Short Communication

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Effect of a Synthetic Pheromone When Applied to Different Models on Male Mating Behaviour in *Musca domestica*

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The mating behaviour of the house fly, *Musca domestica* has frequently been studied [1,2]. The discovery of the presence of species specific volatile chemicals in female house flies that can influence the behaviour of male house flies [3], and subsequent isolation, identification and synthesis of a female sex pheromone called (Z)-9-tricosene (a synonym of *cis*-9-tricosene) or "muscalure" [4], led to the demonstration that the male house flies apparently become sexually stimulated on exposure to this synthetic or natural pheromone. Later on, it was confirmed that (Z)-9-tricosene is one of the cuticular hydrocarbons of *M. domestica* and is only identifiable in females but not in males [5]. So far, however, four components of house fly sex pheromone have been reported [6].

In the experiment reported here, we tested whether the synthetic pheromone, *cis*-9-tricosene topically applied to three different models, *viz.*, live females, dead females or dead males would increase male attractiveness towards them. Two strains of *M. domestica*, Cooper and Boxted, obtained as larvae from the Field Station of the University of Newcastle upon Tyne, UK where they are being maintained for the last 6-7 year, were used. Within 8 hrs of emergence, males and females were separated, held in the constant room temperature at 22 ± 1°, 70+5% RH, and offered with 5% sugar solution soaked in cotton pads. For behavioural assays, flies aged 2-3 days from eclosion were used. *Cis*-9-tricosene was procured from Sigma Chemical Co., USA and 2% and 4% solutions were prepared in acetone. The stock material working solutions were stored in a deep freezer at - 20° until use. With a micropipette 2 µl of each of the concentrations were applied per fly. The live females were treated with pheromone under carbon dioxide anaesthesia. Two µl acetone only were applied to flies used as controls. After treatment, the flies were set aside for 5 mins so that the live ones could regain consiousness and the acetone solvent could evaporate from all. The treated flies were marked with a white spot (vinyl matt emulsion, lily white colour) on the thorax to distinguish them from the control ones. Three flies: untreated male, pheromone-treated (hence marked) live female or dead female/male, and the control live female or dead female/male, were transferred to a 9-cm-diameter Petri dish used as a test chamber. For each set of observations, matings/mating attempts were recorded for about one hour or until mating were recorded in all the replicates though multiple matings were noted in some of the test chambers, only the first matings/mating attempts were taken into consideration. All observations were carried out in room temperature (25±5°) in day light between 0900-1500 hrs. After each assay, the test chambers were thoroughly washed in hot water with detergents and dried. The Chi-square values were calculated from the observed numbers of matings/mating attempts between the untreated males and the live or dead, control or treated flies that

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Table 1. Attractiveness of the Male House Flies to *Cis*-9-tricosene Applied to Different Fly Models.

Tricosene conc.	N	Copper strain matings/ attempts (♂ x ♀)	X^2 values	N	Boxted strain matings/ attempts (♂ x ♀)	X^2 values
2%	30	Ux T =12 U x C =18	1.20ns	20	Ux T =9 U x C =11	0.20ns
	30	U x Tdf=14 U x C =16	0.13ns	20	U x Tdf=8 U x C =12	0.80ns
	30	U x Tdm=13 U x C =17	0.53ns	20	U x Tdm=8 U x C =12	0.80ns
	20	U x Td =16 U x Cd =4	7.20**	20	U x Td=14 U x Cd =6	3.20ns
4%	50	U x T =28 U x C =12	13.52***	20	U x T =15 U x C =18	5.00*
	20	U x Tdf=14 U x C =6	3.20ns	40	U x Tdf=22 U x C =18	0.40ns
	20	U x Tdm=12 U x C =8	0.80ns	40	U x Tdm=25 U x C =15	2.50ns
	20	U x Td =14 U x Cd =6	3.20ns	20	U x Td =17 U x Cd =3	9.80**

N = number of replication: U = untreated males; T = tricosene-treated live females; C=control (acetone-treated) live females; Tdf=tricosene-treated dead females; Tdm = tricosene-treated dead males; Td = tricosene-treated dead, males or females; Cd=control dead, males or females; ns=not significant (P > 0.05); * = P<0.05; **= P<0.01; ***= P<0.001; all values at 1 df.

gave an estimate of mating behaviour of the male house flies in respose to *cis*-9-tricosene under laboratory conditions.

Results of the experiment are presented in Table 1. In both strains, the males were attracted by a greater number of control, live females than the 2% tricosene-treated live females or dead females/males, though the observed number of mating combinations in favour of the control females were not found to be significant statistically. In contrast, live females treated with 4% tricosene attracted a significantly greater number of males ($P<0.001$ in Cooper and $P<0.05$ in Boxted, respectively) than did the control females or dead flies. It is apparent from the Table that a greater number of males were attracted by the 4% tricosene-treated dead females/males than the live, control flies. It is also remarkable to note that in both strains the treated dead flies attracted a greater number of males than the control, dead flies.

Behavioural experiments with house flies have shown that throughout most of its life, the male house fly is sexually aggressive, and it will attempt to mate with a previously mated or unmated females, with a male or even occasionally with a dead fly of its own or another species, [3,4]. Moreover pseudofly assays indicate that the synthetic sex pheromone, (Z)-9-tricosene causes sexual stimulation in the male house flies, and mating strikes and subsequent copulation increase by the pheromone treatment [6-8]. Our results are in well agreement with these findings.

Though the results of the present study are somewhat difficult to interpret, partly because of the use of only two concentrations of the synthetic pheromone, and partly because of the simplicity of the bioassays, nevertheless the data indicate that the mating strikes and copulatory behaviour of the males increased significantly after a higher concentration of the tricosene treatment to all model flies. However, it should be kept in mind that the male attractiveness towards the synthetic pheromone source in the wild might be quite different from that recorded in the small artificial arena used in the present study. Besides, our results imply that a large scale test of house fly control concept in nature would be worthwhile, and use of suitable synthetic pheromone-baited traps under field conditions should be considered.

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Key words: *Musca domestica,* Synthetic pheromone, Male mating behaviour.

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