

A field trial of pyriproxyfen-treated targets as an alternative method for controlling tsetse (Diptera: Glossinidae)

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Abstract

A juvenile hormone mimic (pyriproxyfen) was used with odour-baited targets to assess its suitability for controlling tsetse flies (*Glossina* spp.). In August 1991, 41 odour-baited targets identical to those used with insecticide in tsetse control operations, were each treated with 4 g of pyriproxyfen and deployed near Rekomitjie Research Station, Zambezi Valley, Zimbabwe, in a 12.3 km² block of woodland habitat of *Glossina morsitans morsitans* Westwood and *G. pallidipes* Austen. After three months, emergence rates from puparia of the two species collected in the block fell to 34% and 20% of control levels; 50 and 70%, respectively, of puparia of the two species collected were found, on dissection, to show arrested development. Changes in mean ovarian age and wing-fray category in the tsetse population during the trial were due partially to the pyriproxyfen and partially to high mortality, in the larval/pupal stages and in young adult flies, which occurs each year in the hot/dry season. Chemical analysis of cloth samples indicated that after four months 68–85% of the pyriproxyfen had been lost, a large proportion apparently dripping off the bottom of the target. If the technical problem of persistence can be solved pyriproxyfen could substitute for pesticides in target-based tsetse control operations.

Introduction

In an earlier paper (Hargrove & Langley, 1990) it was established that odour-baited traps treated with the juvenile hormone mimic, pyriproxyfen (Sumitomo Chemical Company) could be used to produce high levels of sterility in field populations of the tsetse flies *Glossina morsitans morsitans* Westwood and *G. pallidipes* Austen. Two questions needed to be answered before embarking on large scale field trials to test pyriproxyfen as a chemical for tsetse control. Firstly, can pyriproxyfen be transferred effectively from males to females during mating in the field? This is a prerequisite if the chemical is to have any advantage over the insecticides currently in use (Langley & Weidhaas, 1986). Secondly, can

pyriproxyfen be used on targets instead of traps and, in particular, will it be sufficiently durable to compete with pyrethroids in terms of the cost of re-treating targets? The first question will be answered elsewhere (Hargrove & Langley, unpublished data); here we report on the results of a small scale field trial using pyriproxyfen treated targets against the same populations of tsetse as for the earlier trapping trial.

Materials and methods

Treatment block and method

The treatment area (fig. 1) was described by Hargrove & Langley (1990). The 'targets' (Vale *et al.*, 1985) consisted of a central panel of black cotton, 70 cm wide by 100 cm high, with a 50 cm panel of terylene netting on either side. The material was tied to an 8 gauge

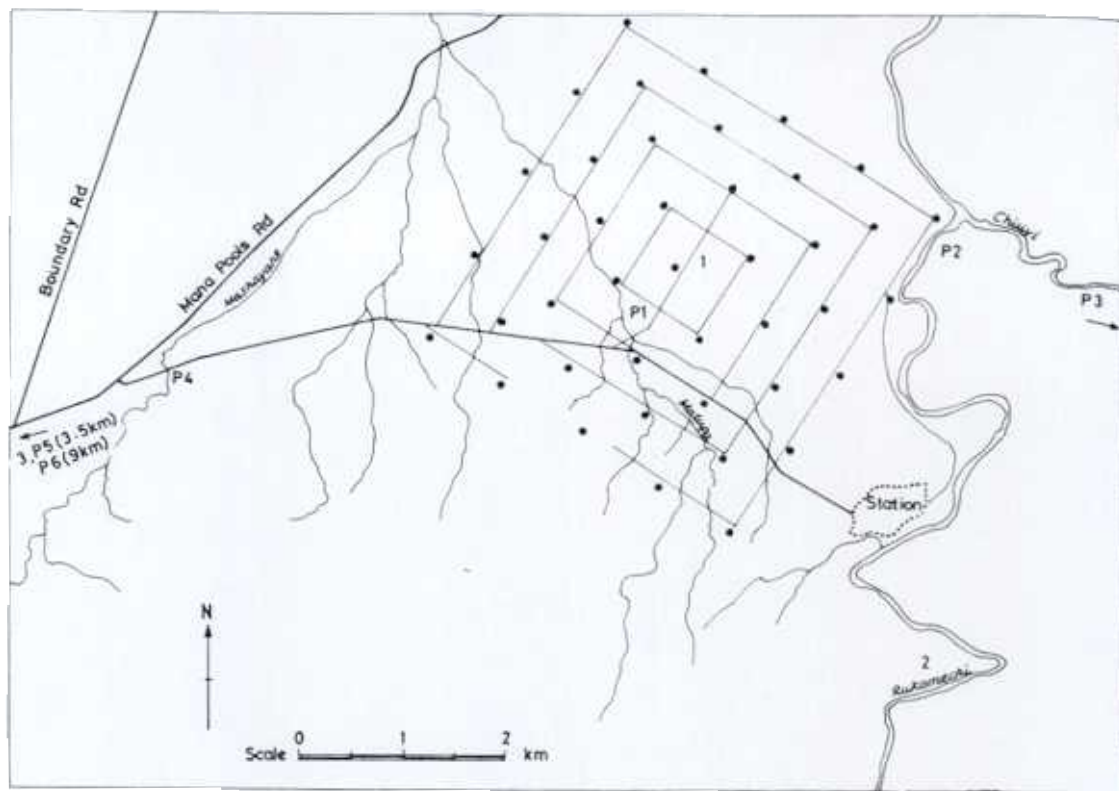


Fig. 1. Map of the study area in the region of Rekomitjie Research Station, Zambezi Valley, Zimbabwe. Odour-baited targets treated with pyriproxyfen were deployed at the sites indicated by dots. Puparia were collected along dry river beds in the location of the sites marked P1–P6. Adult females for ovarian dissection were sampled using odour-baited traps or the VET in the region of Sites 1 (centre of square) and 3 (Ruyese River).

wire frame, asymmetrically pivoted on a steel support which allowed the target to rotate freely into the wind. Prior to deployment the target fabrics were immersed in a mixture of 50% Cereclor S45 (Imperial Chemical Industries UK) in acetone containing 1% pyriproxyfen. This deposited about 4 g of pyriproxyfen on the target — distributed in the approximate ratio 3:1 between the cloth and netting (Langley *et al.*, in press). The targets were deployed at exactly the same sites as for the trap trial and baited, as before, with acetone, 1-octen-3-ol

(octenol), 3-n-propyl phenol and 4-methyl phenol as olfactory attractants (Vale *et al.*, 1988).

Collection of puparia

Puparia were collected each month from the dry beds of six rivers in the Rekomitjie area (table 1; fig. 1) by a team of five workers. Emergence of tsetse, or parasitoids, from the puparia was monitored and the unhatched puparia were dissected when no further emergence of tsetse could be expected. The stage of

Table 1. Details of puparial collections. Disposition of sites and their distances from the centre of the treatment block.

Site	River	Distance from centre (km)		Dates searched
		range	mode	
P1	Matupa	0.8–1.5	1	28.7, 2.8, 25.8, 5.9, 27.9, 5.10, 25.10, 3.11
P2	Rukomechi	2–4.5	2.5	1.8, 30.8–1.9, 1.10, 4.10, 26.10, 31.10
P3	Chiuyi	3–6	4	31.7, 28.8, 29.8, 4.9, 29.9, 30.9, 3.10
P4	Mashayene	3.5–6.5	5	30.7, 27.8, 2.9, 28.9, 28.10
P5	Ruyese	10–12	11	29.7, 3.8, 26.8, 3.9, 24.9, 2.10, 27.10, 2.11
P6	Mukondore	16–17	16.5	5.9, 23.9, 1.11

development inside the puparial case (see Langley *et al.*, 1988) was judged according to the method of Bursell (1959) and the results were lumped into the following broad categories of development: 1. 'Nil'. 2. 'Arrested' at Bursell's stage III 3. Beyond this stage (classified as 'complete').

The probability of emergence (from unparasitized puparia) for each site was corrected by dividing by the probability of emergence for puparia collected from the site most distant from the treatment block. Searching for puparia was abandoned after heavy rains flooded local rivers in late November. In January to February 1992 female *G. pallidipes* were collected from bait oxen near Site 2 (fig. 1) and near the centre of the treatment block. The flies were maintained in the laboratory, fed on oxen,

and the puparia produced were monitored for emergence rates.

Sample age distributions

The ages of tsetse sampled in the Rekomitje area were judged by ovarian dissection (Challier, 1965) and the degree of wing fray (Jackson, 1946). Tsetse were caught using odour-baited 'epsilon' traps (Hargrove & Langley, 1990) or an electric net mounted on the back of a Land Rover truck — this system is termed here the 'VET'. A fuller understanding of the effect of pyriproxyfen treated baits (i.e. traps as used in 1989 and targets as used in the present study) was derived from the

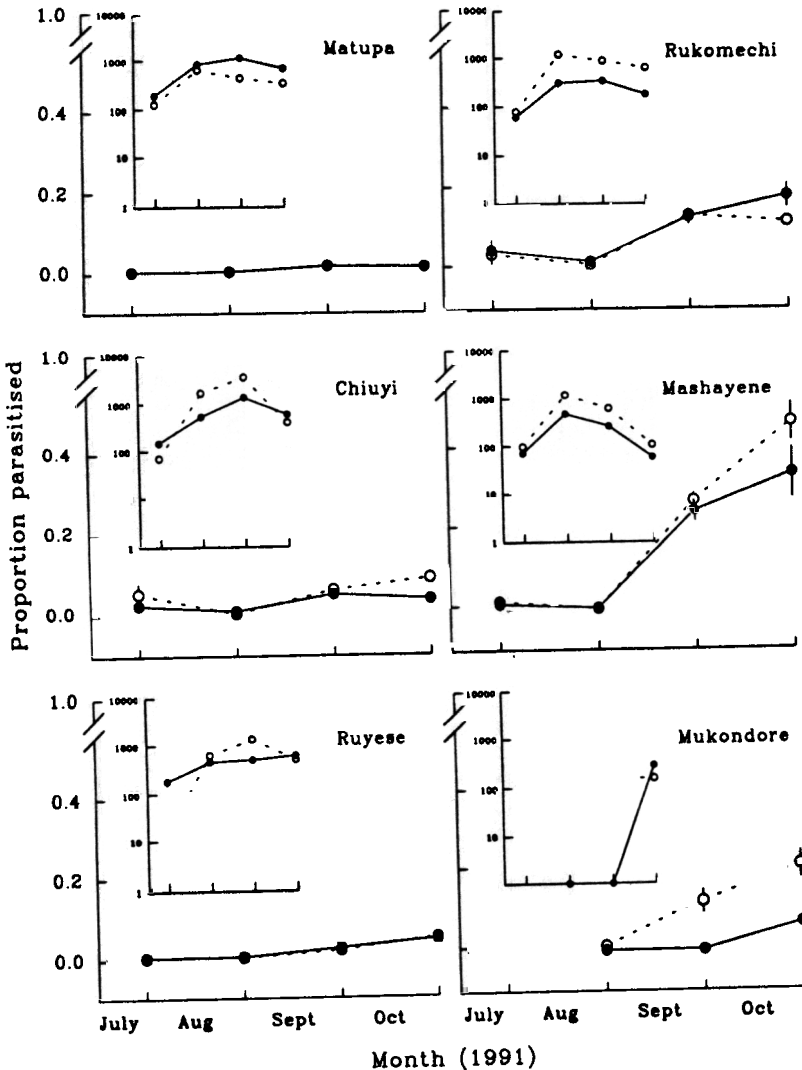


Fig. 2. Rates of parasitism for puparia of *Glossina m. morsitans* (●) and *G. pallidipes* (○) collected along various dry river beds in the vicinity of Rekomitje Research Station. Inset: total puparia collected each month.

mean ages of samples of flies caught in various areas between the end of 1988 and the middle of 1992. The analysis is based on the dissection of >80,000 flies.

Chemical assays of target fabric for loss of pyriproxyfen

In December 1991 pieces of black cloth and netting (ca. 25x25cm) were cut from targets deployed in the block and sent to Dr B. Mauchamp, Insect Physiology Laboratory, INRA, Versailles, France, for assay of the authentic pyriproxyfen remaining on the targets.

Results

Emergence rates from field-collected puparia

A total of 9625 *G. m. morsitans* and 15,417 *G. pallidipes* puparia were collected. Numbers generally increased from July to September and decreased at the end of October (fig. 2, insets). The proportion parasitized

(judged by the emergence of parasites and by puparial dissection) varied between sites, but always increased as the hot season advanced (fig. 2). The corrected emergence probability (p_i) was >0.95, for each site, for *G. m. morsitans* puparia collected in July-August, before the targets were deployed (fig. 3); for *G. pallidipes* p_i was more variable, but still >0.75. As the year progressed p_i for puparia collected along the Matupa declined progressively, to 0.34 for *G. m. morsitans* and 0.20 for *G. pallidipes* by the end of October. For the control areas (the Ruyese and Mukondore Rivers >10 km from the centre of the square) absolute emergence probabilities were >0.75 throughout the treatment period.

For *G. m. morsitans* puparia collected along the Rukomechi River (the closest river to the edge of the treatment block) p_i declined by ca. 40% during the trial but, for the more distant rivers, there was no measurable effect on adult emergence from puparia. For *G. pallidipes*,

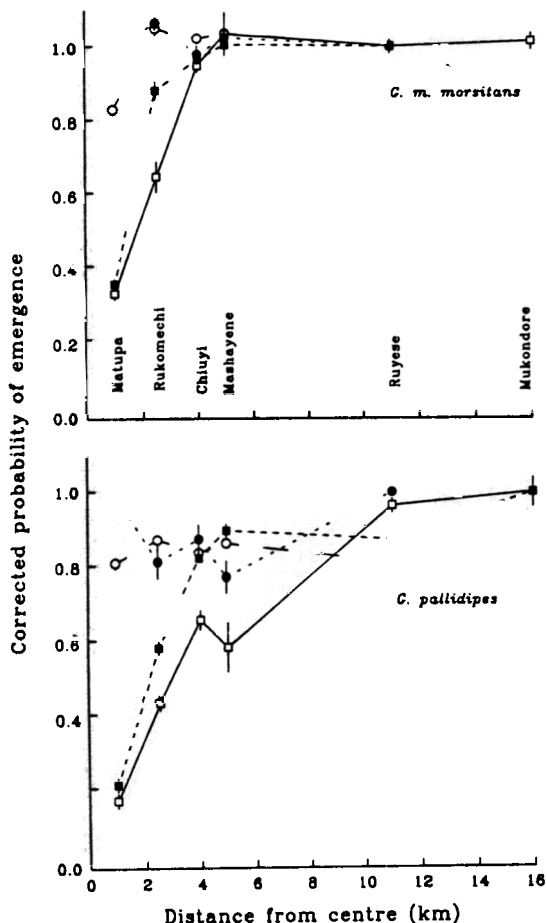


Fig. 3. The probability of emergence (\pm one standard deviation) from puparia collected in the area of Rekomitjje Research Station in 1991, plotted against the approximate mode of the distance of the collection sites from the centre of the treatment block. Key for collection times: July/August, ●; August/September, ○; September/October, ■; October/November, □.

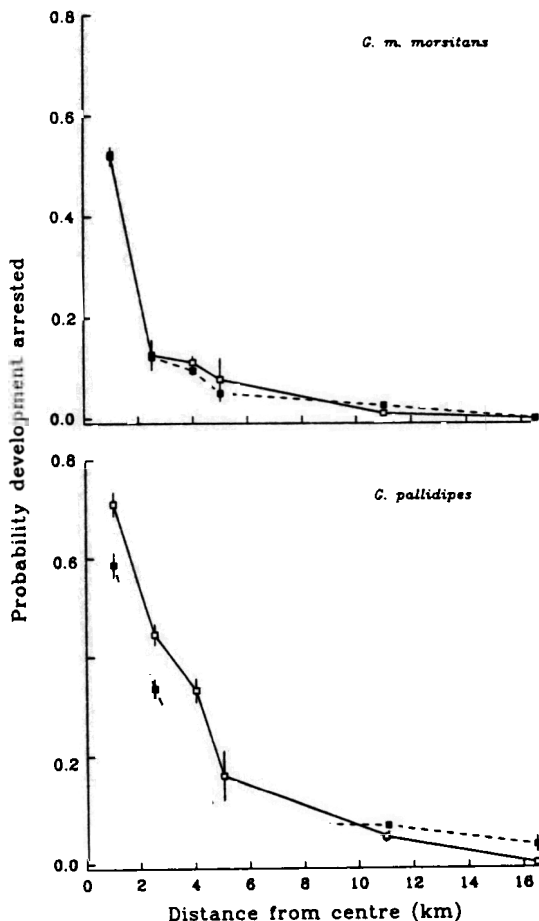


Fig. 4. The proportion of unparasitized puparia which failed to produce an adult tsetse and which were found, on dissection, to be in Bursell's (1959) stage III 3 of development. Key for collection times: September/October, ■; October/November, □.

Table 2. Emergence of *Glossina pallidipes* from laboratory produced puparia. Unhatched puparia were dissected to assess the degree of development of the enclosed fly. Flies were classified as having completed development if they had emerged or if they had developed beyond Bursell's (1959) stage III 3 but had not emerged. Flies which were found to be at stage III 3 when the puparia were dissected were classified as showing arrested development.

Site	Puparia	Development					
		emergence		complete		arrested	
		no.	%	no.	%	no.	%
2 (Control)	174	54	31.0	158	90.8	5	2.9
1 (Centre)	201	26	12.9	119	59.2	52	25.9

however, p_c for puparia collected from the Chiuyi and Mashayeni Rivers was ca. 40% less than for puparia collected at the control sites. For puparia of both species, collected along the Matupa River, p_c did not change between 6 and 10 weeks after the deployment of the targets.

Degree of arrested development exhibited in dissected puparia

Pupal dissections indicated, as expected, that reductions in emergence of tsetse from unparasitized puparia were largely due to an increase in the proportion of puparia exhibiting arrested development (fig. 4). As with the emergence rates there was little apparent effect on *G. m. morsitans* outside the block, and less difference between the two months than observed in *G. pallidipes*. The results confirm, for the latter species, that by late October a significant proportion of the females depositing larvae as far as 5 km from the centre of the block (>2 km from the nearest edge) had been treated effectively with pyriproxyfen.

Emergence rates from laboratory produced puparia

The rate of emergence from puparia produced in the laboratory by field-collected adult female *G. pallidipes* was 2.4 times higher for flies collected at Site 2 (fig. 1) than it was for those collected at the centre (table 2). The emergence rate even for the 'control' site was unexpectedly low. Since Site 2 was only 4.5 km from the centre of the block, and 2 km from the nearest target, it was possible that these flies had been severely affected by the presence of the targets. Dissection of all of the unhatched puparia showed, however, that the majority for both sites had developed beyond Bursell's (1959) stage III (table 2), suggesting that their failure to emerge was due to some feature of the laboratory conditions, rather than an effect of pyriproxyfen. However, at the centre, the proportion of puparia which had completed development was only 65% of that for puparia from the 'control' area. Furthermore, arrested development was shown by 26% of the *G. pallidipes* puparia from the centre (ca. nine times the percentage for Site 2) indicating that the targets were still partially active five months after deployment.

Sample age distributions

The mean ovarian ages for *G. pallidipes* caught near the centre of the treatment block, using traps or the VET, at various times between the end of 1988 and mid-1992 show sharp rises during the times when pyriproxyfen treated traps or targets were deployed in the area (fig. 5). The average ages from the two methods are highly

correlated; the VET generally produced samples with lower mean ages (fig. 5, inset). Between September and December 1991 the mean ovarian ages increased by 81 and 93%, respectively, for the two capture methods.

At the time of the trap treatment in 1989 the ovarian dissection capability was limited, so that average ages of flies collected from sites far from the treatment block were not obtained. Present results show that for *G. pallidipes* collected using traps or the VET near the Ruyese, the mean ovarian age increased by 22 and 38%, respectively, between September and December 1991 (fig. 6). Since emergence rates from wild-collected puparia (see

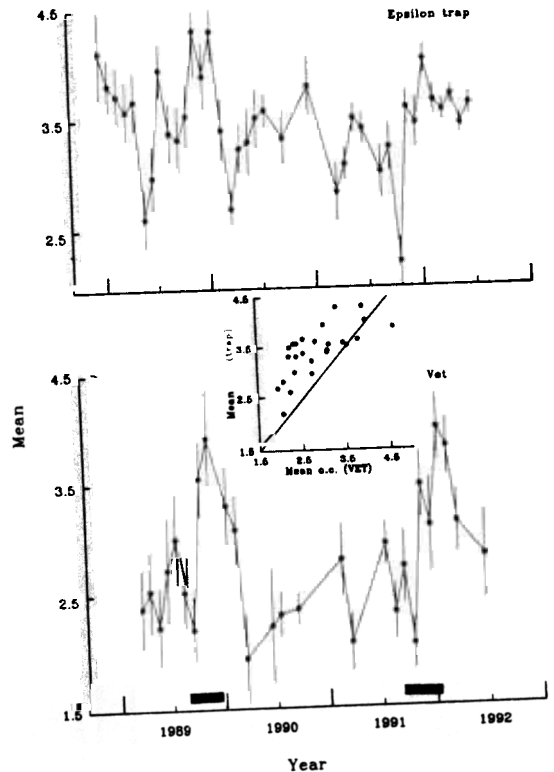


Fig. 5. The mean ovarian category (\pm two standard errors) of female *Glossina pallidipes* caught near the centre of the treatment block. Bars above the abscissa denote treatment periods. Inset: mean ovarian ages from the two methods plotted against each other.

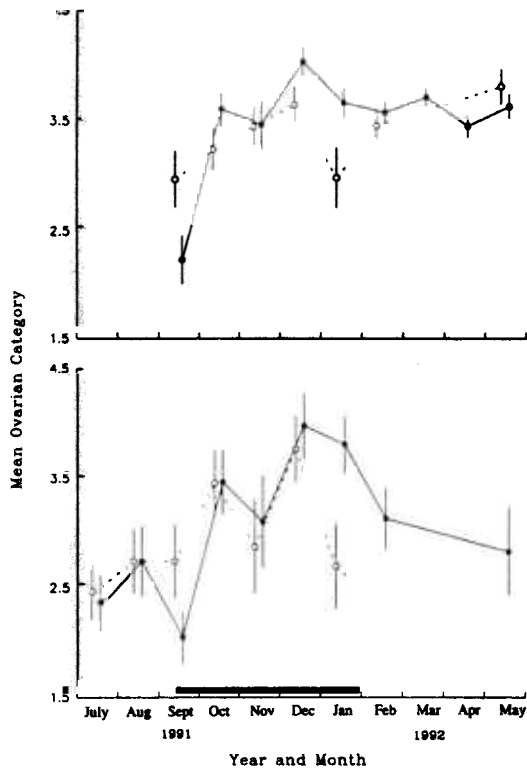


Fig. 6. The mean ovarian category (\pm two standard errors) of female *Glossina pallidipes* caught near Site 1 (●—●) and Site 3 (Ruyese River; ○—○). Bar above the abscissa denotes treatment period.

above) show that the population near the Ruyese was not measurably affected by the targets, the increase in average age appears to be due to the season of the experiment as well as the effect of the pyriproxyfen. The same conclusion may be drawn from the mean wing-fray categories of the samples of flies from the VET (fig. 7): there is a strong correlation between the indices of mean age arising from the two techniques (fig. 7a, inset).

The mean ovarian ages of female *G. pallidipes* caught at Site 2 in 1988 and 1990 (when pyriproxyfen treated baits were not used at Rekomitjie) confirm the tendency for average ages of this species to increase towards the end of each year as the hot dry season progresses (fig. 8). Less extensive evidence suggests the situation may differ in *G. m. morsitans*; for females caught using the VET in 1991 there was no increase in mean ovarian age between September and December (fig. 9) near the Ruyese River but an increase of 29% for flies caught near the centre.

Loss of pyriproxyfen from targets

The amount of pyriproxyfen remaining on the targets, four months after treatment, is strongly dependent on the type of fabric where the chemical was deposited and the position on the target (table 3). For the black cloth there was nearly twice as much pyriproxyfen on

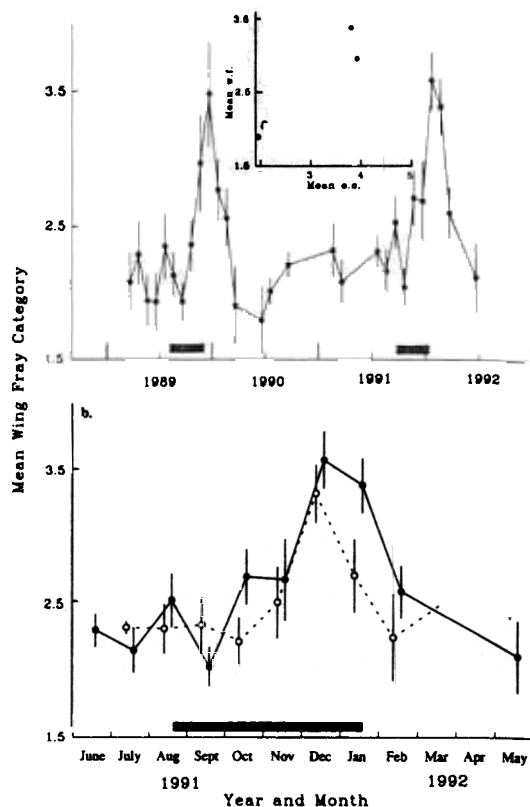


Fig. 7a. The mean wing fray category (\pm two standard errors) of female *Glossina pallidipes* caught in the region of Site 1 using the VET. Inset: comparison of mean ovarian category and mean wing-fray for flies in main graph.

Fig. 7b. The mean wing frays at Sites 1 (centre; ●) and 3 (near the Ruyese River; ○) for female *G. pallidipes* caught on the VET. Bars above the abscissa denote treatment periods.

the bottom third of the target as there was on the top third and for the netting nearly four times as much. The central portion had intermediate amounts for each fabric. There was ca. 6–14 times as much pyriproxyfen on the cloth as on the netting. The total area of netting and cloth in the target was 1.0 and 0.7 m², respectively, from which it is estimated that the total pyriproxyfen remaining was 70–264 mg on the netting and 545–1033 mg on the cloth, giving a total of 615–1297 mg on the whole target. Since 4000 mg of pyriproxyfen was initially deposited on each target, the total loss over the four month period was 60–85%.

Discussion

Interpretation of changes in mean ovarian age

The rapid increase in mean ovarian age between September and December (in years when no pyriproxyfen trials were carried out) suggests, at first glance,

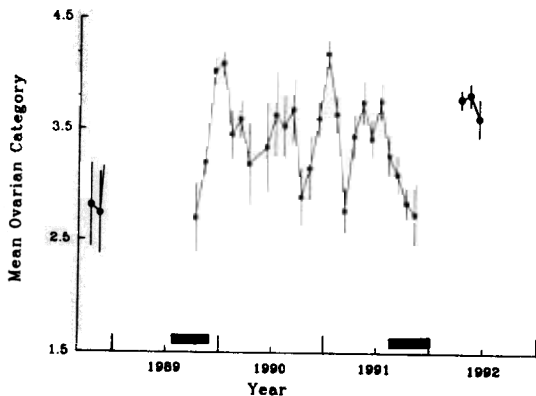


Fig. 8. The mean ovarian category (\pm two standard errors) of female *Glossina pallidipes* caught near Site 2 using an odour-baited epsilon trap. Bars above the abscissa denote treatment periods.

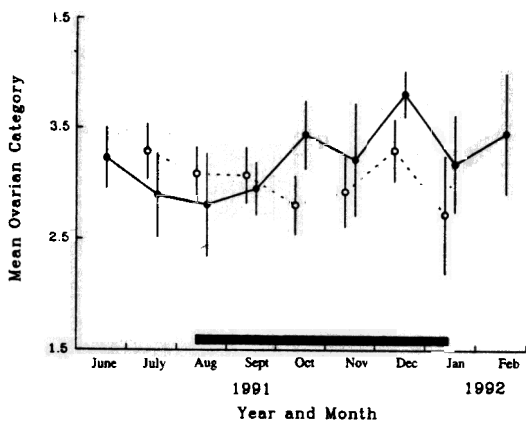


Fig. 9. The mean ovarian category (\pm two standard errors) of female *Glossina m. morsitans* caught near Sites 1 (●) and 3 (○) using the VET. Bar above the abscissa denotes treatment period.

that mortality is decreasing over this period. Previous work suggests that the hot dry season is a time of stress for tsetse populations (Phelps & Clarke, 1974; Vale & Cumming, 1976) and there is no suggestion of an increase in total population numbers which would be expected if mortalities were, in truth, dropping rapidly. If anything one would expect mortalities to be increasing. We suggest that the increases in mean age in the late dry season are indeed due to increases in mortality, but that these increases are predominantly in the immature stages and in young adults. This natural effect is accentuated by the pyriproxyfen treatments.

Increases in the numbers of puparia collected as the hot dry-season progresses are presumably indicative of increasing puparial densities in selected deposition sites. They are matched by increasing levels of parasitism (fig. 2) and these may be expected to be matched, in turn, by increasing levels of pupal predation. Phelps & Clarke

Table 3. Loss of pyriproxyfen from fabric targets in the field. Concentration of authentic pyriproxyfen ($\mu\text{g cm}^2$) in cloth and netting samples from targets deployed in the field for three months.

	Top	Middle	Bottom
Netting			
Cloth			

(1974) have also shown that, for male *G. m. morsitans* in Zimbabwe, small flies in the youngest age classes are selectively removed — particularly in November. Dransfield *et al.* (1989) found similar effects for *G. pallidipes* in Kenya and, again, the greatest mortalities occurred at the end of the dry season.

The full theoretical implications of the changes in population age structure will be considered elsewhere. We note only that Van Sickle & Phelps (1988) have warned of the dangers of estimating adult mortalities from age distributions when the mortalities are fluctuating with season and the age distributions are never truly stable. The present study underlines that danger. It suggests further that changes in mortalities of the larval/pupal and newly emerged adult classes at certain seasons may be sufficiently large to cast doubt both on the level and the general trend of adult mortalities as estimated from ovarian dissection data in the manner suggested by Rogers *et al.* (1984) and Van Sickle & Phelps (1988).

Pyriproxyfen as an alternative to insecticides for tsetse fly control

Hargrove & Langley (1990) calculated that odour-baited traps treated with pyriproxyfen and deployed at 4 km² could reduce a tsetse population to less than a millionth of its original level in a year. The current trial marks an advance in that odour-baited targets were substituted for traps and set up exactly as they would be for an insecticide-based control programme. The results have, in one respect, been even more promising; the initial rate of decline in emergence from puparia collected near the centre of the treatment block was faster than when traps were used. This was expected because tsetse, particularly *G. m. morsitans*, are more likely to contact a target than to pass through a trap (Vale *et al.*, 1986). If the high rate of sterilization could be maintained there is little question that pyriproxyfen-treated targets could be used for tsetse eradication. Even if pyriproxyfen is not immediately preferred over pyrethroids currently in use, its entirely new mode of action offers a valuable alternative means of control in the event of insecticide resistance appearing in tsetse populations.

The important remaining problem is the high loss rate of pyriproxyfen; by comparison deltamethrin applied at 0.6% of active ingredient showed no decline in activity over 210 days (Torr *et al.*, 1992). The accumulation of the chemical at the bottom of the targets accords with the visual observation that Cereclor migrated down the targets and dripped off the bottom. By the time the assay was performed the efficacy was clearly reduced and it is not surprising that the initially rapid decline in the emergence rate of field-collected puparia was not sustained. In the earlier trial with traps (Hargrove & Langley, 1990) the treated cones of material

were replaced each month and the emergence rate for *G. pallidipes* continued to decline throughout the treatment period, reaching 0.03 after three months. The concentration on the treated cones was presumably always close to the initial value of 2 mg cm⁻², 14–26 times as high as on the target cloth at the time of assay. The present results therefore indicate the importance of developing a better formulation of pyriproxyfen for use on odour-baited targets.

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