Chapter 8

Summary and general discussion

Introduction
Houseflies, *Musca domestica* L., are cosmopolitan insects commonly associated with man and livestock. In warm environments they remain active and reproduce throughout the year. Houseflies feed on many different food sources such as decomposing substrates as well as human or livestock food. In doing so, they may be vectors of pathogens and transmit several diseases. In addition, high population densities of houseflies can cause nuisance to such an extent that livestock production decreases. Therefore, houseflies constitute a major problem in farming industries as well as in food processing industries. Several methods for housefly control have been developed, but a completely effective control has not yet been accomplished. Improvement of the efficacy of traps and baits with attractive odours is promising. The most effective attractants are decaying natural products, which are not practical for use in traps and baits. Presumably synthetic chemical mixtures that attract houseflies are be better applicable and could enhance the attractiveness of traps and baits that are currently in practice.

This thesis is aimed at the improvement of housefly pest control using attractive odours in traps and baits. To achieve this aim, a further understanding is required of how houseflies detect and discriminate odours and how their olfactory sensitivity depends on physiological circumstances. Furthermore, the identity of compounds making up the fragrance of attractive substances is investigated.

Insects have developed specialized structures, olfactory sensilla, with which they smell odours. These olfactory sensilla are innervated, hollow hairs with pores in their walls. Odour molecules diffuse through the pores towards the dendrites of the receptor cells. When odour molecules bind to the receptors in the membrane of the dendrite, the receptor cell responds by depolarization and increasing the production of action potentials (spikes) or by hyperpolarization and decreasing its spike frequency. With
electrophysiological techniques we can measure the responses of olfactory receptor cells to odour stimuli. By placing an electrode on the cuticular surface at the base of a sensillum (and the indifferent electrode into the haemolymph), we can record the spikes generated by the receptor cells nearby the electrode and determine the response of a receptor cell to an odour stimulus. To interpret these responses, we first have to know more about the specific morphology of the olfactory organs of houseflies.

Types and distribution of sensilla
In Chapter 2 we describe the morphology of the olfactory organs of houseflies. The olfactory organs are the antennae and the maxillary palps. We investigated the morphology of these organs using scanning electron microscopy and transmission electron microscopy. The antennae consist of a scape, pedicel, funiculus and arista. The funiculus is densely covered with several types of hairs, most of which have an olfactory function, as is suggested by their morphology and as is shown in the following chapters by electrophysiology. Apart from non-innervated microtrichia, several types of olfactory hairs are present on the surface of the antennae: trichoid sensilla, basiconic sensilla, grooved sensilla and clavate sensilla. Three pits are present in the funiculus: two of them contain grooved sensilla and several sensilla without wall-pores, and one pit contains numerous clavate sensilla. Trichoid sensilla are 12-20 µm long, thick-walled hairs and they are the most numerous sensilla on the funiculus (1500-2000 per funiculus; 70% of the sensillar population). They are present at all sides of the funiculus except for the proximal 1/4 of the ventral side. Basiconic sensilla are 4-11 µm long, thin-walled sensilla. Their number on the funiculus is about 550-700, and they are distributed over the whole funicular surface. About 90-110 small (1.5-4 µm) grooved sensilla and a few 9-11 µm long clavate sensilla are present on the funicular surface. The palps bear microtrichia, long grooved bristles and some olfactory basiconic sensilla. The number of basiconic sensilla present on the palps is 75-90, their density increasing towards the palpal tip.

No differences in number and distribution of sensilla exist between males and females. It appeared that small houseflies, having small antennae and palps, possess less sensilla than large flies of the same strain. The sensilla of small and large flies are similar in size.

Based on this study of the morphology and distribution of olfactory hairs, showing that the different sensillum types on the antennal surface are densely intermingled, we know that electrophysiology on the antennal surface will give responses from several different types of olfactory hairs. Further electrophysiological studies were performed on olfactory hairs on all sides of the funiculus and on the distal half of the palps of large houseflies of both sexes.
Characterization of olfactory celltypes

In Chapter 3 we measured the electrophysiological responses of olfactory cells on the antennae and palps to a set of odours from different chemical classes. In the antennae, a large variation in response profiles of olfactory cells is present. Most cells respond to several odours and few specialist cells exist. 1-Octen-3-ol, amyl acetate, 2-pentanone, 3-methylphenol, and R-limonene induce the largest responses. Cluster analysis identified ten clusters of antennal cells with similar response profiles in the multidimensional odour space (each odour representing one dimension). Cells with similar response profiles are distributed over the whole funicular surface; no regionalization in receptive fields for different odours is present in housefly antennae. In the palps, the clusters of cells that respond mainly to 3-methylphenol or 2-pentanone are clearly separated from the cells responding to other substances. Receptor cells with similar or with different response profiles can reside within one and the same sensillum.

Because few specialist cells are present and most cells respond to several odours, we continued our research using some odours that elicit clear responses in most cells.

Sensitivity of olfactory cells

Effective pest control requires that all individuals of the population of houseflies, including young and adult flies of both sexes, respond to the attractive stimuli to be used in traps and baits. Therefore, we investigated the olfactory sensitivity of the different groups of houseflies by comparing dose-response curves (Chapter 4). Very few differences in the sensitivity of olfactory cells of newly emerged flies versus mature flies are found. Also differences between males and females are virtually absent. However, large differences between antennal and palpal sensitivity for several odours exist.

These results show that houseflies of all ages and both sexes can detect attractive odours and may be lured towards traps and baits.

Background odour

So far most substances were tested as single odour stimuli. However, in nature, air is always loaded with some ambient background odour. For attractants or repellents to be effective, the flies have to be able to distinguish these chemicals from the ambient odours. Therefore, we tested the responses of single odour stimuli in the presence of different background odours (Chapter 5).

The presence of a background odour (1-octen-3-ol or manure) has a limited influence on the sensitivity to different test odours (1-octen-3-ol, 2-pentanone and R-limonene). Only high concentrations of a background odour of 1-octen-3-ol induced slight adaptation and sensitisation: responses to high doses of test odour were decreased and
responses to low doses of test odour were increased compared with the responses in clean air. No effect of manure or low doses of 1-octen-3-ol as a background odour on the sensitivity to the test odours was found.

Thus, using attractive odours in smelly, odour-loaded environments may be effective in luring animals to control fly populations.

**Electroantennography**

Single olfactory cells respond to a limited spectrum of odours. When measuring a summated response from a population of olfactory cells, belonging to different clusters, the spectral sensitivity will enhance, and a faster discrimination of biologically important odours will be possible. Therefore, we used electroantennography in our further research.

We show that EAGs measured from the cuticle of the globular antennae of houseflies represent the summated receptor potentials of a population of olfactory cells near the electrode (Chapter 6). The size of an EAG reflects the initial concentration step of the stimulus. Longer stimulation does not increase the EAG. After flushing the antenna with clean air for some time, complete recovery of the olfactory cells may occur.

High doses of strong acids induce electrochemical artifacts that interfere with physiological responses, making the interpretation of the EAG to these stimuli unreliable.

**Components of the odour of attractive natural substances**

Chicken manure, pork meat and moistened bread were shown to be very attractive natural substances for houseflies (Smallegange et al., 1999). We identified some chemicals from these substances by gas chromatography-mass spectrometry, combined with EAG measurements (Chapter 7). Furthermore, we made EAG dose-response curves for these chemicals and for other chemicals present in manure, meat and bread. Based on the sensitivity to these chemicals, expressed by the threshold dose, we identified the chemicals that may contribute most to the attractive odour of the natural substances for the housefly. The main components of the odour of manure are 2-methylpropanol, dimethyldisulfide, 3-methylbutanoic acid, 3-methylphenol and skatole; meat-odour may be formed by 3-methylbutanal, dimethyldisulfide, 3-(methylthio)-propanal, 3-methylbutanoic acid, benzaldehyde, 1-octen-3-ol and skatole; and in odour of bread 2-methylpropanol, 3-methylbutanal, 1,1-diethoxyethane, dimethyldisulfide and benzaldehyde may be the most important odours.

**Electrophysiology and detection of attractants and repellents**

For detecting which odours may be biologically important for the housefly, electrophysiology is very suitable. Most cells are generalist cells, only a few specialist
cells were found. No cells were found that responded exclusively to an odour that was not detected by other cells. Therefore, the chance of missing cells that respond to an important odour is small.

With our optical setup it was impossible to observe from which sensillum type electrophysiological responses were measured. Therefore, we cannot assign cells from certain clusters to certain sensillum types. We assume that with the surface contact technique, we measure electrophysiological responses from cells from all the different sensilla in a similar ratio as their presence on the antennae. On the palps, all olfactory cells are located in basiconic sensilla, as no other olfactory sensilla are present.

As EAGs represent the summated receptor potentials the olfactory cells near the electrode, cells from all sensillum types participate. There is no regionalization of cells with specific response profiles, so EAGs measured at different spots on the antennae of houseflies all give similar results. EAGs may have a lower sensitivity than single cell measurements, but they have a broader response spectrum and, therefore, also are useful in testing odours.

Electrophysiology provides a method for quick screening of odours. We can assume that substances that do not evoke any electrophysiological response are not detected by the housefly and may be disregarded. High electrophysiological responses prove that an odour is detected by the housefly. Components from attractive substances are likely to contribute to the attractiveness of the substance. However, electrophysiology does not give information on the biological function of an odour, being attractive, repellent or indifferent. Therefore, after making a preselection with electrophysiology, behavioural essays should be performed.

The odours that evoke the highest responses all seem to be of biological importance: 1-octen-3-ol is attractive to many Diptera, amyl acetate and 3-methylphenol are attractants for houseflies and R-limonene is a repellent. The chance that an odour evokes high responses but is indifferent to the fly is low. No behavioural studies were done with 2-pentanone (which evokes high responses), but this compound is present in food, and it may contribute to the attractive odour of food. Some false positive results are found when testing acids: high doses of strong acids evoke large positive EAG-like potentials, but these are artificial electrochemical effects. At lower doses these electrochemical potentials are small enough to be able to interpret the physiological responses.

For some chemicals that are known to be attractive, the electrophysiological responses are rather low. Single cell responses to, for instance, muscalure, skatole and acetic acid suggest only a moderate biological function, but they were shown to be strong attractants in literature (Carlson and Beroza, 1973; Frishman and Matthysse, 1966). Small
responses to these odours could be expected in our experiments. The volatility of muscalure is very low and therefore, only a low concentration in air could be tested. Skatole is difficult to dissolve and only low doses of this substance could be made. Therefore, the concentrations tested of skatole in air also are low (see Chapter 4, Fig. 2B). Still, in nature the concentrations to these odours will not be higher. For selecting behaviourally important odours, it may be best to compare electrophysiological responses to all stimuli with similar low concentrations in air. For acetic acid, there is another problem. As shown in Chapter 6, acid stimuli generate electrochemical artifacts that interfere with the physiological responses. These artifacts are larger at more acidic stimuli. At high doses of acetic acid, the physiological response may be completely overruled by the artifact, at low doses the artifact is small and a physiological response can be seen. Still, however, a small artifact interferes with the response, that consequently is reduced in size. Therefore, substances evoking small electrophysiological responses should not consequently be considered to have only small behavioural effects.

High doses of test stimuli are useful to quickly determine if an odour is detected by the animal, using electrophysiological techniques. To elicit behavioural responses, the concentration of an odour may have to be chosen in a range that causes low or moderate spike responses as was shown for *Triatoma infestans* (Guerenstein and Guerin, 2001). Attractive substances may even become repellent when the concentration is too high (Frishman and Matthysse, 1966).

**Composition of synthetic attractive mixtures**

Based on the results from Chapter 7 we may compose synthetic mixtures, mimicking manure, meat and bread. Considering that acetic acid is described to have high attractiveness to houseflies, we might add this compound to the manure mix. Several chemicals with low threshold doses are present in more than one of the natural substances, e.g. 2-methylpropanol, 3-methylbutanal, dimethyldisulfide, 3-methylbutanoic acid, benzaldehyde and skatole. Apart from the three mixtures described in Chapter 7, a mix of these chemicals presumably is also attractive to houseflies and should be tested in behavioural assays.

**Applicability of baits**

There are few differences in sensitivity to odours between males and females. Therefore, we may assume that an attractive odour mix may lure both the males and females from a housefly population. Moreover, the olfactory system of newly emerged houseflies is already functional and also these young flies may be lured to an attractive food-mimick. By catching flies before they mate and multiply, housefly control can become more
Summary and general discussion

Small flies have less sensilla than normal (large) flies. We did not find a lower sensitivity of single olfactory cells of small flies. But because of a smaller sensitive surface, small flies could have a lower overall sensitivity of the olfactory system than large flies. However, if all types of olfactory cells are present on the antennae and palps, and these all project to their glomeruli in the antennal lobe, similar olfactory information (detected by less sensilla) could reach the fly brain as in large flies. As small flies have the same food preferences as large flies an attractive odour mix will lure both large and small flies.

We have shown that the presence of a background odour (for instance the smell of manure in a stable) has little effect on the sensitivity of the olfactory system. It even may enhance the sensitivity to low concentrations of new odour puffs, while the sensitivity to high concentrations of odour puffs, still evoking large responses, may be diminished.

We conclude that the application of the proposed attractants will be effective to enhance the control of the whole population of houseflies, young and mature, males and females, small and large in clean as well as in smelly environments.

Integrated control

For effective control of housefly pests in livestock habitats, integration of the different control methods is necessary (Axtell and Arends, 1990). Firstly the environment should be kept hygienic. Manure should be kept dry, and during cleanout a base of old manure should be left to conserve fly parasites and predators. Pest management should be based on the use of baits and traps and on biological control. Application of pesticides should be limited to the most heavily infested areas only, to prevent extinction of natural enemies. The pesticides used should be varied to prevent the development of resistance. Good monitoring is necessary to evaluate the fly management and to time new interventions. Development of new insecticides, biological control methods and attractive visual and olfactory cues can improve future pest management.

References

Guerenstein, P.G. and Guerin, P.M. (2001) Olfactory and behavioural responses of the blood-