5 Responses of female New World Screwworm flies to coloured targets in the laboratory

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5.1 Summary

The responses of unmated female New World screwworm flies, *Cochliomyia hominivorax*, to visual targets were studied in a wind-tunnel. Both activity and frequency of contacts with targets increased greatly when the screwworm attractant mixture swormlure-4 was added to the airstream. Target-orientated responses depended on target colour, with red and black targets being preferred over blue, white and yellow ones; this preference was much greater in the presence of odour than in its absence. No preference was detected for different shapes and orientations of red targets, all of equivalent surface area. Omitting different components from swormlure-4 generally resulted in a large reduction in activation and target contacts. Attempts to substitute 1-octen-3-ol for the butanol fraction were unsuccessful, but skatole may substitute to some extent for indole; the two isomers of butanol normally present in swormlure-4 may substitute partly or completely for each other. This type of measurement forms a suitable bioassay in the development of attractive targets for monitoring and control of wild adult screwworm populations.

5.2 Introduction

Laboratory studies formed an integral part of the development of baits used against tsetse flies (Bursell *et al.*, 1988), as they permit rapid evaluation of different elements of the system under controlled conditions. Warnes and Green (Chapter 4) demonstrated activation and changes in flight behaviour in female *C. hominivorax* in a wind-tunnel when exposed to swormlure-4. This Chapter reports on orientation of female *C. hominivorax* to targets in the presence of swormlure-4 and derived mixtures, in an attempt to develop a bioassay for the development of a target-based control system for this species.

5.3 Materials and Methods

Flies

Flies were received and matured as described in 4.3. Unmated female flies were used when between 4-9 days post-emergence.

Observation chamber and recording techniques

Behaviour of groups of 35 flies at a time was recorded using video apparatus (Cohu 6712-2000 CCD camera, Panasonic AG-6702 S-VHS time lapse video recorder and For.A VTG-22 timer) in an observation chamber. The chamber was similar to that described by Bursell (1990), except that the ceiling and floor were made from 'Perspex' (ICI; 0X02 grade, 5mm) covered with 'Benchkote' (Whatman International Ltd.), and the walls were covered with grey card (Daler-Rowney no.51) (Fig. 5.1). Illumination was provided by four 20 W tungsten halogen bulbs, positioned 0.4 m above the chamber; the Benchkote on the ceiling acted as a light diffuser. The chamber had a flow of clean air (passed through a charcoal filter) at 0.2-0.5 m/s. The chamber, odours and camera were sited in a windowless room maintained at 28x, with the video recorder and observer in an adjacent room.

Table 6.1 Composition of swormlure-4

Targets

Two targets were present in all experiments, in the positions indicated in Fig. 5.1; unless otherwise stated, they were 240mm high squares, one covered with red cloth and the other yellow cloth. Black, red, white and yellow cloths were similar to those described by Wall *et al.* (1992); the blue was a 'phthallogen' blue, similar to sample [40] in Green (1989).

For the experiment on target shape, flies had a choice between the standard target (a red upright square), and one of five different targets: square (standard), diamond (square rotated through 45x), upright oblong, oblong on its side, and circle (Fig. 5.4), each covered in red cloth.

Odours

Odours were supplied to the chamber by passing a stream of clean air through a U-tube containing the mixture to be tested, and then out through pipes in the upwind section of the chamber (Fig. 5.1; NB. flies are separated from this section by a netting screen). For earlier experiments with standard swormlure-4, outlets were 3 silicone rubber tubes terminating within 30 mm of each other, and the air flow rate was 1 l/min, giving a release rate (measured by weight loss in the U-tube) of 15 mg/min. For experiments on target colour and shape, fly behaviour was recorded over 15 min; odour was supplied over minutes 6-10. A further 15 min was left before the next treatment.

For experiments testing different odour mixtures, a number of measures were introduced to combat both habituation and cross-contamination, both shown to be problems in early trials. The air flow rate was reduced to 0.2 l/min (giving a variable odour release rate depending on the odour substances). Odour release time was reduced to 4 minutes. Outlet tubes were changed to PTFE plastic, and each odour was given an individual tube, and U-tube. Between experiments, PTFE tubes were cleaned with a sequence of dichloromethane, acetone, water and air; other tubing, U-tubes and plastic connectors were discarded. Contamination of upstream equipment (pump, tubing etc.) was prevented by occluding upstream pipes between odour pulses.

Experimental design

Both the experiment with colours, and that with shapes, made use of a 5 X 5 Latin square design, in which the 5 treatments were presented during each of 5 days in varying sequences. In the odour experiments, a replicate consisted of a run with the control odour (swormlure-4), followed by the other treatments in random order, finishing up with a further control run.

5.4 Results

Effect of swormlure-4 on different aspects of fly behaviour

Flights were generally slow and meandering, with relatively little activity in the absence of odour (see also Chapter 4). Flies at rest in the field of view of the camera at different positions in the chamber were scored every 30 s, and changes monitored over a twelve-minute period, including six minutes when swormlure-4 odour was blown into the chamber. Relative numbers at different positions in the chamber did not change significantly over this period, and so were pooled into the category of total at rest; these are shown in Fig. 5.2A. Flies at rest declined significantly during minutes 4-9 in the presence of odour. Totals in flight were also scored every 30 s, and these increased greatly during minutes 4-9 (Fig. 5.2B).

A continuous record of contacts of flies with the two targets was made. Those longer than 0.3 s were scored separately and are termed 'landings'. Totals during each 30 s period are shown in Fig. 5.2C for the red target and Fig. 5.2D for the yellow target. The frequency of both landings and briefer contacts on the red target increased greatly during minutes 4-9. Relatively few contacts were observed on the yellow target.

Fig. 6.1 RELEASE OF SWORMLURE FROM OPEN BOTTLES
(5 ml in bottles with 13 mm or 3 mm openings; 21oC)

The totals of flies at rest and those in flight give the total numbers in the field of view at 30 s intervals. Only upwind flies were visible to the camera and since the number of flies in the chamber did not alter, changes in the number of flies visible allow estimates of the downwind or upwind movement of flies. In the first minute there was a small increase in flies in view, and therefore moving upwind, but this was not significant. In the first 30 s after odour switch-off (min 10) the number of flies in the field of view dropped from 30 to 16, implying a rapid movement of flies downwind.

The effects of odour on the different aspects of behaviour measured appears to be subject to some time-lag, especially in the target-orientated responses; the frequencies of these behaviours did not reach a maximum until several minutes after odour-on, and did not decline immediately on odour-off.

Effect of target colour

Five differently coloured targets were tested, each being paired with a yellow control target. Total contacts with the test targets over a 15 min session are shown in Fig. 5.3, with odour on for minutes 6-10. Black and red targets received many more contacts than the other colours. Red appeared to be more attractive than black, but this could be an artefact, as the flies were difficult to see against a black background on video, and some flies may have been missed. Once again there appeared to be a time-lag in the starting and stopping of odour-mediated activity relative to the stimulus.

Statistical analysis shows overall effects of colour and odour on the frequencies of total contacts and landings. It also shows a significant colour X odour interaction ($P<0.001$ for all main effects and interactions). This reflects the much greater effect of target colour on behaviour when odour is present compared to when it is absent.

Contacts with the control targets were also analysed. These were generally similar to contact frequencies on the blue, white and yellow treatments. They also increased in the presence of odour.

Effect of target shape

Contacts and landings on the test targets during odour stimulation (minutes 9 and 10 only), along with diagrams of the shapes tested, are given in Fig. 5.4. There was no significant effect of shape on either overall contacts, or landings, either during odour stimulation (as shown on the bar-chart), or during other minutes.

Importance of different components of swormlure-4

A series of tests were conducted to check the importance of the various swormlure-4 components in mediating specifically target-orientated responses. The response of flies to red targets (described above) was chosen as a suitable assay. A yellow target was placed opposite the red in all tests, in case of an odour-mediated change in colour preferences, but this was never observed. Both overall contacts and landings for the different mixtures tested are given in Table 5.1.

Standard swormlure-4 is a mixture of dimethyldisulphide; sec and iso butanols; acetic, butyric, valeric and benzoic acids; phenol and 4-methyl phenol; and indole. In the first two experiments, these different groups of compounds were omitted in turn, and all were shown to be necessary to give a full response, as the fractions all produced significantly lower levels of contacts with the red target than did complete swormlure-4.

In experiment 3, a simple substitution was attempted by omitting each isomer of butanol, whilst increasing the other to compensate. The results suggested that the two butanols might indeed be able to substitute for each other (although sec butanol did give a response that was just significantly lower than the standard mixture).

Experiments 4 and 5 investigated two compounds which produce strong

Fig. 6.3 RELEASE OF SWORMLURE COMPONENTS FROM OPEN BOTTLE (5 ml in 3 cm x 1.3 cm glass bottle; 21oC)

—+— iso Butyl alcohol —□— Dimethyl disulphide electroantennogram (EAG) responses, 1-octen-3-ol (octenol) and 4-methyl indole (skatole) (see Chapter 3). Octenol was tested in place of the butanols, to which it shows the greatest chemical similarity. None of the three concentrations tested gave any indication of increasing the target-orientated responses above the level of swormlure-4 without both butanols. Skatole was tested in place of indole at two concentrations, one similar to the standard indole concentration, and one at 0.1 times this concentration. Skatole does appear to substitute for indole to a great extent, although target contact frequency was still significantly less with skatole than with either indole (standard swormlure-4), or with swormlure-4 with a reduced indole concentration.

Among the partial swormlure-4 mixtures, some gave a much higher level of response than others. Apart from the butanols (where it may be possible to substitute them for each other without reducing the response), the fraction giving the highest response was that omitting dimethyldisulphide, which gave over 50 % of the control response. Omitting indole alone gave results of 54 % and 27 % of control in different experiments. Omitting larger groups of chemicals gave the greatest reductions in target-orientated responses.

On occasions where the same fractions were re-tested, the responses showed a reasonable degree of stability (for example, swormlure-4 less butanols gave 4.1%, 8.3% and 4.6% of control in separate tests), even though comparisons were based on relatively few repetitions. Activity levels were not measured, but generally corresponded closely with levels of target-orientated activity.

Landing frequencies followed the frequencies of overall contacts, making up about one-third of the total contacts.

5.5 Discussion

The most striking changes observed in female *C. hominivorax* when stimulated by swormlure-4 were an increase in flight activity, and an increase in target-orientated behaviour. A high level of fly contacts was observed on red and black targets in the presence of swormlure-4 odour; relatively few contacts were observed in the absence of odour, or on blue, white and yellow targets. The importance of target colour indicates that orientation to visual stimuli was involved, and not just the incidental results of increased activity. Whether the response is truly to colour (in the sense of differential reflectivity at different wavelengths) is doubtful. Red wavelengths are generally poorly perceived by Diptera (Menzel, 1979), and *C. hominivorax* may perceive red objects as dark, like black. There was no indication of colour preferences among blue, yellow, or white, as is often found among other Diptera (eg. Green & Flint, 1986; Kirk, 1984). The response observed here may be to an object showing dark-contrast with a background. It is not known what natural stimuli swormlure-4 imitates (it was developed simply as a replacement for the rotting liver previously employed as an attractant; Jones *et al.*, 1976), but if swormlure-4 imitates wound odours, the visual response might be part of the wound-locating strategy, as wounds might appear dark, or reddish, against a background of fur or fleece.

No evidence was found of preference for some shapes of targets over others. This contrasts with similar studies on Glossina (Doku & Brady, 1989), in which a marked preference for vertically-orientated shapes was demonstrated. This may indicate a different priority for visual and olfactory stimuli in host-location between tsetse and screwworm flies.

The increase in flight activity observed in the presence of swormlure-4 odour was probably due both to activation of individual flies, and an increase in the time spent by individuals in flight (Warnes & Green, 1992). The latter study also described a tendency to upwind flight in the presence of odour, and downwind flight when odour was turned off, as was observed here.

Fig. 6.5 RELEASE OF SWORMLURE FROM POLYTHENE SACHET (50 sq.cm; 120u thick; 21oC)

—+— release (gm) —□— release (%)

Odour experiments demonstrated that this experimental protocol is suitable for the rapid assessment of candidate attractant mixtures. Although not exhaustive, these experiments did show that most if not all of the components in swormlure-4 are necessary to produce maximum response to targets. Swormlure-4, in common with earlier versions of swormlure, was developed in the field using traps, but these experiments show that it is probably suitable in its present form for use with targets, also. The literature on swormlure development does not allow much assessment of the contributions of individual components to overall swormlure attraction. An exception to this is the study of Coppedge *et al.* (1977), in which dimethyl disulphide was added to an earlier version of the swormlure mixture, leading to a two-fold increase in catches of screwworm flies (sex unspecified). It is interesting that in the present experiments, a halving of target contacts was produced by omitting this compound from swormlure-4.

The strong visual element in the response, demonstrated by the present study, is encouraging for the use of target technology in controlling *C. hominivorax*; similar principles might also be used for a population monitoring system, for example with sticky targets. Most contacts observed here were brief (over 60% less than 0.3 s), and it remains to be seen whether insecticide formulations can be developed giving a satisfactory kill rate for insecticidal devices (although contacts may be repeated many times, giving much longer cumulative contacts). It may be possible to develop further olfactory or gustatory stimuli to keep flies in contact with targets for longer, as contacts of female flies with wounds are much more prolonged (Guillot *et al.*, 1977). Improved attractants based on extracts of wound fluid or blood (Hammack & Holt, 1983) may also be developed for use with targets, and the present assay would seem highly suitable for this type of bait development.

Fig. 6.7 RELEASE OF SWORMLURE FROM THIN SACHET 21oC (10ml in 10x5 cm 45u thick)

Table 5.1

Contacts of female *C. hominivorax* with a red target when stimulated by swormlure-4, and by swormlure-4 with different components deleted or substituted (mean contacts/min for mins 2-4 of 4 min observation periods, replicated as indicated).

Mixture (S4 = swormlure-4)	Contacts (Landings)	% control
Experiment 1 (3 replicates)		
S4	30.1 (13.5)	(100.)
S4 - dimethyl disulphide	15.4 (10.3)	51.3 ***
S4 - butanols	1.2 (0.7)	4.1 ***
S4 - acids	2.0 (0.7)	6.6 ***
S4 - indole & phenols	3.3 (1.1)	11.1 ***
Experiment 2 (4 replicates)		
S4	18.8 (7.5)	(100.)
S4 - indole & phenols	3.6 (1.4)	19.1 ***
S4 - indole	10.2 (4.8)	54.1 ***
S4 - phenols	4.8 (2.2)	25.7 ***
Experiment 3 (3 replicates)		
S4	28.2 (9.1)	(100.)
S4 - butanols	2.3 (0.7)	8.3 ***
S4 - iso butanol+	25.6 (8.3)	90.6
S4 - sec butanol+	22.7 (5.2)	80.3 *
Experiment 4 (1 replicate)		
S4	28.7 (9.0)	(100.)
S4 - butanols	1.3 (0.3)	4.6 ***
S4 - butanols + octenol (1%)	4.0 (1.3)	14.0 ***
S4 - butanols + octenol (5%)	0.7 (0)	2.3 ***
S4 - butanols + octenol (10%)	0.7 (0.3)	2.3 ***
Experiment 5 (4 replicates)		
S4	23.1 (8.6)	(100.)
S4 (indole at low dose++)	22.7 (6.7)	98.0
S4 - indole	6.3 (2.8)	27.3 ***
S4 - indole + skatole	17.5 (6.0)	75.7 **
S4 - indole + skatole (low dose)++	16.3 (5.2)	70.3 ***

Difference from control (X2) * P <0.5 ** P <0.01 *** P <0.001

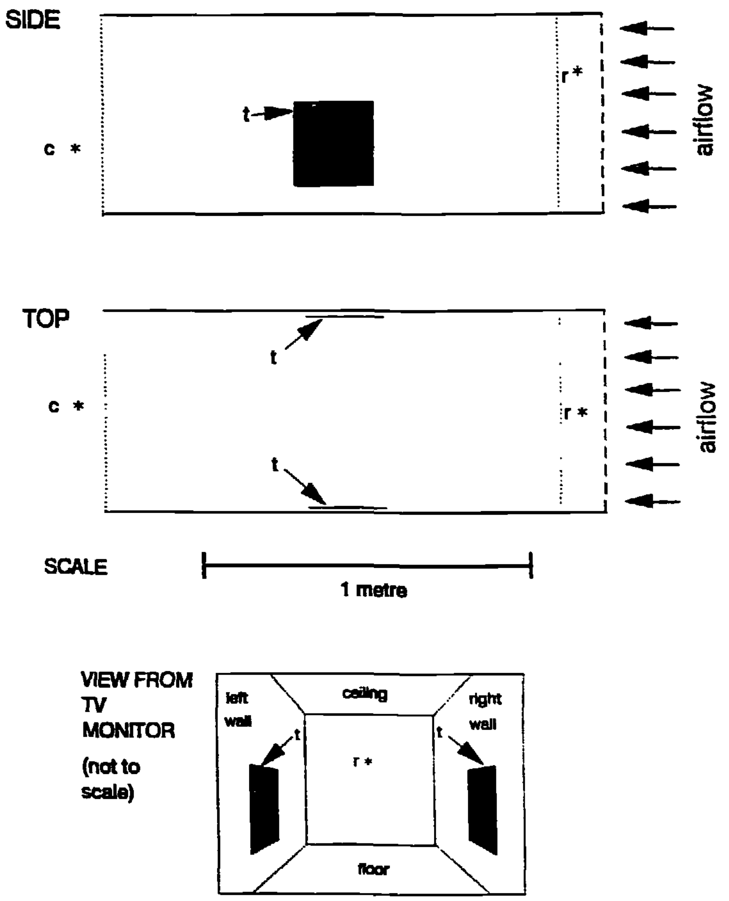
+ When only one butanol was omitted, amounts of the other were doubled

++ 'low-dose' was 0.1 of normal amount

Fig. 6.9 RELEASE OF DIMETHYL DISULPHIDE FROM DRIP

Fig. 5.1

Diagram of flight space of observation chamber, and view of chamber through video apparatus (c= video camera, r= odour delivery rosette, t= target; dotted lines indicate netting, dashed lines the baffle plate).



7 Laboratory investigations of insecticide

Fig. 5.2

Behaviour in a group of 35 female *C. hominivorax* during a single 12-minute observation period. A: flies at rest in field of view at end of each 30 s period; B; flies in flight at end of each 30 s period; C: total landings/brief contacts with red target during each 30 s period; D: total landings/brief contacts with yellow target during each 30 s period.

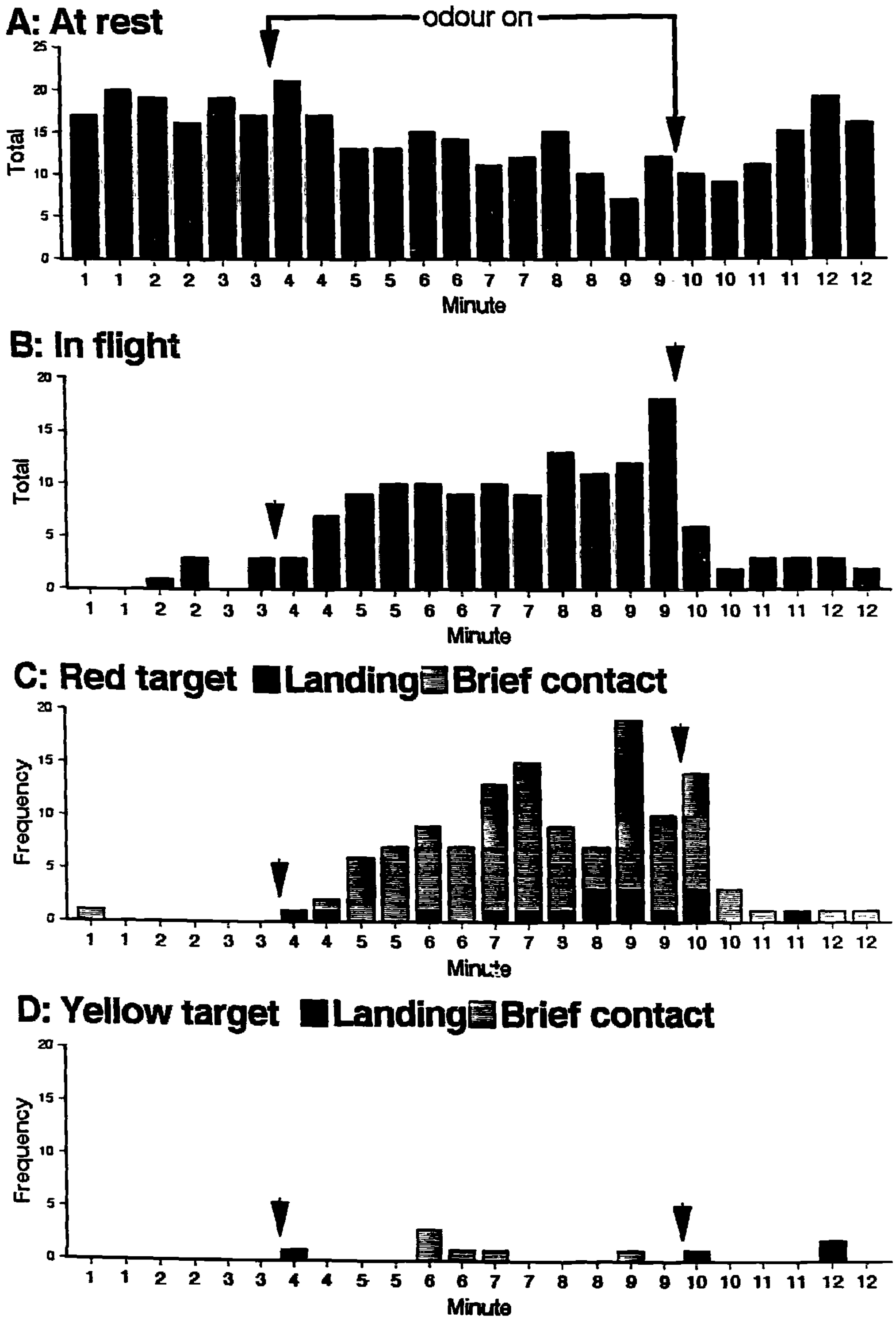
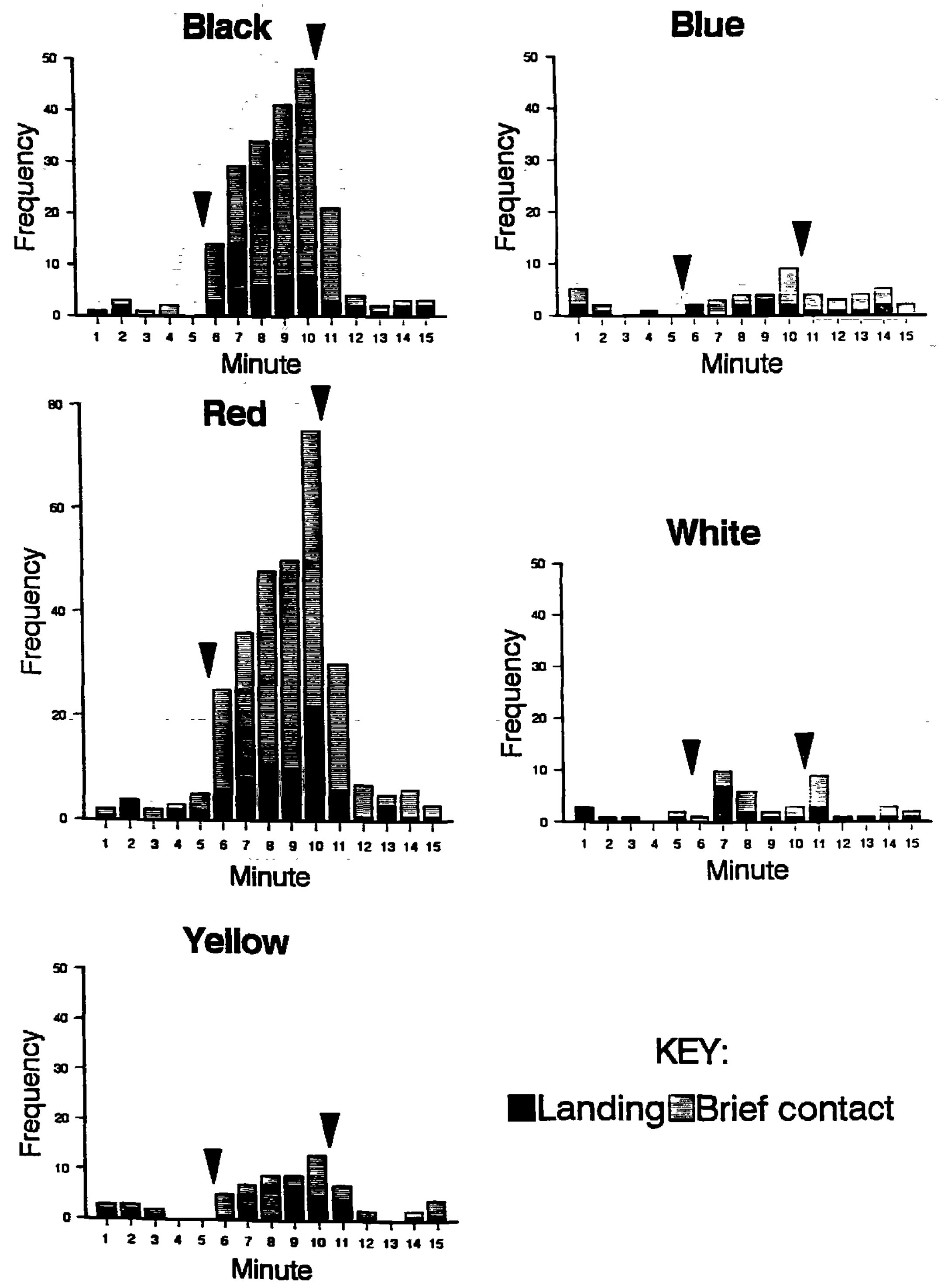


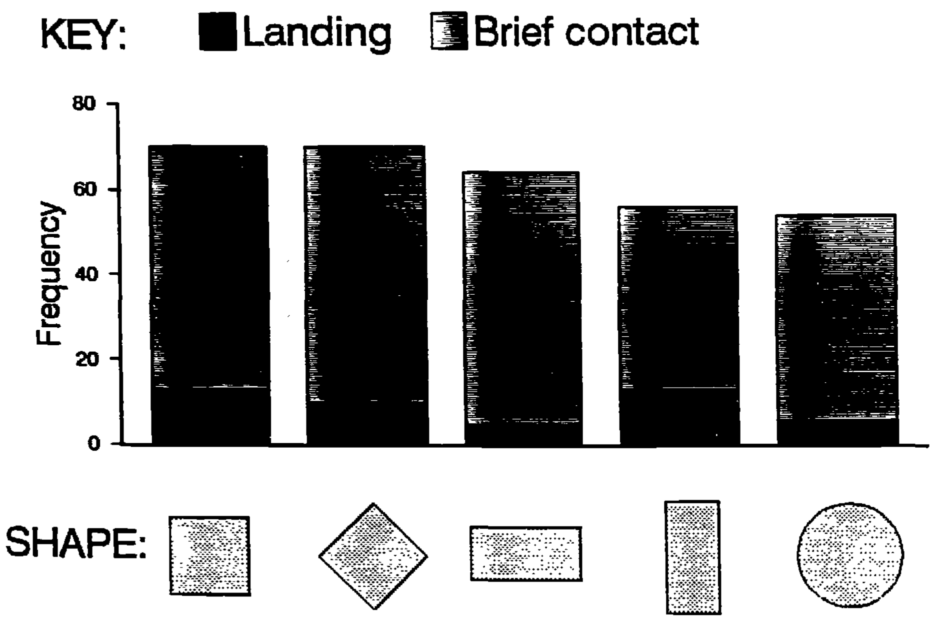
Fig. 5.3

Landings/brief contacts of female *C. hominivorax* over a 15 minute period, with odour for minutes 6-10 (marked by arrows), on targets of 5 different colours; totals from 5 replicates.



Tests were also carried out to investigate the possibility of reduced toxicity after a treated test piece had been contacted by a large number of

Fig. 5.4
Summary of landings/brief contacts of female *C. hominivorax* made with red targets of different shapes, but equal surface area, during two minutes with odour stimulation; totals from 5 replicates.



fly directly, unlike tsetse which usually circle the target before landing (Vale, 1974). Even if screwworm flies circle a target the addition of a