Evaluation of a proposed odour-baited target to control the tsetse flies *Glossina brevipalpis* and *Glossina austeni* (Diptera: Glossinidae) in South Africa

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ABSTRACT

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The most effective odour attractant for *G. brevipalpis* Newstead, namely a combination of octenol released at *c.* 9,1 mg/h, 4-methyl phenol released at *c.* 15,5 mg/h and acetone released at *c.* 350 mg/h, when used together with the smallest recommended colour target (as determined in previous studies), namely a 1,75 m wide x 1 m high black/pthalogen-blue/black target, was evaluated for the control of *G. brevipalpis* and *G. austeni* Newstead. This combination increased the catches of *G. brevipalpis* by 3,5 fold when compared to the number of those caught on a 1,5 m wide x 1 m high black target baited with a synthetic ox-odour as was used in a trial to control this species in the Hluhluwe-Umfolozi Game Reserve in 1992. There was an indication that odour (olfaction) plays a far more important role in attracting *G. brevipalpis* than does colour (vision). For *G. austeni* visual attraction appears to play the major role as the odours used were relatively unattractive to them. The odour-baited target should, however, attract *G. austeni* in sufficient numbers (visually) to achieve control to the fly.

Keywords: Control target, *Glossina austeni, Glossina brevipalpis,* odour-bait, South Africa, tsetse flies

INTRODUCTION

In South Africa trypanosomosis or nagana occurs only in the north-eastern areas of KwaZulu-Natal Province where the two tsetse fly species, *Glossina brevipalpis* and *G. austeni* are its vectors. These flies are confined to evergreen forests and thickets, often associated with water-courses and other densely or semi-forested areas. In South Africa they are responsible for the transmission of various trypanosome species to livestock. These are the severely pathogenic tsetse-transmitted *Trypanosoma* species *T. brucei* Plimmer & Bradford (in horses), *T. vivax* Ziemann (in cattle), *T. congolense* Broden (in cattle) and *T. simiae* Bruce *et al.* (in pigs) (Connor 1994).

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The main nagana problem areas of KwaZulu-Natal Province are in the magisterial districts of Ingwavuma, Ubombo, Hlabisa and Nongoma in the north-east (De Waal, Carter, Matthee & Bagnall 1998). The area is confined to some 16 000 km^2 and contains 426 000 humans, 130 000 small ruminants (De Waal *et al.* 1998) and *c.* 350 000 cattle belonging to developing farmers in communal farming areas and *c.* 9 000 on commercial farms. This number of cattle amounts to *c.* 10,8 % of those in the entire KwaZulu-Natal (A. Ilemobade, unpublished report 1997).

In 1990 an outbreak of nagana contributed to severe cattle mortalities in the communal areas of the magisterial districts mentioned above, involving *T. vivax* and *T. congolense* (De Waal *et al.* 1998). Between 1990 and 1994 surveys showed cattle served by 77 of 132 diptanks to be infected with nagana. Emergency control measures, which consisted of the treatment of cattle with homidium bromide ("Ethidium"—

Hoechst Roussel Vet. (Pty) Ltd, P.O. Box 6065, Halfway House, 1685 South Africa) and diminazene ("Berenil"—Hoechst Roussel Vet.) as well as the weekly to fortnightly dipping of cattle in a pyrethroid, cyhalothrin ("Grenade"—Hoechst Roussel Vet.) brought the disease under control (Kappmeier, Nevill & Bagnall 1998). This dipping was maintained for only 2 years so that ticks would not develop resistance as they have to the chlorinated hydrocarbons. Since these control measures are costly, a long-term solution for the control of the disease, through the control or eradication of tsetse flies from the infested areas, is being sought.

Improved understanding of visual and olfactory stimuli responsible for the host-seeking behaviour of tsetse flies, has provided motivation for the development of artificial bait technology. In recent years reliance for the control of tsetse flies in parts of Africa has increasingly been placed on attracting them to insecticide-impregnated odour-baited targets of the right colour (Vale, Lovemore, Flint & Cockbill 1988; Willemse 1991; Knols, Willemse, Flint & Mate 1993; Van den Bossche 1997). In 1992 odour-baited targets were also used in South Africa in a trial to control *G. brevipalpis* in the northern parts of the Hluhluwe-Umfolozi Game Reserve (Kappmeier *et al.* 1998). Use was made of the target technology then used in Zimbabwe for the control of *G. morsitans morsitans* Westwood and *G. pallidipes* Austen (Vale *et al.* 1988). The target consisted of a 1,5 x 1 m black cloth baited with 3-*n*-propyl phenol, 1-octen-3-ol, 4-methyl phenol and acetone. Research conducted in KwaZulu-Natal between 1993 and 1996 has, however, indicated a target design of pthalogen blue (p.blue) flanked by black (black/p.blue/black) to be 2,1–2,9 times more effective for *G. brevipalpis* than an all-black target (Kappmeier & Nevill 1999a). This research also indicated that the odour-bait, which was used in the Hluhluwe-Umfolozi control trial, could be improved on for *G. brevipalpis* by omitting 3-*n*-propyl phenol, which had no effect on the catches, and by increasing the doses of octenol and 4-methyl phenol by four to 16 times (Kappmeier & Nevill 1999b). This dose increment increased the catches of this species significantly by 2,3–2,8 times compared to the mixture used in the 1992 control trial, and attracted 10,1–12,3 times more flies than with 'no odour'. Although odours were unattractive to *G. austeni* (Kappmeier & Nevill 1999b) the 2 m black/p.blue/black targets were found to be 3,8–5,7 x more attractive than the 1,5 m plain black target (Kappmeier & Nevill 1999a).

All previous studies on odours undertaken at the Hellsgate Tsetse Research Station, which is situated on the shores of Lake St Lucia (Kappmeier 1997), were conducted with a standard target and all studies on colour targets were baited with a standard odour (Kappmeier & Nevill 1999a; 1999b). The current study was, therefore, conducted to evaluate the combined effect of the best South African (SA) odour (for *G. brevipalpis*), namely octenol (released at *c.* 9,1 mg/h), 4-methyl phenol (released at *c.* 15,5 mg/h) and acetone (released at *c.* 350 mg/h), together with the smallest (narrowest) recommended colour target, namely a 1,75 m wide x 1 m high black/p.blue/black target (i.e. 75 cm p.blue flanked by two 50 cm black panels) (Kappmeier & Nevill 1999a, 1999b) for both species. This would indicate whether the newly developed target/odour combination would indeed be effective for *G. brevipalpis* and *G. austeni*, and also how it would compare with that used in 1992 against *G. brevipalpis.*

METHODS

Studies were conducted at the Hellsgate Tsetse Research Station, described in Kappmeier (1997), where both *G. brevipalpis* and *G. austeni* occur in large numbers. Tests were carried out from midday until dark during which time the two species are active (Kappmeier, unpublished information 1999).

The various standard (control) treatments used in earlier (Kappmeier & Nevill 1999a; 1999b) colour and odour studies, namely a 1,5 m wide x 1 m high black target (in colour experiments) and a 1 x 1 m p.blue target flanked by 0,5 m x 1 m netting (in odour experiments), each baited with the then standard Zimbabwe odour mixture (Zim-mix), were combined and tested in one experiment and compared with the best odour (for *G. brevipalpis*) (Kappmeier & Nevill 1999b) and the smallest recommended colour target (Kappmeier & Nevill 1999a). The various treatments and details of the composition of these targets and odour baits are given in the following table:

Target conformation (all 1,0 m high)	Odour-bait and release rates
1* 1,5 m black 2 1,5 m p.blue/net (1 m p.blue flanked by 0,5 m net) 3 1,75 m black/p. blue/black (1:1,5:1) (smallest recommended target	Zim-mix (standard odour): 1:4:8 (3-*n*-propylphenol at 0,1 mg/h: octenol at 0,4 mg/h: 4-methylphenol at 0,8 mg/h) plus acetone at 350 mg/h (6 mm diameter opening)
4 1,5 m black 5 1,5 m p.blue/net (1 m p.blue flanked by 0,5 m net) 6 1,75 m black/p.blue/black (1:1,5:1) (smallest recommended target)	Best recommended South African (SA) odour 1:2 (octenol at 9,1 mg/h: 4-methylphenol at 15,5 mg/h) plus acetone at 350 mg/h (6 mm diameter opening)

* The 1,5 m black target baited with the Zim-mix as was used during the 1992 *G. brevipalpis* control trial acted as the control treatment

Each target system under comparison was fitted into a framework of electrified copper wiring (electric grids) under which was a sticky tray so that flies settling on the electrified target or colliding with the flanked electrified netting were electrocuted and then retained on the tray, following the methods given by Vale (1974a). The flies could then be identified, sexed and counted. Electrified targets were set in sand forest, 200 m apart.

The treatments were incorporated into a series of Latin squares of treatments x days x sites. Eighteen replicates were carried out from May to October 1996, with one replicate being one treatment tested at one site for one day. The daily catches (n) were subjected to an analysis of variance (Anova) following an estimated skewness for normal distribution and a $\log_{10}(n + 1)$ transformation. The Anova was followed by Bonferroni's multiple range test to compare treatment means. The general test level was P = 0,05. Male and female catches were analyzed separately for *G. brevipalpis*, but pooled catches were used for *G. austeni*, due to low numbers caught.

RESULTS

The results of the various treatments under comparison are summarized in Fig. 1A and B for *G. brevipalpis* and *G. austeni* respectively. These are expressed as the indices of increase, which were estimated by expressing the overall electric grid catch of each treatment as a proportion of the catch of the control treatment, using detransformed mean log (n) daily fly catches. Females and male catches are indicated separately, where the numbers were adequate, also relative to the control catch. Treatments that are significantly different from the control are indicated with an asterisk in Fig. 1.

For *G. brevipalpis* (Fig. 1A) the smallest recommended target plus best SA odour blend increased the total catches of this species 3,5 times (for totals) when compared to the standard 1,5 m black target baited with the Zim-mix (i.e. the 1992 control target). The smallest recommended target plus best SA odour attracted a geometric mean of 52,0 flies per test period (22,5 females) for the combined Latin squares with a maximum of 111 (46 females) flies/day. (Note that the studies were conducted during the cold season and that in summer much larger catches are obtained.) Furthermore, all three targets baited with the best SA odour were significantly more effective than all three targets baited with the Zim-mix (for total, female and male catches) increasing their catches, over their corresponding Zim-mix baited targets, by *c.* 2,2–2,7 fold. The three targets (when baited with the same odour) did not, however, differ significantly from each other in this experiment so that it seems that odour is far more important in the attraction of this species than is colour.

For *G. austeni* (Fig. 1B) only the total catches were analyzed due to the low number of flies of this species that were caught during all three replicates. Geometric means for the combined squares were as low as 5,3 flies/day (maximum 17 flies/day), however, catches of this species in winter are usually very low relative to what can be caught in the warm season. None of the treatments differed significantly from the standard control (1,5 m wide black target baited with Zim-mix). The two p.blue/net targets baited with both the odour treatments (Zim-mix or best SA odour) and the 1,75 m wide black/p.blue/black target baited with

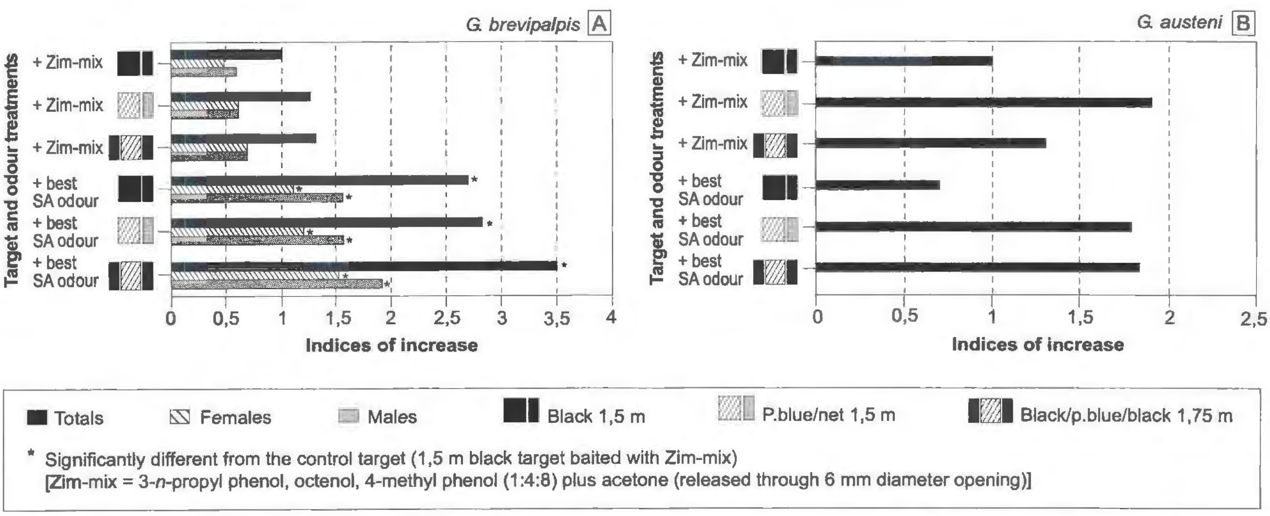

FIG. 1 Comparison of some standard target and odour systems with the new recommended best South African (SA) odour combined with the smallest recommended target for (A) *G. brevipalpis* and (B) *G. austeni*

the best SA odour still increased catches by *c.* 1,8–1,9 x, compared to the control. These were also found to be significantly different from the 1,5 m black target baited with the best SA odour. The results suggest that the odours used did not play an important role in the attraction of this species. Black remained equally unattractive when using the Zim-mix or the best SA odour, and no significant increase was obtained in the catches when alternating the odours with the remaining, more attractive, colour targets. This suggests that vision plays a more important role than do the odours that were used.

DISCUSSION

The purpose of controlling tsetse vectors is to reduce or eliminate their contact with humans and/or livestock. The control of *Glossina* has become increasingly dependent upon the use of insecticides. Ground and aerial application of DDT, HCH, dieldrin and endosulfan were widely used in Africa and have proved to be very effective for the management of crisis situations (Du Toit 1954; Davies 1981; Turner 1984). These methods are, however, comparatively expensive and nowadays not favoured because of logistical requirements and for environmental reasons. Vector control, by applying insecticide to cattle in the form of cattle dips or pour-ons, is technically feasible and is a promising technique in tsetse-infested areas where cattle occur (Thomson 1987; Thomson, Mitchell, Rees, Shereni, Schonefeld & Wilson 1991; Fox, Mmbando, Fox & Wilson 1993; Bauer, Amsler-Delafosse, Clausen, Kabore & Petrich-Bauer 1995) and could also be used together with targets to maintain tsetse fly barriers (Warnes, Mudenge, Chihiya, Van den Bossche, Shereni & Chadenga 1997). Therefore, these methods are more likely to be effective on commercial ranches where the density of wild hosts is usually low (Williams & Williams 1992). They are, however, inappropriate when it comes to tsetse fly control in game reserves or other tsetse-infested areas where the flies cannot, for obvious reasons, come into contact with insecticide-treated cattle. The most recent approaches towards tsetse fly control are more selective and environmentally friendly. The use of pyrethroid-impregnated targets is, therefore, one of the more refined approaches towards tsetse control (Vale, Hargrove, Cockbill & Phelps 1986; Vale *et al.* 1988; Willemse 1991; Knols *et al.* 1993; Van den Bossche 1997).

In the present study, a practical and effective combined target and odour system was developed which, it is considered, could prove effective for the control of both *G. brevipalpis* and *G. austeni* when used in the field. The combination of the best developed SA odour (Kappmeier & Nevill 1999b), namely octenol (released at *c.* 9,1 mg/h), 4-methyl phenol (released at *c.* 15,5 mg/h) and acetone (released at *c.* 350 mg/h), together with the smallest recommended colour target (Kappmeier & Nevill 1999a), namely a 1,75 m black/p.blue/black target (i.e. 75 cm p.blue flanked by two 50 cm black panels), appears to be an effective combination to employ as a control device for both species. In experiments this target and odour combination was found to be significantly more attractive for *G. brevipalpis* than the target used in the 1992 trial (Kappmeier *et al.* 1998). The new system increased the catches of *G. brevipalpis* by 3,5 fold when compared to the 1992 target system. The present results also indicate that olfactory attraction plays a more important role in attracting this species than does vision. It is, therefore, concluded that the present available odours are important attractants for *G. brevipalpis* in the South African situation.

It has been suggested that when odour is present, colour and other visual cues are less important in attracting tsetse flies to a target (Vale 1974b; Hargrove & Vale 1978; Jordan & Green 1984). Results in the present study indicate, however, that for *G. austeni* visual attraction could be more important than olfactory attraction (with the present used odours), as the targets baited with the best SA odour did not increase catches significantly over those baited only with the Zim-mix, which has been shown to be no better than an unbaited target (Kappmeier & Nevill 1999b). Although the 1,75 m wide recommended black/p.blue/black target (baited either with the Zim-mix or the best SA blend) did not significantly increase the catches compared to those of the black target, its catches were still up to 2,5 times higher. In previous studies (Kappmeier & Nevill 1999a) it was shown that this target increased catches by nearly four times compared to those of the black target. Since the use of odours is ineffective, improvement of the visual attractiveness of targets will, therefore, be particularly important to attract *G. austeni* to their vicinity. The 1,75 m wide black/p.blue/black target is still effective for capturing *G. austeni*, even in the absence of any odours.

The best odour-baited target system, as evaluated in this study, is therefore recommended for the control of *G. brevipalpis*. Where *G. austeni* occurs allopatrically, the target need not be baited with synthetic odours. It is considered that this target could be used with good results in infested parts of KwaZulu-Natal, particularly those areas that have a framework of roads, where alternative control approaches, e.g. the use of insecticide-treated cattle are not feasible. These areas include the KwaZulu-Natal Nature Conservation Service's game and nature reserves situated in northeastern KwaZulu-Natal, namely the Hluhluwe-Umfolozi, Mkuzi, Tembe and Ndumu Game Reserves and the St Lucia Lake, Kosi Bay and other coastal conservation areas, as well as private game ranches and natural State Forest areas, all of which maintain single or mixed populations of *G. brevipalpis* and *G. austeni* (Nevill 1997). Cattle belonging to communal farmers often graze right up to the fenced or unfenced boundaries of these reserves. The use of the target system to control tsetse flies in these reserves, especially those that maintain large populations of game and tsetse flies and which therefore act as a major source of infection, should benefit small-scale farming in the immediate surrounding communal areas through a decreased risk of trypanosome infection. The optimal density of targets that should be used for the control of the two tsetse species, as well as the use of target barriers to prevent emigration of the flies from infected areas, still needs to be investigated. In the communal areas and on commercial cattle ranches alternative control approaches, e.g. the use of insecticide-treated cattle, may be more feasible and effective. In certain areas where fly numbers are very low or have been reduced to minimal levels or where topography and vegetation do not permit the use of targets, eradication, using the Sterile Insect Technique, might be an option.

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