossina pallidipes* Austen (Diptera: Glossinidae) were released from a box at 10, 20 and 30 m downwind of an odour source. Tsetse were recaptured the same day on a 9 m wide wall of electric nets at 4 m upwind of the release box, or at the inside of an incomplete ring of eight nets at 4 m around the box. The incomplete ring was only used when tsetse were released at 10 m downwind of the source. Four odours were tested: natural ox odour; an artificial blend of acetone, octenol and phenols; the same blend with carbon dioxide and carbon dioxide alone. When tsetse were released at 10 m downwind of a source of natural ox odour, they avoided flying upwind. Dense vegetation did not influence their flight direction. Artificial odour did not influence the flight direction of tsetse released at 10 to 20 m downwind of the source in open vegetation, but tsetse avoided entering dense vegetation and preferred flying into crosswind oriented 'game trails'. About 35 % of the recaptured tsetse released at 30 m from the source of the artificial blend with or without carbon dioxide, flew within 20° of the prevailing wind direction. Tsetse released in the absence of odour or in the presence of the other odours flew away at random. It appears that tsetse change from straight fast upwind flight to more sinuous and slower flight when they approach the source.

Introduction

Tsetse flies (Diptera: Glossinidae) probably locate their hosts by a random biassed walk (Brady *et al.*, 1990; Williams, 1994). The bias towards the source is the result of upwind take-off (Bursell, 1987) and changes in flight direction in response to wind direction when odour is encountered (Gibson & Brady, 1988; Brady & Griffiths, 1993). The percentage of tsetse that reacts to wind direction when they detect odour, depends on the distance from the source (Gibson *et al.*, 1991). It also depends on the composition of the odour (Brady & Griffiths, 1993), at least at distances up to 15 m downwind of the source.

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Flight direction of tsetse at greater distances from the odour source has not been studied yet, because of practical problems.

Flight behaviour of tsetse was studied mostly in open vegetation and concentrated on the effects of odour (e.g. Vale, 1977; Gibson & Brady, 1988; Torr, 1988). Most experiments made use of a visual target as well (eg Gibson & Brady, 1988). However, flight behaviour of tsetse is also affected by vegetation and visual stimuli (Vale, 1974b; Torr, 1989). Tsetse follow game trails (Paynter & Brady 1992) and avoid "hedges" (Vale, unpubl.). Furthermore, wind speed and wind direction are influenced by vegetation (Brady *et al.*, 1989) so that vegetation is likely to affect the flight behaviour of tsetse indirectly.

In this chapter I discuss studies on the effect of odour composition and vegetation on the flight behaviour of tsetse. Tsetse were released from a box at 10 to 30 m downwind of the odour source; no visual stimuli were present.

Materials & Methods

Experiments were conducted near Rekomitjie Research Station, Zambezi Valley, Zimbabwe, where *G. pallidipes* is abundant. Tsetse were collected, marked and released in 'mopane' woodland during the dry season as described in Chapter 4. Electric nets (Vale, 1974a) of 1.5 x 1.5 m were used to intercept tsetse after they were released from the box. Release from a box has no great effect on the subsequent behaviour of marked tsetse (Griffiths *et al.*, 1995; Chapter 4). Two different set-ups of eight electrified nets were used: (1) the 'wall' and (2) the 'ring of nets' (Vale, 1977).

The 'wall' experiment

Six electric nets were placed perpendicular to the main wind direction, forming a 'wall' 9 m long. The release box was placed 4 m downwind of the centre of the 'wall'. Two other electric nets were placed 4 m downwind of the release box (Fig. 1). The distribution of the catch over the six upwind nets indicated the accuracy with which tsetse respond to wind direction and the downwind nets gave an indication of the proportion of the tsetse that did not react to the

odour. This experiment was placed in open vegetation; there was no undergrowth or grass and there were a few leafless mopane trees (*Colophospermum mopane* Kirk ex Benth.). Odour puffs could thus travel in relatively straight lines (David *et al.*, 1982, 1983). Odour was dispensed at 10, 20 and 30 m upwind of the release box.

The 'ring' experiment

Eight electric nets covered half the circumference of a circle with a radius of 4 m. The release box was in the centre (Fig. 2A). This experiment was conducted at the same site as the wall experiment, and also in dense riverine vegetation. In the latter, shrubs (*Combretum* spp.) were standing on the outside of the 'ring' about 0.5 m from four of the nets. The bushes were about 2 - 2.5 m high. To the human eye, there were four obvious trails, separated by dense vegetation (Fig. 2B). Odour was dispensed from 10 m upwind of the release box.

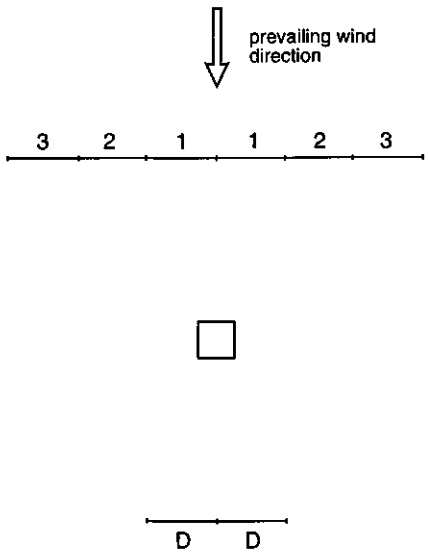

Figure 1: The long wall experiment. Catches from nets 1,2,3 and D(own) were pooled. Square = release box.

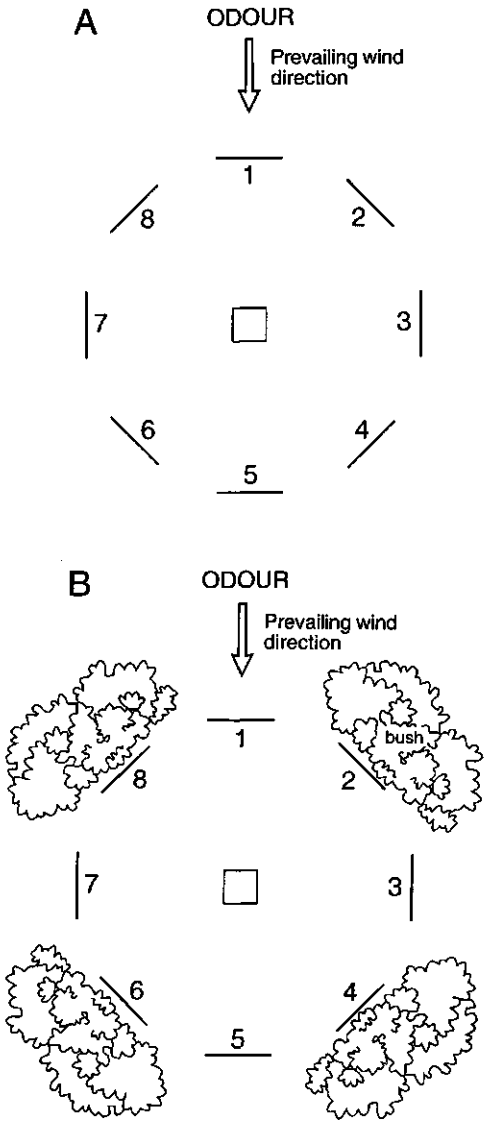

Figure 2: The incomplete ring of nets experiment in open (A) and dense (B) vegetation. Square = release box

Odour

Five odour treatments were tested with both set-ups: (1) natural ox odour (OX), dispensed from the fan powered ventilation shaft of a roofed pit (Vale, 1974b) which contained a live ox of 550 kg; (2) Complete Artificial Odour (CAO), which consisted of carbon dioxide (480 l/h), acetone (0.2 g/h), released from a polythene sachet (Laveissière *et al.*, 1990) and a mixture of 1-octen-3-ol (0.16 mg/h), 4-methylphenol (0.32 mg/h) and 3-n-propylphenol (0.04 mg/h), released from another polythene sachet; (3) Artificial Odour (AO), which contained no carbon dioxide, but was otherwise identical to CAO. (4) carbon dioxide (C) only at 480 l/h; (5) No Odour (NO) was used as a control. The rate at which CAO was dispensed, produces catches that are about double the catches with OX (Vale & Hall, 1985).

Recapture and analysis

The odour dispensers and the electric nets were switched on 10 minutes before the release box was opened. Video data from chapter 4 showed that 95 % of the tsetse had left the box in 20 min, and therefore the experiment was stopped 30 min after the release of the tsetse.

A randomized block design was used, each block constituting of five days. Each treatments was randomly allocated to one day within each block. An arcsine transformation was used to stabilize the variance in the recapture percentages. When more than two means were compared, a Least Significant Difference test was used. Differences in distribution of the recaptures over the nets were assessed with a Chi-square test.

The catches of Nets 8 and 2, Nets 7 and 3 and Nets 6 and 4 of the Ring Experiment were pooled because those pairs of nets caught tsetse that flew at the same angle to the wind. To assess the effect of vegetation, the nets in front of vegetation (2,4,6,8) and those in front of trails (1,3,5,7) were pooled to make two groups.

Results

About 5 % of the tsetse did not leave the release box, irrespective of the treatment. These tsetse were ignored in the analysis. Males and females showed no differences in behaviour (χ^2, P>0.1, df= 4) and the sexes were therefore pooled for the analysis.

The 'Wall' experiment

When AO or CAO was dispensed, a significantly higher percentage of the tsetse released at 10 m and 30 m downwind of the source were recaptured on all nets than when other odours were dispensed (Table 1). When tsetse were released at 20 m, there was a similar tendency, but the difference was not significant. When C or OX were used, the recapture percentage was similar to that of No Odour. For each odour, the distance from the odour source did not affect the percentage of tsetse recaptured.

Table 1: Mean untransformed percentages of tsetse recaptured on all nets of the long-wall-experiment. Means in the same column followed by different letters differ significantly from each other.

Odour	N	Release distance		
		10	20	30
NO	10	11.4^{b}	11.4^{ab}	11.4^{bc}
CAO	10	18.4^{c}	21.0^{b}	18.6^{bc}
AO	10	18.8^{c}	16.0^{ab}	20.3^{c}
C	8	9.3^{a}	11.5^{a}	10.9^{ab}
OX	8	11.7^{ab}	11.3^{a}	10.1^{a}
P		<0.001	0.07	<0.05

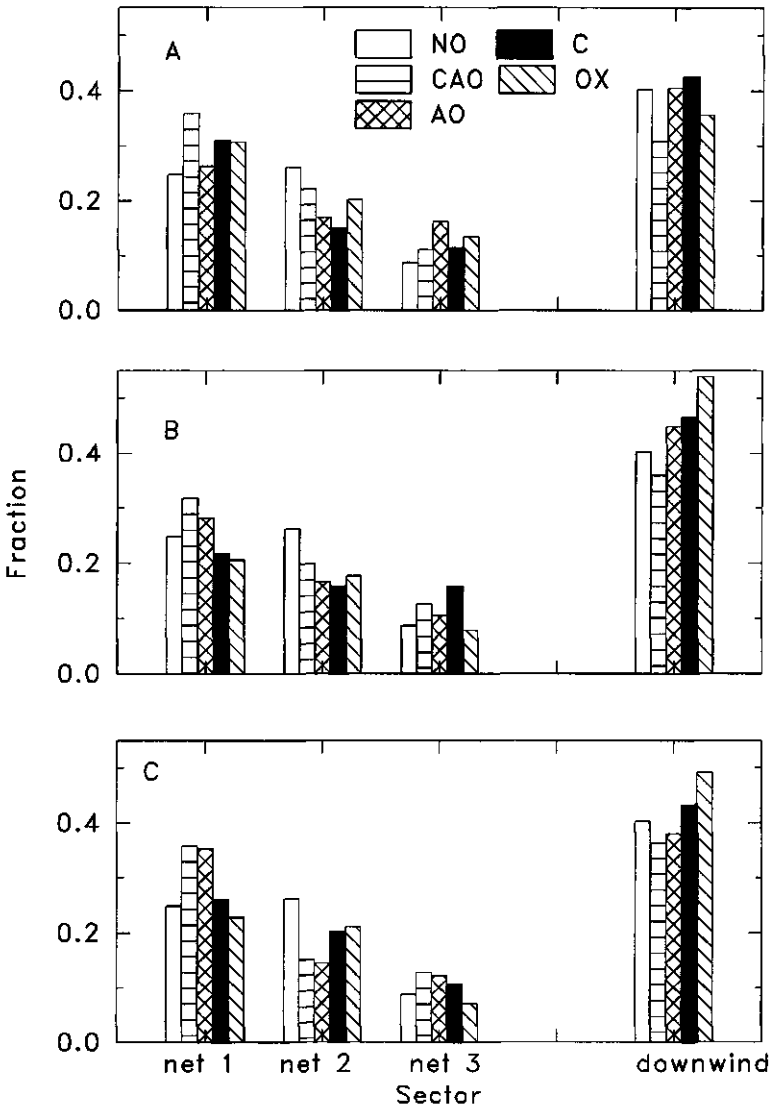

Figure 3: Distribution of recaptures over the nets of the long wall experiment when odour is released at 10 (A), 20 (B) and 30 m (C) upwind of the release site.

When tsetse were released at 10 or 20 m downwind of the odour source, there were no differences between treatments in distribution of the recaptures over the upwind nets (Fig. 3A-C). For releases at 10 m, CAO decreased the catch at the downwind nets, as compared to AO (χ^2, $P<0.025$, df=3). CAO caused a significant shift to the upwind nets in recaptures of tsetse released at 20 m, as compared to OX (χ^2, $P<0.025$). When tsetse were released at 30 m downwind of the source, C, OX and NO caused a similar distribution of recaptures over both the upwind and downwind nets. AO and CAO caused a significant concentration of the upwind catch at the central nets, compared to NO (χ^2, $P<0.05$, df=3) and OX (χ^2, $P<0.025$, df=3). Within each odour treatment, the distribution of the recaptured tsetse over the nets did not differ significantly with release distance.

The 'Ring' experiment

In 'open' vegetation, the recapture percentage of tsetse at all nets did not vary with treatment (Table 2). In dense vegetation, 13.9 % of the marked tsetse were

Table 2: Mean untransformed percentage (%) and mean number (n) of tsetse recaptured on all nets of the incomplete ring experiment. Means in the same column followed by different letters differ significantly from each other ($P<0.05$; LSD-test).

Odour	N	Open vegetation		Dense vegetation	
		%	n	%	n
NO	5	13.7^{ab}	18.2	5.8^{a}	6.2
CAO	5	13.5^{ab}	16.8	10.5^{ab}	11.2
AO	5	12.8^{ab}	10.4	6.0^{a}	6.4
C	5	16.2^{a}	19.4	4.5^{a}	5.1
OX	15	8.7^{b}	10.3	13.9^{b}	17.1

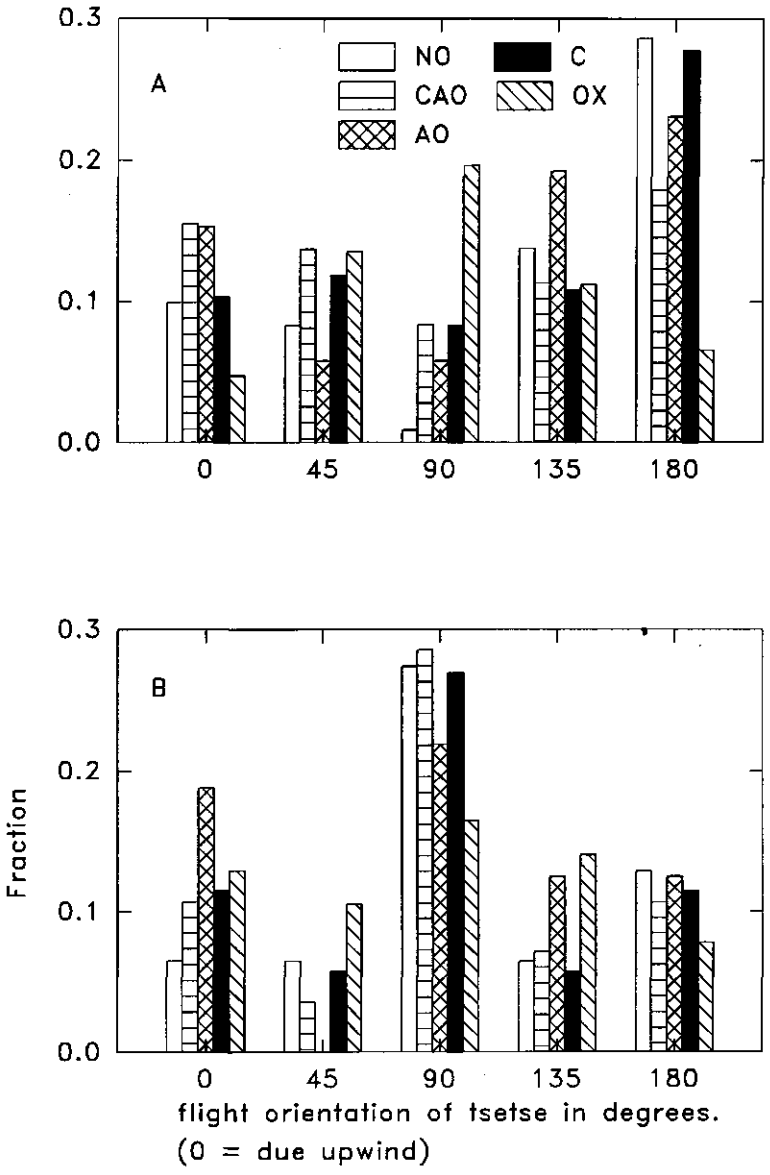

Figure 4: Distribution of recaptures over the nets of the incomplete ring of net experiment in open vegetation (A) and dense vegetation (B).

recaptured when OX was dispensed, more than the 6.7 % when other odours were used (LSD-test, $P<0.05$) (Table 2). When OX was dispensed, a larger percentage of the released tsetse flew crosswind as compared to the other treatments (χ^2, $P<0.001$, df=4), which might explain why few tsetse were recaptured at the upwind nets of the 'wall' when OX was dispensed (Table 1). There was no difference in the distribution of catches over the nets when NO, CAO, AO or C were dispensed (Fig. 4A).

Vegetation did not affect the distribution of recaptures when OX was dispensed. In the presence of other odours and in the absence of odour, more tsetse flew crosswind in dense vegetation than in open woodland (χ^2, $P<0.001$, df=4) (Fig. 4). The vegetation caused a shift in the distribution of the catch towards the nets placed on a 'game trail' when CAO, AO or C was dispensed (χ^2, $P<0.05$, df=1).

Discussion

Other work has shown that several factors affect the distribution of the catch of electric nets. First, tsetse react instantaneously to wind direction, but with a large variety of turn sizes (Gibson & Brady, 1988). As a result, about 50 % of the tsetse fly within 35° of due upwind (Torr, 1988). The place where a tsetse first hits the electric net is therefore the result of variation in turn size, the place where the odour was detected, the wind direction and possibly the odour composition. Second, the electric nets sampled tsetse during 30 minutes during which the wind changes direction frequently (Brady *et al.*, 1989). Third, electric nets kill about 50% of the tsetse the first time they hit the net (Packer & Brady, 1990; Griffiths & Brady, 1994).

An upwind bias in flight directions was found for tsetse released at 30 m when CAO or AO was used. There was no evidence that other odours caused this behaviour, perhaps because they did not produce enough detectable odour pockets to cause a significant concentration of recaptures. When tsetse were released at 10 or 20 m from the source, there was no upwind bias in flight direction strong enough to be discerned from the background

noise (see above). In the presence of ox odour, tsetse released at 10 m downwind of the source, seemed to avoid upwind flight and orientated across the wind direction. I therefore conclude that tsetse react instantaneously to wind direction when they encounter odour at 26-30 m downwind of the source.

High release rates of odour increase the sinuosity of tsetse flight in a wind tunnel (Warnes, 1990; Paynter & Brady, 1993), and in the field (Torr, 1990, his Table 2). Extending the range of components used to attract tsetse also seems to increase the variation in flight direction (Brady & Griffiths, 1993). The avoidance of upwind flight near a source has been observed previously (Gibson *et al.* 1991). In addition, tsetse also reduce their flight speed with increasing odour dose (Warnes, 1990; Gibson *et al.*, 1991; Paynter & Brady, 1993).

Tsetse could use several odour plume characteristics to change from fast, straight flight to slower, more sinuous flight. Mean flux, dose and peak value of flux in each burst all decrease with distance from source, but at different rates (Murlis *et al.*, 1992). However, tsetse may encounter odour of sources that vary from a small solitary warthog to a herd of buffaloes. Responding to the fastest declining plume characteristic, flux or dose of the odour (in the sense of Murlis *et al.*, 1992) in each 'burst', offers the best chance of locating a source without wasting time and energy in slow, sinuous flight far from the source. Studies of tsetse responses to large doses of odour suggested that the flux or the dose of odour is important (Hargrove *et al.*, 1995). The perceived flux or dose increases with release rate and perhaps also with the number of kairomones present in the odour. It seems that the absence of an odour component in the mixture can only partly be compensated for by a higher release rate of the components that are present in the mixture.

Tsetse attracted by odour only show an imprecise orientation to the source (Vale, 1974b). We did not use a visual target in this experiment. Tsetse respond to visual stimuli near an odour source (Torr, 1989; Gibson *et al.*, 1991). The flight behaviour of tsetse near the source might reflect a search for visual cues which should result in a greater probability that a hidden and camouflaged host is found. The results from the experiment in dense

vegetation are in agreement with this hypothesis. When artificial host odour was present, tsetse followed game trails, but avoided upwind flight. This is similar to earlier findings (Paynter & Brady, 1992). When ox odour, which contains at least one more attractive component (Torr *et al.*, 1995) was used, tsetse did not avoid dense vegetation. but still avoided upwind flight. This may reflect an even stronger tendency to search for a visual cue, rather than an odourous one.

The percentage of box-released tsetse recaptured at the 'long wall' in upwind flight did not depend on the distance from source or the odour composition, but the percentage recaptured at the source declines with increasing distance of release (Griffiths *et al.*, 1995; Chapter 5). Thus, near the source even the tsetse that do not orientate accurately to the wind direction, somehow find it. This is partly due to the fact that these tsetse have a higher probability of encountering an odour plume when they are released close to the source and partly because at close range tsetse probably increase the sinuosity of their flight and decrease their flight speed, and thus increase their chance to detect another odour pocket. Tsetse also turn when they do not encounter odour for some time, which would bring them closer to the source again.

When compared with flight and search behaviour of other insects, the searching behaviour of tsetse appears to be similar to that of male moths, but on a larger scale. Both insects react instantaneously to an odour pocket by turning upwind (Gibson & Brady, 1988; Mafra-Neto & Cardé, 1995) and both restrict their area of search by slowing down and changing their orientation to the wind (Willis *et al.*, 1991; Warnes, 1990).

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8. The physiological state of tsetse flies (Diptera: Glossinidae) caught with odour-baited targets: a mark-release-recapture study

Abstract

In the field in Zimbabwe, *G. pallidipes* were marked and released from refuges and boxes placed at 10, 20 and 30 m downwind of an odour baited visual target. The physiological state of all tsetse recaptured on the same day, samples of the populations from which the marked tsetse originated and a sample of unmarked tsetse caught together with the marked tsetse, was assessed by analysis of their fat and haematin content. Before fat and haematin analysis, the females were dissected to assess their ovarian age and pregnancy stage. Unmarked tsetse caught with a target had on average fed 2.5 days previously. Among the females from the refuge, the variance in haematin decreased with progressing pregnancy and the females in the last stage of the pregnancy all had a high haematin content, indicating that most had fed less than 24 h previously. Tsetse recaptured from the refuge had a higher haematin content than unmarked tsetse. Refuge leaving females that were recaptured, were younger than unmarked females. The fat and haematin content were not related to the distance from which females were recaptured with the target. The females caught at a target baited with acetone, 1-octen-3-ol and phenols contained less fat than females caught at a target baited with the same odour plus CO_2. The fat content of recaptured males leaving from a refuge, increased with distance of release. There was no such correlation with box-released males. It is concluded that resting tsetse with high energy reserves will feed from a host that comes close by and that the threshold of response to host cues decreases with decreasing energy reserves.

Introduction

Tsetse flies (Diptera: Glossinidae) feed only on the blood of vertebrates. The blood meal is largely converted to fat, which serves as the energy reserve for all metabolic processes (Bursell & Taylor, 1980). The fat is used for reproduction and as an energy reserve until the next blood meal is obtained. Males use their fat reserves for mate and host location. Females produce a larva every nine days that weighs about as much as the mother. The time that

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has passed since a tsetse fly obtained its last blood meal can be determined by measuring the amount of fat and haematin, a blood meal residue, present in the fly (e.g. Ford *et al.*, 1972; Randolph & Rogers, 1978).

The behaviour of tsetse flies changes during the digestion of a blood meal and the depletion of the fat reserves derived from the blood meal (e.g. Brady, 1975; Randolph *et al.*, 1991; Hargrove & Packer, 1993). The antennal olfactory sensitivity increases with time since feeding (Den Otter *et al.*, 1991), as does the spontaneous activity of *Glossina morsitans* Westw. in the laboratory (Brady, 1972a, 1972b, 1975). If this also occurs in the field, then the probability that a tsetse encounters a host cue in the field increases. The probing responses also increases with time since feeding (Brady, 1972a, 1972b, 1975) and thus the chance that a tsetse takes a blood meal from a located host increases as well. The feeding interval is probably flexible (Hargrove & Packer, 1993) and can be influenced by the density of host animals or their cues (Langley & Wall, 1990).

Tsetse use odour cues in long range host location (Vale, 1974b). Odour moves through the air as a series of odour pockets (Murlis & Jones, 1981). The number of detectable odour pockets in a plume depends on the release rate and possibly on the odour composition (Murlis & Jones, 1981; Chapter 5 & 6). An odour baited catching device that produces relatively few detectable odour pockets should thus mainly attract tsetse that have fed long ago, whereas a device that produces many detectable odour pockets should also catch tsetse that have fed more recently.

The nutritional status of the captured tsetse may also be influenced by the catching device itself. Male *G. pallidipes* Austen that approach a trap, but do not enter it, contained more fat than the males that entered the trap; but there was no consistent difference in fat content between entering and non-entering females (Langley & Wall, 1990). Females caught with an electric net in front of a visual target contain more fat than females caught with a trap (Griffiths *et al.*, 1995). However, the fat content of a female tsetse is closely correlated with her pregnancy cycle (Randolph *et al.*, 1991) and catches of F3-traps (Hargrove & Langley, 1990) are biased towards females in the early stage of pregnancy (Chapter 3). Whether target catches are biased towards a certain pregnancy stage is unknown.

In this chapter I present a study of the physiological state of tsetse caught with a target baited with different odours. Marked tsetse were released at increasing distances from the source and the physiological state of the recaptured tsetse was determined.

Materials & Methods

Experiments were conducted from October 1993 to October 1994 near Rekomitjie Research Station, Zambezi Valley, Zimbabwe. Study sites were at the edge of the riverine vegetation (described by Vale, 1971) and in 'mopane' woodland (described by Vale, 1974a). *G. pallidipes* were released from a release box or a refuge (Chapter 4 & 5). Samples from tsetse subjected to different treatments were dissected and their fat and haematin content was measured.

The 'Refuge Leaving' Experiment

During the hot seasons, September to October, of 1993 and 1994, tsetse were collected from artificial refuges (Vale, 1971) from 13.00 to 13.30 h. After collection, the tsetse were kept in cages in a cool box with a damp cloth until they were marked. The mark consisted of two small spots of artist's oil paint which indicated the day and the distance 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 placed in a release refuge. These refuges were placed at 10, 20 and 30 m downwind of an odour source. Tsetse that had not settled 15 minutes after the last marked tsetse was put inside, were removed. These tsetse will henceforward be termed 'restless'. After that, the refuge was opened without disturbing the tsetse resting inside (For details see Chapter 5).

An electrified standard target (Vale, 1993) was placed at the source of the odour. The target consisted of a 1 x 1 m black cloth with a 1 x 0.5 m sheet of fine netting on each flank. The visual target was used because tsetse attracted by odour only, show an imprecise orientation to the odour source (Vale, 1974b). Cloth and netting were covered by an electrified grid of fine copper

wires; flies contacting the grid were killed or stunned and fell on a tray of corrugated iron. The tray was not coated with glue, because tsetse covered in glue cannot be analyzed for fat and haematin content. Consequently, tsetse that recovered from their electrocution, could escape. The experiment started around 15.00 h and lasted until sunset.

The 'Box Release' Experiment

Tsetse were caught with epsilon traps (Hargrove & Langley, 1990) from 10.00 to 15.00 h. The traps were baited with acetone (0.5 g/h), released from a small bottle, 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). A non-return cage of netting was fitted on the trap. The captured tsetse were then handled like the tsetse used in the 'Refuge Leaving' experiment.

The marked tsetse were released from a box (Chapter 4). Flies were released at distances of 10, 20 and 30 m downwind of the odour source. The flies were released 10 minutes after the odour dispensers and the electric target were switched on. There were not always enough flies to fill all release tubes, the number released per box varied from 35 to 125. The experiment was conducted in May and August 1994. The experiment was conducted during the last two hours preceding sunset (approx. 18.00h).

Odour

Two odours were tested: (1) Complete Artificial Odour (CAO), which consisted of carbon dioxide (480 l/h), released from a pressurized cylinder, and 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; (2) Artificial Odour, (AO), which contained no carbon dioxide, but was otherwise identical to CAO. The dose rate of CAO used here results in catches that are about twice as large as catches with the odour of one ox (Vale & Hall, 1985).

Analysis of physiological state

Tsetse taken from the refuge and those that would not settle in the release refuge were killed within two hours of collection. Tsetse caught at the target

obviously were dead when collected at the end of the experiment. I analyzed the reproductive and nutritional state of (1) all recaptured tsetse; (2) all restless tsetse; (3) samples of the tsetse caught with a trap or from a refuge, and (4) a sample of unmarked tsetse caught with the target.

The legs and wings of each fly were excised and discarded. Females were dissected to determine the ovarian age category (Challier, 1965) and the pregnancy stage (Hargrove, 1994). Males with a residual dry weight of less than 7 mg were considered to be immature (Hargrove & Packer, 1993). The residual dry weight and the fat content of the female and the offspring it carried, were determined as described by Potts (Mulligan, 1970).

After removal of the fat, the haematin content was measured as described by Ford *et al.* (1972). The optical density was read at 417 or 558 nm, depending on the density of the solution. It is assumed that both sexes lose 1 log unit of haematin per 30 h (see Langley, 1966; Randolph *et al.*, 1991).

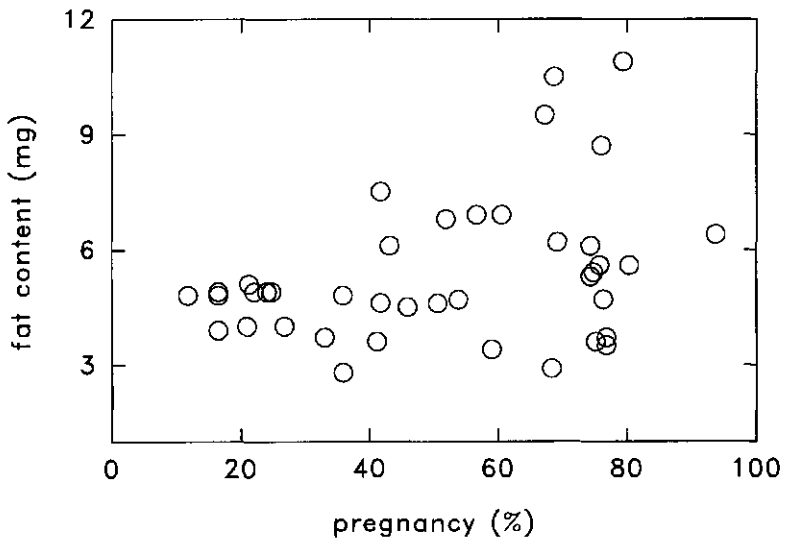

Figure 1: The fat content of recaptured female *G. pallidipes* that came from the refuge 10 m downwind of the odour source.

The fat content of the female plus offspring increases with the progression of the pregnancy (Rogers & Randolph, 1978). A preliminary analysis showed that the variance in the data increased with fat content, a representative example is given in Figure 1. Therefore, I performed a linear regression of the log of the fat content on the progression of the pregnancy and compared the elevation and slope of the regression correlations of each sample. A difference in elevation indicated a difference in fat content during the whole pregnancy cycle between the samples. A difference in slope indicated that females of the sample with the steeper slope were building up fat reserves more quickly during their pregnancy.

Results

The 'Refuge Leaving' experiment

Females. The highest haematin content of a female from the refuge was 190 * 10^3 ng (log value 5.28024). Assuming that the fly had entered the refuge between nine and ten in the morning, when the temperature rose above 32°, it had fed at least 4 hours before dissection. The fly lost about 0.133 log units of haematin during that time (see Materials & Methods) and its haematin content at feeding would have been 259 * 10^3 ng (log value (5.2802+0.133)). This corresponds with a blood meal of about 70 mg (Hargrove & Packer, 1993), which is similar to the estimate of 76 mg for the average blood meal size of female *G. pallidipes* (Taylor, 1976). The latter value was used as indication for the average blood meal size in estimations of time since feeding of (re)captured females.

The haematin content of females from the refuge and restless females or those that were recaptured were similar (Fig. 2). Wild-caught females contained less haematin than recaptured females or females taken from a refuge (χ^2, $P<0.005$, df=5). Among the refuge sample, the variance in haematin content decreased with progressing pregnancy (Fig. 3). This decrease in variance was due to the absence of low values in the later stages of pregnancy.

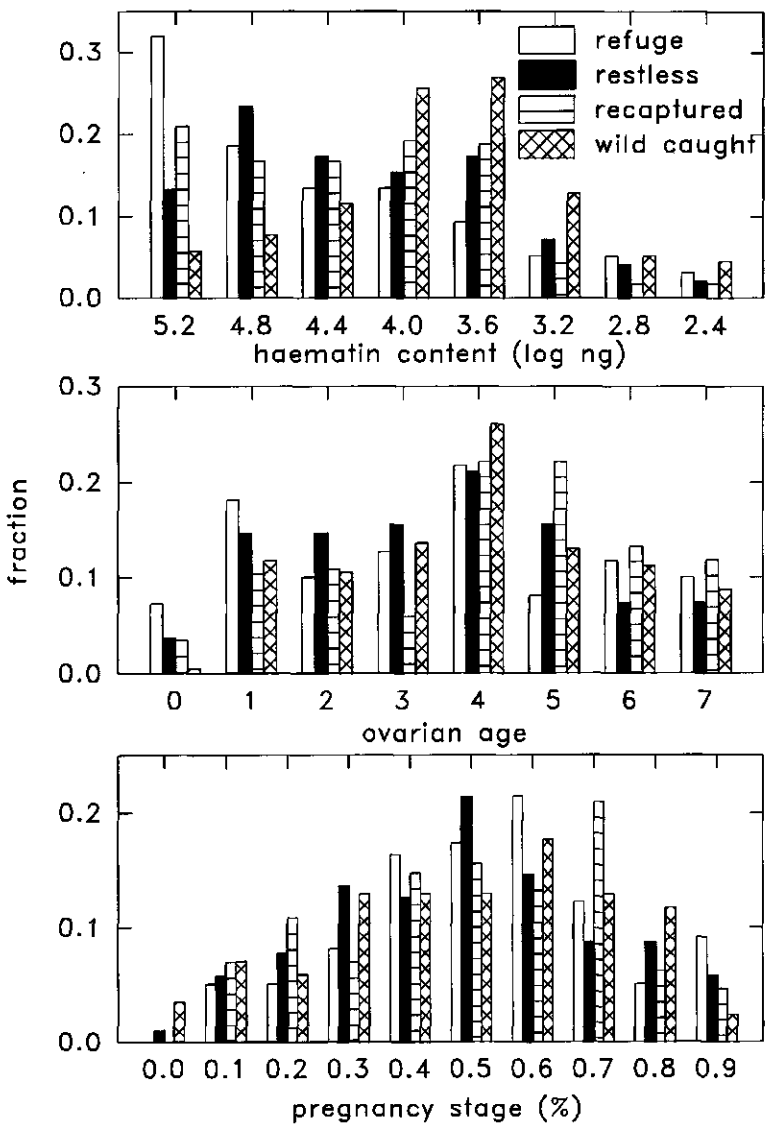

Figure 2: Haematin content, ovarian age and pregnancy stage of female *G. pallidipes* from the refuge leaving experiment.

The tsetse (re)captured at targets baited with different odours had the same haematin content.

The samples had a similar distribution over the pregnancy stages (Fig. 2) and there was no correlation between pregnancy and haematin content.

The females from the refuge were younger than the females recaptured at the target (χ^2, $P<0.05$, df=6). The unmarked tsetse caught at the target were of the same age as the ones from the refuge.

Females recaptured from 10 m at a target baited with CAO contained more fat (4.1 mg; detransformed intercept) than females from the refuge and restless females (2.4 mg; detransformed intercept) (Table 1). Restless females and those taken from the refuge had a similar fat content. Unmarked females caught at a CAO-baited target were building up fat more slowly during their pregnancy than the females from a refuge and the restless females. Unmarked females caught at an AO-baited target had a lower fat content than females from the refuge or females that would not settle.

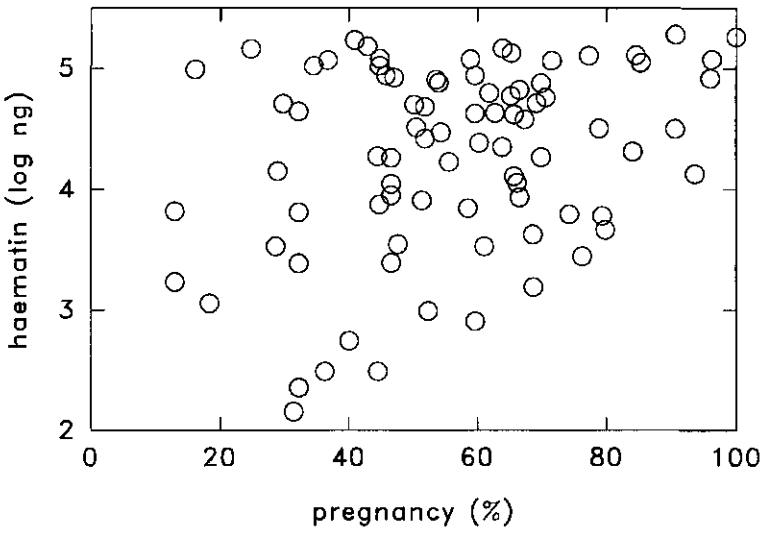

Figure 3: Haematin content against the pregnancy stage of female *G. pallidipes* taken from a refuge.

Table 1: Slopes and intercepts of regressions of log (fat) on the progression of the pregnancy (%) of refuge leaving female *G. pallidipes*. F-test; P<0.025 in all cases.

catch method	elevation	slope	r^2
refuge	0.384[a]	0.0061[a]	0.35
restless	0.423[a]	0.0054[a]	0.35
CAO 10 m	0.611[b]	0.0019[b]	0.10
CAO unmarked	0.589[a]	0.0020[b]	0.03
AO unmarked	0.324[c]	0.0058[a]	0.42

The regressions of fat on pregnancy of females explained more of the variation in females caught at the AO-baited target than in females caught at the CAO-baited target.

Males. The highest haematin content of a male captured in the refuge was 146 * 10^3 ng (log value 5.28024). With the same assumptions as for females, this gave an estimated blood meal of 54 mg, the same as the average blood meal size for male *G. pallidipes* found by Taylor (1976). Again, I used Taylors (1976) value for estimations of time since feeding for (re)captured males.

The haematin content of males recaptured from various distances was the same. Males from a refuge contained more haematin than restless males and males that were (re)captured at a target (χ^2, P<0.025, df=5) (Fig. 4). The majority of the males caught at a target baited with CAO had fed 2 to 2.5 days previously, significantly more recently than males caught at a target baited with AO, the majority of which had fed 2.5 to 3 days earlier (χ^2, P<0.05, df=5). This result was almost entirely due to the absence of males that had fed the previous day. Males recaptured from 10 m distance contained 2.52 $\pm$ 0.647 mg fat (average $\pm$ 95 % confidence interval), significantly less than males recaptured from 30 m, that contained 3.52 $\pm$ 0.600 mg. The fat content of males caught from 20 m, 2.75 $\pm$ 0.641, and of unmarked males, 2.85 $\pm$ 0.552 mg, were not different from that of the other samples.

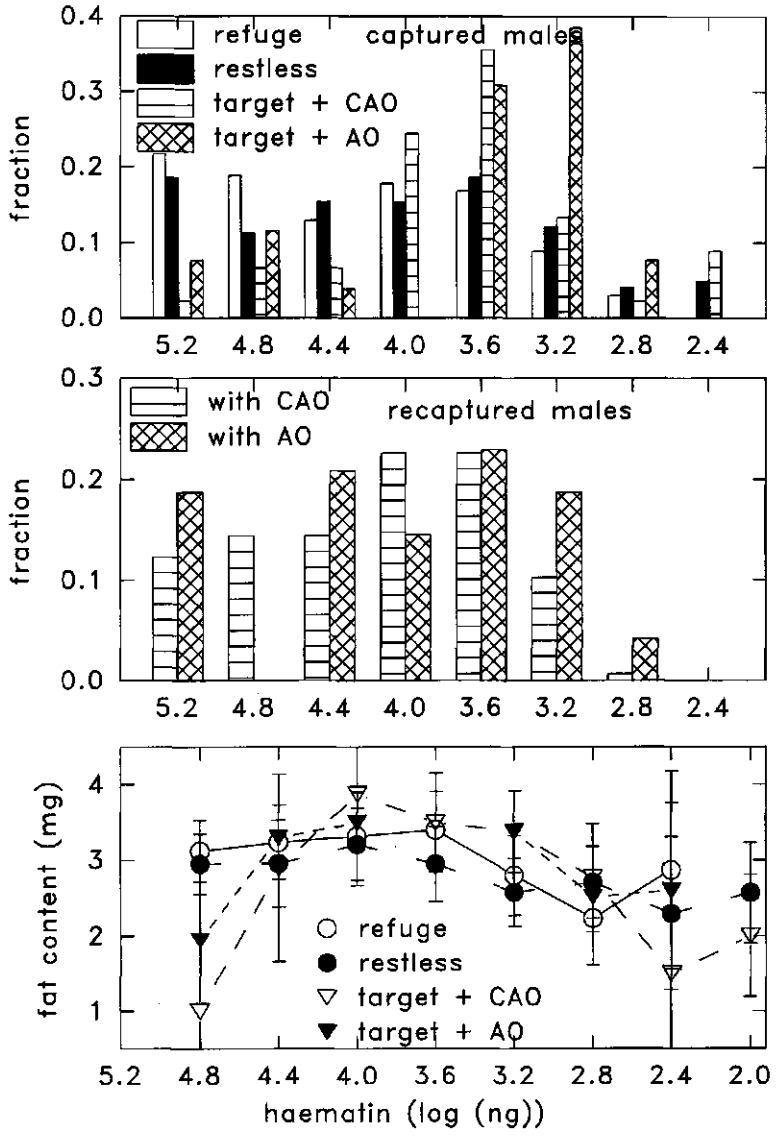

Figure 4: Haematin and fat content of male *G. pallidipes* from the refuge leaving experiment.

The 'Box Release' Experiment

Females. Unmarked females caught at the target baited with CAO had a larger haematin content, i.e. had fed more recently, than trap-caught females (χ^2, $P<0.005$, df=4). The difference in haematin content between the trap catch and the unmarked catch at a target baited with AO (χ^2, $P<0.01$, df=4), was almost completely due to the larger fraction of target-caught tsetse that had fed less than one day earlier. The composition of the odour had no effect on the haematin content of recaptured females and the recaptures were pooled. Among the recaptured females, those that had fed approximately 60 hours earlier were over-represented compared to the trap catch (χ^2, $P<0.005$, df=4). Females that had fed earlier or later, were under-represented (Fig. 5). Unmarked females caught at the CAO-baited target contained more haematin (χ^2, $P<0.005$, df=4) than unmarked females caught at the AO-baited target.

When CAO was used as bait with the target, the females recaptured from 30 m were in an earlier stage of the pregnancy than those recaptured from shorter distances (χ^2, $P<0.05$, df=4): among the females recaptured from 10 m, there were three peaks: in the early stage (10 % of the pregnancy), halfway and at around 70 % of the pregnancy. Among females recaptured from 20 m, the early peak was small and the last peak more pronounced. Among females recaptured from 30 m, the early peak was more pronounced, while the last peak was absent (Fig. 5). Females caught in the trap, at the AO-baited and at the CAO-baited target, had a similar distribution over the pregnancy stage (Fig. 5). Females recaptured at the AO-baited target were also similar to the aforementioned groups of females and the distance of release made no difference. The distributions of recaptured females over the pregnancy stage were the same as those of the unmarked females caught at the target. The haematin content was not correlated with the pregnancy.

The distribution of females from these samples over the ovarian age categories were the same (Fig 5).

Females recaptured from 30 m had less fat than those captured with a trap. The fat content of recaptured females was similar to that of unmarked females when the target was baited with AO. Unmarked females caught at a target baited with CAO contained more fat than recaptured females or

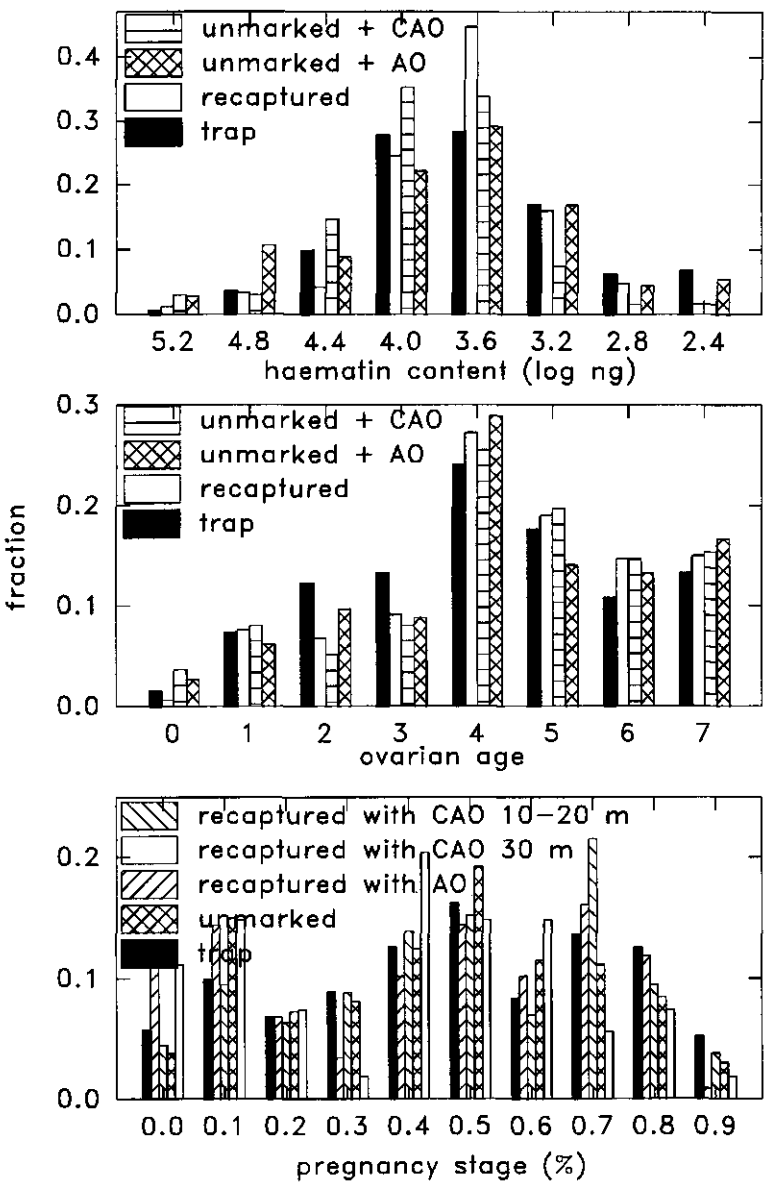

Figure 5: Haematin content, ovarian age and pregnancy stage of female *G. pallidipes* from the box release experiment.

unmarked females captured with a target baited with AO (Table 2). The regression explained more of the variation in fat content of females caught at the AO-baited target than of the variation in fat content in females caught at the CAO-baited target. There was no correlation between haematin content and pregnancy stage.

Males. The haematin content of males caught with different methods was the same. Males that had fed two previously and were caught in a trap, contained more fat than similar males caught with a target, irrespective of the odour (Fig. 6).

Table 2: Slopes and intercepts of regressions of log (fat) on the progression of the pregnancy (%) of box-released female *G. pallidipes*. F-test; $P<0.05$ in all cases.

catch method	elevation	slope	r^2
trap	0.424^a	0.0049^a	0.26
CAO 30 m	0.347^b	0.0051^a	0.10
AO 30 m	0.222^a	0.0082^b	0.47
CAO unmarked	0.476^b	0.0050^a	0.05
AO unmarked	0.403^a	0.0050^a	0.23

Discussion

From these data it appeared that *G. pallidipes* caught at a target, fed with intervals of 48 to 60 hours. This interval is similar to the earlier estimate of the feeding interval of tsetse in this area (Hargrove & Packer, 1993; Hargrove & Williams, 1995), but is shorter than that found in Kenya (Randolph *et al.*, 1991). The samples of trap- and target-caught females did not differ enough to

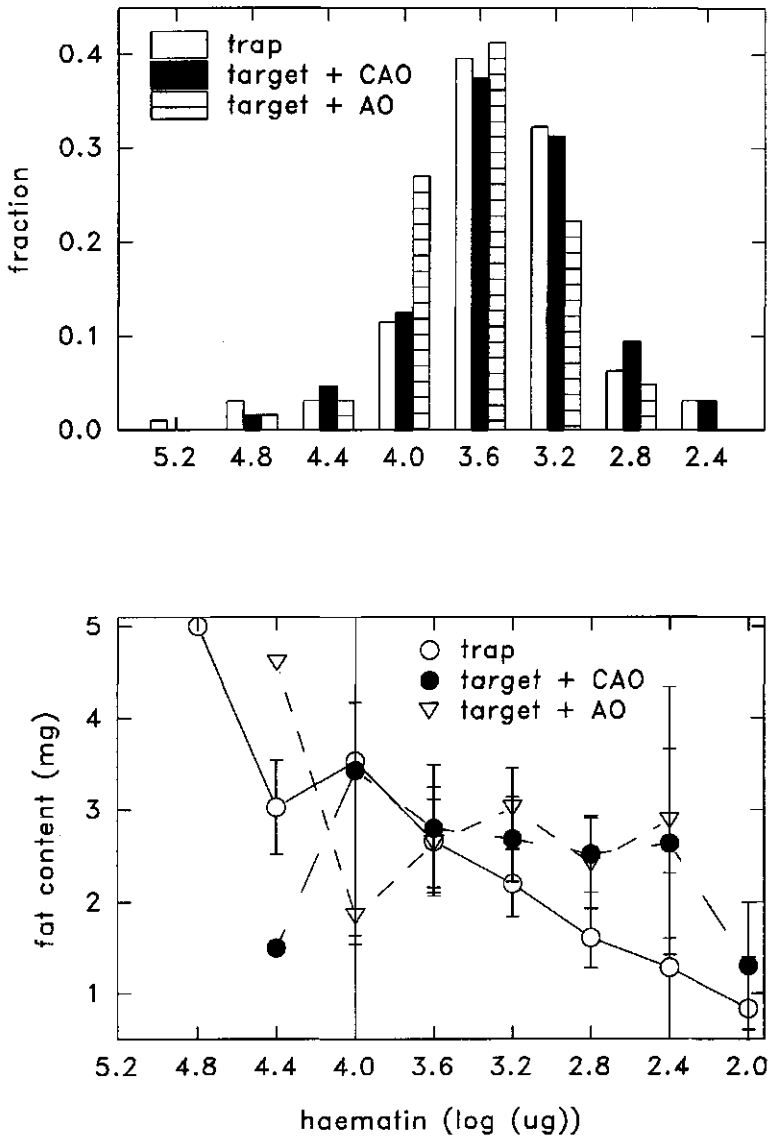

Figure 6: Haematin and fat content of male *G. pallidipes* from the box release experiment.

assume a difference in feeding interval of twelve hours. The difference between my estimate and that of Randolph *et al.* (1991) can therefore not be attributed to the difference in sampling methods. Perhaps the two populations have different feeding strategies.

The variance in haematin content decreased with progressing pregnancy among females from the refuge sample. It can be assumed that the refuge sample is not biassed towards females with a high haematin content (Hargrove & Packer, 1993). The low values of haematin content gradually disappeared from about the time that the larva hatched, at around 40 % of the pregnancy (Fig 3). Apparently, females that carry an egg, have the possibility to vary the feeding interval to a larger extend than females that carry a larva. When the larva is growing fast, females need to take blood meals at short intervals.

The females that had completed 90 % of their pregnancy all had a high haematin content. Judging from the haematin content, four out of five of these females took a blood meal of average size just before they entered the refuge. It is almost impossible that these females took a blood meal in the late afternoon of the previous day, because three of those females would than have taken a blood meal of more than 200 mg, i.e. more than 1.5 times the previously recorded maximum (Taylor, 1978).

This is different from laboratory studies, in which females often did not feed during the last two days of their pregnancy (Boyle, 1971; Denlinger & Ma, 1974, Langley & Pimley, 1974). It does, however, agree with field studies of Taylor (1976), who found that 40 % of the largest blood meals were taken by females carrying a third instar larva. The females will also need this blood meal, if 4 mg of larval dry weight must be produced during the last day of the pregnancy (Randolph *et al.*, 1991).

Females in the late stage of pregnancy do take blood meals, but these females were not captured by traps and targets. The catches of traps and targets were thus biassed against females in the last 10-20 % of pregnancy, compared to refuge catches. A bias in trap catches towards females in the early stage of pregnancy was already shown earlier (Van Sickle & Phelps, 1988; Chapter 3). The cues of targets and especially traps, although attractive, might indicate a high-risk situation to tsetse (Langley & Wall, 1990). If so, it appears

that females in the early stage of pregnancy tend to extend their non-feeding period and 'accept' high risks when feeding (Hargrove & Williams, 1995). Females in the later stages of pregnancy feed more frequently, but nevertheless avoid high-risk situations. The females in the later stages of pregnancy would then probably have to use active search more frequently than females in the earlier stage of pregnancy. The results of chapter 3 do suggest that that is the case.

Refuge-leaving females recaptured from 10 m with CAO contained more fat than females recaptured from further away, or unmarked females caught with a target baited with CAO or AO. All refuge-leaving tsetse contained more haematin than wild caught tsetse. This was in accord with earlier suggestions that tsetse with large fat and haematin reserves will only feed from a host that comes close to the resting site (Hargrove & Packer, 1993; Chapter 3). The data also suggests that females with large fat reserves only react if the odour contains carbon dioxide, which is known to activate tsetse (Bursell, 1984, 1987). Females recaptured from a refuge were also younger than unmarked females captured at the same time. This agrees with earlier findings that young females are less likely to use active search as a host-location mechanism than older females (Hargrove, 1991; Chapter 3).

Females in the last stages of their pregnancy (Randolph *et al.*, 1991) or with a large residual blood meal (Fig. 2) have a smaller probability of being caught in a trap. When using trap-caught females for box releases, the sample is thus slightly biased, compared to the population of tsetse that is 'ranging' (Vale, 1980). Something similar happens with the recaptures. Among the recaptured tsetse, females which had fed 2.5 days before recapture were over-represented, compared to the sample of females that were released. Studies that use same-day recaptures of female *G. pallidipes* (Griffiths *et al.*, 1995; Chapter 5 & 6), are thus referring to a small, well defined part of the population.

Females caught at the target baited with CAO contained more fat and had a larger variation in fat reserves, than those caught at the target baited with AO. This supports the suggestion that the response threshold decreases with fat content. However, it is not clear from this experiment whether the

females with larger fat and haematin content did not respond to the odour at long distance, or located the source but did not get close enough to be captured.

The males caught at the target baited with CAO had fed more recently than those caught at the target baited with AO. This suggests that males that are looking for a blood meal, avoid the target with fewer host cues (the absence of CO_2) if they have fed recently. With males the fat content increased with increasing distance. Perhaps these well fed males were searching for mates rather than for a blood meal. It is unknown where *G. pallidipes* mates, but a host seems an obvious meeting point (Bursell, 1961).

In conclusion, haematin, or the time since feeding, appeared to be the most important factor in the regulation of host seeking behaviour of male and female *G. pallidipes*. The probability that a female was captured with a target or a trap decreased with increasing fat content or pregnancy stage. These factors apparently influence the risk associated with feeding that tsetse 'find acceptable'. Females in the last stage of pregnancy or with a high fat content, did respond to a target placed close to their resting site. Females that are young were less likely to use active search as a host-location strategy, and were under represented in the catches of stationary baits.

Acknowledgements

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9. General Discussion

Foraging behaviour and host location behaviour of tsetse are influenced by several factors. In this thesis, the effect of olfactory cues and physiological state have been studied. More specifically, it was not yet known how kairomones affected the distance from which tsetse can find a host and the host-location efficiency. The effects of fat content, haematin content and pregnancy on foraging and host-location behaviour were also unknown.

A detailed discussion of the results has been given in each chapter. Here I present and discuss an overview of the results from this research.

Foraging strategies

The tsetse flies *G. pallidipes* and *G. m. morsitans* are mainly active in the early morning and in the late afternoon (e.g Pilson & Pilson, 1967; Dean *et al.*, 1969; Van Etten, 1982; Hargrove & Brady, 1992; Chapter 1). The rhythm found with an unbaited net - the net which did not attract tsetse - was supposed to give the best representation of the daily rhythm in tsetse activity. But the activity rhythms found with odour-baited targets were similar to those with the unbaited net (Chapter 1). The catch of *G. pallidipes* at the mobile ox also showed a sharp peak in the late afternoon. However, a mobile bait mainly attracts the tsetse that are sitting on the vegetation along the path of that bait (Vale, 1974). It thus seems that *G. pallidipes* only responds to it during its activity period and may at other times not react to its main target host, even when the host is in view.

Tsetse are active during the time of the day that the atmospheric boundary thermal stratification is neutral to stable (Meixner, 1995). This means that there is little exchange between layers of air with different temperature, a situation that occurs when the lower air layers are colder than the higher ones. In this situation odour remains in the layer where it is produced during dispersion and is not diluted quickly. A neutral or stable thermal stratification is thus an optimal environment for host location by means of odour. Under these circumstances, carbon dioxide exhaled by 2 oxen can be distinguished

from the background fluctuations at 32 to 64 m downwind from the source in the dry season (Meixner, 1995). In the rainy season the background fluctuations in carbon dioxide may be larger; the photosynthetic activity of vegetation can cause fluctuations of more than 0.01 % (Desjardins *et al.*, 1982). Carbon dioxide from hosts is then more difficult to discern from background fluctuations.

In much of the tropics, unstable atmospheric conditions occur during daytime, from about 8.30 to 16.00 h. During this time, the soil becomes hot due to irradiation. The lowest layers of air are heated by the ground and mix with other layers. In this mixing process, odour is diluted quickly. Under these circumstances, carbon dioxide from hosts can only be discerned from the back ground concentration at shorter distances, probably less than 20 m (Meixner, 1995). Tsetse thus use active search when the conditions are optimal.

To keep the risks associated with feeding to a minimum, tsetse should feed as infrequently as possible, but must obviously obtain a blood meal before they starve. Tsetse appear to be very efficient in finding a host, once they have detected its odour (Vale, 1980). However, the percentage of the tsetse that obtains a blood meal after having found the host varies from 60 % on an ox to 2 % on a bushbuck (Vale, 1977; Torr, 1994). Therefore, tsetse probably cannot wait until the last possible moment to obtain a blood meal and must balance the risk of feeding with the risk of starvation (Hargrove, 1988).

In the laboratory, the spontaneous activity increases with the time since feeding. There is a good correlation between the number of active tsetse and the number of responding tsetse both during the time of the day (Chapter 2) and in different seasons and vegetation types (Chapter 3). It appears that in the field, this spontaneous activity is the most important strategy of tsetse to find stationary hosts, as suggested earlier (Hargrove, 1991). For males, the time since feeding appears to be the only factor influencing the spontaneous activity.

The majority of the tsetse that were caught at a target had fed 48 to 60 hours earlier (Chapter 8). However, some of the refuge-leaving tsetse that were recaptured, had fed less than 24 hours earlier. This supports the suggestion of Hargrove & Packer (1993) that males may take a blood meal from a host that

walks past their resting site. It seems that the same is true for females, because females recaptured from the refuge had a higher haematin content than unmarked females that were caught at the same time and place (Chapter 8). The time since feeding appears to influence whether the females remain stationary or start to search for a blood meal.

In both sexes, the fat content has a secondary influence on the foraging behaviour. The fat content seems to regulate whether tsetse will try to obtain a blood meal at a host they have located. Males caught with a trap have a lower fat content than males that arrived near the trap but were not caught (Langley & Wall, 1990). Females caught with a target baited with CO_2, acetone, 1-octen-3-ol (henceforth termed octenol), 3-n-propylphenol and 4-methylphenol, contained more fat than the females caught with a target baited with the same odour without CO_2 (Chapter 8).

In females, the pregnancy stage seems to play a similar role in foraging behaviour as the fat content. Females in the last stage of their pregnancy appear to be as active as other females (Chapter 3), but few are caught on a target or in a trap. It remains unknown whether the females with the higher fat content or further developed pregnancy do not react to the odour without CO_2 at long distance, or locate the source, but do not get caught at the target. The results with trap catches of males indicate that it might be the latter effect (Langley & Wall, 1990).

Early in the pregnancy, females can delay taking a blood meal for some time. But when the egg hatches and the larva starts to grow, the time between two blood meals seems to decrease and virtually all females in the last day of their pregnancy had fed one day previously. This is probably necessary, because the larva has to put on about 4 mg of dry weight during the last day of its development (Randolph *et al.*, 1991).

With females, age also plays a role in foraging behaviour. Older females are more likely to use spontaneous activity than younger females. This is probably the effect of the lower flight capacity of young females (Hargrove, 1975).

Locating the host

In a tsetse habitat, the wind may change direction at 20° per second. The odour pockets that make up an instantaneous plume, are thus dispersed in almost all directions (Brady, 1989). Host location by means of a 'random biassed walk' seems the best option under such circumstances (Brady, 1989; Williams, 1994; Griffiths *et al.*, 1995). In this model the bias is provided by upwind take off and upwind turns when odour is detected. It is now clear that tsetse react instantaneously to the wind direction at distances up to 30 m downwind of the odour source (Chapter 7). The success of location a source increases with the bias of the movement in the direction of the source. When the bias is larger than 20 %, the chance that the searcher finds the source is close to unity (Fisher & Lauffenburger, 1987).

It appears that tsetse increase their source location success by turning when they lose the odour plume (Gibson & Brady, 1988). When the odour flux increases, which indicates that the tsetse approaches the odour source, tsetse change their flight behaviour: they slow down, increase the turning rate and orientate less precisely to the wind direction (Warnes, 1990; Brady & Griffiths, 1993, Chapter 7). The latter seems to be counter productive, because it lowers the bias in flight direction. However, this behaviour would keep a tsetse in the vicinity of the odour source (Warnes, 1990). Tsetse react to visual stimuli when flying up an odour plume (Torr, 1989). It is possible that they are 'on the look-out' for visual stimuli when they slow down and increase their turning rate.

In my opinion, the odour flux in an odour pocket is most important for the long range host location of tsetse. The odour flux determines whether a tsetse detects the odour pocket and how it changes its behaviour if it detects the odour pocket. The flux of odour in an odour pocket is determined by the number of components and the concentration of these components in the odour pocket. It seems unlikely that tsetse have specific behavioural patterns that are a reaction to specific kairomones. This suggests that the absence of one kairomone in an odour mixture can at least partly be compensated for by an extra dose of other kairomones. However, the maximum effect of a kairomone might be limited by the number and the sensitivity of receptor cells on the

antenna that respond to that particular kairomone, which may limit the compensatory effect of a kairomone.

The research described in this thesis has mainly focused on the effects of CO_2. It remains to be seen whether the effect of other odours on the flight behaviour of tsetse is equally strong. Work on acetone, octenol, 3-n-propylphenol and 4-methylphenol indicates that these odours have less impact than CO_2 (Brady & Griffiths, 1993). There is at least one more as yet unidentified attractive component in ox odour (Torr *et al.*, 1995), and this component seems to have a strong effect on tsetse behaviour. Ox odour had a measurable effect on tsetse released at 100 m from the source (Chapter 5), while this was not the case with an artificial odour that was released at a much higher rate (Chapter 6). Other components may thus have as strong an effect on tsetse behaviour as CO_2.

An important question is why the number of kairomones in the odour is more important than the dose of the kairomones present? It can be argued that the reliability of the cue increases with the number of kairomones. Carbon dioxide produced by hosts can be difficult to discern from fluctuations in the background concentration of CO_2 and it is not produced by host animals of tsetse only. Octenol is also produced by organisms that are not hosts of tsetse (Buttery & Kamm, 1980). The above mentioned phenols are the product of bacterial activity in the urine of hosts and thus do not necessarily indicate that hosts are close by. If all these odours occur together, however, then it is likely that a host is present.

The odour flux declines with distance from the source (Murlis *et al.*, 1992). I suggest that tsetse use the odour flux to estimate distance from the source. The apparent flux of the odour seems to increase with the number of kairomones present and the release rate of the odour. If this suggestion makes sense, one would expect that at the same distance from the true source, tsetse should show straighter flight paths that are more closely orientated towards the wind direction when an odour with a low flux is present than when odour with a high flux is present. By monitoring tsetse on video, the straightness and orientation of their flight paths can be measured (Gibson & Brady, 1985). The

effect would off course show up most clearly when no visual targets are present.

Increasing the catch of a target?

From the above studies it becomes clear that not all tsetse that are spontaneously active, are caught with the target currently in use. It is possible that these tsetse locate the target, but do not come into contact with it and the insecticide deposited thereon. This is in part due to the absence of CO_2, which seems to 'deter' a well defined group: females with a higher fat content and in the later stage of pregnancy. The calculations that showed that an additional daily mortality of 4 % is enough to eradicate tsetse, assumed that each tsetse had the same chance of being killed by a target (Hargrove, 1988). It is now clear that this is not the case. Especially the assertion by Hargrove (1991) and the results shown in chapter 3, that young females have a smaller probability of making contact with a target, is worrying. The fact that females with a large fat content do not land on the targets also gives rise to concern. The latter effect could make the eradication or control of tsetse by means of targets difficult in areas where many hosts are available. These same groups of tsetse are also caught with low probability by a trap used to monitor the progress of a control operation.

These effects themselves do not put a control programme in which odour-baited, insecticide-treated targets are used, in direct jeopardy. It is possible though, that, due to these effects, the tsetse population remains present at low numbers for quite a long time after the placement of the targets. During this time, some targets become ineffective (Willemse, 1991; Knols *et al.*, 1993). And, due to the absence of tsetse in monitoring traps, control personnel may think that the tsetse have been eradicated when they are not. This could lead to a premature stop of the control programme.

The daily mortality needed to eradicate a tsetse population, needs to be recalculated, accounting for the differences in availability of females in different life stages. New target designs do catch larger numbers of tsetse

(Vale, 1993). It is advisable to determine whether these targets catch more of the same tsetse or also tsetse that were caught with a small probability previously.

Host location in other blood feeding insects: a comparison

Host-location behaviour of other blood feeding insects other than tsetse has been reviewed by Takken (1991) and Sutcliffe (1987). Both reviews show that until now host-location behaviour of tsetse has been studied with more success than that of most other blood feeding insects. All blood feeding insects are activated by and attracted to CO_2, but few other attractive compounds have been identified for other blood feeding insects. Lactic acid is attractive to many species of mosquitoes (Takken, 1990), but this compound is probably repellent to tsetse (Vale, 1979). Octenol (Takken & Kline, 1989) and as yet unidentified compounds, probably short-chain fatty acids and methanethiol, that sweaty feet and cheese have in common (Knols & De Jong, 1996), attract mosquitoes. The difference in reaction to various components is probably due to the differences in hosts. The mosquito species that were used in the above mentioned studies, are mainly, in some cases almost exclusively, attracted to man, whereas *G. pallidipes* is not host specific (Vale, 1977) and repelled by humans (Vale, 1979).

Several tabanids that occur in Zimbabwe, are attracted to the same odours as tsetse, a combination of octenol, acetone, 3-n-propylphenol and 4-methylphenol (Phelps & Holloway, 1992). Work in this thesis showed that the tabanid *Philoliche (Stenophara) zonata* Walker (Diptera: Tabanidae) was attracted to these components as well (Chapter 1). The stable fly *Stomoxys calcitrans* (L.) (Diptera: Muscidae) is also attracted to octenol and acetone (Warnes & Finlayson, 1985; Holloway & Phelps, 1991). Tabanids and the stable fly are, like tsetse, not host specific. It appears that acetone, octenol and the phenols appear in the odour of all mammals and thus attract blood feeding Diptera with catholic feeding habits.

The range of attraction of carbon dioxide to mosquitoes and of ox odour to female *Tabanus* spp. is about 45 m and that of female *Philoliche (Stenophara)*

zonata Walker (Diptera: Tabanidae) to ox odour is about 80 m (Gillies & Wilkes, 1969, 1972; Phelps & Vale, 1975), distances similar to those found for tsetse (Chapter 5). Snow (1980) found a correlation between host weight and range of attraction that holds for both tsetse and mosquitoes. These similarities suggests that the maximum distance from which a host can be located by insects, is to some extent determined by characteristics of the wind. For insects using the 'biassed random walk', to locate a host, the variability in the wind direction is important (Chapter 6). The higher the variability in wind direction, the lower the probability that the wind direction indicates the true direction to the source when odour is detected. This results in a lower bias in the "biassed walk' and thus in a lower probability that the source is found.

Odour source location in other insects

Many species of insects use odours to find food sources (Visser, 1986; Ridgeway *et al.*, 1990), but there are only a few species for which the host-location behaviour is known in detail (Murlis *et al.*, 1992). Only the flight behaviour of several species of male moths towards a pheromone source has been studied in as much detail as the flight behaviour of tsetse (eg David *et al.*, 1983; Willis *et al.*, 1991; Mafra-Neto & Cardé, 1994).

Male moths appear to fly almost straight upwind in the field (David *et al.*, 1983). In a wind tunnel, moths were frequently observed to fly in zigzag patterns, but in a turbulent plume they fly straight upwind (Mafra-Neto & Cardé, 1994). When moths lose contact with a plume of pheromones, they start a zigzag pattern until they contact odour again (David *et al.*, 1983). When the moths approach the source, they slow down and steer more across the wind (Willis *et al.*, 1991). The pattern of flight is similar to that of tsetse. The main difference is that tsetse make rather shallow turns both upwind and downwind when they lose contact with an odour plume (Gibson & Brady, 1988), whereas moths hardly move upwind or downwind when they have lost contact with the plume (David *et al.*, 1983). This might be an adaptation to the difference in ecology of moths and tsetse flies. In the forests where the moths are

searching, the wind does not change direction as often as in tsetse habitat (cf Elkinton *et al.*, 1987 and Brady *et al.*, 1989).

It has been suggested, as I have for tsetse, that moths do not react to specific odours with specific behaviour, but that the complete blend of pheromones elicits the same behaviour at lower threshold than an incomplete mixture (Linn & Roelofs, 1989). Other workers suggested that specific pheromone components elicit specific behaviours (Cardé *et al.*, 1975; Bradshaw *et al.*, 1983). However, use was made of incomplete blends or of components that were not present in the natural pheromone blend and the specific behaviour was related to mating, not to host location.

The percentage of male moths that were recaptured at a source, increased with proximity of source and moths were recaptured from up to 120 m away (Elkinton *et al.*, 1987). Male moths were only released when odour was present at the release site, and about 45 % of the males released at 20 m was recaptured. It seems that moths can locate a source from a similar distance as tsetse, which supports the suggestion that wind characteristics partly determine the distance from which an odour source can be located. Male moths seem less efficient in locating an odour source than tsetse. Tsetse may achieve a host location efficiency of 80 to 100 % when they detect the host at a distance of 30 m or less (Chapter 5).

Epilogue

The research described in this thesis has resulted in an improved understanding of the foraging and host-location behaviour of tsetse. It is now clear that tsetse can detect a host at 100 m distance and that long range host location depends on odour flux rather than the presence or absence of individual kairomones in the odour mixture. Furthermore, the effect of the physiological state of the tsetse on its behaviour is now more clearly understood. These findings have important consequences for tsetse eradication programmes based on target technology: Control operations that use odour-baited, insecticide-impregnated targets, must be sustained for a long time, even

when stationary baits no longer catch tsetse. It is advisable to develop an additional monitoring system that does catch well fed tsetse.

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Summary

The tsetse flies *Glossina pallidipes* Austen and *G. m. morsitans* Westw. (Diptera: Glossinidae) are obligatory blood feeding insects that do not live in close association with their hosts (mainly mammals). Tsetse flies are relatively long lived insects and have to take a blood meal regularly. Tsetse flies use smell and vision to find their hosts. In the last decade, many aspects of tsetse foraging and host-location behaviour have been elucidated. A range of kairomones has been identified. These kairomones can be used to which increase the number of tsetse caught with a trap or a target. However, some aspects of tsetse behaviour remained unclear. Not much known about the effects of the identified kairomones on the host-location behaviour of tsetse at long distance for example. Also, the effect of the physiological state of a tsetse on its foraging behaviour, was unclear.

This thesis describes a field study, conducted in the Zambezi valley, Zimbabwe, that aimed at clarifying these questions. Chapter 2 and 3 describe studies of the foraging behaviour of tsetse. Studies of tsetse host location behaviour are presented in the following five chapters.

Foraging behaviour

In the field, *G. pallidipes* and *G.m. morsitans* are mostly caught in the early morning and the late afternoon. Spontaneous activity of *G. morsitans* in the laboratory also showed peaks in the morning and evening. How the activity patterns in the field are and what the effects of sampling methods on the apparent rhythm are, was not clear, however. In **chapter 2**, I describe studies in the diurnal rhythm in catches with different devices.

From before sunrise until after sunset, hourly catches of *G. m. morsitans* and *G. pallidipes* were made from a stationary unbaited electric net; an ox fly-round; an electrified target; an epsilon trap and a biconical trap. The latter three were baited with artificial host odour, consisting of acetone, 1-octen-3-ol (henceforth termed octenol), 3-n-propylphenol and 4-methylphenol.

Catches of tsetse were low from dawn to early afternoon, peaking just before sunset. Despite the broad similarity in diurnal patterns, there were some consistent differences between the sampling methods. The fraction of the daily catch caught in the afternoon with the target or the traps was larger for males than for females. The catch at the unbaited electric net probably gave the best estimate of the diurnal rhythm of flight activity of tsetse. Compared to the net catch, trap catches of tsetse were relatively high during the middle of the day and low in the early morning and late afternoon. The differences were attributed to sampling biases of traps. The pattern of the target catches was not significantly different from the pattern of unbaited net catches. The catch pattern at the mobile bait was significantly different from that of the net. This is attributed to the response of tsetse sitting on vegetation, which were not sampled by the unbaited net, to the ox fly-round. It is concluded that target catches can be used to monitor diurnal rhythms in tsetse activity. The pattern of *Stomoxynae*-catches on targets resembled that of tsetse. However, no peak was evident in the electric net catches. Catches of the tabanid *Philoliche (Stenophara) zonata* Walker showed a sharp peak in the early afternoon.

The daily patterns in catches at the unbaited net and the baited target were similar. This suggested that active search was the most important strategy for tsetse to find a host. In **chapter 3**, the catches of targets and unbaited nets and the tsetse sitting on vegetation were studied in more detail to obtain evidence for this suggestion.

The catches of *G. pallidipes* and *G. m. morsitans* at a target baited with odour (acetone, octenol and two phenols) were positively correlated with catches of the same species at an unbaited net. No correlation existed between target catches and hand net catches of tsetse flies sitting on the vegetation. *G. pallidipes* females caught at a target and at an unbaited net were older than those caught from vegetation. Of the female *G. pallidipes* caught at the target, 46 % were in the first 3 days of pregnancy. Of those caught at the unbaited net, significantly fewer, 21 %, were in this stage. *G. pallidipes* males caught from vegetation contained more fat (3.07 $\pm$ 0.333 mg) than those caught at the unbaited net (2.06 $\pm$ 0.339 mg) or at the target (2.19 $\pm$ 0.218 mg). The target catches consisted mostly of tsetse that were already in flight when they sensed

the stimuli from the target. Target catches were biased towards female *G. pallidipes* in the first 3 days of pregnancy.

Host-location behaviour

To investigate the effects of odour composition and odour release rate on host-location behaviour of tsetse, mark-release-recapture studies were used. Before these experiments were started, the behaviour of tsetse just after their release was studied at 10 m downwind of an odour source with a video camera and with electric nets. This work is described in **chapter 4**. Tsetse were collected with traps, marked and released. Only tsetse that were recaptured on the same day were analyzed. The study focused on *G. pallidipes*, because it is more readily caught in traps than *G. m. morsitans*.

Video studies showed that in the absence of odour, 46 % of the released *G. pallidipes* turn downwind and 32 % turned upwind. Tsetse left the release box at a constant rate and appeared to avoid each other. When an artificial odour mixture containing carbon dioxide, acetone, octenol and phenols was used, 35% turn downwind, significantly less than in the absence of odour, and 37 % turned upwind. Tsetse left the release box later than in the absence of odour and not at a constant rate. Tsetse did not avoid each other.

When the release box was placed in a complete ring of electrified nets, only natural ox odour changed the distribution of tsetse over the electric nets compared to the no odour treatment. The artificial odour mixture, with and without carbon dioxide, had no effect on the distribution of tsetse over the electric nets. Most tsetse were caught while flying in a downwind direction.

The electric nets have an efficiency of about 50 %. In this experiment, with a complete ring of nets, the tsetse that survive their first contact with the net, could thus be killed on another net. The difference between the video study and the electric net study is attributed to this effect. The small differences between the odour treatment and the control was probably due to frequent changes in wind direction. Odour did not always reach the box

because of these changes. However, if odour is present at the box, tsetse released or departing from the box react to the presence of odour immediately.

Marked *G. pallidipes* were released downwind of an odour source and the percentage recaptured at the source on the same day was measured. The influence of release distance, odour composition (**chapter 5**) and odour release rate (**chapter 6**) on the recapture percentages were studied.

In the absence of odour, 1.3 % of the marked tsetse released from a box or refuge were recaptured, independent of the distance between release point and odour source. When natural ox odour or a blend of carbon dioxide, acetone, octenol and phenols was dispensed, untransformed recapture percentages of box-released tsetse decreased from 18 % for tsetse released at 10 m to 2 % for tsetse released at 100 m. Recapture percentages were significantly higher than in the absence of odour at all release distances for ox odour and for release distances up to 75 m downwind for the artificial odour. When a combination of acetone, octenol and phenols or carbon dioxide on its own was dispensed, recapture percentages decreased from 6 % for tsetse released at 10 m to 0 % for tsetse released at 100 m. With these odours, recapture percentages were higher than in the absence of odour when tsetse were released at 20 m from the source, but were lower than recaptures in the presence of ox odour or the artificial mixture with carbon dioxide. Recapture percentages of flies leaving a refuge were higher than those of box-released tsetse. Proximity of source had no effect on the recapture percentage of refuge-leaving tsetse.

The odour mixture consisting of carbon dioxide, acetone, 1-octen-3-ol and phenols was released at three different rates. The medium odour release rate, was identical to the rate used in the comparison of odour composition and the results were similar. The low odour release rate, a quarter of the medium rate, only attracted tsetse from 20 m and less; the recapture percentage declined from 5% at 10 m to 2 % at 30 m. The decrease in odour release rate thus caused a decline in plume length and a decline in host location efficiency or recruitment of tsetse to the plume. When the medium release rate was increased fourfold, the resulting high odour release rate did

not increase the recapture percentages from the same distances, nor did the distance from which tsetse were recaptured increase, compared to the medium rate.

From the experiment described above, we can conclude that: (1) tsetse have an efficient host-location mechanism, 80 to 100 % of the tsetse that detect the odour at less than 30 m from the source found the source; (2) tsetse can detect an ox at 100 m distance downwind.

An odour plume consists of 'pockets' of odour that are moved by the wind. The flux of kairomones in an 'odour pocket' might be the only cue used by tsetse. The flux is determined by the dose and the number of kairomone compounds present in the mixture. The number of kairomone compounds in the odour pocket seems to be more important than the dose of the components.

To elucidate the differences in recapture percentages between different odour treatments, I studied the effects of odour composition on the flight behaviour of released tsetse. This work is described in **chapter 7**.

Marked *G. pallidipes* were released from a box at 10, 20 and 30 m downwind of an odour source. Tsetse were recaptured the same day on a 9 m wide wall of electric nets at 4 m upwind of the release box, or at the inside of an incomplete ring of eight nets at 4 m around the box. The incomplete ring was only used when tsetse were released at 10 m downwind of the source. Four odours were tested: natural ox odour; an artificial blend of acetone, octenol and phenols; the same blend with carbon dioxide and carbon dioxide alone.

When tsetse were released at 10 m downwind of a source of natural ox odour, they avoided flying upwind. Dense vegetation did not influence their flight direction. Artificial odour did not influence the flight direction of tsetse released at 10 to 20 m downwind of the source in open vegetation, but tsetse avoided entering dense vegetation and preferred flying into crosswind oriented 'game trails'. About 35 % of the recaptured tsetse released at 30 m from the source of the artificial blend with or without carbon dioxide, flew within 20° of the prevailing wind direction. Tsetse released in the absence of odour or in the presence of the other odours flew away at random.

The results suggest that tsetse change from straight fast upwind flight to more sinuous and slower flight when the flux of kairomones increases.

In **chapter 8** I studied the effect of the physiological state of tsetse on their host-location behaviour. *G. pallidipes* were marked and released from refuges and boxes placed at 10, 20 and 30 m downwind of an odour baited visual target. The physiological state of all tsetse recaptured on the same day, samples of the populations from which the marked tsetse originated and a sample of unmarked tsetse caught together with the marked tsetse, was assessed by analysis of their fat and haematin content. Before fat and haematin analysis, the females were dissected to determine their ovarian age and pregnancy stage.

Unmarked tsetse caught with a target had on average fed 2.5 days previously. Among the females from the refuge, the variance in haematin decreased with progressing pregnancy. Females in the last stage of their pregnancy all had a high haematin content, indicating that most had fed less than 24 h ago before being captured. Tsetse recaptured from the refuge had a higher haematin content than unmarked tsetse. Recaptured refuge-leaving females were younger than unmarked females. The fat and haematin content were not related to the distance from which females were recaptured with the target. The females caught at a target baited with acetone, 1-octen-3-ol and phenols contained less fat than females caught at a target baited with the same odour plus CO_2. The fat content of recaptured males leaving from a refuge, increased with distance of release. There was no such correlation with box-released males.

It appears that haematin or the time since the last blood meal controls the search strategy of tsetse. Resting tsetse with high energy reserves will feed from a host that comes close by, but do not search actively for a host. The fat content, and in females also the pregnancy stage, influence whether tsetse will take a blood meal from a host that has been found.

The research described in this thesis has resulted in an improved understanding of the foraging and host location behaviour of tsetse. It is now clear that tsetse can detect a host at 100 m distance and that long range host

location behaviour of tsetse is influenced by odour flux rather than individual kairomones in the odour mixture. Furthermore, the effect of the physiological state of the tsetse on its behaviour is now more clearly understood. Targets do not catch all tsetse that are searching for a host. To ensure success, target control operations must be sustained, even when stationary baits no longer catch tsetse.

Samenvatting

De tseetseevliegen *Glossina pallidipes* Austen en *G. m. morsitans* Westw. (Diptera: Glossinidae) voeden zich alleen met het bloed van gewervelde dieren. Ze leven relatief lang en moeten regelmatig een gastheer vinden om een bloedmaaltijd te bemachtigen. Om hun gastheer te vinden, gebruiken tseetseevliegen hun reuk- en gezichtsvermogen. Op korte afstand is het gezichtsvermogen belangrijk: tseetseevliegen worden aangetrokken door eenvoudige vormen die contrasteren met de omgeving en landen bij voorkeur op een zwart object. Op grotere afstand maken tseetseevliegen gebruik van kairomonen.

Gedurende de laatste tien jaar is er veel bekend geworden over het gedrag van tseetseevliegen. Een aantal van die kairomonen is geïdentificeerd en wordt gebruikt om tseetseevliegen te vangen in een val of op een zwarte katoenen doek van 1 m^2 , een "scherm". De effecten van de verschillende kairomonen op het zoek-gedrag van tseetseevliegen op grote afstand van de bron zijn nog grotendeels onbekend. Ook het effect van de fysiologische status van de tseetseevliegen op hun foerageergedrag staat nog volop ter discussie.

Dit proefschrift beschrijft veldonderzoek dat is uitgevoerd in de Zambezievallei, Zimbabwe, met als doel het beantwoorden van de bovenstaande vragen. Na een inleiding, worden in hoofdstuk 2 en 3 studies van het foerageer-gedrag van tseetseevliegen beschreven. De volgende vijf hoofdstukken beschrijven studies van het zoekgedrag van tseetseevliegen.

Foerageergedrag

G. pallidipes en *G.m. morsitans* worden meestal gevangen in de vroege ochtend en laat in de middag. Het is echter niet zeker of dit komt doordat de omstandigheden dan het gunstigst zijn of omdat tseetseevliegen dan het meest actief zijn. In **hoofdstuk 2** beschrijf ik een experiment waarin het patroon in vangsten van o.a. vallen en een scherm gedurende de dag worden vergeleken.

Van vijf uur 's morgens (voor zonsopkomst) tot zeven uur 's avonds (na zonsondergang) werd elk uur de vangst geteld. Vliegen werden gevangen met een geëlectrificeerd net zonder geuren, met een vlindernetje vanaf een lopende

os, met een geëlectrificeerd scherm, een epsilon val en een biconische val. De drie laatstgenoemden waren voorzien van de kairomonen aceton, 1-octen-3-ol (verder aangeduid als octenol), 3-n-propylfenol en 4-methylfenol.

De vangsten waren klein in de ochtend en bereikten hun maximum vlak voor zonsondergang. De vangstpatronen van de verschillende methoden leken in grote lijnen op elkaar, maar er waren een paar consistente verschillen. Met vallen en het scherm werd 's middags een groter percentage van de mannelijke dan van de vrouwelijke tseetseevliegen gevangen. Het patroon van vangsten met het geëlectrificeerde net gaf vermoedelijk de beste schatting van de dagelijkse activiteit van tseetseevliegen. Vergeleken met het net vingen de vallen relatief weinig tseetseevliegen in de vroege ochtend en de late middag. Dit verschil was te wijten aan een vertekening van het vangstpatroon in de vallen. Het patroon van de schermvangsten week niet af van dat van het geelectrificeerde net. De vangsten van de os weken wel significant af. Dat komt omdat vooral tseetseevliegen die op vegetatie zitten, op de lopende os afkomen. Stilzittende tseetseevliegen worden uiteraard niet bemonsterd met het geëlectrificeerde net. Schermen kunnen dus gebruikt worden om het patroon in dagelijkse activiteit van tseetseevliegen te onderzoeken. Het patroon in de vangst van stalvliegen (*Stomoxynae*) leek op dat van tseetseevliegen, maar de vangst met het geëlectrificeerde net had geen piek in de middag. Vangsten van de daas *Philoliche (Stenophara) zonata* Walker waren geconcentreerd in de vroege middag. Deze resultaten gegevens suggereren dat actief zoeken het belangrijkste foerageergedrag van tseetseevliegen is.

In **hoofdstuk 3** zijn de vangsten van geëlectrificeerde netten en schermen in detail bestudeerd om deze suggestie te steunen danwel te weerleggen.

Vangsten van *G. pallidipes* en *G. m. morsitans* met een geëlectrificeerd scherm voorzien van kairomonen waren gecorreleerd met vangsten van dezelfde soort met een geëlectrificeerd net. Er was geen correlatie tussen de aantallen tseetseevliegen die met een vlindernetje werden gevangen van vegetatie en de aantallen die werden gevangen met het geëlectrificeerde scherm.

Vrouwelijke *G. pallidipes* gevangen met het scherm of het net waren ouder dan vrouwelijke vliegen gevangen van vegetatie. Verder was 46 % van

de vrouwtjes in de eerste drie dagen van hun dracht. Van de vrouwtjes gevangen met het net was een kleiner percentage, 21 %, in dat stadium. De mannelijke *G. pallidipes* gevangen van vegetatie hadden grotere vetvoorraden (3.07 ± 0.333 mg) dan mannetjes gevangen met een net (2.06 ± 0.339 mg) of een scherm (2.19 ± 0.218 mg). De schermvangsten bestonden dus voornamelijk uit vliegen die actief op zoek waren naar een gastheer. Met een scherm worden minder vrouwelijke vliegen in de latere stadia van de dracht gevangen dan dat er rondvliegen.

Zoekgedrag

Voordat experimenten met het loslaten van gemerkte vliegen begonnen, werd het gedrag van tseetseevliegen die een loslaatdoos verlieten bestudeerd. De doos was 10 m benedenwinds van een geurbron geplaatst en het gedrag van de losgelaten tseetseevliegen werd bestudeerd met een videocamera of met geëlectrificeerde netten. Alleen vliegen die werden teruggevangen op dezelfde dag als waarop ze werden losgelaten, werden bestudeerd. Dit experiment wordt beschreven in **hoofdstuk 4**. Alleen *G. pallidipes* werd onderzocht omdat die soort makkelijker is te vangen dan *G. m. morsitans*.

Video-opnamen lieten zien dat 46 % van de losgelaten vliegen windafwaarts draaide en 32 % windopwaarts. Als er wel geuren werden verspreid, draaiden significant minder vliegen windafwaarts, 35 %. Ongeveer 37 % draaide windopwaarts. Als er geen geur was, bleef het percentage vliegen dat de doos per tijdseenheid verliet, constant. De vliegen leken elkaar in dit geval ook te vermijden. Was er wel geur aanwezig, dan verlieten de tseetseevliegen de doos wat later en vermeden ze elkaar niet.

Als de loslaatdoos volledig omringd was met geëlectrificeerde netten, werden meer tseetseevliegen gevangen op de windafwaarts staande netten als er natuurlijke ossegeur aanwezig was, dan wanneer er geen geur aanwezig was. Kunstmatige mengsels van kairomonen hadden geen effect op de verdeling.

De geëlectrificeerde netten doden ongeveer 50 % van de tseetseevliegen die ermee in aanraking komen. In dit experiment kunnen de tseetseevliegen die niet het overleven later op een ander net wel gedood worden. Dit verklaart vermoedelijk het verschil tussen de studies met de netten en met de video. Het kleine verschil tussen de behandelingen werd vermoedelijk veroorzaakt door de frequente verandering in windrichting. Hierdoor bereikte de geur niet altijd de loslaatdoos. Uit de resultaten blijkt dat de losgelaten tseetseevliegen direct op de geuren reageerden.

Gemerkte vliegen werden windafwaarts van een geurbron losgelaten en het percentage dat dezelfde dag op een scherm bij de geurbron werd teruggevangen, werd bepaald. De invloed van de loslaatafstand, de geursamenstelling (**hoofdstuk 5**) en de dosis van de geuren (**hoofdstuk 6**) werden bepaald.

Zonder geur werd ongeveer 1.3 % van de losgelaten vliegen teruggevangen, onafhankelijk van de afstand waarop ze werden losgelaten. Als ossegeur of een mengsel van kooldioxide, aceton, octenol en twee fenolen werd gebruikt, werd 18 % teruggevangen als de vliegen 10 m windafwaarts werden losgelaten. Het terugvangpercentage nam af tot 2 % als de afstand waarop de vliegen werden losgelaten, toenam tot 100 m. De terugvangpercentages waren significant hoger dan die zonder geur op alle afstand voor ossegeur en voor loslaatafstanden tot 75 m voor het kunstmatige mengsel. Als het kunstmatige mengsel zonder kooldioxide of als kooldioxide alleen werd gebruikt, werd 6 % van de vliegen die werden losgelaten op 10 m terug-gevangen. Van de vliegen losgelaten op 100 m werd er niet één teruggevangen. Voor vliegen losgelaten op 20 m was het terugvangpercentage significant hoger dan dat van vliegen losgelaten in de afwezigheid van geuren en significant lager dan dat van vliegen losgelaten als ossegeur of een mengsel met kooldioxide gebruikt werd.

Gemerkte tseetseevliegen die een kunstmatige en koele schuilplaats verlieten tijdens de hete tijd, werden vaker teruggevangen dan tseetseevliegen die uit een loslaatdoos vertrokken. De afstand tussen de schuilplaats en de geurbron had geen effect op het terugvangpercentage.

Drie verschillende doses van een mengsel van kooldioxide, aceton, octenol en de fenolen werden getest. De middelste dosis was hetzelfde als de dosis die bij de vergelijking van samenstellingen werd gebruikt en de terugvangpercentages waren dan ook ongeveer gelijk. De lage dosis, een kwart van de middelste dosis, werd alleen gevonden door tseetseevliegen losgelaten op 20 m afstand of minder. Het terugvangpercentage nam af van 5 % voor vliegen losgelaten op 10 m tot 2 % voor vliegen losgelaten op 30 m of meer. Tseetseevliegen konden de bron van een kleine dosis geuren niet zo goed lokaliseren als de bron van een grotere dosis. Een kleine dosis werd ook op kortere afstand niet meer gedetecteerd dan een grote dosis. Als de middelste dosis vier keer zo groot werd gemaakt, werden er niet meer tseetseevliegen teruggevangen van dezelfde afstand en er werden ook niet meer vliegen teruggevangen van een grotere afstand.

Uit bovenstaande experimenten blijkt dat: (1) tseetseevliegen hun gastheren met grote waarschijnlijkheid vinden als ze de geur hebben gedetecteerd: bijna alle vliegen die de geur detecteren op 30 m of minder van de bron, vinden de bron; (2) tseetseevliegen kunnen de geur van één os op 100 m afstand waarnemen.

Een geurpluim bestaat uit 'bellen' geurstof die door de wind worden verplaatst. De flux (de hoeveelheid geur die per tijdseenheid langs de antenne komt) van kairomonen in zo'n 'geurbel' lijkt het belangrijkste kenmerk van een geurpluim te zijn voor tseetseevliegen. De flux is afhankelijk van het aantal kairomonen dat aanwezig is in het mengsel en de dosis van elk kairomoon. Het aantal aanwezige kairomonen lijkt belangrijker dan de hoeveelheid van elk kairomoon.

Uit het voorgaande bleek dat de zoekefficiëntie van tseetseevliegen afhankelijk is van de geursamenstelling. Hoe dat verband precies ligt, is in **hoofdstuk 7** nader onderzocht.

Gemerkte tseetseevliegen werden losgelaten op 10, 20 en 30 m windafwaarts van een geurbron. Op 4 m windopwaarts van de loslaatdoos stond een 9 m lange 'muur' van geëlectrificeerde netten dwars op de windrichting. In een ander experiment werd de doos geplaatst in het centrum van een in-

complete cirkel van 8 geëlectrificeerde netten met een middellijn van 8 m. De incomplete cirkel werd alleen gebruikt als tseetseevliegen op 10 m afstand van de geurbron werden losgelaten. Vier geuren werden getest: natuurlijke ossegeur, een kunstmatig geurmengsel van aceton, octenol, fenolen, met of zonder kooldioxide en alleen kooldioxide.

Tseetseevliegen die 10 m windafwaarts van een bron van ossegeur werden losgelaten, vlogen niet windopwaarts en hun vliegrichting werd niet beïnvloed door dichte begroeiing. Kunstmatige geuren hadden geen invloed op de vliegrichting van tseetseevliegen die op 10 en 20 m windafwaarts van de geurbron werden losgelaten. Wel vermeden tseetseevliegen dichte vegetatie als kunstmatige geuren werden gebruikt.

Ongeveer 35 % van de tseetseevliegen die 30 m windafwaarts van een kunstmatige geur met of zonder kooldioxide werden losgelaten, had een vliegrichting die minder dan 20° afweek van een koers tegen de heersende windrichting in. Als andere geuren werden losgelaten, vlogen de tseetseevliegen weg in een willekeurige richting.

Waarschijnlijk reageren tseetseevliegen op een toenemende flux van kairomonen door langzamer te gaan vliegen en vaker een draai te maken.

In **hoofdstuk 8** heb ik de effecten van de fysiologische conditie van de tseetseevliegen op hun zoekgedrag bestudeerd.

G. pallidipes werden gemerkt en losgelaten vanuit loslaatdozen en kunstmatige schuilplaatsen. De fysiologische status werd bepaald van 1) alle tseetseevliegen die werden teruggevangen; 2) steekproeven van de gemerkte vliegen die niet werden losgelaten; 3) steekproeven van ongemerkte vliegen die gelijktijdig met de gemerkte vliegen gevangen werden. Van deze vliegen werd het vet- en haematinegehalte bepaald. Het haematinegehalte neemt af met het verstrijken van de tijd sinds de laatste bloedmaaltijd. De vrouwelijke vliegen werden voor deze analyse plaatsvond gedissecteerd om het ontwikkelingsstadium van de dracht en leeftijd te bepalen.

Ongemerkte vliegen hadden gemiddeld 2.5 dag voor ze gevangen werden hun laatste bloedmaaltijd genomen. Bij de vrouwelijke vliegen uit de schuilplaatsen, nam de variatie in haematinegehalte af met de progressie van

de dracht. Alle vrouwtjes die langer dan zeven dagen drachtig waren, hadden minder dan 24 uur voor ze gevangen werden nog een bloedmaaltijd genomen. Gemerkte vliegen die losgelaten waren uit een schuilplaats en werden teruggevangen, hadden een hoger haematinegehalte dan ongemerkte vliegen. De teruggevangen vrouwtjes waren bovendien jonger dan de ongemerkte vrouwtjes.

Er was geen verband tussen de afstand waarop tseetseevliegen werden losgelaten en het haematinegehalte van de teruggevangen vliegen. De teruggevangen mannetjes die van verder weg kwamen hadden echter grotere vetreserves dan de teruggevangen mannetjes die van dichtbij kwamen. Een dergelijk verband was er niet bij mannetjes die uit een doos werden losgelaten. Vrouwelijke vliegen die werden gevangen op een scherm dat voorzien was van een kunstmatige geur zonder kooldioxide, hadden kleinere vetreserves dan vrouwtjes die werden teruggevangen op een scherm dat voorzien was van een kunstmatige geur met kooldioxide.

Het haematinegehalte, of de tijd die is verstreken sinds de laatste bloedmaaltijd, bepaalt kennelijk de zoekstrategie van tseetseevliegen. Rustende vliegen met een hoog haematinegehalte zullen wel een bloedmaaltijd nemen van een passerende gastheer, maar gaan niet actief op zoek naar een gastheer. Hoe groter de vetreserves, en hoe verder de dracht gevorderd is bij vrouwtjes, des te kleiner is de kans dat de tseetseevlieg land op een gevonden gastheer.

Het onderzoek dat in dit proefschrift is beschreven, geeft een beter inzicht in het foerageer- en zoekgedrag van tseetseevliegen. Het is duidelijk geworden dat tseetseevliegen een gastheer van 100 m afstand kunnen vinden. Zoekgedrag op lange afstand wordt vermoedelijk alleen beïnvloed door de flux van de geur en niet door individuele kairomonen. Verder is nu duidelijker wat het effect van de fysiologische status van tseetseevliegen op hun zoek- en foerageergedrag is. Met insecticiden geïmpregneerde schermen voorzien van lokstoffen vangen niet alle tseetseevliegen die op zoek zijn naar een gastheer. Om tseetseevliegen met succes te bestrijden, dienen de schermen lang operationeel te blijven, nog lang nadat geen tseetseevliegen meer worden gevangen tijdens bemonsteringen met vallen.

List of Publications

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Curriculum vitae

Cornelis Aart Groenendijk werd geboren op 1 mei 1965 te Maasland. Hij ging in 1977 naar het Oranje Nassau College in Zoetermeer en vanaf 1978 naar het Interconfessioneel Westland College in Naaldwijk. Daar behaalde hij in 1983 het VWO-diploma. Daarna ging hij Materiaalkunde studeren in Delft. Na een jaar zag hij in dat dat geen goede keuze was en in 1984 begon hij de studie Biologie in Wageningen. Hij deed drie afstudeervakken, bij respectievelijk Entomologie, Natuur- en Weerkunde en Theoretische Productie Ecologie. Hij liep zes maanden stage in Kenya, bij het I.C.I.P.E. Daar maakte hij kennis met de medische en veterinaire entomologie en met de tseetseevliegen. In 1990 haalde hij het ingenieursdiploma.

Tijdens zijn middelbare school- en studietijd is hij achtereenvolgens werkzaam geweest als krantenbezorger, tomatenplukker, winkelbediende, barman, postbode, verkiezings-oproeprondbrenger, student assistent, sjouwer, nachtportier en produktiemedewerker in een broodfabriek en een drukkerij. Mede daardoor is hij zijn bank niets verschuldigd.

In 1990 gaf hij een maand onderwijs in het gebruik van simulatiemodellen in Myanmar (Birma). Daarna werd hij tijdelijk medewerker bij de vakgroep Theoretisch Productie Ecologie en ontwikkelde Computer Ondersteund Onderwijs voor het vak Populatiedynamica. In 1991 begon hij aan het in dit proefschrift beschreven onderzoek.

Curriculum vitae

Cornelis Aart Groenendijk was born on 1 May 1965 in Maasland, The Netherlands. He received is secondary education at the Oranje Nassau College at Zoetermeer and the Interconfessioneel Westland College at Naaldwijk, where he graduated in 1983. He first studied material science at the Technical University Delft. After one year he admitted to himself that that was not a good choice and he changed to Biology at the Wageningen Agricultural University. He did three Msc-projects at the departments of Entomology, Physics and Meteorology and Theoretical Production Ecology respectively. He did a practical period at I.C.I.P.E. in Kenya, where he was introduced to medical and veterinary entomology and to tsetse flies. He obtained his degree as 'landbouwkundig engineer (comparable to M.sc.) in 1990.

During his secondary education and his study he had jobs as paper boy, tomato picker, shop-assistant, bar-tender, postman, polling card roundsman, student assistant, porter, night porter and as unskilled worker in a bread factory and a printing company. He is therefore not indebted to his bank.

In 1990 he conducted a roving seminar on the use of simulation models in Myanmar (Birma). After that he became a temporary lecturer at the department of Theoretical Production Ecology, where he developed computer aided education for the subject Population Dynamics. In 1991 he started the research described in this thesis.