FINAL TECHNICAL REPORT

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Preliminary studies of the effects of host physiology on the efficacy of cattle as baits for tsetse control

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Executive summary

Background

Tsetse infest *ca.* 11 million square kilometres of Africa where they are vectors of trypanosomiasis in man and domestic livestock. This disease is a major constraint to agricultural production in Africa, preventing or greatly reducing the productivity of animals. An increasingly important method of controlling trypanosomiasis is based on treating cattle with an insecticide to kill tsetse; in areas where cattle exist, the technique is often the cheapest disease control option. There is little information on the density of dipped cattle required to control tsetse, or how this density is affected by variables in both cattle and tsetse populations.

Purpose

The purpose of the project was to develop and promote sustainable and cost effective strategies for the control of trypanosomiasis in Zimbabwe and tsetse-infested countries of sub-Saharan Africa by promoting the use of insecticide-treated cattle. The project aimed to contribute to this purpose by quantifying how fly-cattle contact is influenced by major physiological variables in cattle.

Research activities

In Zimbabwe, field studies were made of the attraction and feeding responses of tsetse to cattle of different age, sex and nutrition. At NRI, the physiological basis for differences in the attractiveness of cattle was determined by chemical analyses of host odours collected in the field.

Outputs

The odour from a mature ox was at least as attractive as the blend of synthetic attractants used with odour-baited targets in current control operations in Zimbabwe.

Attraction to the odour from different types of cattle decreased in the order ox > cow > calf; oxen were *ca*. twice as attractive as calves (<6 months). The difference was positively correlated with liveweight and the host's rate of carbon dioxide production.

The feeding success of tsetse decreased in the order ox > cow > calf. The effect was most marked for calves aged < 6 months where <10% of tsetse fed successfully compared to 50-80% for mature oxen.

There was a significant seasonal variation in the feeding success of tsetse. This was positively correlated with the frequency of host defensive behaviour and the abundance of *Stomoxys*. Seasonal changes in nutrition had no significant effect on attraction or feeding responses.

Contribution of outputs

The results suggest that mature Mashona oxen distributed evenly at densities of 4 km^{-2} can control tsetse populations. However, since communal herds in Zimbabwe typically comprise 30% young animals and 50% females, and cattle are not evenly distributed spatially or temporally, the minimum density of insecticide-treated cattle required to control tsetse will be greater than 4 km^{-2}. Further work is required to quantify the effect of cattle management practices on the use of insecticide-treated cattle.

The work was undertaken with TTCB and RTTCP scientists in Zimbabwe and these organisations will be the main uptake pathways. Scientists from Kenya, South Africa, Tanzania and Uganda also showed interest in the work. A paper on the physiological status of tsetse attracted to Mashona oxen and a major review of the host-orientated responses of biting Diptera were accepted for publication by *Medical and Veterinary Entomology*.

Background

Tsetse flies infest over 11 million square kilometres of Africa where they are vectors of trypanosomiasis in man and domestic livestock. Animal trypanosomiasis is a major constraint to agricultural production in many of the more deprived areas of Africa, preventing or greatly reducing the productivity of animals.

Throughout Africa, an increasingly important method of controlling trypanosomiasis is based on treating cattle with an insecticide to kill tsetse; in areas of Zimbabwe where cattle exist, the technique is the cheapest option for controlling trypanosomiasis (Barrett, 1994). There is little information on the density of dipped cattle required to control tsetse, or how this density is affected by variables in both cattle and tsetse populations. Consequently there is little rational basis for planning and managing control operations based on this approach. Accordingly, this project has carried out research to determine how fly-cattle contact is influenced by major physiological variables in cattle.

Project purpose

The purpose of the project was to develop and promote sustainable and cost-effective strategies for the control of trypanosomiasis in Zimbabwe and tsetse-infested countries of sub-Saharan Africa, in particular by promoting the use of insecticide-treated cattle.

To predict the effect of insecticide-treated cattle on tsetse populations, it is necessary to establish what proportion of a tsetse population contacts a treated animal per day. The specific aim of this project was to quantify the effects of cattle nutrition, age and sex on tsetse-cattle contact, and to establish general methodologies for investigating the effects of other host factors on tsetse-cattle contact.

The specific objectives of the project were:-

1. To establish preliminary quantitative relationships between host nutrition and age and cattle-tsetse contact.

2. To make recommendations on methodology and benefits of further research on factors affecting cattle-tsetse contact.

3. To make recommendations on the effects of stock composition and density on the efficacy of cattle as baits for tsetse control.

Research Activities

All field studies were carried out at Rekomitjie Research Station in the Zambezi Valley of Zimbabwe where *Glossina pallidipes* Austen and *G. morsitans morsitans* Westwood occur. Experiments carried out under the auspices of the present project were undertaken between November 1995 and March 1998. Previously unpublished data from relevant experiments were also analysed as part of this project and are reported here. The timing of these experiments are indicated as footnotes.

Attractiveness of different types of cattle

The attractiveness of odour from various cattle was assessed by placing different types of cattle in a roofed pit and exhausting the air from the pit at 2000 l/min via a ventilation shaft (25 cm dia.) fitted with a 12 V co-axial fan (Vale, 1974a). The pit was cleaned daily to minimise the accumulation of phenolic materials present in ox excreta. To gauge the numbers of tsetse attracted to various odours, an electric net (Vale, 1974b), 1.5 x 1.5 m, was placed 1 m downwind of the odour source. The net was mounted on a corrugated tray coated with polybutene. Flies that struck the net were killed or stunned and fell onto the tray where they became stuck. Tsetse orientate imprecisely to an odour source unless it is marked by a visual stimulus (Vale, 1974a). Consequently, a target, consisting of a panel of black cloth, 0.75 x 0.75 m, was sewn on to the centre of the electric net.

Synthetic ox odour

In some experiments, natural ox odour was compared with a synthetic ox odour comprising carbon dioxide (2 l/min), acetone (5 mg/h), 1-octen-3-ol (0.01 mg/h), 4-methylphenol (0.5 mg/h) and 3-n-propylphenol (0.01 mg/h) dispensed following the methods of Vale & Hall (1985) and Torr *et al.* (1997).

Air sampling for attractants

The rates of production of kairomones produced naturally by cattle were measured using a variety of collection and analytical methods.

Carbon dioxide.- Air was drawn at 300 ml/min *via* a tube (3 mm i.d.) inserted through a port (1 cm dia) in the pit ventilation shaft. The concentration of carbon dioxide was measured using an infra-red gas analyser (EGM-1, PP Systems, Hitchin, UK). The output from the analyser was recorded continuously on an integral logger and the data were subsequently downloaded onto a personal computer for analysis. The logger recorded the mean levels of carbon dioxide at 1 min intervals.

Octenol and phenols.- Volatiles were collected on Porapak filters (200 mg; 50-80 mesh) at 2 l/min for approximately 2 hr. The Porapak was pre-purified by soxhlet extraction with dichloromethane and further washing with dichloromethane after making up the filters. At NRI trapped volatiles were eluted with dichloromethane (3 x 0.5 ml) and decyl acetate (2 μg) added as internal standard. The solution was analysed by gas chromatography (GC) coupled directly to mass spectrometry (MS) using a Finnigan ITD 700 ion trap detector operated in electron impact mode. The GC column was fused silica (25 m x 0.25 mm i.d.) coated with polar CPWax52CB (Chrompack) with helium carrier gas (0.5 kg/cm^2) and oven temperature held at 50°C for 2 min then programmed to 240°C at 6°C/min. Components were identified by their GC retention times and mass spectra and comparison with synthetic standards where possible. Components were quantified against the internal standard and rates of production calculated from the flow rates used.

Carbonyls.- Odour was collected on silica SepPak cartridges impregnated with 2,4-dinitrophenyl-hydrazine (Waters; 360 mg) at 0.5 l/min for approximately 2 hr. Before and after sampling, great care was taken to avoid contamination of the filters, maintaining them as far as possible in heat-sealed foil bags. At NRI, the trapped 2,4-dinitrophenylhydrazones (DNPHs) were eluted with 3 ml of HPLC grade acetonitrile and the eluate made up to 5 ml. Analyses used a Spherisorb5 ODS2 column (25 cm x 4.6 mm; HPLC Technologies) eluted with a 60:40 acetonitrile/water mixture at 1 ml/min. The eluate was monitored by a UV detector at 350 nm. DNPHs of acetone, butanone, formaldehyde and acetaldehyde were synthesised at NRI and amounts in the test samples were quantified by external standard.

Carboxylic acids.- Samples were collected at 2 l/min for approximately 2 hr on filters containing Chromosorb P AW impregnated with 2.5% tetrabutylammonium hydroxide (200 mg). At NRI the tetrabutylammonium salts were eluted with acetone (3 x 0.5 ml) and benzyl bromide (2 µl) added to convert the salts to the benzyl esters. After standing for 2 hr, an internal standard, e.g. decyl acetate (2 µg), was added and the solution analysed by GC-MS as above. Benzyl esters were detected by single ion scanning at m/z 91 and 108. Synthetic standard benzyl esters were prepared *via* the tetrabutylammonium salts on a preparative scale. The internal standard was calibrated against these and amounts of benzyl esters converted to amount of acid trapped.

Urine samples.- The phenolic attractants produced by cattle occur naturally in the urine (Bursell *et al.*, 1988). Urine samples (10 ml) were absorbed onto ToxElut TE3010 filters (Varian) and organic components eluted with dichloromethane (25 ml). Decyl acetate (1 mg) was added as internal standard and the solution analysed by GC-MS as above.

Experimental design and analysis

All field experiments were carried out during the 3 h preceding sunset when tsetse are most active (Hargrove & Brady, 1992). In comparisons of different cattle, the various treatments were incorporated into a series of replicated Latin squares consisting of days x sites x treatments. The catches (n) were normalised using a $\log_{10}(n+1)$ transformation and subjected to analysis of variance.

Tsetse behaviour

Studies were made of the responses of tsetse to different types of Mashona cattle placed individually at the centre of an incomplete ring (8 m dia) of six electric nets (Vale, 1974b), following the method of Vale (1977). The cattle were retained in a large (2.5 m wide x 3.5 m long) crush at the centre of the ring to prevent them from touching the nets but otherwise allowing them freedom of movement. The electric nets were mounted on either corrugated trays coated with polybutene or metal hoppers (Vale & Hargrove, 1979). Flies that struck the net were killed or stunned and fell onto the tray or hopper where they were retained. Tsetse were separated according to the side of the net where they were caught and classed as fed or unfed according to the presence or absence of fresh red blood visible through the abdominal wall. Flies caught on the outside or the inside of the ring were presumed to be approaching or leaving the ox respectively (Vale, 1977; Torr, 1994). Following Vale (1977), feeding efficiency was estimated as the number of fed tsetse on the inside of the ring of nets expressed as a percentage of the total catch from the inside of the ring.

Tsetse physiology

In one experiment[1], flies were collected at 30 min intervals from the metal hoppers, placed in single tubes and subsequently kept in the dark at 2-10^{o}C. Female flies were dissected within 18 h and assigned to ovarian categories as described by Challier (1965). Following Hargrove (1995), the lengths of the two largest oocytes and any uterine inclusion were recorded. For male tsetse, the legs and head of each fly were excised and discarded. The carcass was dried over calcium chloride, the abdomen and thorax were placed separately in labelled gelatine capsules and sent to the Tsetse Research Laboratory, Langford, Bristol for analysis of fat and residual dry weight (Langley *et al.*, 1990).

[1] Experiment undertaken in 1992

Oxen

Mashona cattle were used in all studies. To prevent trypanosomiasis, adult cattle at Rekomitjie were treated at three-month intervals with isometamidium (1 mg/kg; Trypamidium, Rhône Mérieux). Any animals that developed trypanosomiasis were treated with diminazene aceturate (3.5 mg/kg; Berenil, Hoechst) and then treated with isometamidium 14 days later.

The Packed Cell Volume (PCV) of the herd at Rekomitjie was measured at 10-20 day intervals. Blood was collected from an ear vein into a heparinized capillary tube and the PCV of the blood was measured after spinning the sample in a haematocrit centrifuge for 5 min. The weights of cattle were recorded at 10 day intervals and condition score was measured monthly. Newly born calves were not treated with either isometamidium or diminazene aceturate until their first infection with trypanosomes was detected.

Ox behaviour

The defensive behaviour of oxen in the ring of nets was observed directly. To avoid problems associated with the effect of human odours and visual stimuli affecting the behaviour of tsetse (Vale, 1974a), all observations were made from a tower (Torr, 1994). Four types of behaviour were recorded:- 1. twitching the skin; 2. flicking the tail above the back height; 3. kicking the lower torso with rear feet and 4. stamping. Observations were made for 10 minute periods, followed by a 20 minute rest for the observers, giving a total of 50 minutes observation per afternoon.

Alighting responses

The numbers, position and resting time of tsetse alighting on an ox were observed from a tower or from an observation pit (Hargrove, 1976) with an ox placed 1 m away from the window. For observations from the tower, the species, location and duration of alighting tsetse were recorded. The observers also noted whether the fly was:- (i) disturbed by ox behaviour; (ii) disturbed by other flies; (iii) obtained a full fee; or (iv) none of these. Observations of alighting flies were made during the last hour of the afternoon.

For observations made from the observation pit[2], the species and location of the tsetse on an ox were recorded every 10 minutes and classed into one of six regions: 1. upper leg, above the hock or knee; 2. middle leg, above the fetlocks; 3. lower leg, below the fetlock; 4. within 10 cm of the umbilicus; 5. torso below the midline and 6. head and torso above the midline. The sex of alighting flies was not distinguished.

Experimental design and analysis

Experiments involving electric nets were carried out during the 150 min preceding sunset, and observations of tsetse landing on oxen were made in 60-90 minutes periods immediately after sunrise and before sunset, these being the periods when tsetse are most active (Hargrove & Brady, 1992). In any one experiment only one site was used. Groups of adjacent days were regarded as different blocks and treatments were allocated randomly to days within these blocks.

All experiments were analysed using GLIM4 (Francis *et al.,* 1993) which fits models using a maximum likelihood method. To analyse changes in catch, the catches (n) were transformed to $\text{Log}_e(n+1)$ and then subjected to analysis of variance. To analyse the proportions of tsetse feeding or landing at various sites, a binomial model with a logit link was used and the significance of changes in deviance were assessed by χ^2 or by an F-test after re-scaling (by dividing Pearson's χ^2 by the degrees of freedom) if the data displayed a small amount of overdispersion (Crawley, 1993). To analyse experiments where data were classed into different categories (*e.g.* landing site, reproductive status), a model with a Poisson error and log link was specified and the significance of changes in deviance was assessed on the basis of a G-test. Means are accompanied by their standard errors unless stated otherwise.

Review of chemical and physical attractants for haematophagous flies.

The editors of *Medical and Veterinary Entomology* invited Drs Torr and Gibson to write a review of "Chemical and physical attractants for haematophagous flies" and this was jointly funded by the Animal Health and Livestock Production programmes. The review (Appendix

[2] Experiment undertaken in 1982.

1) has been accepted and will be published in the January 1999 issue of *Medical and Veterinary Entomology* .

Outputs

Feeding responses of tsetse to mature Mashona oxen

The numbers of tsetse attracted to the odour from a single ox or a blend of acetone (500 mg/h), 4-methylphenol (3.2 mg/h), 3-*n*-propylphenol (0.4 mg/h) and octenol (1.6 mg/h) were compared[3]. The results (Fig. 1) show that natural ox odour attracts as many *G. pallidipes* and *G. m. morsitans* as the synthetic blend. The synthetic blend is *ca*. twice the dose typically used for targets and traps in control operations (Torr *et al.* 1996) and thus it seems fair to assume that a large (400-500 kg) Mashona ox attracts at least as many *G. pallidipes* as a target.

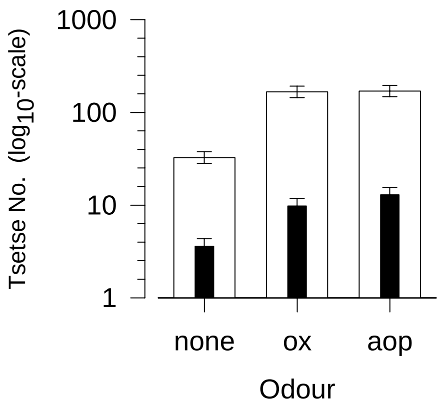

Figure 1. Mean catch (±S.E., 12 replicates) of *G. pallidipes* (open bars) and *G. m. morsitans* (solid bars) from unbaited electric targets (none) or targets baited with natural ox odour (ox) or a blend of acetone, octenol and phenols (aop).

[3] Experiment undertaken in 1992

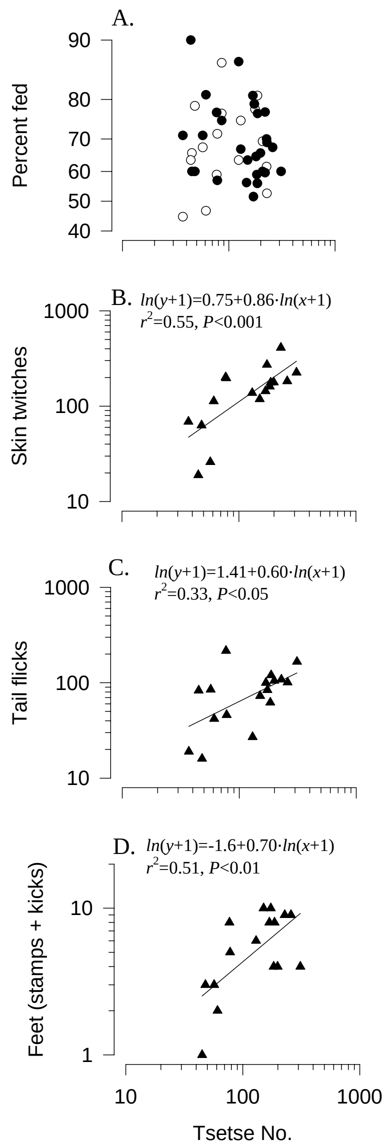

Figure 2. Scatterplots of (A.) the percentage of *G. pallidipes* (solid circles, males; open circles, females) feeding and the number of tsetse caught on an incomplete ring of nets surrounding an ox, and (B-D) the frequency of various types of ox behaviour.

Studies of the landing and feeding responses of *Glossina pallidipes* on mature Mashona oxen showed that for *G. pallidipes*, *ca.* 70% of those approaching an ox fed; increasing densities of *G. pallidipes* were correlated with an increase in the grooming responses of the ox but the ox’s behaviour had no significant effect on the percentage of tsetse that engorged (Fig. 2).

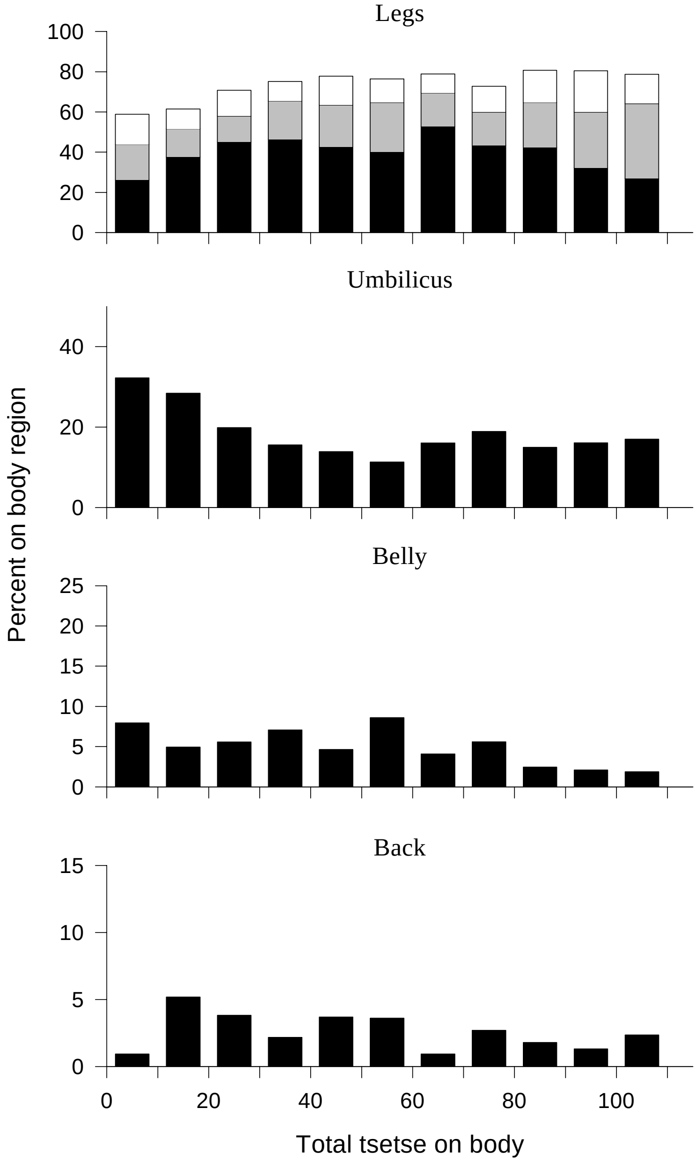

Figure 3 The percentage of *G. pallidipes* on various parts of the body of an ox at various densities of tsetse. Umbilicus = area within 10 cm of umbilicus; Belly = ventral surface of torso except umbilicus. For tsetse landing on the legs, the percentage on the top, middle and bottom of the legs are shown as open, shaded and solid respectively. Tick marks on the x axis delimit densities of 0-9, 10-19, etc flies/ox/observation. Sample sizes for 0-9, 10-12, etc. categories were 214, 405, 287, 411, 216, 221, 318, 518, 775, 379, 212.

These data, data along with those of Vale (1977), Baylis (1996) and Torr *et al.*(1996) suggest that ca. 50% of tsetse approaching a host feed on it and Hargrove (1976) estimated that *ca*. 90% of tsetse alighted on an ox. Thomson's (1987) estimates of the efficacy of various insecticides applied to cattle was based on an alighting time of only a few seconds. Thus if an ox is treated with an effective dose of insecticide over its entire body, an ox may kill >90% of tsetse approaching it. This compares with efficiencies of 60%-70% for traps and targets (Hargrove *et al.*, 1995; Torr *et al.*, 1996) currently used to control *G. pallidipes* and *G. m. morsitans*.

Studies of *G. pallidipes* alighting on an ox showed that the landing site varied with density with *ca*. 50% landing on the legs at low densities (<20 flies/ox), compared to *ca*. 80% at densities >40 flies/ox (Fig. 3). The large percentage of *G. pallidipes* observed on the lower legs underlines the importance of ensuring that there is sufficient insecticide there if cattle are being treated with insecticide. Thomson (1987) reported that for cattle fitted with an ear tag impregnated with deltamethrin, 90% of tsetse died if they alighted on the head and neck compared to <20% on the lower legs. She suggested that the reduced mortality on the legs was due to a lower amounts of insecticide there. Research recently undertaken by Dr Vale (RTTCP) in Zimbabwe has highlighted this problem. During the wet season, the persistence of insecticides on the legs of cattle is markedly shorter than that reported by Thomson (1987).

Tsetse physiology.- In studies of the nutritional status of male *G. pallidipes* feeding on an ox, it was found that the mean blood-meal size was 37 mg which is markedly smaller than previous estimates (Taylor, 1976; Hargrove & Packer, 1993; Loder, 1997). The discrepancy may be due, in part, to the presence of men. In Taylor's and Loder's experiments, flies were collected off an ox by men using handnets. Vale (1974b) has shown that men adjacent to an ox repel tsetse, and this repellency is less marked for 'hungrier' flies. Thus Taylor's and Loder's samples of flies were probably biased towards those with low fat contents and the present results suggest that tsetse with low fat contents take larger meals.

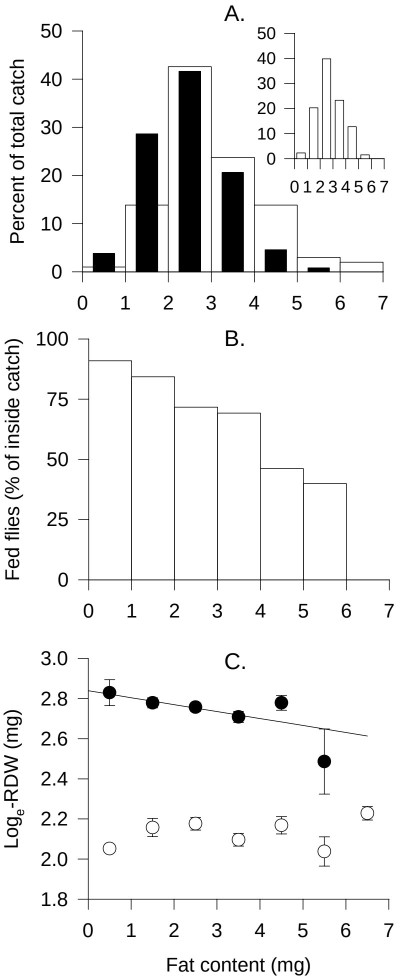

Figure 4. Frequency distribution of (A) the fat contents of fed (solid bars, n=262) and unfed (open bars, n=101) male *G. pallidipes* from the inside of the ring of nets and unfed flies (n=133) from the outside of the ring (inset), (B) the mean percentage of flies feeding and (C) the mean (±S.E.) Log_e-RDW of fed (solid circles) and unfed (open circles) flies for each fat category and the regression line Log_e-RDW (mg) = 2.84-0.03·Fat (mg). Y-axis for fig. B is logit-transformed.

Fed flies had significantly lower fat contents (Fig. 4A) and the probability of feeding was negatively correlated with fat content declining from 91% for flies with <1 mg fat to <50% for flies with >4 mg fat (Fig. 4B). Blood-meal size was also negatively correlated with fat content (Fig. 4C); the regression equation relating blood-meal size and fat content indicating that the mean wet weight declined from 42 mg for flies with 1 mg of fat to 31 mg for flies with 5 mg of fat.

The biting rate has been identified as one of the most important parameters in the epidemiology of trypanosomiasis (Rogers, 1988; Milligan & Baker, 1988). The estimates of blood meal size from these experiments suggest that the degree of tsetse-cattle contact may be higher than previously supposed. In this regard, one needs to consider two possibilities:

Firstly, the mean blood-meal size for male *G. pallidipes* is 37 mg wet weight; and flies feed to completion from a single animal, so that the biting rate can be estimated from the inverse of the feeding interval (Rogers, 1988). Hargrove & Packer (1993) estimated the feeding interval in male *G. pallidipes* at 54 - 65 h assuming a blood-meal of 84 mg wet weight. If the present estimate of 37 mg wet weight is assumed their method gives a feeding interval of no more than two days. The implied biting rate (1/2 = 0.5) is then twice as high as estimated by Rogers (1988). However, the errors in such estimates are unknown and, moreover, there is no general agreement on how best to estimate feeding intervals from nutritional data, and very similar data has been used to produce a wide variety of such estimates (Langley & Wall, 1990; Randolph *et al*., 1991; Hargrove & Packer, 1993; Baylis & Nambiro, 1993).

Secondly, it may be that we *under*estimated the mean blood-meal size because a proportion of the 'fed' flies had not completed their meal; a 'feed' really consists of a number of small feeds undertaken over a short period. Since most tsetse hosts are found in groups, repeated attempts to feed are likely to result in a given tsetse fly feeding off a number of different individuals in a short space of time. This scenario, therefore, also leads to an increase in transmission rate.

For females, the probability of feeding was not significantly affected by age as determined by ovarian category but there was a paucity of young (ovarian category 0) flies attracted to the ox (Fig. 5A). Pregnancy status had no significant effect on the probability of feeding but samples of flies attracted to the ox showed a relative dearth of females approaching larviposition and a preponderance just after (Fig. 5B). These data suggest that the physiological biases of tsetse attracted to and feeding on a stationary ox are similar to those caught by traps and targets, characterised by a paucity of young and well-fed flies (Hargrove, 1995; Hargrove & Packer, 1993), and females in the late stages of pregnancy (Hargrove, 1995).

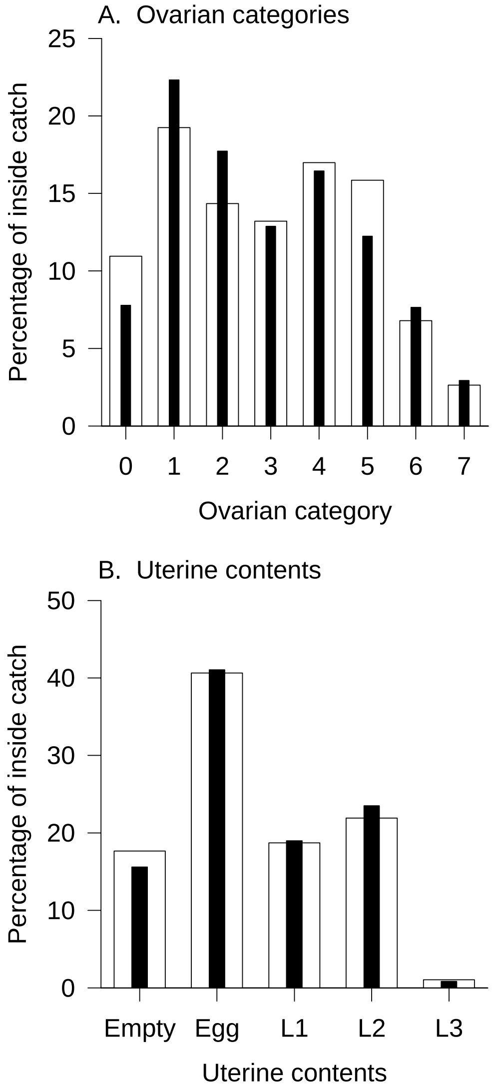

Figure 5. Frequency distribution of ovarian categories (A) and uterine contents (B) of fed (solid bars) and unfed (open bars) *G. pallidipes* caught from the inside of an incomplete ring of electric nets surrounding an ox. Sample sizes of ovarian category data for fed and unfed flies were 265 and 784 respectively and for uterine content data, the samples sizes were 283 and 821 respectively.

Taken together, these results suggest that an insecticide-treated ox is at least as effective as the odour-baited targets currently used in control operations in Zimbabwe (Vale, 1993). Currently, densities of 4 targets/km^2 evenly distributed can eradicate populations of tsetse (Vale, 1993) and presumably cattle evenly distributed at a similar density would be equally effective. Cattle densities in tsetse-affected areas are very variable, but for

Zimbabwe the potential *capacity* for such areas is estimated at *ca*. 8-20 cattle/km^2 (Barrett, 1992) and in Zambia and Ethiopia the stocking rates in areas controlled using insecticide-treated cattle are *ca*. 5 cattle/km^2 (P. Van den Bossche, unpublished data) and 10-20 cattle/km^2 (Leak *et al.* 1995), respectively. In such areas the cattle densities appear to be adequate for effective control, but in practice cattle are, of course, not stationary, not evenly distributed, and not all in the same size and condition. These factors alone could have a profound effect on the efficacy of the technique and thus studies were undertaken to investigate some of these matters.

Effect of host nutrition on the attraction of tsetse to cattle

Experimental animals at Rekomitjie are normally maintained on a supplementary diet during the dry season to keep their condition constant throughout the year. Cattle in communal areas, on the other hand, are subject to seasonal changes in nutrition which could alter the attractiveness of tsetse to cattle by four-fold (Vale, 1981). Accordingly, studies were made of the attractiveness and behaviour of two groups of four Mashona oxen. One group was grazed naturally on the veld without any supplementary feeding, in a regime similar to that used in the communal areas of Zimbabwe, while the second group was given concentrate during the dry season. The experiment commenced in November 1995 and continued until March 1997.

At the commencement of the study, there was no significant difference in the mean weight of the two groups but in April - May, at the end of the wet season, the veld-fed group lost weight such that by October 1996 the mean weight of the veld-fed group was 373 kg (±7.5, s.e.) compared to 428 kg (±8.2) for the supplement-fed group(Fig. 6 A.).

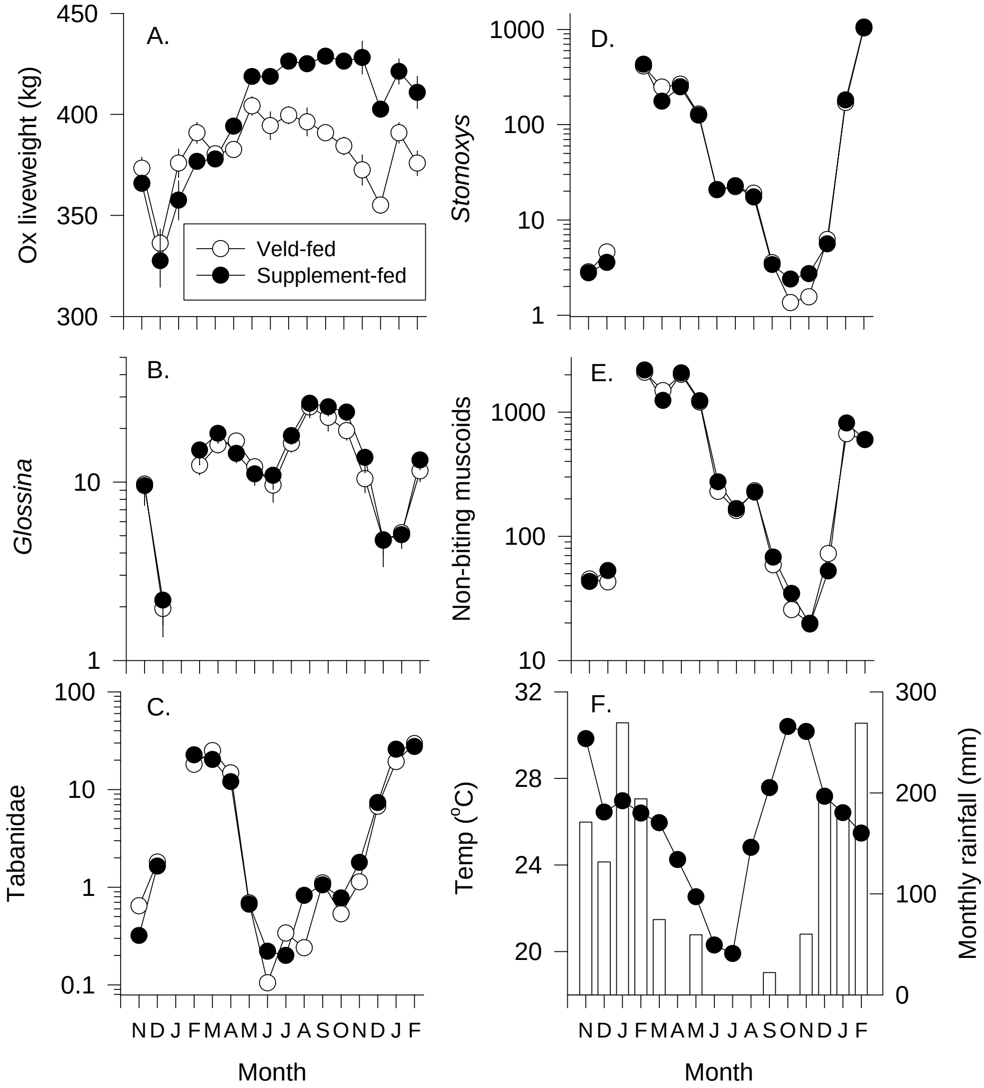

Figure 6. The weight of cattle allowed to feed naturally on the veld or fed a supplement during the dry season (June-September) (A), the mean daily catches of tsetse (B), Tabanidae (C), Stomoxyinae (D) and non-biting muscoids (E) attracted to the two groups of cattle, and the mean monthly temperature and monthly rainfall between November 1995 and February 1997.

Studies of the numbers of flies attracted to the different types of cattle (Fig. 6, B-E) showed that there was no significant difference in the numbers of tsetse and other Diptera attracted to cattle from the two groups. Similarly there were no significant differences in the levels of attractants produced by cattle from the different groups.

There were however significant (P<0.05) differences in the mean catches of tsetse and other Diptera from different oxen and these differences were significantly (P<0.05) correlated with weight (Fig. 7); the smallest ox (mean weight = 285 kg) had a detransformed mean catch of 9.1 *Glossina*/day compared to 21.1 from the largest ox (mean weight = 482 kg). There were also similar significant correlations between weight and the numbers of *Stomoxys* spp. and non-biting muscoids attracted to the oxen (Fig. 7). These data suggest that although the attractiveness of an ox to tsetse is correlated with weight, the relatively modest (*ca.* 50 kg) seasonal changes in weight do not result in any significant change in attractiveness.

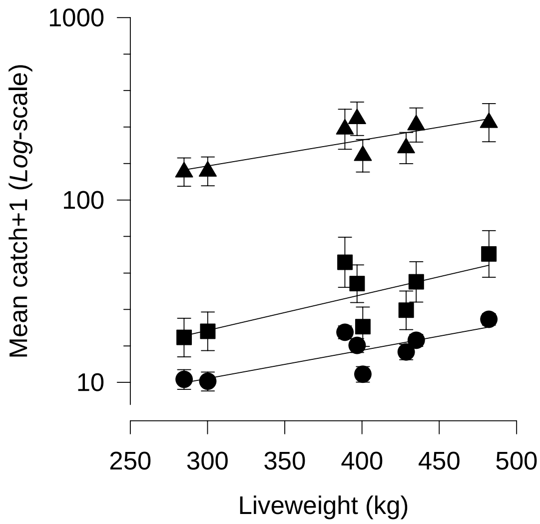

Figure 7. Mean (±SE) catch of *Glossina*, *Stomoxys* and non biting muscoids from Mashona oxen of different weight.

In studies undertaken between May and November, the catch from different oxen was compared with a blend of synthetic ox odour. For these studies the daily catch of *Glossina* from an ox was expressed as a proportion of the catch from the synthetic blend. The transformed indices were normalised using a Log_{10} transformation and a mean catch index for all ox-SO comparisons was calculated. The indices were subjected to regression analysis with indices weighted by the reciprocal of their respective variances and weight as an explanatory variable. The results (Fig. 8) show that there was a significant increase in the index with weight. The regression equation relating catch index and weight indicates that an animal weighing *ca*. 350 kg has a detransformed catch index of 1.0, i.e. a 350 kg ox is as attractive as a blend of synthetic ox odour.

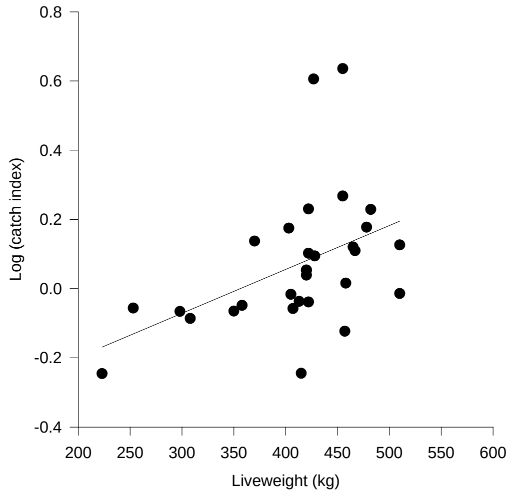

Figure 8. Catch of tsetse attracted to the odour from oxen of various weights, and expressed as a proportion of the catch from synthetic ox odour.

Effect of host age and sex on the attraction of tsetse to cattle

Studies were made of the numbers of tsetse and other Diptera attracted to the odour from young and female cattle. The various cattle were compared with large (400-500 kg) mature Mashona oxen. The detransformed mean catches of tsetse from a target baited with calf- or cow-odour were expressed as a proportion of the detransformed mean catch from a target baited with ox-odour and this proportion is termed the catch index.

Calves attracted consistently fewer tsetse than oxen ($P<0.05$); for instance, in six separate comparisons of different calves (aged *ca.* 6 months) the mean catch index was 0.49 (range 0.37-0.71). Young animals were significantly less attractive than mature oxen up to *ca.* 18 months of age but there was no significant difference in the attractiveness of the odour from standard oxen and 2-year old bull (mean catch index = 0.87, range 0.60-1.33) which weighed *ca.* 250 kg (Fig. 9). In comparisons between mature male and female cattle, generally fewer tsetse were attracted to females but the difference was relatively slight (mean catch index = 0.84, range 0.54-1.11) and generally not significant.

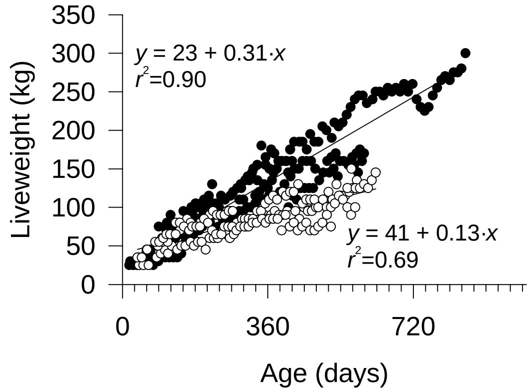

Figure 9. Scatterplot of age and weight of male (solid circles) and female (open circles) Mashona cattle.

Kairomone production: the effect of host physiology

Effects of nutrition.- There was no significant difference in the rates of production of different kairomones between veld- and supplement-fed animals. These results supported those from the entomological studies showing that there was no significant effect of seasonal changes in host nutrition and weight on the attractiveness of cattle to tsetse (Fig. 6). There were however significant differences in the odours produced by animals of different age (Table 1).

Firstly, young calves produced lower levels of all attractants, in particular phenols in their urine and carbon dioxide (Table 1). The reduced levels of kairomones in calf odour are in accord with the finding that calves attract significantly fewer tsetse than an ox. The slight reduction in ketone production is unlikely to have any effect on the attractiveness of a host (Torr, 1990; Torr *et al.,* 1997) but the differences in phenols and carbon dioxide are sufficiently large to produce significant differences in attraction (Vale *et al.,* 1988; Torr, 1990)

Table 1. Levels of various kairomones produced by calves and oxen. The data are based on studies of 8 oxen aged >3 years and 7 calves aged <6 months.

	Oxen	Calves
Ox kairomones[3.]		
Carbon dioxide (l/min)	2.2	0.3
Acetone (mg/h)	8.8	1.2
1-Octen-3-ol (mg/h)	none detected	none detected
4-Methylphenol (mg/l)	1199	194
3-n-Propylphenol (mg/l)	38	4
2-Methoxyphenol (mg/l)	0.5	0.1
Valeric acid (mg/h)	0.02	0.04

Levels of kairomones are means based on samples collected between either 0800-1000 h or 1600-1800 h from a ventilated pit containing oxen or calves except for the phenols which are based on samples of urine.

Phenol production.- To determine the biological significance of differences in the phenol content of urine, studies were made of the effect of baiting a trap with either calf or ox urine. The results (Table 2) show that adding any of the urines to an AO-baited trap increased the catch of tsetse significantly but there was no significant difference between the urines. These results suggest that although the levels of phenols in calf urine are lower, the concentrations are still sufficient to be biologically active.

Table 2. Mean catches of *G. pallidipes* from Epsilon traps baited with acetone (A) and octenol (O) ± urine from various types of cattle or 4-methylphenol + 3-n-propylphenol (P). Means followed by the same letter do not differ at the $P<0.05$ level of probability.

	Males		Females	
Odour	Mean catch	Detransformed mean	Mean catch	Detransformed mean
AO+ox urine (veld-fed)	0.985a	8.6	1.340a	20.9
AO+ox urine (supplement fed)	0.896a	6.8	1.328a	20.2
AO+calf urine	0.886a	6.7	1.347a	21.2
AOP	0.891a	6.7	1.479a	29.1
AO	0.672b	3.7	1.091b	11.3
SE	0.056		0.068	
P	<0.001		<0.001	

For animals greater than 6 months old, the levels of phenols in whole animal odour (Fig. 10) and urine (Fig.11) were similar to the levels found in ox odour and urine. Thus the differences in the attractiveness of tsetse to young animals does not appear to be due to differences in phenol levels.

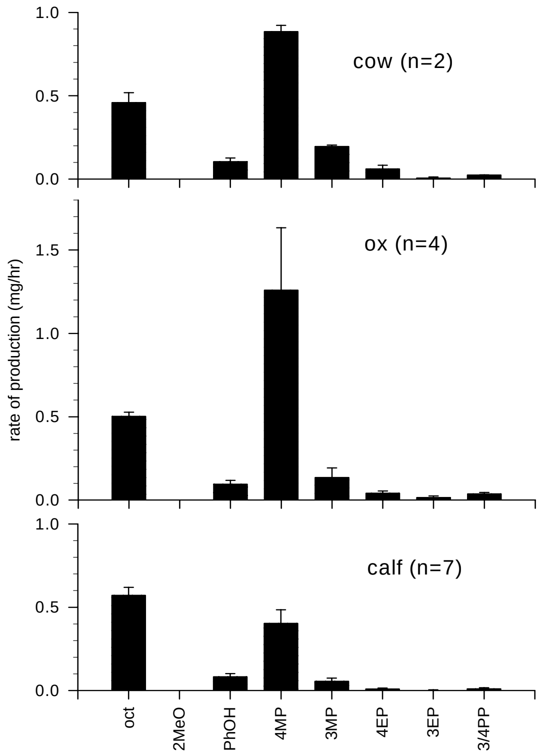

Figure 10. Compositions of volatiles trapped on Porapak from cows oxen and calves (*ca*. 12 months) from Zimbabwe (oct = octenol; 2MeO = 2-methoxyphenol; PhOH = phenol; 4MP = 4-methylphenol; 3MP = 3-methylphenol; 4EP = 4-ethylphenol; 3EP = 3-ethylphenol; 3/4PP = 3- and 4-propylphenol).

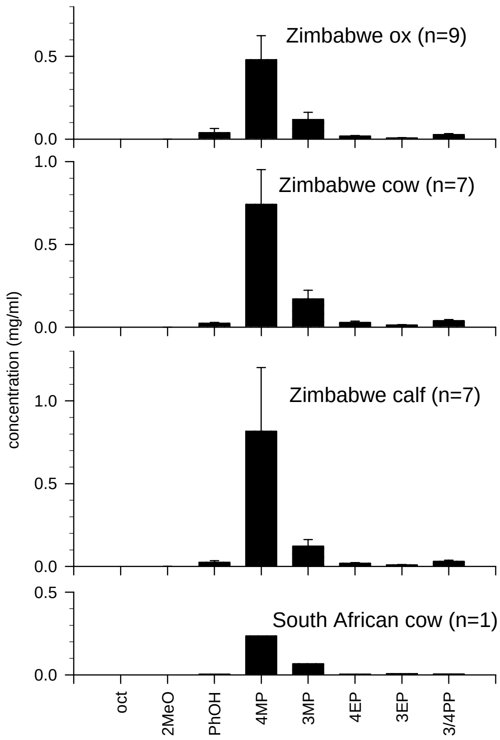

Figure 11. Compositions of urines of oxen, cows and calves from Zimbabwe or South Africa (oct = octenol; 2MeO = 2-methoxyphenol; PhOH = phenol; 4MP = 4-methylphenol; 3MP = 3-methylphenol; 4EP = 4-ethylphenol; 3EP = 3-ethylphenol; 3/4PP = 3- and 4-propylphenol)

Carbon dioxide.- Measurements of carbon dioxide production from different cattle were undertaken either in the morning (0800 - 1000 h) or the afternoon (1600 - 1800 h). The rate of carbon dioxide was Log_{10} transformed and subjected to analysis of covariance (ANCOVA) with weight as an explanatory variable and time of day as a factor. The best fit to the data was obtained by subjecting the weight of the animals to a Log_{10} transformation. The results (Fig. 12) show that the (Log_{10}) rate of carbon dioxide production was highly correlated with weight of the animal suggesting that the increase in attractiveness of cattle with weight (Fig. 7 & 8) and age is simply a consequence of increased carbon dioxide production. The rate of carbon dioxide production was also significantly greater in the afternoon and this afternoon increase appeared to be related to feeding; oxen that were allowed to graze had morning and afternoon rates of 1.3 and 2.6 l/min respectively compared to 1.3 and 1.3 l/min respectively for animals that were prevented from feeding.

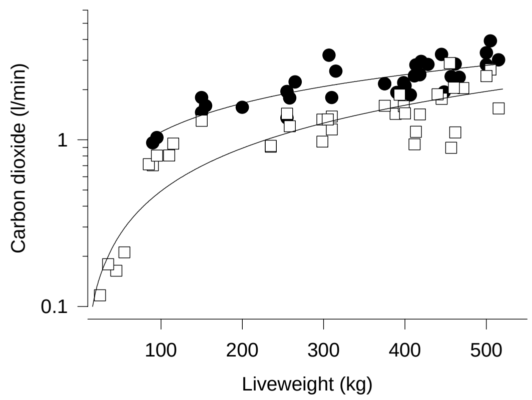

Figure 12. Carbon dioxide production from cattle of various weights in the morning (open squares) or the afternoon (solid circles).

These findings suggest that any process that increases metabolic rate, and hence carbon dioxide production, will increase the catch of tsetse. Intriguingly, studies by Roberts (1972) reported that lactation increased carbon dioxide production. However, the present studies found that lactating cows were generally less (but not significantly) attractive than oxen (Table 3), and that they produced lower levels of carbon dioxide, presumably due to their smaller size.

Table 3. Detransformed mean (transformed in brackets) catches of various Diptera from oxen and lactating cows, their respective transformed standard errors (SE) and the probability (P) that the catches from cows and oxen differ significantly.

Diptera	Ox	Cow	SE	P
Glossina	15.0 (1.21)	8.1(0.96)	0.08	ns
Stomoxys	5.5 (0.81)	2.4(0.53)	0.20	ns
Muscoids	36.4 (1.57)	32.0(1.52)	0.15	ns
Tabanidaes	1.2 (0.34)	2.0(0.47)	0.08	ns

Effect of host physiology on feeding responses

Seasonal effects.- There was no significant difference in the feeding responses of tsetse to veld- and supplement-fed cattle (Fig. 13A) but feeding success was significantly higher in October-November when numbers of biting Diptera (Fig. 13 B,C) and host-defensive movements (Fig. 13 D) were lower.

The data were analysed to determine the relationships between fly density, ox behaviour and the feeding behaviour of tsetse. The results showed that there was no significant effect of fly abundance on the percentage of tsetse that engorged (Fig. 14) but the percentage was correlated significantly with the number of leg movements.

All ox defensive behaviours were correlated with numbers of Diptera; tail flicks, skin ripples and ear flicks were correlated with the numbers of *Stomoxys* and *Glossina* but leg movements were correlated with numbers of non-biting muscoids, Stomoxys and Tabanidae but *not* tsetse.

These data suggest that seasonal changes in cattle nutrition do not affect the feeding responses of tsetse but that nonetheless there is a significant seasonal change in feeding success. This variation is caused by seasonal changes in ox behaviour which, in turn, is determined by the abundance of other biting Diptera.

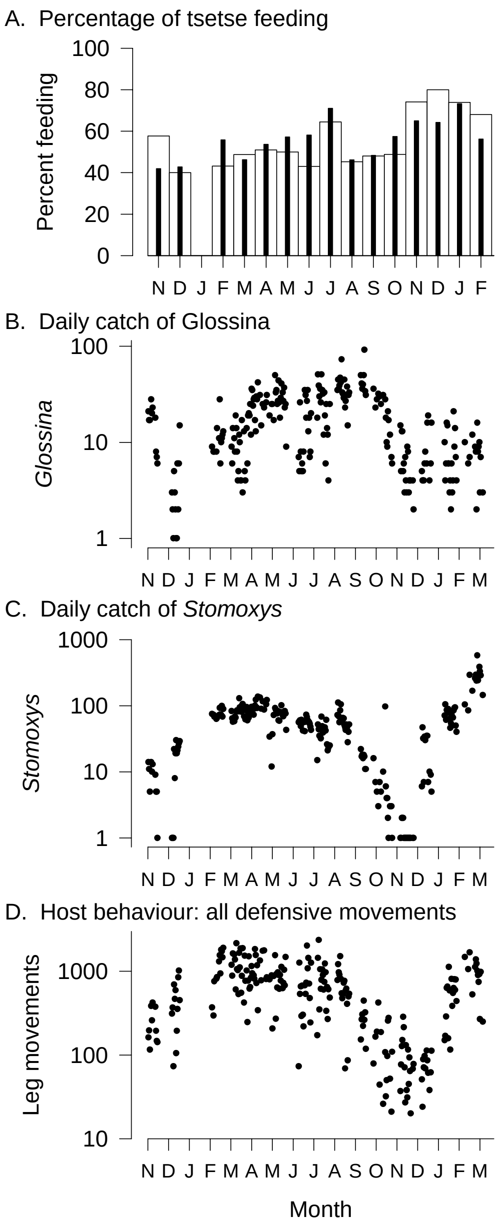

Figure 13. Mean percentage of tsetse feeding successfully on veld- (open bars) or supplement-fed (solid bars) (A), daily catch of *Glossina* (B), *Stomoxys* (C) from an incomplete ring of nets surrounding a mature Mashona ox, and the total defensive movements (D) of the ox. No data for January 1996

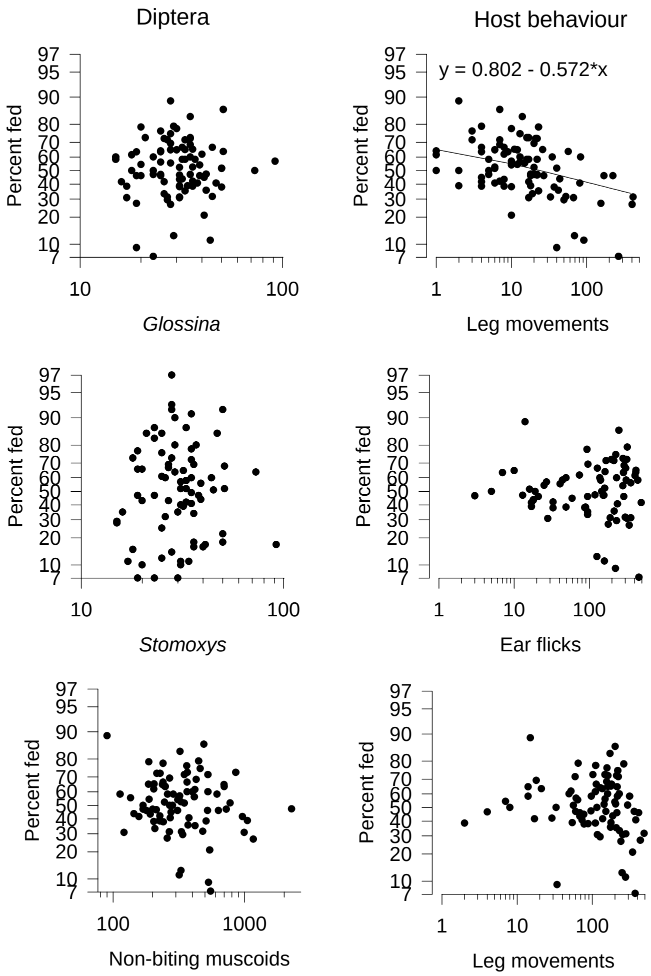

Figure 14. Scatterplots of percentage feeding success of tsetse and the numbers of various Diptera (left-hand column) or the frequency of various ox defensive behaviours (left-hand column). Only the frequency of leg movements was significantly correlated with feeding success.

Effect of host sex and age on the feeding responses of tsetse

Feeding success decreased in the order: mature oxen (59.6% feeding success, *n*=1496), cows and heifers (45.4%, *n*=1575), young (1-2 year olds) cattle (19.3%, *n*=1065) and calves (9.6%, *n*=291). The results for studies undertaken in 1997-98 (Fig. 15) suggest that feeding success varied through the year with a peak in October - November.

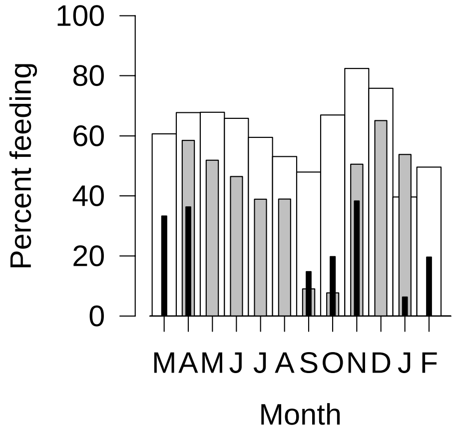

Figure 15. Percentage of tsetse feeding successfully on mature oxen (open bars), cows (grey bars) and 1-2 year old calves (black bars) between March 1997 and February 1998. No studies were made of cows in March 1997 and February 1998 and calves were studied in March-April 1997, September - November 1997 and January - February 1998.

As with studies undertaken in 1995-96, the October - November peak in feeding success coincides with relatively low numbers of biting Diptera and consequently low rates of defensive behaviour (Fig. 16).

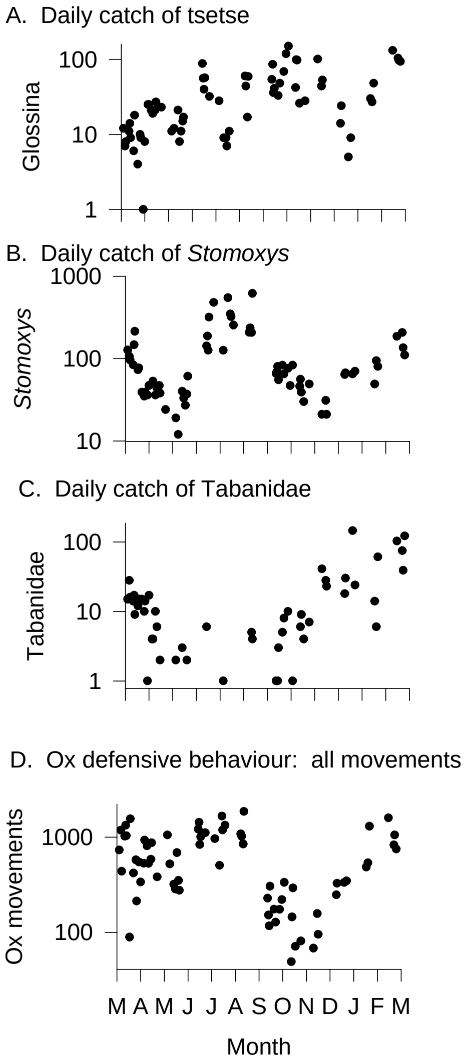

Figure 16. Daily catch of *Glossina* (A), *Stomoxys* (B) and Tabanidae (C) from an incomplete ring of nets surrounding a mature Mashona ox between March 1997 and February 1998 and the total defensive movements (D) of the ox.

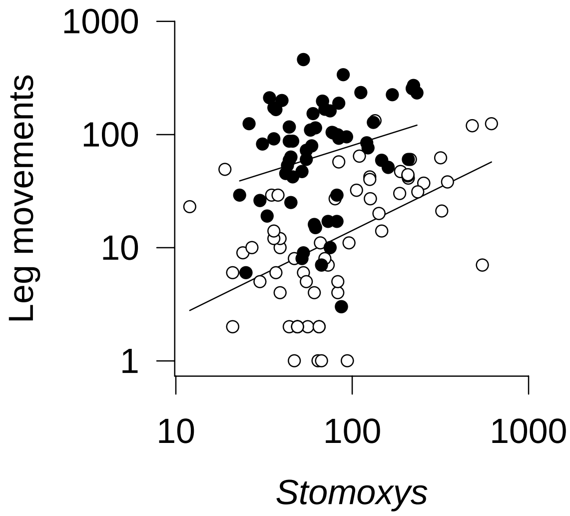

Figure 17. Scatterplot of cattle leg movements and daily catch of *Stomoxys* for calves (solid dot) and mature oxen (open dots).

The differences in success of tsetse attempting to feed on different types of cattle appeared to be correlated with the frequency of the host defensive behaviour; the detransformed mean rate of leg movements per afternoon for oxen, cows and 1-2 year old calves were 10.4, 25.9 and 62.2 respectively, and as with the 1995-97 study of oxen, the frequency of leg movements was highly correlated with numbers of *Stomoxys* (Fig. 17).

Observations of tsetse landing on cattle showed that 58% (*n*=276) of *G. m. morsitans* landed on the torso compared to only 8% (*n*=1500) of *G. pallidipes*. Of those flies that landed, <10% were observed to feed, most flies being disturbed by either the host's defensive behaviour or by other Diptera. For the "disturbed" flies, the mean time on a host was 15 s and the probability of such a fly remaining on the host declined exponentially (Fig. 18). For those flies that fed successfully, the mean time on the host was 93 s and the probability density function for these flies exhibited a more normal distribution. These results suggest that most flies do not take a meal at their first attempt but move between sites on the host, and possibly between hosts, to obtain a feed.

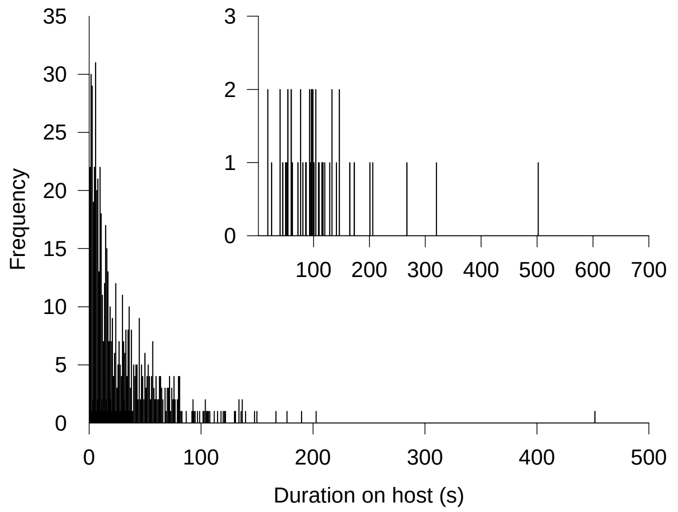

Figure 18. Frequency distribution of residence durations for *Glossina* alighting on a mature ox. Main graph shows tsetse that failed to obtain a complete feed (n=646), inset shows *Glossina* that completed a feed (n=50).

Somewhat surprisingly, the mean duration of landing was not significantly different between different types of cattle. There were however differences in the cause of disturbance: the ratio of tsetse disturbed by either ox- or insect-behaviour was 2.9:1 for a calves compared to 1.2:1 for oxen.

These results suggest that young animals are intolerant of tsetse and are thus not effective baits. Moreover, since most tsetse attempting to feed on a host are disturbed by either the host or other Diptera, it seems that a bloodmeal may consist of a number of small feeding attempts. This has potentially important implications for both disease epidemiology and tsetse control. Firstly, if the feeding attempts are made on different animals, as may be the case where hosts are in groups, then disease transmission will be increased by feeding interruptions. Secondly, flies that are disturbed as they attempt to feed on an intolerant animal within a herd may subsequently attempt to feed on a more tolerant individual within a herd. Thus the larger, more attractive and tolerant animals within a herd are fed on more frequently, and it is these large animals that are the most effective baits.

Contribution of outputs

Progress towards project outputs

Output 1. Preliminary quantitative relationships between host nutrition and age and cattle:tsetse contact established.

Quantitative relationships between host nutrition and age and cattle-tsetse contact were established. The attractiveness of cattle to tsetse is correlated with the weight of the host with small and young animals being less attractive than mature oxen. The relatively slight (*ca.* 50 kg) annual fluctuations in weight caused by seasonal changes in nutrition do not produce significant changes in attractiveness. Young animals were less tolerant of feeding tsetse with only 10% of tsetse feeding successfully on calves compared to >50% on oxen. Consequently, the numbers of tsetse feeding on calves is *ca.* 5% of that feeding on mature oxen. Differences in attraction appear to be due largely to variation in carbon dioxide production, and variation in feeding success is governed by host behaviour, in particular the frequency of leg movements.

Output 2. Recommendations on methodology and benefits of further research on factors affecting cattle:tsetse contact.

The methodologies adopted for this project were able to distinguish differences in the attractiveness and the feeding responses of tsetse to different types of cattle. The analyses of host odours supported the biological studies and provided a rational basis for understanding the physiological cause of the differences. Accordingly, we now have a sound means of predicting the attractiveness of a host to tsetse. In short, any physiological process that elevates carbon dioxide production (e.g. body mass, work, food intake) increases host attractiveness.

Output 3. Recommendations on the effects of stock composition and density on the efficacy of cattle as baits for tsetse control.

The project established that an insecticide-treated ox would attract and kill as many tsetse as the type of odour-baited target used to control *G. pallidipes* and *G. m. morsitans* in Zimbabwe. Accordingly, densities of 4 oxen/km^2 evenly distributed would be sufficient to control tsetse. Cows, and calves in particular, are less effective as baits and consequently higher densities of cattle would be required in practice. The aggregation of cattle caused by herding would also greatly affect the efficacy of cattle since the curvilinear dose response of tsetse to host odours (Torr, 1990; Hargrove *et al.,* 1996) means that the number of cattle attracted to a group of oxen is less than the sum of the numbers attracted to each individual ox.

Progress to purpose

Sustainable and cost effective strategies for the control of trypanosomiasis in Zimbabwe and tsetse-infested countries of sub-Saharan Africa developed and promoted.

The project made a significant contribution towards quantifying the densities of insecticide-treated cattle required to control tsetse. The TTCB is currently treating cattle with insecticide to control tsetse in NE Zimbabwe. The technique has successfully controlled tsetse in some parts of Zimbabwe but has recently been found to be unable to prevent tsetse from invading areas previously cleared of tsetse (Warnes *et al.,* 1998). The reasons for the variation in efficacy are still uncertain but a combination of low and unevenly distributed populations of cattle is the most likely cause.

Uptake pathways

The work was carried out in close collaboration with TTCB and RTTCP and thus the project outputs are directly available to these authorities. Moreover, during the course of the project, links were established with tsetse control authorities in Kenya, South Africa, Tanzania, Uganda and Zambia, and the ouputs have been made available to them. Insecticide-treated cattle are being used to control tsetse in all the aforementioned countries, and the outputs are thus particularly germane to current control activities. To date, one paper has been published and a second accepted for publication in *Medical and Veterinary Entomology* and hence these outputs will be available to tsetse control authorities throughout Africa. A further three papers are in preparation, and we anticipate that these will be published in *Bulletin of Entomological Research* or *Medical and Veterinary Entomology*. Finally, the project outputs will be disseminated through the continuing professional links between NRI, TTCB and RTTCP.

Further work

A number of further research projects are required for this particular tsetse control technique to be developed adequately.

Firstly, further work is required to establish the efficacy of various insecticide formulations for use as pour-ons and dips, and the effect of these formulations on cattle-tsetse contact. This work is currently being undertaken in Zimbabwe by Dr Vale (RTTCP) *et al.* with funding from the TTCB, RTTCP and various insecticide companies. To date the results show that the efficacy of different insecticide formulations varies with season, but none has an effect on the behavioural interaction of tsetse and cattle.

Secondly, work is required to quantify the effects of cattle movement on tsetse-cattle contact; the work undertaken by this project was concerned primarily with stationary animals. Studies of the responses of tsetse to mobile baits are currently being undertaken by Drs Vale (RTTCP) and Hargrove (DFID-supported) with funding from RTTCP, TTCB and DFID.

Thirdly, work is also required to determine how cattle management practices affect the efficacy of using insecticide-treated cattle. This is currently the subject of a project being undertaken by NRI, UEA, TTCB and various Tanzanian authorities, funded in part by the DFID Animal Health Programme.

Finally, there are currently no data on the direct environmental impact of treating cattle with insecticides for controlling tsetse, and we suggest that this should be the subject of an investigation. As far as we are aware, no funding agencies are currently considering supporting such work.

Dissemination

The following overseas visits were undertaken wholly or partly supported by Project R6559.

Country	Scientist(s)	From	To
Zimbabwe	Torr & Hall	8 March 1996	21 April 1996
Zimbabwe	Torr	6 September 1996	18 October 1996
Zimbabwe	Torr & Hall	21 February 1997	18 April 1997
Zimbabwe	Torr	21 July 1997	1 August 1997
Zimbabwe	Torr	16 September 1997	6 October 1997
South Africa	Torr	6 October	17 October 1997
Germany	Torr	17 November 1997	28 November 1997
UK	Mangwiro	1 December 1997	14 December 1997
Zimbabwe	Torr	26 January 1998	13 February 1998
Zimbabwe	Torr & Hall	23 March 1998	24 April 1998

The following presentations were produced:-

Zimbabwe

- Three discussion papers, one specifically related to the AHP-funded project, were presented at the RTTCP Research & Development Workshop, Harare, Zimbabwe 12 - 14 March, 1996.
- Presentation on various aspects of the tsetse-host physiology project to the Zimbabwe Under-secretary for Agriculture and staff of the Zimbabwe Tsetse and Trypanosomiasis Control Branch (TTCB) (April 1997).
- Presentation to the Commissioner of the Ugandan Department of Entomology during his visit to Zimbabwe (February 1998). Subsequently the Commissioner was provided with copies of reports and papers outlining the project activities and results.

Ethiopia

Hall, D.R. & Torr, S.J. (1997). Improving cost-effectiveness of traps and targets. Paper presented at “The Way Forward”, FITA research workshop, Axum & Addis Ababa, Ethiopia, 3 - 7 February 1997.

USA

Torr, S.J. (1996). Host-orientated responses of tsetse. Paper presented at the Biting Fly Symposium, New Orleans, La. USA. 13 - 14 May 1996

South Africa

A visit, funded by the RTTCP, was made to the Hellsgate tsetse research station of the Onderstepoort Veterinary Institute (OVI), South Africa. During the visit, presentations on aspects of the project were made to tsetse entomologists from South Africa and Zambia, and South African scientists were trained in methods for collecting samples of host odour for chemical analysis.

Germany

A visit, funded by the German National Science Foundation (DFG), was made to the Johannes Gutenberg University in Mainz, Germany, to present a seminar to graduate students and staff on the host-orientated responses of tsetse. The Max Planck Institute for Chemistry, which is on the University of Mainz campus, has assisted this project by providing advice on robust methods for measuring host carbon dioxide.

UK

A presentation on aspects of the host physiology project was made to a group of South African academics who visited NRI in December and Professor Hall presented an inaugural Professorial lecture which included some elements of NRI's work on the host-orientated behaviour of tsetse.

Internal reports to TTCB & RTTCP

Six visit reports, six quarterly reports and two annual reports were produced for senior scientists and managers within TTCB and the Regional Tsetse and Trypanosomiasis Control Programme (RTTCP). The reports provided updates of the project work and the practical implications of the findings.

Papers published

Gibson, G. & Torr, S.J. (1999). The visual and olfactory responses of biting Diptera to host stimuli. *Medical and Veterinary Entomology* **13**, 000-000. (submitted January 1998, accepted April 1998, anticipated publication January 1999)

Hall, D.R. (1998). *A rose by any other name?* Inaugural Lecture Series, University of Greenwich, UK.

Torr, S.J. & Hargrove, J.W. (1998). Factors affecting the landing and feeding responses of *Glossina pallidipes* Austen to a stationary ox. *Medical and Veterinary Entomology* **12**, 196-207.

References cited

Barrett, J. (1992). The economic role of cattle in communal farming systems in Zimbabwe. *Pastoral Development Network* **32**(b), 1-35.

Barrett, J. (1994). Economic issues in trypanosomiasis control: case studies from Southern Africa. Ph.D thesis, University of Reading.

Baylis, M. (1996). Effect of defensive behaviour by cattle on the feeding success and nutritional state of the tsetse fly *G. pallidipes* (Diptera: Glossinidae). *Bulletin of Entomological Research* **86**, 329-336.

Baylis, M. & Nambiro, C.O. (1993). The nutritional state of male tsetse flies, *G. pallidipes*, at the time of feeding. *Medical and Veterinary Entomology* **7**, 316-322.

Bursell, E., Gough, A.E., Beevor, P.S., Cork, A., Hall, D.R. & Vale, G.A. (1988) Identification of components of cattle urine attractive to tsetse flies, *Glossina* spp. (Diptera:Glossinidae). *Bulletin of Entomological Research*, **78**, 281-292.

Challier, A. (1965). Amélioration de la methode de détérmination d l'âge physiologique des glossines. *Bulletin de la Societé de Pathologie Exotique* **58**, 250-259.

Crawley, M.J. (1993). GLIM for ecologists. Blackwell Scientific Publications, Oxford, UK. 379 pp.

Francis, B., Green, M., & Payne, C. (1993). The GLIM System (Release 4 Manual), Clarendon Press, Oxford, UK 821 pp.

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

Hargrove, J.W. (1976) The effect of human presence on the behaviour of tsetse (*Glossina* spp) (Diptera: Glossinidae) near a standing ox. *Bulletin of Entomological Research* **66**, 173-178.

Hargrove, J.W. (1995). Towards a general rule for estimating the stage of pregnancy in field-caught tsetse flies. *Physiological Entomology* **20**, 213-223.

Hargrove, J. W. & Brady, J. (1992). Activity rhythms of tsetse flies (*Glossina* spp.) Diptera: Glossinidae) at low and high temperatures in nature. *Bulletin of Entomological Research* **82**, 321-326.

Hargrove, J.W., Holloway, M.T.P., Vale, G.A., Gough, A.J.E. & Hall, D.J. (1995) Catches of tsetse flies (*Glossina* spp.) (Diptera : Glossinidae) from traps baited with large doses of natural and synthetic host odour. *Bulletin of Entomological Research*, **85** 000-000.

Hargrove, J.W. & Packer, M.J. (1993). Nutritional status of male tsetse flies (*Glossina* spp) caught in odour baited traps and artificial refuges: models for feeding and digestion. *Bulletin of Entomological Research* **83**, 29-46.

Langley, P.A. & Wall, R. (1990). The implications of hunger in the tsetse fly, *Glossina pallidipes*, in relation to its availability to trapping techniques. *Journal of Insect Physiology* **36**, 903-908.

Langley, P.A., Hargrove, J.W. & Wall, R.L. (1990). Maturation of the tsetse fly *Glossina pallidipes* (Diptera: Glossinidae) in relation to trap-orientated behaviour. *Physiological Entomology* **15**, 179-186.

Leak, S.G.A., Woudyalew, M., Rowlands., & D'Ieteren, G.D.M. (1995). A trial of cypermethrin 'pour-on' to control *Glossina pallidipes, G. fuscipes fuscipes* and *G. morsitans submorsitans* (Diptera: Glossinidae) in south-west Ethiopia. *Bulletin of Entomological Research* **85**, 241-251.

Loder, P.M.J. (1997). Sizes of blood meals taken by tsetse flies (*Glossina* spp.) (Diptera: Glossinidae) correlates with fat reserves. *Bulletin of Entomological Research*, **87**, 547-549

Milligan, P.J. & Baker, R.D. (1988). A model of tsetse-transmitted animal trypanosomiasis. *Parasitology* **96,** 211-239.

Randolph, S.E., Rogers, D.J. Dransfield, R.D. & Brightwell, R. (1991). Trap-catches, nutritional condition and the timing of activity of the tsetse fly *Glossina longipennis* (Diptera: Glossinidae). *Bulletin of Entomological Research* **81**, 455-464.

Roberts, R.H. (1972) Relative attractiveness of CO_2 and a steer to Tabanidae, Culicidae and *Stomoxys calcitrans* (L.). *Mosquito News,* **32**, 208-211.

Rogers, D.J. (1988). A general model for the African trypanosomiases. *Parasitology* **97**, 193-212.

Taylor, P. (1976). Blood-meal size of *G. m. morsitans* Westw. and *G. pallidipes* Austen (Diptera: Glossinidae) under field conditions. *Transactions of the Rhodesia Scientific Association* **57**, 29-34.

Thomson, M.C. (1987). The effect on tsetse flies (*Glossina* spp.) of deltamethrin applied to cattle either as a spray or incorporated into ear-tags. *Tropical Pest Management* **33**, 329-335.

Torr, S.J. (1990) Dose response of tsetse flies (*Glossina*) to carbon dioxide, acetone and octenol in the field. *Physiological Entomology,* **15**, 93-103

Torr, S. J. (1994). Responses of tsetse flies (Diptera: Glossinidae) to warthog (*Phacochoerus aethiopicus* Pallas). *Bulletin of Entomological Research* **84**, 411-419.

Torr, S.J., Hall, D.R., Phelps, R.J. & Vale, G.A. (1997). Methods for dispensing odour attractants for tsetse flies (Diptera: Glossinidae) *Bulletin of Entomological Research* **87**, 299-311.

Torr, S.J. & Hargrove, J.W. (1998). Factors affecting the landing and feeding responses of *Glossina pallidipes* Austen to a stationary ox. *Medical and Veterinary Entomology* **12**, 196-207.

Torr, S.J., Mangwiro, T.N.C & Hall, D.R. (1996). Responses of *Glossina pallidipes* (Diptera: Glossinidae) to synthetic repellents in the field. *Bulletin of Entomological Research,* **86**, 609-616.

Vale, G. A. (1974a). The responses of tsetse flies (Diptera: Glossinidae) to mobile and stationary hosts. *Bulletin of Entomological Research* **64,** 545-588.

Vale, G.A. (1974b). New field methods for studying the response of tsetse flies (Diptera: Glossinidae) to baits. *Bulletin of Entomological Research* **64,** 199-208.

Vale, G.A. (1977). Feeding responses of tsetse flies (Diptera: Glossinidae) to stationary hosts. *Bulletin of Entomological Research* **67**, 635-649.

Vale, G.A. (1981) An effect of host diet on the attraction of tsetse flies (Diptera: Glossinidae) to host odour. *Bulletin of Entomological Research*, **71**, 259-265.

Vale, G.A. (1993) Development of baits for tsetse flies (Diptera: Glossinidae) in Zimbabwe. *Journal of medical Entomology,* **30,** 831 842.

Vale, G.A. & Hargrove, J.W. (1979). A method for studying the efficiency of traps for tsetse flies (Diptera: Glossinidae) and other insects. *Bulletin of Entomological Research* **69**, 183-193.

Warnes, M.L., Van den Bossche, P. Chihiya, J., Mudenge, D. Robinson, T.P., Shereni, W. & Chadenga, V. (1999). Trial to investigate the efficacy of insecticide-treated cattle as a barrier to re-invasion of tsetset to cleared areas in north-eastern Zimbabwe. *Medical and Veterinary Entomology* (submitted May 1998).