

University of Groningen

## Pheromones of the housefly

Noorman, N

**IMPORTANT NOTE: You are advised to consult the publisher's version (publisher's PDF) if you wish to cite from it. Please check the document version below.**

*Document Version*

Publisher's PDF, also known as Version of record

*Publication date:*

2001

[Link to publication in University of Groningen/UMCG research database](#)

*Citation for published version (APA):*

Noorman, N. (2001). *Pheromones of the housefly: A chemical and behavioural study*. [Thesis fully internal (DIV), University of Groningen]. s.n.

**Copyright**

Other than for strictly personal use, it is not permitted to download or to forward/distribute the text or part of it without the consent of the author(s) and/or copyright holder(s), unless the work is under an open content license (like Creative Commons).

The publication may also be distributed here under the terms of Article 25fa of the Dutch Copyright Act, indicated by the "Taverne" license. More information can be found on the University of Groningen website: <https://www.rug.nl/library/open-access/self-archiving-pure/taverne-amendment>.

**Take-down policy**

If you believe that this document breaches copyright please contact us providing details, and we will remove access to the work immediately and investigate your claim.

*Downloaded from the University of Groningen/UMCG research database (Pure): <http://www.rug.nl/research/portal>. For technical reasons the number of authors shown on this cover page is limited to 10 maximum.*

# Chapter 3

## SEXUAL ACTIVITY AND EAG RESPONSES OF HOUSEFLIES (*MUSCA DOMESTICA* L.) FROM STRAINS WITH DIFFERENT MUSCALURE QUANTITIES

### Abstract

Muscalure, (Z)-9-tricosene, is thought to be the major component of the cuticular contact sex pheromone of the female house fly *Musca domestica*. (Z)-9-heneicosene is also supposed to be of importance in inducing sexual behaviour in the flies. The amount of muscalure present on the cuticle of female flies of a WHO laboratory strain was found to be considerably higher than that on females of two wild type strains, which is possibly due to selection in subsequent generations of isolated populations. We investigated whether these differences in muscalure quantities are reflected in the sexual activity of the males.

The results show that male sexual activity was higher towards females with higher amounts of muscalure. In addition, males from strains with higher amounts of muscalure on the females appeared to be more sexually active. This indicates that selection in laboratory cultures increases both muscalure production in the females and sexual activity of the males.

EAG recordings indicated that males as well as females of all three strains are able to detect (Z)-9-tricosene and (Z)-9-heneicosene, which suggests that differences in sexual behaviour were not due to differences in ability to smell these substances.

## Introduction

(Z)-9-tricosene (muscalure), one of the cuticular hydrocarbons of female houseflies, *Musca domestica*, plays an important role in inducing sexual behaviour in male houseflies (Carlson *et al.*, 1971; Uebel *et al.*, 1976, 1978). However, several other (Z)-9-unsaturated hydrocarbons also appear to evoke sexual behaviour in *M. domestica* males. For example, a mixture of (Z)-9-tricosene and (Z)-9-heneicosene induced and maintained high excitement and mating behaviour in males (Mansingh *et al.*, 1972).

Experiments of La-France *et al.* (1989) suggested that a certain amount of muscalure on females is required for inducing sexual activity in males. These authors washed dead females with petroleum ether and then loaded them with (Z)-9-tricosene. They found that males were sexually excited by females loaded with 10 µg (Z)-9-tricosene, but not by those containing 5 µg of this substance. However, chemical analyses by Nelson *et al.* (1981), Dillwith *et al.* (1983) and Ahmad *et al.* (1989) have shown that on female houseflies from different laboratory strains a maximum as low as 1.2, 1.5 and 3.5 µg muscalure, respectively, is present. Hence, one would expect that amounts lower than 5 µg may also evoke sexual behaviour in males. Noorman and Den Otter (2001) showed that females of wild-type strains hardly contain any muscalure, in contrast to strains with had been reared in the laboratory for several generations. Nevertheless, no differences in reproduction capacity were observed between the strains. This suggests that muscalure is not indispensable for mating.

The present paper presents results of studies on the effects of females from strains containing different muscalure quantities on the sexual behaviour of the males. In addition, in order to determine the ability of the flies to smell these chemicals, electroantennograms (EAGs) were recorded from males and females from different strains on stimulation with (Z)-9-tricosene and (Z)-9-heneicosene

## Materials and methods

### *Insects*

Experiments, electrophysiological and behavioural, were performed with *Musca domestica* L. flies from 3 different strains: A laboratory strain (WHO Ij2), obtained from

the Statens Skadedyrlaboratorium, Lyngby (Denmark) and cultured in the laboratory since 1961, and two wild-type strains obtained from a poultry breeding (Van Diermen) and a cow-house with pig-sty (Pesse) in The Netherlands, respectively. The latter two strains had been cultured in the laboratory for 12 (Van Diermen-12) and 6 (Pesse-6) months, respectively. The flies were reared in cages (30 x 30 x 40 cm) in a L12:D12 regime at 25 °C and r.h. 60%. They were fed (ad libitum) a mixture of sugar, powdered milk and yeast (5 : 5 : 1 by weight). In addition, a fountain filled with tap water was present.

### *Bioassay*

Flies used in behavioural tests originated from pupae, which had been kept individually in glass tubes (height: 5 cm, i.d. 1.5 cm). Immediately after emergence males and females were put in separate cages. For an experiment 1 virgin male and 1 virgin female (5 - 15 days of age) were put in a Petri dish (i.d. 9 cm) through a hole in the centre of the cover, after which the hole was closed. The numbers of copulation attempts ('strikes': Tobin and Stoffolano, 1973) and successful copulations were recorded during 15 minutes. Each couple of flies was monitored only once. After each experiment the dishes were thoroughly cleaned in hot water containing detergent, rinsed in distilled water and then dried. For electroantennogram recordings, flies, 5 - 10 days of age, were taken from cages containing both males and females. Experiments were carried out between 9 a.m. and 4 p.m. at 25 °C and r.h. 60%.

### *Electroantennogram recordings*

Electroantennograms (EAG's) were obtained at 25°C and r.h. 60% from intact, living flies using the technique described by Den Otter *et al.* (1988). A fly was fixed in a plastic pipette tip with its head protruding out of the tip's narrow end. The tip of a tungsten electrode was inserted into the head of the fly. The tip of a glass pipette/Ag-AgCl electrode was brought into contact with the distal end of the funiculus of one of the antennae. This pipette was filled with Beadle-Ephrussi saline containing 10% by volume polyvinylpyrrolidone K90 (Fluka Chemie AG, Buchs, Switzerland). The electrodes were connected to a high-impedance DC amplifier, the output of which was recorded on a PC. EAG's were analysed using the software programme EAG<sup>TM</sup> 2.6 (SYNTECH, Hilversum, The Netherlands).

Stimuli were 0.01, 0.05, 0.1, 1, 5, and 10 µg (Z)-9-tricosene and (Z)-9-

heneicosene dissolved in 25  $\mu\text{l}$  silicon oil. The solutions were pipetted onto pieces of filter paper (1  $\text{cm}^2$ ). In addition, papers loaded with 1  $\mu\text{g}$  amylacetate in 25  $\mu\text{l}$  silicon oil (reference stimuli) and with 25  $\mu\text{l}$  silicon oil (control stimuli) were prepared. Each individual paper was put into a Pasteur pipette. The pipette served as an odour cartridge.

Stimulation was achieved by injecting, during 0.1 s, 1.5 ml of the vapour content of an odour cartridge into a continuous, charcoal-filtered, humidified airstream (1 m/s) passing over the antennae. Recordings were made from only one antenna per fly. Each fly was used in one experiment only.

The various substances were applied in random sequence, each substance in ascending intensity. Reference stimuli were applied before and after each stimulus. Control stimuli were applied 3 times per experiment. The EAG values were corrected for changes in antennal sensitivity by normalising the data to the reference values.

#### *Chemical analysis*

Two females of the same strain were immersed in 0.4 ml hexane; the whole was shaken during 1 min, after which the flies were kept in this fluid for at least 1 hour. Gas chromatography was performed on a Shimadzu GC-17A. One  $\mu\text{l}$  of the solution was injected into a 10 m x 0.32 mm CP-Sil-5 CB column (Chrompack) with the injector at 250  $^{\circ}\text{C}$  and the FID at 300  $^{\circ}\text{C}$ . The flow rate of the helium carrier gas was approx. 1 ml/min. GC oven temperature was programmed from 50 to 300  $^{\circ}\text{C}$  at 10  $^{\circ}\text{C}/\text{min}$ . 2-Nonanone was used as an internal standard. (Z)-9-tricosene was identified by comparing the retention time with those of reference runs of synthetic (Z)-9-tricosene. Quantities of (Z)-9-tricosene were expressed in  $\mu\text{g}$ .

## **Results**

*Sexual behaviour.* Figure 1 shows the amounts of muscalure on female flies used in the behavioural experiments. As appears from the figure, WHO females had about 3 times more muscalure on their body than Pesse-6 females, and about 15 times more than Van Diermen-12 females (no muscalure was present on first generation laboratory Pesse and Van Diermen females). These differences were statistically significant (t-test, WHO x Pesse:  $p < 0.01$ , WHO x Van Diermen:  $p < 0.001$ , Pesse x Van Diermen:  $p < 0.005$ ).

Figure 2 shows the percentages of males of each of the three strains which had performed strikes and successful copulations. Copulation was always preceded by a strike; a strike was not always followed by copulation. A strike took 1-2 seconds while the average duration of copulation was about 90 min. The WHO males were sexually most active, closely followed by the Pesse-6 males; the Van Diermen-12 males showed the lowest sexual activity. Males of all three strains copulated mostly with WHO females, less with Pesse and hardly with van Diermen females (Fig. 2). In the experiments males of the Van Diermen strain did not copulate at all with females of their own strain. The strike latencies (interval between the beginning of the experiment and the first strike) of the three strains were indistinguishable. Comparison of Figures 1 and 2 shows that the percentages of successful copulations increases with the amounts of muscalure on the females. On comparing the numbers of strikes and copulations of males of different age classes (5-7, 8-10, 11-13 and 14-15 days old) we did not find differences in sexual activity of males of different age (Chi Square test,  $p > 0.05$ ).

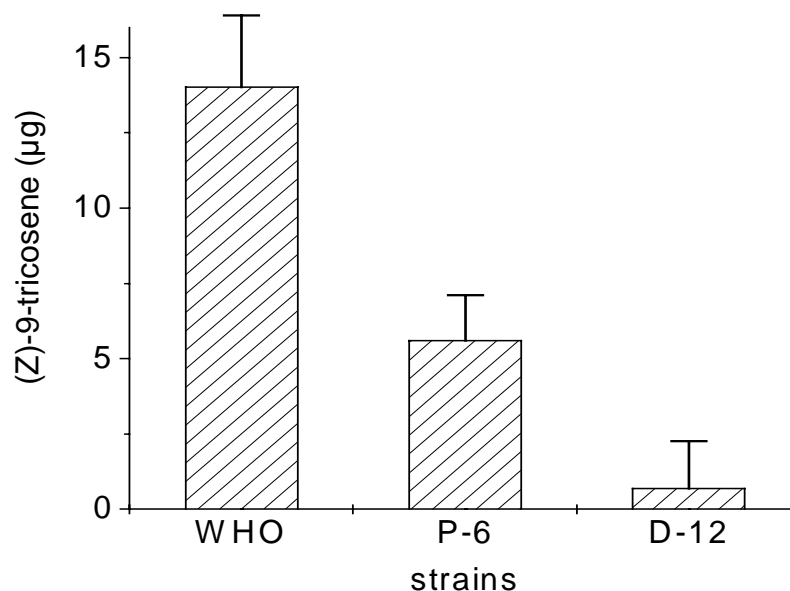


Figure 1. Amounts of (Z)-9-tricosene on WHO, Pesse-6 and Van Diermen-12 females used in the behavioural experiments.  $n = 12$  for each strain. Error bars denote SEM.

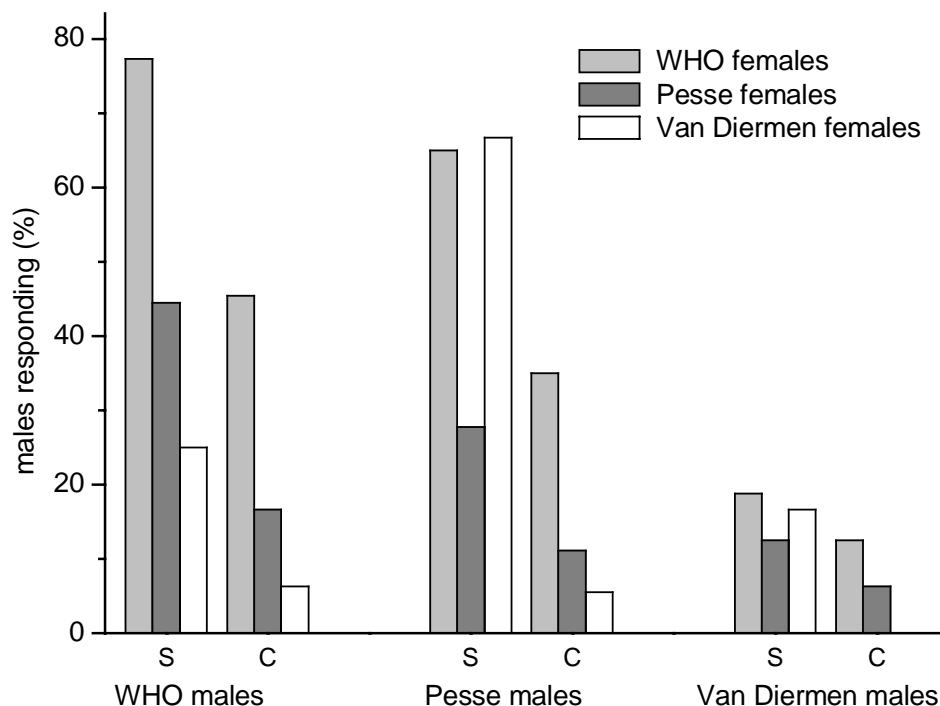


Figure 2. Percentages of males from the three different strains which performed strikes (S) and copulations (C) with females of the various strains. The number of virgin couples tested varied from 18-22.

In Fig. 3A the average number of strikes without and with subsequent copulation performed by males of the different strains is given irrespective of the origin of the females. Again it can be seen that males of the Van Diermen strain were considerably less sexually active than males of the other two strains. WHO males were the most active (Mann Whitney U test,  $a > c: p < 0.01$ ;  $a > b: p < 0.05$ ;  $b > c: p < 0.05$ ). Figure 3B shows the average number of strikes performed on females of the different strains irrespective of the origin of the males. Although the amount of musculature on the Van Diermen females is low compared to the amounts found on the females of the WHO and Pesse strain, the average numbers of strikes followed by copulation were about the same in all three strains. However, the number of strikes on WHO females is higher than those on females of the other strains when no copulation is achieved (Mann Whitney U test,  $a > b: p < 0.05$ ).

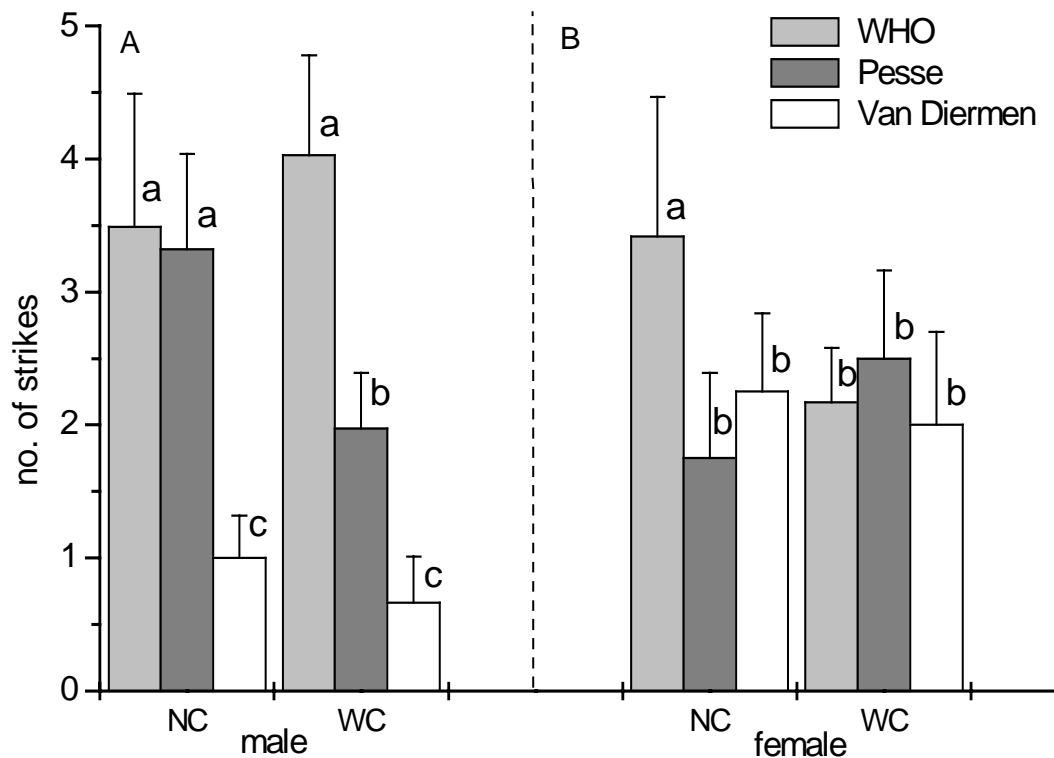


Figure 3. Average number of strikes without (NC) and with (WC) subsequent copulation. A. Strikes of males of the three strains irrespective of the origin of the females. B. Strikes on females of the three strains irrespective of the origin of the males. Columns with different letters are significantly different. Error bars denote standard deviations.

*Electrophysiology.* EAG responses (Figure 4) demonstrated that both males and females of all three strains are able to detect (Z)-9-tricosene and (Z)-9-heneicosene. (Z)-9-heneicosene evoked significantly higher EAG responses than (Z)-9-tricosene in both male and female flies of all three strains. However, females showed significantly higher responses to these substances than males (Anova repeated measurements;  $p < 0.01$ ). All three strains were equally sensitive to (Z)-9-tricosene and (Z)-9-heneicosene, respectively.



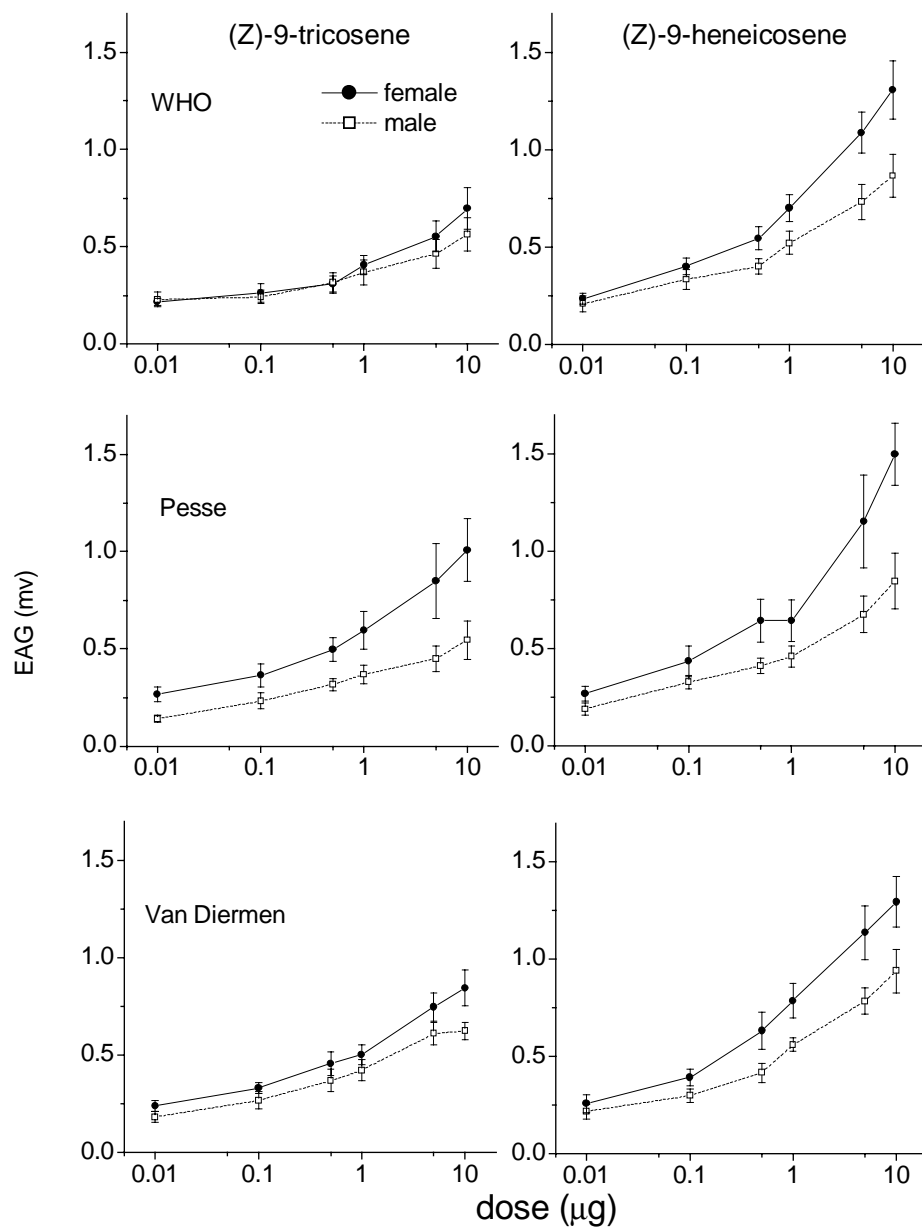


Figure 4. Mean EAG responses to (Z)-9-tricosene and (Z)-9-heneicosene of males and females (n=8) of the WHO, Pesse-6 and Van Diermen-12 strains. Error bars denote standard deviations.

## Discussion

The present study confirms our previous findings (Noorman and Den Otter, 2001) that muscalure quantities on female *Musca domestica* may differ considerably among strains from different origin and may change in the course of time. We observed that on females

of the WHO strain, reared in the laboratory for almost 40 years, relatively high amounts of muscalure were present, whereas on first generation laboratory females originating from larvae taken from the field (Pesse and Van Diermen strains) hardly any muscalure was found. However, after several generations in the laboratory, the amounts of muscalure on females of the latter strains had increased considerably. This led us to assume that selection in subsequent generations of isolated populations may lead to increased production of muscalure by the females. This is also suggested by results of Adler *et al.* (1984) who found that 10-day-old females of 4 different laboratory strains (1–20 years reared in the laboratory) contained 5 to 11 times more muscalure (percentage of total cuticular components) than first and third laboratory generation wild type strains. In the present study with Pesse and Van Diermen flies, originating from newly collected larvae, we observed that first generation laboratory females did not contain muscalure. However, after, 6 (Pesse) and 12 (Van Diermen) generations in the laboratory females had already around 1 and 5  $\mu\text{g}$ , respectively, muscalure on their body. On females of the WHO we found an average of 15  $\mu\text{g}$  muscalure with a maximum of 20  $\mu\text{g}$ . This is a higher amount of muscalure production than compared to the amounts other authors found on laboratory females.

Several behavioural experiments have been carried out to examine the role of muscalure on sexual behaviour of male houseflies (Carlson *et al.*, 1974; Adams and Holt, 1987; Islam and Port, 1994). In all these experiments muscalure was applied artificially to living or dead females or to dummies. A clear positive relationship existed between the presence of muscalure and the intensity of sexual activity of male flies towards the females or dummies even when the doses were extraordinarily high (up to 80  $\mu\text{g}$  muscalure /female). In our experiments the females had naturally acquired quantities of muscalure on their cuticle and here also male sexual activity was higher towards females with higher amounts of muscalure. Even males from the Pesse-6 and Van Diermen-12 strains were sexually more excited by females of the WHO strain than by females of their own strain; the latter contained relatively low amounts of muscalure. Our experiments also indicated that males of the WHO strain are sexually more active than males of the other two strains, males of the Van Diermen strain showing the lowest sexual activity (Fig. 3A). The reason for this phenomenon is not known. Possibly, selection in laboratory cultures also increases sexual activity. It thus seems that though muscalure increases male courtship behaviour, other factors may also be important in sexual communication.

The males of the three strains tested did not show significant differences in EAG responses to (Z)-9-tricosene. This may indicate that the differences in sexual behaviour were not due to differences in ability to smell muscalure. Our EAG recordings also indicate that females are able to smell muscalure suggesting that muscalure may possibly not only affect male but also female behaviour. In addition, (Z)-9-heneicosene evoked EAG responses in males as well as females. That the responses to this substance were even higher than those to (Z)-9-tricosene may result from the fact that (Z)-9-heneicosene, being 2 C atoms shorter, is somewhat more volatile than (Z)-9-tricosene. We also found that both (Z)-9-tricosene and (Z)-9-heneicosene evoked higher EAG's in females than in males. This may be due to the females being generally bigger than males and having bigger antennae. Kelling (personal communication) showed that the number of sensilla per antenna increases with increasing antenna surface. Because bigger antennae contain more odour receptor cells than smaller ones females may have higher EAG responses.

So far, the effects of (Z)-9-tricosene and (Z)-9-heneicosene on the behaviour of houseflies are not well known. Mansingh *et al.* (1972) found that a mixture of (Z)-9-tricosene and (Z)-9-heneicosene induced and maintained higher excitement and mating activity in male flies than (Z)-9-tricosene alone. However, this could not be confirmed by Carlson *et al.* (1974) and Richter (1974). To find out the exact effects of muscalure and (Z)-9-heneicosene, the behaviour of both male and female flies of different strains has to be studied in the presence of different doses of these chemicals.

### **Acknowledgements**

We thank Dr J. B. Jespersen of the Danish Pest Infestation Laboratory, Lyngby (Denmark), for providing us with pupae of the WHO Ij2 strain.

This research was supported by the Technology Foundation (STW).