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PRELIMINARY LABORATORY AND FIELD TRIALS OF A HEATED PHEROMONE TRAP FOR THE SANDFLY *LUTZOMYIA LONGIPALPIS* (DIPTERA: PSYCHODIDAE)

RICHARD D. WARD; IAN E. MORTON; REGINALDO P. BRAZIL*; SHEILA TRUMPER & ALDA L. FALCÃO**

Department of Medical Entomology, Liverpool School of Tropical Medicine, Pembroke Place, Liverpool, L3 5QA, Great Britain *Departamento de Parasitologia, Universidade Federal do Rio de Janeiro, Cidade Universitaria, 21941 Rio de Janeiro, Brasil **Centro de Pesquisas René Rachou – FIOCRUZ, Caixa Postal 1743, 30190 Belo Horizonte, MG, Brasil

A heated pheromone trap for the sandfly Lutzomyia longipalpis *was tested in the laboratory using filter paper or plastic vial dispensers. Male pheromone extracted from 8 male tergal glands and absorbed on to filter paper dispensers attracted 82/120 (68.3%) of virgin females released in cages. Similarly plastic vial dispensers baited with the extract of 24 males caught 73/120 (61%). In field trials carried out near Januária, Minas Gerais, Brazil using the plastic dispensers baited with extracts of 50 male tergal glands only 70 female* L. longipalpis *were captured. Over 1000 male flies were, however, caught during 6 nights, with greater numbers in the unbaited control traps than in the pheromone baited test traps. It is concluded that at excessive concentrations male* L. longipalpis *pheromone may act as a repellent to conspecific males.*

Key words: pheromone trap – sandfly – *Lutzomyia longipalpis*

Male sandflies of the *Lutzomya longipalpis* complex produce pheromones which attract females from a distance to the host or to structures surrounding the host where mating subsequently takes place (Morton & Ward, 1989; Ward et al., 1989). The pheromones are produced in glands beneath the fourth, and in some populations, also the third tergites (Lane & Bernades, 1990) and the chemical nature of the pheromones has been broadly classified as farnasene/homofarnasene-like and diterpenoid-like (Lane et al., 1985), although an exact chemical structure for these substances has yet to be elucidated. Other attractant chemical cues, which appear to act synergistically with the pheromone in nature, include host semio-chemicals that may be carried initially on the convection currents created by the body of the host (Nigam & Ward, in press). Previously, Morton & Ward (1990) were able to demonstrate that extracts of male tergal gland could be used on the upper panels of laboratory cages to trap female flies on sticky surfaces. When pheromone was presented on the lower part of the cage, however, females failed to respond and descend to the stimulus. In the present paper we give a full description of preliminary laboratory (Brazil, et al., 1989) and field experiments to combine a battery powered heat source and male pheromone extracts to trap female flies on an adhesive surface.

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MATERIALS AND METHODS

Laboratory experiment with filter paper dispensers – In the preliminary laboratory experiments 2.8 x 3.8 cm ceramic heat blocks were removed from commercially produced insecticide vaporizers (Figs 1, 2) (Spira 'No-Bite' NB012). These portable 12 volt 'mosquito killer' vaporizers are normally sold with bio-allethrin insecticide tablets for use in caravans, tents, motor homes and boats. When the vaporizer is powered at 12 volts the ceramic block encased in an outer plastic support frame heats to a temperature of 140 °C, thus vapo-rizing the insecticide and killing any flying insects. For our purposes, however, we wished to reproduce a heat source which was similar to the normal body temperature of a potential sandfly host. Experimentation showed that a combination of 3 x 1.5 volt alkaline batteries

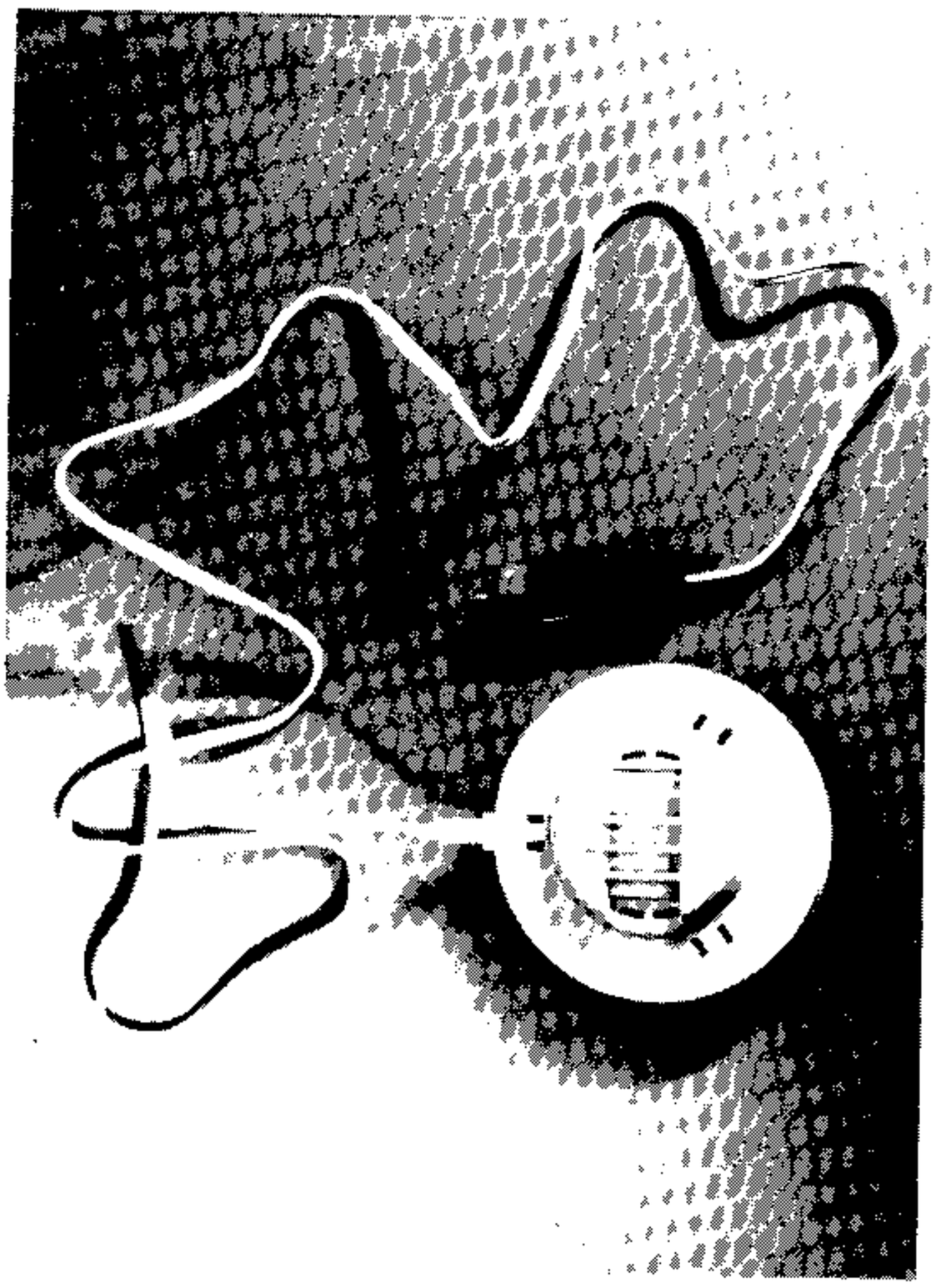
(4.5 volts) in series was sufficient to heat the ceramic block to 37.5-40.5 °C for up to 36 h. Two blocks were mounted in 6 x 18 x 2 cm polystyrene supports and placed parallel, 12 cms apart, on the floor of a 55 cm^3 cage (Fig. 3) in a humidified insectary at 85% RH and 27 °C. The experimental and control blocks each supported the lower half of a 9 cm diameter petri dish, the inner base of which was coated in castor oil. At the centre of each dish was a small square of aluminium cooking foil which supported either male pheromone or a control solvent blank. In a first series of six laboratory trials the pheromone treatment consisted of a 2 cm diameter filter paper disc on to which had been absorbed a 200 µl hexane extract of eight male sandfly tergal glands. To prepare the extract, eight 4-6 day-old males were used from a colony of sandflies originating from individuals first collected in L'Aguila, Melgar, Tolima, Colombia. The abdominal segments containing the tergal glands from 8 individuals were dissected, placed in 1.0 ml extraction vials and immersed in 200 µl of spectrophotometric hexane for 10 min before the extract was placed on the filter paper disc. The control dish supported a similar filter paper disc soaked in only 200 µl of pure spectrophotometric hexane. Experimental and control discs were allowed to dry before use and the positions of the discs were alternated between replicates to eliminate any environmental bias resulting from position. Twenty 4-6 day old, virgin female sandflies were released into the cage 1 h before the start of the experiment which was run overnight between 17.00 h and 09.00 h. The number of female flies caught in the experimental and control dishes, and those not captured, was then counted each day.

Laboratory experiment with plastic dispensers – In a second experiment, extracts of 24 male tergal glands were made in the manner described above and placed in 1.5 ml plastic 'azlon' slow release dispensers of the type used by some commercial pheromone trap manufacturers (Pheromone International). The control trap vials were loaded with 200 µl of pure spectrophotometric grade hexane. In these experiments the vials were stored for 24 h at ambient temperatures (24-26 °C) before use to be certain that the pheromone had sufficient time to diffuse through the wall of the dispenser. Twenty virgin sandfly females were released in the experimental cage as before and the numbers of flies captured on the dishes and those not caught were counted the following morning.

Both experiments each consisted of a series of 6 replicates. The net cages were washed between replicates, and the heated blocks and supports cleaned with hexane to remove any contamination. Results were analyzed for significance using the Wilcoxon signed rank test.

Field experiment – The laboratory experiments led to a preliminary field trial of the heated traps in February 1989. The study was carried out in the village of Tabua about 10 km south of Januária, Minas Gerais State (15°25'S, 44°25'W). A previous visit to this site in 1988 had established that *L. longipalpis* of the 2 main pheromone types were present (farnasene/homofarnasene and diterpenoid-like) and abundant in and around chicken coops in the yards of many houses. Sandflies were captured each night to obtain a supply of males, using CDC light traps set inside chicken coops and houses. The pheromone trap was modified for field use by leaving the heated ceramic block encased in its plastic support (Fig. 1), which was attached by 4 'Velcro' strips to a 25 cm^2 plastic tray coated in castor oil (Fig. 4).

Plastic 'azlon' slow release dispensers were used in the trap and attached to the plastic grid above the heated block by a rubber band. The test trap dispenser was baited with an extract of 50 male tergal glands from 1 spot (farnasene/homofarnasene-like), wild caught flies extracted in 200 µl of hexane for 10 min. Earlier trial experiments using vials baited with extracts from 20 flies had failed to catch female flies and, therefore, we subsequently increased the number of glands in the extract to 50. The control trap was baited with a dispenser containing only hexane. Extractions were carried out about 5-6 h before the start of each experiment. The traps were set at 19.00 h and the operating temperature was checked at dusk and dawn using a digital thermometer. Over the first 6 trapping nights the control and test traps were placed alternately on the tops of 2 small stone-built chicken coops situated about 3 m apart (designated coop 1 and 2 respectively). The coops were in the yard of a house where sandflies were numerous and where human and canine cases of visceral leishmaniasis had been recorded.



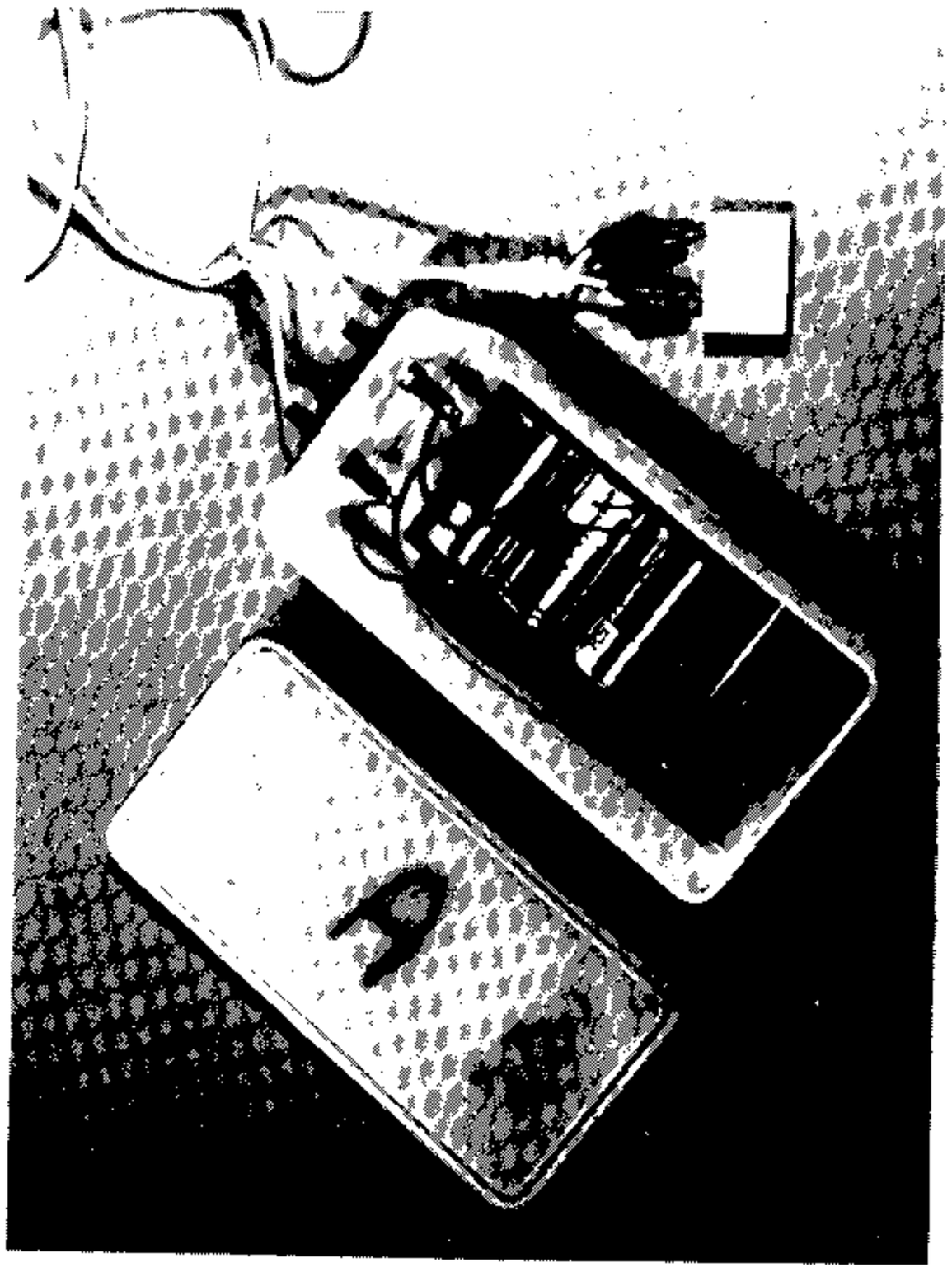


Fig. 1: Spira 'No Bite' insecticide vaporizer for use in caravans, cars tents and motor homes. Fig. 2: the ceramic heat pad removed from the insecticide vaporizer and powered with 3 x 1.5 volt alkaline batteries. Fig. 3: the ceramic heat pad mounted in a polystyrene support beneath a sticky petri dish baited with male *Lutzomyia longipalpis* pheromone on a filter paper dispenser. Fig. 4: the Spira 'No Bite' insecticide vaporizer attached by "Velcro" strips to a sticky sandwich box lid and baited with a plastic azlon vial containing the extract from 50 male *Lutzomyia longipalpis* tergal pheromone glands.

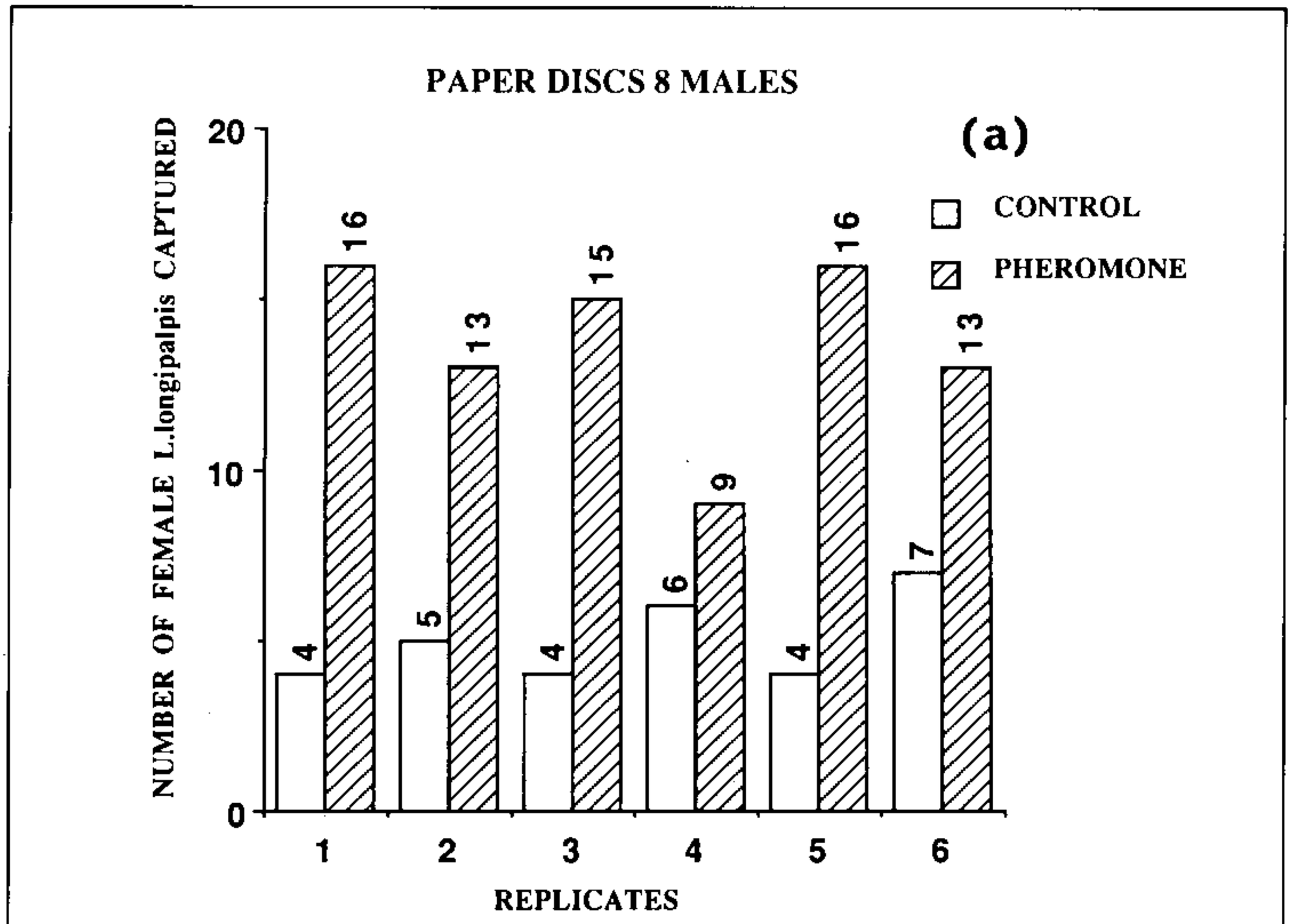


Fig. 5: (a) nightly laboratory catches of *Lutzomyia longipalpis* females on a heated pheromone trap baited with an extract of 8 male tergites absorbed on a filter paper dispenser; (b) nightly laboratory catches of *L. longipalpis* females on a heated pheromone trap baited with an extract of 24 male tergites in a plastic "azlon" vial dispenser.

During a second period of 6 trapping nights, 2 heated, unbaited traps that had not previously been used for pheromone trapping were also alternated between the 2 coops as a double control experiment. The numbers of male and female flies captured were recorded and the female flies dissected to determine insemination rates. Results were analyzed for statistical significance using log-transformed analysis of variance.

RESULTS

Laboratory experiments with filter paper dispensers – Over 6 nights 82 (68.3%) of the 120 female *L. longipalpis* released were captured in the dishes baited with pheromone, in contrast with only 30 (25%) in the controls (P = 0.036). The remaining 8 flies (6.7%) were recaptured from the cage. Nightly catches on the experimental and control dishes are shown in Fig. 5a (replicates 1-6).

Laboratory experiments with plastic dispensers – In this series of replicates 73 (61%) of 120 females released were captured in the dishes baited with pheromone vials. However, only 26 (21.6%) were caught in the controls (P = 0.036) and 21 (17.4%) were not captured in either trap (Fig. 5b replicates 1-6).

Field experiments – During the first 6 nights only 70 female *L. longipalpis* were caught representing 6.4% of the 1,096 adults captured, of which 30 were on the pheromone traps and 40 on the controls. There was therefore no indication that at the concentration of pheromone used females were attracted to the trap (P = 0.885). Out of 64 females dissected only 12 (18.75%) were uninseminated and 4 (6.25%) were unclassified. There was therefore no evidence for selective trapping of virgin flies.

In the double control only 97 females were captured which represents 5.3% of the 1,826 adults captured, 46 in one trap and 51 in the other (P = 0.944).

Much higher numbers of males were, however, trapped in both series of catches with 1,022 in the first 6 nights and 1,729 in the second series of (double control) catches (Table). The

TABLE

Distribution of 1 spot and 2 spot male *Lutzomyia longipalpis* caught in chicken coops in Januária, Minas Gerais, Brazil using heated pheromone and control traps.

Test and Control

	COOP 1				COOP 2			
	Pheromone		Control		Pheromone		Control	
Morphotype	1	2	1	2	1	2	1	2
Nights								
1	73	15					32	27
2			138	28	13	16		
3	65	8					28	14
4			76	20	19	11		
5	88	34					66	11
6			137	62	34	7		
Total ♂♂	226	57	351	110	66	34	126	52

Double Control

	Control		Control		Control		Control	
	1	2	1	2	1	2	1	2
1	123	16					40	11
2			71	8	24	12		
3	160	24					85	12
4			303	24	67	17		
5	251	22					83	22
6			267	36	42	9		
Total ♂♂	534	62	641	68	133	38	208	45

population of males in coop 1 was significantly higher than that in the smaller coop 2 (P = 0.000). Interestingly the catches over the first 6 nights showed that the pheromone traps on both coops consistently caught lower numbers of males than in the control traps (P = 0.015). In contrast when the 2 control traps were set during the second 6 night period, the differences in the male catches in the 2 traps were not significant (P = 0.484). The concentration of farnasene-like pheromone from 50 one spot flies did therefore appear to reduce the number of all males caught in the traps. This effect was, however, only statistically significant for the reduction in 1 spot farnasene-like flies (P = 0.034) and not for 2 spot males with the diterpenoid-like pheromone (P = 0.103). The numbers of 1 spot farnasene-like flies caught in the 2 traps used in the double controls were not significantly different (P = 0.417). Similarly the numbers of 2 spot diterpenoid-like flies in the 2 double control traps were not significantly different (P = 0.872).

DISCUSSION

The laboratory traps, when operated with extract of 8 male glands on filter paper were clearly effective in trapping a high proportion of virgin 4-6 day-old female flies. This concentration of pheromone was chosen on the basis of previous work which had indicated that under laboratory conditions female reponse was highest at this dosage when compared to extracts of 1, 2 and 16 male glands (Ward et al., 1989; Morton unpublished observations). Although we also showed that aged pheromone of the diterpenoid type elicited an even greater response from females than fresh extract, Nigam & Ward (in press) were unable to detect an increased response to aged farnasene-like pheromone. Since the flies used in this series of laboratory experiments also producr the farnasene-like pheromone (Ward et al., 1988) no attempts were made to use aged pheromone in these traps. Previous bioassays have also indicated that inseminated flies are less responsive to the pheromone than virgin individuals. The factors involved in changes in sexual receptivity may be either chemicals such as peptides or proteins in the male ejaculate, or result from female hormonal release following the stimulation of stretch receptors during copulation. Similar loss of female response to male pheromone following insemination has been observed in the Queensland fruit fly *Dacus tryoni* (Fletcher & Giannakakis, 1973) in which some females become responsive to pheromone again after a period of several weeks. Paper disc pheromone dispensers are not sufficiently durable for field application and for this reason we decided to experiment with a plastic vial dispenser. There are, however, a wide range of potential commercial dispensers including plastic laminates, hollow polymer fibres, gelatin microcapsules, polyvinyl chloride rods and even cigarette filters (Jutsum & Gordon, 1989). The results of the second laboratory experiment showed that the farnasene-like pheromone was diffusing through the wall of the 'azlon' vial and was attractive to virgin *L. longipalpis*. As a result of these encouraging preliminary trials we decided to test the same system under field conditions using the modified trap described.

The low proportion of females captured was disappointing and may reflect a seasonal population imbalance between the sexes. It is nonetheless a feature often observed in chicken coops. Thus the proportions of female/male *L. longipalpis* recorded in chicken coops in Jacobina, Bahia by Sherlock and Guitton (1969) was similar to our own result with 26/506 (5.1%) females, and in animal houses in Ceará Deane (1956) recorded 5,910/20,278 (29.1%) females. This preponderance of males over females adds support to the idea that the coops act as a meeting site for the sexes in which males await for prolonged periods the arrival of females who visit the coops only fleetingly for a bloodmeal and sexual encounters. More recently Lainson et al., (1990) caught almost equal proportions of the sexes of *L. longipalpis* in Marajó, but these results may reflect some trap selectivity for females since CDC light traps as well as chickens were employed. It is well established that in light trap catches for mosquitoes there is a sexual bias in the catches in favour of unfed females (Service, 1977). Equal proportions of males to females are more commonly encountered in natural resting site catches in locations such as under rocks where, for example, Sherlock & Guitton (1969) recorded, 2,603 females/2,719 males in Bahia, and Deane (1956) caught 587 females/680 males in a similar situation in Ceará. Interestingly the ratio of females to males inside houses were also almost equal in Ceará with 5,586 female/ 4,735 males (Deane, 1956). But in contrast in Bahia, Sherlock & Guitton (1969) recorded a predominance of males inside houses with 312 females/2,195

males. These contrasting observations may simply reflect seasonal variations or, more excitingly, might indicate that one female member of the *L. longipalpis* complex is more endophilic than the other.

The reduction in males caught on our pheromone baited traps was probably due to the fact that pheromone extracted from 50 male glands was too concentrated and resulted in a repellent effect. The repellency appeared also to have been selective in that 1 spot (farnasene-like pheromone) flies were significantly fewer in the traps and 2 spot diterpenoid flies were not significantly repelled. Pheromone repellency has also been observed in studies on the synthetic female attractant Trimedlure with males of the Mediterranean fruit fly *Ceratitis capitata* (Beroza et al., 1961; Nakagawa, et al. 1971). In addition some male moths such as *Pseudaletia unipuncta* (the 'army worm'), are also known to produce pheromones which act as aphrodisiacs for females but which at the same time inhibit the approach of other males (Hirai et al., 1978). In discussing the dual function of some semiochemicals, Prokopy et al. (1984) postulated that too many males in a single lek might be "maladaptive in terms of individual male probability of procuring a mate within a lek as well as in terms of efficient utilization of available lekking sites within a habitat harboring females". He also added that "there is no evidence as yet of individual males being repelled by pheromone emanating from a large lek". Our results were not due to a large male lek but the high concentration we used appears to have acted to produce the same effect. Khalil et al. (1983) have also noted how tick pheromones in high concentrations may function as repellants. Therefore a novel control approach to future tick control might be through 'treatment of livestock with excessive concentrations of sex pheromone to repel homo- and heterospecific males'. Mwase et al. (1990) suggested that this strategy might serve to disrupt mating and tick reproduction without the use of insecticides and it is clearly a potential sandfly control technique that should not be ignored in future studies. In conclusion these preliminary results demonstrate the need for further studies on the chemical nature of the pheromones involved and the entrainment of fly volatiles to determine release rates and isomeric blend composition. In the long term it may then be possible to design highly specific traps for monitoring *L. longipalpis* populations for use during endemic disease control programmes.

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