Evaluation of coloured targets for the attraction of *Glossina brevipalpis* and *Glossina austeni* (Diptera: Glossinidae) in South Africa

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ABSTRACT

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Studies on the attractiveness of various coloured targets for *Glossina brevipalpis* and *G. austeni* in South Africa showed black and pthalogen blue (p.blue) combinations to be the most effective for both species. A 2 m wide (all targets 1 m high) black/p.blue/black (colour ratio 1:2:1) conformation caught nearly three times more *G. brevipalpis* and nearly five times more *G. austeni* than a 1,5 m wide black standard control target. For *G. brevipalpis* the black/p.blue/black (1:2:1) target should be at least 2 m wide in order to increase catches significantly while a 1,5–2,0 m wide target is optimal for *G. austeni*. The p.blue section of a 2 m black/p.blue/black target should not make up less than 20 % of the total target width for either species. The most effective combination of practical target sizes and colour ratios were a 1,75 m wide black/p.blue/black (1:1,5:1) or 2 m wide target (1,5:1:1,5). Between 61–95 % of *G. brevipalpis* and 34–90 % of *G. austeni* that were attracted, settled first on the black section of black/p.blue targets (> 1 m wide). Further studies revealed that for *G. brevipalpis* only the black parts of the 2 m wide target need to be treated with insecticide, while the entire 1,75 m wide target should be treated. For *G. austeni* the total width of either target should be treated with insecticide since this species readily settles on both blue and black.

Keywords: Colour targets, *Glossina austeni*, *G. brevipalpis*, South Africa, tsetse flies, visual attraction

INTRODUCTION

The control of tsetse flies has relied increasingly on attracting them to odour-baited insecticide-impregnated cloth targets of the right shape, size and colour (Vale 1993a, 1993b). These have been applied successfully in a number of African countries (Vale, Hargrove, Cockbill & Phelps 1986; Vale, Lovemore, Flint & Cockbill 1988a; Willemse 1991; Knols, Willemse, Flint & Mate 1993; Van Den Bossche 1997). Tsetse flies respond to stationary visual stimuli over a short range (Snow 1980). These include form (shape) (Vale 1974a; Torr 1989), size (Hargrove 1980), contrast with the background (Turner & Invest 1973; Gibson 1992), colour (spectral sensitivity) (Buxton 1955; Green 1993) and movement (Vale 1969, cited in Green 1994), all of which will be perceived by tsetse flies in an unobstructed view. These stimuli are not only important in attracting flies towards a target once they are in the visual range, but also play a role in the alighting responses of the flies (Green 1989; 1993), which is fundamental for the targets to be effective.

Many workers have tested several colours for their attractiveness to various tsetse species (Lambrecht 1973; Challier *et al.* 1977, cited by Challier 1982; Owaga 1981; Dransfield, Brightwell, Onah & Okolo

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1982; Jordan & Green 1984; Green 1986, 1988, 1990; Green & Flint 1986; Torr, Parker & Leigh-Browne 1989). All showed that the flies were attracted to dark or royal blue, especially that produced by a pthalogen blue pigment, followed by black and to a lesser extent light blue, white and grey. Visual attractiveness is controlled by spectral reflectivity. Blue wavelengths increase the attractiveness, while ultraviolet and green-yellow diminish it (Green 1994). The strongest landing responses of tsetse flies occur on either dark surfaces (Barrass 1960) or those strongly reflective in the ultraviolet wavelength range (300–400 nm) (Green 1988; 1994). The pthalogen blue pigment has high reflectivity (30–40 %) at 460 nm (mid-blue), little ultraviolet reflectivity and relatively little green-yellow reflectivity (Green 1988; 1994) so that, although this blue is visually very attractive, the landing responses of the flies are inhibited.

The use of electric nets to intercept tsetse flying near and around targets shows that tsetse often circle targets without landing on them (Vale 1974a; Hargrove 1980). To improve target efficacy, insecticide-treated mosquito-netting side-panels were added to the colour targets (Filledier & Politzar 1985, cited by Green 1990; Green 1988; 1989) and were also used in control trials (Vale, Bursell & Hargrove 1985). However, field trials revealed that the netting is easily damaged and deteriorates much faster than the cloth. To overcome this, Green (1989) suggested the combination of an attractive colour (pthalogen blue), which does not induce landing responses, with one (black) that does, therefore, dispensing with the need for impractical and costly mosquito-netting panels. Vale (1993a) proposed that the target size be increased to compensate for the replacement of the netting.

In 1992 a small-scale target trial was carried out to control *G. brevipalpis* in the Hluhluwe-Umfolozi Game Reserve in KwaZulu-Natal Province of South Africa (Kappmeier, Nevill & Bagnall 1998). No studies had been conducted previously on the use of targets for this species in South Africa or elsewhere so that the target then in use in Zimbabwe, for the control of *G. pallidipes* and *G. morsitans*, was chosen. This was a 1,5 m wide x 1 m high black cloth target baited with synthetic ox-odour (Vale 1991) and impregnated with deltamethrin. The *G. brevipalpis* trial was unsuccessful but this could have been due to a variety of reasons (Kappmeier *et al.* 1998).

The present work attempted to evaluate and improve on the attractiveness of the black target for *G. brevipalpis* and to find an attractive target for *G. austeni*. Various colours of proven attractiveness for other species were tested in a number of combinations and conformations for *G. brevipalpis* and *G. austeni*. Further variations were made to the best colour conformations and sizes to develop an attractive yet economical target for the control of these two species, taking into account the landing responses of the flies on different colours and the need to restrict, to a minimum, the surface area to be treated with insecticide.

MATERIALS AND METHODS

Experiments were conducted in north-eastern KwaZulu-Natal at the Hellsgate Tsetse Research Station (28°02'40"S, 32°25'50"E), described in detail in Kappmeier (1997). Electrified grids (Vale 1974b) of two sizes (1 x 1 m and 0,5 x 1 m) were used which could be combined into various dimensions (all targets tested in this study were 1 m high). Framed coloured cloth screens of various sizes and conformations were inserted between the two electrified surfaces of the grids. Since not all tsetse flies, attracted to a visual target, settle on it, a non-visual electric net panel (0,5 m wide) was sometimes added to one side of the visual target to intercept circling flies. This configuration is referred to as a flanked target (Fig. 1A). The grids were operated with a 12 V battery and an electric unit that increased the voltage to 20 000 V. Electrocuted flies were retained on a tray placed

FIG. 1A Electrified grids consisting of a visual 1 x 1 m pthalogen blue (p.blue) and non-visual 0,5 x 1 m net incorporated to form a flanked (p.blue/net) target

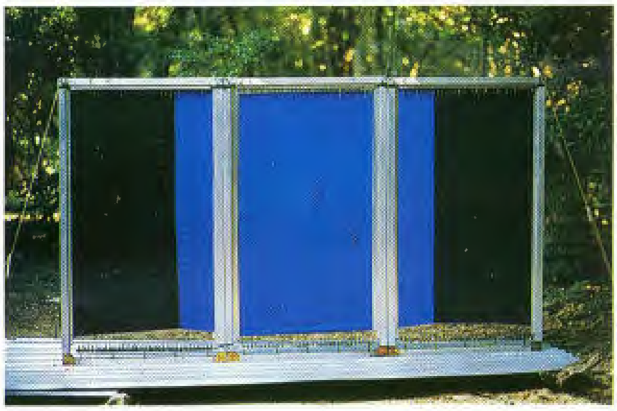

FIG. 1B Electrified grids with colour panels incorporated into black/p.blue/black bicoloured targets which could vary in size beneath the grids and painted with polybutene (sticky substance).

The electrified colour and/or net panels of the flanked or bicoloured targets were placed vertically and immediately adjacent to each other to be incorporated in the targets which were tested on their own as simple targets (a single colour target), flanked targets (Fig. 1A) or bicoloured targets which could also vary in size (Fig. 1B). Various conformations of the most attractive colour combination were also tested of which the best was then tested for optimal target size(s) and colour proportions (ratios). These were then incorporated to develop a practical target, which would attract and kill both *G. brevipalpis* and *G. austeni*.

All targets were baited with the synthetic ox-odour mixture used in Zimbabwe (Vale, Hall & Gough 1988b), which was also used in the small-scale *G. brevipalpis* control trial (Kappmeier *et al.* 1998), and later tested extensively at Hellsgate (Kappmeier & Nevill 1999). This bait consisted of a mixture of 3-*n*-propyl phenol, octenol and 4-methyl phenol in a 1:4:8 ratio dispensed by diffusion from a heat-sealed polythene sachet (wall thickness 150 microns, surface area 75 cm^2) as well as acetone dispensed from a glass bottle with a 6 mm diameter hole in the lid. These baits were placed approximately 40 cm downwind of the targets. The electrified grids were operated from noon until dark, which covered the diurnal activity of both species at Hellsgate (K. Kappmeier, unpublished information). Unless stated otherwise, a 1,5 m wide electrified black target served as the control to be certain that any new target would be significantly better than the black target originally used against *G. brevipalpis* in the Hluhluwe/Umfolozi control trial.

In a number of separate experiments the results obtained for each type of target were compared with those of this control target, to give an index of effectiveness that could be compared with the indices for other experimental targets tested at other times. The targets that were tested in the same experiment were positioned at least 100 m apart so that they did not interfere with each other. All target treatments under comparison were incorporated into a series of Latin squares of treatments x days x sites. A minimum number of five treatments was tested in one such square (Van Ark 1981). The squares were conducted at least one to three times so that treatments had a total of 5–24 replicates (one replicate being one treatment operating 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 $\log_{10}(n)$ or, where zero catches were obtained, 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.

To determine the attraction vs. settling responses of the flies, separate scores were kept for the flies that were recovered on the sticky tray under the net and under the variously coloured panels of the flanked and bicoloured targets. The "landing score" (in the case of flanked targets) was calculated as the number of flies on the tray under the colour panel expressed as a percentage of the overall catch. The "landing bias" (in the case of bicoloured targets) was calculated as the proportion of flies that landed on one (the darkest) of the two-coloured sections expressed as a percentage of the overall target catch.

EXPERIMENTS AND RESULTS

The results obtained for the various treatments tested in the experiments are presented in Fig. 2–7 and Tables 1 and 2. Unless stated otherwise, these are given as indices of increase, i.e. the catch index was estimated by expressing the detransformed mean catch as obtained with the test treatment as a proportion of the detransformed mean control catch (index = 1). Thus catch indices significantly greater than the index of the 1,5 m wide black control target indicated improved attraction. Indices less than one showed that attraction was less than obtained by the control. Female and male catches are indicated separately relative to the total catch. The landing score or bias is also given, where applicable, in Tables 1 and 2.

Attractiveness of various coloured targets

Studies on the development of a suitable target system for the control of *G. brevipalpis* and *G. austeni* started in January 1993. Preliminary experiments with single colour targets (flanked and non-flanked) of various sizes, as well as bicoloured targets of various conformations and sizes, were conducted to provide an indication as to which target designs should be further investigated. The experiments were only performed once (five to eight replicates) and the results obtained for each treatment were compared with those of the control. The results are summarized in Fig. 2A and B and in Table 1.

Experiment 1

The attractiveness of some colours that were attractive to tsetse flies elsewhere in Africa was tested. These were 1 x 1 m squares of black, white, pthalogen blue (p.blue), light blue (l.blue) and brown, each flanked by a 0,5 m wide electrified net to measure attractiveness. Although brown is not known to be attractive it was included in the experiment due to observations that *G. brevipalpis* seemed to be attracted to the brown interior of the vehicle used in the field. For male, female and total catches of *G. brevipalpis* [Fig. 2A, (1)], the results showed that white and brown were significantly unattractive (*c.* 3,4 and 4,8 x poorer respectively) when compared to the black flanked target. The catches obtained with the l.blue and p.blue colours were, however, not significantly different from those obtained with black for both sexes, although p.blue was nearly 1,9 x more attractive than black. For *G. austeni* [Fig. 2B, (1)] brown was significantly (3,9 x) less attractive than black (for totals and females), while the l.blue and white targets were just as attractive for both sexes. P.blue was significantly (3,3 x) more attractive than the black target (for totals).

Most flies of each species were caught on the flanking net. Therefore, although p.blue was very attractive for both species, the landing score for the total catches of *G. brevipalpis* on the p.blue target (2,8 %) was very low compared to the landing score on the black target (30,8 %), followed by brown, then light blue and then white (Table 1). A landing score of 30,2 % was obtained for *G. austeni* totals on the p.blue section against the 45,4 % that landed on the black section of the flanked targets. After black, *G. austeni* settled more readily on p.blue than did *G. brevipalpis*, followed by brown, then white and then light blue (Table 1).

Experiment 2

The attractiveness of p.blue and black flanked targets was compared with that of single targets of the same colour but of different sizes (1 and 1,5 m wide). This was done to determine whether an increase in the size of a single colour target (from 1 to 1,5 m wide) would eliminate the need for a net panel as was proposed by Vale (1993a). The results for *G. brevipalpis* [Fig. 2A, (2)] showed that the majority of flies which circled a black and especially p.blue target, never landed on it. It seems that an increase in the width (from 1,0 to 1,5 m) of the black target encouraged landing (34,1 % for totals) and could eliminate the need for a net side-panel. However, this was not found to be the case for p.blue, since *G. brevipalpis* does not readily settle on this colour [landing score = 10,0 % for totals (Table 1)]. The results for *G. austeni* [Fig. 2B, (2)] showed that the net panel could be eliminated when the black target was increased to 1,5 m wide (landing score on black = 44,2 % for totals). The flanking of p.blue did not significantly improve catches whether 1 m or 1,5 m wide, probably due to the relatively high landing response on p.blue (56,6 % for totals) (Table 1).

Experiment 3

The suggestion of Green (1989) to combine two colours, i.e. one for attraction and one for settling, was investigated. If successful, it would eliminate the need to use a net side-panel. The standard 1,5 m wide black target was compared to 2 m wide bicoloured targets of combinations of black/p.blue, black/l.blue and p.blue/l.blue (colour ratio 1:1). For comparison the target which produced the best results in the previous experiment (i.e. a p.blue flanked target) was included here and in following experiments, especially due to its efficiency for *G. austeni*. The results showed that black and blue, when used together, were very effective for attracting *G. brevipalpis* males but not significantly so for females [Fig. 2A, (3)] and that this target was not only significantly (2,4 x) better than the control (black 1,5 m), but also significantly better (2,9 x) than the all-blue (p.blue/l.blue) target. For both sexes of *G. austeni* [Fig. 2B, (3)] all bicoloured targets were found to be superior to the control black target but not to the p.blue flanked target. When compared to the control, total catches were increased significantly as follows: p.blue flanked target (4,2 x), black/p.blue (3,0 x) and bicoloured all-blue targets (3,2 x).

It became evident from the landing biases in this experiment (Table 1) that *G. brevipalpis* preferred to settle first on dark surfaces. Eighty-two percent of the *G. brevipalpis* that were caught with the black/p.blue target, first alighted on the black section, and in the case of the p.blue/l.blue bicoloured target, 82,5 % of the total catches landed on the darker section. This was, however, not shown to be the case for *G. austeni* whose landing biases in this experiment were found to be higher on the p.blue sections of the bicoloured targets (65,5–84,7 %). Black was, however, preferred for settling on the black/l.blue target (71,2 %). The basis on which the p.blue and black combination targets work is, therefore, synergistic. The p.blue attracts flies strongly to the target, while the black induces landing responses.

Experiment 4

In this experiment selected black/p.blue target conformations were tested. The control (black 1,5 m) target was compared to the flanked p.blue target (which was previously found to be best for *G. austeni*) and the 2 m wide black/p.blue targets (previously found to be the best bicoloured target for *G. brevipalpis*) of three different conformations. These were black/p.blue (colour ratio 1:1), p.blue/black/p.blue (1:2:1) and black/p.blue/black (1:2:1). The black/p.blue/black conformation target was found to be significantly (2,0 x) better than the control for *G. brevipalpis* totals [Fig. 2A, (4)]. The other targets did not differ significantly from each other or from the control for both sexes. The landing biases (Table 1) on the bicoloured targets indicated that 67,8–91,0 % of this species settled on the black section(s) first. For *G. austeni* [Fig. 2B, (4)] all targets except the p.blue/black/p.blue were significantly (*c.* 2,9–3,9 x) better than the control target for female and total catches. The landing biases (Table 1) indicated that between 39,3 and 51,7 % of this species first settled on the p.blue parts of the targets, therefore, contradicting the previous experiment's findings where their landing preference on p.blue was greater.

Experiment 5

The subsequent experiment was conducted to compare the standard black control with targets of two sizes (1 and 2 m wide) consisting of the two best black/p.blue conformations of the previous experiment (black/p.blue and black/p.blue/black). For *G. brevipalpis* [Fig. 2A, (5)] the 2 m wide black/p.blue/black target was significantly better than the control for female and total catches. The 2 m wide targets were also significantly better than the 1 m wide targets which was an indication for this species that target size plays an important role and that seemingly the bigger it is the better it is. Landing biases were 50,6–95,4 % on black (Table 1). For *G. austeni* totals [Fig. 2B, (5)] the 2 m wide bicoloured conformations were significantly (± 3,5 x) better than the control. The 2 m wide targets did not, however, increase the catches of males or females significantly compared

TABLE 1 Summary of results of preliminary experiments to find a colour combination target suitable for *G. brevipalpis* and *G. austeni* (total catches)

Target conformation	*G. brevipalpis*			*G. austeni*		
	Landing score/bias[L]	Indices of increase	S.D. from control*	Landing score/bias[L]	Indices of increase	S.D. from control*
Experiment 1						
Black 1 m / net 0,5 m	30,8	1,0		45,4	1,0	
White 1 m / net 0,5 m	5,0	0,3	NS	10,4	1,0	NS
P.blue 1 m / net 0,5 m	2,8	1,9		30,2	3,3	
L.blue 1 m / net 0,5 m	10,8	0,8		4,8	1,0	
Brown 1 m / net 0,5 m	19,2	0,2		29,2	0,2	
Experiment 2						
Black 1,5 m (control)		1,0			1,0	NS
	Black 1 m		0,7			0,5
Black 1 m / net 0,5 m	34,1	1,1		44,2	0,9	
P.blue 1,5 m		0,7			1,5	
P.blue 1 m		0,4	*		1,6	
P.blue 1 m / net 0,5 m	10,0	1,6		56,6	2,2	
Experiment 3						
Black 1,5 m (control)		1,0			1,0	
P.blue 1 m / net 0,5 m	11,9	1,8		57,2	4,2	*
Black 1m / p.blue 1 m	82,3	2,4	*	34,5	3,0	*
Black 1m / l.blue 1 m	87,3	1,7		71,2	2,2	
P.blue 1 m / l.blue 1 m	82,5	0,8		84,7	3,2	*
Experiment 4						
Black 1,5 m (control)		1,0			1,0	
P.blue 1 m / net 0,5 m	20,4	0,7		47,2	2,9	*
Black 1 m / p.blue 1 m	86,5	1,6		50,8	3,3	*
P.blue 0,5 m / black 1 m / p.blue 0,5 m	91,0	1,2		60,7	2,1	
Black 0,5 m / p.blue 1 m / black 0,5 m	67,8	2,0	*	48,3	3,9	*
Experiment 5						
Black 1,5 m (control)		1,0			1,0	
Black 1 m / p.blue 1 m	89,2	1,9		48,4	3,8	*
Black 0,5 m / p.blue 0,5 m	95,4	0,8		52,5	1,9	
Black 0,5 m / p.blue 1 m / black 0,5 m	85,5	2,6	*	51,3	3,3	*
Black 0,25 m / p.blue 0,5 m / black 0,25 m	50,6	0,4		61,1	1,3	
Experiment 6						
Black 1,5 m (control)		1,0			1,0	NS
Black 0,5 m / p.blue 1 m / black 0,5 m	84,3	2,6	*	61,7	3,3	
Black 0,25 m / p.blue 0,5 m / black 0,25 m	70,4	0,5		77,8	2,1	
Black 0,5 m / p.blue 0,5 m	87,9	1,0		60,2	2,8	
Black / p.blue 1 m (horizontal division—upper black)		0,5			2,4	
Black / p.blue 1 m (horizontal division—upper p.blue)		0,7			2,3	
Black / p.blue 1 m (diagonal division—upper black)		0,7			2,8	
Black / p.blue 1 m (diagonal division—upper p.blue)		1,0			2,8	

L Landing score/bias = catch from darkest colour as a percentage of overall target catch

* Significantly different from control treatment in the same experiment ($P < 0,05$)

NS No significant difference between treatments in the same experiment

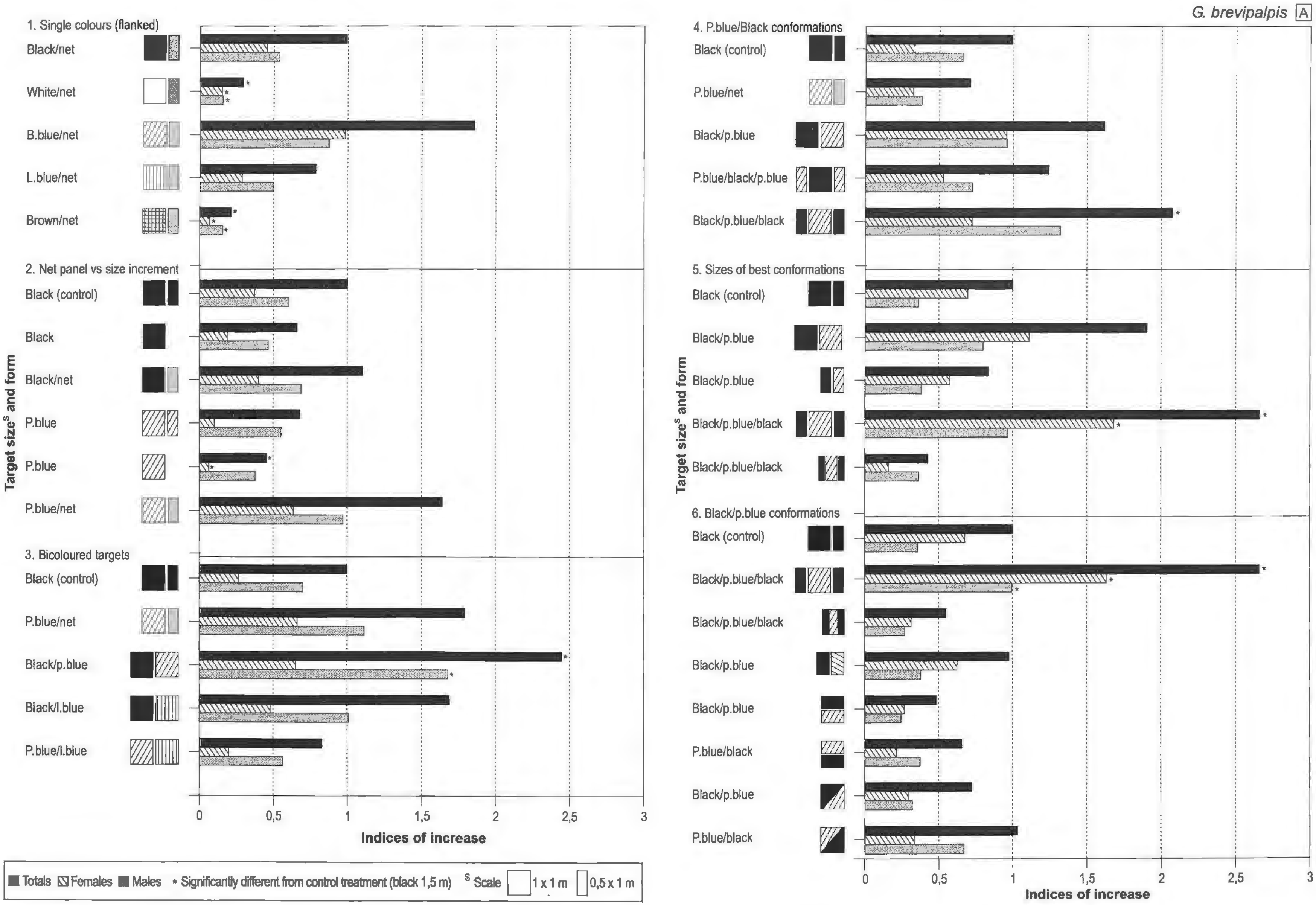

FIG. 2 Electric grid catches of (A) *G. brevipalpis* on single- and bicoloured target sizes and colour conformations (preliminary experiments 1–6)

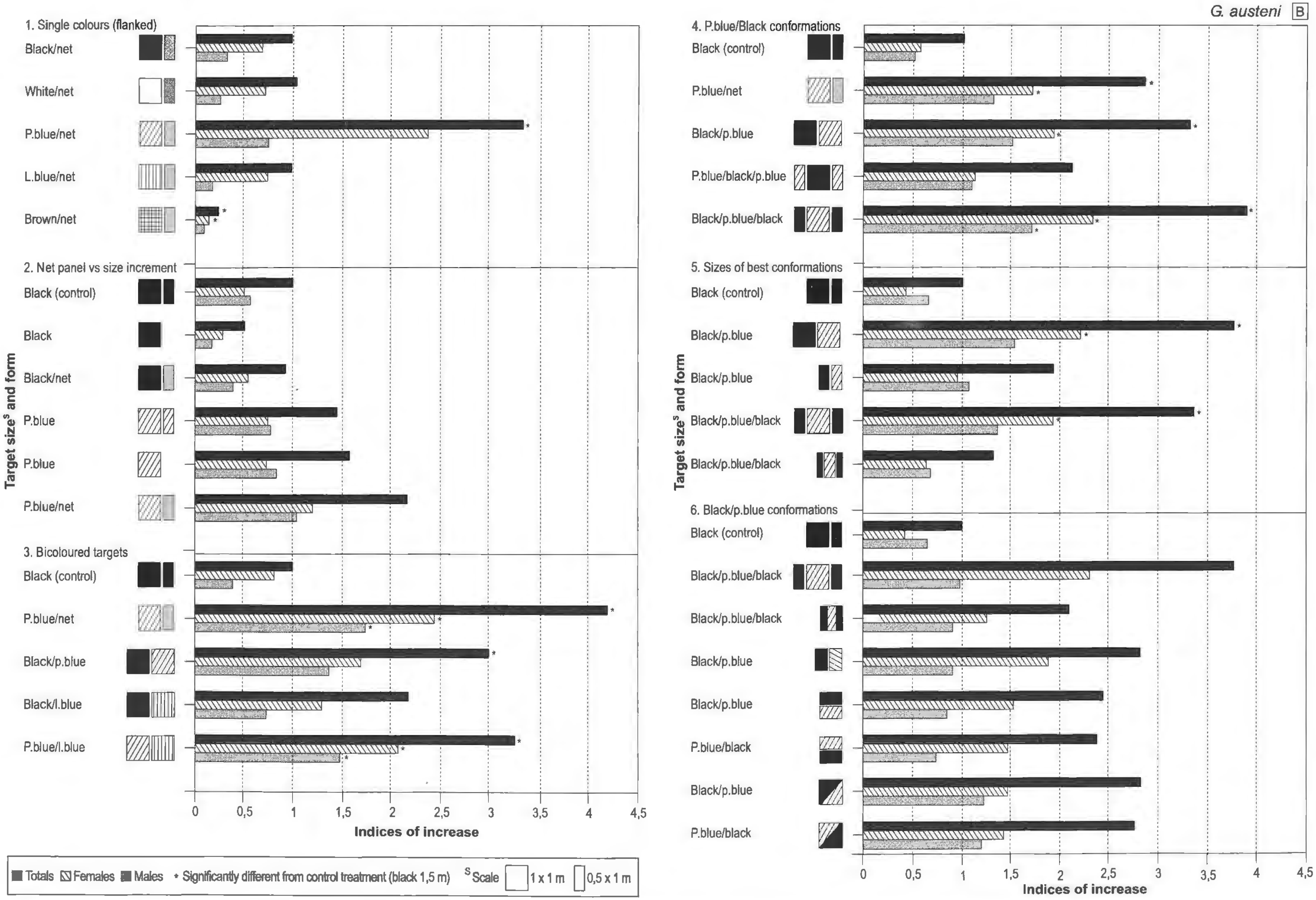

FIG. 2 Electric grid catches of (B) *G. austeni* on single- and bicoloured target sizes and colour conformations (preliminary experiments 1–6)

TABLE 2 Summary of results for experiments on best target designs, optimal sizes, optimal colour proportions and alighting responses for *G. brevipalpis* and *G. austeni* (total catches)

Target conformation	*G. brevipalpis*			*G. austeni*		
	Landing score/bias[L]	Indices of increase	S.D. from control*	Landing score/bias[L]	Indices of increase	S.D. from control*
Refinement of target designs						
Black 1,5 m (control)		1,0			1,0	
Black 2 m		1,7	*		1,2	
P.blue 1 m / net 0,5 m	14,0	1,9	*	31,3	4,8	*
P.blue 1 m / l.blue 1 m	66,3	1,1		85,4	2,1	*
Black 1 m / l.blue 1 m	89,1	1,8	*	90,4	2,1	
Black 1 m / p.blue 1 m	83,1	2,8	*	51,0	3,2	*
P.blue 0,5 m / black 1 m / p.blue 0,5 m	86,0	2,5	*	69,1	3,6	*
Black 0,5 m / p.blue 1 m / black 0,5 m	79,2	2,9	*	65,0	4,9	*
Optimal target size of best target						
Black 1,5 m (control)		1,0			1,0	
0,5 m	54,2	0,1		75,0	0,3	
1,0 m	66,4	0,4		67,8	1,8	
1,5 m (Black/p.blue/black (1:2:1) combination targets)	72,5	1,4		73,7	3,8	*
2,0 m (Black/p.blue/black (1:2:1) combination targets)	77,5	2,5	*	60,9	5,8	*
2,5 m (Black/p.blue/black (1:2:1) combination targets)	77,2	2,3	*	61,0	5,3	*
3,0 m (Black/p.blue/black (1:2:1) combination targets)	77,6	2,9	*	66,8	6,2	*
Optimal colour proportions of best target						
Black 1,5 m (control)		1,0			1,0	
1:1:1 (33 % p.blue) (Ratios of 2 m Black/p.blue/black target)	84,8	2,8	*	74,3	5,5	*
1:2:1 (40 % p.blue) (Ratios of 2 m Black/p.blue/black target)	75,4	2,2	*	55,1	5,2	*
1:3:1 (60 % p.blue) (Ratios of 2 m Black/p.blue/black target)	61,6	2,1	*	53,4	5,7	*
2:1:2 (20 % p.blue) (Ratios of 2 m Black/p.blue/black target)	90,3	2,6	*	85,9	4,1	*
3:1:3 (14,3 % p.blue) (Ratios of 2 m Black/p.blue/black target)	94,4	2,0		89,8	3,8	*
Practical target sizes and colour proportions	–		NS	–		NS
1,5 m (1:1:1) (Black/p.blue/black target)		1,2			0,6	
1,75 m (1:1,5:1) (Black/p.blue/black target)		1,1			0,9	2,0 m
(1,5:1:1,5) (Black/p.blue/black target)		1,0			1,1	
2,0 m (1:2:1) (control) (Black/p.blue/black target)		1,0			1,0	
2,25 m (1:1:1) (Black/p.blue/black target)		1,4			0,7	
Alighting responses	–			–		
Black/p.blue/black (1,75 m):						
all panels electrified (control)		1,0			1,0	
only black panels electrified		0,4			0,6	
only p.blue panels electrified		0,3	*#		0,1	*#
Black/p.blue/black (2,0 m):						
all panels electrified (second control)		0,8			1,1	
only black panels electrified		0,9			0,3	*#
only p.blue panels electrified		0,4			0,5	

L Landing bias = catch from darkest colour as a percentage of overall target catch
* Significantly different from control treatment in the same experiment ($P < 0,05$)
# Significantly different from second control
NS No significant difference between treatments in the same experiment

to the 1 m targets although at least a doubling in the catch size with a doubling of target size, especially for females, was indicated. Landing biases of total catches were 48,4–61,1 % on black (Table 1).

Experiment 6

In the last of the six preliminary experiments various black/p.blue target configurations of 1 m wide, as described by Green (1989), were tested. These were compared to the control target and the best target (for *G. brevipalpis*) from the previous experiments, namely the 2 m wide black/p.blue/black target. The colours on the 1 m wide black/p.blue targets were vertically, horizontally and diagonally divided as indicated in Fig. 2 (6). For *G. brevipalpis* [Fig. 2A, (6)] the 2 m wide black/p.blue/black target was solely significantly superior to the 1,5 m black control target and also to the 1 m wide target configurations, excepting the vertical black/p.blue and the diagonal p.blue/black

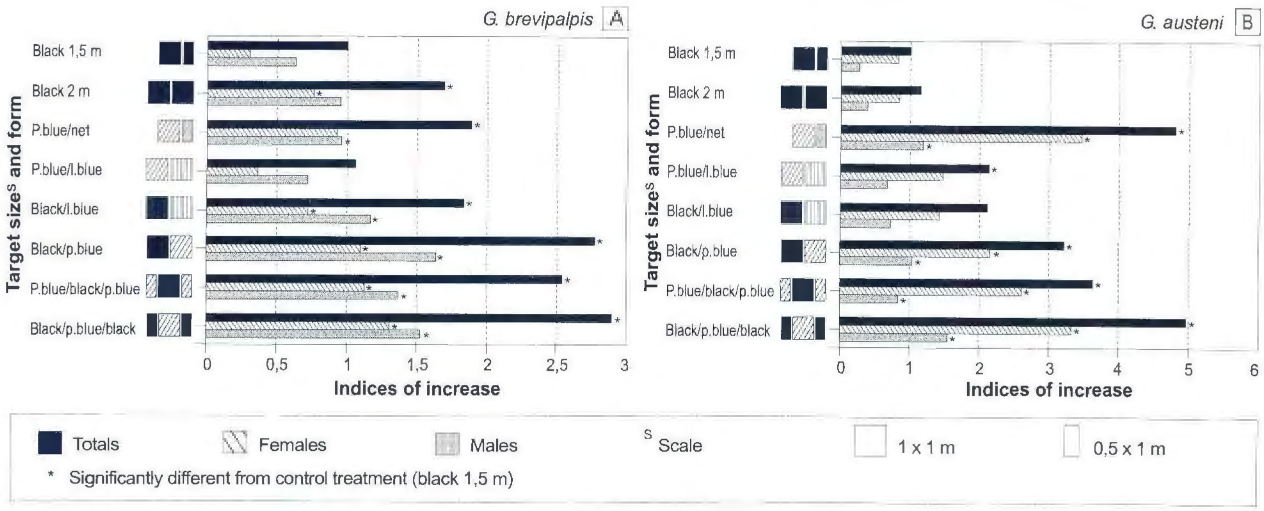

FIG. 3 The comparative efficacy of the best preliminary target designs for (A) *G. brevipalpis* and (B) *G. austeni*

(top blue) targets. Again this species preferred to settle on the black part of the target (70,4–87,9 %) (Table 1). For *G. austeni* males and females [Fig. 2B, (6)] no significant differences were found between the treatments, although an increase of over 3,5 x for females and totals was still obtained with the 2 m wide black/p.blue/black target when compared to the control target. The landing biases showed that 60,2–77,8 % of the flies settled on the black section (Table 1).

Refinement of target design

Previous experiments indicated four to seven designs that could be considered for use in campaigns to control *G. brevipalpis* and *G. austeni*. The designs were incorporated into one experiment and repeated three times in different seasons of the year during 1994–1995 so that each treatment had a total of 24 replicates. The treatments were: 1,5 m wide black target as the control, 1,5 m wide flanked p.blue target (p.blue/net), and 2 m wide targets of all-black, black/p.blue, black/l.blue, p.blue/l.blue, p.blue/black/p.blue and black/p.blue/black (1:1 and 1:2:1 colour ratios).

The results are given in Fig. 3A and B for *G. brevipalpis* and *G. austeni* respectively and are summarized in Table 2. For *G. brevipalpis* (Fig. 3A) all treatments were significantly better (*c.* 1,7–2,9 x) than the control and the p.blue/l.blue targets. The best target for female and total catches of this species was the 2 m wide black/p.blue/black target which was also significantly better (*c.* 1,7 x) than the 2 m wide black target. This emphasized that p.blue should be used in combination with black. The catch with the black/p.blue/black target was, however, not significantly different from the catches of the other blue/black target conformations for both sexes. For *G. austeni* (Fig. 3B) the flanked p.blue and the black/p.blue/black targets were shown to be best for both sexes and they increased catches significantly when compared to those of all the other targets (*c.* 2,3–4,9 x) except the p.blue and black target conformations. All p.blue and black target conformations and the flanked p.blue target were, however, significantly better than any of the two sizes of the black targets (1,5 and 2 m wide). The p.blue/l.blue target was also significantly better than the 1,5 m control target.

This experiment showed that *G. brevipalpis* settled more readily on black first (79,2–89,1 %) when black is used in combination with p.blue and l.blue, and on p.blue (66,3 %) when this colour is used in combination with l.blue. The landing scores indicated that *G. austeni* has a slight preference to settle on black (51,0–69,1 %) over p.blue when these colours are used in combination. This species does not, however, settle readily on l.blue (9,6–14,6 %) (Table 2).

Optimal target size of best target

The optimal size of the best target found in previous experiments, namely the 2 m black/p.blue/black target conformation with 1:2:1 colour ratios, was investigated during 1995. The sizes ranged from 0,5–3,0 m widths and were tested in 0,5 m intervals. The proportions of the black/p.blue/black sections were kept constant at a 1:2:1 ratio. Twenty-one replicates were carried out and the treatments were compared to the 1,5 m wide black control target.

The results are given in Fig. 4A and B for *G. brevipalpis* and *G. austeni* respectively and summarized in Table 2. It was found that the 2,0–3,0 m targets improved the catches of *G. brevipalpis* females, males and totals significantly when compared to the control (Fig. 4A). These treatments were *c.* 2,3–2,9 x

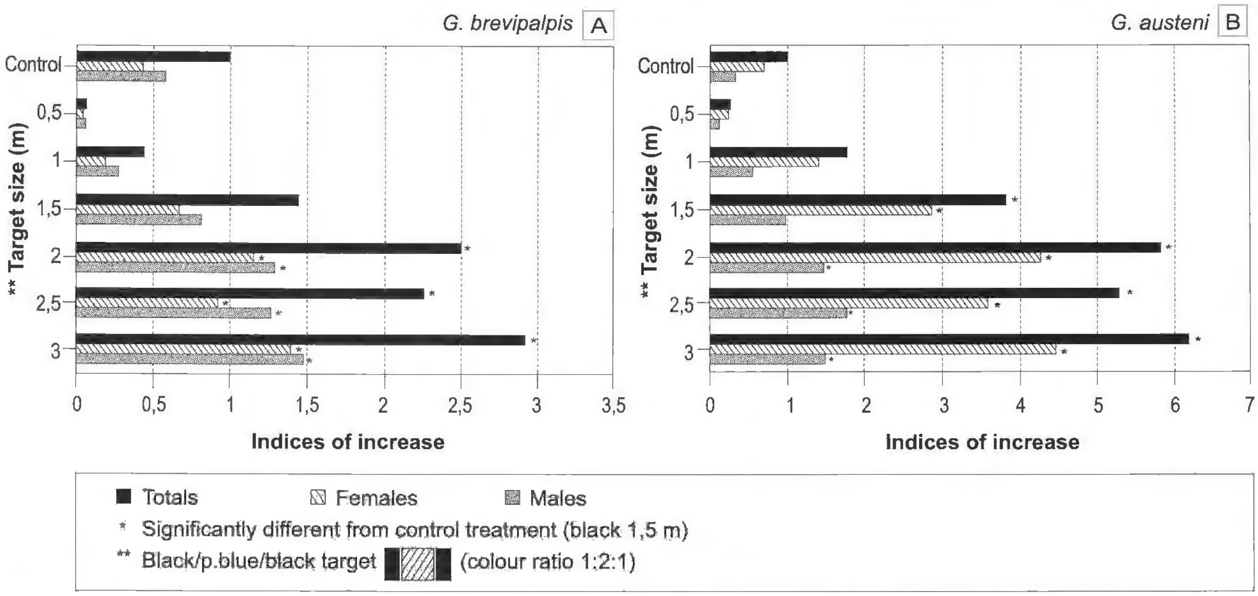

FIG. 4 The effect of target size on the attraction of (A) *G. brevipalpis* and (B) *G. austeni*

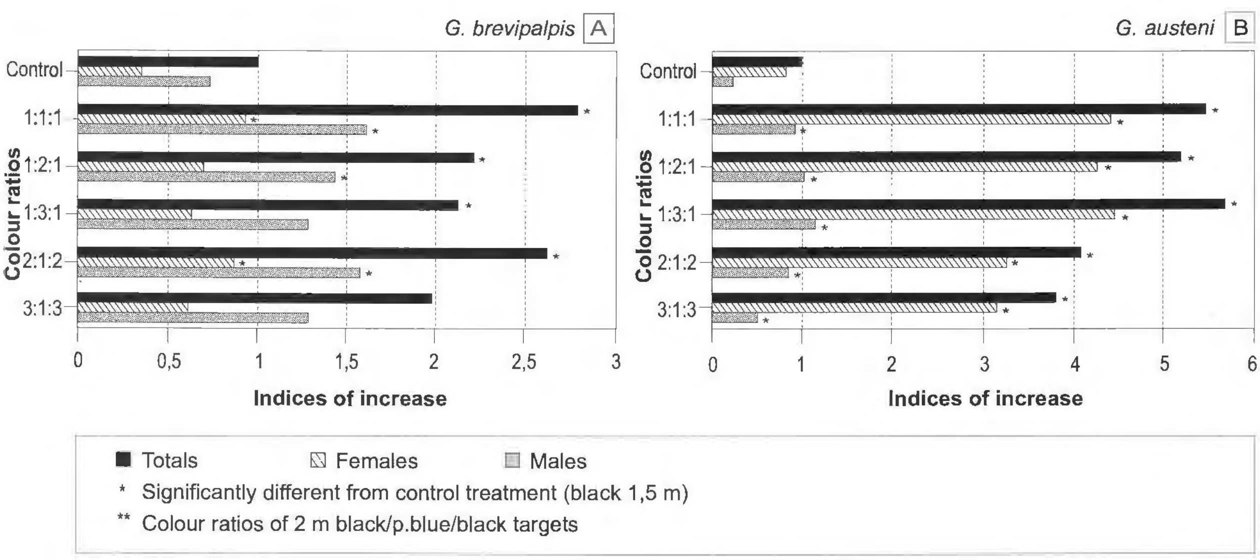

FIG. 5 The effect of colour proportions of black/p.blue/black targets on the attraction of (A) *G. brevipalpis* and (B) *G. austeni*

more attractive than the control and even more so than the 0,5 and 1 m wide targets. The results indicated that since there were no significant differences in catches between the three largest target sizes, the 2 m wide target can be considered a suitable size target for this species.

The landing biases (Table 2) for the three biggest targets also showed that *c.* 77,2–77,6 % of the flies that were attracted, first settled on the black section of the targets. The landing biases were lower on the black portion in the smaller targets (0,5–1,5 m wide). For *G. austeni* (Fig. 4B) the 1,5–3,0 m wide targets increased the female and total catches significantly (3,8–6,2 x) when compared to the control. No significant differences were found in the catches between the 1,5–3,0 m wide targets, which indicated that a target size of 1,5–2,0 m wide would be suitable for this species. The landing biases (Table 2) also showed that the highest settling response was on the black sections (60,9–73,7 %) of the four largest targets (> 1 m).

Optimal colour proportions of best target

In an attempt to improve further on the 2 m wide black/p.blue/black (1:2:1) target, different ratios of these

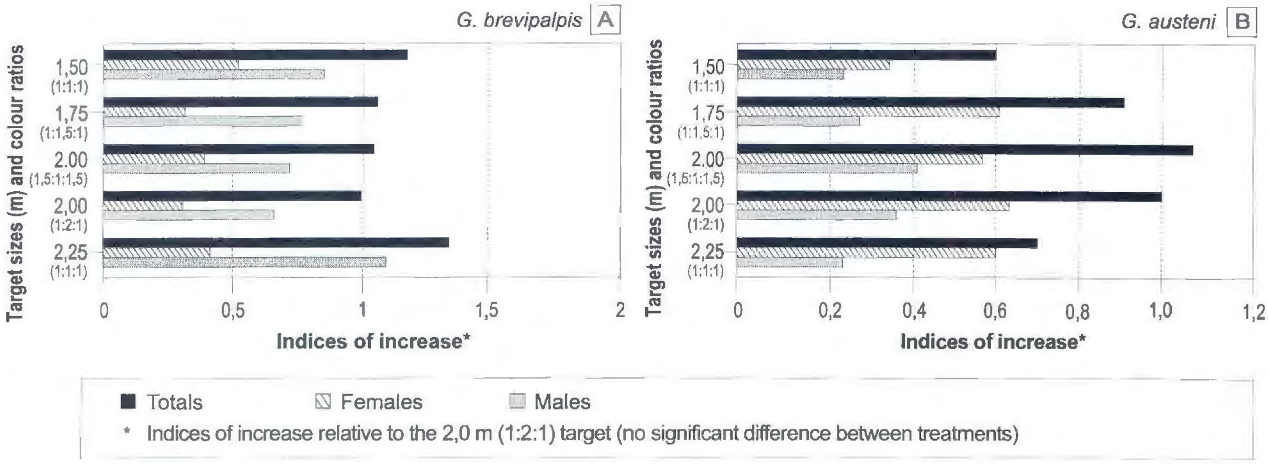

FIG. 6 The effect of combined practical black/p.blue/black target sizes and colour proportions on the attraction of (A) *G. brevipalpis* and (B) *G. austeni*

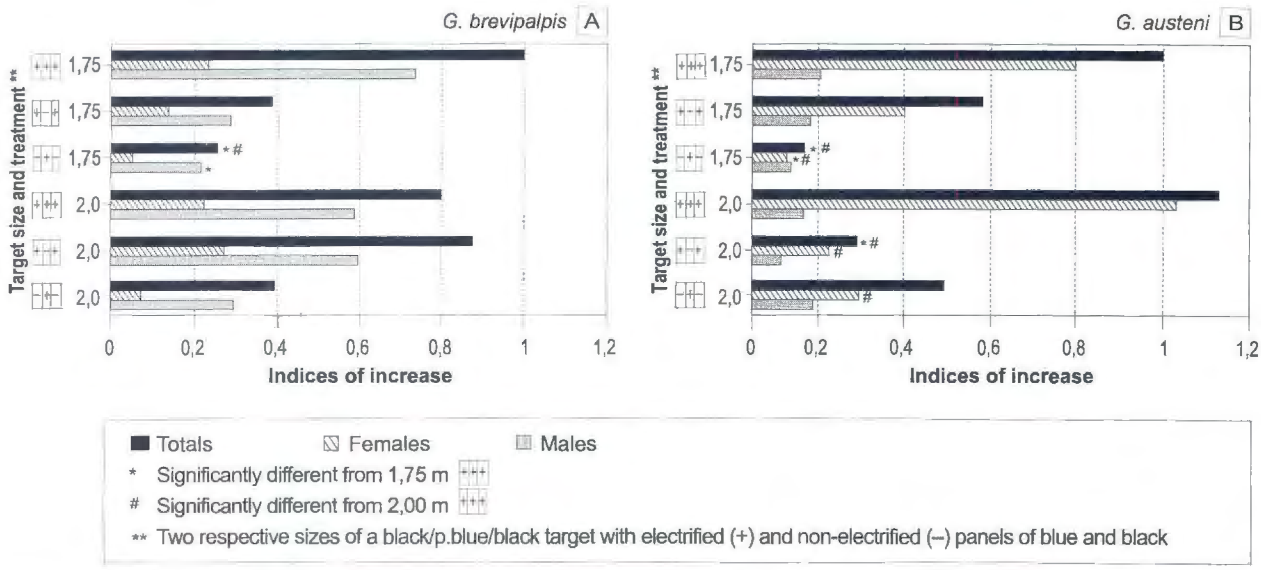

FIG. 7 Comparison of two sizes of black/p.blue/black targets and alighting responses of (A) *G. brevipalpis* and (B) *G. austeni* with a view to optimal insecticide use

colours were tested during 1995 to see if any variations would make a difference to the catch sizes. Two metre targets consisting of black/p.blue/black colour panels in the ratios 1:1:1, 1:2:1, 1:3:1, 2:1:2 and 3:1:3 were tested and compared to the control target.

The results obtained in 18 replicates are given in Fig. 5A and B for *G. brevipalpis* and *G. austeni* respectively and summarized in Table 2. For *G. brevipalpis* (male, female and total catches) (Fig. 5A) no significant differences were found between the various ratios. All targets, except for the 3:1:3 target, increased the total catches significantly (2,1–2,7 x) when compared to those of the control target. This indicates that the target becomes less effective when the blue part becomes too small (less than 20 % of total width). However, due to this species' positive alighting response on black (Table 2), a high landing bias of *c.* 90,3–94,4 % was obtained on the black portions of the 2:1:2 and 3:1:3 targets. For *G. austeni* (male, female and total catches) (Fig. 5B) no significant differences were found between the various colour ratios. All treatments were found significantly 3,8–5,7 x better than the control. The landing biases (Table 2) also showed that a higher proportion (85,9–89,8 %) of the flies settled on the black part of the 2:1:2 and 3:1:3 targets in which p.blue covered only a relatively small part (i.e. 14,3–20,0 %).

Practical target sizes and colour proportions

To simplify target-making and reduce wastage, the width of rolls of cloth material was taken as a starting point in selecting practical but effective target sizes. The p.blue and black cloth, which is used for targets, is available from Bonar Industries [Bonar Industries (Private) Ltd, P.O. Box ST 580 Southerton, Harare, Zimbabwe] in 150 cm widths. The practical widths of colour panels to use would thus be 75, 50, 30 cm, etc. The optimal target size(s) and colour ratio(s), as determined previously, were combined with these practical sizes of the blue and black target panels. The 2 m wide (1:2:1) black/p.blue/black target was here used as the control. The target sizes, colour ratios and panel widths were as follows:

	Size (width) m	Ratio	Panel widths cm	P.blue %
1.	1,5	1:1:1	50/50/50	33,0
2.	1,75	1:1,5:1	50/75/50	42,8
3.	2,0	1,5:1:1,5	75/50/75	25,0
4.	2,0	1:2:1	*50/100/50	50,0
5.	2,25	1:1:1	75/75/75	33,0

(* Not practical—included for comparison as the control target)

The results obtained for each of these targets (six replicates) are given in Fig. 6A and B for *G. brevipalpis* and *G. austeni* respectively and summarized in Table 2. There were no significant differences between any of the targets for female, male or total catches of *G. brevipalpis*, indicating that any of the ratio and size combinations could be used. This also applied to female, male or total catches of *G. austeni* al-though the 1,75–2,0 m targets appeared to improve catches for both species (Fig. 6A and B).

Alighting responses

The foregoing experiments showed that the landing biases (Table 2) of both species on black/p.blue targets were highest on the black section of the target, i.e. 61,6–94,4 % for *G. brevipalpis* and 34,5–89,8 % for *G. austeni* (on targets > 1 m). This suggests, especially for *G. brevipalpis*, that only the black parts of a target need be treated with an insecticide when used in campaigns to control these flies. It could then be a more cost-effective target. However, before its use could be unequivocally advocated or adopted, it was necessary to determine what proportion of flies that initially settle on the blue part will eventually settle on the black part.

Two of the practical targets (as obtained in previous experiment) were used for this experiment so as to make a final decision on a target which could be used to control both species. These two targets were incorporated in an experiment (7 replicates), which was designed to determine whether the flies that first settled on the blue part of the target, could eventually settle on the black part and vice versa. This was done by electrifying only the required colour section (blue or black) of the 1,75 m and 2,0 m wide practical targets. The number of flies electrocuted on these sections was compared with those from the overall electrified targets as set out in Fig. 7.

The results of the experiment are given in Fig. 7A and B for *G. brevipalpis* and *G. austeni* respectively and summarized in Table 2. The female, male and total catches of *G. brevipalpis* electrocuted on the 1,75 m and the 2,0 m wide targets which were electrified overall, did not differ significantly from each other. The two targets in which only the p.blue (centre) was electrified were relatively ineffective. When comparing the targets with only the black parts (flanks) electrified, the 2 m wide target was as effective as the two sizes of fully electrified targets, while the 1,75 m wide target was found to be ineffective compared to the fully electrified targets. This indicates that only the black parts of a 2 m wide blue/black target need be treated with insecticide for *G. brevipalpis* (Fig. 7A) whereas the entire 1,75 m wide target should be treated. For *G. austeni* (Fig. 7B) it was shown that both the 1,75 m and 2 m wide targets, fully electrified, were equally effective. However, these two targets were ineffective when only the black or blue parts were electrified so that for this species the entire target, irrespective of size, would need to be treated with insecticide.

DISCUSSION

The present work succeeded in greatly improving on the attractiveness of the 1,5 m black target, used in a trial in the northern parts of the Hluhluwe-Umfolozi Game Reserve (Kappmeier *et al.* 1998), for *G. brevipalpis*, as well as in developing a target which is very effective for *G. austeni*. It was also confirmed that the behaviour of the two tsetse fly species towards colour targets is in some ways similar to that of other species elsewhere in Africa.

Pthalogen blue (p.blue) proved to be nearly twice as attractive as black for *G. brevipalpis*. For *G. austeni* black was relatively unattractive and p.blue was at least three times more attractive than either black, white or light blue. Various other *Glossina* species favour blue over black as visual attractants and to a lesser extent white (Owaga 1981; Jordan & Green 1984; Green 1986; Green & Flint 1986; Green 1988, 1989; Torr *et al.* 1989; Green 1990). Low attractiveness to black was also found for *G. austeni* in Zanzibar, where either white (Hall 1986) or light blue (Madubunyi 1990) was suggested as the most attractive colour. A blue target was used to control *G. austeni* in the Jozani forest on the Unguja island of Zanzibar prior to applying the Sterile Insect Technique (Tanzania Government/FAO/IAEA 1994).

Vale (1974a) and Hargrove (1980) found that tsetse flies often circle objects without landing on them. This behaviour occurs in species such as *G. p. palpalis,* *G. tachinoides, G. m. submorsitans, G. m. morsitans* and *G. pallidipes* (Merot & Filledier 1985, cited by Vale 1993a; Green 1986, 1990). In the present study this circling behaviour was also found with *G. brevipalpis* and *G. austeni* where most flies were caught on a flanking net when placed adjacent to a coloured target. The addition of a net side-panel to a 1 m wide black or p.blue target increased the catch of *G. brevipalpis* two to three times and for *G. austeni* only when it was placed next to a p.blue target, due to this species' non-attraction to black. *G. brevipalpis* in Kenya was also attracted optimally to a 1 x 1 m black or blue cloth target with a 0,5 x 1 m mosquito netting panel next to it (Kiragu, Green, Stevenson & Makumi 1997).

Although black mosquito-netting side-panels, added to a colour target, have previously been used to control the flies (Vale 1982; Vale *et al.* 1985, 1986, 1988a) it is suggested that they be avoided due to their easy deterioration and impracticality for use in the field. An increase in size of a black-only target or the addition of p.blue to black overcame the use of mosquito-netting for the control of *G. pallidipes* and *G. m. morsitans* in Zimbabwe (Vale 1993a). For *G. brevipalpis*, the addition of a black panel to increase the target width from 1 m to 1,5 m, eliminated the need for a net panel, since black encourages landing in this species. The same increment in the size of a p.blue target had no effect, probably due to the low landing responses, which were obtained on p.blue for this species. The opposite was the case for *G. austeni*, in that a bigger p.blue target (and not a bigger black target) compensated for the net panel.

By combining the two different stages of fly orientation to targets, i.e. initial attraction and landing, the use of a net panel could also be eliminated. The 2 m wide bicoloured target conformations of black and p.blue were found most effective for *G. brevipalpis* and *G. austeni*. Of these, the most effective was the black/p.blue/black combination (colour ratio 1:2:1) which caught nearly three times more *G. brevipalpis* and five times more *G. austeni* than the 1,5 m black control target. Black induced a stronger alighting response in the 2 m wide targets for *G. brevipalpis* (67,8–91,0 %) as opposed to p.blue. *G. austeni* also alighted readily on black (34,5–61,7 %) on these targets, but also to a lesser extent on p.blue. This supported some findings with other species such as *G. m. morsitans, G. m. submorsitans, G. pallidipes, G. p. palpalis* and *G. tachinoides* (Merot & Filledier 1985, cited in Vale 1993a; Laveissiere, Couret & Grebaut 1987, cited by Vale 1993a; Green 1990; Vale 1993a, 1993b).

The optimal size of the black/p.blue/black target (colour ratio 1:2:1) for the two Kwazulu-Natal species was at least 2 m wide for *G. brevipalpis* and 1,5–2,0 m wide for *G. austeni*. These targets increased the catches of both species 2,5 and 3,8–5,8 fold respectively compared to the 1,5 m wide black target. The landing biases of these species to settle on black over p.blue with the above targets was 77,5 % for *G. brevipalpis* and 60,9–73,7 % for *G. austeni* respectively. In general the bigger targets were more attractive for *G. brevipalpis* than the smaller ones (also for single coloured targets), but for *G. austeni* size did not seem to play as important a role. It was recently shown that blue/black combination targets could be slightly superior to black in Zimbabwe for *G. pallidipes* when targets are large (Vale 1993a). Hargrove (1980) also noted that large objects are more attractive than small objects for the savannah species.

No significant differences were found in the catch size of *G. brevipalpis* and *G. austeni* when changing the proportions of the colour panels of a 2 m wide black/p.blue/black target. However, when the attractive part of the target (p.blue section) made up less than 20,0 % of the total target width for *G. brevipalpis* and less than 14,3–20,0 % for *G. austeni*, fly catches were the same as with the standard all-black target, even of the same size. Where the proportion of black was increased in these targets, the landing biases on black compared to p.blue was also very high, namely 90,3–94,4 p.blue was also very high, namely 90,3–94,4% for *G. brevipalpis* and 85,9–89,8 % for *G. austeni*.

When practical target sizes and colour ratios were taken into account it appeared that a 1,75 m wide black/p.blue/black target of colour ratio 1:1,5:1 (i.e. 75 cm p.blue centre flanked by two 50 cm black panels) was nearly as attractive as a 2 m wide (optimal) target (1:2:1 or 1,5:1:1,5) for *G. brevipalpis* and even more attractive than a 1,5 m wide (1:1:1) target for *G. austeni*. This target could, therefore, be used in future control operations. This 1,75 m wide target differs from the one which has more recently been developed for *G. pallidipes* and *G. m. morsitans* in Zimbabwe, namely a 1,7 m wide p.blue/black/p.blue target (i.e. 70 cm black centre flanked by 50 cm p.blue panels) which is now interchangeably used with a 1,7 m all-black target in control campaigns in Zimbabwe and Zambia (Vale 1993a). The difference in colour conformations (i.e. p.blue vs. black centre) between the Zimbabwean and South African targets is due to the two savannah species' preference for landing on the centre (black). As is the case with the two Zululand species, *G. p. palpalis* also prefers a target where blue is in the centre and not at the sides due to their preferred alighting position on the sides of targets (Laveissiere *et al.* 1987, cited in Vale 1993a).

The main practical considerations are, however, the economy and flexibility of target construction. Therefore, reduction in the quantity of insecticide required will be one of the relevant factors in reducing the cost of target technology. It was shown that only the black parts of a black/p.blue/black target, when it is at least 2 m wide, need be treated with insecticide when using the target for *G. brevipalpis* control, whereas the entire 1,75 m wide target must be treated. For *G. austeni* it is essential that the whole target be impregnated for control purposes, irrespective of size.

CONCLUSIONS

The 1,5 m black target used for the control of *G. m. morsitans* and *G. pallidipes* in Zimbabwe, which was also used in the first trials against *G. brevipalpis* in the Hluhluwe-Umfolozi Game Reserve, is not the most effective target for *G. brevipalpis*. Its attractiveness could be greatly improved (*c*. 1,3–2,9 x) by the addition of p.blue. The standard black target was even less attractive for *G. austeni* and, although a p.blue target was found very attractive for this species, it is clear that a black/p.blue combination would increase (*c*. 2,1–6,2 x) kills of this species.

For the simultaneous control of both *G. brevipalpis* and *G. austeni* it is recommended that a 1,75 m wide (i.e. 75 cm centre p.blue panel flanked by two 50 cm black panels) x 1 m high black/p.blue/black target be used. The entire target should be treated with a residual insecticide. Where *G. brevipalpis* occurs allopatrically the entire target still needs to be treated with insecticide, unless a 2 m wide target (i.e. 50 cm p.blue centre panel flanked by two 75 cm black panels) is used, in which case only the black parts need to be treated with insecticide.

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