RIJKSUNIVERSITEIT GRONINGEN

Pheromones of the housefly

A chemical and behavioural study

Proefschrift

ter verkrijging van het doctoraat in de
Wiskunde en Natuurwetenschappen
aan de Rijksuniversiteit Groningen
op gezag van de
Rector Magnificus, dr D.F.J. Bosscher,
in het openbaar te verdedigen op
dinsdag 26 juni 2001
om 13.15 uur

door

NICOLAAS NOORMAN
geboren op 3 juni 1949
te Groningen

Promotores: Prof. dr D.G. Stavenga

Prof. dr J.M. Koolhaas

Referent: Dr C.J. den Otter

Beoordelingscommissie:

Prof. dr S. Daan

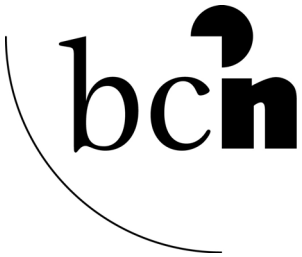
Prof. dr W. van Delden

Prof. dr K-E. Kaissling

ISBN: 90-367-1440-0

“Will you walk into my parlor?” said the spider to the fly;
“‘Tis the prettiest little parlor that ever you did spy.
The way into my parlor is up a winding stair,
And I’ve many curious things to show when you are there.”

From “ The Spider and the Fly ”
by Mary Howitt (1789-1888)



Financial support by SYNTECH, Hilversum, the Netherlands, and by DENKA International, Barneveld, the Netherlands, for the publication of this thesis is gratefully acknowledged.

The research reported in this thesis was performed at the Department of Animal Physiology of the University of Groningen, the Netherlands. This study was funded by the Technology Foundation (grant GBI33.2997) of the Netherlands Organization for Scientific Research.

Cover : Nico Noorman and Dick Visser

Printed by Van Denderen, Groningen

Contents

Chapter 1

GENERAL INTRODUCTION

Introduction

Chemicals play an important role in communication between insects. Chemicals that mediate interactions between organisms (inter- or intraspecific) are called semiochemicals. They can be divided into two major groups: allelochemics and pheromones. Allelochemics are chemicals that are secreted by an organism and cause a reaction in a receiving organism of a different species. Pheromones are substances secreted by an organism that cause a specific reaction in a receiving organism of the same species. Pheromones can be classified on the basis of the kind of behaviour they evoke, such as sexual, oviposition, aggregation, dispersion or alarm behaviour (Hendrikse, 1990).

Since Butenandt *et al.* (1959) identified trans-10, cis-12-hexadecadienol (bombykol) as the female sex pheromone of the silkworm moth *Bombyx mori*, sex pheromones of hundreds of insect species have been identified.

In Diptera, pheromones are often alkenes with a double bond Z-configuration at an odd position. For example, the female sex pheromones of *Drosophila melanogaster*, *Fannia canicularis*, *F. femoralis* and *F. pusio* are (Z,Z)-7,11-heptacosadiene (Antony and Jallon, 1982), Z)-9-pentacosene, (Z)-11-hentriacosene and (Z)-11-hentriacosene (Uebel *et al.*, 1977, 1978) respectively. In *Musca autumnalis*, however, apart from (Z)-13-nonacosene and (Z)-13-heptacosene also (Z)-14-nonacosene with the double bond at an even position is a component of the sex pheromone (Uebel *et al.*, 1975). It became apparent that these sex pheromones could play an important role in the control of insects.

This thesis focuses on the role semiochemicals play in the behaviour of the

housefly *Musca domestica* L. Differences are described between the cuticular hydrocarbon composition of laboratory and wild-type strains. The reasons for these differences are investigated.

The research is part of a project funded by the Technology Foundation of the Netherlands Organization for Scientific Research called "Environmentally friendly control of houseflies using combined visual and chemical stimuli".

Biology of the housefly

Musca domestica L. (Diptera: Muscidae), the common housefly, is one of the most widespread fly species in the world. The insects belong to a group of domestic flies often called "filth flies". They can be found at almost every place where people live. They have adapted their lifestyle to the human lifestyle by using waste products of human communities to live on and breed in. They can breed in animal faeces, garbage, rotting fruits and vegetables, and in other decomposing organic material. In addition, everything people eat seems to be interesting to houseflies as well. Consequently, houseflies are a nuisance in human and livestock habitations (West, 1951). Moreover, they may be responsible for the transmission of over 100 different pathogens (Pospischil, 1994). They may transmit intestinal worms, or their eggs, and are potential vectors of pathogens of dysentery, gastroenteritis, typhoid, cholera, foot and mouth disease and tuberculosis.

Losses caused by *M. domestica* in poultry houses were reported to be in excess of 60 million US dollars per year in the United States (Anonymous, 1976). High population densities of *M. domestica* in poultry units may not only cause irritation and annoyance to employees but may also considerably reduce egg production (Miller *et al.*, 1993).

The adult fly is about 6-8 mm long. It has a single pair of membranous wings. The hind wings have been modified to balancing organs, the halteres. The fly's thorax is grey with 4 longitudinal dark stripes. Prominent parts of the head are the non-biting suctorial mouth parts (proboscis) and large compound eyes. The female is usually bigger and has more space between the eyes than the male.

Houseflies are sexually mature 2-3 days after emergence. Females only mate once, whereas males try to mate with several females. A female lays 5-6 batches of about 90 eggs during her lifetime. The eggs hatch 12-24 hours after oviposition. The larvae (maggots), which are yellowish white, reach a length of 8-11 mm in about a

week, the ultimate size depending on the quantity and quality of the food substrate. Then, the larvae pupate and after 7-10 days the flies emerge from the pupae. At 25 °C, the entire development from egg to adult is completed in 14-18 days. As a consequence, several generations can occur during the period of one summer. In our winter houseflies can hibernate as pupae or adults. However, in (sub)tropical countries and in warm environments houseflies remain active and reproduce throughout the year. Organic waste materials and the relatively high temperatures at livestock farms promote rapid development and the continuous presence of flies (Howard and Wall, 1996).

Pheromones of houseflies

The presence of a sex pheromone on female houseflies which induces courtship behaviour in males was first reported by Rogoff *et al.* (1964). Studies of Carlson *et al.* (1971) led to the conclusion that this pheromone consists of (Z)-9-tricosene ("muscalure"). Since then, many scientists concentrated their research on several aspects of (Z)-9-tricosene, including its biosynthesis and behavioural functions. Dillwith and Blomquist (1982) and Dillwith *et al.* (1986) studied the biosynthesis of (Z)-9-tricosene. Using isolated tissue and radio tracer techniques they demonstrated that the female sex pheromone is synthesized by epidermal tissue. (Z)-9-tricosene formation occurs by elongation of oleoyl-CoA to a 24 carbon fatty acyl moiety, which is then converted to an alkene which is one carbon shorter (Blomquist *et al.*, 1993). The highest elongation activity is in the abdominal epidermal tissue (Vaz *et al.*, 1989).

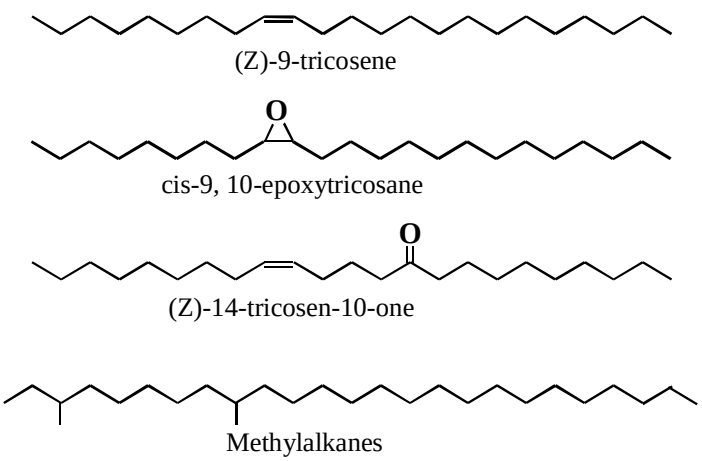


Figure 1. Components of the sex pheromone of the female housefly.

Several oxidation products of (Z)-9-tricosene, such as (Z)-9,10 epoxytricosane and (Z)-14-tricosene-10-one and some methylalkanes were also found on female houseflies (Fig. 1). These substances were shown to enhance male sexual activity in combination with (Z)-9-tricosene (Uebel et al., 1978; Rogoff et al., 1980).

Control of houseflies

Several techniques are used in an attempt to control the flies (reviewed by Howard and Wall, 1996).

Chemical control

Since the forties chlorinated hydrocarbons and organophosphates were used for many years as insecticides. These insecticides were applied to surfaces at which flies prefer to rest. DDT and Lindane, for instance, appeared to be highly effective against adults and larvae of *M. domestica* and other fly species (Lindquist *et al.*, 1945; Tanada *et al.*, 1950). However, these pesticides are toxic to a large spectrum of animal species, also killing non-target organisms. In addition, these substances cannot be metabolized by the organisms and residuals of pesticides persisted in the environment, entered the food chains and accumulated in the body tissue of non-target organisms, including humans (Lancaster and Simco, 1969; Pimental and Perkins, 1980). A further problem is the development of resistance to the killing power of the insecticides (Pospischill *et al.*, 1996). Since a limited number of genetic factors are involved in the development of resistance, which are not strictly bound to specific molecules, cross-resistance to novel insecticides exists (Plapp, 1986). In 1985, Chapman reported that a strain of *M. domestica* collected from a farm in England was resistant to 18 toxicants. Nowadays synthetic pyrethroids are used as insecticides that are rather safe for mammals, although they may kill crustaceans and fish (Eliot *et al.*, 1978; Hill, 1985). Moreover, several of these substances are biologically degradable.

In the last decades, the search for chemicals other than insecticides has increased. Insect growth regulators (IGRs) have been developed that do not directly act on the nervous system as conventional insecticides do, but principally affect embryonic, larval and nymph development and thus disrupt metamorphosis and reproduction (Howard and Wall , 1996). These so-called third generation pesticides do not usually kill the target pest immediately. Using these substances it takes longer to reduce insect populations than with nerve insecticides. IGRs show some selectivity

(Myamoto *et al.*, 1993) and can be divided into three categories: juvenile hormones, chitin synthesis inhibitors and 'others' (Howard and Wall, 1996). Widespread resistance against IGRs, however, also develops (Silhacek *et al.*, 1976; Pap and Farkas, 1994).

Biological control

Besides insecticides and IGRs considerable attention has been given to biological control of flies. Especially in livestock units predators, parasitoids and parasites may be used to control fly populations. Renn (1995) studied the mortality of eggs and larvae of houseflies in artificial diet and chicken manure after exposure to encapsulated entomopathogenic nematodes (*Steinernema felitae* and *Heterorhabditis megidis*). It appeared that slow release of the nematodes to the manure may control housefly infestations. However, further investigations are required to determine the optimum application of such a formulation. Renn (1998) also compared the efficacy of the same nematodes with that of the carbamate insecticide methomyl in pig units and showed that nematode baits offer a practical and effective alternative to conventional insecticides for the control of housefly populations in intensive animal units. The nematode *Paraiotonchium muscadomesticae* appears to have considerable promise as biological control agent for houseflies. The fly larvae are highly susceptible to infection, resulting in either their death or parasitic castration of the adult fly (Geden, 1977).

Johnson *et al.* (1998) reported the use of *Bacillus thuringiensis* as a safe and effective way for controlling agricultural pests and especially houseflies. The δ-endotoxins responsible for the activity of the bacteria are members of the so-called Cry IB class of protoxins. These are produced in a few strains of *B. thuringiensis* only.

King (1997) investigated the effectiveness of the parasitoid wasps *Spalangia cameroni* and *Muscidifurax raptor* in controlling fly populations. *S. cameroni* alone appeared to be consistently more effective in killing fly pupae than *M. raptor* alone or than the 2 species combined, regardless of fly age and burial (pupae uncovered or covered with 2 cm of larval medium). Greene *et al.* (1998) reported about mass-released *Spalangia nigroaenea,* which attacks the pupae, for biological control of *M. domestica* in western Kansas. The parasitoid-induced mortality (PIM) varied from 23 to 58 % depending on the parasitoid-to-host ratio.

Watson *et al.* (1996) used the entomopathogenic fungi *Beauveria bassiana* and *Entomophthora muscae*, concurrently with sawdust bedding, to control the housefly in calf hutches on New York dairy farms. Combined mycoses from both fungi infected 60 and 54% of the fly population at two farms respectively. Kuramoto and Shimazu (1997) introduced a small number of flies infected with *E. muscae* in experimental poultry houses and found that 90% of the initially present flies were killed 33 days after introduction. After introduction of conidia-discharching fly cadavers 90% of the flies were killed within 20 days.

Predator flies may also function in the natural control of fly pests. Farkas and Jantnyik (1992) studied the role of *Hydrotaea aenescens* (*Ophyra aenescens*) as predator of housefly larvae. Second and third instar *Hydrotaea* larvae showed to be effective in an experimental setup. One *Hydrotaea* larva is capable of destroying at least 5 *Musca* larvae during its larval development. Tsankova and Luvchiev (1993) and Luvchiev and Tsankova (1994) reported that second and third instar larvae of *Ophyra capensis* can kill up to 17 housefly larvae depending on the larval instar and culture density. Betke *et al.* (1989) used *O. aenescens* for the control of *M. domestica* in pig fattening units. The authors stated that introduction of laboratory-raised *O. aenescens* resulted in definite elimination of the *M. domestica* stable population. *O. capensis* and *O. aenescens* can, however, become pests themselves (Axtell and Arends, 1990).

Mullens *et al.* (1996) studied the effect of *Machrocheles* mites and predacious Coleoptera on the presence of *M. domestica* and *Fannia* species. On 3 southern California caged-layer poultry facilities two manure-handling systems were compared during 2 years: all manure rows were removed, or half of the manure was left undisturbed to conserve a part of the predator population. The authors concluded that the slight increase in fly control as a result of alternate manure removal is overshadowed by the required time and effort involved.

Sterilization

The release of sterilized male flies to control fly populations (sterile insect technique; SIT) into a wild population may drive the wild population to extinction and was succesfully applied in Lybia to eradicate the New World screwworm fly, *Cochliomyia homonivora* (Lindquist *et al.*, 1992).

In general, however, the use of SIT is limited by its expense and logistic complexity and, as is the case with houseflies, the release of huge numbers of the pest around human dwellings, although sterile, would exacerbate the nuisance problem at least for a short while. In addition, sterilized males must be sexually competitive with the naturally occurring males (Howard and Wall, 1996).

Howard and Wall (1996a,b) used the chitin synthesis inhibitor triflumuron for autosterilization of the house fly. Sugar-baited targets with this chemical can be applied to reduce fly population in conjunction with the release of insect predators or parasitiods.

Light traps

Many insects are sensitive to UV light with a wavelength of approximately 350 nm. Deay and Taylor (1962) showed that wavelengths between 320-380 nm were most attractive to *M. domestica*. However, Burkhard (1962) and McCann and Arnett (1972) found that there were two peaks in the visual systems of houseflies one of 350 nm and the other around 500 nm. Bellingham and Anderson (1993) even found three spectral peaks flies at 350, 450-550 and 630 nm.

Nowadays light traps (lamps emitting attractive wavelengths) in combination with electric grids which kill the flies) are commonly used for capturing flies. Morgan and Pickens (1968) tested several types of lamps with spectra between 310 and 720 nm at temperatures between 19 and 32 °C for their attractiveness to houseflies. Males were shown to be most responsive to green and orange light at lower temperatures. Females responded best to green, blue and ultraviolet light at 32 °C. According to Syms and Goodman (1987) flickering UV light (100 or 120 Hz) is more attractive to *M. domestica* than non-flickering light. Rutz *et al.* (1988) evaluated the effectiveness of insect-electrocutor black light devices in cage-layer poultry facilities. The addition of (Z)-9-tricosene (25 to 100 mg/device) increased the total number of flies caught by about 30%. The authors concluded that these devices, particularly when operated with (Z)-9-tricosene, could be an effective component in an integrated fly management programme in poultry facilities. Veal *et al.* (1995) carried out experiments to compare the efficiency of electrocuting traps in which a green and ultraviolet lamp were combined against traps containing lamps emitting blue and ultraviolet. They found that the green + ultraviolet lamp caught 30% more houseflies than the blue + ultraviolet lamp. However, the authors also concluded that it is hardly possible to

predict the effectiveness of electrocuting traps because many different factors are involved, such as design and siting of the traps, and light wavelengths and brightness.

The role of (Z)-9-tricosene (muscalure) in controlling *Musca domestica*.

Laboratory studies

Rogoff *et al.* (1964) first demonstrated the presence of a sex pheromone on female houseflies. Olfactometers baited with live females or frozen females attracted significantly more males than olfactometers without females or baited with males. Both males and females did not attract females. Female-extract-impregnated pseudo flies (a piece of shoelace) caused sexual excitation in male flies. These results were confirmed by Murvosh *et al.* (1965) and Mayer and Thaggard (1966). The latter authors showed that dead females remained attractive for 8 days.

Carlson *et al.* (1971) were able to isolate, identify and synthesize the female pheromone. This substance, (Z)-9-tricosene, was called muscalure. The attractant was obtained from sexually mature, laboratory-reared, female houseflies by surface washing with hexane or ether. A 50 μg sample of the synthetic (Z) isomer attracted more flies than 200 μg of the (E) isomer. Other cuticular monoolefins (C27 and C29) were weakly active. The authors conclude: "Though not a potent attractant as compared to some sex pheromones, (Z)-9-tricosene is expected to be inexpensive to manufacture and it may have good potential for reducing the amount of insecticide needed to control the ubiquitous housefly."

Mansingh *et al.* (1972) investigated the effects of a series of (Z)-9-alkenes with 19-25 carbon atoms on the behaviour of male houseflies. It appeared that a large number of these substances showed biological activity. The most potent was a 7:3 mixture of (Z)-9-tricosene and (Z)-9-heneicosene, which induced and maintained high excitement and mating behaviour in most male flies. However, Richter (1974) found that moving dummies loaded with (Z)-9-heneicosene or a mixture of (Z)-9-heneicosene and (Z)-9-tricosene did not induce more mating strikes in males than unloaded moving dummies.

Carlson *et al.* (1974) determined the effects of structural changes of the (Z)-9-tricosene molecule on the activity of male flies in olfactometers. Structural changes included variations in carbon chain length and in the position of the double bond, substitution of double bond by triple bond, cis-trans isomerism and methyl

branching at different points in the molecule. Compounds with double bonds at the 7 and 11 positions and those with a trans configuration rated poorly. The length of the longer chain next to the unsaturated bond seemed to be crucial. The most active compounds contained in their longer chains mostly C14 and some C13 groups. (Z)-9-heneicosene, having a C12 chain, showed low activity. Methyl branching at the second position on the short chain elicited high activity. The finding of Mansingh *et al.* (1972) that a 7:3 mixture of (Z)-9-tricosene and (Z)-9-heneicosene increased courtship behaviour in males could not be confirmed.

In contrast to Carlson *et al.* (1974), Uebel *et al.* (1976) observed little response in male flies to pseudo-flies when these were loaded with (Z)-9-tricosene alone. Unfractionated cuticular hydrocarbon fractions washed from females induced much higher activity (mating strikes) in males. Washings from males were ineffective. Saturated and unsaturated female cuticular hydrocarbons tested separately elicited low responses. However, these substances were combined at a ratio of 65%:35% saturated:unsaturated, induced male activity. When methylheptacosanes and methylnonacosenes were combined with (Z)-9-tricosene male activity strongly increased compared to (Z)-9-tricosene alone. The two compounds producing the highest activity in combination with (Z)-9-tricosene were 4,8-dimethylheptacosene and 13-methylnonacosene.

Rogoff *et al.* (1980) confirmed the findings of Uebel *et al.* (1976) that the several synthetics tested, being less active than (Z)-9-tricosene when tested alone, markedly increased male sexual activity when combined with (Z)-9-tricosene. However, they found that this also occurred without (Z)-9-tricosene when three of the materials were mixed together. They conclude that: “It appears that what has been referred to as “the” housefly sex pheromone may actually be a complex mixture of materials.”

Blomquist *et al.* (1984) showed that on all bodyparts of both male and female houseflies (Z)-9-tricosene is metabolized to an epoxide and ketone. Adams and Holt (1987) found that these substances had different roles in male courtship behaviour. (Z)-9-tricosene increased male mating strike activity towards females and other males. The non-hydrocarbon fraction including both the epoxide and the keton decreased the number of homosexual mating strikes when (Z)-9-tricosene was present. The authors thus concluded that the non-hydrocarbon fraction contained sex recognition factors. Both the methylalkanes and the non-hydrocarbon fraction increased the number of

copulatory attempts made by males. It was concluded that the methylalkane fraction acted as an arrestant and increased the amount of time spent with a treated model.

La-France *et al.* (1989) confirmed that (Z)-9-tricosene is the main active component of the sex pheromone produced by the female housefly, initiating striking activity in males. The (Z)-9-alkenes present in the cuticular lipid layer probably act synergistic to (Z)-9-tricosene. The n-alkanes and methylalkanes showed low to medium activity and the addition of (Z)-9-tricosene did not enhance it.

Field studies

Carlson and Beroza (1973) carried out a study on the use of (Z)-9-tricosene as an attractant for *M. domestica* in the field. Panels containing adhesive paper strips and sugar bait in pans and electric grids were used as traps. The addition of (Z)-9-tricosene increased the number of flies caught 3 to 12 times. (Z)-9-tricosene-baited traps caught about equal numbers of males and females. This was in contrast to olfactometer studies in the laboratory in which only males were attracted to (Z)-9-tricosene.

In experiments in open poultry houses, Mitchell *et al.* (1975) showed that (Z)-9-tricosene-baited traps caught 2-14 times more flies than unbaited traps. The traps were most effective when situated on the ground adjacent to manure. The sex ratios of the flies caught were the same in baited and unbaited traps. Traps containing both (Z)-9-tricosene and toxic substances (pheromone-toxicant devices; PTD's) were evaluated in the field for control of the housefly by Carlson and Leibold (1981). Traps loaded with both permethrin and (Z)-9-tricosene were used, and their catches were compared with those of traps containing permethrin only. It appeared that the (Z)-9-tricosene treated devices captured 1.4 to 2.0 more flies than the untreated controls. However, the pheromone apparently attracted flies from surrounding areas in such numbers that the flies were not effectively controlled by the toxicant. All (Z)-9-tricosene in the traps had disappeared within 2 to 3 weeks and all permethrin around 60 days after the start of the experiment. Chapman *et al.* (1998) carried out field trials comparing the effectiveness of toxic targets impregnated with different formulations of (Z)-9-tricosene. Targets baited with (Z)-9-tricosene caught significantly larger numbers of males and females of *M. domestica* than control targets. The introduction of these toxic targets suppressed the density of adult *M. domestica* populations up to 13 weeks.

General conclusions

Control of houseflies requires an integrated pest management approach, considering all available pest control tactics and evaluating the potential interaction among them (Axtell and Arends, 1990). In stables the manure should be kept as dry as possible. The manure should also not be totally removed in a brief span of time. Some old manure should be left to conserve fly predators and parasites. Chemical techniques can be used as a supplement to cultural and biological methods. However, one should exercise due care in applying insecticides. The use of insecticides should be limited to small areas of manure containing high numbers of fly larvae, because most insecticides are toxic to predators and parasites. In addition, there is the risk of the development of resistance to insecticides, even against the degradable pyrethroids and specific IGRs. Finally, a good monitoring system to evaluate re-invasion and to depict the moment of control measures as well as fly dispersal should be incorporated in integrated pest management (IPM).

The role of semiochemicals and particularly (Z)-9-tricosene in the behaviour of houseflies is not unambiguous. Moreover, behavioural studies have almost exclusively been carried out on flies of laboratory cultures. Therefore, if semiochemicals are used for controlling houseflies, one should also focus on possible differences in behaviour between laboratory and wild-type strains.

Outline of the thesis

The present work is part of the project "Environmentally friendly control of houseflies using combined chemical and visual stimuli" which comprises behavioural studies on the responses of *Musca domestica* to different light sources and olfactory stimuli, electrophysiological studies on the responses of olfactory cells of *M. domestica* to natural and synthetic volatiles, and studies on the production of cuticular hydrocarbons by *M. domestica* and the role of these in the behaviour of the flies. The work reported in this thesis focuses on the latter part.

Almost all knowledge on the production of cuticular hydrocarbons and on their role in the behaviour of houseflies has been collected from laboratory strains of houseflies. However, in order to be able to control the flies in their natural environments, studies on wild-type flies are also necessary. In this thesis attention is paid to houseflies which had been kept in culture in the laboratory for 40 years (WHO strain of flies) and flies obtained from a poultry breeding (Van Diermen strain) and a

cow-house with pig-sty (Pesse strain). The latter two strains have been cultured in the laboratory for several generations.

In Chapter 2 gas chromatographical studies are described which showed striking differences between the cuticular hydrocarbon composition of females of the WHO strain and females of the Van Diermen and Pesse strains. On WHO females hydrocarbons with 23-25 C atoms constituted about 65% of the total hydrocarbons, whereas on first-generation laboratory wild-type females these compounds made up less than 2 % of the total amount. (Z)-9-tricosene ('muscalure'), the alleged sex pheromone of the female housefly, comprised up to 20-30% of the total hydrocarbons on 5-20-day-old WHO females, whereas less than 0.5% of the total hydrocarbons on the wild-type femalesconsisted of muscalure. Furthermore, it appeared that on the wild-type strains the amounts of muscalure had increased considerably after some tens of generations in the laboratory.

In Chapter 3 we investigated whether these differences in muscalure quantities were reflected in the sexual activity of the males. We found that sexual activity of males of all three strains 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. EAG recordings indicated that both males and females of all three strains were able to perceive (Z)-9-tricosene, which suggested that differences in sexual behaviour were not due to differences in ability to smell these substances.

The next step was to find out whether environmental circumstances like temperature, humidity and population density affected the hydrocarbon composition on the cuticle of the flies with special attention to the production of (Z)-9-tricosene. The results of this study are presented in Chapter 4. Male and female flies produced more hydrocarbons at 35 °C than at 20 °C. However, no indication was found that the relative humidity had a disinct effect on the production of (Z)-9-tricosene by females than on the production of the other hydrocarbons. On females the relative amounts of nonacosane, and methyl- and dimethylnonacosanes were significantly higher at 35 °C than at 20 °C. Female flies produced some (Z)-9-tricosene after 8 generations at low population density, in contrast to females at high population density which did not produce muscalure.

In Chapter 5 a new technique is introduced to apply semiochemicals on test flies in a more natural way than is commonly used. It appeared that hydrocarbons were

taken up by flies walking on a filter paper onto which the pure chemicals had been pipetted. In this way, the substances were distributed in a more natural way over the body than on flies onto which the chemicals, solved in hexane, had been pipetted. Using this new 'self-loading' technique it appeared that (Z)-9-heptacosene stimulated copulation when present in relatively high amounts on females, whereas (Z)-9-pentacosene did not affect male sexual behaviour.

Another new technique is proposed in Chapter 6. A radar-Doppler actometer is described which allows the recordings of movements of individual body parts of the flies. It is shown that head movements of the fly can be used as a behavioural detection mechanism for semiochemicals. Although flies can smell both (Z)-9-heneicosene and (Z)-9-tricosene as shown by EAG studies, head movement reactions to these chemicals, however, occur to (Z)-9-tricosene but not to (Z)-9-heneicosene, which is in accordance with male sexual responses to these substances. Comparison of our results with those of field experiments described in the literature lead us to suggest that (Z)-9-tricosene may function as an aggregation pheromone bringing males and females together and as a female sex pheromone inducing mating behaviour in males.

Finally in Chapter 7 we present strong indications that an oviposition pheromone is deposited together with the eggs. This pheromone appears to disappear within a short period of time after oviposition either because it is very volatile or disintegrates.

Chapter 2

THE EFFECTS OF LABORATORY CULTURING ON MUSCALURE QUANTITIES ON FEMALE HOUSEFLIES

Abstract

Using gas chromatography the relative amounts of (Z)-9-tricosene (muscalure) and some other hydrocarbons on the cuticle of 1-20-day-old houseflies (*Musca domestica* L.) from different strains were determined. Flies from a WHO strain, in culture since 1961, and first-generation laboratory-cultured flies from two wild-type strains from a poultry breeding and a cow-house with pigsty, respectively, were compared.

On WHO females hydrocarbons with 23-25 C atoms constituted about 65% of the total hydrocarbons, whereas on wild-type females less than 2 % of these compounds was present. Muscalure comprised up to 20-30% of the total hydrocarbons on 5-20-day-old WHO females, whereas less than 0.5% muscalure was present on the wild-type females.

It is suggested that in mixed populations (both sexes together in a cage) in the course of time muscalure is transferred from females to males and (Z)-9-heptacosene from males to females.

We also compared the amounts of muscalure and some other hydrocarbons on female houseflies, kept in culture in the laboratory for several generations. It appeared that whereas on first-generation wild-type females hardly or no muscalure could be detected, the amounts of this substance had increased considerably after some tens of generations in the laboratory. It is suggested that this was due to selection in subsequent generations of high-density populations. Production of (Z)-9-tricosene and of tricosane was shown to be closely linked. Selection did not affect the production of other cuticular hydrocarbons by the females.

It is concluded that reproduction ability of houseflies does not primarily depend on the amounts of (Z)-9-tricosene on females, although higher amounts of this substance may increase contacts between males and females.

Introduction

The cuticular hydrocarbons of insects provide a barrier to water diffusion (Wigglesworth, 1945; Beament, 1945; Gibbs *et al.*, 1991; Gibbs, 1995) and also play an important part in mate selection by means of chemical communication (Silhacek *et al.*, 1972; Nelson, 1978; Howard & Blomquist, 1982; Blomquist *et al.*, 1987).

Rogoff *et al.* (1964) showed that on the cuticle of female houseflies, *M. domestica*, chemicals are present which influence the behaviour of male houseflies. Since the identification of (Z)-9-tricosene (muscalure) by Carlson *et al.* (1971) and the confirmation that this substance is a part of the female sex pheromone of the house fly by Rogoff *et al.* (1973), many studies have been carried out to reveal the precise effect of the various female cuticular chemicals on the sexual activity of male flies. In 1976 and 1978, Uebel *et al.* showed that cuticular methyl alkanes and the non-hydrocarbon oxidation products of muscalure, (Z)-9,10 epoxytricosene and (Z)-14-tricosen-10-one, also play a role in inducing sexual activity of males towards females. Adams and Holt (1987) found that the cuticular non-hydrocarbon fraction contains sex recognition factors and that the methyl alkane fraction increases contact time between males and females and, thus, has an arresting effect; the latter fraction does not induce mating striking behaviour in males.

Nelson *et al.* (1981) determined the composition of the cuticular hydrocarbon fraction on both females and males and found three groups of long-chain hydrocarbons: (Z)-9-alkenes, *n*-alkanes and methyl alkanes. Methyl alkanes were more abundant in females than in males. Of the total hydrocarbons present on males and females about 97% were components with 23-31 carbon atoms. (Z)-9-tricosene was only present on females, whereas (Z)-9-heptacosene comprised about fifty percent of the total hydrocarbons on adult males and about three percent on females. Nowadays it is widely accepted that the substance inducing sexual behaviour in male *M. domestica* consists of several components, although (Z)-9-tricosene is believed to be the major component of this sex pheromone.

The above studies were all done with flies which had been kept in culture for several years. However, studies of Adler *et al.* (1984) on North American houseflies have shown that 4-11 times less muscalure is present on 10-day-old females than on laboratory-reared females of the same age. It is conceivable that in laboratory-cultured

houseflies and in flies living in more or less isolated environments (e.g. stables) production of cuticular hydrocarbons and sexual behaviour may have changed by genetic drift. In addition, it is known that the production of cuticular hydrocarbons in insects may be affected by environmental factors (Gibbs *et al.*, 1991, Gibbs & Mousseau, 1994, Toolson *et al.*, 1990, Hadley, 1978). Laboratory conditions often differ considerably from environmental conditions in nature and thus may contribute to the differences in production of cuticular hydrocarbons. Thus, for controlling houseflies in, e.g., houses and stables using cuticular hydrocarbons as an attractant, possible differences between laboratory and wild-type strains of houseflies have to be studied.

The present paper reports results of studies on the amounts of hydrocarbons present on houseflies originating from different strains – a WHO laboratory strain and wild-type strains from different sites in The Netherlands - and of different sex and age. In addition, we compared the amounts of hydrocarbons on flies from strains which had been kept in culture for different numbers of generations. One of the main aims of these studies is to find out whether or not selection may play a role in (Z)-9-tricosene production and if and to what extent the production of this substance is related to that of other cuticular hydrocarbons.

Materials and methods

Insects

Experiments were done with *Musca domestica* L. flies from a laboratory strain (WHO Ij2) obtained from the Statens Skadedyrlaboratorium, Lyngby (Denmark), and from wild-type strains obtained from a poultry breeding (Van Diermen) and a cow-house with pig-sty (Pesse) in The Netherlands, respectively. In Experiment 1, flies from the WHO strain (in culture since 1961) and first-generation laboratory-cultured flies of the wild-types were used; in Experiment 2 flies from the wild-type strains were used which had been reared in the laboratory for 37, 25, 12 and 12 generations, respectively (‘Diermen 37', ‘Pesse 25', ‘Diermen 12' and ‘Pesse 12' strains). The flies were reared in cages (30 x 30 x 40 cm) in a L12:D12 regime at 25 ^{0}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.

Chemical analyses

In Experiment 1 cuticular hydrocarbons were determined on flies 1, 3, 5, 8, 12 and 20 days after emergence. We mainly concentrated on (Z)-9-tricosene, the major component of the female sex pheromone, and on (Z)-9-heptacosene, which is the most abundant hydrocarbon on males. The flies were taken from cages containing about 30 males and 30 females ('mixed populations'), and from cages containing either about 30 males or 30 females ('isolated populations'). In the latter cases each sex was kept separately from emergence.

Two samples were prepared each containing two flies of the same strain, sex and age in 0.4 ml hexane. Each sample was shaken during 1 min, after which the flies were kept in the fluid for at least 1 hour.

In Experiment 2 cuticular hydrocarbons were determined on single females. The flies were taken from cages containing about 30 males and 30 females. 10 female flies, 8 days old, from each strain were used for analysis. The weight of each fly was determined after which the flies were individually immersed in 0.2 ml hexane. Each preparation was shaken during 1 min, after which the fly was kept in the fluid for at least 1 hour.

Gas chromatography was performed on a Shimadzu GC 17A gas chromatograph. One µl of a solution was injected into a 10 m, 0.32 mm CP-Sil-5 CB column (Chrompack) with injector at 250 ^{0}C and FID at 300 ^{0}C. The flow rate of the helium carrier gas was approx. 1 ml/min. GC oven temperature was programmed from 50 to 300 ^{0}C at 10 ^{0}C/min. 2-Nonanone was used as an internal standard. In each sample hydrocarbons were identified by comparing the retention time with reference runs of alkanes and (Z)-9-alkenes, or with data from literature, and by mass spectrometry. Test runs with C24-C38 n-alkanes demonstrated that all these long-chain chemicals could readily be recovered using the above technique. The lower detection level of the individual hydrocarbons was in the order of 5 ng. The quantities of the hydrocarbons were expressed as percentages of the total quantity of hydrocarbons or in micrograms.

Results

Experiment 1: Figure 1 shows the relative amounts of cuticular (Z)-9-tricosene and

(Z)-9-heptacosene as a function of age for females and males from the isolated and mixed populations of the WHO laboratory strain, and the average amounts of (Z)-9-heptacosene on the Van Diermen and Pesse strains. The amounts of (Z)-9-tricosene on the latter two strains are not shown, since on males of these strains (Z)-9-tricosene could never be detected and on the females the amounts of this substance were very low or below detection level, and did not increase with age.

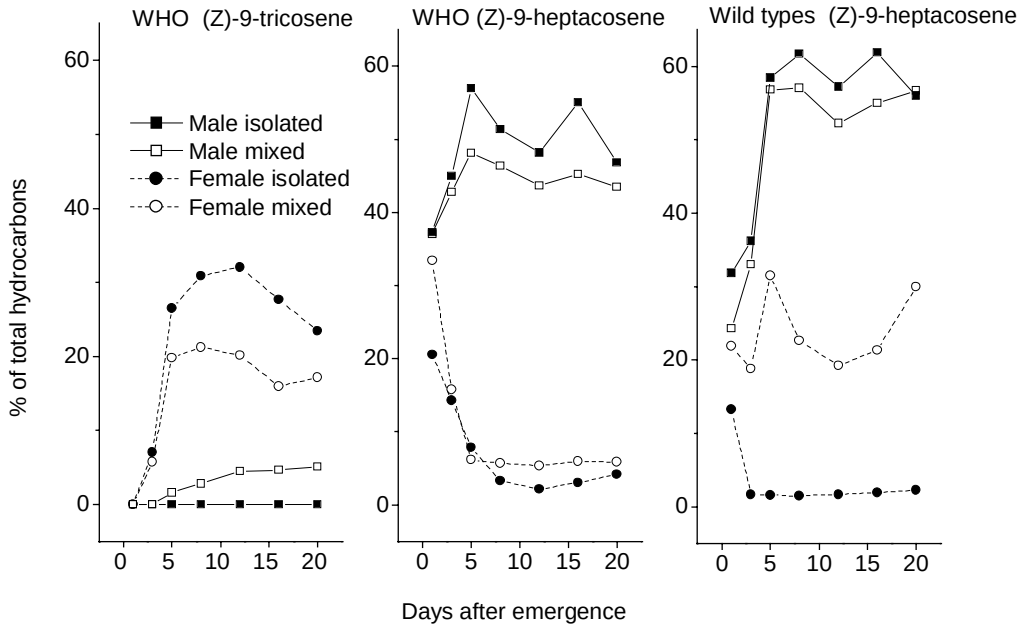


Figure 1. Quantities of (Z)-9-tricosene and (Z)-9-heptacosene as a percentage of the total cuticular hydrocarbons on isolated and mixed populations of the WHO and the wild type strains.

As appears from Fig. 1, on the cuticle of 1-day-old females of both WHO populations no muscalure was present. The relative amounts of (Z)-9-tricosene on females increased with age. On females from both the isolated and mixed populations the relative amount of (Z)-9-tricosene increased up to day 5, remained at about the same level up to day 12 and then decreased slightly. Interestingly, on females of the mixed population the relative amounts were lower than on those of the isolated population ($p < 0.03$; Wilcoxon signed ranks test; 2-tailed). Cuticular washes of males of the isolated WHO population never contained (Z)-9-tricosene. However, on males from the mixed population a small amount of (Z)-9-tricosene was found on day 5 after emergence, which gradually increased with age.

On 1-day-old males from both the mixed and isolated WHO populations relatively

large amounts of (Z)-9-heptacosene were present, which increased up to day 5. Beyond that day the relative quantities slightly decreased with age. (Z)-9-heptacosene was found in larger amounts on males of the isolated population than on males of the mixed population ($p < 0.02$; Wilcoxon signed ranks test; 2-tailed). One-day-old WHO females from both populations also contained relatively high amounts of (Z)-9-heptacosene. However, thereafter these amounts gradually decreased on both females of the mixed and the isolated populations up to day 8 and remained at a very low level up to day 20. On females from the mixed population (Z)-9-heptacosene was present in higher quantities than on females from the isolated population ($p < 0.03$; Wilcoxon signed ranks test; 2-tailed).

On 1-day-old males from both the mixed and isolated wild-type populations relatively large amounts of (Z)-9-heptacosene were present, which increased up to day 5. Beyond that day the relative quantity remained about the same up to day 20. (Z)-9-heptacosene was found in larger amounts on males of the isolated than on males of the mixed population ($p < 0.03$; Wilcoxon signed ranks test; 2-tailed). One-day-old wild-type females from both mixed and isolated populations also contained relatively high amounts of (Z)-9-heptacosene. On females from the mixed population the amounts of this substance remained on this relatively high level up to day 20. However on females of the isolated populations the amounts of (Z)-9-heptacosene were much lower on day 3 and remained at that low level up to day 20.

In Fig. 2 the GC traces of 8-day-old males and females from the three different strains are shown. The peaks of the substances with more than 29 C atoms are not considered because they comprised only about 2 to 3% of the total hydrocarbons. It is evident that compared to females of the WHO strain, the hydrocarbons with up to about 27 C atoms were underrepresented on females of both wild-type strains. (Z)-9-tricosene was even virtually absent on females of the wild-types. It is also clear that the amounts of pentacosene and (Z)-9-heptacosene on females of the three strains were higher in mixed than in isolated populations. No striking differences are seen between the amounts of the hydrocarbons on the males of the various strains. However, the figure shows that (Z)-9-tricosene was not present on WHO males of the isolated populations, but occurred -in small amounts- on males of the mixed populations.

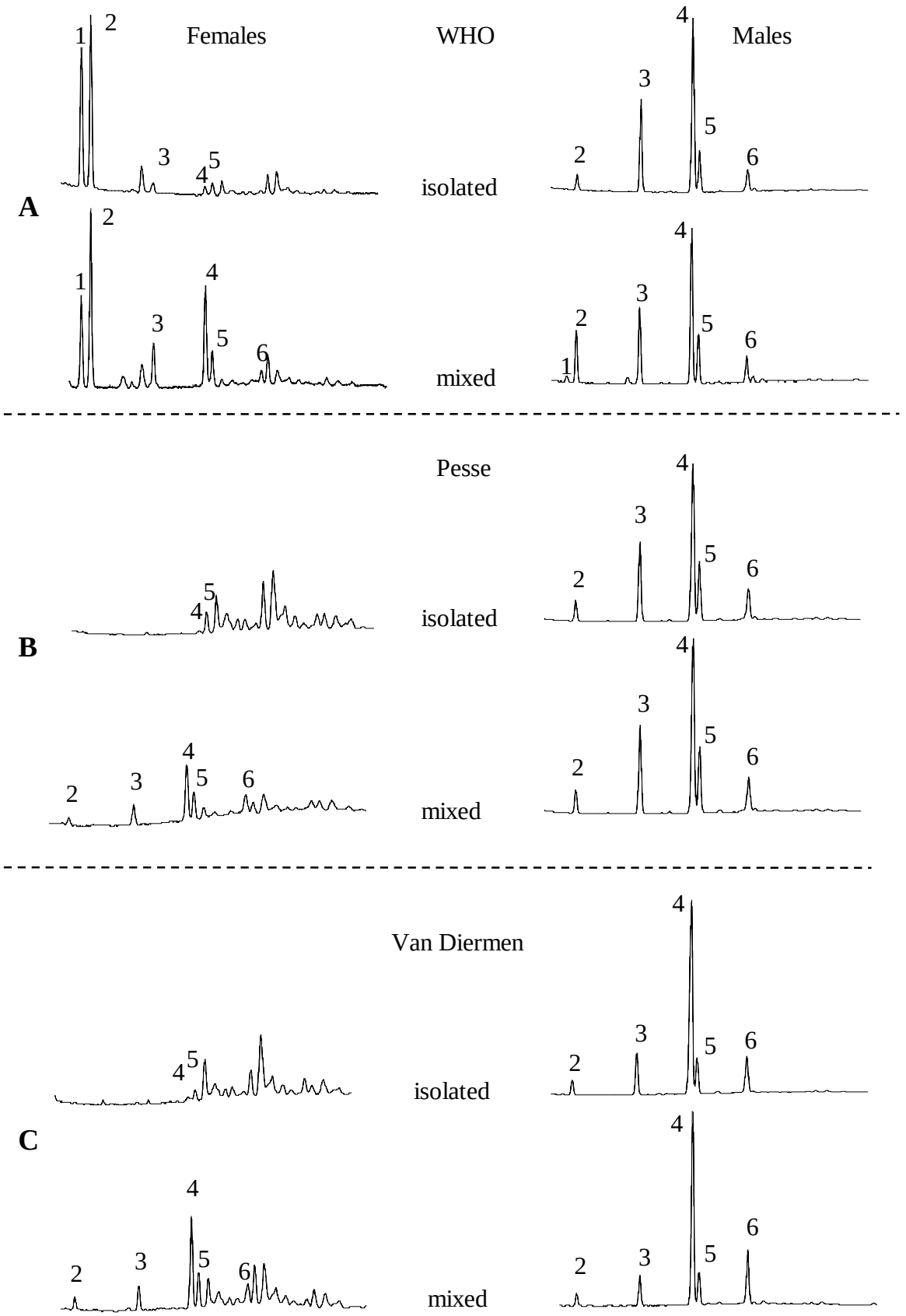


Figure 2. GC traces of the cuticular hydrocarbons of 6-day-old male and female *Musca domestica* from isolated and mixed populations of the 3 strains. A : WHO, B: Pesse, C: Van Diermen.
1: (Z)-9-tricosene, 2: tricosane, 3: pentacosane, 4: (Z)-9-heptacosene, 5: heptacosane, 6: (Z)-9-nonacosene.

Table 1. Relative amounts of a number of hydrocarbons on first-generation laboratory-cultured wild-type and on WHO 8-day-old male and female *M.domestica* from isolated populations.

	WHO		Pesse		Van Diermen	
	♂♂	♀♀	♂♂	♀♀	♂♂	♀♀
(Z)-9-tricosene	0.0	28.2	0.0	0.0	0.0	0.3
tricosane	4.0	34.0	4.9	0.0	3.3	0.0
pentacosane	24.3	2.8	20.6	0.0	10.9	0.8
(Z)-9-heptacosene	52.2	1.6	45.2	1.3	62.9	1.4
heptacosane	11.2	2.9	15.3	6.1	9.5	3.3
(Z)-9-nonacosene	7.5	1.1	9.4	1.3	10.6	0.9
nonacosane	0.9	4.0	0.8	14.3	0.4	8.3
methyl- and dimethylheptacosanes	<0.5	2.8	<0.5	12.6	<0.5	12.4
methyl- and dimethylnonacosanes	<0.5	5.6	<0.5	25.8	<0.5	25.7

In Table 1 the relative amounts of a number of alkanes and alkenes are shown which were found on 8-day-old males and females of the three strains. It appears that on females of the WHO strain the group of hydrocarbons with 23 up to 25 C atoms constituted 65 % of the total hydrocarbons, whereas on females of the wild-type strains less than 2 % of these compounds were present. On females of the wild-type strains nonacosane, and the methyl and dimethyl heptacosanes and nonacosanes were the most abundant chemicals. Males of both laboratory and wild-type strains contain only very small amounts of the methyl and dimethyl heptacosanes and nonacosanes. (Z)-9 heptacosene and pentacosane are the most abundant hydrocarbons on male flies. As in Fig. 2, the table does not show striking differences between the relative amounts of the various hydrocarbons on the bodies of males of the laboratory and wild-type strains.

Experiment 2: Figure 3 shows the relative quantities of muscalure on females from the different strains. It appears that females of the WHO strain had about 10 times more muscalure on their skin than females of the Pesse 12-, Van Diermen 37- and Van Diermen 12-generation strain (Mann-Whitney U test, $p<0.001$) and about 3.5 times more than the

females of the Pesse 25 strain (Mann-Whitney U test, $p<0.001$). The females of the Pesse 25 strain had produced significantly more muscalure than the Pesse 12 and the Diermen

12 and 37 strains (Mann-Whitney U test, $p < 0.05$). No significant differences in muscalure quantities occurred between the Pesse 12, and the Diermen 37 and 12 strains.

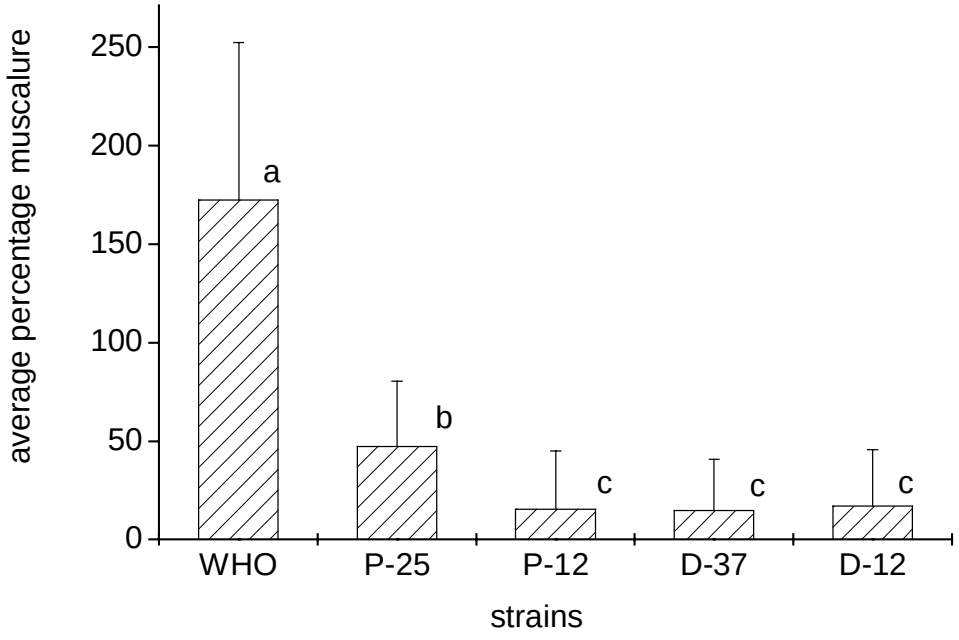


Figure 3. Average percentages (relative to internal standard) of muscalure on females of the WHO strain and the wild-type Pesse 25- and 12-, and Diermen 37- and 12-generation strains. Averages labeled with different letters differ significantly. n=10 for each strain.

In Fig. 4 the correlation coefficients (Pearson correlation test) of the quantities of (Z)-9-tricosene and those of other hydrocarbons on females of the wild-type strains (A) and of the WHO strain (B) are presented. It can be seen that on the wild-type females a high correlation only existed between the amounts of muscalure and tricosane. Quantities of the remaining hydrocarbons showed a low positive or even a negative correlation with muscalure. In the females of the WHO strain, however, the amount of muscalure was significantly correlated to the amounts of 14 out of 22 hydrocarbon GC peaks (Fig. 4B). Here again the correlation between (Z)-9-tricosene and tricosane was by far the highest. Figure 5 shows the relation between the amounts of (Z)-9-tricosene and tricosane on the 40 individual females of the wild-type (A) and the 10 females of the WHO laboratory strains (B).

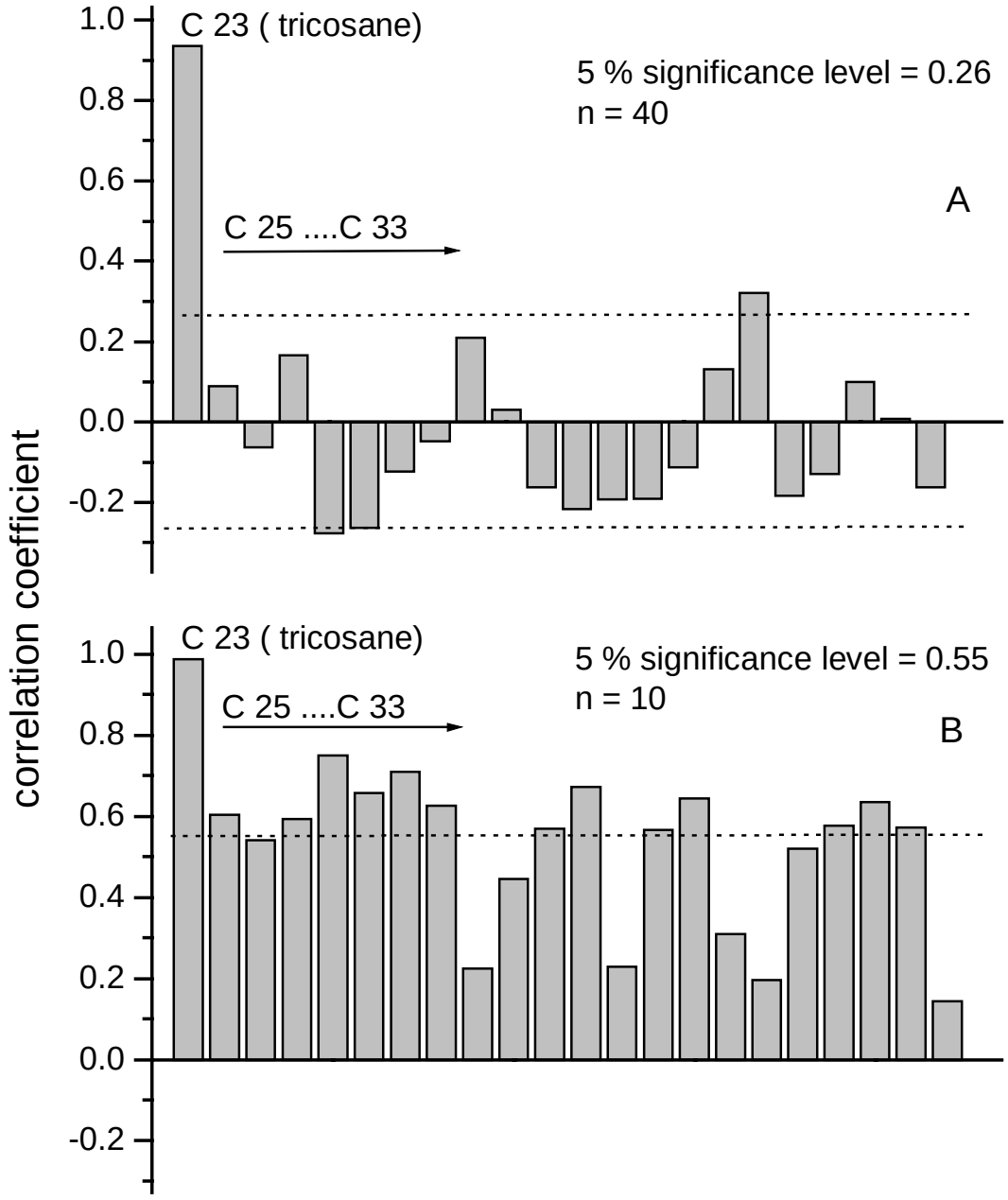


Figure 4. Correlation between (Z)-9-tricosene and other hydrocarbons on the cuticle of 8-day-old females of the wild-type strains (A) and of the WHO strain (B). n=10 for each strain.

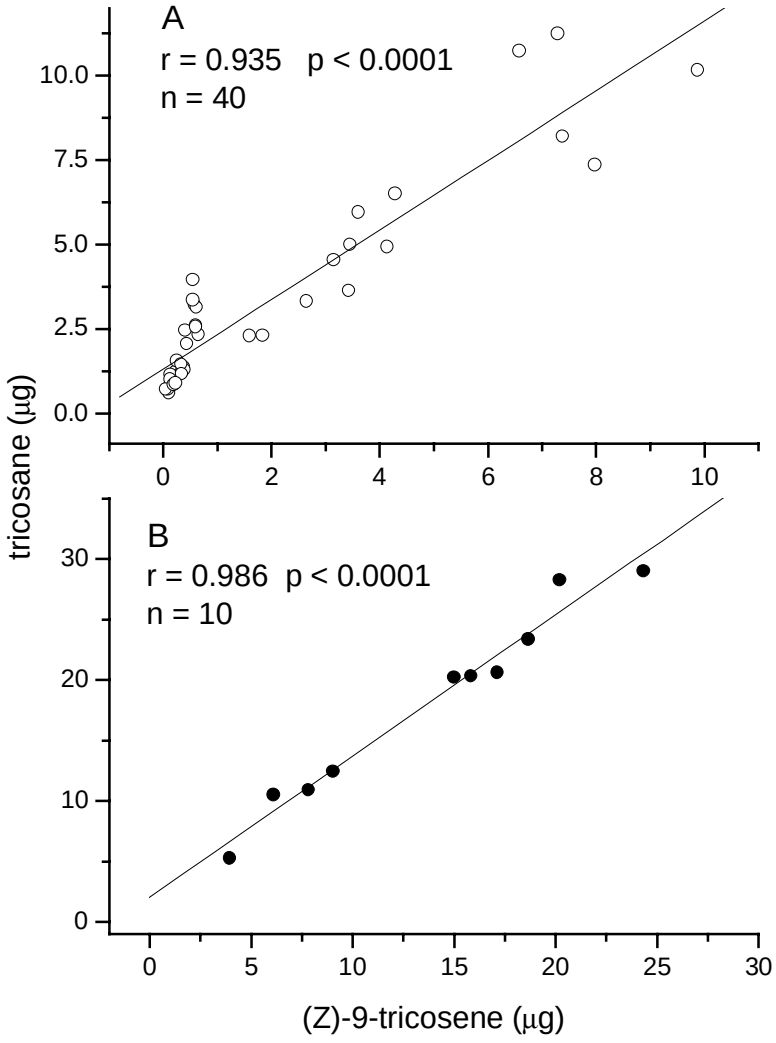


Figure 5. Relationship between the amounts of (Z)-9-tricosene and tricosane on individual females of the wild-type (A) and WHO strains (B).

In Fig. 6 the coefficients of variation (standard deviation as a percentage of the mean) of the hydrocarbons of females of every strain separately are presented. The variation in quantity of (Z)-9-tricosene and to a lesser extent of tricosane was high in Pesse 12 and Van Diermen 12 and 37, the strains in which muscalure production was low (Figure 3). In Pesse 25, in which muscalure production was three times higher, the variations in (Z)-9-tricosene and tricosane quantities were much lower and in the WHO strain these variations were even of the same low order of magnitude as those of the other hydrocarbons identified on the females.

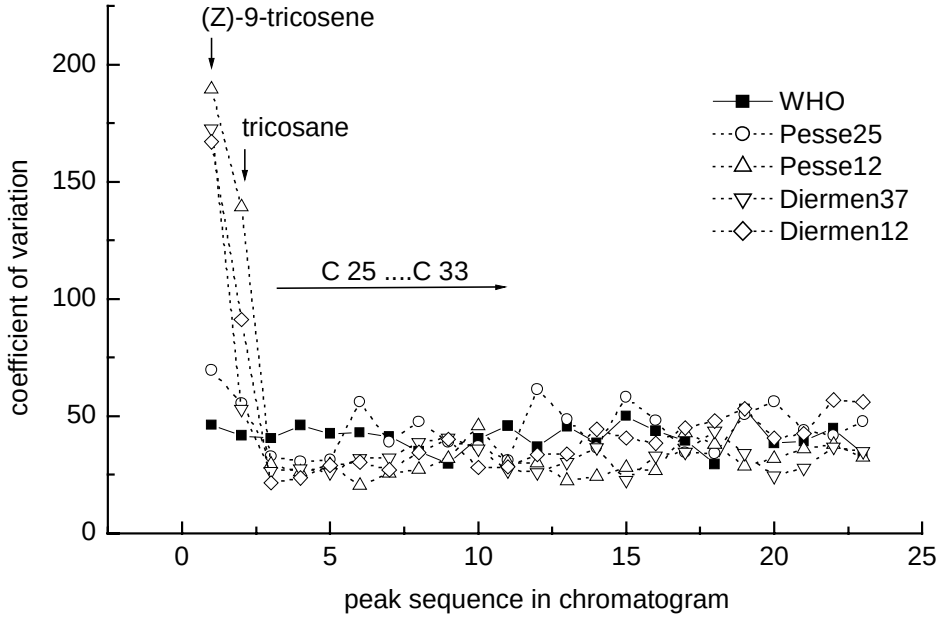


Figure 6. Coefficients of variation of the relative quantities of the hydrocarbons on female flies of the wild-type (dotted lines) and of the WHO strain (solid line). n=10 for each strain.

Cuticular hydrocarbons provide the primary barrier to water loss in terrestrial insects (Beament, 1945; Gibbs *et al.*, 1991; Gibbs, 1995). Therefore, a positive relationship may be expected between the surface of the cuticle of the flies and the quantities of hydrocarbons present on their cuticles. Toolson and Hadley (1979) calculated the surface of scorpions using the equation $S = 15\ (M)^{0.68}$ in which S is the surface area in cm^2 of a scorpion of mass M g. Hadley and Schultz (1987) used the equation $S = 12\ (M)^{0.67}$ as proposed by Edney (1977) to estimate the surface of beetles (M is the mass of a beetle in g and S its surface area in cm^2). We applied both equations on our data but within the measured range of weights (10 to 35 mg) the estimated surface areas showed a strong linear relationship with the body weights of the flies ($r = 0.998$, n=40). Therefore, we chose the body weight of the flies instead of the surfaces as a measure of size.

Figure 7 shows the relationship between the total quantity of cuticular hydrocarbons and bodyweight of 8-day-old females of the wild-type strains. It appears that there is a significant correlation between the weight of the flies and the total amount of hydrocarbons present on their bodies (Fig. 7A). This correlation is a bit stronger when (Z)-9-tricosene and tricosane are excluded (Figure 7B).

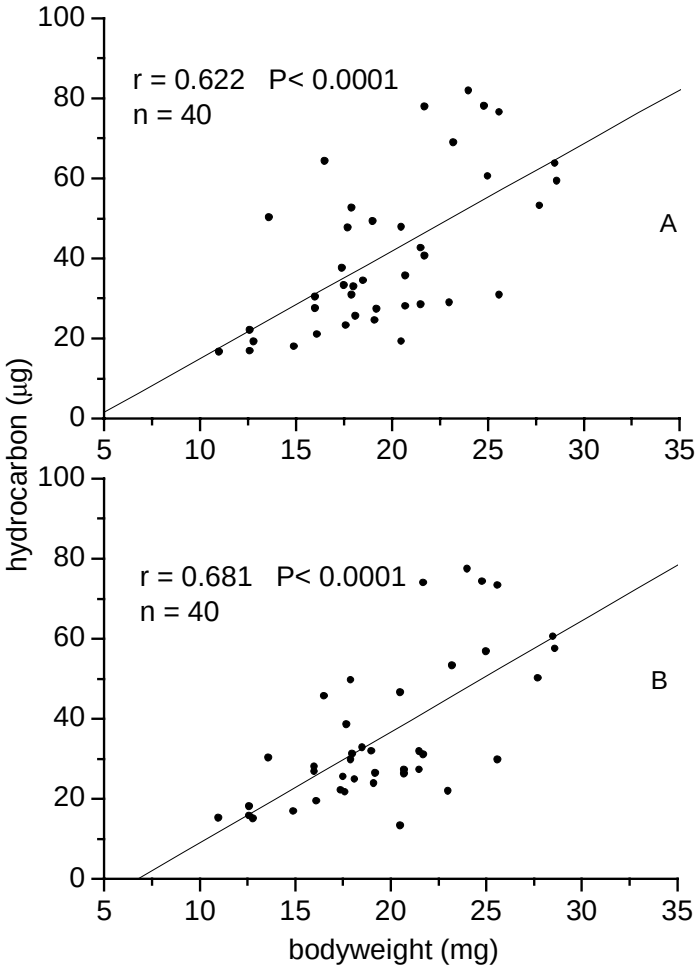


Figure 7. The total quantities of cuticular hydrocarbons (A) and the quantities of cuticular hydrocarbons with (Z)-9-tricosene and tricosane not taken into account (B) as a function of bodyweight of 8-day-old female wild-type houseflies.

To get more insight into the relation between body weight and individual cuticular hydrocarbons we calculated the correlation coefficients of the quantities of 23 cuticular hydrocarbons peaks and body weight of 8-day-old wild-type females (Figure 8). The weight of the flies and the amount of each of cuticular hydrocarbon (or hydrocarbon group) are positively correlated except for (Z)-9-tricosene and tricosane. The latter pair showed a negative although not significant correlation with bodyweight (muscalure: r = -0.148, tricosane: r = -0.009, n = 40). Correlation between bodyweight and cuticular hydrocarbons for females of the WHO strain is not presented, since the range in bodyweight of these flies was to small to draw any conclusions.

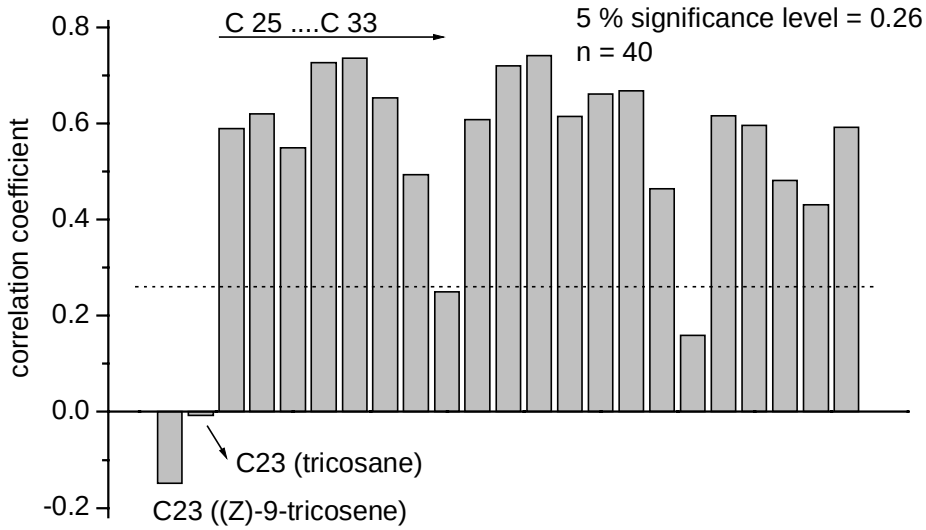


Figure 8. Correlation between the amounts of cuticular hydrocarbons and bodyweight in 8-day-old wild-type female flies (10 flies from each strain).

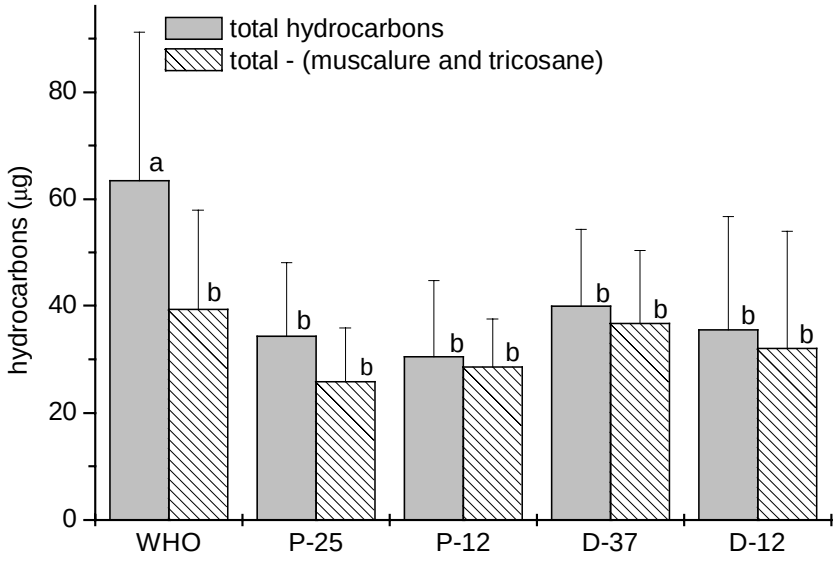


Figure 9. Average relative hydrocarbon quantities on laboratory WHO and wild-type female (*Musca domestica*) corrected for bodyweight. Averages labeled with different letters differ significantly (10 flies from each strain).

Figure 9 presents data for the total amounts of cuticular hydrocarbons and for the total amounts minus (Z)-9-tricosene and tricosane on the body of females from each

strain. For a reliable comparison the amounts are corrected for bodyweight. Significant differences only occurred between the total hydrocarbon amounts of the WHO strain and the four wild-type strains. However, Fig. 9 also shows that these differences were caused by the high amounts of muscalure and tricosane present on the skin of females from the WHO strain compared to the wild-type strains.

Discussion

We found remarkable differences in the composition of cuticular hydrocarbons of females from the WHO laboratory strain on the one hand and from two wild-type strains on the other hand. Whereas in the cuticular washes of 5-20-day-old females of the WHO strain (Z)-9-tricosene comprised up to 20-30 % of the total cuticular hydrocarbons, no (Z)-9-tricosene was found on females of the Pesse strain and less than 0.5 % on those of the Van Diermen strain. These differences were even larger than those observed by Adler *et al.* (1984) who found 4-11 times more muscalure on laboratory strains of houseflies than on those of wild-type strains. The amounts may differ between laboratory strains as well. Nelson *et al.* (1981), e.g., found 4.3 % (Z)-9-tricosene on 4-day-old females of a laboratory strain, whereas around 10% of this substance was present on our WHO females of that age.

Adams and Nelson (1990) studied the effect of diet on pheromone production in female houseflies of the so-called 'Orlando Regular' strain. These authors found that the amounts of (Z)-9-tricosene were not affected by the food offered to the flies, which consisted of either sugar or protein. This was in contrast to the amounts of (Z)-9-heptacosene, which appeared to be significantly higher on females fed with sugar than on those fed with protein. Since all our strains were fed the same mixture of sugar, lipids and proteins, it is very unlikely that the differences in (Z)-9-tricosene quantities between WHO and wild-type females were due to differences in essential food constituents. Assuming that females with higher quantities of (Z)-9-tricosene on their bodies are more attractive to males leads to the suggestion that selection over the years in laboratory cultures may have led to the relatively high production of (Z)-9-tricosene in the laboratory strain.

The amounts of (Z)-9-tricosene on WHO females from the mixed populations were

lower than those on WHO females from the isolated populations, whereas some (Z)-9-tricosene was present on males of the mixed populations but absent on males of the isolated populations. The amounts of (Z)-9-tricosene on males gradually increased and those on females decreased in the course of time, which suggests that transfer of (Z)-9-tricosene from females to males took place due to physical contact between the sexes. This agrees with the results of Ahmad *et al.* (1989) who showed that during copulation an average of 4.1 % topically applied radiolabelled (Z)-9-tricosene on the abdomen of a female was transferred to the body of a male. A similar effect can be observed for the most abundant hydrocarbon in males, (Z)-9-heptacosene. In this case transfer may have taken place from males to females. This is strongly suggested by the fact that higher amounts of this substance were present on females from mixed populations than on those from isolated populations. The large differences in relative amounts of (Z)-9-heptacosene on females from the mixed and isolated wild-type strains compared to those on the mixed and isolated WHO females (Fig. 1) is due to the very low absolute amounts of (Z)-9-tricosene on wild-type females. The relatively high amounts of (Z)-9-heptacosene we found on 1-day-old males and females are in accordance with the findings of Tillmann-Wall *et al.* (1992). These authors found that microsomes prepared from 1-day-old males and females produced (Z)-9-heptacosene as the major alkene. So far, the behavioural function of (Z)-9-heptacosene is unknown. The large differences in the amounts of this substance between the sexes suggest some role in sexual behaviour. Although Rao *et al.* (1990) suggested that (Z)-9-heptacoseneis a component of the male sex pheromone the data presented by these authors, in our opinion, does not support that conclusion. It is not clear why the quantities of hydrocarbons with backbones consisting of 23 to 25 C atoms found on 8-day-old wild-type females are much lower than those found on WHO females. This difference also holds true for flies of other ages (chromatograms not shown).

Transfer of hydrocarbons between the sexes in the course of time, as appears to occur with (Z)-9-tricosene and (Z)-9-heptacosene may result in modification of the original male and female cuticular hydrocarbon composition. Possibly this enables one sex to recognise whether the other sex has already copulated or not.

Both the Van Diermen and Pesse strains easily survived in their ‘natural’ environments and, in addition, can readily be maintained in the laboratory. This questions the role (Z)-9-tricosene may actually play in mating behaviour. (Z)-9-tricosene enhances

sexual activity in male houseflies (Mansingh *et al.*,1972; Uebel *et al.*, 1976; Adams and Holt, 1987; La-France *et al.* 1989; Lemke *et al.*,1990; Islam and Port, 1994) which may explain its relatively high production by the females in the laboratory where environmental conditions are constant and not extreme and were selection pressure is high. However, our results suggest that it is not decisive for mating. To investigate this, studies on the sexual behaviour of the various strains have been initiated.

From the results of Experiment 2 it can be concluded that after some tens of generations in the laboratory the amounts of (Z)-9-tricosene had increased considerably. Now, females of the Pesse 25-generation contained only about 3.5 times less (Z)-9-tricosene and those of the Pesse 12- and Van Diermen 12- and 37-generation about 10 times less (Z)-9-tricosene than the WHO females (Fig. 3). This appears to confirm our suggestion that selection in subsequent generations of high-density populations may lead to increased production of (Z)-9-tricosene by the females. Apparently, higher amounts of this substance increase the attractiveness of females to males.

As already said above, Adams and Nelson (1990) showed that sucrose-fed and protein-fed *M. domestica* females did not differ in amount of cuticular (Z)-9-tricosene. However, the sugar-fed females contained significantly higher quantities of (Z)-9-heptacosene. In our laboratory cultures, both the wild-type and WHO flies were fed the same diet, containing proteins as well as sucrose, and they were reared at the same temperature and r.h. Thus, food and environmental factors are not likely to account for the differences in the production of (Z)-9-tricosene which we observed between the Pesse 25- and the Van Diermen 37-generation. Obviously, selection pressure differed between these two strains.

Toolson *et al.* (1990) found that on both male and female *Drosophila majovensis* collected from the field, lower quantities of total hydrocarbons were present than on flies from laboratory strains. Gibbs *et al.* (1991) showed that variation in melting temperature of cuticular lipids of the grasshopper *Melanoplus sanguinipes* was mostly determined by geographic distribution, followed by family effects and rearing regimes. Their results provided evidence for genetic differences in the biophysical properties of surface lipids in natural populations. Toolson and Hadley (1979) observed marked seasonal changes in the relative abundance of cuticular hydrocarbons of the scorpion *Centruroides sculpturatus*, the percentage of *n*-alkanes in the epicuticular hydrocarbon decreasing considerably in warmer months, whereas the proportion of branched alkanes increased.

These seasonal changes in epicuticular hydrocarbon composition probably account for much of the concomitant changes in cuticular permeability to water. Hadley and Schultz (1987) also observed a correlation between water loss rates and quantities and composition of cuticular hydrocarbons. In the tiger beetle *Cicindela obsoleta*, a summer-active species that inhabits dry grasslands, the cuticular hydrocarbon fractions only contains saturated hydrocarbons, whereas on the body of *C. oregona*, a spring- and fall-active species which lives along water courses, about 50% of the hydrocarbons are unsaturated. The presence of unsaturated hydrocarbons is thought to increase the permeability of the hydrocarbon layer to water. Indeed, Hadley and Schultz (1987) found a significant negative correlation between water loss rate and the quantity of saturated hydrocarbons. Gibbs *et al.* (1995) suggested that a potential consequence of pheromone production by female *Musca domestica* may increase cuticular transpiration because the pheromone components are unsaturated or methyl-branched. This tends to lower melting temperatures. It may be that in nature, where high temperatures and low humidities may occur, the production of (Z)-9-tricosene by female houseflies is low or absent, as the presence of this substance may negatively interfere with the water barrier function of the cuticular lipid layer.

In both the wild-type and WHO strains the quantities of (Z)-9-tricosene and tricosane on the females were strongly correlated (Table 1, Figs. 4 and 5). Although in the WHO females the amounts of (Z)-9-tricosene also showed a significant positive correlation with several other hydrocarbons, the correlation between (Z)-9-tricosene and tricosane was by far the highest. In addition, we found no positive correlation between the weight of the females and the (Z)-9-tricosene and tricosane quantities on their bodies, whereas a positive correlation did exist between female weight and the amounts of other cuticular hydrocarbons (Figures 5 and 6). Comparison of the total cuticular hydrocarbons quantities on females of the various strains, after correction for body weight, revealed that selection led tot an increase in both (Z)-9-tricosene and tricosane quantities but did not affect the total amount of the other cuticular hydrocarbons (Figure 7). Moreover, the variation in (Z)-9-tricosene and tricosane quantities between females decreased and ultimately reached the same, relatively low level of that of the other hydrocarbons on the females (Figure 7). These results again strongly suggest that the production processes of (Z)-9-tricosene and tricosane are closely linked and confirm that (Z)-9-tricosene is only synthesized in large amounts under certain environmental conditions and/or selective

pressure. This, however, does not seem to affect the production of other cuticular hydrocarbons by the females. Since the quantities of these substances are positively related with size (body weight) of the females, we assume that these chemicals may (mainly) act as a water barrier preventing desiccation of the flies.

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).

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^{TM} 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 µl silicon oil. The solutions were pipetted onto pieces of filter paper (1 cm^2). In addition, papers loaded with 1 µg amylacetate in 25 µl silicon oil (reference stimuli) and with 25 µl 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 µl of the solution was injected into a 10 m x 0.32 mm CP-Sil-5 CB column (Chrompack) with the injector at 250 °C and the FID at 300 °C. The flow rate of the helium carrier gas was approx. 1 ml/min. GC oven temperature was programmed from 50 to 300 °C at 10 °C/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 µg.

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, $p > 0.05$).

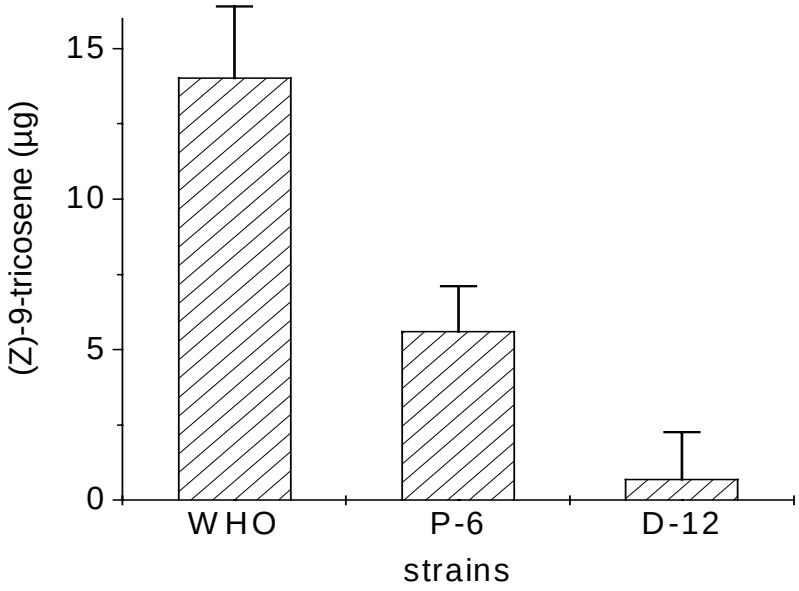


Figure 1. Amounts of (Z)-9-tricosene on WHO, Pesse-6 and Van Diermen-12 females used in the behavioural experiments. n = 12 for each strain. Error bars denote SEM.

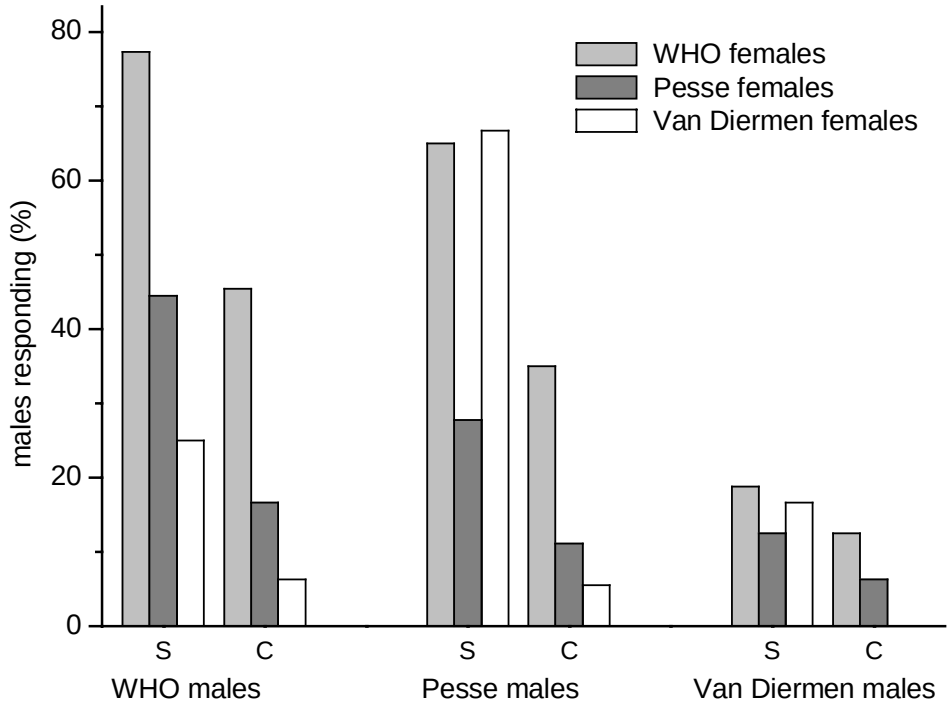


Figure 2. Percentages of males from the three different strains which performed strikes (S) and copulations (C) with females of the various strains. The number of virgin couples tested varied from 18-22.

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 muscalure 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: $p < 0.05$).

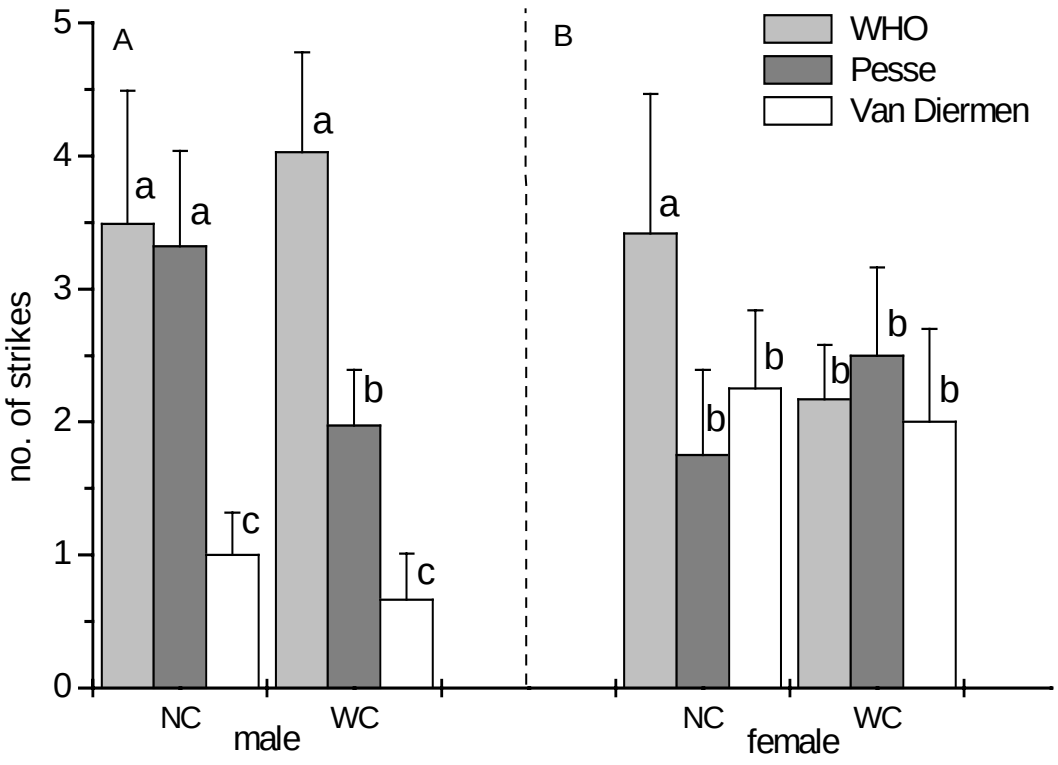


Figure 3. Average number of strikes without (NC) and with (WC) subsequent copulation. A. Strikes of males of the three strains irrespective of the origin of the females. B. Strikes on females of the three strains irrespective of the origin of the males. Columns with different letters are significantly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

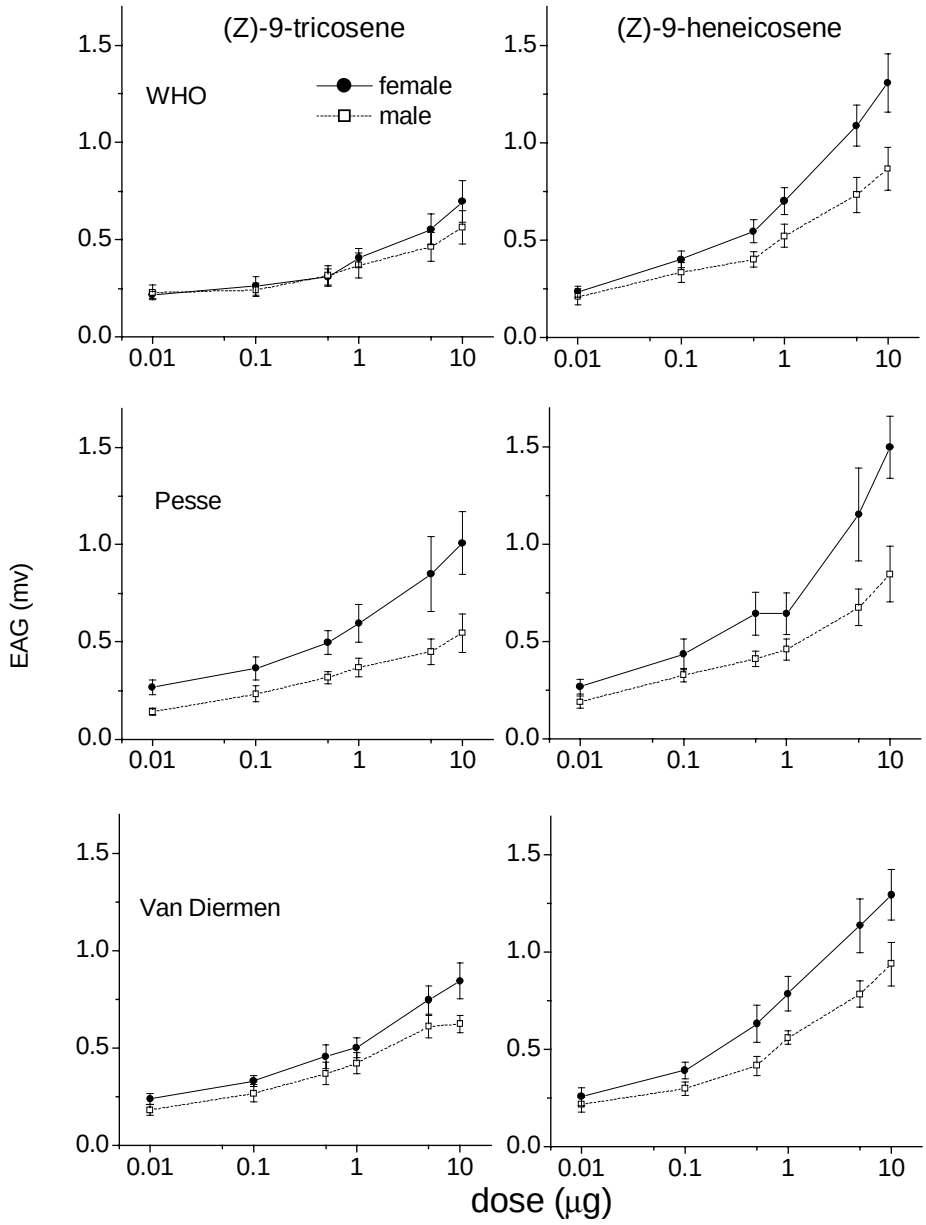


Figure 4. Mean EAG responses to (Z)-9-tricosene and (Z)-9-heneicosene of males and females (n=8) of the WHO, Pesse-6 and Van Diermen-12 strains. Error bars denote standard deviations.

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 µg, respectively, muscalure on their body. On females of the WHO we found an average of 15 µg muscalure with a maximum of 20 µg. 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 µg 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 phenomenom 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).

Chapter 4

EFFECTS OF RELATIVE HUMIDITY, TEMPERATURE AND POPULATION DENSITY ON THE PRODUCTION OF CUTICULAR HYDROCARBONS IN THE HOUSEFLY *MUSCA DOMESTICA* L.

Abstract

The production of cuticular hydrocarbons by both males and females of *Musca domestica* after emergence under very wet conditions (90% r.h.) compared to the production at 50 and 20% r.h. is delayed up to at least 3 days. Eight days after emergence, however, males contain the same amounts of hydrocarbons at all three r.h. values, whereas females at 90% r.h. still possess less of these substances than at 50 and 20% r.h. It is suggested that this is due to the fact that males, being more active than females, need more cuticular hydrocarbons to prevent water-loss than females. No indication is found that the r.h. has a different effect on the production of muscalure by females than on the production of the other hydrocarbons.

Male and female flies produce more hydrocarbons at 35 °C than at 20 °C. On females, the relative amounts of nonacosane and methyl- and dimethylnonacosanes are significantly higher at 35 °C than at 20 °C.

Female flies produce some (Z)-9-tricosene after 8 generations at low population density in contrast to females at high population density which did not produce muscalure. We suggest that because of the relatively large contribution to the total population, the properties of a small number of females are likely to be expressed sooner in the next generations of small populations than in those of large populations.

Introduction

The cuticular hydrocarbons of insects provide a barrier to water diffusion, thus preventing desiccation of the animals. However, some of these substances may also act as semiochemicals and may play a role in mating behaviour. In houseflies, several hydrocarbons on the body of females induce sexual behaviour in males. (Z)-9-tricosene is considered to be the main component of this sex pheromone (Carlson *et al.*, 1971, 1974; Mansingh *et al.*, 1972; Richter *et al.*, 1976; Uebel *et al.*, 1976; Rogoff *et al.* 1980; La-France *et al.*, 1989; Adams *et al.*, 1995).

It is known that environmental factors may affect the production of cuticular hydrocarbons (Hadley, 1977, 1978; Toolson *et al.*, 1990; Gibbs *et al.*, 1991). In several species of arthropods it has been found that at higher temperatures and lower humidities the proportion of cuticular n-alkanes is higher than that of branched alkanes and/or unsaturated hydrocarbons. These differences in hydrocarbon composition account for concomitant differences in cuticular permeability to water (Toolson and Hadley, 1979; Hadley and Schultz, 1987). Gibbs *et al.* (1995) suggested that production of sex pheromone by *Musca domestica* females may increase cuticular transpiration. Because the pheromone components are unsaturated or methyl-branched this might lead to higher permeability to water.

In previous studies (Noorman and Den Otter, 2001) we found that (Z)-9-tricosene comprised up to 20-30 % of the total hydrocarbons on females of a strain that had been cultured for many generations in the laboratory at constant temperature and humidity. On wild-type females, however, less than 0.5 % of this component was present. It appeared that on females of subsequent generations of laboratory populations the quantities of both (Z)-9-tricosene and tricosane –the production processes of which were shown to be closely linked- increased, whereas the amounts of the other cuticular hydrocarbons remained the same. This led us to assume that, although temperature and humidity may affect the production of all cuticular hydrocarbons, temperature and humidity mainly determine the quantities of the non-pheromonal components. (Z)-9-tricosene, however, may primarily be produced as a result of a selection process in high-density populations in isolated environments, females with higher quantities of this substance on their body being more attractive to males. These females may be the first to mate and oviposit fertile eggs. Their progeny may therefore survive in higher numbers in the next generation than

that of females producing lower amounts of (Z)-9-tricosene. It may be expected that this selection proceeds faster in high-density than in low-density populations.

This paper presents results of studies designed to test these hypotheses. We investigated the effects of relative humidity, temperature and population density on the production of various hydrocarbons in males and females of *Musca domestica*.

Materials and Methods

Insects

Experiments were carried out with *Musca dom*estica L. the larvae of which had been obtained from a cow-house with pig-sty (Pesse strain) and a poultry breeding (Van Diermen strain) in the Netherlands, respectively.

To investigate the effects of relative humidity on cuticular hydrocarbon production experiments were carried out with Pesse flies cultured in the laboratory for about 40 generations (Pesse-40) at 25 °C, r.h. 50 % and L12 : D12. Eight-day-old females of this strain contained about 3 μg muscalure/fly.

Immediately after emergence 10 male and 10 female flies were placed together in a cage (15 x 8 x 5 cm). Six cages were used, 2 of which were kept at about 90 % r.h., 2 at 50 % r.h. and 2 at about 20 % r.h. In order to obtain 90 % r.h., the cages were placed on a rack in the rearing room in a Perspex container (50 x 50 x 50 cm) the bottom of which was covered with a layer of tap water. Ventilation took place through holes in the walls of the container. To achieve 20 % r.h. the bottom of the container was covered with oven-dried silicagel, which was replaced before it was saturated with water. For 50 % r.h. the cages were placed in an empty container of which the upper cover was removed. Every day the actual temperature and r.h. in the cages were recorded. The temperature in the cages appeared to be 25.8 ± 0.5 °C, and r.h. was 19.1 ± 1.5 %, 47.2± 1.4 % and 91.8 ± 2.0 % respectively. Hydrocarbon quantities were determined on flies of each of these groups 3 and 8 days after emergence.

To establish the effects of temperature, Pesse flies were used from a strain reared for about 30 generations (Pesse-30) in the laboratory at 25 °C, r.h. 50 % and L12 : D12. Eight-day-old females of this strain contained about 1 μg muscalure/fly. Hydrocarbon quantities of 8-day-old flies from two cultures were compared. Immediately after

emergence, the flies of these cultures had been placed at 20 and 35 °C, respectively, in cages (30 x 30 x 40 cm) containing about 100 males and females.

The effects of population density on cuticular hydrocarbon composition were studied using Van Diermen flies. Flies originating from the 1st generation grown in the laboratory were reared for 8 successive generations (F1-F8) at 25 °C, r.h. 50 % and L12 : D12 in cages (30 x 30 x 40 cm) containing either less than 20 or more than 300 flies. Hydrocarbon quantities on 8-day-old flies of the F1 and F8 generations were compared.

All flies were fed a mixture of powdered milk, sugar and yeast (5 : 5 : 1 by weight). Water was present ad libitum in a tube filled with cotton.

In the first 2 series of experiments flies of only 1 generation, that already produced considerable amounts of muscalure were studied, because we expected an immediate effect on cuticular hydrocarbon production when changing the abiotic factors temperature and relative humidity. Since, however, the amounts of (Z)-9-tricosene production is supposed to be primarily the result of a selection process in isolated high-density populations, we compared in the third series flies from two generations, starting with a culture that did not produce detectable amounts of (Z)-9-tricosene.

Chemical analysis

Individual flies were immersed in 0.2 ml hexane, the whole was shaken during 1 min, after which the fly was kept in this fluid for 1 hour. Gas chromatography was performed on a Shimadzu GC-17A. Gas chromatography was performed on a Hewlett Packard 5890 series II gas chromatograph. Two µl of the solution was injected into a WCOT fused-silica CP-Sil-5 CB column (25 m x 0.32 mm i.d., film thickness, 0.25 µm; Chrompack) with injector at 250 ^{o}C and FID at 300 ^{o}C. The flow rate of the nitrogen carrier gas was 26 cm/s. The split ratio was 56:1. GC oven temperature was programmed from 50 to 300 ^{o}C at 10 ^{o}C/min. 2-Nonanone was used as an internal standard. Muscalure and the other hydrocarbons were identified by comparing the retention time with reference runs of alkanes and (Z)-9-alkenes, or with data from the literature. Quantities of the hydrocarbons were expressed as percentages of the internal standard or as micrograms.

Results

Figure 1 shows the total amounts of cuticular hydrocarbons on males and females, 3 and 8 days old, which had been kept at different relative humidities from emergence. It appears that on both 3-day-old males and females the amounts of hydrocarbons were higher at 20 and 50 % r.h. than on those kept at 90 % r.h. (Mann-Whitney U test, $p < 0.01$). Eight days after emergence the total hydrocarbon quantities on the flies had become significantly higher at all r.h. levels compared to day 3 (Mann-Whitney U test, $p < 0.01$). In addition, the amounts on 8-day-old males at 90% r.h. were no longer lower than on those kept at 50 and 20 % r.h. This is in contrast to the females of which 8-day-old specimens at 90 % r.h. still contained less hydrocarbons than those kept at the lower r.h.'s (Mann-Whitney U test, $p < 0,05$).

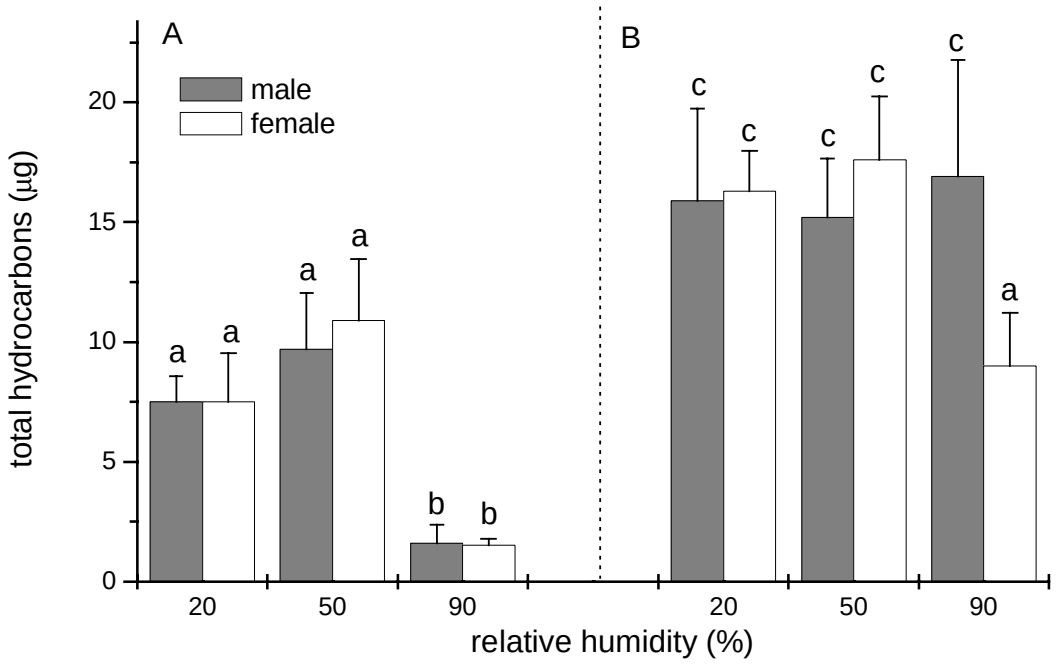


Figure 1. Amounts of total cuticular hydrocarbons of males (n=5) and females (n=10) of *M. domestica*, 3 (A) and 8 (B) days after emergence, at different relative humidities. Error bars represent standard deviations. Averages labeled with different letters differ significantly.

Figure 2 shows the amount of the most abundant hydrocarbon molecules present on the cuticle of the flies. These molecules cover about 90 % of the total cuticular hydrocarbons. The hydrocarbon 'profiles' of males and females differed considerably, males having relatively high quantities of (Z)-9-heptacosene, whereas on females relatively high amounts of (Z)-9-tricosene, tricosane and (Z)-9-heptacosene were present. On day 8 the amounts of hydrocarbons were higher than on day 3, but the hydrocarbon profiles of the males and females were still about the same. The amounts of (Z)-9-tricosene plus tricosane on females are about 15% of the total cuticular hydrocarbon quantity on day 3 after emergence and about 30% of the total on day 8 at each of the three r.h.'s.

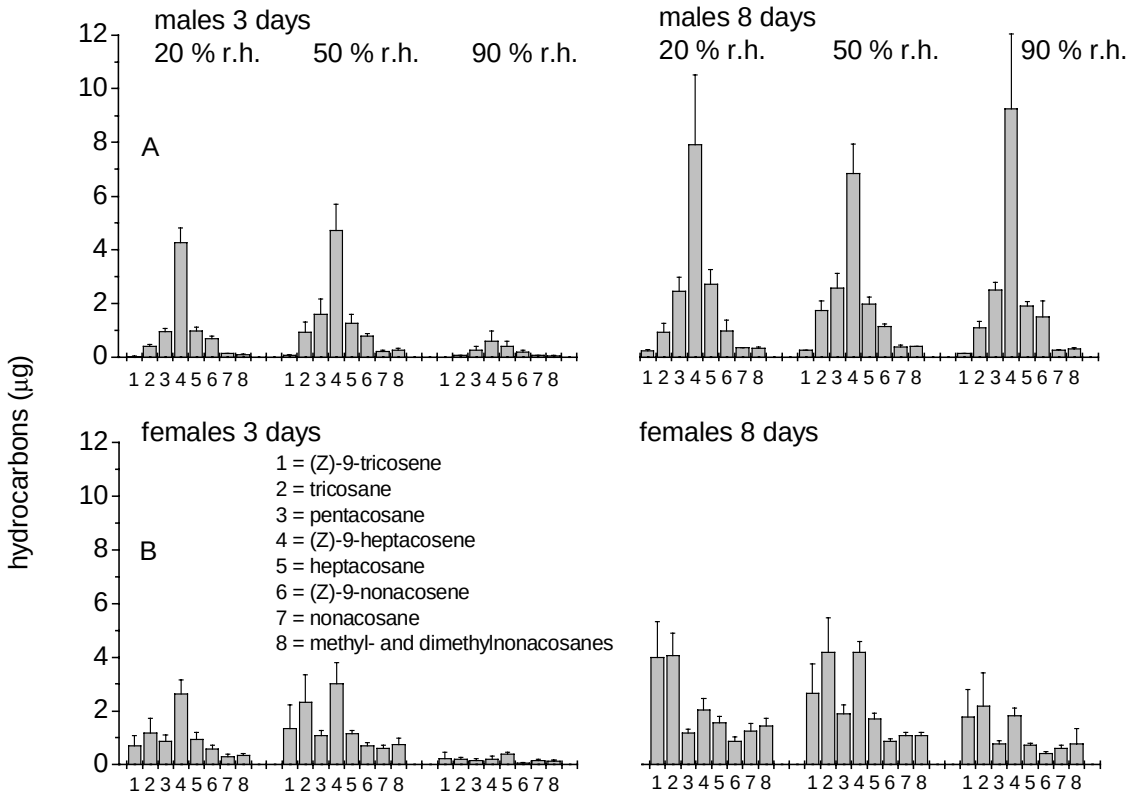


Figure 2. Amounts of cuticular hydrocarbons on males (A, n=5)) and females (B, n=10) of *M. domestica*, 3 and 8 days after emergence, at different relative humidities. Error bars denote standard deviations.

Figure 3 shows the hydrocarbons present on 8-day-old male and female flies at different temperatures. Striking differences in the total amounts of hydrocarbons were present between flies kept at 20 °C and at 35 °C. Males at 35 °C produced about 80 % more hydrocarbons than males kept at 20 °C (19.5 and 10.7 µg, respectively); females at 35 °C produced about 50 % more than females kept at 20 °C (21.2 and 13.9 µg, respectively). In male flies the relative amounts of the various hydrocarbons produced at the two temperatures did not differ significantly, whereas in female flies the relative amounts of nonacosane, and of the methyl- and dimethylnonacosanes were significantly higher at 35 °C than at 20 °C (t-test, $P<0.01$). Although at 20 °C females produced less muscalure than at 35 °C (1.0 vs. 1.4 µg) this difference was not significant. The relative amounts of the remaining hydrocarbons of females kept at 20 °C and at 35 °C did not differ. Hence, the production of muscalure is not deviant from the production of these hydrocarbons at the two temperatures.

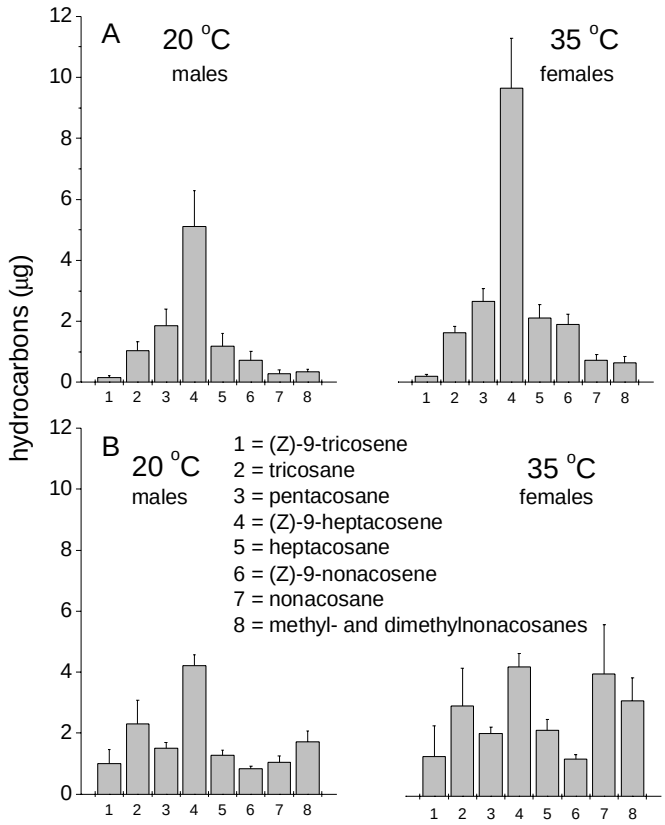


Figure 3. Amounts of cuticular hydrocarbons on 8-day-old males (A, n=5) and females (B, n=10) of *M. domestica* at 20 and 35 °C. Error bars denote standard deviations.

Figure 4 shows the results of the selection experiment with Van Diermen flies.

The total amounts of the hydrocarbons on the first generation males (11.9 µg) and females (16.5 µg) did not differ significantly from those on the 8th generation flies kept at low or high density (males 11.4 and 14.3 µg, females 19.2 and 14.0 µg, respectively (Mann-Whitney U test, $p > 0.05$). The hydrocarbon profiles of the males were similar to those of the males of the Pesse strains used in the previous experiments (cf. Figs. 2A , 3A and 4A), whereas those of the females differed considerably from the Pesse females (cf. Figs. 2B, 3B and 4B). On 8-day-old Van Diermen females, nonacosane and methyl- and dimethylnonacosanes were the most abundant hydrocarbons. Females of the low-density population contained (Z)-9-tricosene, although the amounts were low.

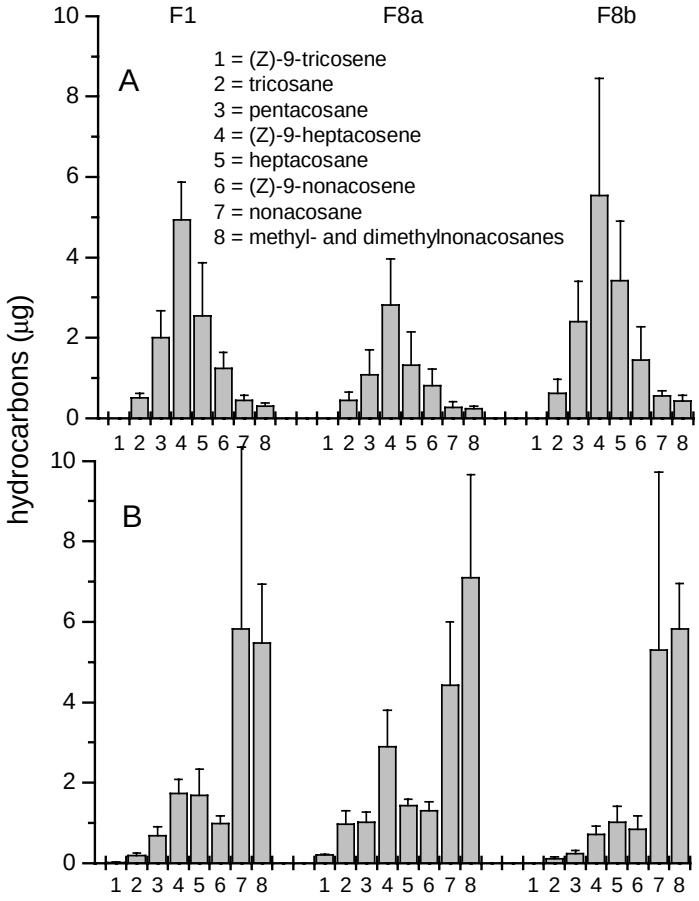


Figure 4. Amounts of cuticular hydrocarbons on 8-day-old 1st laboratory generation (F1) males (A) and females (B) of *M. domestica* and on males and females of the same strain kept in culture for 8 generations at different population densities (F8a <20 and F8b >300 flies/cage). n=10 for each generation. Error bars indicate standard deviations.

Discussion

Evaporation of water through the cuticle can be expected to be lower at higher r.h. Therefore, fewer cuticular hydrocarbons may be needed to protect the flies from desiccation. Toolson and Hadley (1979) observed marked seasonal changes in the relative abundance of cuticular hydrocarbons of the scorpion *Centruroides sculpturatus,* the percentage of *n*-alkanes in the epicuticular hydrocarbon decreasing considerably in warmer months, whereas the proportion of branched alkanes increased. Hadley and Schultz (1987) showed a correlation between water loss rates and quantities and composition of cuticular hydrocarbons. In the tiger beetle *Cicindela obsoleta*, a summer-active species that inhabits dry grasslands, the cuticular hydrocarbon fraction only contains saturated hydrocarbons, whereas on the body of *C. oregona*, a spring- and fall-active species which lives along water courses, about 50% of the hydrocarbons are unsaturated. The presence of unsaturated hydrocarbons is thought to increase the permeability of the hydrocarbon layer to water. Indeed, Hadley and Schultz (1987) found a significant negative correlation between water loss rate and the quantity of saturated hydrocarbons.

We found that both males and females of 3 days old reared at 90 % r.h. possessed remarkably lower amounts of saturated as well as unsaturated hydrocarbons than those reared at 20 and 50% r.h. Eight days after emergence, however, males kept at 90% r.h. contained the same amounts of hydrocarbons as those kept at 20 and 50% r.h., whereas females still had less hydrocarbons on their cuticle. A possible explanation may be that, in general, males are more active than females and as a consequence may need more cuticular hydrocarbons to prevent water-loss than the less active females. The hydrocarbon profiles of the females cultured at different r.h. are about the same. This strongly suggests that our hypothesis that the production of (Z)-9-tricosene, the main component of the female sex pheromone, may not be affected by changes in humidity to the same extent as that of the other hydrocarbons does not hold true.

The melting temperature of the alkenes, methylalkanes and alkanes isolated from 4-day-old houseflies are respectively –0.2, 29.9 and 41.5 °C (Gibbs *et al.*, 1995). The melting temperature (midpoint of phase transition) of the mixture of all cuticular hydrocarbons was found to be 36.8 °C in females and 39.4 °C in males. However, the transition of lipids from the solid to the liquid phase ranges over 10-15 °C. The necessity to keep the melting temperature of the cuticular lipid-mixture within a certain range can

probably affect the amounts in which certain hydrocarbons are produced, depending on different environmental circumstances. We observed that the relative amounts of nonacosane and methylnonacosanes on females were higher at 35 °C than at 20 °C. This may have led to an increase of the melting temperature of the whole mixture of cuticular hydrocarbons. The hydrocarbon profiles on male flies were, however, the same at the two temperatures. The melting temperature of all cuticular hydrocarbons is higher in males than in females (Gibbs *et al.*, 1995). Probably there is no reason to change the amount and composition of hydrocarbons on males in an environmental temperature range of 20 °C to 35 °C.

In the low-density population cultures (<20 flies/cage) all females of the 8th generation produced a low amount of muscalure, whereas no muscalure was found on the high-density (>300 flies/cage) females. We were enable to compare 8 generation but, nevertheless, after this limited number of generations differences in (Z)-9-tricosene production between the two populations could already be observed. We found (Z) 9 tricosene on all the females of the analysed low-density population where each fly contained about the same amount of this substance. Surprisingly, none of the females of the high-density populations produced detectable amounts of this substance. Probably this difference developed by chance. The properties of one or a few females are likely to be expressed in relatively more individuals in the next generations of small (laboratory culture populations) than in large (wild-type) populations. It is clearly shown that selection can sneak in very rapidly. This effect should always be a point of major attention in laboratory colonies.

The clear difference in the hydrocarbon profiles between 8-day-old females of the Pesse-30 and -40 strains and the Van Diermen strain may be explained by focussing on the main function of cuticular lipids in general, i.e. to provide a barrier to water loss. The insects will need a certain amount of cuticular lipids in order to regulate the water-balance in an effective way. When (Z)-9-tricosene and tricosane in female houseflies are only present in small amounts, this paucity is probably compensated for by other hydrocarbons (nonacosane and methyl- and dimethylnonacosanes). This assumption seems to be plausible when considering the total amount of hydrocarbons on females of the Pesse-40 and Van Diermen strains in the experiments carried out at the same temperature and humidity (25 °C, r.h. 50%). Females of both populations produced about the same amount of total hydrocarbons irrespective of the hydrocarbon profiles. In

previous studies Noorman and Den Otter (2001) also found that both first-generation Van Diermen and Pesse females in the laboratory produced very low or no detectable amounts of (Z)-9-tricosene and relatively high amounts of nonacosane and methyl- and dimethylnonacosanes. Since no significant differences in the hydrocarbon profiles between these first-generation females of the Pesse and Van Diermen strains existed, strain differences do not appear to be responsible for the differences in hydrocarbon profiles.

The overall conclusions of the experiments are that relative humidity and temperature have a prominent effect on the production of cuticular hydrocarbons and that the effects on the production of muscalure and the other hydrocarbons are similar. The population-density experiment showed that in a small number of generations selection may give rise to differences in muscalure production between populations. The fact that in contrast to high-density populations females of the low-density population cultures produced some muscalure suggests that population density is not the main factor for inducing muscalure production.

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 and Dr R. Bos from the Department of Pharmaceutical Biology of the University in Groningen for the use of equipment and assistance.

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

Chapter 5

‘SELF-LOADING’ WITH SEMIOCHEMICALS BY HOUSEFLIES (*MUSCA DOMESTICA* L.): A NEW TECHNIQUE

Abstract

It was shown that application of cuticular semiochemicals (long-chain hydrocarbons) dissolved in hexane or acetone to the thorax of houseflies (*Musca domestica* L.) may strongly affect the flies’ condition. In addition, the distribution of these substances over the various body parts may deviate from the natural distribution. It appeared that hydrocarbons, when liquid at room temperature, are also taken up and that they are distributed over the body in a more natural way when the flies are walking on a filter paper onto which the pure chemicals had been pipetted. The higher the amounts of hydrocarbons on the paper and the longer the flies walked on it, the higher the amounts of chemicals taken up.

Using this new “self-loading” technique, females were loaded with (Z)-9-heptacosene or (Z)-9-pentacosene. The former substance is the most abundant hydrocarbon on the cuticle of male houseflies and hardly occurs on females. (Z)-9-pentacosene is absent on houseflies, but acts as a female sex pheromone of the little housefly *Fannia canicularis*, which often is sympatric with *M. domestica*. We hypothesize that both (Z)-9-heptacosene and (Z)-9-pentacosene may inhibit sexual behaviour in male houseflies. However, we now show that in contrast to this (Z)-9-heptacosene stimulates copulation when present in relatively high amounts on females, whereas (Z)-9-pentacosene did not affect male sexual behaviour.

Introduction

Studies to reveal the role of cuticular semiochemicals on sexual behaviour of *Musca domestica* often imply topical application of these chemicals on the flies. The chemicals are usually dissolved in an organic solvent like hexane, acetone or petrol-ether after which a few microliters of the solution are pipetted onto the dorsal part of the fly. In this way the amount of semiochemical applied is exactly known (Adams *et al.*, 1987; La France, 1989; Islam and Port, 1994). This technique, however, has a number of disadvantages. The aggressive solvents may disturb the natural chemical matrix of hydrocarbons present on the cuticle or may partly disrupt the cuticle itself (Beament, 1945). In addition, the distribution of the applied chemicals over the body parts may differ from the natural distribution of these chemicals. These effects are very likely to influence the normal behaviour of the flies.

In the present study we investigated possibilities to apply semiochemicals in a non-aggressive, more natural way and, as a result, to obtain a more natural starting point for behavioural studies. We compared the distribution of hydrocarbons on untreated female flies with the distribution of these substances after they had been taken up from a filter paper on which the females had been walking or after these substances had been applied topically in a solvent.

(Z)-9-Heptacosene is the most abundant hydrocarbon present on the cuticle of male flies. Unmated females have this substance only in small amounts. Rao *et al.* (1988, 1990) stated that (Z)-9-heptacosene is a part of the male sex pheromone of *M. domestica* and is attractive to female houseflies. Schlein *et al.* (1981) found that hexane-soluble substances on the cuticle of male flies terminate courting by other males on contact. The inhibitory activity of these "abstinons" follows from the fact that males did not mate with females treated with male extract. We hypothesized that (Z)-9-heptacosene may repel males, thereby preventing homosexual contacts between them. As a consequence, a relatively high amount of (Z)-9-heptacosene on females might have an inhibitory effect on mating behaviour of male flies.

The cuticular (Z)-9-alkenes produced by female houseflies all possess carbon chains with an odd number of C atoms ranging from 21 to more than 31. In this range only (Z)-9-pentacosene is absent (Nelson *et al.*, 1981). Mansingh *et al.* (1972) found weak behavioural responses of male flies to (Z)-9-pentacosene in olfactometer tests when

compared to those to (Z)-9-tricosene, (Z)-9-tetracosene and a 7:3 mixture of (Z)-9-heneicosene and (Z)-9-tricosene. (Z)-9-pentacosene constitutes 66% of the cuticular lipids of females of the little housefly *Fannia canicularis* and induces copulatory responses in males of this species towards pseudoflies treated with 100 or 200 µg of this substance (Uebel *et al.*, 1977). Other mono-olefins, including (Z)-9-heptacosene and (Z)-9-tricosene, do not elicit responses in male *F. canicularis*. Therefore, the authors concluded that (Z)-9-pentacosene is used by male *F. canicularis* as a species- and sex-recognition pheromone. Since the habitats of *M. domestica* and *F. canicularis* overlap, this led us to assume that (Z)-9-pentacosene may play a role in reproductive isolation of these two species and may have an inhibitory effect on courtship behaviour of male *M. domestica.* To investigate this, we studied courtship behaviour of male houseflies towards females which had taken up (Z)-9-heptacosene or (Z)-9-pentacosene from a filter paper.

Materials and methods

Insects

Experiments were done with *Musca domestica* L. flies from two different strains: A laboratory strain (WHO Ij2) which was obtained from the Statens Skadedyrlaboratorium, Lyngby (Denmark), and a wild-type strain obtained from a cow-house with pig-sty (Pesse strain) in The Netherlands. Flies of the WHO strain were in culture since 1961, flies of the Pesse strain had been reared in the laboratory for about 2 years. The WHO females contained relatively high amounts of (Z)-9-tricosene (approx. 10 µg/fly), whereas the Pesse females produced relatively low amounts of this substance (approx. 1 µg/fly). Depending on the objectives of the experiments either WHO flies or Pesse flies were used. The flies were kept in cages (30 x 30 x 40 cm) in a L12 : D12 regime at 25 ^{o}C and r.h. 60%. They were fed a mixture of sugar, powdered milk and yeast (5 : 5 : 1 by weight). In addition, tap water was present. Experiments were done with flies, which were 4 or 8 days old.

Experiments

Survival test: Sixty female WHO flies, 4 days of age, were immobilized by low

temperature (≈ 2 min at –5 °C). On 20 flies 1 µl hexane and on another 20 flies 1 µl acetone was pipetted onto the dorsal part of the thorax. On the remaining control group of 20 flies no chemical was applied. The three groups of flies were put in separate cages to recover. After 1 hour the condition of the females was determined.

Application of semiochemicals

Application and behavioural tests: The tested chemicals were applied on female flies in two ways: Topically on the thorax ((Z)-9-heneicosene) or by uptake from filter paper on which the flies were walking ((Z)-9-heneicosene, (Z)-9-tricosene, (Z)-9-pentacosene, (Z)-9-heptacosene and (Z)-9-nonacosene). Topical appliance was done by pipetting 1 µl hexane containing 10 µg of the test chemical onto the dorsal part of the thorax. Uptake of the semiochemicals from filter paper was established by putting the females in a Petri dish (9 cm diameter) the bottom of which was covered with filter paper. One or 5 µl of the pure chemical had been applied to the filter paper about 0.5 cm from the edge of the dish (flies prefer walking along the edge) at two opposite locations. The flies stayed in the dish for 30, 60 or 120 min and were then placed back in a cage. Within 1 to 5 hours after treatment the flies were used in behavioural experiments.

Behavioural experiments

The behaviour of 8-day-old Pesse flies was observed in Petri dishes (9 cm diameter). One virgin male and 1 virgin female were put in a dish 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 10 minutes. After each experiment the dishes were cleaned in hot water containing detergent, rinsed in distilled water and then dried.

Chemical analyses

Cuticular hydrocarbons were determined on 8-day-old females. The flies were killed by freezing after which the head, legs, wings, thorax and abdomen were separated under a stereomicroscope using a forceps and scalpel. Each body part was separately immersed in 0.2 ml hexane, the whole was shaken during 1 min, after which the parts were kept in the fluid for at least 1 hour.

Gas chromatography was performed on a Hewlett Packard 5890 series II gas

chromatograph. Two µl of the solution was injected into a WCOT fused-silica CP-Sil-5 CB column (25 m x 0.32 mm i.d., film thickness, 0.25 µm; Chrompack) with injector at 250 ^{o}C and FID at 300 ^{o}C. The flow rate of the nitrogen carrier gas was 26 cm/s. The split ratio was 56:1. GC oven temperature was programmed from 50 to 300 ^{o}C at 10 ^{o}C/min. 2-Nonanone was used as an internal standard. The most abundant hydrocarbons (alkanes and (Z)-9-alkenes) were identified by comparing their retention times with those of reference runs, with data from literature, and by mass spectrometry. No attempts were made to chemically identify the other hydrocarbons. The lower detection level of the individual hydrocarbons was in the order of 5 ng. Quantities of the hydrocarbons were expressed as micrograms.

Results

From the 20 flies treated with 1 µl hexane 45% died and only 20% fully recovered. 35 % of the flies did not fully recover, i.e., the flies were still alive but their mobility was very low or uncontrolled. From the 20 flies treated with 1 µl acetone 15% died and 80% fully recovered. All 20 untreated control flies fully recovered from chilling (Fig. 1).

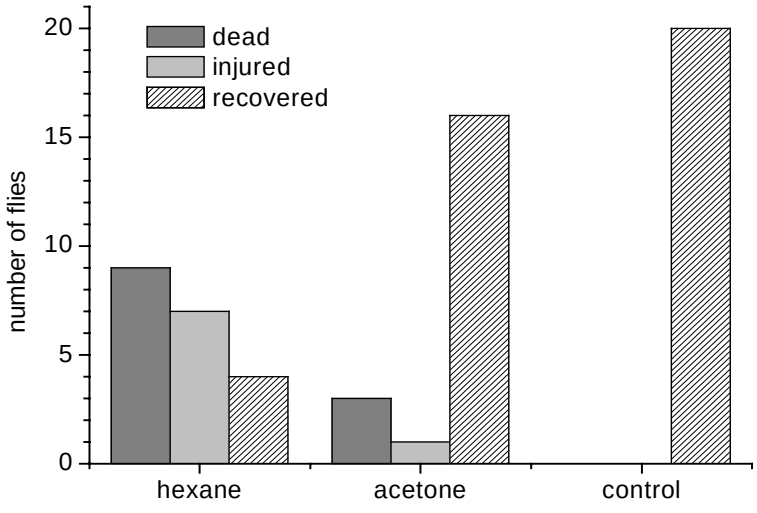


Figure 1. Condition of 4-day-old WHO female houseflies 1 hour after immobilization by low temperature (control), and after immobilization and subsequent treatment with 1 µl hexane or acetone. n=20 for each group.

The natural distribution of the most abundant alkanes and alkenes on the various

body parts of 8-day-old WHO females is presented in Figure 2. Figure 2A shows the amounts in micrograms. It appears that on the head the total amount of the hydrocarbons was considerably lower than on the other parts of the body. In order to compare the hydrocarbon profiles on the various body parts (which differ considerably in size), the amounts of the various hydrocarbons are presented in percentages in Figure 2B. This figure shows that the hydrocarbon profiles were about the same for the legs, wings, thorax and abdomen. The distribution of the hydrocarbons on the head, however, differed from that on the other body parts. This difference was mainly caused by a shift in the alkane/alkene ratio. Figure 3 shows this difference in more detail. The ratio n-alkane/alkene for the various body parts did not differ for the legs, wings, thorax and abdomen. However, on the head this ratio was significantly higher (Mann Whitney U test, C23: $p < 0.05$, C27: $p < 0.001$, C29: $p < 0.001$).

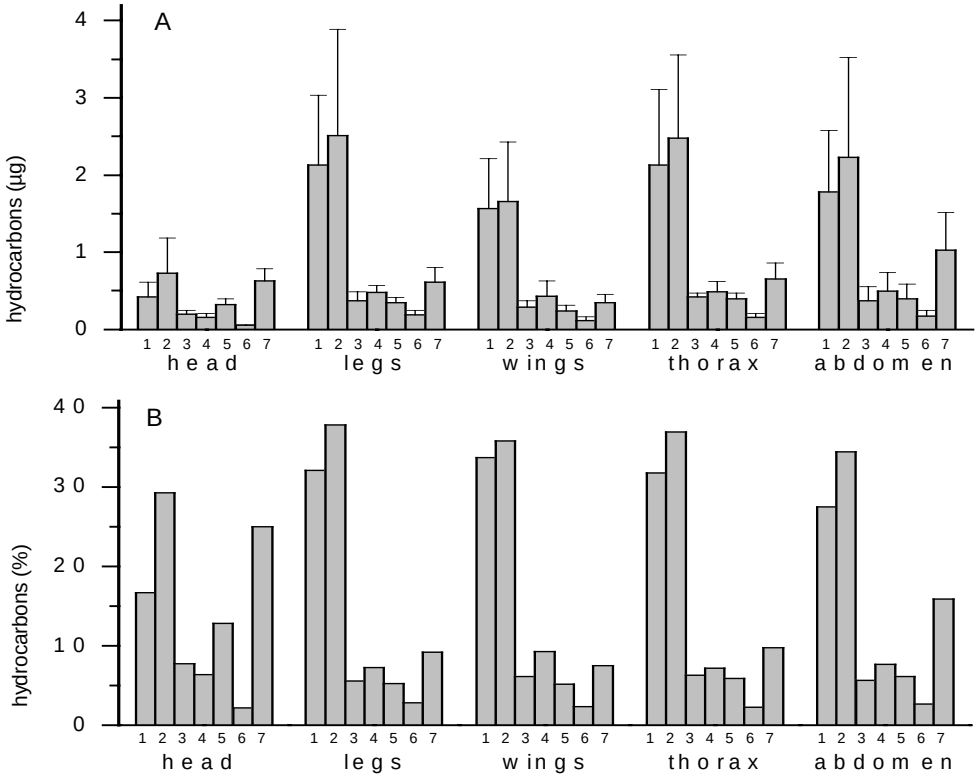


Figure 2. Natural distribution of the most abundant hydrocarbons on WHO females of *Musca domestica* (n=10). A: amounts, B: percentages on head, legs, wings, thorax and abdomen. 1=(Z)-9-tricosene, 2=tricosane, 3=pentacosane, 4=(Z)-9-heptacosene, 5=heptacosane, 6=(Z)-9-nonacosene and 7=nonacosane. Error bars denote standard deviations.

Figure 4 shows the amounts of (Z)-9-tricosene on Pesse females immediately after they had been removed from the rearing cage and on females of the same strain which

had been walking, during 1 h, on filter paper onto which 2 drops had been pipetted of, 1 and 5 µl (Z)-9-tricosene, respectively. It is clear that during walking uptake of (Z)-9-tricosene had taken place and that the amounts taken up were higher the more of this substance was present on the filter paper. Compared to the control females an additional uptake of about 1 and 5 µg had occurred at doses of 1 and 5 µl, respectively.

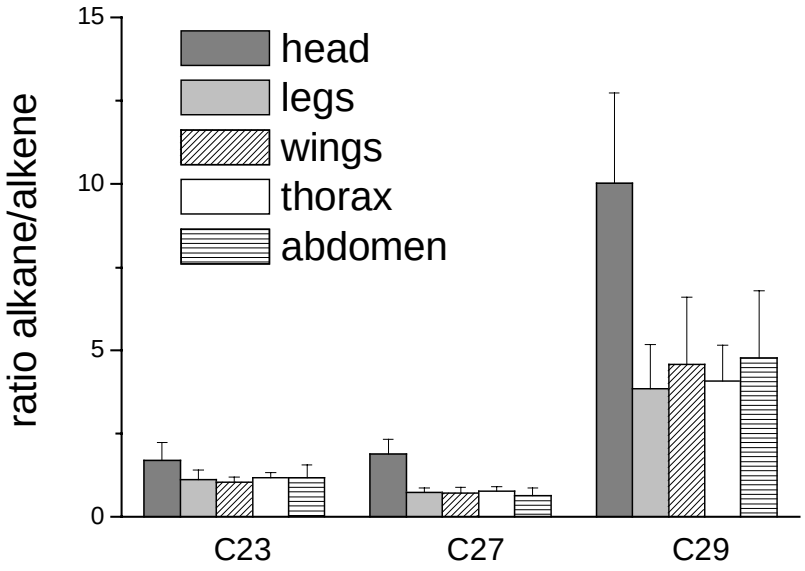


Figure 3. Ratio of alkanes and corresponding (Z)-9-alkenes on different body parts of female WHO *Musca domestica*. C23: tricosane/(Z)-9-tricosene, C27: heptacosane/(Z)-9-heptacosene, C29: nonacosane/(Z)-9-nonacosene (n=10). Error bars denote standard deviations.

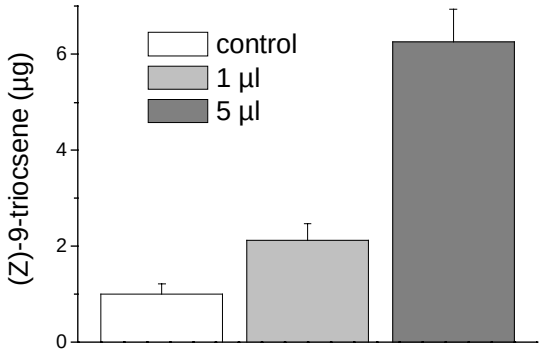


Figure 4. Total amounts of (Z)-9-tricosene on Pesse females which had been walking, during 1 hour on filter paper containing 0 (control) or 2 drops of 1 and 5 µl (Z)-9-tricosene, respectively. n=5 for each group. Error bars denote standard deviations.

Figure 5 shows the distribution on 8-day-old WHO females of (Z)-9-heneicosene when this substance had been applied directly to the thorax and when it had been taken

up from filter paper. The natural distribution of (Z)-9-tricosene on 8-day-old WHO females served as a control. There was no significant difference in the distribution over the body parts between naturally occurring (Z)-9-tricosene and (Z)-9-heneicosene taken up from filter paper. However, when (Z)-9-heneicosene had been applied topically on the thorax significantly lower amounts of this substance were present on the legs and higher amounts on the wings compared with the amounts taken up from filter paper (Mann-Whitney U test, legs: $p < 0.001$, wings $p < 0.001$). These results indicate that in the latter case the semiochemicals were distributed over the body in a more natural way than when applied in a solvent to the thorax of the fly.

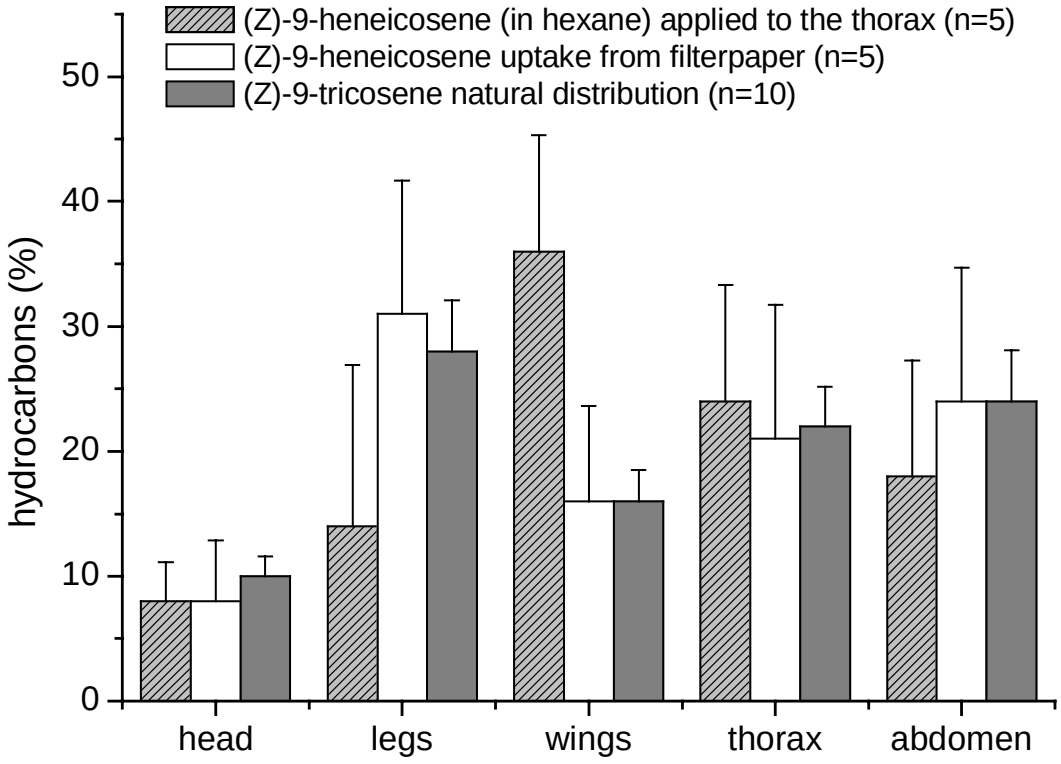


Figure 5. Distribution on 8-day-old WHO females of (Z)-9-heneicosene after topical application and after uptake from filter paper compared to the natural distribution of (Z)-9-tricosene. Error bars denote standard deviations.

In order to find out whether flies could also take up chemicals with high melting points from a filter paper, we tested the uptake of (Z)-9-heptacosene (liquid at room temperature) and (Z)-9-nonacosene (solid at room temperature). For this purpose 2 drops

of 5 µl (Z)-9-heptacosene or (Z)-9-nonacosene (made liquid by heating) was pipetted onto filter paper. Figure 6 shows the amounts of (Z)-9-heptacosene and (Z)-9-nonacosene on 8-day-old WHO females which had been walking, for 1 h, on filter paper loaded with one of these substances. It appears that flies, which had been walking on filter paper with (Z)-9-heptacosene, had taken up an average of about 1 µg of this substance when compared to the amounts present on control flies. However, on flies which had been in contact with filter paper containing (Z)-9-nonacosene the amounts of this substance had not increased compared to the amounts of (Z)-9-nonacosene on the control flies.

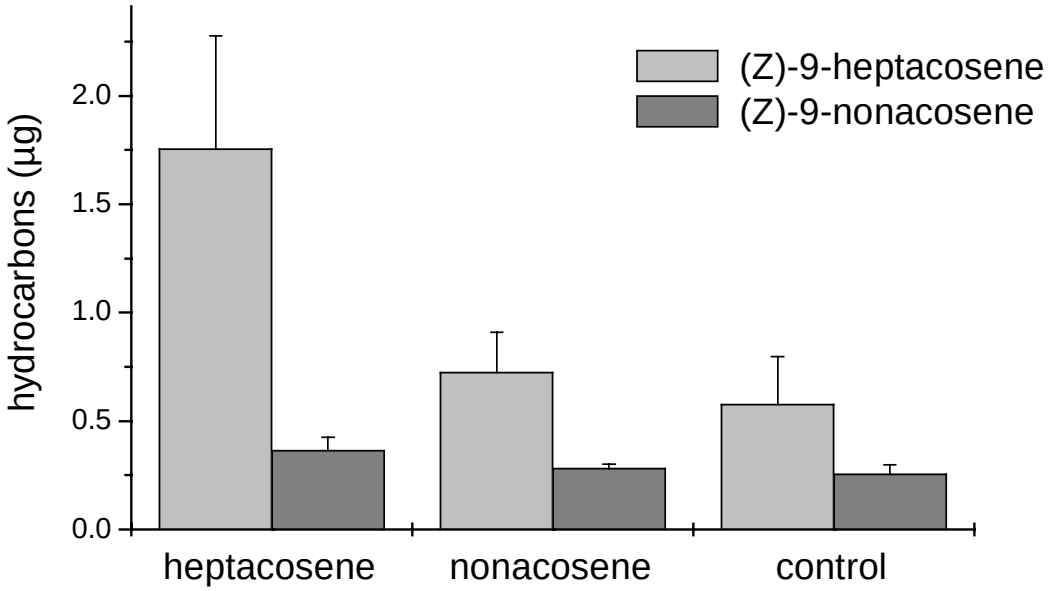


Figure 6. Amounts of (Z)-9-heptacosene and (Z)-9-nonacosene taken up by 8-day-old WHO female *Musca domestica* which had been walking, during 1 hour, on filter paper containing 2 drops of 5 µl of one of these hydrocarbons. Control flies had been walking on clean filter paper. n=10 for each group. Error bars indicate standard deviations.

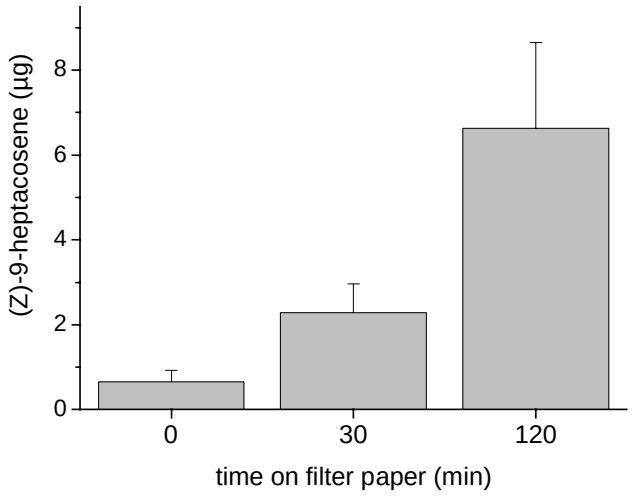


Figure 7. Amounts of (Z)-9-heptacosene present on 8-day-old virgin Pesse females which had been walking on filter paper loaded with 2 drops of (Z)-9-heptacosene during 0, 30 and 120 min. n=6 for each group. Error bars indicate standard deviations.

Figure 7 shows the amounts of (Z)-9-heptacosene are shown present on 8-day-old virgin Pesse females which had been walking on filter paper loaded with 2 drops of 5 µl of this substance for 0, 30 and 120 min, respectively. It is clear that the longer the flies had been walking on the paper the more (Z)-9-heptacosene had been taken up.

We carried out experiments in which each of the 6 females of each group was coupled with an 8-day-old virgin male in a Petri dish and observed if copulations occurred. It appeared that within 10-min periods no copulations took place when the females contained less than 1 µg (Z)-9-heptacosene. Two out of 6 males mated with females which had been on the filter paper for 30 min and contained 3.1 and 3.2 µg, respectively. All couples copulated when the females had been on the filter paper for 120 min; these females contained between 3.6 and 9.7µg (Z)-9-heptacosene. Hence, all eight females with which copulation had taken place contained more than 3 µg of (Z)-9-heptacosene.

We also investigated the sexual behaviour of Pesse males towards 10 Pesse females loaded with amounts of (Z)-9-pentacosene taken up from filter paper ranging from 0.3 to 10.1 µg/female. We found that copulation occurred with 3 females containing 0.4, 1.3 and 4.2 µg (Z)-9-pentacosene, respectively. From a control group of 10 Pesse females not containing (Z)-9-pentacosene 4 females copulated. It thus seems that the amount of (Z)-9-pentacosene on a female does not play a significant role in male sexual behaviour.

Discussion

Application of cuticular semiochemicals dissolved in rather aggressive solvents to female *Musca domestica* flies is a common method to study the effects of these substances on the behaviour of males. However, we showed that application of 1 µl hexane or acetone to the thorax of the females flies may strongly affect the condition of the insects. Only 20 % of the flies treated with hexane and 80 % of the flies treated with acetone fully recovered. The remaining flies died or lost control of mobility. The fact that acetone is less harmful than hexane may be due to the fact that the long-chain apolar cuticular hydrocarbons hardly or not dissolve in the more polar acetone.

Table 1. Percent distribution of (Z)-9-tricosene, (Z)-9,10-epoxytricosane (C23 epoxide) and (Z)-14-tricosen-10-one (C23 ketone) on different body parts of female laboratory-cultured *Musca domestica*.

	Noorman (2000) (Z)-9-tricosene	Dilwith (1982) (Z)-9-tricosene	Blomquist (1984) C23 epoxide	Blomquist (1984) C23 ketone
Head	10	8	13	7
Legs	28	31	33	38
Wings	16	15	13	14
Thorax	22	20	17	17
Abdomen	24	26	24	24

On determination of the natural distribution of various hydrocarbons over the body parts of the WHO flies we obtained results similar to those found on laboratory-cultured flies by other authors (Table 1). However, on application of these substances in a solvent to the flies' thorax the distribution of the chemicals over the various body parts deviates from the natural distribution, high amounts of the substances being present on the wings and only small amounts on the legs. In behavioural experiments this unnatural distribution may affect the flies' behaviour. Therefore we attempted the flies to take up cuticular hydrocarbons by having them walk on a filter paper onto which these substances had been pipetted. Indeed it appeared that in this -non-aggressive- way hydrocarbons were taken up and distributed over the flies' body in conformity with the distribution of naturally occurring hydrocarbons. The higher the amounts of hydrocarbons on the filter paper and

the longer the flies walked on the paper, the higher the amounts of the chemicals taken up. Larger insects may need to be exposed for longer times to higher amounts of chemicals to be adequately loaded.

'Self-loading' of test chemicals can only be done with substances which are liquid at room temperature. (Z)-9-heptacosene, which is liquid at room temperature, was taken up from the filter paper, in contrast to (Z)-9-nonacosene, which at room temperature is still solid.

(Z)-9-heptacosene is the most abundant hydrocarbon present on the cuticle of male houseflies (about 10µg/fly). Females produce this substance in relative small amounts only (0.4 –1.0 µg/fly) (Nelson *et al.*, 1981; Noorman and Den Otter, 2001). The latter authors also showed that substantial amounts of (Z)-9-heptacosene are transferred from males to females (Chapter 1) due to physical contacts between the sexes. We hypothesized that mating behaviour of the males might be inhibited when high amounts of this substance would be present on the females. However, in contrast to this, (Z)-9-heptacosene appeared to stimulate copulation when present in relatively high (> 3 µg/fly) amounts on females and thus it seems not to indicate to males that females have mated before. According to Rao *et al.* (1988, 1990), (Z)-9-heptacosene is a part of the male sex pheromone of *M. domestica* and that it attracts the females. La-France *et al.* (1989) showed that mixtures of (Z)-9-alkenes (including (Z)-9-tricosene and (Z)-9-heptacosene) enhanced sexual activity in males and they suggested a synergistic effect of certain (Z)-9-alkenes.

Our hypothesis that (Z)-9-pentacosene might have an inhibitory effect on the sexual activity of male *M. domestica* clearly does not hold true. Adult females which had taken up (Z)-9-pentacosene from filter paper were not less or more attractive than females without (Z)-9-pentacosene. Probably (Z)-9-pentacosene plays no role in sex-recognition by *M.domestica.*

Finally, the results of this study indicate that the natural uptake of semiochemicals from filter paper can be a good alternative for the application of chemicals solved in rather aggressive oxidising solvents as, for instance, hexane. A more natural starting point for conducting behavioural studies is achieved by this new method.

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 and Dr R. Bos from the Department of Pharmaceutical Biology of the University in Groningen for the use of equipment and assistance.

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

Chapter 6

RADAR-DOPPLER AS A TOOL TO MEASURE BEHAVIOURAL RESPONSES OF FEMALE HOUSEFLIES

Abstract

A radar-Doppler actometer is described with which movements of individual body parts of *Musca domestica* flies can be recorded. Movements of the insect's head were used to monitor the behavioural responses of houseflies to (Z)-9-tricosene, (Z)-9-heneicosene and a 7:3 mixture of these two chemicals. A comparison was made with The results were compared with EAG recordings obtained previously on stimulation with the same substances. It was found that, although (Z)-9-heneicosene evoked high EAG responses in males as well as females, both sexes did not show behavioural responses to this substance. Our results compared with those of field experiments described in the literature suggest that (Z)-9-tricosene may function as an aggregation pheromone bringing males and females together and as a female sex pheromone inducing mating behaviour in males.

Introduction

Buchnan and Sattelle (1979) and Buchnan and Moreton (1981) were the first to use actometers equipped with a radar-Doppler detector for quantitative analysis of insect activity. They were able to monitor the locomotor activity of several insect species (*Periplaneta americana* L., *Musca domestica* L., *Calliphora erythrocephala* Mg. and *Drosophila melanogaster* Mg). Since then the locomotor activity of several other insect species have been studied with various types of actometers fitted with radar-Doppler systems. These actometers have been shown to be sensitive enough to record flight and locomotor activity of individual insects, such as tsetse flies (Van der Goes van Naters and Den Otter (1992), moths (Den Otter *et al.*, 1996; Renou *et al.*, 1998), *Anopheles* mosquitoes (Van de Broek *et al.*, 1999) and fruit flies (Knoppien *et al.*, 2000).

In this study we describe a radar-Doppler actometer with which movements of individual body parts of (fixed) *Musca domestica* flies can be recorded. We have used this actometer to measure behavioural responses of the flies to semiochemicals. For that purpose we recorded the head movements occurring after application of (Z)-9-tricosene, (Z)-9-heneicosene and a 7:3 mixture (by weight) of these substances. (Z)-9-tricosene is supposed to function both as a sex attractant and a sex stimulant in males (Carlson *et al.*, 1971, 1974). According to Mansingh *et al.* (1972) (Z)-9-heneicosene may induce and maintain sexual behaviour in male *Musca domestica* when combined with (Z)-9-tricosene.

Materials and methods

Insects

Behavioural experiments were done with *Musca domestica* L. flies from 2 different strains: A laboratory strain (WHO Ij2), which was obtained from the Statens Skadedyrlaboratorium, Lyngby (Denmark) and is cultured in the laboratory since 1961, and a wild-type strain obtained from a cow-house with pig-sty (Pesse) in The Netherlands. The latter strain had been cultured in the laboratory for 6 (Pesse-6) months. 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.

Registration of the insects' movements.

The movements of the flies were detected by an actometer (Syntech, Hilversum, The Netherlands) consisting of a radar-Doppler sensor (Alpha Industry, Type GOS 2780), the output of which (24 GHz, 3 µW) was directed to the insect through a 4-cm-long wave-guide (Fig. 1). Opposite of the outlet of the wave-guide a microwave absorber was placed. Both the sensor and absorber were mounted on magnetic clamps fixed to a metal base plate.

The dorsal side of the thorax of the fly was fixed to the head of a pin using super glue, and the fly positioned horizontally in front of the wave-guide's outlet with its head at about 1 cm from the latter (Fig. 1). In this way the combined movements of the legs, wings and head could be recorded.

To study the movements of the legs, wings or head separately, body parts which were not subject to examination, were immobilized with glue. For recording head movements during olfactory stimulation the fly was positioned in a plastic pipette tip with the head protruding from the tip's narrow end. The fly was placed vertically with the head up and the lateral side of the head directed to the wave-guide outlet.

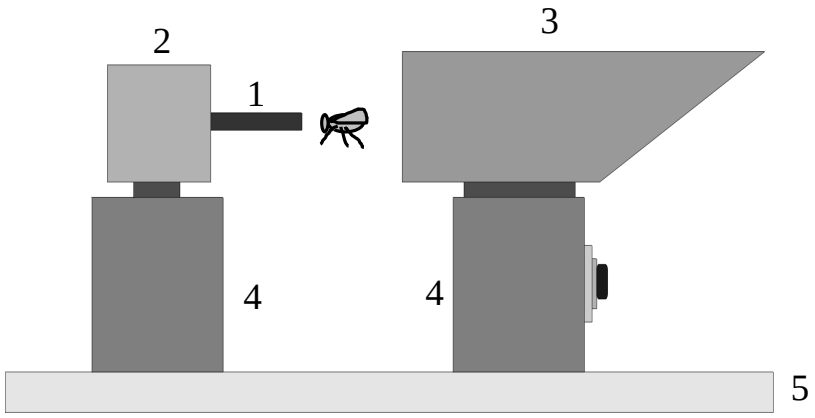


Figure 1. radar-Doppler actometer (Syntech, Hilversum, The Netherlands).
1: wave-guide, 2: radar sensor, 3: microwave absorber, 4: magnetic clamps,
5: base plate.

The microwave beam passing over and along the insect was partially reflected by its body. The frequency and phase shifts of the reflected waves induced by the insect's movements were detected by mixing with the emitted wave. The signal from the detector was electronically filtered (bandwidth 0.1-10 Hz), amplified, rectified and integrated (time constant 0.5 s). The signal was digitized (IDAC, Syntech), stored and evaluated with the software programme EAD (Syntech).

Stimuli

Stimuli were 0.01 and 10 µg (Z)-9-tricosene, (Z)-9-heneicosene and a 7:3 mixture (by weight) of these two chemicals dissolved in 25 µl silicon oil. The solutions were pipetted onto pieces of filter paper (1.5 cm^2). In addition, papers loaded with 25 µl 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 1.5 s, the vapour content of an odour cartridge into a continuous, charcoal-filtered, humidified airstream (0.8 m/s) passing over the insect's head. Each fly was stimulated with 10 pulses of one and the same substance with intervals of 15 s, after which 10 pulses of another substance were applied. First all lower doses of each substance were tested. Before the series of lower and before that of the higher doses 10 pulses from the cartridge with silicon oil and from a cartridge containing clear air were given.

Results

'Spontaneous' movements

Figure 2A shows an actogram of 'spontaneous' head movements of a female WHO fly of which the legs and wings were fixed. Every time the head moved, a steep, high peak was seen followed by peaks which gradually declined in height. The fly spontaneously moved its head about 16 times per minute with intervals of about 2.25 s. The average duration of a head movement was 1.5 s. Figure 2B shows the movements of the six legs of a female fly with wings and head fixed. It appears that the number, duration and interval of the leg movements were about the same as those of the head. The shape of the leg actogram peaks, however, was different from that of the head movements. A high peak occurs at the start of the movements which stays at

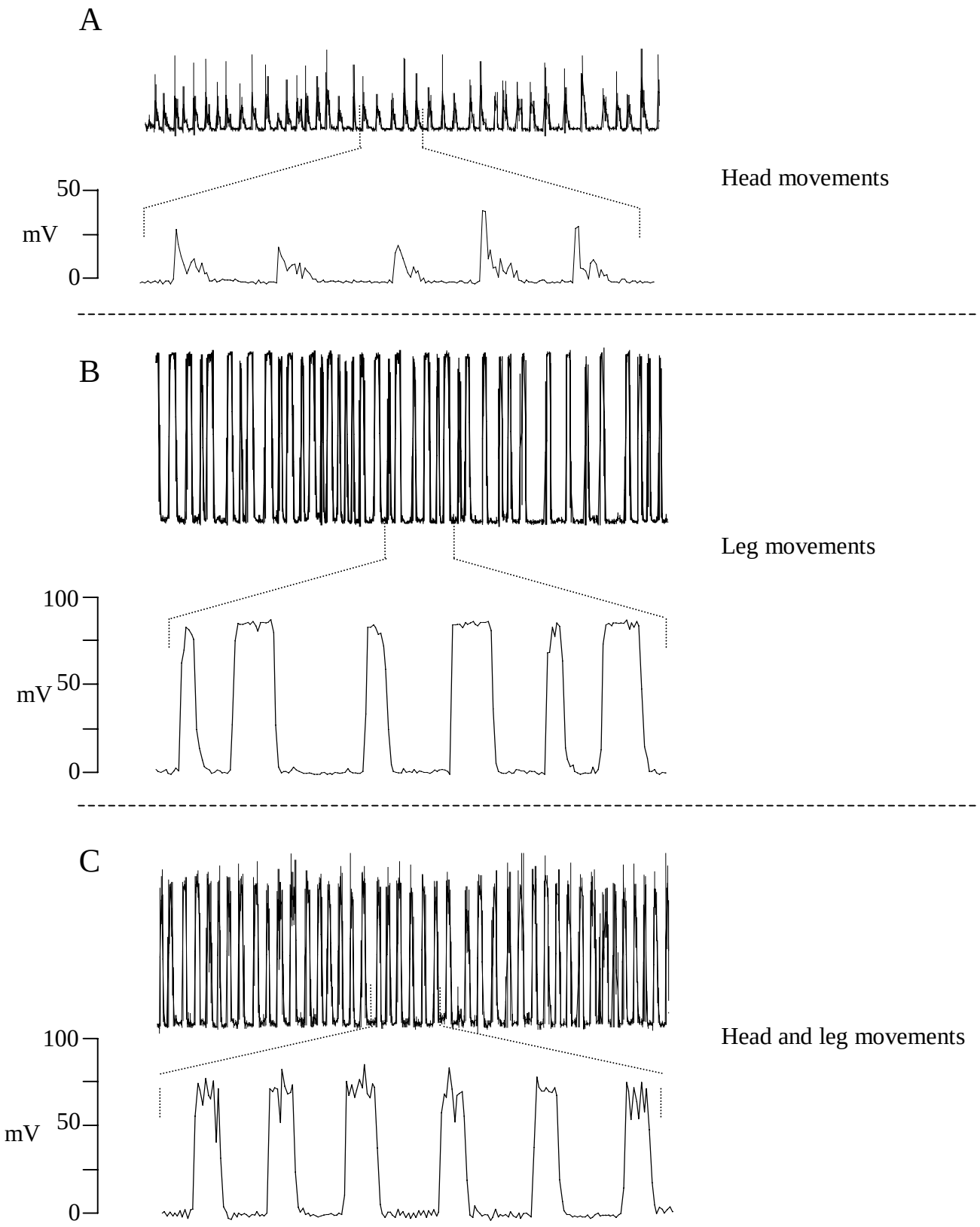


Figure 2. Actographs of spontaneous movements of head (A), legs (B), and head and legs (C) of individual *Musca domestica* females during periods of 150 s (upper traces). Lower traces show some individual movements in more detail. In A legs and wings, in B head and wings and in C the wings of the fly were fixed.

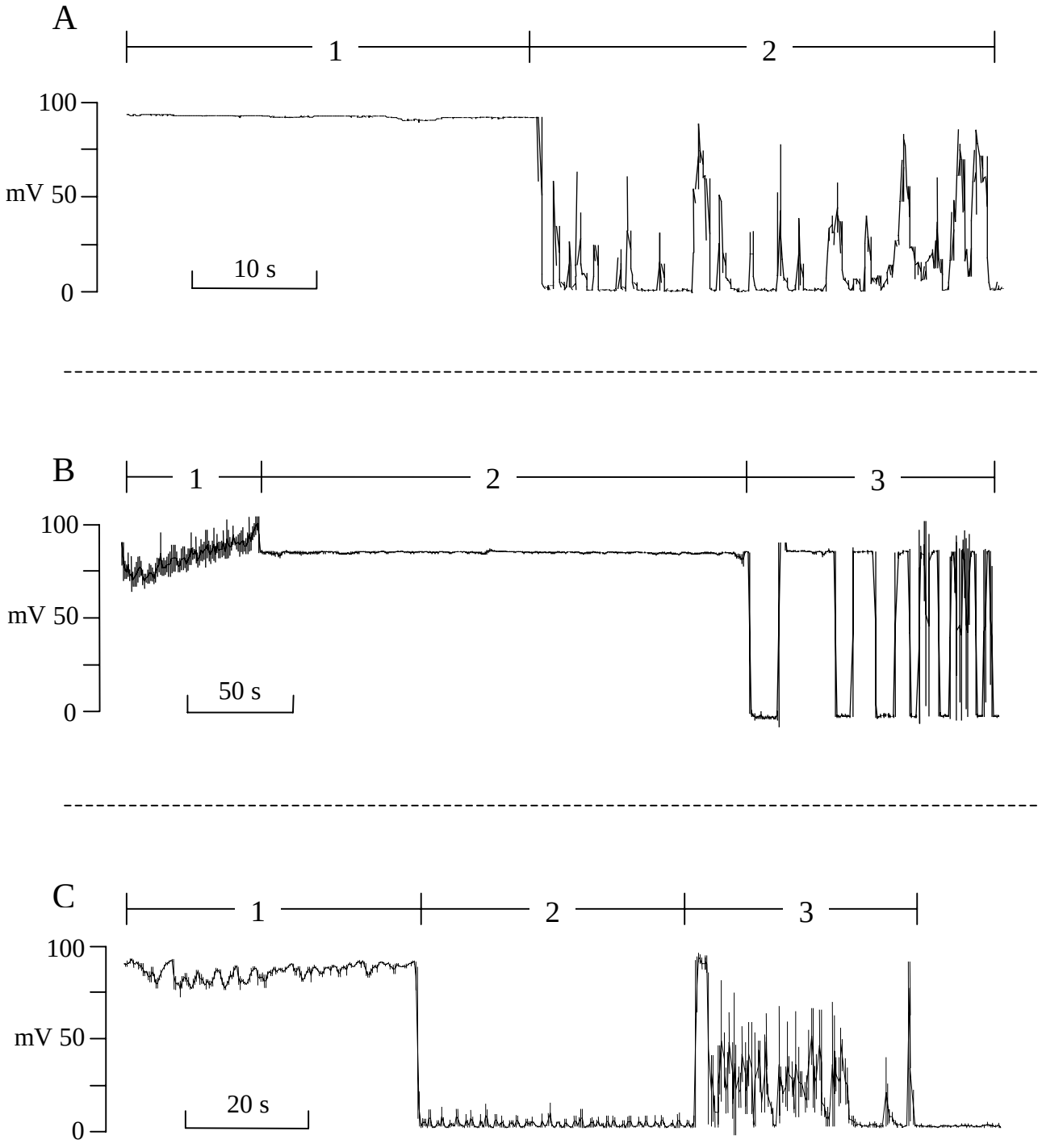


Figure 3. Spontaneous movements of wings (A), wings and legs (B), and wings, head and legs (C) of individual *Musca domestica*.
A: Continuous flight (1) and short movements of wings (2) of a female. 70 seconds recording.
B: Continuous flight of male with dangling legs (1), continuous flight (2) and short periods of flight followed with short flights combined with leg movements (3). 400 seconds recording.
C: Continuous flight of male with dangling legs (1), head movements (2) and combined wing and leg movements (3). 120 seconds recording.

a relatively constant high level after which its falls sharply to the original level. In Fig. 2C simultaneous movements of legs and head are shown of a female of which the wings were fixed. Visual observation of the movements learned that the legs and head moved synchronously. This is also reflected in the actogram, in which now clear peaks are seen.

Figure 3A shows the actogram of wing movements of a female fly with the legs and head immobilized during constant flight and when the wings were moving during short periods. In Fig. 3B recordings of leg and wing movements of a male fly with immobilized head are shown. Figure 3C presents actograms of a male fly able to move head, wings and legs.

Short movements of the wings (Fig. 3A:2) can be clearly distinguished from the wing movements during flight. In the latter case the actogram shows a nearly straight line at a relatively high level (Figs. 3A:1, 3B:2). When flight was combined with 'uncontrolled' dangling movements of the legs, small deviations of the almost straight line occur (Figs. 3B:1, 3C:1), whereas flight and 'controlled' leg movements resulted in a combination of the high-peak flight and leg movement recordings (cf. Figs. 2B and 3B:3). Short movements of wings plus 'controlled' leg movements resulted in a series of sharp, relatively high peaks (Fig. 3C:3). The head movement's actograms in Fig. 3C:2 show the same characteristics as in Fig 2A.

The results show that movements of the various body parts of a fly can be separately detected by the actometer. We have used this technique to investigate whether behavioural reactions (head movements) to semiochemicals occur and we have compared these results with those of EAG recordings (Chapter 3).

Head movements on stimulation with semiochemicals

Figure 4 shows that head movements occur on application of pulses of (Z)-9-tricosene. Responses to the control (silicon oil) and pure air were small and varied from 0 to 2 per 10 pulses. In Fig. 5 the average numbers of head movements of Pesse males and females of different age to 10 stimulus pulses are shown. For both males and females of all ages tested, the responses to the low doses of (Z)-9-tricosene, (Z)-9-heneicosene and a 7:3 mixture of these two chemicals did not significantly differ from each other and also not from the silicon oil control (not shown) (paired t-test) (Fig. 5A). At the higher doses, however, the responses to (Z)-9-tricosene and to the mixture of (Z)-9-tricosene and (Z)-9-heneicosene were

significantly higher than the responses to (Z)-9-heneicosene alone; the latter did not differ significantly from the control (paired t-test, $p<0.01$)(Fig. 5B). No significant differences existed between the responses to (Z)-9-tricosene and the mixture. In addition, the responses of males and females of the same age did not differ (t-test). It is obvious that the responses decreased when the flies grew older than about 6 days. In 12 days and older flies the responses to (Z)-9-tricosene and the mixture were no longer different from the control (paired t-test).

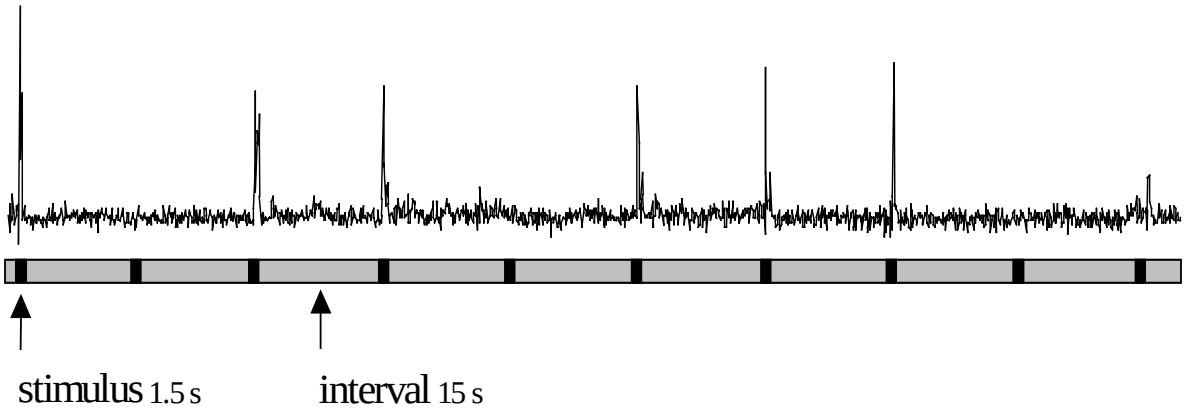


Figure 4. Head movement recording of a 6-day-old female Pesse fly in response to 10 µg (Z)-9-tricosene.

Figure 6 presents results of similar experiments with WHO flies. It can be seen that the responses of WHO flies tended to be somewhat higher, although not significantly (t-test), than those of the Pesse flies of the same age. Again the responses to the lower doses did not differ from those to the control, as was also the case with the higher doses of all three substances for 1-day- and 9-day-old flies. As in the Pesse flies (Z)-9-heneicosene never evoked significant responses in WHO flies. For flies of all ages the responses to (Z)-9-heneicosene did not differ from the control. The responses of flies 2-5 days of age were equally high.

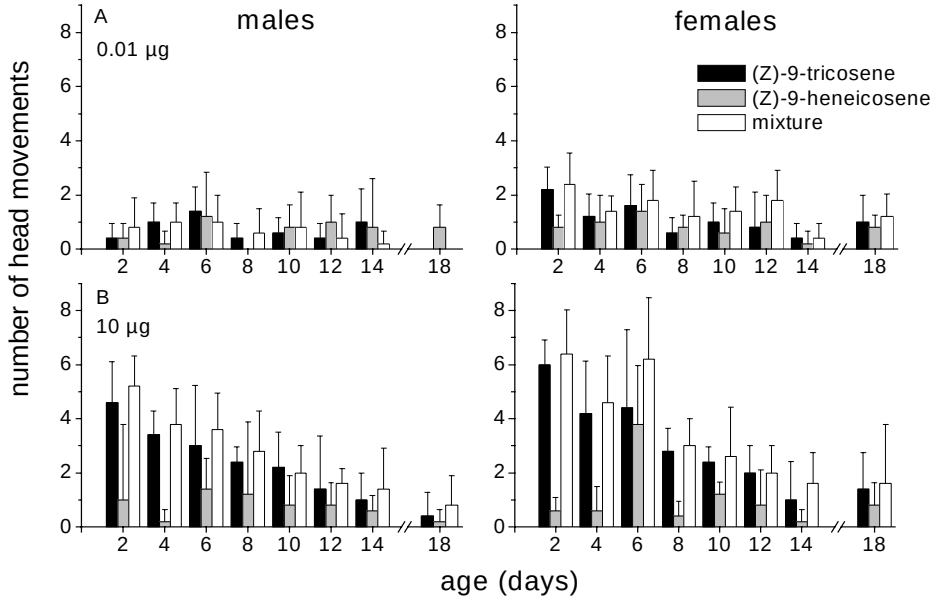


Figure 5. Average numbers of head movements of 2- to 18-day-old Pesse males and females to 10 pulses of the vapours of 0.01 µg and 10 µg (Z)-9-tricosene, (Z)-9-heneicosene and a 7:3 mixture of these two chemicals. n=8 for both sexes of all ages tested. Error bars denote standard deviations.

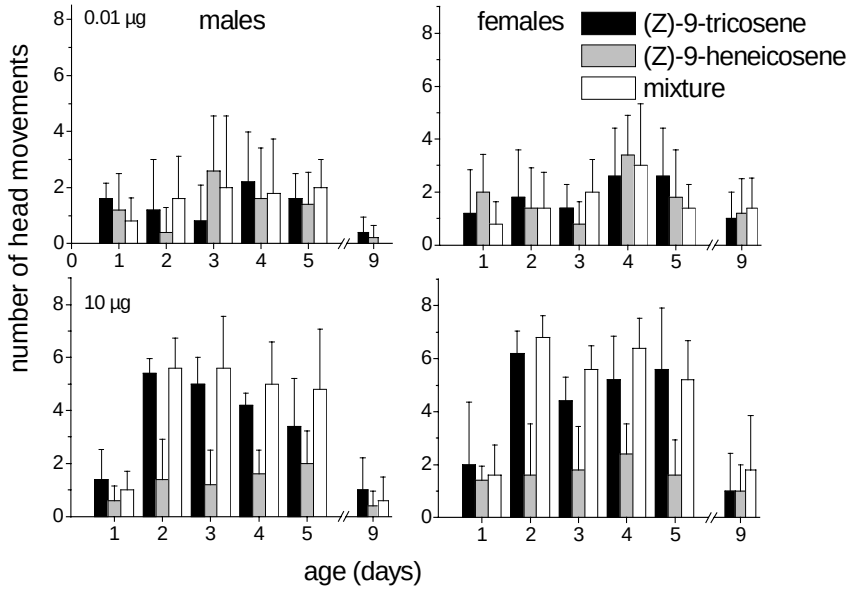


Figure 6. Average numbers of head movements of 1- to 9-day-old WHO males and females to 10 pulses of the vapours of 0.01 µg and 10 µg (Z)-9-tricosene, (Z)-9-heneicosene and a 7:3 mixture of these two chemicals. n=6 for both sexes of all ages tested. Error bars denote standard deviations.

Discussion

Although EAG's indicate which chemicals an insect can smell no conclusions about the insect's behaviour towards those chemicals can be drawn from these recordings. In the present study we used the movements of the insect's head in order to obtain an indication of the behavioural responses of *M. domestica* to certain semiochemicals by means of an actometer equipped with a radar-Doppler detector. This technique has several advantages: it works at low light intensity or in complete darkness, the radar beam can pass through glass and plastic walls of containers without interference, it has a low sensitivity to noise, it is cheap and easy to handle and, moreover, it provides very reliable recordings of the timing and sequence of responses (Renou *et al.*, 1999).

In the present study we tested the same chemicals ((Z)-9-tricosene and (Z)-9-heneicosene) as we used before in EAG recordings (Chapter 3) in order to be able to compare between the two techniques. Comparison of the EAG recordings and the actograms shows that, although (Z)-9-heneicosene evoked high EAG responses, no behavioural responses to this substance occurred. (Z)-9-tricosene, however, evoked high responses in both the EAG and actometer studies, as did the 7:3 mixture of (Z)-9-tricosene and (Z)-9-heneicosene. As to the males, these results agree with the common opinion that (Z)-9-tricosene attracts and sexually excites male houseflies (see e.g. Carlson *et al.*, 1971, 1974). It also may explain the results of Mansingh *et al.* (1972) who found that the mixture of (Z)-9-tricosene and (Z)-9-heneicosene induced high excitement and mating responses in male flies, whereas only low responses were found to (Z)-9-heneicosene alone. Carlson *et al.* (1974), in olfactometer tests, also found that (Z)-9-heneicosene was not very attractive to males. These authors, however, could not confirm the findings of Mansingh *et al.* (1972) that this substance, when mixed with (Z)-9-tricosene enhanced sexual activity in males. Finally, La-France *et al.* (1989) showed that mixtures of 5 :g of (Z)-9-tricosene and 5 :g of other (Z)-9-alkenes when applied to washed dead females induced the same striking activity in males as compared to females that were treated with 10 :g of (Z)-9-tricosene alone.

The sensitivity of female houseflies to (Z)-9-tricosene may explain that in the field, traps loaded with this substance not only may attract males but also about equal numbers of females (Carlson and Beroza, 1973; Mitchell *et al.*, 1975; Chapman *et al.*, 1998). We suggest that (Z)-9-tricosene may function as an aggregation pheromone bringing males and females together and as a female sex pheromone inducing mating

behaviour in males. So far, the effects of (Z)-9-heneicosene and of the mixture of (Z)-9-tricosene and (Z)-9-heneicosene on the behaviour of females is unknown.

The finding that the head responses to (Z)-9-tricosene and a mixture of (Z)-9-tricosene and (Z)-9-heneicosene declined with age, may be contributed to a general decline in olfactory sensivity with age as has also been found in several other insect species (Den Otter *et al.*, 1991; Roelofs and Comeau, 1971). However, Kelling (2000) found that the response of single olfactory cells of 1-day-old male and female *M. domestica* did not differ significantly from those of flies up to 28 days of age. A reason for the age-related decrease in head responses may be due to an increasing number of inoperative number of cells as has also been found for labellar taste hairs in the fly *Phormia regina* (Rees, 1970; Stoffolano, 1973). The fact that the number of head responses to (Z)-9-tricosene and a mixture of (Z)-9-tricosene and (Z)-9-heneicosene of 1-day-old WHO males and females did not differ from the control may be due to the fact that 1-day-old flies are still sexually immature.

We have demonstrated that movements of different body parts of a fly can be distinguished by actometry, even when they occur simultaneously. The new head movement measuring technique is a relatively easy way to investigate behavioural reactions towards odours. More detailed studies are necessary, however, in order to relate these responses to the behaviour of free moving insects and such studies may lead to discrimination of actograph patterns evoked by repellent and attractive 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. We are indebted to Dr J. Van der Pers for supplying equipment.

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

Chapter 7

THE PREFERENCE FOR OVIPOSITION SITES AND THE PRESENCE OF AN OVIPOSITION PHEROMONE IN THE HOUSE FLY

Abstract

Houseflies prefer to deposit their eggs in clusters at one and the same site in crevices in rough substrates, especially when these have a smell of decaying animal products, such as fly eggs or dead flies. Clustering of eggs is not induced by the physical presence of the eggs. We found strong indications that an oviposition pheromone is deposited together with the eggs. This pheromone appears to disappear within a short period of time after oviposition either because it is very volatile or because it disintegrates.

Introduction

Laying eggs in clusters is known to occur in several insect species. Eggs of egg-clustering butterflies are often aposematically coloured and distasteful to vertebrate predators. Depositing eggs in clusters may be of advantage to the eggs, the larvae as well as the adults (Stamp, 1980). Egg clustering decreases the exposed surface. It thus reduces desiccation and the accessibility to parasitoids and predators. In addition, larvae that feed, rest and moult synchronously may grow faster than those that live singly, and also the protection from parasites and predators may be better than when larvae stay apart. Aggregated larvae were shown to be four times as active than solitary larvae, spent 25 % more time feeding, had a higher fat content, and pupated sooner (Long, 1953). Finally, the adults have to spend less time to find mates and to search for suitable oviposition sites when they are close together than when they live widely separated.

In several dipteran species it has been found that females aggregate at oviposition sites. In the blackfly *Simulium damnosum* the phenomenon of aggregated oviposition was investigated under controlled laboratory conditions (McCall *et al.*, 1994, 1995). It appeared that significantly more females oviposited on substrates already containing eggs or volatiles from freshly laid eggs than on control substrates without these substances. Moreover, the females landed more quickly when more eggs were present on the substrate. The authors suggested that specific attractants were emanating from freshly laid eggs.

In the sandfly *Lutzomyia longipalpis*, Elnaiem and Ward (1990) found that the presence of eggs resulted in higher oviposition rates than when eggs were absent. Eggs washed in hexane and water did not induce oviposition indicating that the positive response was due to chemicals present on the eggs (Elnaiem and Ward, 1991). In addition, Dougherty *et al.* (1994) showed that chemicals extracted with hexane from eggs of *L. longipalpis* attracted gravid females of this species for oviposition. The females oviposited earlier and laid more eggs on substances containing these chemicals than on substances on which these substances were absent. Dougherty and Hamilton (1997) identified dodecanoic acid as the oviposition pheromone of the sandfly.

Females of *Culex* spp. lay their eggs in boat-shaped clusters on the surface of stagnant, organically rich water. The egg rafts attract gravid females of *C. tarsalis, C. pipiens molestus* and *C. quinquefasciatus* and lead them to lay at the same sites (McCall

and Cameron, 1995). Dawson *et al.* (1989) showed that eggs of *C. quinquefasciatus* emit a volatile pheromone that induces gravid females to oviposit near freshly laid eggs.

In this study we examined the possible presence of an oviposition-stimulating chemical in *Musca domestica*. Aggregation of insects during oviposition gives the opportunity to catch gravid females and thus to reduce the numbers of insect in a population drastically.

Materials and methods

Insects

Experiments were done with *Musca domestica* L. flies from the laboratory strain WHO Ij2 1961, which was obtained from the Statens Skadedyrlaboratorium, Lyngby (Denmark). The flies, about 300 per cage, were reared in cages (30 x 30 x 40 cm) in a L:D 12:12h regime at 25 °C and r.h. 70%. Light was provided by 2 white fluorescent tubes (Philips TL 40W/33). The flies 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. A mixture of yeast, powdered milk, agar and water (5:5:1:25 by weight) was used as ovipostion substrate and larval food.

Bioassay

All experiments were carried out in rearing cages in the culture chamber. In a preliminary series of experiments 4 oviposition substrates were offered, simultaneously in one cage, in Petri dishes (5 cm i.d.) containing (respectively) wet smooth filter paper impregnated with fly food, wet grains of kilned clay (3 mm diam.) impregnated with fly food, a smooth layer of larval food, and a rough layer of larval food.

In the subsequent experiments oviposition occurred in glass tubes (7 cm high, 1.5 cm i.d.) filled with clay grains (3 mm diam.) and tap water except for the upper 2 cm which contained a folded strip of filter paper (3 mm wide, 30 cm long); the lower 7-cm-long end of the filter paper extended to the bottom of the tube. The folded paper, which remained humid by capillary attraction, was offered as oviposition substrate either without or with additional stimuli (see Results section).

Estimation of the number of eggs deposited was done by putting the eggs in 1 litre

tap water. After thorough shaking, 4 samples of 1 ml of the mixture were taken in which the eggs were counted. An estimation of the total number of eggs was made by multiplying the average number of eggs present in the samples by 1000. The number of females which had been ovipositing was calculated by dividing the number of eggs by 85 (observed average number of eggs deposited by 1 female during oviposition).

Results

Preliminary experiments

Two experiments were done on successive days using the same flies. Table 1 shows the results of these studies. By far the most eggs were deposited on clay grains and rough larval food. About 15 females used these substrates as oviposition sites on the first day and about 80 females on the second day. Smooth filter paper and smooth larval food were far less attractive to the females. Only 3 females deposited their eggs on these substrates. The results suggest that the females strongly prefer to lay their eggs on substrates with crevices above smooth substrates. Therefore, the oviposition experiments were continued with wet folded filter paper in glass tubes, as described in Material & Methods section.

Table 1. Number of eggs on different substrates.

	Fly's food on		Larval food	
	Smooth paper	clay grains	smooth	rough
Day 1	0	300	90	1000
Day 2	80	5000	100	2000

Filter paper experiments

Experiment 1:

Flies were offered, during one day, 5 standard tubes containing wet filter paper without additional substrates. The next day 5 fresh tubes were placed in the same cage. The tubes were randomly distributed over the bottom of a cage. It appeared that on both days all eggs were deposited on the filter paper in one and the same tube. Which tube was chosen was determined by the female that started to oviposit first, and this did not depend on the

position of the tube in the cage. On the first day about 80 females laid 6750 eggs in one tube, and on the second day 120 females deposited 10,000 eggs in a tube at another place on the cage's bottom.

Experiment 2:

In this experiment the effects of the presence of fresh and old housefly eggs and of the smell of the latter on oviposition in the tubes was tested. Flies in one cage were offered 8 tubes of which one was loaded with fresh eggs (2-6 h old). This experiment was repeated 3 times with the same flies on successive days. In another cage 8 tubes were placed of which 1 contained old eggs (3 days old, decaying). This experiment was repeated the next day. In a third cage, during one day, 8 tubes were present of which one contained the smell of old eggs (eggs had been present in the tube for 3 days).

Table 2 shows that visual or tactile stimuli from the eggs or the smell of fresh eggs did not stimulate gravid females to deposit their eggs in tubes where these stimuli were present. On the 3 successive days all females (about 150) laid their eggs in tubes were no fresh eggs were present. The general tendency of flies to lay their eggs at one and the same site is, however, unquestionable shown. However, when a tube contained old decaying eggs or only the smell of these, all females (about 325) except for 1 chose for the tubes with these stimuli.

Table 2. Number of eggs deposited in tubes with (shaded) and without stimulus.

	Tubes							
Stimulus	1	2	3	4	5	6	7	8
Fresh eggs 1	0	0	0	0	5000	0	0	0
Fresh eggs 2	0	0	0	0	0	0	85	0
Fresh eggs 3	0	0	0	0	0	80	7500	0
Old eggs 1	0	85	9500	0	0	0	0	0
Old eggs 2	0	0	0	0	0	10000	0	0
Smell old eggs	0	0	0	0	8000	0	0	0

Experiment 3:

In this experiment the smell of 3-day-old dead flies was tested for its attractiveness to gravid females. To exclude visual or tactile stimuli 10 dead flies were placed in the test tube under the water surface between the clay grains. On two successive days 8 tubes (7 without, 1 with dead flies) were presented to flies in one and the same cage. On the first day about 95 females (8000 eggs) chose for the baited tube and only 1 female (75 eggs) oviposited in the unbaited one. On the second day about 4 flies (350 eggs) chose for the tube with stimulus and none for the unbaited tubes.

Experiment 4:

In this experiment larval food was added as oviposition substrate. The food was offered fresh (newly made) or 2 days after preparation when it had started to decay. Two experiments were carried out on 2 successive days with flies in one and the same cage. The results are shown in Table 3. On the first day about 20 females deposited their eggs in the tube with fresh larvae food, whereas about 90 females laid their eggs in a tube without this substrate. On the second day about 150 females chose for the tube with fresh larval food and none for the seven tubes without this substrate. Decaying larval food attracted about 120 females on the first day and no females on the second day. In the latter case 35 females deposited their eggs in a tube without substrate. The results do not indicate a preference for either fresh or old larval food. Again the clustering effect in egg deposition is unmistakable present.

Table 3. Number of eggs in tubes with (shaded) and without stimulus.

	Tubes							
Stimulus	1	2	3	4	5	6	7	8
Fresh larval food 1	0	1800	0	0	0	0	7500	0
Fresh larval food 2	0	0	0	0	0	13000	0	0
Old larval food 1	0	0	0	10000	0	0	0	0
Old larval food 2	0	3000	0	0	0	0	0	0

Experiment 5:

To determine whether egg clustering would also occur when more tubes with an attractive stimulus were offered, 3 experiments on 3 successive days with the same population of flies were done. Each day 10 tubes, 5 baited with dead flies (in the same way as in experiment 3) and 5 unbaited, were placed randomly on the bottom of the cage. The results are presented in Table 4. On the first day most of the females were not yet ready to oviposit but the few females that laid eggs deposited them in 3 out of the 5 tubes baited with dead flies. On the second day about 60 females deposited their eggs in only one tube baited with dead flies. On the third day about 80 females chose for one and the same baited tube whereas only 1 female oviposited in a tube without dead flies.

Table 4. Number of eggs in tubes with (shaded) and without stimulus (dead flies).

	Tubes									
	1	2	3	4	5	6	7	8	9	10
Day 1	0	0	0	0	200	0	0	70	60	0
Day 2	0	0	5200	0	0	0	0	0	0	0
Day 3	0	0	0	0	0	0	0	7000	50	0

Experiment 6:

The presence of eggs 2–6 hours old did not enhance oviposition (Experiment 2). This suggested that, if an oviposition stimulating pheromone is deposited together with the eggs, this pheromone may be very volatile or may disintegrate within a short period of time after oviposition. Therefore, in a final experiment we investigated whether the smell of very fresh eggs may attract gravid females.

In 3 different cages one oviposition tube was placed, which was removed within 30 minutes after egg deposition in the tube had started. About 1000 eggs were collected from each tube, put into 3 new tubes and covered with the humid filter paper strip in such a way that the flies could not notice the physical presence of the eggs. Then, in each of the 3 cages one of these new tubes was placed together with 4 unbaited ones; the 5 tubes were randomly distributed over the bottom of the cages. It appeared that oviposition immediately started again and without exception all eggs were laid in the tubes with the fresh eggs.

Discussion

The described experiments clearly demonstarte that gravid females have a strong drive to lay their eggs in clusters at one and the same site and in crevices on rough substrates, even when no additional visual or chemical stimuli are present. Smooth surfaces do not seem to be a suitable place for females to deposit eggs. The reason for this may be that eggs laid in crevices and in clusters are more protected against desiccation and predation. Moreover, as already said before, clustering of eggs may have several advantages resulting in higher survival rates for the eggs, larvae as well as the adults. We also found strong indications that the clustering strategy secures survival of the fittest when temporary food shortage occurs. In places with low availability of food, eggs and young larvae may serve as a food source for older larvae. In fact, we frequently observed that without additional food source a number of larvae in a cluster survived and pupated by eating eggs and other larvae.

The smell of decaying organic material attracts gravid females and induces oviposition, probably because these substrates may be suitable food sources for the larvae. In addition, we have strong indications that the odour of eggs collected within 30 minutes after oviposition is attractive to gravid females and stimulates ovipostion, suggesting the presence of an oviposition stimulating pheromone. However, 2-6 h old eggs do not enhance oviposition, suggesting that the pheromone may disappear shortly after oviposition. This agrees with the results of McCall (1995), who found that significantly more blackflies (*Simulium damnosum*) oviposited on substrates baited with freshly laid eggs than on control substrates. Substrates baited with 12-hour-old eggs were not significantly more attractive

Application of an oviposition stimulating pheromone at suitable locations in the flies' habitat provides new perspectives to environmentally friendly control of houseflies. The possibility to control flies at moments within their life cycle when new generations are created would provide a most effective way of attacking the problem, namely the nuisance of the flies. Removal of individuals from the population at a moment they do not cause annoyance and are not yet able to propagate is a more efficient way of control than removal of adult individuals. The challenge of further is to identify the oviposition pheromone.

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).

Chapter 8

GENERAL DISCUSSION

The housefly is a highly successful insect species and creates considerable problems especially in the agricultural sector. The substantial production losses, occurrence of stress by their irritating presence (Axtell and Arends, 1990), and the fact that flies can act as transmitters of many bacterial and viral pathogens (Pospischil, 1994), has strongly stimulated the demand for an adequate control of the flies. The customary approach is to apply insecticides. However, the rapidly spreading resistance of *Musca domestica* to the environmentally unfriendly insecticides (Chapman and Morgan 1992, Pospischil et al. 1996), has led to more frequent application of increasingly higher doses of insecticides. This has caused severe chemical contamination of the environment.

The main questions we were dealing with in the present study were:

1. Does the production of cuticular hydrocarbons that affect the behaviour of the flies, differ in different fly populations (laboratory versus wild-type strains) and, if so, do the behavioural responses of these strains to these hydrocarbons differ?
2. Does an oviposition-stimulating pheromone exist in *M. domestica?*
3. Can we use these semiochemicals for environmentally friendly control of houseflies?

We found that the behavioural role of one of the cuticular substances, (Z)-9-tricosene (‘muscalure’), as a female sex pheromone is overestimated. (Z)-9-tricosene is commonly considered to be the most important component of the female sex pheromone of the housefly since behavioural studies have shown that it is attractive to male *M. domestica* and induces sexual behaviour in males. However, our results showed that wild-type strains produce muscalure hardly or not at all (Chapter 2). As a consequence, we concluded that this chemical is not indispensable for reproduction of

M. domestica. This implies that, in contrast to what has been advanced by Uebel et al. (1978) and Adams and Holt (1987), the oxidation products of muscalure, (Z)-9,10-epoxytricosane and (Z)-14-tricosen-10-one, also may not be essential to induce sexual activity in male flies. We propose that, if present, (Z)-9-tricosene, being the most volatile of the cuticular hydrocarbons of the females, is perceived easier by the males than other less volatile hydrocarbons of females and therefore may be the most 'suitable' hydrocarbon for short-range attraction. Other hydrocarbons may also play a role in inducing sexual behaviour in males. In contrast to experiments in the laboratory where a single hydrocarbon can be tested for its behavioural effects on males, this situation will not be met in nature. In natural conditions, a male fly approaching a female, will never be exposed to a single hydrocarbon. It will always occur in combination with other components of a complex blend. We therefore considered the idea that the males only respond to one chemical out of a complex mixture of chemicals present on the cuticle of females rather questionable. Single-cell responses to muscalure do not markedly differ from the responses to other cuticular hydrocarbons. In fact, Kelling (2001) showed that single cells in the antennae of both male and female *M. domestica* respond to many different chemicals and therefore all cells are probably generalists. This situation is totally different from that in, for example, several species of moths where specialist cells in the antennae detect sex pheromones. This specificity for pheromone molecules is not present in houseflies. We showed that EAG responses to (Z)-9-tricosene were somewhat lower than to (Z)-9-heneicosene, which is 2 C atoms shorter, and higher than the responses to (Z)-9-heptacosene, which is 4 C atoms longer. This again suggests that the perception of substances, and thereby the behaviour of the males, may be determined to a considerable extent by their volatility. These findings suggested that a mixture of cuticular lipids may form the most suitable attractant to be used in control methods. For practical purposes a mixture of a small number of chemicals should be composed that approximates the natural blend as close as possible and that can be produced easily. A good starting point for field experiments should be a mixture of (Z)-9-tricosene and (Z)-9-heptacosene, because these chemicals have proven to induce sexual behaviour and are relatively easily to synthesize (Chapters 3 and 5). The addition of (Z)-9-heneicosene, which lowers the melting point and makes the mixture more volatile, should be considered.

We found that selection processes in isolated housefly populations may lead to the production of higher amounts of muscalure by the females (Chapters 2 and 3) and that relative humidity and temperature may affect the production of cuticular hydrocarbons by both males and females (Chapter 4). Lower relative humidity and higher temperatures may lead to higher amounts of these substances on the flies and to a relative increase of long-chain saturated hydrocarbons. These changes may account for concomitant changes in cuticular permeability to water, as has been found in several species of arthropods (Toolson and Hadley, 1979; Hadley and Schultz, 1987). In the tiger beetles, *Cicindela obsoleta* and *C. oregano*, for example, Hadley and Schultz (1987) showed a significant negative correlation between water loss rate and the quantity of saturated hydrocarbons. Gibbs *et al*. (1995) suggested that a potential consequence of pheromone production by female houseflies may be that cuticular transpirationis increased because the pheromone components are unsaturated or methyl-branched, which tends to lower melting temperatures. We propose that in an environment where rapid changes in temperature and humidity may occur, the presence of muscalure may negatively interfere with the water barrier function of the cuticular layer. In our opinion, this is the main reason why muscalure was not or hardly produced by the wild-type flies we collected in stables in The Netherlands. In laboratory cultures that are usually kept at a constant temperature of about 25 $^{\circ}$C this negative effect will not be met, even at a rather high production level of muscalure. Females living in the laboratory in successive generations will, therefore, produce this substance with its relatively high volatility and attractiveness to males, in higher amounts. This leads to the conclusion that results obtained from laboratory housefly populations cannot simply be considered valid for wild-type populations. In addition, differences in hydrocarbon composition may be expected between housefly populations living at different climatic circumstances and feeding from substrates of different composition. As to the latter, it has been observed that the amounts of (Z)-9-heptacosene are significantly higher on females fed with sugar than on those fed with protein (Adams and Nelson, 1990).

It may be assumed that males may be more sensitive to blends of hydrocarbons that are in close harmony with the cuticular hydrocarbon composition of the females of their own strain. This implies that, if these semiochemicals are used for control purposes, the composition of the hydrocarbon blend has to be adjusted to the local situation.

Houseflies prefer to deposit their eggs in clusters at one and the same site in crevices in rough substrates, especially when these have a smell of decaying animal products. We found strong indications that an oviposition pheromone is deposited together with the eggs (Chapter 7). Identification and synthesis of this oviposition-stimulating pheromone should be one of the main goals in future research. Attracting pregnant females using pheromones to places where they can be killed may be a very effective way of controlling the flies.

The new 'self-loading' technique for semiochemicals described in Chapter 5 can also be used in behavioural studies on other insects. The use of aggressive solvents, which may affect the condition and behaviour of the animals is avoided. Moreover, using this technique, the distribution of the semiochemicals over the various body parts mimics the natural distribution better than when these substances are applied in a solvent to the thorax of the insect.

Up till now hardly any attention has been paid to the behaviour of females during copulatory attempts of males. Without protrusion of the ovipositor by a female copulation cannot be established. It is still unknown why some males are immediately 'accepted' by females and other males not or only after they have made several strikes. The described technique allows, for example, males to be loaded in a non-aggressive way with different amounts of various semiochemicals for studying the sexual behaviour of the females to these males.

The radar-Doppler technique (Chapter 6) provides a relatively easy way to find out which semiochemicals induce behavioural responses in flies. In this way biologically active and non-active substances can be separated relatively easily before the above experiments are carried out. The technique is also suitable for studies on other insect species.

REFERENCES

Adams, T.S., Holt, G.G., 1987. Effect of pheromone components when applied to different models on male sexual behaviour in the housefly *Musca domestica.* J. Insect Physiol. 33**,** 9-18.

Adams, T.S., Nelson, D.R., 1990. The influence of diet on ovarian maturation, mating and pheromone production in the housefly, *Musca domestica*. Invert. Reprod. Develop. 17, 193-201.

Adams, T.S., Nelson, P.R., Fatland, C.L., 1995. Effect of methyl alkanes on male house fly *Musca domestica*, sexual behaviour. J. Insect Physiol. 41, 443-449.

Adler, V.E., Uebel, E.C., Schwarz, M., 1984. Production of (Z)-9-tricosene by colonized and feral female house flies. Southwest. Entomol. 9, 223-226.

Ahmad, S., Mackay, M.E., Blomquist, G.J., 1989. Accumulation of the female sex pheromone and its transfer to, and metabolism in, the male housefly, *Musca domestica L*., during courtship and mating. J. Insect Physiol. 35, 775-780.

Anonymous, 1976. Control of insects affecting livestock. USDA Agricult. Research Service Natural Research Program no. 2048.

Antony, C., Jallon, J.M., 1982. The chemical basis for sex recognition in *Drosophila melanogaster*. J. Insect Physiol. 28, 873-880.

Axtell, R.C., Arends, J.J., 1990. Ecology and management of arthropods pests of poultry. Annu. Rev. Entomol. 35, 101-126.

Beament, J.W.L., 1945. The cuticular lipids of insects. J. Exp. Biol. 21, 115-131.

Bellingham, J., Anderson, M., 1993. Variations and sexual differences in the spectral sensitivity of the compound eye of the housefly *Musca domestica* (L) and the lesser housefly *Fannia canicularis*. In: Proc. of the Int. Conf. on Insects Pests in the Urban Environment p. 480. Cambridge.

Betke, P., Hiepe, T., Mueller, P. Ribbeck, R., Schultka, H., Shumann, H., 1989. Biological control by means of *Ophyra aenescens* of *Musca domestica* on pig farming units. Monatshefte für Veterinärmedizin 44, 842-844.

Blomquist, G.J., Dillwith, J.W., Pomonis, J.G., 1984. Sex pheromone of the housefly, metabolism of (Z)-9-tricosene to (Z)-9, 10-epoxytricosane and (Z)-14-tricosene-10-one. Insect Biochem. 14, 279-284.

Blomquist, G.J., Nelson, D.R., De Renobales, M., 1987. Chemistry, biochemistry, and physiology of insect cuticular lipids. Arch. Insect Biochem. Physiol. 6, 227-265.

Buchnan, P.B, Moreton, R.B., 1981. Flying and walking of small insects (*Musca domestica*) recorded differentially with a standing-wave radar actograph. Physiol. Entomol. 6, 149-155.

Buchnan, P.B., Satelle, A.R.C., 1979. A radar-Doppler autocorrelation analysis of insect activity. Physiol. Entomol. 4, 103-109.

Burkhardt, D., 1962. Spectral sensitivity and other response characteristics of single visual cells in the arthropod eye. Symp. Soc. Exp. Biol. 16, 86-109.

Butenandt, A., Beckmann, R., Stamm, D., Hecker, E., 1959. Ueber den Sexual-Lockstoff des Seidenspinners *Bombyx mori*. Reinanstellung und Konstitution. Zeitschrift Naturforsch. B. 14, 283-284.

Carlson, D.A., Beroza, M., 1973. Field evaluation of (Z)-9-tricosene, a sex attractant pheromone of the house fly. Environ. Entomol. 2, 555-559.

Carlson, D.A., Doolitle, R.E., Beroza, M., Rogoff, W.M., Gretz, G.H., 1974. Muscalure and related compuonds. I. Response of houseflies in olfactometer and pseudofly tests. J. Agr. Food Chem. 22, 194-197.

Carlson, D.A., Leibold, C.M., 1981. Field trials of pheromone-toxicant devices containing muscalure for house flies (Diptera: Muscidae). J. Med. Entomol. 18, 73-77.

Carlson, D.A., Mayer, M.S., Silhacek, D.L., James, J.D., Beroza, M., Bierl, B.A., 1971. Sex attractant pheromone of the housefly: isolation, identification and synthesis. Science 174, 76-78.

Chapman, J.W., Howse, P.E., Knapp, J.J., Goulson, D., 1998. Evaluation of three (Z)-9-tricosene formulations for control of *Musca domestica* (Diptera: Muscidae) in caged-layer poultry units. J. Econ. Entomol. 91, 915-922.

Chapman, P.A., 1985. The resistance to eighteen toxicants of a strain of *Musca domestica* L. collected from a farm in England. Pestic. Sci. 16, 271-276.

Chapman, P.A., Morgan, C.P., 1992. Insecticide resistance in *Musca domestica* L. from eastern England. Pestic. Sci. 36, 35-45.

Dawson, G.W., Laurence, B.R., Pickett, J.A., Pile, M.M., Wadhams, L.J., 1989. A note on the mosquito oviposition pheromone. Pestic. Sci .27, 277-280.

Deay, H.O., Taylor, J.G., 1962. Response of the housefly *Musca domestica* to electric lamps. Proc. Ind. Acad. Sci. 72, 161-166.

Den Otter, C. J., De Cristofaro, A., Voskamp, K. E., Rotundo, G., 1996. Electrophysiological and behavioural responses of chestnut moths, *Cydia fagiglandana* and *C. splendana* (Lep., Tortricidae), to sex attractants and odours of host plants. J. Appl. Entomol. 120, 413-421.

Den Otter, C. J., Tchicaya, T., Schutte, A.M., 1991. Effects of age, sex and hunger on the antennal olfactory sensitivity of tsetse flies. Physiol. Entomol. 16, 173-182.

Den Otter, C. J., Tchicaya, T., Van den Berg, M.J., 1988. Olfactory sensitivity of five species of tsetse (*Glossina* spp.) to 1-octen-3-ol, 4-heptanone, 3-nonanone and acetone. Insect Sci. Applic. 9, 213-218.

Dillwith, J.W., Adams, T.S., Blomquist, G.J., 1983. Correlation of housefly sex pheromone production with ovarian development. J. Insect Physiol. 29, 377-386.

Dillwith, J.W., Blomquist, G.J., 1982. Site of sex pheromone biosynthesis in the female house fly, *Musca domestica*. Experientia 38, 471-473.

Dilwith, J.W., Blomquist, G.J., Nelson, D.R., 1981. Biosynthesis of the hydrocarbon components of the sex pheromone of the housefly, *Musca domestica* L. Insect. Biochem. 11, 247-253.

Dougherty, M.J., Hamilton, J.G.C., 1997. Dodecanoic acid is the oviposition pheromone of *Lutzomyia longipalpis*. J. Chem. Ecol. 12, 2657-2671.

Dougherty, M.J., Hamilton, J.G.C., Ward, R.D., 1993. Semiochemical mediation of oviposition by the phlebotomine sandfly *Lutzomyia longipalpis*. Med. Vet. Entomol. 7, 219-224.

Dougherty, M.J., Hamilton, J.G.C., Ward, R.D., 1994. Isolation of oviposition pheromone from the eggs of the sandfly *Lutzomyia longipalpis*. Med. Vet. Entomol. 8, 119-124.

Edney, E.B., 1977. Water balance in land arthropods. Springer, Berlin.

Elliot, M., Janes, N.F., Potter, C., 1978. The future of pyrethroids in insect control. Ann. Rev. Entomol. 23, 443-469.

ElNaiem, D.A., Ward, R.D., 1990. An oviposition pheromone on the eggs of sandflies (Diptera: Psychodidae). Trans. Royal Soc. Trop. Med. Parasit. 84, 456-475.

ElNaiem, D.A., Ward, R.D., 1991. Response of the sandfly *Lutzomyia longipalpis* to an

oviposition pheromone associated with conspecific eggs. Med. Vet. Entomol. 5, 87-91.
Farkas, R., Jantnyik,T., 1992. Laboratory studies on *Hydrotaea aenescens* as predator of house fly larvae (Diptera: Muscidae). Parasitol. Hungar. 23, 103-108.
Geden, C.J., 1997. Evaluation of *Paraiotonchium muscadomesticae* (Nematoda: Tylenchida: Iotochiidae), a potential biological control agent of the housefly (Diptera: Muscidae). Biological Control 10, 42-47.
Gibbs, A., 1995. Physical properties of insect cuticular hydrocarbons: model mixtures and lipid interaction. Comp. Biochem. Physiol. 112, 667-672.
Gibbs, A., Kuenzli, M., Blomquist, G.J., 1995. Sex- and age-related changes in the biophysical properties of cuticular lipids of the housefly, *Musca domestica*. Arch. Insect Biochem. and Physiol. 29, 87-97.
Gibbs, A. , Mousseau, T.A., 1994. Thermal acclimation and genetic variation in cuticular lipids of the lesser migratory grasshopper (*Melanoplus sanguinipis*): Effects of lipid composition on biophysical properties. Physiol. Zool. 67, 1523-1543.
Gibbs, A., Mousseau, T.A., Growe, J.H., 1991. Genetic and acclimatory variation in biophysical properties of insect cuticle lipids. Proc. Nat. Acad. Sci.USA88, 7257-7260.
Gibbs, A., Pomonis, J.G., 1995. Physical properties of insect cuticular hydrocarbons: The effect of chain length, methyl-branching and unsaturation. Comp. Biochem. Physiol. 112B, 243-249.
Greene, G.L., Guo, Y., Chen, H., 1998. Parasitization of house fly pupae (Diptera: Muscidae) by *Spalangia nigroaenea* (Hymenoptera: Pteromalidae) in cattle feedlot environments. Biological Control 12, 7-13.
Hadley, N.F., 1977. Epicuticular lipids of the desert tenebrionid beetle, *Eloedes armata*: seasonal and acclimatory effects on composition. Insect Biochem. 7, 277-283.
Hadley, N.F., 1978. Cuticular permeability of desert tenebroid beetles: correlations with the epicuticular hydrocarbon composition. Insect Biochem. 8, 17-22.
Hadley, N.F., Schultz, T.D., 1987. Water loss in three species of tiger beetles (*Cicindela*): correlations with epicuticular hydrocarbons. J. Insect Physiol. 33, 677-682.
Hendrikse, A., 1990. Sex-pheromone communication and reproductive isolation in small ermine moths. Thesis. University of Leiden, The Netherlands.
Hill, I.R., 1985. The pyrethroids insecticides (J.P. Leahey, Ed.), pp 151-262.
Howard, R.W., Blomquist, G.J., 1982. Chemical ecology and biochemistry of insect hydrocarbons. Ann. Rev. Entomol. 27, 149-172.
Howard, J., Wall, R., 1996. Control of the housefly *Musca domestica* in poultry units: Current techniques and future prospects. Agricult. Zool. Rev. 7, 247-265.
Howard, J., Wall, R., 1996a. Autosterilization of the housefly, *Musca domestica*, using the chitin synthesis inhibitor triflumuron on sugar-baited targets. Med. Vet. Entomol. 10, 97-100.
Howard, J., Wall, R., 1996b. Autosterilization of the house fly, *Musca domestica* (Diptera: Muscidae) in poultry houses in north-east India. Bull. Entomol. Res. 86, 363-367.
Islam, M.S., Port, G.R., 1994. Effect of a synthetic pheromone when applied to different models on male mating behaviour in *Musca domestica*. Pak. J. Sci. Ind. Res. 37, 160-161.
Johnson, C., Bishop, A.H., Turner, C.L., 1998. Isolation and activity of strains of *Bacillus thuringiensis* toxic to larvae of the housefly (Diptera: Muscidae) and tropical blowflies (Diptera: Calliphoridae). J. Invert. Pathol. 71, 138-144.
King, B.H., 1997. Effects of age and burial of house fly (Diptera: Muscidae) pupae on parasitism by *Spalangia cameroni* and *Muscidifurax raptor* (Hymenoptera: Pteromalidae). Environ.

Entomol. 26, 410-415.
Knoppien, P., Van der Pers, J.N.C., Van Delden, W., 2000. Quantification of locomotion and the effect of food deprivation on locomotor activity in *Drosophila*. J. Insect Behav. 13, 27-43.
Kuramoto, H., Shimazu, M., 1997. Control of house fly populations by *Entomophtora muscae* (Zygomycotina: Entomophtorales) in a poultry house. Appl. Entomol. Zool. 32, 325-331.
La-France, D., Shani, A., Margalit, J., 1989. Biolological activity of synthetic hydrocarbon mixtures of cuticular components of the female housefly (*Musca domestica* L.). J. Chem. Ecol. 15, 1475-1490.
Lancaster, J.L.Jr., Simco, J.S., 1969. House fly control by chemical sterilization with Apholate. Arkansas Experimental Station, Bulletin 737, 12 pp.
Lemke, L.A., Koehler, P.G., Patterson, R.S., 1990. Laboratory method for measuring the attractiveness of pheromones to adult *Musca domestica* (Diptera: Muscidae). J. Med. Ent. 27, 1062-1064.
Lindquist, A.W., Madden, A.H., Wilson, H.G., Knipling, E.F., 1945. DDT as a residual type treatment for control of houseflies. J. Econ. Entomol 38, 257.
Lindquist, D.A., Abusowa, M., Hall, M.J.R., 1992. The New World screwworm fly in Libya: a review of its introduction and eradication. Med. Vet. Entomol. 6, 2-8.
Long, D., 1953. Effects of population density on larvae of Lepidoptera. Trans. R. Entomol. Soc. Lond. 104, 543-585.
Luvchiev, V.I., Tsankova, R.N., 1994. *Ophyra capensis* in Bulgaria and the possibilities for its use as an antagonist of *Musca domestica* L. Ekolgiya Sofia 25, 117-124.
Mansingh, A., Steele, R.W., Smallman, B.N., 1972. Pheromone effects of *cis*-9 long chain alkenes on the common house fly : An improved sex attractant combination. Can. Ent. 104, 1963-1965.
Mayer, M.S., Thaggard, C.W., 1966. Investigations of an olfactory attractant specific for males of the housefly, *Musca domestica*. J. Insect Physiol. 12, 891-897.
McCall, P.J., 1995. Oviposition aggregation pheromone in the *Simulium damnosum* complex. Med. Vet. Entomol. 9, 101-108.
McCall, P.J., Cameron, M.M., 1995. Oviposition pheromones in insect vectors. Parasitology Today 11, 352-355.
McCall, P.J., Trees, A.J., Walsh, J.F., Molyneux, D.H., 1994. Aggregated oviposition in the *Simulium damnosum* complex is mediated by eggs in a laboratory bioassay. Med. Vet. Entomol. 8, 76-80.
McCann, G.D., Arnett, D.W., 1972. Spectral and polarisation sensitivity of the dipteran visual system. J. Gen. Phys. 59, 534-558.
Menn, J.J., Hollingworth, R.M., 1985. Comprehensive Insect Physiology, Biochemistry and Pharmacology in: Insect Control, Pergamon Press, Oxford 12, 1-8.
Miller, R.W., Pickens, L.G., Potts, W.E., 1993. Comparison of traps and an integrated program to manage house flies and stable flies on dairy farms. J. of Agricult. Entomol. 10, 189-196.
Mitchell, E.R., Tingle, F.C., Carlson, D.A., 1975. Effect of muscalure on house fly traps of different color and location in poultry houses. J. Georgia Entomol. Soc. 10, 168-174.
Miyamota, J., Hirano, M., Takimoto, Y., Hatakoshi, M., 1993. Insect growth regulators for pest control, with emphasis on juvenile hormone analogs. Present status and future prospects. ACS Symposium Series 524, 144-168.

Morgan, N.O., Pickens, L.G., 1968. Influence of air temperature on the attractiveness of electric lamps to house flies. J. Econ. Entomol. 61, 1257-1259.

Mullens, B.A., Hinkle, N.C., Szijj, C.E., 1996. Impact of alternating manure removal schedules on pest flies (Diptera: Muscidae) and associated predators (Coleoptera: Histeridae, Staphylinidae: Acvarina: Macrochelidae) in caged-layer poultry manure in southern California. J. Econ. Entomol. 89, 1406-1417.

Murvosh, P.B., LaBreque, G.C., Smith, C.N., 1965. Sex attraction in the house fly *Musca domestica* L. Ohio J. Sci. 65, 68-71.

Nelson, D.R., 1978. Long-chain methyl branched hydrocarbons: occurrence, biosynthesis, and function. Adv. Insect Physiol. 13, 1-33.

Nelson, D.R., Dillwith, J.W., Blomquist, G.J., 1981. Cuticular hydrocarbons of the house fly *Musca domestica.* Insect Biochemistry 11, 187-197.

Noorman, N., Den Otter, C. J., Biancaniello, G., 1998. Variations in sexual activity, electrophysiological responses and muscalure quantities between different strains of houseflies. Chem. Senses 24, 97.

Pap, L., Farkas, R., 1994. Monitoring of resistance of insecticides in house fly (*Musca domestica*) populations in Hungary. Pesticide Science 40, 245-258.

Pimental, D., Perkins, J.H., 1980. Pest control: Cultural and environmental aspects. AAAS selected symposium, 43, Westview Press, Colorado. 243 pp.

Plapp, F.W. Jr., 1986. Genetics and biochemistry of insecticide resistance in arthropods: Prospects for the future. In: Pesticide Resistance: Strategies and Tactics for Management. National Academy Press, Washington. pp. 74-86.

Pospischill, J., 1994. *Musca domestica* in livestock and poultry farming: problems and solutions. Public. Health, Bayer 9, 14-17.

Pospischill, J., Szomm, K., Londershausen, M., Schroder, I., Turberg, A., Fuchs, R., 1996. Multiple resistance in the larger house fly *Musca domestica* in Germany. Pestic. Sci., 48, 333-341.

Rao, K.V., Kaiser Jamil, Machwe, V.G., Tamaki, Y., 1990. Identification of three chemical components from male pheromone glands of *Musca domestica* L. Journal of Reprod. Biol. Comp. Endocrinol. 2, 88-93.

Rao, K.V., Machwe, V.G., Kaiser Jamil, 1988. Extraction and isolation of sex pheromone of male *Musca domestica* (L). Journal of Reprod. Biol. Comp. Endocrinol. 8, 68-73.

Rees, C.J.C., 1970. Age dependency of response in an insect chemoreceptor sensillum. Nature 227, 740-742.

Renn, N., 1995. Mortality of immature houseflies (Musca domestica L.) in artificial diet and chicken manure after exposure to encapsulated entomopathogenic nematodes (Rhabditida: Steinernematidae, Heterorhabditidae). Biocontrol Science and Technology 5, 349-359.

Renn, N., 1998. The efficacy of entomopathogenic nematodes for controlling housefly infestations of intensive pig units. Medical and Veterinary Entomology 12, 46-51.

Renou, R., Berthier, A., Desbarats, L., Van der Pers, J., Querrero, A., 1999. Actographic analysis of the effects of an esterase inhibitor on male moth responses to sex pheromone. Chemical Senses 24, 423-428.

Richter, I., 1974. Sex attraction of the house fly by moving dummies is not increased by pheromones. Naturwissenschaften 61, 365.

Richter I., Krain H., Mangold, H. K., 1976. Long-chain (Z)-9-alkenes are 'psychedelics' to houseflies with regard to visually stimulated sex attraction and aggregation. Experientia 32, 186-188.

Rogoff, W.M., Beltz, A.D., Johnsen, J.O., Plapp, F.W., 1964. A sex pheromone in the housefly *Musca domestica* L. J. Insect Physiol. 10, 239-246.

Rogoff, W.M., Gretz, G.H., Jacobson, M., Beroza, M., 1973. Confirmation of (Z)-9-tricosene as a sex pheromone of the house fly. Ann. Ent. Soc. Am. 66, 739-741.

Rogoff, W.M., Gretz, G.H. Sonnet, P.F., Schwarz, M., 1980. Responses of male house flies to muscalure and to combinations of hydrocarbons with and without muscalure. Environ. Entomol. 9, 605-606.

Rutz, D.A., Scoles, G.A., Howser, G.G., 1988. Evaluation of fly-electrocuting black light devices in caged-layer poultry facilities. Poultry Science 67, 871-877.

Silhacek, D.L., Carlson, D.A., Mayer, M.S., James, J.D., 1972. Composition and sex attractancy of cuticular hydrocarbons from houseflies: Effects of age, sex, and mating. J. Insect Physiol. 18, 347-354.

Silhacek, D.L., Oberlander, H., Zettler, J.L., 1976. Susceptibility of malathion-resistant strains of *Plodia interpunctella* to juvenile hormone treatments. J. Stored Prod. Res. 20, 201-203.

Stamp, N. E., 1980. Egg deposition patterns in butterflies: why do some species cluster their eggs rather than deposit them singly? The American Naturalist 115, 367-380.

Stoffolano, J.G., 1973. Effect of age and diapause on the mean impulse frequency and failure to generate impulses in labellar chemoreceptor sensilla of *Phormia regina*. J. Gerontol. 28, 35-39.

Syms, P.R., Goodman, L.J., 1987. The effect of flickering U-V light output on the attractiveness of an insect electrocutor trap to the house-fly, *Musca domestica.* Entomol. Exp. Appl. 43, 81-85.

Tanada, Y., Holdaway, F.G., Quisenberry, J.H., 1950. DDT to control flies breeding in poultry manure. J. Econ. Entomol. 43, 30

Tillman-Wall, J.A., Vanderwel, D., Kuenzli, M.E., 1992. Regulation of sex pheromone biosynthesis in the housefly, *Musca domestica*: Relative contribution of the elongation and reductive steps. Arch. Biochem. Biophys. 299, 92-99.

Tobin, N.E., Stoffolano, Jr. J.G., 1973. The courtship of *Musca* species found in North America. 1. The house fly, *Musca domestica.* Ann. Ent. Soc. Am. 66, 1249-1257.

Toolson, E.C., Hadley, N.F., 1979. Seasonal effects on cuticular permeability and epicuticular lipid composition in *Centruroides sclupturatus* Ewing 1928 (Scorpiones Buthidae). J. Comp. Physiol. 129, 319-325.

Toolson, E.C., Markow, T.A., Jackson, L.L., Howard, R.W., 1990. Epicuticular hydrocarbon composition of wild and laboratory-reared *Drosophila mojavensis* Patterson and Crow. Ann. Entomol. Soc. Am. 83, 1165-1176.

Tsankova, R.N., Luvchiev, V.I., 1993. Laboratory investigations on the larval zoophagy of *Ophyra capensis*: An antagonist of *Musca domestica*..Appl.Parasitol. 34, 221-228.

Uebel, E.C., Sonnet, P.E., Menzer, R.E., Miller, R.W., Lusby, W.R., 1977. Mating-stimulant pheromone and cuticular lipid constituents of the little house fly, *Fannia canicularis* (L.). J. Chem. Ecol. 3, 269-278.

Uebel, E.C., Sonnet, P.E., Miller, R.W., 1976. House fly sex pheromone: Enhancement of mating strike activity by combination of (Z)-9-tricosene with branched saturated hydrocarbons. J. Econ. Entomol. 5, 905-908.

Uebel, E.C., Sonnet, P.E., Miller, R.W., Beroza, M., 1975. Sex pheromone of the face fly, *Musca autumnalis* De Geer. (Diptera: Muscidae). J. Chem. Ecol. 1, 195-202.

Uebel, E.C., Schwarz, M., Lusby, B.R., Miller, R.W., Sonnet, P.E., 1978. Cuticular non-hydrocarbons of the female housefly and their evolution as mating stimulants. Lloydia 41, 63-67.

Van der Broek, I.V.F., Den Otter, C.J., Van der Pers, J.N.C., 1999. Actometers for evaluating mosquito activity: daily activity and stimulus-induced activation. Proc. Exper. & Appl. Entomol., N.E.V. Amsterdam 10, 85-90.

Van der Goes van Naters, W.N., Den Otter, C.J., 1992. Olfactory sensitivity of tsetse flies: a daily rhythm. Chem. Senses 23, 351-357.

Vaz, A.H., Brownson, A., Blomquist, G.J., Reitz, R.C., 1989. Sex pheromone biosynthesis in the housefly: Evidence for the regulation of the fatty acyl-CoA desaturation and elongation system by 20-hydroxyecdysone. Arch. Insect Biochem. Physiol. 12, 173-186.

Veal, L., Bath, C., Hutcheson, D., 1995. A comparison of the attractiveness towards house flies of two lamps used in insect electrocuting traps. Int. J. Environm. Health. Res. 5, 247-254.

Watson, D.W., Rutz, D.A., Long, S.J., 1996. *Beauveria bassiana* and sawdust bedding for the management of the house fly, *Musca domestica* (Diptera: Muscidae) in calf hutches. Biological Control 7, 221-227.

West, L.S., 1951. The housefly, its natural history, medical importance and control. New York. 584 pp.

Wigglesworth, V.B., 1945. Transpiration through the cuticle of insects. J. Exp. Biol. 21, 97-114.

SUMMARY

Houseflies (*Musca domestica* L.) often make themselves a nuisance in human and livestock habitations. Moreover, these flies may transmit over a hundred different pathogens. They can transmit intestinal worms or their eggs, and are potential vectors of pathogens of, for example, dysentery, gastroenteritis, typhoid, cholera and tuberculosis. Organic materials and the relative high temperatures present in animal and human dwellings promote rapid development and allow the continuous presence of the flies. The main goal of the studies described in this thesis was to reveal the role that certain semiochemicals produced by *M. domestica* could play in environmentally friendly control of the flies.

According to the literature of the last three decades, a pheromone is present on the body of housefly females which induces sexual behaviour in males. The main component of this female sex pheromone is supposed to be (Z)-9-tricosene ('muscalure'), one of the more than 150 cuticular hydrocarbons of females. (Z)-9-tricosene was found to be present in relatively high amounts on the females. This knowledge on the production of cuticular hydrocarbons and on their role in the behaviour of the flies has mainly been collected from laboratory strains of the flies. However, in order to be able to control the flies in their natural environments, we considered studies on wild-type flies also necessary. Therefore, we paid attention to houseflies which had been kept in culture in the laboratory for 40 years (WHO strain of flies), but also to flies obtained from a poultry breeding (Van Diermen strain) and from a cow-house with pig-sty (Pesse strain). The latter two strains had been cultured in the laboratory for different numbers of generations.

In Chapter 2 gas chromatographical studies are described with showed striking differences between the cuticular hydrocarbon composition of females of the WHO strain and females of the Van Diermen and Pesse strains. It appeared that on WHO females hydrocarbons with 23-25 C atoms constituted about 65% of the total hydrocarbons, whereas on first-generation wild-type females less than 2% of these compounds was present. Muscalure comprised up to 20-30% of the total hydrocarbons on 5-20-day-old WHO females, whereas less than 0.5% muscalure was present on the wild-type females. On males of both the WHO and wild-type strains (Z)-9-heptacosene was the most abundant hydrocarbon; males did not produce muscalure. It was found that in the course of time in 'mixed populations' (both sexes

in one and the same cage) muscalure was transferred from females to males and (Z)-9-heptacosene from males to females. It is suggested that this transfer of hydrocarbons between the sexes may result in modification of the original male and female cuticular hydrocarbon composition and may enable one sex to recognize whether the other sex has already copulated or not.

The marked differences in muscalure quantities on WHO and first-generation wild-type females led us to suggest that selection in subsequent generations of high-density populations may lead to increased production of muscalure by the females. Indeed, we found that after several generations in the laboratory the amounts of muscalure on females had increased considerably (Chapters 2 and 3). Apparently, higher amounts of this substance increased the attractiveness of females to males. Selection did not affect the production of other cuticular hydrocarbons by the females apart from that of tricosane, the production of which was shown to be closely linked to that of muscalure.

Despite the low quantities of muscalure on wild-type females no differences in reproduction capacity were observed between the different strains, which suggested that muscalure is not indispensable for mating. To investigate the role of muscalure in more detail we studied the effects of females from strains containing different muscalure quantities on sexual behaviour of the males. In addition, we determined the olfactory sensitivity of males and females from the different strains to muscalure by recording the electrical responses (electroantennograms) of their antennal olfactory cells (Chapter 3). The results showed that male sexual activity was higher towards females with higher amounts of muscalure on their cuticle. Moreover, males from strains with more muscalure on the females appeared to be more active, indicating that selection in laboratory cultures not only increases muscalure production in females but also sexual activity of males. The electroantennogram recordings indicated that males as well as females of all three strains were equally able to detect muscalure, which suggested that differences in sexual behaviour were not due to differences in ability to smell this substance.

Apart from acting as pheromones, cuticular hydrocarbons of insects provide a barrier to water diffusion, thus preventing desiccation of the animals. It is known that environmental factors may affect the production of cuticular hydrocarbons. As was remarked above, on females of subsequent generations of laboratory populations, cultured at constant temperature and humidity, the quantities of both muscalure and

tricosane increased, whereas those of the other cuticular hydrocarbons remained the same. This led us to assume that temperature and humidity may mainly determine the quantities of the non-pheromonal components, whereas muscalure may primarily be produced as a result of a selection process in high-density populations in isolated environments. We expected that selection proceeds faster in high-density than in low-density populations.

In Chapter 4 we studied the effects of relative humidity, temperature and population density on the production of cuticular hydrocarbons. The results showed that the production of these substances by both males and females of *M. domestica* was delayed up to at least 3 days after emergence under very wet conditions (90% r.h.) compared to the production at 50 and 20% r.h. Eight days after emergence, however, males contained the same amounts of hydrocarbons at all three r.h. values, whereas females still possessed less of these substances at 90% r.h. than at 50 an 20% r.h. In our opinion this was due to the fact that males, being more active than females, need more cuticular hydrocarbons to prevent water-loss than females. No indication was found that the r.h. has a different effect on the production of muscalure by females than on the production of the other hydrocarbons. As to the effects of temperature, it appeared that male and female flies produced more hydrocarbons at 35 °C than at 20 °C. In male flies the relative amounts of the various hydrocarbons produced at the two temperatures were the same. In females the production of muscalure at the two temperatures did not differ. However, the relative amounts of nonacosane and of the methyl- and dimethylnonacosanes were significantly higher at 35 °C than at 20 °C. We proposed that this had led to an increase of the melting temperature of the whole mixture of cuticular hydrocarbons, preventing extreme loss of water. Since the melting temperature of the whole mixture of hydrocarbons is higher in males than in females (39.4 and 36.8 °C, respectively: Gibbs *et al*., 1995), we supposed that in males there was no need to change the composition of cuticular hydrocarbons when the temperature is raised to 35 °C. In low-density population cultures (< 20 flies/cage) all females of the 8^{th} generation produced a low amount of muscalure, whereas no muscalure was found on the high-density (> 300 flies/cage) females. Hence, our hypothesis that the production of muscalure may not be affected by changes in humidity and temperature to the same extent as the production of other hydrocarbons did not hold true. The production of muscalure was affected in a similar way by these factors as that of the other hydrocarbons. The population-density

experiments showed that selection may sneak in very rapidly and should always be a point of major attention in laboratory colonies. We assume that because of the relatively large contribution to the total population the properties of a few females are likely to be expressed sooner in the next generations of small populations than in those of large populations.

Studies to reveal the role of cuticular semiochemicals on sexual behaviour of *M. domestica* often imply topical application of the chemicals to the flies when the chemicals are dissolved in an organic solvent like hexane or acetone. In Chapter 5 we showed that this way of application of semiochemicals may strongly affect the flies' condition. In addition, the distribution of these substances over the various body parts may deviate from the natural distribution. We showed that hydrocarbons, when liquid at room temperature, are also taken up and are distributed over the body in a more natural way when the flies are walking on a filter paper onto which the pure chemicals had been pipetted. The higher the amounts of hydrocarbons on the paper and the longer the flies walked on it, the higher the amounts of chemicals taken up.

Using this new "self-loading" technique, females were loaded with (Z)-9-heptacosene or (Z)-9-pentacosene. The former substance is the most abundant hydrocarbon on the cuticle of male houseflies and hardly occurs on females. (Z)-9-pentacosene is absent on houseflies, but acts as a female sex pheromone of the little housefly *Fannia canicularis*, which often is sympatric with *M. domestica*. We hypothesized that both (Z)-9-heptacosene and (Z)-9-pentacosene may inhibit sexual behaviour in male houseflies. However, we found that in contrast to this (Z)-9-heptacosene stimulated copulation when present in relatively high amounts on females, whereas (Z)-9-pentacosene did not affect male sexual behaviour.

In Chapter 6 a radar-Doppler actometer is described with which movements of individual body parts of the flies can be recorded. With this actometer movements of legs, wings and head could be distinguished. Discrimination between movements was possible by comparing the shapes and amplitudes of the recordings visualized in an actogram. Movements of the insect's head were used to obtain an indication of the behavioural responses of houseflies to (Z)-9-tricosene, (Z)-9-heneicosene and a 7:3 mixture of these two chemicals. A comparison was made with the results of EAG recordings obtained in a previous study (Chapter 3) on stimulation with the same substances. It was found that, although (Z)-9-heneicosene evoked high EAG responses in males as well as females, both sexes did not show behavioural responses to this

substance. Comparison of our results with those of field experiments described in the literature led us to suggest that (Z)-9-tricosene may function as an aggregation pheromone bringing males and females together and as a female sex pheromone inducing mating behaviour in males.

In Chapter 7 we describe studies on the oviposition behaviour of female houseflies. We found that the females preferred to deposit their eggs in clusters at one and the same site in crevices on rough substrates even when no additional visual or chemical stimuli were present. The reason for this may be that eggs laid in crevices and clusters are less susceptible of desiccation and predation. The smell of decaying organic material attracted gravid females and induced oviposition, probably because these substrates may be suitable food sources for the larvae. In addition, we obtained strong indication that the odour of eggs collected within 30 min after oviposition was attractive to gravid females and stimulated oviposition suggesting the presence of an oviposition stimulating pheromone. However, eggs which were 2-6 h old, did not enhance oviposition, which suggested that this pheromone may disappear shortly after oviposition because it is very volatile or may disintegrate.

As far as we are aware this was the first indication of the presence of an oviposition pheromone in the housefly. The application of an oviposition pheromone at suitable locations in the flies' habitat may offer new possibilities for environmentally friendly control of houseflies. It is evident that control of the flies at moments within their life cycle when new generations are created may attack the problem -nuisance of the flies- in the most effective way. Removal of individuals from the population at a moment they do not cause annoyance and are not yet able to propagate is a more efficient way of control than removal of adult individuals. Further studies are necessary to identify the pheromone.

SAMENVATTING

Huisvliegen (*Musca domestica* L.) bezorgen mens en dier veel overlast. Bovendien kunnen deze vliegen meer dan 100 pathogenen overbrengen. Ze kunnen vectoren zijn van maag- en darmwormen, of van hun eieren, en van pathogenen van ziekten als dysenterie, gastroenteritus, thyfus, cholera and tuberculose. De aanwezigheid van organisch materiaal en de relatief hoge temperaturen die in stallen en woningen kunnen heersen bevorderen een snelle ontwikkeling en maken mogelijk dat de vliegen het hele jaar door aanwezig zijn. Het hoofddoel van het onderzoek beschreven in dit proefschrift was na te gaan welke rol bepaalde vliegeigen signaalstoffen (semiochemicaliën) kunnen spelen bij een milieuvriendelijke bestrijding van huisvliegen.

Volgens de literatuur van de laatste drie decennia is er op het lichaam van vrouwtjes van huisvliegen een feromoon aanwezig dat sexueel gedrag bij de mannetjes opwekt. Men veronderstelt dat (Z)-9-tricoseen ('muscalure'), een van de meer dan 150 stoffen die op de cuticula van vrouwtjes voorkomen, de belangrijkste component is van dit vrouwelijk sexferomoon. (Z)-9-tricoseen wordt in relatief grote hoeveelheden op de vrouwtjes aangetroffen.

Het onderzoek naar de produktie van cuticulaire koolwaterstoffen en naar hun rol in het gedrag van de vliegen is tot nu toe voornamelijk verricht aan laboratoriumstammen. Willen we echter de vliegen in hun natuurlijke omgevingen kunnen bestrijden dan is onderzoek aan wild-type-vliegen uiteraard ook nodig. Daarom hebben wij niet alleen onderzoek verricht aan vliegen van een zgn. WHO-stam, vliegen die al 40 jaar in het laboratorium in kweek zijn, maar ook aan vliegen afkomstig van een kippenopfokbedrijf (Van Diermen-stam) en van een rundveehouderij annex varkensfokkerij (Pesse-stam).

In hoofstuk 2 worden gaschromatografische studies beschreven waaruit bleek dat er grote verschillen bestonden tussen de samenstelling van huidstoffen van vrouwtjes van de WHO-stam en van de Van Diermen- en Pesse-stam. Het bleek dat bij de WHO-vrouwtjes koolwaterstoffen met 23-25 C-atomen 65% van de totale hoeveelheid cuticulaire koolwaterstoffen uitmaakten. Op eerste-generatie wild-type-vrouwtjes kwam echter niet meer dan 2% van deze stoffen voor. Op 5-20 dagen oude WHO-vrouwtjes maakte (Z)-9-tricoseen 20-30% van de totaal aanwezige koolwaterstoffen uit, terwijl dit bij wild-type-vrouwtjes minder dan 0.5% was. Op

mannetjes van zowel de WHO- als de wild-type-stammen was (Z)-9-heptacoseen de meest voorkomende koolwaterstof. Mannetjes produceerden geen (Z)-9-tricoseen. Verder werd aangetoond dat in de loop van de tijd in gemengde populaties (mannetjes en vrouwtjes tezamen in een kooi) door onderlinge contacten (Z)-9-tricoseen van vrouwtjes naar mannetjes werd overgebracht en (Z)-9-heptacoseen van mannetjes naar vrouwtjes. Door deze overdracht verandert de oorspronkelijke cuticulaire koolwaterstofsamenstelling bij mannetjes èn vrouwtjes, wat wellicht de ene sexe in staat stelt vast te stellen of de andere sexe gecopuleerd heeft of niet.

Bij vergelijking van de hoeveelheden (Z)-9-tricoseen en een aantal andere koolwaterstoffen op vliegen die een verschillend aantal generaties in kweek waren gehouden bleek dat, terwijl er op eerste-generatie laboratorium wild-type-vrouwtjes nauwelijks of geen (Z)-9-tricoseen voorkwam, de hoeveelheid van deze stof aanzienlijk was toegenomen na 10 of meer generaties in het laboratorium (hoofdstuk 2 en 3). Blijkbaar wordt de aantrekkelijkheid van vrouwtjes voor mannetjes hoger naarmate er meer (Z)-9-tricoseen op vrouwtjes aanwezig is. Wij veronderstelden dat bij hoge populatiedichtheden in opeenvolgende generaties selectie optreedt. Deze selectie had echter geen invloed op de produktie van andere koolwaterstoffen door de vrouwtjes, behalve op die van tricosaan waarvan de produktie aan die van (Z)-9-tricoseen gekoppeld bleek te zijn.

Ondanks de kleine hoeveelheden muscalure op wild-type-vliegen werden geen verschillen in reproduktiecapaciteit van huisvliegen van verschillende stammen aangetoond. Dit suggereert dat copulatiegedrag niet alleen wordt bepaald door de hoeveelheid (Z)-9-tricoseen. Om de rol van muscalure nader te onderzoeken hebben we het sexuele gedrag van mannetjes bestudeerd ten opzichte van vrouwtjes van verschillende stammen die verschillende hoeveelheden muscalure bevatten. Verder hebben we de gevoeligheid voor muscalure bepaald van antennale reukcellen van mannetjes en vrouwtjes van de verschillende stammen door de electrofysiologische reacties (electroantennogrammen = EAG's) van deze cellen te meten bij prikkeling met muscalure (hoofdstuk 3). De resultaten toonden aan dat de sexuele activiteit van mannetjes hoger was naarmate de vrouwtjes meer muscalure op hun cuticula hadden. Bovendien bleek dat mannetjes afkomstig van stammen waarvan de vrouwtjes meer muscalure bevatten ook actiever waren. Dit gaf aan dat selectie in laboratoriumkweken niet alleen de produktie van muscalure door de vrouwtjes verhoogt maar ook de (sexuele) activiteit van de mannetjes. Uit de

electrofysiologische reacties bleek dat zowel mannetjes als vrouwtjes van de drie verschillende stammen (muscalure) even goed konden ruiken. Dit suggereert dat verschillen in sexueel gedrag niet bepaald worden door een verschil in reukvermogen.

Behalve als feromoon hebben de cuticulaire koolwaterstoffen van insecten een belangrijke functie als waterbarrière, waardoor uitdrogen van de dieren wordt voorkomen. Het is bekend dat omgevingsfactoren de produktie van cuticulaire koolwaterstoffen kunnen beïnvloeden. Zoals hierboven al vermeld nemen bij een constante temperatuur en relatieve vochtigheid de hoeveelheden van zowel muscalure als tricosaan op vrouwtjes van opeenvolgende generaties laboratoriumpopulaties toe, terwijl die van de andere koolwaterstoffen gelijk blijven. Dit bracht ons ertoe te veronderstellen dat temperatuur en vochtigheid hoofdzakelijk de hoeveelheden van de niet-feromooncomponenten bepalen en dat muscalure primair geproduceerd wordt als gevolg van een selectieproces bij hoge populatiedichtheden in geïsoleerde omgevingen. We verwachtten dat deze selectie sneller zou verlopen bij hoge dan bij lage populatiedichtheden.

In hoofdstuk 4 beschrijven we de invloed van relatieve vochtigheid, temperatuur en populatiedichtheid op de produktie van cuticulaire koolwaterstoffen. De resultaten tonen aan dat bij zowel mannelijke als vrouwelijke vliegen de produktie van deze stoffen onder zeer vochtige omstandigheden (90% r.h.) wordt vertraagd tot tenminste 3 dagen na uitkomen uit de pop. Acht dagen na uitkomen bevatten vrouwtjes nog steeds minder van deze stoffen bij 90% r.h. dan bij 50 en 20% r.h., dan op de mannetjes bij alle drie relatieve vochtigheden evenveel koolwaterstoffen aanwezig zijn. Vermoedelijk houdt dit verband met het feit dat mannetjes, die actiever zijn dan vrouwtjes, meer cuticulaire koolwaterstoffen nodig hebben om waterverlies tegen te gaan dan vrouwtjes. Er werden geen aanwijzigingen gevonden dat de relatieve vochtigheid de muscalureproduktie anders beïnvloedt dan dan de produktie van de andere koolwaterstoffen. Wat betreft de invloed van de temperatuur bleek dat zowel mannetjes als vrouwtjes bij 35 °C meer koolwaterstoffen produceren dan bij 20 °C. Bij mannetjesvliegen zijn de relatieve hoeveelheden geproduceerde huidstoffen bij de twee temperaturen niet verschillend. Muscalure wordt door de vrouwtjes bij beide temperaturen in even grote hoeveelheden aangemaakt, maar de relatieve hoeveelheden methyl- en dimethylnonacosanen waren bij de vrouwtjes significant hoger bij 35 °C dan bij 20 °C. Wij veronderstellen dat hierdoor het smeltpunt van het totale mengsel van huidstoffen wordt verhoogd, waardoor bij relatief hoge temperaturen waterverlies

wordt vermeden. Doordat het mengsel van koolwaterstoffen op mannetjes een hoger smeltpunt heeft dan dat op vrouwtjes (respectievelijk 39,4 and 36,8 °C; Gibbs *et al*., 1995) nemen we aan dat er bij mannetjes geen aanleiding bestond de samenstelling van de cuticulaire koolwaterstoffen te veranderen toen de temperatuur wordt verhoogd tot 35 °C. Bij lage populatiedichtheden (<20 vliegen/kooi) produceren alle vrouwtjes van de achtste generatie in het laboratorium geringe hoeveelheden muscalure, terwijl de aanwezigheid van muscalure niet werd aangetoond op vrouwtjes van populaties met hoge dichtheden (>300 vliegen/kooi).

Uit het bovenstaande blijkt dat onze hypothese dat de produktie van muscalure niet in dezelfde mate wordt beïnvloed door veranderingen in vochtigheid en temperatuur als die van de andere cuticulaire koolwaterstoffen niet juist was: de produktie van alle koolwaterstoffen wordt in dezelfde mate beïnvloed door deze factoren. De dichtheidsexperimenten tonen aan dat selectie zeer snel kan optreden en dat hiermee terdege rekening moet worden gehouden bij laboratoriumkweken. We veronderstellen dat in kleine populaties, door hun relatief grote bijdrage aan de totale populatie, de eigenschappen van weinig vrouwtjes sneller tot uitdrukking komen in volgende generaties dan in grote populaties.

In experimenten waarbij de invloed van cuticulaire semiochemicaliën op het sexueel gedrag van *M. domestica* wordt onderzocht, worden deze stoffen meestal plaatselijk op de thorax van het insect aangebracht in een organisch oplosmiddel zoals hexaan of aceton. In hoofstuk 5 laten we zien dat deze manier van toedienen van semiochemicaliën de conditie van de vlieg sterk kan aantasten. Bovendien kan dan de verdeling van deze stoffen over de verschillende onderdelen van het vliegenlichaam afwijken van de natuurlijke verdeling. Wij hebben aangetoond dat koolwaterstoffen die vloeibaar zijn bij kamertemperatuur, op een niet-agressieve manier door de vliegen worden opgenomen wanneer ze over filtreerpapier lopen waarop de pure stoffen zijn gepipetteerd. De verdeling van deze stoffen over het lichaam is dan veel meer in overeenstemming met de natuurlijke verdeling van stoffen over het lichaam. Hoe hoger de hoeveelheden koolwaterstof op het filtreerpapier en hoe langer de vliegen op het filtreerpapier verblijven des te hoger de hoeveelheden die worden opgenomen.

Met deze nieuwe ‘zelf-opname’-techniek werd (Z)-9-heptacoseen of (Z)-9-pentacoseen op de vrouwtjes aangebracht. De eerste stof is in grote hoeveelheden aanwezig op mannelijke huisvliegen en komt slechts in kleine hoeveelheden voor op de vrouwtjes. (Z)-9-pentacoseen, dat niet op huisvliegen wordt gevonden, is het

vrouwelijk sexferomoon van ‘de kleine huisvlieg’ *Fannia canicularis,* die vaak sympatrisch met *M. domestica* voorkomt. Op grond hiervan veronderstelden we dat zowel (Z)-9-heptacoseen als (Z)-9-pentacoseen een remmende werking zouden kunnen hebben op het sexueel gedrag van mannelijke huisvliegen. We vonden echter dat (Z)-9-heptacoseen een copulatiebevorderend effect heeft en dat (Z)-9-pentacoseen het sexueel gedrag van mannetjes niet beïnvloedt.

In Hoofdstuk 6 wordt een radar-Doppler actometer beschreven, waarmee bewegingen van individuele lichaamsdelen van vliegen kunnen worden geregistreerd. Door de vorm en amplituden van de registraties (actogrammen) met elkaar te vergelijken kan onderscheid worden gemaakt tussen de bewegingen van kop, poten en vleugels. De kopbewegingen die optreden bij prikkeling met (Z)-9-tricoseen, (Z)-9-heneicoseen en met een 7 : 3 mengsel van deze twee stoffen gebruikten we om enig inzicht te krijgen in de gedragsreacties van huisvliegen op deze stoffen. Deze reacties werden, zowel bij mannetjes als bij vrouwtjes, vergeleken met EAG-registraties verkregen in eerder uitgevoerde experimenten (hoofdstuk 3) waarbij dezelfde stoffen werden gebruikt. Hoewel (Z)-9-heneicoseen bij zowel mannetjes als vrouwtjes hoge EAG’s opwekte, traden er bij beide sexen geen gedragsreacties op bij prikkeling met deze stof. Vergelijking van onze resultaten met die van veldexperimenten beschreven in de literatuur deed ons veronderstellen dat (Z)-9-tricoseen zowel als aggregatieferomoon kan functioneren dat mannetjes en vrouwtjes samenbrengt als sexferomoon waarbij mannetjes worden aangezet tot copulatie.

In hoofstuk 7 beschrijven we het ovipositiegedrag van vrouwelijke huisvliegen. We toonden aan dat vrouwtjes bij voorkeur hun eieren in clusters in spleten in een ruwe, vochtige ondergrond deponeren. Veel vrouwtjes leggen hun eieren gestapeld op een en dezelfde plek. We hebben sterke aanwijzigingen gevonden dat de geur van pasgelegde eieren (tot 30 minuten na ovipositie) aantrekkelijk is voor zwangere vrouwtjes en deze aanzet tot ovipositie. Dit suggereert dat tegelijkertijd met de eieren een ovipositieferomoon wordt afgezet. Oudere eieren (2-6 uur oud) induceerden geen ovipositie wat erop wijst dat het feromoon snel na ovipositie verdampt of wordt afgebroken. Ook de geur van rottend organisch materiaal, mogelijke voedselbronnen voor de larven, trok zwangere vrouwtjes aan en zette deze aan tot eileggen.

Voor zover ons bekend is dit de eerste aanwijzing dat huisvliegen gebruik maken van een ovipositieferomoon. Toepassing van dit feromoon op geschikte locaties binnen hun habitat zou nieuwe mogelijkheden kunnen openen voor milieuvriendelijke

bestrijding van de vliegen. Verwijdering van individuen uit de populatie op een moment dat ze nog geen overlast bezorgen en zich nog niet kunnen voortplanten is een meer efficiënte manier van bestrijding dan verwijdering van volwassen individuen. Identificatie van het ovipositieferomoon verdient grote prioriteit.

Dankwoord

Ruim zes jaar heb ik op het Biologisch Centrum rondgelopen, koffie gedronken, sigaren gerookt en ook nog gewerkt. Het waren voor mij geen tropenjaren. De bijna spreekwoordelijke dips, downs en writer blocks die je zou moeten ervaren als je promotieonderzoek doet zijn aan mij voorbij gegaan. Nee, ik heb het bijzonder naar mijn zin gehad. Dat lag aan de plezierige werkomgeving en vooral aan de mensen die daarvan deel uit maakten. Ten eerste ben ik Kees den Otter zeer dankbaar dat hij geen moment twijfelde (althans niet zichtbaar) toen ik na ongeveer twee jaar bij hem kwam met de vraag "Zeg Kees wat zou je er van vinden als ik ook ga promoveren? ". Hij zei meteen "dat is prima, maar je moet er wel wat voor doen natuurlijk". Nou dat heb ik geprobeerd te doen en het is allemaal goed gekomen. Voor een groot gedeelte heb ik dat aan jou te danken Kees. Vooral het laatste half jaar heb je met veel enthousiasme bijzonder veel energie in het afronden van het proefschrift gestoken.
Als je gaat promoveren heb je ook één of meerdere promotors nodig. Doekele Stavenga bleek direct bereid als zodanig op te treden. Hij zei: "Dat wil ik wel, ik houd wel van hemelbestormers". De Dikke van Dale zegt dat een hemelbestormer iemand is met revolutionaire denkbeelden of plannen. Doekele, ik dank je voor je visionaire blik maar bovenal voor de wijze waarop je zeer nauwkeurig en snel het manuscript van commentaar hebt voorzien. Vele kleine ongerechtigheden en verbeteringen heb ik dankzij jou respectievelijk kunnen verwijderen of aanbrengen.
Jaap Koolhaas, mijn andere promotor, bedankt ik voor zijn inspanning de grote lijnen in het onderzoek duidelijk en logisch voor het voetlicht te brengen.
Frits Kelling en Renate Smallegange ben ik dankbaar dat ze mij de ruimte hebben gegeven zelf onderzoek te verrichten. Binnen het NWO project was ik samen met de twee laatstgenoemde OIO's aangesteld als technisch medewerker. Eigenlijk had ik dus steeds in dienst moeten staan van de twee OIO's. Veel meer ondersteuning dan de vliegenkweek bijhouden heb ik ze echter niet verleend. Heel erg schuldig heb ik me daarover gelukkig nooit hoeven te voelen. Het leven is vluchtig en kortstondig dus moet je af en toe van de gelegenheid gebruik maken. Het zou echter aanzienlijk moeilijker voor mij zijn geweest eigen onderzoek te verrichten als jullie wat bredere ondersteuning hadden verlangd of geëist. Ingrid, Renate en Frits, jullie waren fijne collega's.
Frans Maes bedank ik voor de adviezen op statistisch gebied.
Ook de studenten Diliana Welink en Marjon van Meerloo die gedragsexperimenten voor mij hebben uitgevoerd en Roy Bleumink die aan de bewegingsexperimenten

heeft gewerkt hebben hun bijdrage aan dit proefschrift geleverd. Giovanni Biancaniello heeft nauwgezet elektrofysiologische metingen aan de antennes van vliegen uitgevoerd waarvoor ik hem wil bedanken.
Rein Bos van de basiseenheid Farmaceutische Biologie (zo goed Rein?) heeft mij met "zijn" apparatuur uitstekend geholpen en geadviseerd bij het uitvoeren van gaschromatografische- en massaspectrometrische bepalingen. Vooral de laatste twee jaar van mijn onderzoek heb ik daardoor veel tijd gewonnen. Hartelijk dank daarvoor Rein.
Jan van der Pers van de firma Syntech wil ik bijzonder bedanken voor zijn adviezen op technisch gebied en voor het lenen van de radar-Doppler apparatuur. Bovendien was zijn visie op het ambtenarenschap in relatie tot de "echte" wereld altijd heel verfrissend.
De heer den Braber en dr Janusz Moskal van de firma Denka International die deel uitmaakten van de gebruikerscommissie ben ik dankbaar voor hun altijd aanwezige enthousiasme en voor het beschikbaar stellen van gesynthetiseerde vliegenhuidstoffen.
Henk Mastebroek van de vakgroep Biofysica die ook lid was van deze commissie dank ik voor zijn interesse in mijn onderzoek maar vooral ook voor zijn altijd vriendelijke aanwezigheid.
Ik ben iets ouder dan de gemiddelde AIO of OIO en kan derhalve ook iets verder terugkijken. En als ik dat doe dan realiseer ik mij dat de basis voor mijn wetenschappelijke nieuwsgierigheid werd gevormd tijdens mijn werk bij het ID-DLO (voorheen CDI) te Lelystad. Op de afdeling parasitologie was het dr H.J. Over die mij veelal de vrije hand gaf om experimenten naar eigen idee uit te voeren en mij bovendien altijd ondersteunde als ik weer eens een cursus wilde volgen. Hans ik wil je hiervoor hartelijk bedanken.
Dick Visser bedankt voor het helpen met de omslag en de contacten met de drukker.
En dan waren er natuurlijk de collega's op de afdeling. Een bijzondere plezierige werkomgeving en een uitstekende sfeer. Een fijne club mensen om mee en tussen te werken. Bedankt allemaal.
Mijn twee paranimfen Eszter en Esther wil ik bedanken voor hun vriendschap.
Tenslotte Hillie, mijn vrouw, jij was de eerste die tegen mij zei "waarom promoveer je zelf niet, wat die OIO's kunnen kan jij ook wel". Zoals meestal had je weer gelijk. Bedankt.

List of publications

1. Over, H.J., Gaasenbeek, C.P.H., Noorman, N., 1980. Environmental factors influencing the *Oscillatoria-Lymnea truncatula-Fasciola hepatica* complex. Procs. Neth. Soc. Parasit. 32, 1.

2. Over, H.J. Gaasenbeek, C.P.H., Noorman, N., 1982. Environmental factors determining the disappearance of cattle pats. Procs. British Soc. Parasit. 84.

3. Over, H.J. Noorman, N., Gaasenbeek, C.P.H., 1982 The influence of environmental factors on the development of *Fasciola hepatica* in *Lymnea truncatula.* Procs. Britisch Soc. Parasit. 84.

4. Hoskam, E.G., de Graaf, G.J., Noorman, N. Over, H.J., 1982. Zinc poisoning in foals. Tijdschr. Diergeneesk. 107, 672-680.

5. Noorman, N., 1985. Gevaar van leverbotinfectie ligt op veel bedrijven op de loer. Boer en Tuinder, 11, 70-71.

6. Noorman, N., 1988. Voorspelling van leverbotinfecties. NRLO-rapport nr. 90/31, 9-17.

7. Pekelder, J.J., Noorman, N., 1988. Early diagnosis of liver fluke infection in sheep. Tijdschr. Diergeneesk. 113, 865-867.

8. Borgsteede, F.H.M., Jansen, J., Van Nispen tot Pannerden, H.P.M., Van der Burg, W.P.J., Noorman, N., Poutsma, J., Kotter,. J.F., 1990. An investigation of the endoparasitic helminth fauna of roe deer (*Capreolus capreolus*). Zeitschr. Jagdwissch. 36, 104-109.

9. Gaasenbeek, C.P.H., Over, H.J., Noorman, N., De Leeuw, W.A., 1992. An epidemiological study of *Fasciola hepatica* in the Netherlands. Vet. Quart. 14, 140-144.

10. Schreuder, B.E.C., Moll, H.A.J., Noorman, N., Halimi, M., Wassink, G., 1994. Livestock mortality in Afghanistan in districts with and without a veterinary programme: a cost-benefit analysis. The Kenya Veterinarian, special issue, Procs of the 7th ISVEE symposium 18: 513-516.

11. Schreuder, B.E.C., Moll, H.A.J., Noorman, N., Halimi, M., Kroese, A.H., Wassink, G., 1996. A benefit-cost analysis of veterinary interventions in Afghanistan based on a livestock mortality study. Prev. Vet. Med. 26, 303-314.

12. Schreuder, B.E.C., Noorman, N., Halimi, M., Wassink, G., 1996. Livestock mortality in Afghanistan in districts with and without a veterinary programme. Trop. Anim. Heatlh and Prod. 28, 129-136.

13. (comparable to 12.) in: Livestock production and Diseases in the Tropics. 1995. Procs, 8th Conf. Inst. Trop. Vet. Med. Berlin, Germany, 574-581.

14. Voskamp, K.E., Den Otter, C.J., Noorman, N., 1996. Monitoring tsetse host odours in the field using mobile electrophysiological device. Abstr. Congr. Euro. Chemorecept. Res. Org. 25-31.

15. Noorman, N., Den Otter, C.J., 1997. Sex-, age- and strain related quantities of muscalure on the cuticula of the housefly (*Musca domestica*). Proc. Exper. & Appl. Entomol., N.E.V. Amsterdam. 8, 105-108.

16. Noorman, N., Den Otter, C.J., 1997. Varations in muscalure quantitiy and sexual activity between different strains of houseflies. Abstr. 5th Euro. Symp. Insect Taste Olfact. 13.

17. Voskamp, K.E., Den Otter, C.J., Noorman. N., 1997. Eag responses of tsetse to host odours in the field. Abstr. 5th Euro. Symp. Insect Taste Olfact. 14.

18. Noorman, N., Den Otter, C. J., Biancaniello, G., 1998. Variations in sexual activity, electrophysiological responses and muscalure quantities between different strains of houseflies. Chem. Senses 24, 97.

19. Schreuder, B.E.C., Noorman, N., Halimi, M., Van Dommelen, M., Hennecken, M., Wassink, G., 1998. Further observations on the impact of a veterinary programme in Afghanistan on seasonal livestock mortality. Trop. Anim. Health and Prod. 30, 83-89.

20. Voskamp, K.E., Den Otter, C.J., Noorman, N., 1998. Electroantennogram responses of tsetse flies (*Glossina pallipides*) to host odours in an open field and riverine woodland. Physiol. Entomol. 23, 176-183.

21. Voskamp, K.E., Noorman, N., Mastebroek, H.A.K., Van Schoot, N.E.G., Den Otter, C.J., 1998. Neural coding in antennal olfactory cells of tsetse flies (*Glossina* spp.). Chem. Senses. 23, 521-530.

22. Den Otter, C.J. Smallegange, R.C. Kelling, F.J. Noorman, N. 1999. Basic research to uncover stimuli for environmentally sound control of house flies. Wiadom. Parazyt. 45, 545.

23. Noorman, N., Den Otter, C.J., 1999. Variations in muscalure pruduction between and within different strains of houseflies. Abstr. 6th Euro. Symp. Insect Taste Olfact. 61.

Curriculum vitae

Nico Noorman werd geboren op 3 juni 1949 te Groningen. Na het behalen van het MULO-diploma volgde hij van 1967-1971 de opleiding tot Zoölogisch Analist met specialisatie Ecologie. Tijdens zijn stagejaar bij de Rijksuniversiteit van Groningen deed hij onderzoek naar het eetgedrag van ratten en het gedrag van koolmezen. Na het vervullen van de dienstplicht bij de Koninklijke Marine trad hij in 1973 in dienst bij het toenmalige Centraal Diergeneeskundig Instituut, tegenwoordig ID-DLO, te Lelystad. Als analist was hij daar tot 1989 werkzaam op de afdeling Parasitologie en voornamelijk betrokken bij het epidemiologisch onderzoek van de leverbotziekte. Van 1989 tot 1992 werkte hij als analist/programmeur op de afdeling Automatisering van hetzelfde instituut. Daarna verbleef hij een jaar in Pakistan als medewerker bij het 'Dutch Committee for Afghanistan'. Van 1994 tot 2000 was hij als technisch medewerker verbonden aan de afdeling Dierfysiologie van de Rijksuniversiteit te Groningen in het kader van het NWO/STW-project 'Milieuvriendelijke bestrijding van huisvliegen door middel van gecombineerde visuele en chemische prikkels'. Zijn onderzoek binnen dit project heeft geleid tot de totstandkoming van dit proefschrift.

Stellingen behorende bij het proefschrift

Pheromones of the housefly
A chemical and behavioural study

Nico Noorman
26 juni 2001

1. Menige stelling is vaak niet meer dan een stellige mening.
   (proefschriften)

2. Wild-types zijn niet altijd van die gewilde types.
   (dit proefschrift)

3. Genetisch manipuleren: een aangeboren eigenschap.

4. Euthanasie: ook een medisch Godsgeschenk.

5. Het Vaticaan: ongeneeslijk zero-positief.

6. Werken aan de grenzen van het weten: gerommel in de marge.

7. Zeven stellingen is meer dan voldoende.