Identification of behaviour modifying chemicals for *Glossina* spp. from host skin secretions

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Appendix 1: Publications

General Introduction

The original objectives of this project were to use existing wind tunnel and video equipment, and to develop a simple bioassay based on probing, to investigate the behaviour of tsetse flies in response to the skin secretions of cattle (sebum) with a view to isolating active components for use at traps and targets. The laboratory work was to be supported by field investigations undertaken in Zimbabwe. Work on cattle skin secretions was to be followed by investigations on the skin secretions of other hosts.

2. Probing responses of tsetse to skin secretions.

2.1. Introduction

A series of experiments on the probing behaviour of *G.m.morsitans* undertaken in 1989-90 gave variable and inconsistent results (Warnes & Malele, TRL annual report 1990). A major problem with these experiments was the variability of the response of control flies. It was considered that the variability may have been caused by a problem with the colony of *G.m.morsitans* used, and it was decided to stop working with this species and to repeat some of the experiments with *G.pallidipes* (kindly supplied by IAEA, Vienna).

2.2. Materials and Methods

2.2.1. Collection of materials

Cattle hair was collected by shaving freshly slaughtered cattle at the slaughterhouse of the Veterinary School at Langford and at a commercial slaughterhouse situated nearby. The hair was sealed in plastic bags and sent to the laboratories of the Natural Resources Institute (NRI) at Chatham for extraction. Sebum was extracted by washing the hair in a solvent as in Warnes (1990a) except that the solvent used was chloroform instead of dichloromethane. Warthog eye secretions were collected from the 'tame' warthog at the Rekomitjie Research Station in Zimbabwe by rubbing the warthogs snout with clean black cloth. The cloth was transported to Bristol and washed in dichloromethane. The washings were then concentrated in a rotary still under pressure to leave a concentrated solution of warthog eye secretion.

2.2.2. Experimental procedure

A 75x25 mm glass tube containing a hungry fly was inverted onto a cotton drill cloth that had been soaked in either a sebum solution or the solvent only and

allowed to evaporate. Once contact was made with the cloth the fly was observed for 30s. Extension of the proboscis and lowering of the haustellum was recorded as a positive response and failure to do so as a negative response. Approximately 60 flies (30 of each sex) were exposed to the sebum treated cloth and another 60 were exposed to the control cloth. This was repeated with the cloth warmed to 37°C to simulate body temperature. The experiment was undertaken daily over five days during which the flies were not fed.

The same procedure was repeated using black felt instead of the cotton drill to investigate any effect of the nature of the cloth substrate. The felt was brushed with a small brass brush in order to roughen the surface before and after the treatment with either sebum solution or solvent. Approximately 40 flies (20 male and 20 female) were used in each test.

In a further experiment the tube containing the fly was inverted over a wooden peg covered with treated cloth in order to minimise mechanical stimuli. A steel pin in the side of the peg held the bottom of the tube just above the cloth, allowing contact with the cloth as well as some restricted movement by the fly. Using this method the probing response of *G.pallidipes* to warthog eye secretions were investigated. It was not possible to test probing stimulants in combination with an increased temperature with this apparatus and the experiment was undertaken at room temperature. The same criteria for a positive or negative response were used. A total of 45 flies were tested (21 males and 24 females) after 96 and 120 h starvation. The results for both sexes were grouped for purpose of analysis.

The data were compared using a chi-squared test.

2.3. Results

The percentage of flies probing on cotton drill with and without sebum at room temperature and at 37°C, as the period of starvation of the flies increased is shown in Fig. 2.1. The presence of sebum on the cloth at room temperature increased the proportion of flies probing after 48h starvation; the proportion probing remained higher than the control as the period of starvation was increased, but the difference was only significant after 96h starvation ($P < 0.05$). When the experiment was repeated at 37°C the proportion of flies probing at both sebum and solvent treated cloth was much higher, as might be expected because of the temperature stimulus. There was a significant increase in probing on the sebum treated cloth after 24h starvation ($P < 0.05$), but the difference once again was small, and the effect was not consistent as the starvation period of the flies increased, (Fig. 2.1.).

The results using felt instead of cotton drill (Fig. 2.2.) show that more flies probed on the rough cloth compared to the smooth cloth (compare Figs. 2.1. and 2.2.). The effect of sebum at room temperature was not significant but at 37°C probing was significantly reduced after 48 and 96 h starvation when sebum was present

($P < 0.05$). This result is difficult to explain, but observations revealed that many flies avoided contact with the sebum treated cloth, especially when rough cloth was used. The decreased probing response observed when sebum was present at 37°C could therefore be explained by flies avoiding contact with the cloth and therefore the temperature stimulus. Attempts were made to ensure that contact with the cloth was made for 30s, but in order to achieve this the tube had to be tapped with the experimenter's finger. This mechanical stimulus also elicits probing. Therefore a system which avoided this mechanical stimulus and enforced contact with the cloth was developed for the experiment with warthog eye secretion.

The proportion of flies probing on warthog eye secretion or a control after 96 and 120 h starvation are shown in Table 2.1. The presence of warthog eye secretion resulted in a significantly lower proportion of flies probing after 96h starvation, but there was no significant effect after 120h starvation.

2.3. Discussion

A simple probing assay to test sebum and components of sebum is highly desirable as it would be quick and easy to perform. However, these results suggest that it is inappropriate since it is difficult with a free moving fly to obtain a simple +/- response without the complication of recording other behaviours. Results from these and other experiments have been inconsistent and positive results have been difficult to repeat. It is well documented that the temperature of the substrate elicits probing (Brady, 1973) and temperature sensitive receptors on the legs of *Glossina* have been reported (Reinouts van Haga & Mitchell, 1975), but despite early results (Warnes, 1989b), evidence of a probing response by tsetse on contact with sebum is tenuous, and no affirmation of an electrophysiological nature exists.

Vale (1974a) showed that tsetse land on the snout of the warthog in preference to other regions of the body, and suggested that the secretions from the eye in this species may be responsible for the increased landing. It might be reasonable to assume that the secretion from the vicinity of warthog eyes may have a similar effect to sebum on the behaviour of tsetse flies, but this was not the case in this experiment. Recent studies in Zimbabwe (S.J. Torr, pers comm) suggest that the preponderance of flies alighting on a warthog snout is a direct result of fly avoidance behaviour in this species.

Recent field experiments support the hypothesis that the effect of sebum is to act as a close range attractant, eliciting an increase in the landing rate (Warnes & White, 1993; section 5) and it is considered that further investigations on probing behaviour are inappropriate.

3. Activation of three species of tsetse (*Glossina* spp.) in response to host derived-stimuli.

3.1. Introduction

Tsetse flies locate their hosts using both visual and olfactory cues (Vale, 1974b). The nature of these responses has been extensively studied and the results have contributed to the development of odour-baited traps and targets for use in tsetse control and eradication programmes (see reviews by Warnes, 1991; Colvin & Gibson, 1992).

Vale (1974b) concluded that a moving visual stimulus was an important factor in the attraction (and subsequent capture) of *G.m.morsitans*, but not so important for attracting *G.pallidipes*. Further experiments confirmed that the former species is difficult to catch at stationary odour baited traps and targets, whereas *G. pallidipes* is readily available for capture at these devices but tends to be under represented in catches from moving targets (Hargrove and Vale, 1978; Vale, 1980). By contrast little is known of the trap/host orientated responses of *G.austeni* Newstead. Studies in Kenya and on Zanzibar suggest that stationary traps are not effective against this species (Moggridge, 1949; Dr M.J.R. Hall, Pers. Comm.) although both workers reported some attraction to bait animals. These differences in behaviour are surprising since all three species belong to the *morsitans* group of the genus *Glossina*, and in some localities within the savanna woodlands of eastern and western Africa, have overlapping distributions (Jordan, 1986).

The first stage in the host-location process for any species of tsetse must be activation. This may be brought on by endogenous factors and the onset of hunger (Brady, 1972a) or by exposure to external stimuli, such as those emanating from the host (Brady, 1972b). This section concerns the relative importance of these host factors on the activity of these three species of tsetse. In addition, the effect of wind speed on flight activity of *G.m.morsitans* and *G.pallidipes* is reported, and the responses of flies from a new colony of *G.m.morsitans* originating from puparia collected in Zimbabwe in 1991 are compared with those of flies in the colony of this species maintained at Langford since 1969.

3.2. Materials and Methods

G.m.morsitans (originating from Charara, Kariba, Zimbabwe), *G.pallidipes* (originating from Lugala, Uganda), and *G.austeni* (originating from Zanzibar) were collected from laboratory colonies at Langford within 24h of emergence and maintained in the experimental room for 24h, before being fed with defibrinated pigs blood through a silicone rubber membrane (Mews *et al.* 1977). The flies were then returned to the experimental room and deprived of food until one day before 50% died of starvation; 96h for *G.m.morsitans*, 72h for *G.pallidipes*, and 48h for

G.austeni. Thus all flies were observed at approximately the same physiological state and all experiments were undertaken between 15.00 and 17.30 h.

A rack of 30 5x5x5 cm open-ended plastic boxes covered with cotton mesh at either end allowed 30 flies to be observed simultaneously in the presence or absence of odours without visual disturbance from each other. This was fitted to the outlet of a wind tunnel, consisting of a centrifugal fan and a flow straightener in a length of lay-flat plastic tubing. A video camera (COHU 4710) was placed 1 m downwind of the outlet and the whole apparatus was operated inside the wind tunnel described by Warnes (1989a) to ensure that the air was free from organic odours. Olfactory stimuli were added to the wind tunnel from an array of nine outlets placed between the centrifugal fan and the flow straightener.

The speed of the leading fan could be controlled using a rheostat and thus the wind speed passing through the observation cages could be varied. The wind speeds were recorded using a hot wire anemometer. The mean wind speed from six measurements taken at different positions in cross section of the outlet to the cages, were taken at six different rheostat settings. The resulting wind speeds were 0.04, 0.06, 0.19, 0.53, 1.81, and 3.05 m/s. The wind tunnel was operated at a wind speed of 0.53 m/s except in experiments where the effect of wind speed on flight activity was investigated.

In the first course of experiments the behaviour of flies was recorded over a four minute period with an olfactory or visual stimulus added for the duration of the third minute. The olfactory stimuli used were whole ox breath, collected as in Warnes (1990b), added at the rate of 2 l/min, or ox urine odours added by passing laboratory air at 2 l/min through a gas jar containing *c.* 200 ml of fresh urine collected from cattle at Langford. The visual stimulus was a moving stripe on a kymograph rotating at 10 revolutions/min, placed downwind of the rack of flies and visible from each box within the rack.

Two racks of flies were used on each afternoon for three days for each species, or if flies were in short supply experiments continued until sufficient flies had been observed. Males and females were observed concurrently and the position of flies within the rack was determined randomly. Fifteen minutes were allowed to elapse between each four minute test and the order of each test was changed each day in a 3X3 Latin Square design. The video tapes were analysed and the number of active flies and the number of flights per minute were recorded. Significant changes in activity were calculated by comparing the number of flights in each minute with that in the preceding minute using a Mann-Whitney U-test.

In a further series of experiments the activity of male *G.m.morsitans* and both sexes of *G.pallidipes* was recorded at six different wind speeds. The behaviour of the flies was recorded over a 60 minute period with the wind speed being changed every 10 minutes. The order of each wind speed was altered each day for six days to give six replicates of each wind speed. In the case of *G.m.morsitans* ox odour

was added to the airstream during the third minute at each wind speed. The spontaneous activity was then calculated from minutes 1-2 and 5-10 for *G.m.morsitans* and from minutes 1-10 for *G.pallidipes*, and the activity in response to ox breath odours for *G.m.morsitans* was calculated from minute 3 for each wind speed. Activity is expressed as the number of active flies per fly-minute (No. active flies / total flies x total mins), since each fly was observed for more than one minute at each wind speed.

3.3. Results

Flight activity can be recorded either as the number of flights per min or as the total number of active flies per min. The former takes account of the level of activity displayed by individual flies but fails to account for the number of flies responding to a stimulus, while the latter takes account of the number of flies responding but not the level of the response. In practice these measurements are closely correlated (here $r = 0.90$; $P < 0.001$, $n = 36$ min), but the former allows more rigourous statistical analysis (Warnes, 1989a).

The number of flights per minute made by each species are shown in Fig. 3.1. Both male and female *G.m.morsitans* showed a significant increase in activity when ox breath odours were added to the airstream, but the responses were relatively low (14% and 11% for males and females respectively) compared with that to a moving visual stimulus (43% and 33%). Both sexes of *G.pallidipes* responded strongly to ox breath odours (52% and 79% for males and females respectively) but showed no significant increase in activity in response to the moving visual stimulus. In *G.austeni* only the females responded to ox breath odours, but both males and females responded to the moving visual stimulus, although the response was much higher in males, 57% compared to 33% for females. None of the tsetse investigated showed any significant change in activity in response to ox urine odours.

Spontaneous activity can be estimated for each species from the control minutes 1 and 2, before any stimulus was applied. For *G.m.morsitans* spontaneous activity was very low (<3% for females and <7% for males), but both *G.austeni* and *G.pallidipes* displayed higher levels of activity *c.* 20%, except for female *G.pallidipes* where the spontaneous activity was in excess of 40%.

Spontaneous activity of male *G.m.morsitans* and both sexes of *G.pallidipes* was further investigated at a range of windspeeds. The activity of male *G.m.morsitans* was low (5.2%) even at the lowest wind speed (0.04 m/s) and declined to below 1.0% at wind speeds in excess of 0.52 m/s (Fig 3.2a). The number of flies active when ox odour was added to the airstream was also low (<10%) even at a wind speed of 0.04 m/s and showed a similar decline as wind speed increased (Fig 3.2b). The spontaneous activity of both sexes of *G.pallidipes* did not differ significantly in this experiment so the results were combined. Spontaneous activity in this species

was once again much higher than for *G.m.morsitans* (*c.* 45%) at wind speeds below 0.2 m/s and declined to *c.* 4% at a wind speed of 3 m/s (Fig. 3.3). Furthermore, the decline in spontaneous activity as wind speed increased was linear for *G.pallidipes*, but for *G.m.morsitans* the decline was exponential.

The low level of spontaneous activity and the low level of response to host odour in *G.m.morsitans* was further investigated by comparing results for flies from the original laboratory colony at Langford with flies from a newly established colony originating from puparia collected in Zimbabwe in 1991, to determine whether the low level of activity in this species can be explained by a decline in the activity of the laboratory strain. The percentage of active flies from each group is shown in Table 3.1. The only significant difference in activity was a greater response for wild female flies to a moving visual stimulus compared to lab flies. Otherwise the response to ox breath and the spontaneous activity in the newly colonised flies appeared to be lower than for the laboratory strain, but these differences were not statistically significant. However, of 24 comparisons 14 showed greater activity in lab flies and only 4 showed greater activity in 'wild' flies (chi-squared = 5.6, $P<0.02$), the other 6 being about the same.

3.4. Discussion

The results of these laboratory investigations are in agreement with much of the known field behaviour of these species. *G.pallidipes*, being strongly activated by olfactory stimuli but unaffected by a moving visual stimulus, is easily collected at stationary baits, but under-represented on ox fly rounds. The reverse of this is the case for *G.m.morsitans* which is strongly attracted by a moving visual stimulus, but only weakly attracted to stationary baits (Vale, 1980). From these laboratory experiments, *G.austeni* appears to fall between these two extremes. Both sexes respond to a moving visual stimulus, but only females respond to ox breath odour, indicating that its responses to host stimuli are probably more similar to those of *G.m.morsitans*. However, field data for *G.m.morsitans* have shown that it is activated by ox odour (Torr, 1988) and odours used at stationary traps and targets improve catches, although not to the same extent as for *G.pallidipes* (Vale & Hall, 1985). The response to ox breath odours in these experiments was relatively high compared to the level of spontaneous activity, and the low responses and spontaneous activity could be caused by some factors intrinsic to the laboratory.

The spontaneous activity of flies in the Langford colony of *G.m.morsitans* appears to have declined significantly since 1972, and further still since 1984. In a comparable situation Brady (1972b, Fig 4.) reported spontaneous activity in the region of 10-20% of flies active, and later Bursell (1984, Fig 4) reported spontaneous activity levels of *c.* 10-12% of flies from the same colony. In addition Bursell (*loc. cit.*) reported responses to ox odour in the region of 30%. Originally it was considered that a reduction in spontaneous activity and in the response to ox odour in the Langford colony of *G.m.morsitans* might be explained in terms of

genetic drift, as the colony has been closed since 1969 and this is investigated in section 4. The study of the behaviour of flies from the original colony with newly colonised (wild) flies showed that lab flies displayed a higher level of spontaneous activity and a higher response to ox odour, although differences for individual minutes were not significant. However, wild female flies were significantly more responsive to a moving visual stimulus than females from the old laboratory colony. The low level of spontaneous activity and the very low response to olfactory stimuli for this species in the laboratory is at present unexplained, but has been observed in many other experiments not reported here. The absence of regular and repeatable differences that are statistically significant between these two groups of flies suggests that they do not differ greatly in their behaviour and whatever is responsible, it is the same for both groups. This fails to explain the differences between the present results and those of Brady (1972b) and Bursell (1984), but in the context of this comparative study these differences are perhaps unimportant.

The high level of spontaneous activity and response to ox breath odours in *G.pallidipes* suggest that it locates its host employing a ranging strategy, moving through its habitat until host odour is encountered. When odour is encountered it changes kinetic aspects of its flight (Warnes, 1990b), and probably its flight direction (Gibson & Brady 1988) or alternatively, if the fly is resting it commences flight activity. On the other hand, *G.m.morsitans* which displays lower levels of spontaneous activity and shows a high level of response to a moving visual stimulus, probably employs a 'sit and wait' strategy, taking off in response to the visual stimulus provided by a moving host.

Ox urine odours are known to increase trap catches of both *G.m.morsitans* and *G.pallidipes* (Owaga, 1985; Dransfield *et al.*, 1986; Bursell *et al.*, 1988) but in these experiments they failed to elicit activation in either species. This is not inconsistent since different odours probably affect different behaviours involved in the host-location process.

The effect of increasing the wind speed was to reduce spontaneous activity for both *G.m.morsitans* and *G.pallidipes*, and to lower the number of *G.m.morsitans* responding to ox odour. The effect was greater for *G.m.morsitans*, spontaneous activity decreasing exponentially as wind speed increased. Presumably the energetics of flight are less efficient at high wind speeds, reducing spontaneous take-off, but if wind direction is to be used as a cue in the host-location process then host-location would be more efficient at higher wind speeds (Brady *et al.*, 1989). Given the different strategy proposed for *G.pallidipes*, one would expect the inhibitory effect of high wind speed to be reduced, since the positive effects of high wind speed in host-location would balance the extra energy used in flight.

4. An analysis of supernumerary or B-chromosomes of wild and laboratory strains of *G.m.morsitans*.

4.1. Introduction

The Presence of supernumerary or B-chromosomes in tsetse have been reported by several authors (Jones & Rees, 1982), although their effect on the phenotype of the fly has not been studied. However, it has been reported for other insect species that the number of B-chromosomes in individuals is negatively correlated with individual fitness. Population studies on the grasshopper (*Myrmeleotettix maculatus)* showed that B-chromosomes were more frequent towards the centre of a range, where conditions are favourable, whereas on the periphery, where conditions were harsher, B-chromosomes were less frequent or not found at all, since less fit individuals fail to survive (Hewitt & Brown, 1970).

Over the years a decline in certain behavioural parameters, notably the response to ox odour and a moving visual stimulus, in the laboratory colony of *G.m.morsitans* at Langford has been observed (section 3; Warnes, unpublished, John Brady pers. comm.). The present study was designed to investigate the possible role of B-chromosomes in tsetse population fitness, and in the observed behavioural decline in laboratory flies.

4.2. Materials and Methods

Puparia from the laboratory colony of *G.m.morsitans* (originating from Charara, Kariba, Zimbabwe) were collected and maintained in the insectary at Langford at 25 °C and 75% R.H. Wild female *G.m.morsitans* were collected from ox fly rounds near to the Rekomitjie Research Station in the Zambezi valley of Zimbabwe (*c.* 80 km from Kariba). Female flies were maintained in the insectary at Rekomitjie and offered blood daily on an ox. Puparia were collected and maintained in the insectary at Rekomitjie under the same conditions as at Langford.

Five to seven days post deposition puparia were dissected under saline to remove the brain. The brain was placed in culture medium (MEM) containing 1 ug/ml colchicine for 3 h, before fixing in 1% Na citrate for 10 min, and storage in 3:1 absolute methanol:glacial acetic acid mix. Later the brains were removed from storage and macerated in 60% acetic acid on an acid cleaned slide. The acetic acid was removed from the slide on a warming plate and the slides were air dried. The following day the slides were stained with 2% Toluidine blue (pH 6.8) and rinsed in a buffer solution before air drying.

The preparations were examined under a microscope and the number of supernumerary or B-chromosomes was recorded. A total of 398 pupal brains were dissected from laboratory flies in 1979 and 100 in 1991, to investigate any change

in the frequencies of B-chromosomes with time in the laboratory colony. Forty puparia were collected from wild flies in Zimbabwe and brains were fixed and brought to Langford for examination. The frequencies of B-chromosomes in the three groups were compared using analysis of variance.

4.3. Results

All the pupae examined had between two and eight B-chromosomes. The mean B-chromosome number for each group is shown in Fig. 4.1. Analysis of variance revealed significant differences between groups ($P < 0.001$) and multiple range analysis using the least significant difference test shows all three means to be significantly different from each other. The distribution frequency of B-chromosomes number is shown in Table 4.1. For the laboratory flies, 47% had 5 or more B-chromosomes in 1979, and by 1991 this had increased to 63%. However, in wild flies only 17.5% of those flies examined contained five or more B-chromosomes.

4.4. Discussion

Jordan *et al* (1977) showed that fecundity and longevity in a laboratory colony of *G.m.morsitans* originating from Zimbabwe were higher than in a colony originating from Tanzania. These authors attributed the lower productivity of the Tanzanian flies in part to the presence of supernumeraries [presumably B's although referred to as S-chromosomes (see Southern & Pell, 1973)], in the populations from Tanzania, but not from Zimbabwe. Langley *et al* (1984) also related the presence of B-chromosomes to fitness in populations of *G.pallidipes* from Uganda and Zimbabwe. They were able to colonise the flies from Uganda which possessed no B-chromosomes, but were unable to colonise flies from Zimbabwe that possessed up to three B-chromosomes per fly.

In a study on the B-chromosomes of *Glossina morsitans centralis* in Zambia Bushrod (1984) showed that the frequencies of B-chromosomes varied with season, being lower in the cold and wet seasons than in the hot dry season. One might expect that the frequencies of B-chromosomes should be highest in the hot season when the stress on the population is highest, unless there is a generation lag between conditions and B-chromosome frequency.

Tsetse flies are usually maintained in the laboratory under optimal conditions which means that populations of laboratory flies are not subjected to the same selection pressures as wild populations. It is not therefore surprising that the frequency of B-chromosomes is significantly higher in the laboratory flies compared with wild flies, even though wild puparia were collected at a time when B-chromosome frequency should be at its maximum (Bushrod, 1984). Indeed the data show that the

frequency has significantly increased over the past 12 years, and it is very likely that this reflects a reduction in the fitness of the laboratory population.

The increase in the frequency of B-chromosomes in laboratory populations of flies probably only reflects changes due to drift that have taken place since the original stock was collected over 25 years ago. Nevertheless, it suggests that regular back-crossing with wild collected flies would be advantageous since this would prevent genetic or behavioural drift as has been observed in the laboratory colony at Langford (Warnes, 1992; section 3).

5. Responses of *G.pallidipes* to sebum odours in a wind tunnel

5.1. Introduction

Field experiments suggested that an olfactory (or non-contact) response to sebum odours was in part responsible for the increased catch of tsetse at electric nets in the field (Warnes, 1990a). Therefore in order to investigate further a possible bioassay in the laboratory the system described by Warnes (1990b) and further refined by Warnes & Green (1992) was used to investigate the effect of sebum odours on various parameters of flight in *G.pallidipes*.

5.2. Materials & Methods

A number of male flies (depending on availability) that had been deprived of food for 72h were placed inside a cage of narrow depth inside the wind tunnel as described by Warnes (1990b). Concentrated sebum solution (collected as in section 2) was evaporated until no solvent could be detected by human nose, and placed in a gas jar in a water bath at *c.* 37°C. Cleaned laboratory air was passed through the jar at 2 l/min during the third minute of a four minute recording period. The effect of adding sebum odours to the airstream was compared with adding CO_2 at 1.0%, and CO_2 at 1.0% in combination with sebum odours. (N.B. concentration of CO_2 at source; this concentration of CO_2 resulted in a maximum response in previous experiments, Warnes, 1990b.) Three recordings of each test with one group of flies were made on each afternoon and the experiment was repeated on three afternoons. A total of 46 flies was observed over the three afternoons of the experiment. The behaviour of the flies was analysed from video tapes and the flight activity, speed, and sinuosity of flight were recorded. In addition, records were made of the direction of take-off of flies that were resting in the central portion of the cage.

5.3. Results

5.3.1. Activity

The numbers of flights per minute were recorded from the video and the mean flights per min +/- S.E. for each test are shown in Fig. 5.1. Analysis of variance was carried out on the data and showed that there were no significant differences with experiment, but the differences with treatments were significantly different ($P < 0.001$). Multiple range analysis showed that the control minutes and those with sebum odour added were not significantly different from each other, but both periods with CO_2, and CO_2 plus sebum showed a significant increase in activity. The presence of sebum odours did not result in a significant increase in flight activity when presented alone, and when presented with CO_2 the increased flight activity was not significantly greater than the increase in activity with CO_2 alone. However, this method of measuring activity is not ideal because of disturbance caused by other flies in the cage (Warnes, 1989a).

5.3.2. Take-off direction

The take-off direction of all flies that took-off from the central portion of the cage was recorded after the fly had travelled a distance of *c.* 15 cm, although this may not have been the true direction of the resulting flight. The results for both the control minutes, and minutes with odour were analysed to test for any directional bias using both the Rayleigh test, and Hodges and Ajne's test (Batschelet, 1981) (Table 5.1). The take-off directions were not significantly different from random, but when sebum odours were presented alone there was a tendency for the flies to take-off in an upwind direction, although the number of take-offs was low.

5.3.3 Linear velocity

The linear velocity of flight was measured as in Warnes (1990b). Control minutes 1 and 2 were grouped and compared with minutes with CO_2, sebum, and CO_2 + sebum. Mean flight speeds +/- 95% confidence intervals are shown in Fig. 5.2. Analysis of variance showed significant differences between treatments ($P < 0.001$). Multiple range analysis revealed that the linear velocity of flight with CO_2, CO_2 plus sebum, or sebum alone, showed a significant decrease compared to the control. The decrease in flight velocity in the presence of 1% CO_2 (at source) was similar to that recorded for *G.pallidipes* by Warnes (1990b). Addition of sebum odours also reduced the flight speed, but addition of sebum with CO_2 did not result in any significant change in the flight speed compared to the addition of CO_2 alone or sebum alone.

5.3.4 Sinuosity

The rate of turning or sinuosity was also recorded as in Warnes & Green (1992). The results (Fig. 5.3.) showed that the presence of sebum odours or CO_2 resulted in an increase in the sinuosity (^{o}/m) (log n +1 ANOVA, $P < 0.001$). Once again the increase in sinuosity with CO_2 (1% at source) was similar to that reported by

Warnes (1990b) but there was no evidence of any additive or synergistic effect between CO_2 and sebum odours.

5.4. Discussion

The effect of CO_2 on various parameters of flight has been previously reported (Warnes, 1989a; Warnes, 1990b) and the results presented here are in close agreement. The presence of sebum odours also resulted in a significant reduction in flight speed and in sinuosity and there was some evidence that sebum odours affected take-off direction. Warnes (1990b) discussed how kinetic changes in the flight pattern could assist in the host location process and clearly any directional change in take-off might be expected to improve the probability of finding a host assuming the wind direction can be accurately assessed.

These results tend to suggest that sebum odours do indeed have an olfactory effect, as was suggested by Warnes (1990a), but before firm conclusions can be drawn further replications are required since the number of flights included in the analysis in the presence of sebum odours was low. The fact that no additive or synergistic effect was recorded when CO_2 was present may be due to the fact that a maximum reduction in linear velocity was recorded at the same concentration of CO_2 (Warnes, 1990b), and it may be that the addition of sebum with lower concentrations of CO_2 may result in an additive or synergistic response. Sebum odours did not effect the activity of tsetse, but neither did urine odours in section 3, and if sebum odours act as 'close-range' attractants then one might expect that activity levels would not be affected since once close range to hosts *G.pallidipes* might be expected to be already active.

6. Field studies on the effect of cattle skin secretion on the behaviour of tsetse.

6.1. Introduction

The effect of cattle sebum on the behaviour of tsetse flies has been studied previously both in the laboratory (Warnes, 1989b; sections 2 & 5) and in the field (Warnes, 1990a; Packer & Warnes, 1991). The presence of sebum at catching devices in the field always resulted in an increase in catch of both *G.m.morsitans* and *G.pallidipes*, although the results were variable and not always significant, and further investigations on fractions of sebum suggested that the active components were to be found in the phenolic and non-acidic fractions (Warnes, 1990a). Warnes (*loc. cit.*) suggested that the response to sebum was at least in part olfactory (or non-contact) since contact with the treated cloth was not a prerequisite for an increased catch at an electrified target, and laboratory studies (section 5) have

shown that sebum odours result in a decrease in linear velocity of flight and an increase in the sinuosity, as has been shown to occur in the presence of ox odour and CO_2 (Warnes, 1990b). Also in the laboratory Warnes (1989b) showed that tarsal contact with a sebum treated target resulted in a reduction in the duration of contact with the target, but increased the tendency for the fly to return to the target. This was described as "feeding-site searching behaviour" (Warnes, *loc. cit.*). However, analysis of video tapes in the field (Packer & Warnes, 1991) suggested that contact with a sebum coated target resulted in an increased duration of stay as well as a greater tendency to return to the target.

The diversity of components in sebum (Smith *et al.*, 1975: McEwan Jenkinson and Mabon, 1973) probably accounts for the wide range of behaviours recorded in the presence of sebum, and it is probable that sebum contains compounds that are repellent as well as those that act as tsetse attractants. This section describes investigations into the behavioural effect of sebum on tsetse flies in the field as well as some experiments aimed at isolating the active components of sebum.

6.2. Materials and Methods

6.2.1. Experimental sites and general conditions

Experiments were performed at the Rekomitjie Research Station in the Zambezi valley, Zimbabwe, where *G.pallidipes* is abundant and *G.m.morsitans* accounts for between four and ten percent of the daily catch depending on the season. Experiments with intermittently sparking electric nets were undertaken in September, 1991 and observations on the behaviour of tsetse and further experiments with electric nets were undertaken in the following April/May. All experimental treatments were run in the presence of acetone, 1-octen-3-ol, 3-*n*-propyl phenol and 4 methyl phenol as currently used in control operations in Zimbabwe.

6.2.2. Collection of sebum

Cattle sebum was collected as in section 1, and some subsequently fractionated to give acidic, phenolic and neutral (non-acidic non-phenolic) fractions as in Warnes (1990a) except that the solvent used was chloroform instead of dichloromethane.

6.2.3. Experiments with intermittently sparking electric targets

Four $1m^2$ black cloth targets electrified over their whole surface were deployed at four sites. Treatments one and two were operated with sparking devices that ran continuously and treatments three and four were operated with the usual sparking devices controlled by a timing device (Ash Green Electronics, Langford, U.K.) that allowed the net to operate for five second periods (on-period) with a pre-set but variable off-period. Targets used in treatments one and three were treated with 100 ml of solvent and those in treatments two and four with 100 ml of sebum solution prior to each daily run that lasted for two hours just before sunset. Each treatment was moved on each day in a 4 X 4 Latin Square design. The off-period was pre-set

to 30 s for the first eight days (two replicates of the Latin Square) after which it was changed to 75 s for one block of four days and then to 180 s for another block of four days. Thus catches with and without sebum were recorded from electric targets running for 100%, 14.29%, 6.25%, and 2.70% of the time.

6.2.4. Experiments with electric nets and targets

Experiments were undertaken in April/May 1992 in an attempt to isolate active components of sebum. Initially whole sebum was compared on three different net/target arrangements (Fig. 6.1). Arrangements 1 and 2 had been used previously (Warnes, 1990a) but arrangement 3 was new and allowed all flies that landed on the target to make tarsal contact. This experiment was run as a 6 X 6 Latin Square replicated once. A further experiment was undertaken using five net/targets of arrangement 3, with either 100 ml of chloroform (control) or solutions of sebum, and acidic, phenolic, and non-acidic fractions of sebum in chloroform applied to the target each day. The experiment was run as a 5 X 5 Latin Square design replicated once. The results were analysed using a multi-factor ANOVA.

6.2.5. Observations of tsetse on targets

Observations on the behaviour of tsetse flies on a $1m^2$ black target were made from *c.* 16.25 - 17.15 local time, from an observation pit in mopane woodland. Five different target treatments were used and 100 ml of either chloroform (control) or a solution of sebum or acidic, phenolic, or non-acidic fraction of sebum in chloroform, were applied to the target each day five minutes prior to the commencement of observations. One treatment was observed each day in a randomized block of five days. This was replicated twice to give three observations for each treatment. As flies landed on the target they were identified to species and sex and their duration of contact with the target was recorded to the nearest second with a stop watch. If the duration of stay was less than 1 second identification was not possible and these flies were not included in the analysis. If flies were seen to land a second time the duration of the second - *n*th landings were recorded as was the number of landings made by each fly. In addition flies walking on the target were also recorded.

6.3. Results

6.3.1. Experiments with intermittently sparking electric targets

The detransformed mean catch of male and female *G.m.morsitans* and *G.pallidipes* at a 1 m^2 electrified black target with and without sebum as the duration of the off-period increased are shown in Fig. 6.2 a and b. In this experiment electrified targets with sebum consistently caught more flies than those without sebum.

If flies landed only once and for a constant duration, less than the on-period, then the predicted catch would decrease in direct proportion to the duration of the off-period. The predicted catch was calculated for both species and sexes with and without sebum and compared with the observed catch using a chi-squared test. All

observed values differed significantly from the predicted values ($P<0.01$ for *G.m.morsitans* females without sebum, and $P<0.001$ for all other comparisons). The decay in catch as the off-period increased was then compared with and without sebum using an R X C Chi-squared test for each species and sex. The differences were significant for both sexes of *G.pallidipes* ($P<0.001$) but not for *G.m.morsitans*. So, for *G.pallidipes* either the number of landings or the duration of each contact with the target was increased in the presence of sebum, whereas for *G.m.morsitans* this was not the case, although the catch was significantly increased with sebum suggesting that there must be an olfactory or a distant response to sebum in this species.

6.3.2. Experiments with electric nets and targets

In April/May 1992 further experiments were undertaken on the effect of sebum and fractions of sebum on the catch of tsetse at electrified nets and targets in an attempt to isolate the active components of sebum. A preliminary experiment was conducted to investigate three different net arrangements with and without sebum (Fig. 6.1). The results are shown in Table 6.1. In this experiment nets with sebum always caught more tsetse flies than nets without sebum, but the results were variable and not always significant. However, arrangement 3 seemed to produce respectable increases in the catch of both species. Therefore this arrangement was deployed to investigate the three fractions of sebum. In a further experiment five nets with targets attached as in arrangement 3 (Fig. 6.1) were deployed and treated daily with either solvent (control), sebum, or acidic, phenolic, or neutral fractions of sebum. The experiment was run as a 5 X 5 Latin Square replicated once. The results are shown in Table 6.2. None of the treatments resulted in a significant increase in the catch of either species of tsetse although both sebum and the non-acidic fraction of sebum caught significantly more muscids than the control treatment.

6.3.3. Observations of tsetse on targets

The durations of stay on a target by tsetse flies are distributed exponentially. Therefore the data were analysed using a Kruskal-Wallis non-parametric analysis of variance followed by comparisons of individual treatments with a Mann-Whitney U-test. The presence of sebum on the target significantly reduced the duration of stay for both species of tsetse (Table 6.3 and 6.4). Of the three fractions of sebum tested only the non-acids consistently reduced the duration of stay. The phenolic fraction also significantly reduced the duration of stay for *G.m.morsitans* but not for *G.pallidipes* (Table 6.4).

Another method of visually comparing the duration of stay with treatments is to use a log survivorship plot. The slope of this plot at any point is equal to the probability of a fly departing within the next very short period of time. The log survivorship plot for male *G.pallidipes* is shown in Fig 6.3. The results are very similar to data obtained from laboratory investigations (see Warnes 1989b, Fig. 5) and show an increased probability of leaving a target when sebum is present.

The data for the number of repeat landings are underestimated as only one side of the target was observed and many flies would have moved out of view before returning or would have returned to the other side of the target. However, this should not affect comparisons between the treatments since the same conditions prevailed in all cases. The numbers of repeat landings recorded did not differ significantly between the sexes of *G.pallidipes* and too few female *G.m.morsitans* landed more than once to analyse males and females separately and therefore the sexes for each species were grouped (Table 6.5). For *G pallidipes* sebum and the non-acid fraction of sebum significantly increased the number of repeat landing whereas the acid fraction significantly reduced the number of repeat landings, but for *G.m.morsitans* none of the differences were significant although similar trends were apparent.

The proportion of flies walking on the target did not differ between the sexes and they were therefore grouped. However, Chi-squared analysis did not reveal any significant differences with treatments.

6.4. Discussion

The present results underline the variable nature of any effect sebum has on the catch of tsetse at electrified targets. Catches in 1991 were significantly increased by the presence of sebum, whereas in 1992 the increases were variable and not statistically different. However, that sebum has significant effects on the behaviour of tsetse is confirmed from experiments with intermittently sparking electrified targets as well as from direct observations, but why this is not always related to target catch is unexplained.

The increased catch at an intermittently sparking electrified target with sebum present indicates that sebum either increases the duration of stay or increases the number of times a fly lands before leaving a target, either of which would increase the probability of capture (see Green *et al.*, 1993). Direct observations show that the former actually decreases for both species, and therefore any increase in the catch must be caused by a greater tendency for flies to make repeated landings, as indeed was observed for *G.pallidipes*, but not for *G.m.morsitans*. However, the record of the number of repeat landings from direct observations is not accurate as only one side of the target was observed, furthermore the probability of recording an incorrect value increases as the number of landings/fly increases. The number of repeat landings required to explain the results from intermittently sparking electric nets can be calculated from observations and by using the following formula from

Green *et al.* (1993):

(i) $p=\frac{(a+c)}{(a+b)}$

where p= probability of capture

a= on-time of net

b= off-time of net

c= contact time of fly

By substituting the duration of each recorded stay for each fly (assuming each landing is a separate fly) the probability of capture for each fly can be calculated. If the duration of a landing is greater than the off-period (b) then the probability of capture is one. The mean of these probabilities for all contacts observed will give the proportion of flies visiting an intermittent electric net which would be captured if the fly visited only once. Following the reasoning of Green *et al.* (1993), if the mean probability of capture given one contact is P, the proportion actually captured (relative to the continuous net) P', and the number of contacts n is given by:

(ii) $P' = 1-(1-P)^n$ and $n = \frac{\log (1-P')}{\log (1-P)}$

Estimates of n (the number of landings) required at the intermittent sparking electric nets using the data from direct observations are shown in Table 6.6. Since in the observation experiments flies that landed for less than one second were ignored from the analysis, the values of n will be underestimated, thus accounting for estimates of n that are less than one for females of both species. Despite this caveat, it is clear that the number of landings for both species is increased by approximately the same amount when sebum is present (3.3 -3.6 times for *G.m.morsitans* and 1.9 - 2.2 times for *G.pallidipes*).

Direct observations in the field confirm the laboratory observations of Warnes (1989b) in that sebum reduced the duration of each contact, but are at variance with the field video study of Packer & Warnes (1991). Furthermore, observations revealed no differences in the proportions of tsetse walking on targets when sebum was present, as was reported by these authors. Warnes & Finlayson (1985) showed that for *Stomoxys calcitrans* (L.), a muscid fly, the presence of sebum on a target resulted in both increased durations of stay, and flies walking over the target for long periods. While it remains a possibility that the differences between the present results and those of Packer & Warnes (1991) could be caused by differences in the concentration of sebum used, the results of Warnes & Finlayson (1985) suggest that it is more likely that many of the tsetse they recorded were in fact not tsetse but other muscid flies. This suggestion is supported by the plethora of muscids observed in these experiments performing similar behaviour to that reported by

Packer & Warnes (1991). Many of these flies would be easily confused with tsetse on video tapes.

In experiments employing direct observations the non-acidic fraction of sebum had similar results to whole sebum for both species and for *G.m.morsitans* the phenolic fraction also significantly reduced the duration of stay but the acidic fraction had no effect. These observations may explain the results of Warnes (1990a) but none of the three fractions had a significant effect on the catch of tsetse in experiments undertaken concurrently with the observations.

In conclusion sebum clearly has demonstratable effects on the behaviour of both *G.m.morsitans* and *G.pallidipes* causing a decrease in the duration of each landing and an increase in the number of landings per fly. The responsible compounds appear to be present mainly in the non-acidic fraction, and there is evidence at least for *G.pallidipes*, that the acidic fraction actually reduces the number of landings per fly. However, translating these behavioural effects into repeatable increases in trap catch, or increases in the number of flies caught at an electrified target or net has proved to be a very laborious process.

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8. Summary and Conclusions

Section 2. Probing responses of tsetse to skin secretions.

1) Several attempts were made to develop a simple bioassay based on the probing response of tsetse to investigate active fractions of sebum.
2) The presence of sebum on a substrate did not consistently increase the probing response in *G.pallidipes*, but an increase in the substrate temperature did.
3) More flies probed on 'rough' felt than on a smooth cotton drill, but once again there was no significant increase when sebum was present.

4) Warthog eye secretion did not elicit a probing response in *G.pallidipes*.
5) It was concluded that a simple assay such as one based on the probing response is inappropriate since the results are so variable and unreliable.

3. Activation of three species of tsetse in response to host derived-stimuli

6) A system was developed to monitor the activity of 30 flies concurrently without visual disturbance from each other
7) The spontaneous activity of *G.m.morsitans* recorded in a wind tunnel was very low (less than 4% of males and 2% of females active per min during control periods). That of *G.austeni* and *G.pallidipes* was in the region of 20% except for *G.pallidipes* females when in excess of 40% were active during control periods.
8) Addition of ox urine odours to the airstream had no effect on activity in any of the species investigated but addition of ox breath odours to the airstream significantly increased activity of *G.pallidipes* and of *G.m.morsitans*, although for the latter only *c.* 12 % of flies were active. For *G.austeni* the addition of ox breath odours resulted in a significant increase in activity of females but not of males.
9) A moving visual stimulus resulted in a significant increase in the activity of both sexes of *G.austeni* and *G.m.morsitans* but no change in the activity of *G.pallidipes*.
10) The low level of spontaneous activity and the low response to ox breath odours in a strain of *G.m.morsitans* maintained in the laboratory since 1969 was compared with a new colony of this species which originated from puparia collected in Zimbabwe in 1991. No differences in either spontaneous activity or the response to ox breath odour was recorded, but females from the new colony were significantly more responsive to a moving visual stimulus.
11) The activity of *G.m.morsitans* and *G.pallidipes* was recorded at varying wind speeds. For both species, activity decreased as the wind speed increased.
12) It is thought that these results may reflect the different host-location strategies used by these species.

4. An analysis of supernumerary or B-chromosomes of wild and laboratory strains of *G.m.morsitans*.

13) The numbers of supernumerary or B-chromosomes of wild and laboratory strains of *G.m.morsitans* were recorded and compared with those recorded from the Langford colony 12 years ago.
14) All pupae examined had between 2 and 8 B-chromosomes.
15) Wild flies had significantly fewer B-chromosomes than laboratory flies.
16) The number of B-chromosomes in the laboratory flies has increased significantly over the past 12 years.
17) The presence of high numbers of B-chromosomes in laboratory populations probably reflects a reduced population fitness.

5. Responses of *G.pallidipes* to sebum odours in a wind tunnel

19) The odours of sebum in a wind tunnel did not affect the level of activity in *G.pallidipes* but the presence of an increased concentration of CO_2 resulted in an increase in flight activity.
20) Both the odours of sebum and CO_2 resulted in a reduction in the linear velocity of flight and an increase in the sinuosity of flight in *G.pallidipes*.
21) There was some evidence to suggest that sebum odours elicit upwind take-off.

6. Field studies on the effect of cattle skin secretion on the behaviour of tsetse.

22) The effect of sebum on tsetse catches at an electrified target was variable; although the catch of both *G.m.morsitans* and *G.pallidipes* was usually increased this was not always significant.
23) The catch of both species of fly at an intermittently sparking electrified target was greater than would be predicted if all flies landed only once for a consistent short duration.
24) As the off-period of an intermittently sparking electric net increased the catch decreased; the rate of decrease was significantly slower when sebum was present for *G.pallidipes* but not for *G.m.morsitans*, indicating that for *G.pallidipes* sebum either increases the duration of stay on the target or the number of times a fly returns to the target.
25) Direct observations of flies on cloth targets revealed that for both species the presence of sebum reduced the duration of contact and for *G.pallidipes* the number of return contacts was increased.
26) The non-acidic fraction of sebum had the same effect on both species as whole sebum.
27) The results from direct observations on tsetse were used to predict the number of repeat landings that would need to be made by flies in order to account for the catch of tsetse at intermittently sparking electric targets.
28) The biological implications of the results and the possible use of sebum with tsetse traps is discussed.

9. General Conclusion

Unfortunately the original objectives of the project proved to be unachievable. Firstly because of problems encountered with laboratory flies, particularly *Glossina morsitans* Westwood. This difficulty was investigated by an analysis of the B-chromosomes of wild and laboratory flies (see section 4) and by comparing the activity of wild and laboratory flies (section 3); the results have now been published (appendix 1). Secondly it was not possible to develop a reliable probing assay that

gave repeatable results. The variable results obtained from investigations on probing behaviour are reported in section 2. Changes in kinetic aspects of flight in *G.pallidipes* Austen were recorded in response to sebum odours (section 5) and studies were undertaken on the behaviour of tsetse around sebum baited targets in the field (section 6); some of these results have been submitted for publication (appendix 1). Unfortunately the field results showed serious variability in the catches of both *G.m.morsitans* and *G.pallidipes* at a sebum baited targets and it was not possible to identify active fractions or components of cattle sebum. However, the strong behavioural responses recorded by tsetse in response to sebum suggest that further field based studies may prove to be fruitful. Whether these responses could be exploited to increase trap catches or to improve the effectiveness of targets and whether the cost effectiveness of such studies would justify the research remains conjectural.

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Table 2 Percent *G.pallidipes* probing control cloth and cloth treated with warthog eye secretion

h starved	96
Control	.5
Warthog eye secretion	2

Table 3.1: Percent wild and laboratory strain *G.m.morsitans* active per min over three four min periods with olfactory or visual stimuli applied during the third min of each test. * indicates the only significant increase in the total No of flights per min between groups ($P < 0.001$).

Test/Min	Females		Males	
	Lab	Wild	Lab	Wild
No. flies (*n*)	89	85	87	70
Ox breath				
1	1.12	1.18	1.15	2.86
2	2.25	0.00	1.15	0.00
3	11.24	7.06	13.79	5.71
4	3.37	3.53	3.45	1.43
Ox urine				
1	1.12	1.18	5.75	1.43
2	1.12	1.18	6.90	1.43
3	1.12	1.18	3.45	1.43
4	0.00	2.35	4.60	1.43
Moving stripe				
	1.12	0.00	4.60	0.00
2	2.25	2.35	3.45	0.00
3	32.58	52.94*	42.53	37.14
4	12.36	24.71	27.59	20.00

Table 4.1: Distribution frequencies of b-chromosomes in laboratory bred and wild *G.m.morsitans* pupae.

Group	2	3	4	5	6	7	8	Total	
Lab 1979									
n		4	53	154	119	63	4	1	398
%		1.0	13.3	38.7	29.9	15.8	1.0	0.3	100
Lab 1991									
n		1	6	30	43	16	4	0	100
%		1.0	6.0	30.0	43.0	16.0	4.0	0.0	100
Wild 1991									
n		0	9	24	5	2	0	0	40
%		0	22.5	60.0	12.5	5.0	0.0	0.0	100

Table 5.1: Take-off direction (TO) of *G.pallidipes* during control minutes 1,2 and 4 (summed for all tests) and with CO_2, Sebum, and Sebum + CO_2: 90^o is directly upwind.

	n-value	Mean TO direction	Mean vector length (r)	Rayliegh test significance	Hodges & Ajne's significance
min 1	16	4.8	0.073	P>0.900	P>0.900
	23	239	0.189	P=0.480	P=0.818
min 4	8	148	0.339	P=0.410	P=0.875
	12	309	0.256	P=0.454	P=0.645
Sebum	11	63	0.397	P=0.174	P=0.097
Sebum+ CO_2	16	176	0.166	P=0.671	P>0.900

Table 6.1: Detransformed Log mean catch of tsetse and other muscids with electrified target/net arrangements with and without sebum. The catch indices (test catch/control catch) are shown in parentheses and * indicates significant differences at $P < 0.05$.

Grid (Fig. 3.1)	Sebum	Sex	*G.m.m.*	*G.p.*	Other muscids
1		M	16.1	105.7	
		F	14.5	144.5	
		M+F	31.6	252.5	
1	+	M	16.1(0.00)	133.9(1.27)*	
	+	F	15.9(1.10)	186.5(1.29)*	
	+	M+F	32.7(1.03)	323.3(1.28)*	60.8(1.80)*
2		M	18.3	156.8	
		F	24.2	259.6	
		M+F	43.0	447.7	
2	+	M	19.3(1.05)	178.0(1.14)	
	+	F	33.5(1.38)*	328.6(1.27)	
	+	M+F	54.3(1.24)*	511.9(1.14)	95.2(1.76)*
3		M	14.5	103.5	
		F	16.3	207.0	
		M+F	31.8	311.6	
3	+	M	18.4(1.27)	126.3(1.22)	
	+	F	20.5(1.25)	299.6(1.45)*	
	+	M+F	39.1(1.23)	429.5(1.38)*	51.0(2.49)*
Overall effect of sebum					
Treatment					
1-3		M	16.2	119.8	
		F	17.9	205.1	
		M+F	35.1	327.9	33.5
4-6	+	M	17.9(1.10)	144.5(1.21)*	
	+	F	23.2(1.30)	263.9(1.29)*	
	+	M+F	41.1(1.17)	414.0(1.26)*	66.6(1.99)*

Table 6.2: Detransformed Log mean catch of tsetse and other muscids with sebum and fractions of sebum compared with a control of solvent only. The catch indices (test/control) are shown in parenthesis and * indicate significant differences at $P < 0.05$.

Test	Sex	*G.m.m.*	*G.p.*	Other muscids
Control	M	21.07	296.78	
	F	30.34	235.65	
	M+F	52.09	543.38	53.49
Sebum	M	25.42(1.21)	315.73(1.06)	
	F	33.30(1.09)	279.22(1.18)	
	M+F	60.42(1.16)	597.55(1.10)	87.43(1.63)*
Acids	M	19.50(0.93)	296.67(0.94)	
	F	28.82(0.95)	261.60(1.11)	
	M+F	49.59(0.95)	561.08(1.03)	37.57(0.70)
Non-acids	M	23.60(1.12)	263.36(0.80)	
	F	31.04(1.02)	203.26(0.86)	
	M+F	57.04(1.10)	468.79(0.86)	96.07(1.80)*
Phenols	M	21.76(1.03)	274.04(0.92)	
	F	32.88(1.08)	257.46(1.09)	
	M+F	54.45(1.05)	533.69(0.98)	35.85(0.67)

Table 6.3: Median duration of each landing in seconds (with range) for species and sexes of tsetse with various treatments added to the screen prior to observations.

Species	Sex	*n*	Median	Range
Control				
G.pallidipes.	M	103	5	1-118
	F	114	8	1-192
	M+F	217	6	1-192
G.m.morsitans	M	56	21.5	1-494
	F	6	22	5-77
	M+F	62	21.5	1-494
Sebum				
G.pallidipes.	M	157	3	1-160
	F	97	5	1-68
	M+F	254	3	1-160
G.m.morsitans	M	37	6	1-240
	F	17	3	1-67
	M+F	54	5.5	1-240
Acid-fraction				
G.pallidipes.	M	62	5	1-96
	F	107	6	1-352
	M+F	169	6	1-352
G.m.morsitans	M	29	20	1-668
	F	9	2	1-17
	M+F	38	12	1-668
Non-acid fraction				
G.pallidipes.	M	174	4	1-135
	F	117	5	1-125
	M+F	291	4	1-135
G.m.morsitans	M	49	6	1-165
	F	17	4	1-37
	M+F	66	5	1-165
Phenol fraction				
G.pallidipes.	M	90	5	1-73
	F	138	7	1-335
	M+F	228	6	1-114
G.m.morsitans	M	26	12	2-91
	F	13	7	1-222
	M+F	39	7	1-222

Table 6.4: Probability levels of the duration of stay on a target by tsetse flies with treatments. Data for all five treatments are compared using a Kruskal-Wallis non-parametric ANOVA, and individual treatments were compared with the control treatment using a Mann-Whitney U-test. A, control; B, sebum; C, acid fraction; D non-acid fraction; E phenol fraction.

Species	Sex	K-W ANOVA	Mann-Whitney			
			A-B	A-C	A-D	A-E
pallidipes	M	0.001	0.001	N.S.	N.S.	N.S.
	F	0.05	0.05	N.S.	0.01	N.S.
	M+F	0.001	0.001	N.S.	0.01	N.S.
morsitans	M	0.001	0.001	N.S.	0.001	0.05
	F	0.10	0.05	0.05	0.05	N.S.
	M+F	0.001	0.001	N.S.	0.001	0.01

Table 6.5. Number of flies landing *n* times on a black target with treatments of a) control, b) sebum, c) acidic fraction, d) non-acidic fraction, and e) phenolic fraction of sebum. *P*-values refer to R x C chi-squared test for each treatment compared to the control: values for 3-8 landings were grouped.

G.pallidipes

Test	*n*	1	2	3	4	5	6	7	8	*P*-value
a)	156	115	30	7	2	-	1	1		
b)	150	89	37	14	4	3	3	-	-	0.05
c)	139	120	10	7	2	-	-	-	-	0.01
d)	169	101	40	16	6	3	1	1	1	0.01
e)	176	139	26	8	2	1	-			

G.m.morsitans

a)	33	20	8	1	1	-	2	1	
b)	30	16	6	7	1	-			
c)	20	10	5	3	1	1			
d)	36	15	14	5	2	-			
e)	24	17	3	1	2	1			

Table 6.6: Estimated values of *n* (the number of landings) made by flies at an intermittently sparking electric target with and without sebum. The data are calculated from the actual catch at an intermittently sparking target with three different off-periods (Fig. 1 a+b) compared to a continuously sparking electric net, and durations of landings from observations of flies on a target (see text).

species	*sex*	(No.Flies)/Mean *n* (Est. No. landings)	
		Control	Sebum
G.pallidipes	M	56/1.50	37/3.22
G.pallidipes	F	103/0.72	157/1.43
G.m.morsitans	M	56/1.49	37/4.97
G.m.morsitans	F	6/0.60	17/2.14

Fig. 2.1: Percentage *G.pallidipes* probing on black cotton cloth treated with sebum solution or solvent only, at room temperature and at 37°C, with increasing starvation.

Fig. 2.2: Percentage *G.pallidipes* probing on black felt treated with sebum solution or solvent only, at room temperature and at 37°C, with increasing starvation.

Fig 3.1: Total flights per min for three species of tsetse recorded over three four minute periods with olfactory or visual stimuli presented for the duration of the third minute. *n* - values for females and males respectively: *G.m.morsitans* 89 and 87, *G.pallidipes* 76 and 85, *G.austeni* 78 and 86. Significant differences comparing number of flights per fly with the preceding minute are indicated, * $P<0.05$, ** $P<0.01$, *** $P<0.001$.

Fig 3.2: Activity of male *G.m.morsitans* in the wind tunnel at increasing wind speeds. a) Spontaneous activity during control minutes (n = 1392 fly-mins). b) Response to ox breath odour (n = 174 fly mins). Each fly min represents one fly observed for one minute.

Fig 3.3: Spontaneous activity of male and female *G.pallidipes* in the wind tunnel set at increasing wind speeds (n = 360 fly mins). Each fly min represents one fly observed for one minute.

Fig 4.1: Mean frequency of b-chromosomes per fly +/- S.E. from laboratory and wild populations of *G.m.morsitans*. All groups are significantly different from each other (ANOVA $P<0.001$), n-values as in Table 1.

Fig. 5.1: Flight activity of male *G.pallidipes* (number of flights per min) +/- S.E. over a four minute period with test odour added in min 3. a) 1% CO_2 at source b) sebum odour c) sebum odour and 1% CO_2 at source. N = 138 flies.

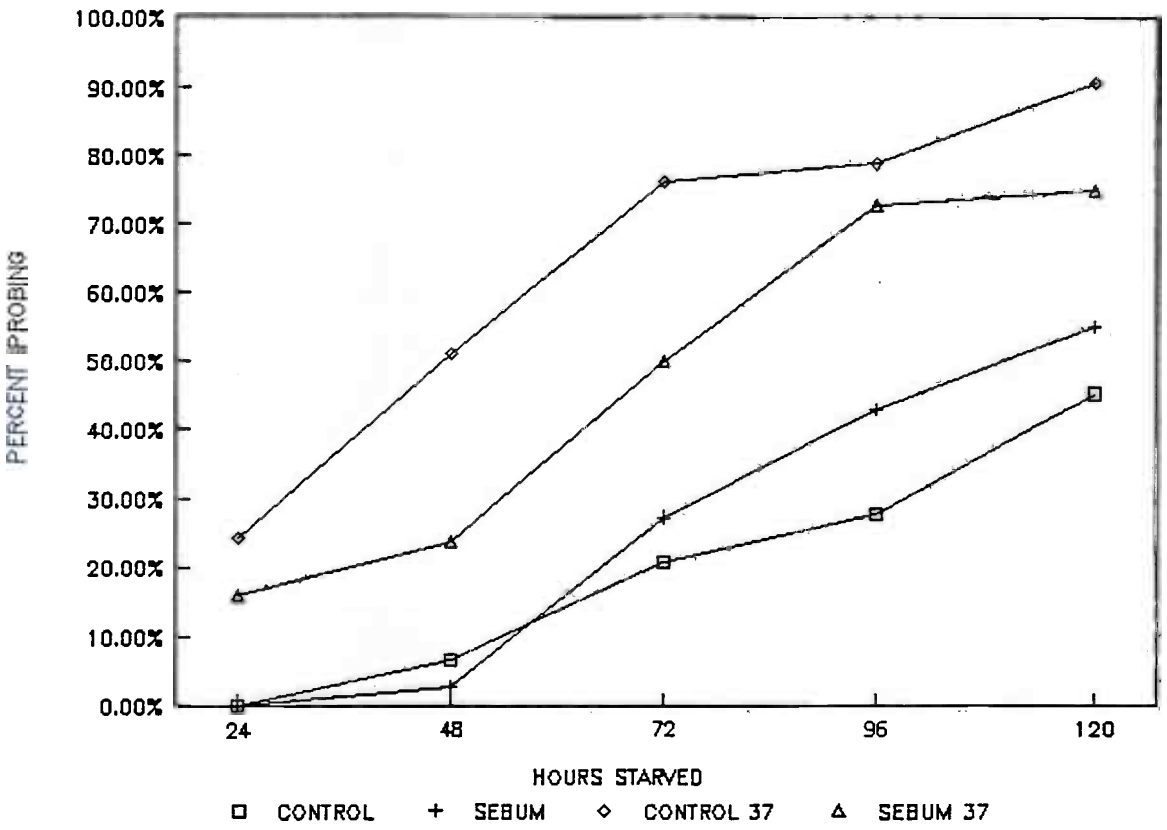
Fig. 5.2: Mean linear velocity of flight of male *G.pallidipes* during control minutes 1+2 and with test odours added to the airstream.

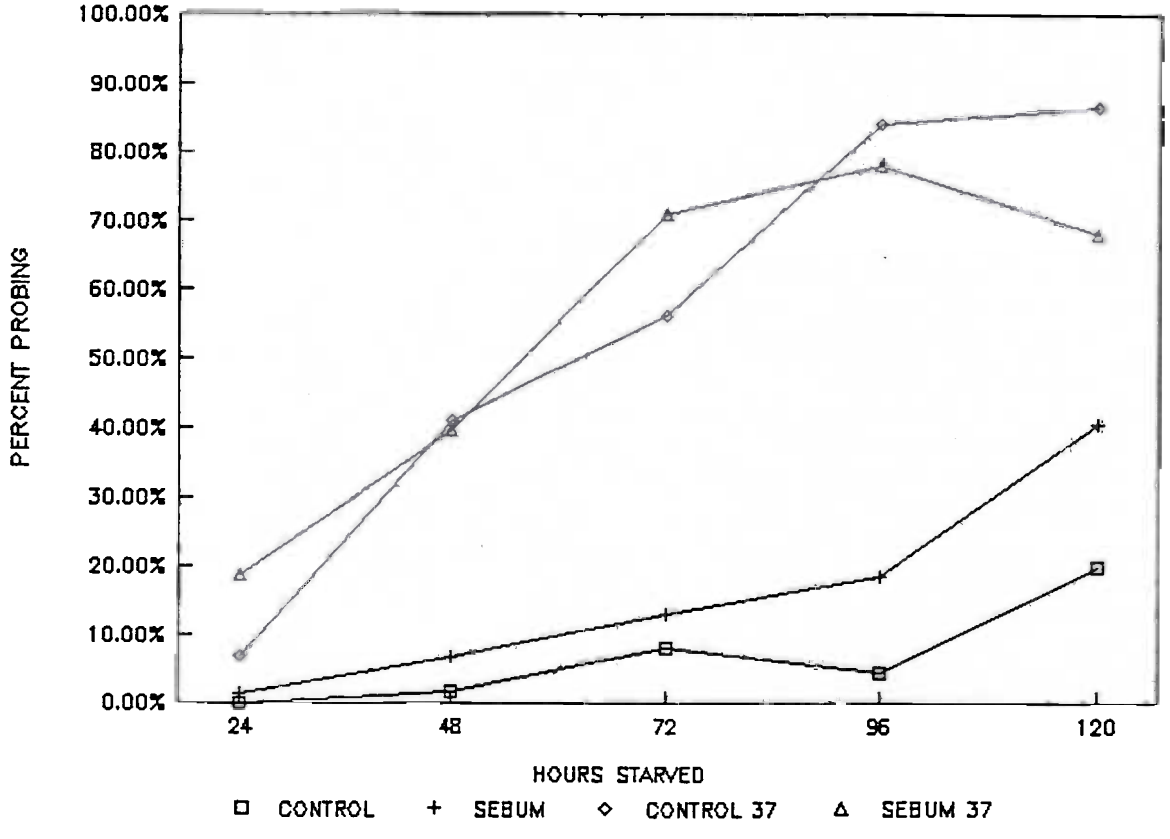
Fig. 5.3: Log (n+1) mean sinuosity (^{o}/m) of male *G.pallidipes* during control minutes 1+2 and with test odours added to the airstream.

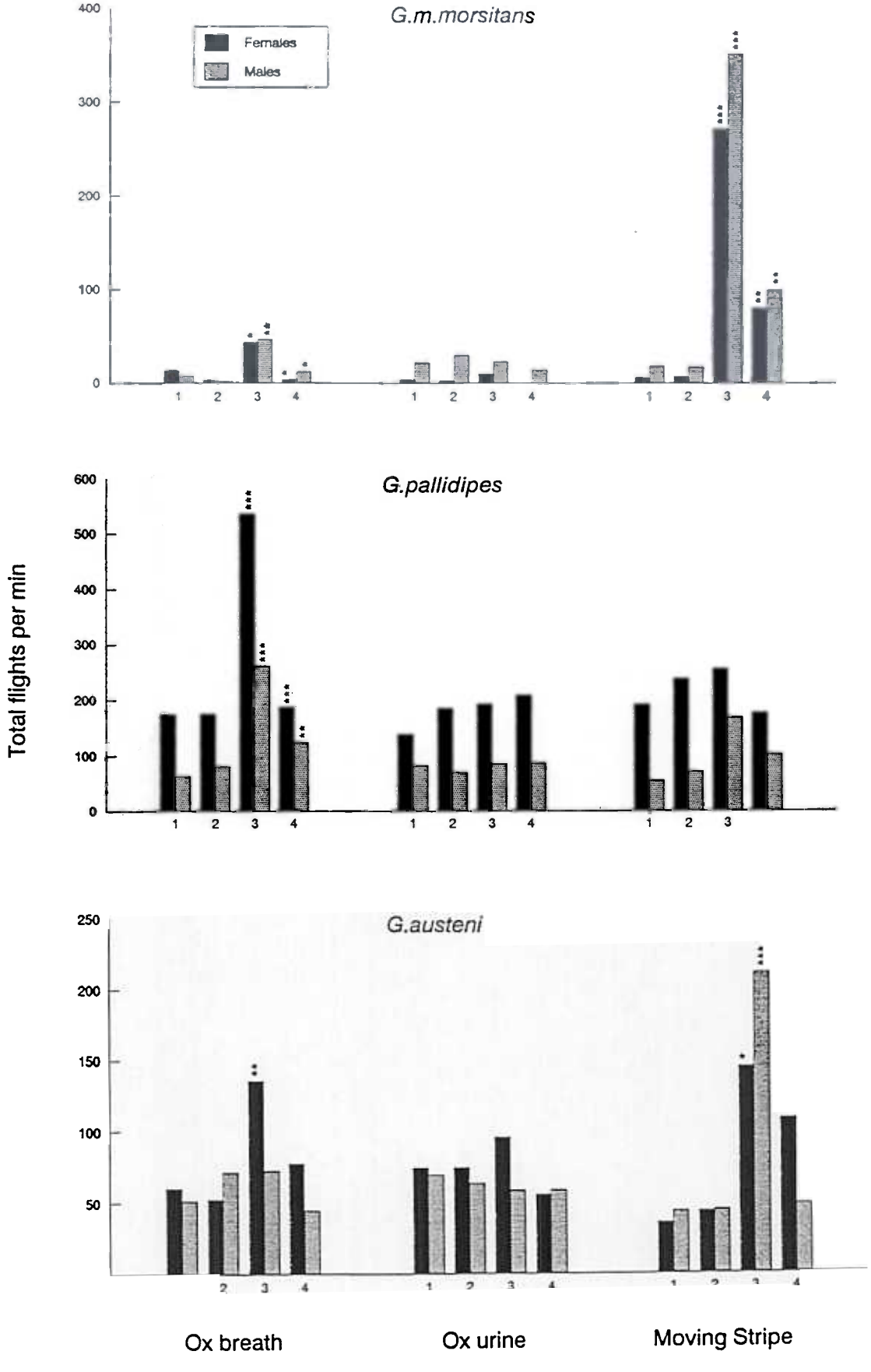
Fig. 6.1: Arrangements of electrified net/targets used. Solid represents black cloth and hatched represents mosquito netting and +/- represents electrified portions. Targets 1-3 were operated with 100 ml solvent (chloroform), and targets 4-6 were operated with 100 ml sebum solution.

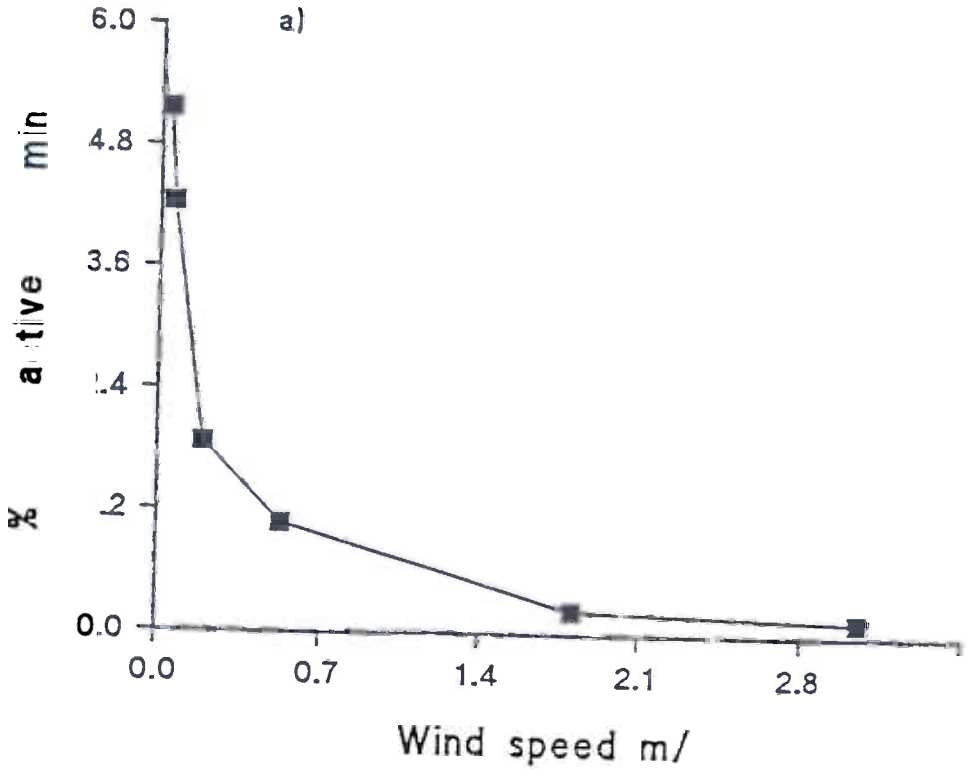
Fig. 6.2: Detransformed mean catch of tsetse +/- 95% C.I. at a 1 m^2 electrified target running intermittently, as the duration of the off-period increases. a) *G.m.morsitans*, b) *G.pallidipes*. Triangles represents results with sebum and squares represents results without sebum.

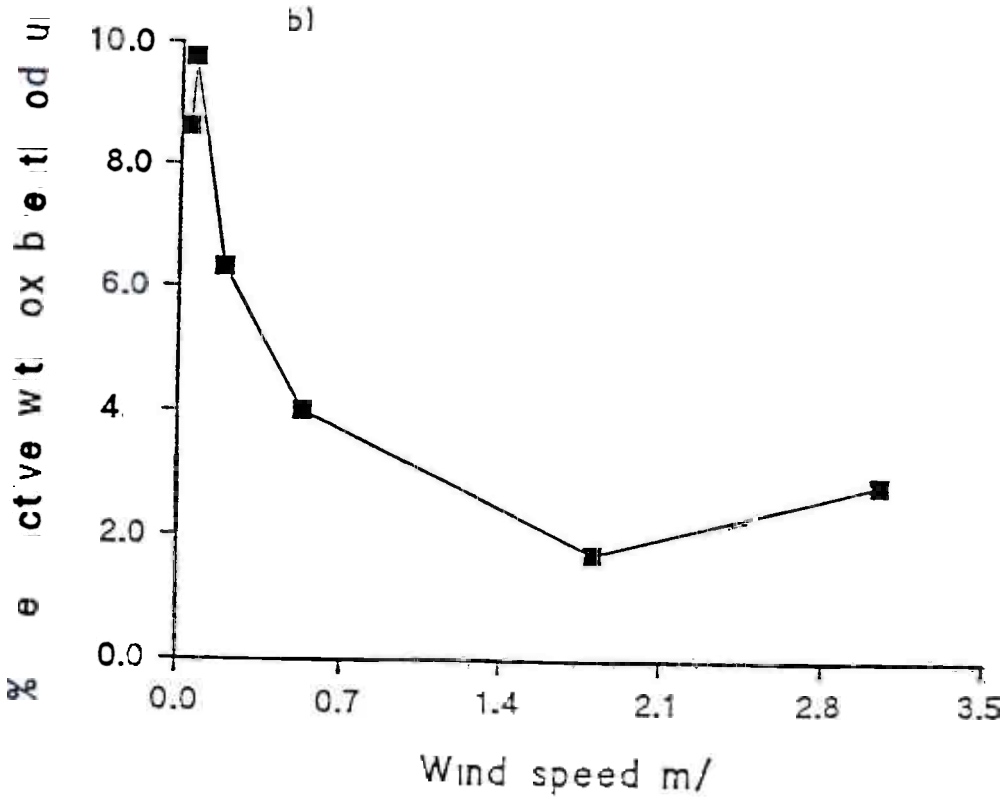
Fig. 6.3: Log survivorship plot of the duration of stay by *G.pallidipes* males on a black cloth target with and without sebum. Triangles represents results with sebum and squares represents results without sebum.

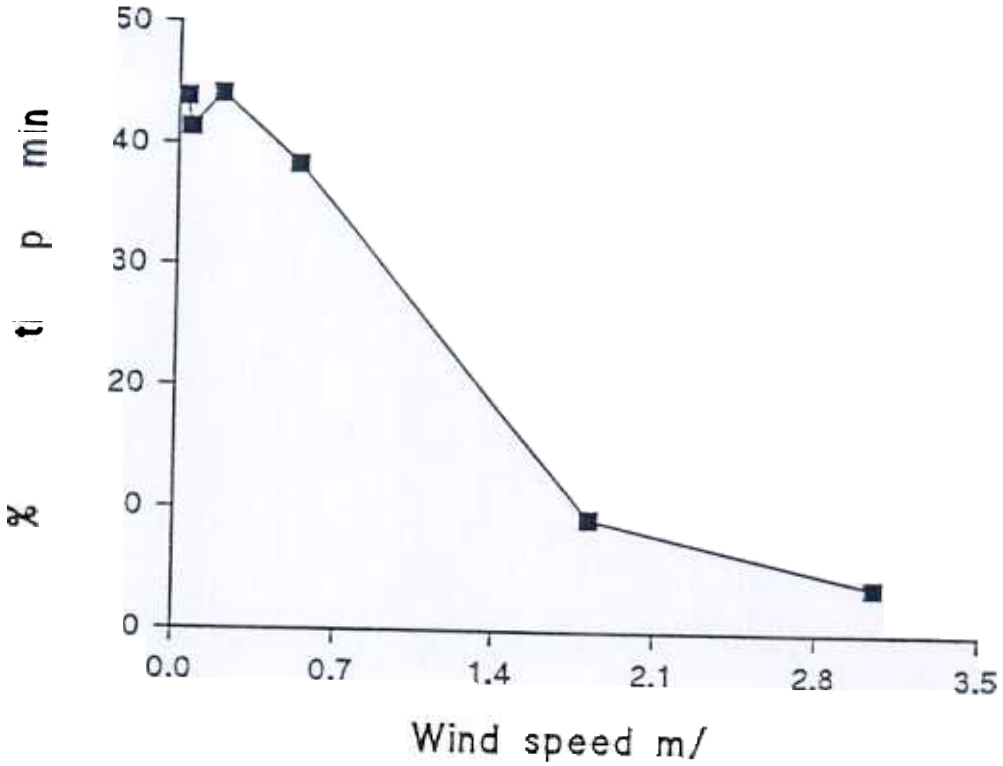

100.00%
90.00%
80.00%
70.00%
60.00%
50.00%
40.00%
30.00%
20.00%
10.00%
0.00%
PERCENT PROBING
24
48
72
96
120
HOURS STARVED
CONTROL
SEBUM
CONTROL 37
SEBUM 37

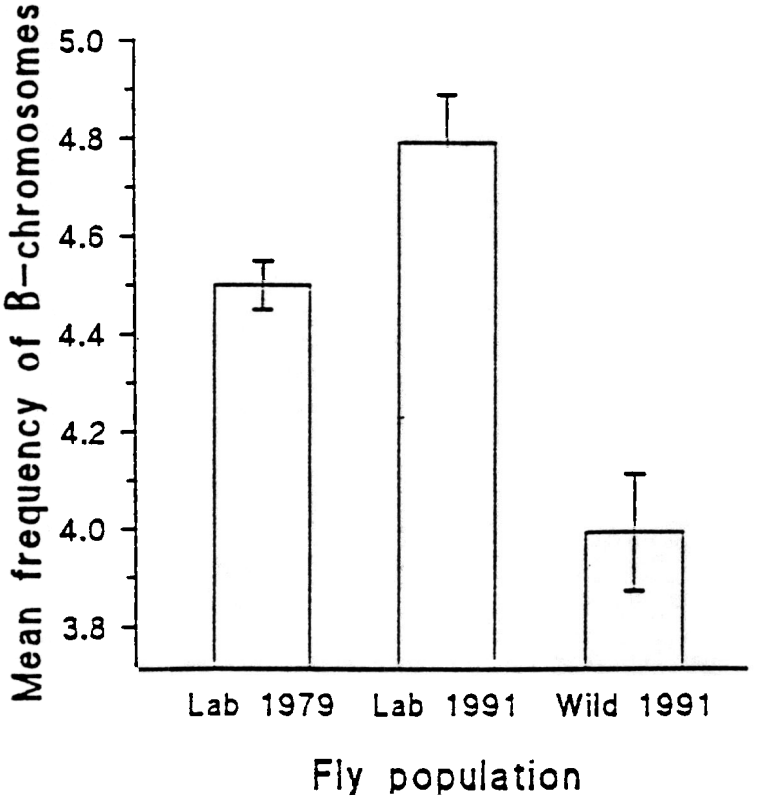

100.00%
90.00%
80.00%
70.00%
60.00%
50.00%
40.00%
30.00%
20.00%
10.00%
0.00%
PERCENT PROBING
24
48
72
96
120
HOURS STARVED
CONTROL
SEBUM
CONTROL 37
SEBUM 37

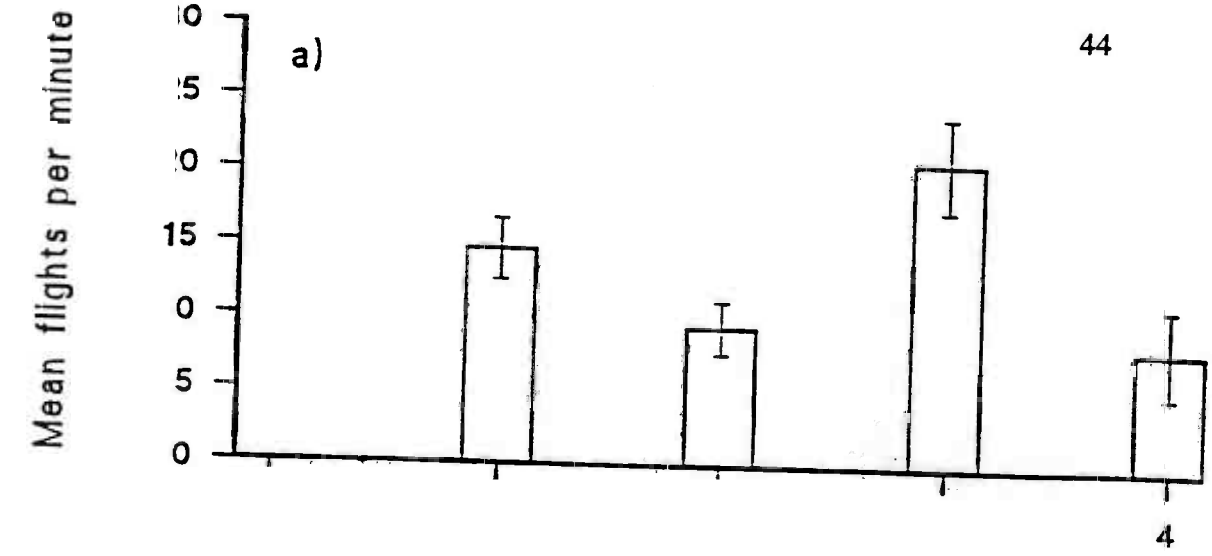

G.m.morsitans
Females
Males
400
300
200
100
0
1 2 3 4 1 2 3 4 1 2 3 4
G.pallidipes
600
500
400
300
200
100
0
1 2 3 4 1 2 3 4 1 2 3
Total flights per min
G.austeni
250
200
150
100
50
2 3 4 1 2 3 4 1 2 3 4
Ox breath
Ox urine
Moving Stripe

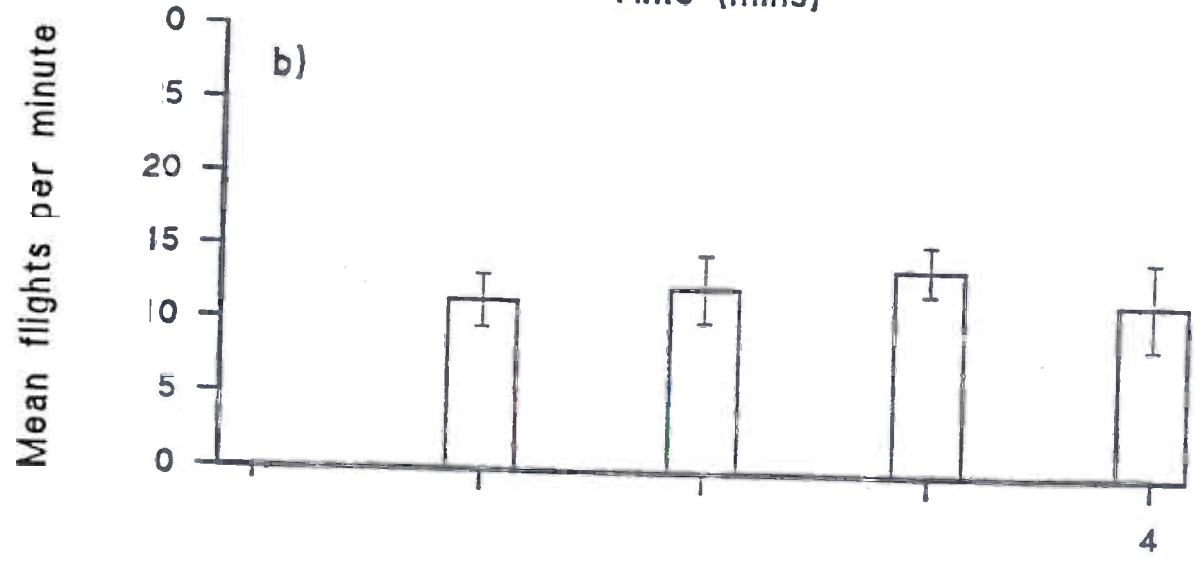

a)
6.0
4.8
3.6
0.0
0.0
0.7
1.4
2.1
2.8
Wind speed m/

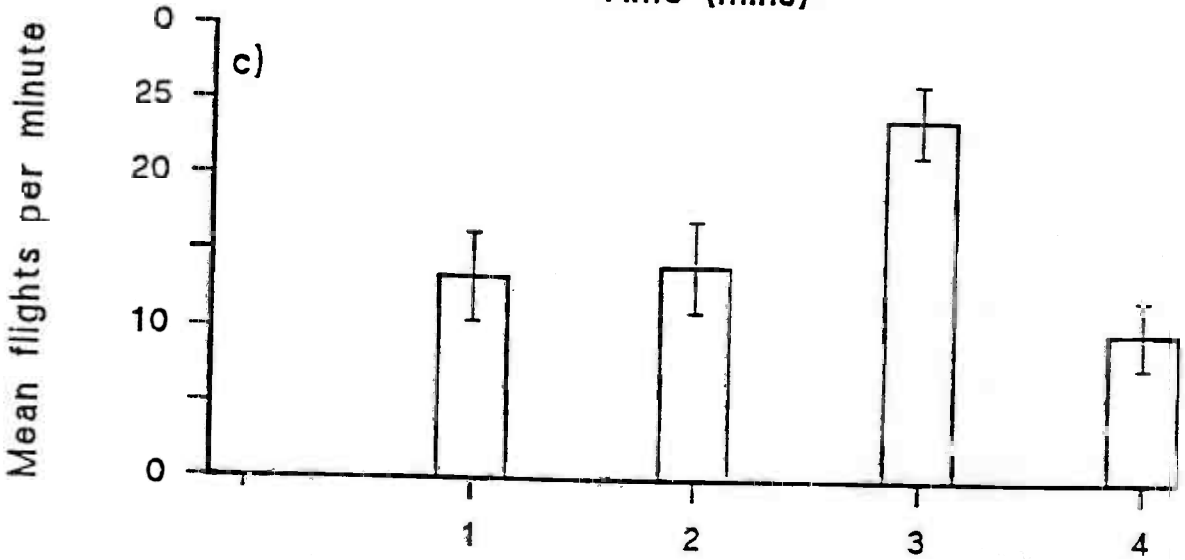

b)
10.0
8.0
6.0
4
2.0
0.0
0.0
0.7
1.4
2.1
2.8
3.5
Wind speed m/

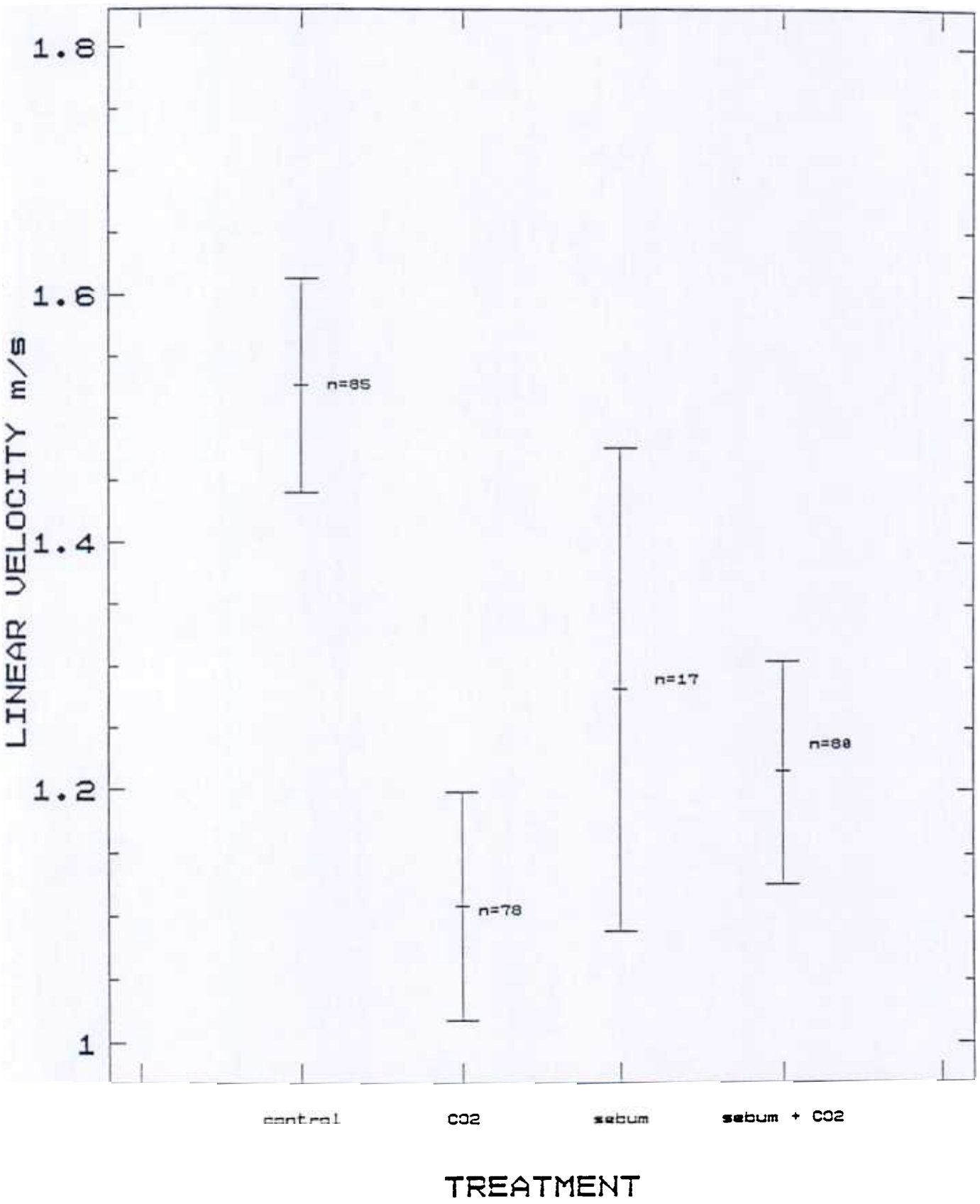

50
40
30
20
0
0
0.0
0.7
1.4
2.1
2.8
3.5
Wind speed m/

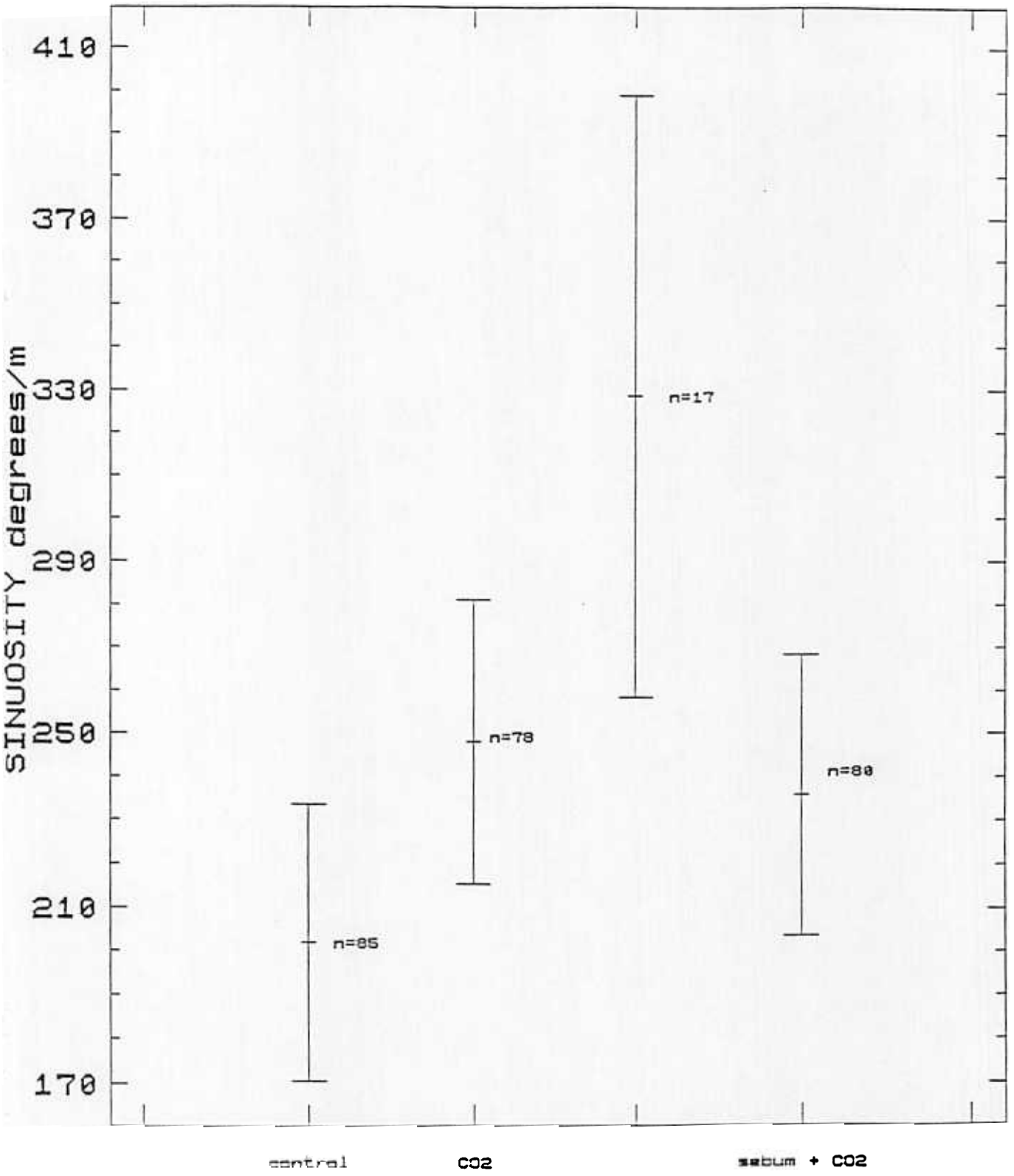

Mean frequency of B-chromosomes
5.0
4.8
4.6
4.4
4.2
4.0
3.8
Lab 1979
Lab 1991
Wild 1991
Fly population


a)
Mean flights per minute
0
5
15
0
5
0
4
Time (mins)

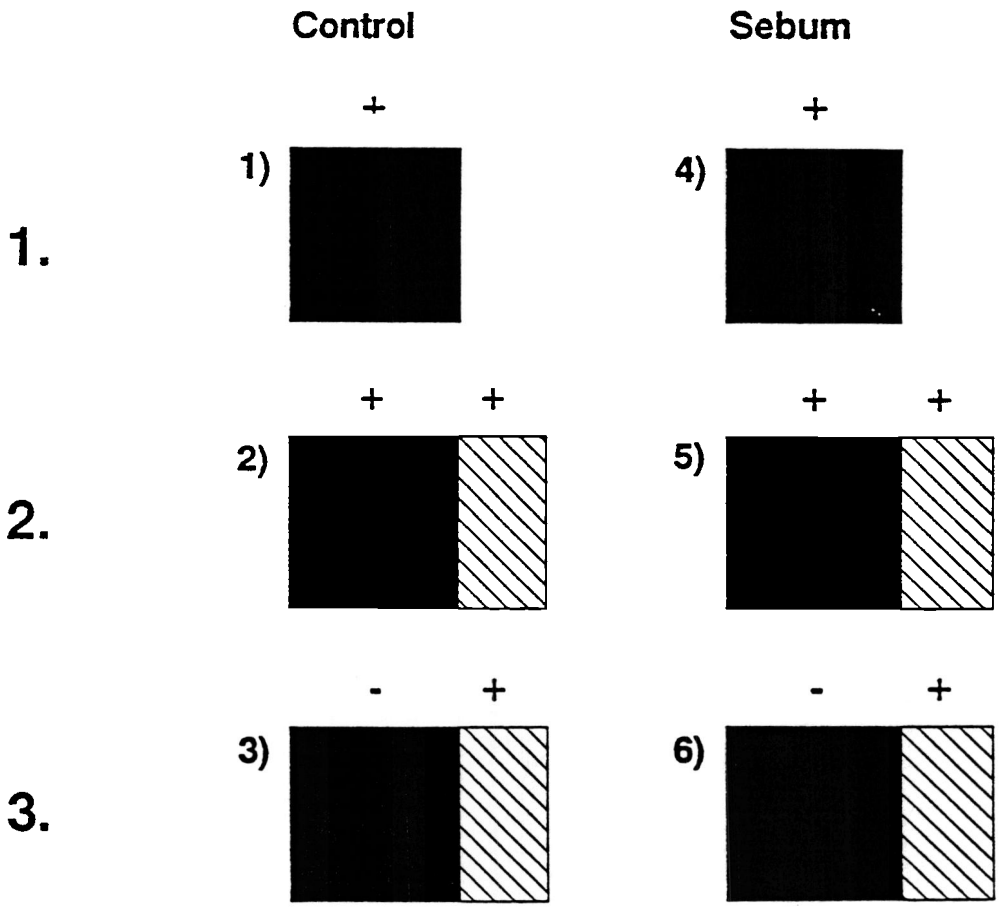

b)
Mean flights per minute
0
5
20
15
10
5
0
4
Time (mins)

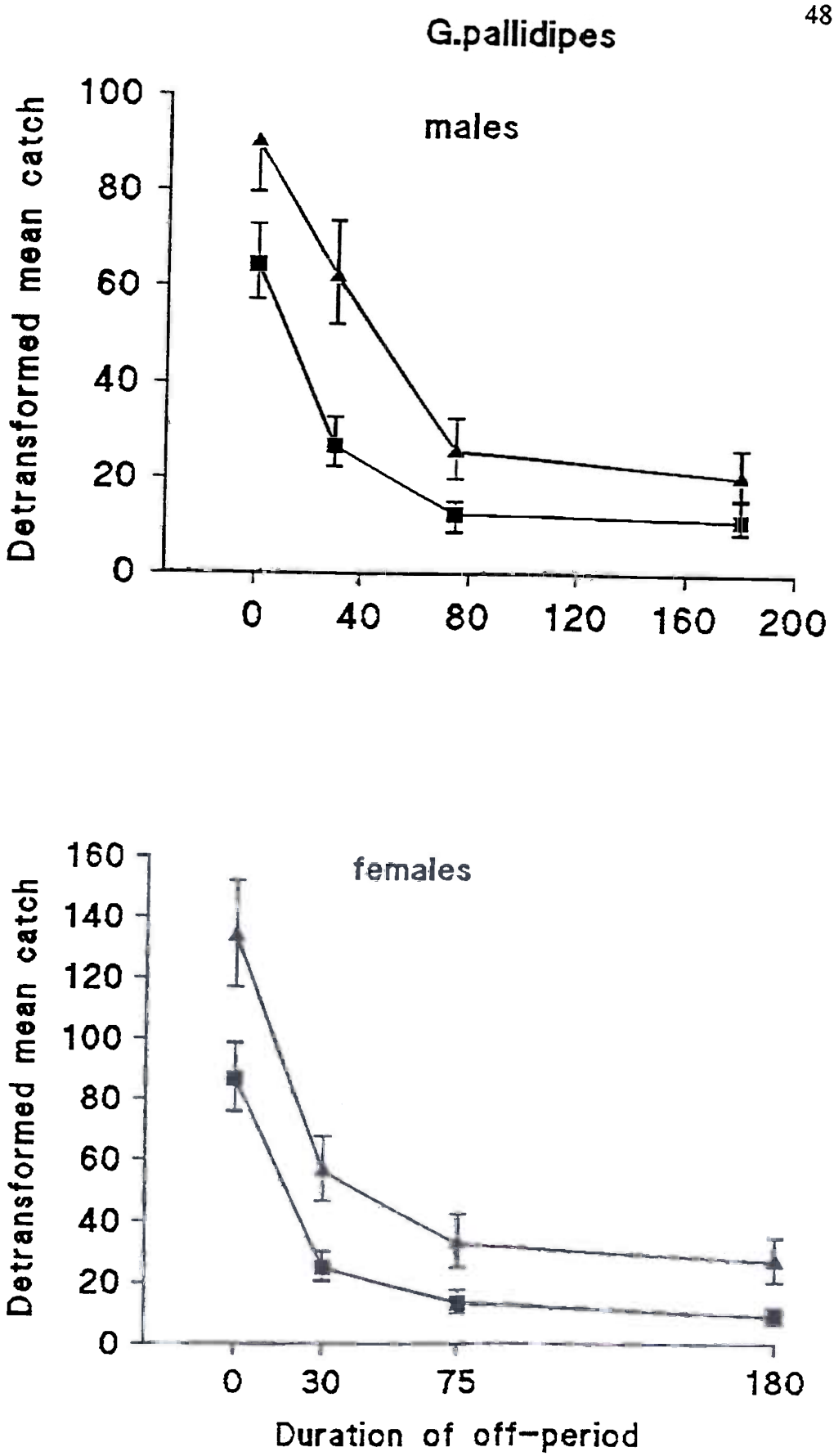

c)
Mean flights per minute
0
25
20
10
0
1
2
3
4
Tim min

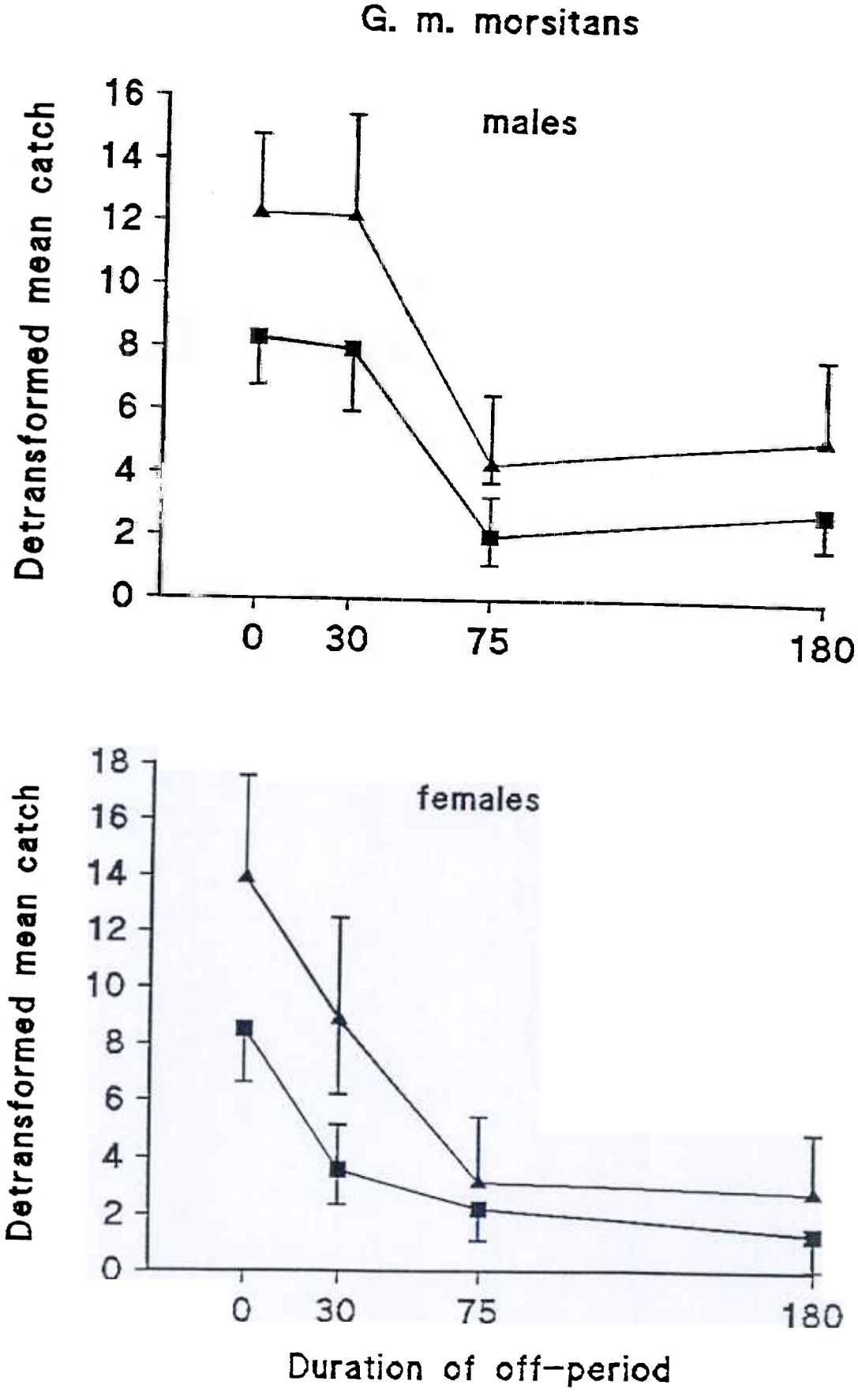

1.8
1.6
1.4
1.2
1
LINEAR VELOCITY m/s
n=85
n=78
n=17
n=80
control
CO2
sebum
sebum + CO2
TREATMENT

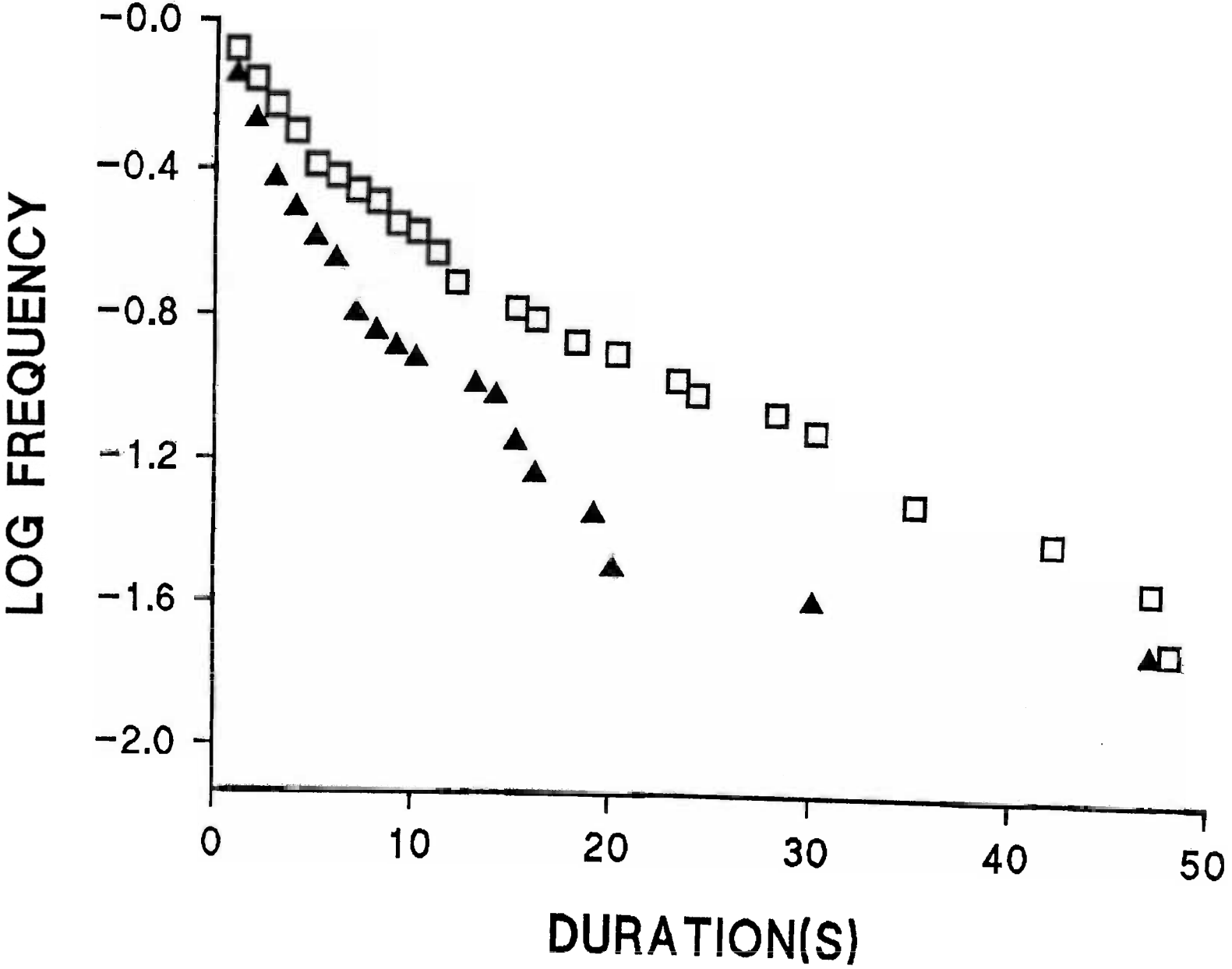

410
370
330
290
250
210
170
SINUOSITY degrees/m
n=85
n=78
n=17
n=80
control
CO2
sebum + CO2
TREATMENT

Net/target arrangements




G.pallidipes
males
Detransformed mean catch
100
80
60
40
20
0
0
40
80
120
160
200
females
Detransformed mean catch
160
140
120
100
80
60
40
20
0
0
30
75
180
Duration of off-period


G. m. morsitans
males
females
Detransformed mean catch
Detransformed mean catch
0
2
4
6
8
10
12
14
16
18
0
30
75
180
Duration of off-period


LOG FREQUENCY
-0.0
-0.4
-0.8
-1.2
-1.6
-2.0
0
10
20
30
40
50
DURATION(S)

Appendix 1

Publications

Original papers:

Packer, M.J. & **Warnes, M.L.** (1991) Responses of tsetse flies to ox sebum - a video study in the field. *Medical and Veterinary Entomology*. **5**, 23-27.

Warnes, M.L. & Green, C.H. (1992) Responses of female New World screwworm flies, *Cochliomyia hominivorax*, to swormlure-4 in the laboratory. *Medical and Veterinary Entomology*. **6**, 98-102.

Green, C.H. & **Warnes, M.L.** (1992) Responses of female New World screwworm flies, *Cochliomyia hominivorax*, to coloured targets in the laboratory. *Medical and Veterinary Entomology*. **6**, 103-109.

Warnes, M.L., and Maudlin I. (1992) An analysis of the supernumerary or B-chromosomes of wild and laboratory strains of *G.morsitans.morsitans*. *Medical and Veterinary Entomology*, **6**, 175-176.

Warnes, M.L. (1992) Activation of three species of tsetse (*Glossina* spp.) in response to host derived-stimuli. *Medical and Veterinary Entomology*, **6**, 000-000.

Revue articles:

Warnes, M.L. (1991) The control of the savanna species of tsetse flies using odour baited traps and targets. *Pesticide Outlook*, **2**, 32-35.

Papers submitted for publication

Warnes, M.L. & White, R.D. - Field studies on the effect of cattle skin secretions on the behaviour of tsetse. - Submitted 01.10.92.