Attractiveness of different light wavelengths, flicker frequencies and odours to the housefly (Musca domestica L.)
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Chapter 1

General Introduction

Introduction
The housefly, *Musca domestica* L. (Diptera: Muscidae), is one of the most important hygiene pests world-wide. The flies not only are a nuisance, irritating people and animals and leaving regurgitation and faecal spots on surfaces, but they also are vectors of pathogens which may cause serious diseases in humans and animals. Therefore, a lot of money is spent on fly control. However, due to their high reproductive rate houseflies have developed resistance against various commonly used insecticides. Also other control methods are not effective enough to reduce fly populations to acceptable levels. Therefore, new or adjusted control methods are needed.

Light traps are considered to be promising devices to control houseflies indoors. These traps are fitted with only one attractive stimulus, light, which may be only effective during certain periods of the flies’ life cycle. This may explain the disappointing control results that are commonly achieved with these traps. Odour-baited traps have the same disadvantage, only relying on an olfactory stimulus. Combining several stimuli may increase the effectiveness of housefly traps. We therefore studied visual and olfactory stimuli, separately and in combination with each other, for their attractiveness to houseflies at different moments of the flies’ life. Because the environment may affect attractiveness, different ambient conditions (illumination, odours) were taken into account. Before starting to describe the results of these experiments an overview will be given of the biology of houseflies as well as of present methods to control them. In addition in this first chapter the aim of our studies and the outline of this thesis are described.
Distribution
The housefly is one of the most common of all insects. It is an endophilic and eusynanthropic species, i.e. it lives closely with humans and is able to complete its entire life cycle within residences of humans and their domestic animals. It thus became world-wide distributed, spread over the inhabited world. It can be found in human dwellings, dairies, poultry houses, horse stables, food processing factories, and other domesticated areas and buildings (Hewitt, 1910, 1912; West, 1951; Hansens, 1963; Lillie and Goddard, 1987; Kettle, 1995).

External morphology
The housefly (Figure 1) is approximately 7 mm long. The female is usually bigger than the male. The body of the fly is composed of three parts: head, thorax, and abdomen. The latter is grey or yellowish with a dark midline on the dorsal side. The thorax is marked with four longitudinal black stripes on its dorsal side and bears three pairs of legs, two halteres or balancing organs, and one pair of wings. As is characteristic of the subfamily Muscinae, the fourth longitudinal wing vein is strongly bent upwards towards the third longitudinal vein when approaching the margin of the wing. The head is dominated by a pair of compound eyes on its lateral sides (Figure 2). The sexes can easily be distinguished by the dorsal space between the eyes which is wider in females. On the dorsal side of the head three ocelli or simple eyes are situated. The mouthparts are configured to a suctorial organ, the proboscis, located ventrally on the head. The proboscis can be folded into a subcranial cavity. Rostrally between the eyes the head bears two antennae consisting of three segments, scapus, pedicellus and funiculus, the latter bearing a feather-shaped arista (Figure 2). The funiculi are covered with olfactory hairs which enable the fly to “smell” (Hewitt, 1910, 1912; West, 1951).

Life cycle
The housefly undergoes a complete metamorphosis in its development from egg to adult (Figure 1). The rate of development depends on food availability and temperature. A female housefly may lay four to six batches of eggs consisting of 75 to 150 eggs each. The eggs are deposited in clumps in cracks and crevices of a moist medium to protect them from desiccation. Manure and spilled food are known to be the principal breeding media for houseflies (Hewitt, 1910; West, 1951; Skoda and Thomas, 1993: see Cossé and Baker, 1996; Kettle, 1995). The pearly-white eggs measure about 1.2 mm in length. They hatch within 24 hours after oviposition. Within approximately a week the whitish, legless, saprophagous larvae (maggots) develop through three larval stages. The full-grown larvae migrate to drier conditions and bury themselves into the substrate where they pupate. After approximately 5 days, the adult emerges from the reddish brown to almost black puparium.
An adult housefly may live 15 to 30 days. Males may already mate on the day of their emergence. Mating readiness of females (which are monogamous contrary to males) is highest when they are three days old (Saccà, 1964). Oviposition takes place a few days after copulation.

During warm weather the life cycle, from egg to egg, takes two to three weeks. Because of this high rate of development and the large numbers of eggs produced by a female, large populations can rapidly build up. In temperate regions of the world ten to twelve generations a year can occur. Breeding is restricted to the warmer months in colder regions, resulting in four to six generations a year. Overwintering takes place in the larval or pupal stage (Hewitt, 1910, 1912; West, 1951; Barnard and Geden, 1993; Kettle, 1995).

Figure 1. The life cycle of the housefly, *Musca domestica* L. (adapted from Pedigo, 1989).
Figure 2. Frontal view of the head of the housefly (adapted from Pedigo, 1989).

Behaviour
The activity of adult houseflies is affected by several physical variables: temperature, humidity, light intensity, air currents, barometric pressure, and electrostatic fields. The flies are diurnal and are more active when temperature is high and humidity low, but they tend to become sluggish when both temperature and humidity are high. Their activity optimum lies around 33°C. Near 45°C symptoms of heat paralysis are evident. The flies remain active at
low temperatures down to about 7°C, regardless of humidity (Dakshinamurty, 1948; see West, 1951; West, 1951; Kettle, 1995).

Houseflies have a flight velocity of about 2 m/s. Males tend to chase moving conspecifics and even non-living, dark, fly-sized moving objects (West, 1951; Vogel, 1956; Wiesmann, 1962: see Arevad, 1965 and Dethier, 1976; Land and Collet, 1974; Wehrhahn et al., 1982). Decreasing light intensities and approaching objects induce a landing response (Fernandez Perez de Talens and Taddei Ferretti, 1970). Indoors, dark (black and red) coloured materials are preferred as resting sites and more time is spent on rough surfaces (Freeborn and Berry, 1935; Hecht, 1963; Arevad, 1965). In contrast, yellow and white have been found to be more attractive outdoors (Hecht et al., 1968; Pickens, 1995). Recently, Howard and Wall (1998) showed that contrast increases the attractiveness of coloured objects.

Adult houseflies are positively phototactic (West, 1951); they are attracted towards light. Each compound eye of a housefly consists of about three thousand hexagonal facets (Hardie, 1986). Each facet functions as a lens, permitting light to stimulate the visual unit (ommatidium). Light is focussed on the visual pigment containing rhabdomeres of the retinula cells by the facet lens and pseudocone. Primary and secondary pigment cells absorb stray light. The spectral sensitivity of the eight photoreceptor or retinula cells (R1-8), which are present in each ommatidium (Figure 3), can be visualized in an electroretinogram (ERG). ERG recordings of *M. domestica* show high responses in the ultraviolet (340-365 nm), the blue-green (450-550 nm), and the red (620-630 nm) (Mazokhin-Porshniakov, 1960; Goldsmith and Fernandez, 1968; Bellingham and Anderson, 1993). Electrophysiological and optophysiological studies demonstrated that the six large, peripheral rhabdomeres belonging to the retinula cells R1-6 are sensitive both to ultraviolet and blue-green light, whereas the rhabdomeres of the central cells R7 and R8 (which rhabdomeres are positioned in tandem) are sensitive to (ultra)violet or green light (Hardie, 1986; Stavenga, 1995). The R1-6 photoreceptors are involved in spatial vision, whereas the R7 and R8 photoreceptors probably allow flies to observe colours (Hu and Stark, 1980; Hardie, 1986; Troje, 1993).

Behavioural studies suggest that light between 300 and 400 nm is the most attractive to both female and male houseflies (Cameron, 1938; Deay and Taylor, 1962; Thimijan et al., 1973; Roberts et al., 1992). However, results of experiments on the phototactic behaviour of houseflies are often contradictory. Cameron (1938), for example, observed that males are less attracted to light than females, whereas Roberts et al. (1992) found no difference between the sexes. Contradiction between results of behavioural experiments with houseflies described in the literature are probably due to the fact that the flies’ sensitivity to light and their phototactic behaviour are affected by several biological and physical factors such as sex, age, nutritional state and searching activity of the flies, ambient temperature, and the presence of other
visual stimuli (Cameron, 1938; Morgan and Pickens, 1968; Pickens et al., 1969; Barker and Cohen, 1971; Pickens and Thimijan, 1986; Skovmand and Mourier, 1986).

Figure 3. Schematic representation of a compound eye unit, the ommatidium (adapted from Stavenga, 1974 and Hardie, 1986). fl: facet lens; ppc: primary pigment cell; psc: pseudocone; sc: semper cell; nu: nucleus; rh: rhabdomeres; retc: retinula cell (R1-R8); spc: secondary pigment cells; cm: central matrix.
Figure 4. Schematic representation of an insect antennal olfactory sensillum (adapted from Van der Pers, 1980).
The eyes of male houseflies have a dorsal frontal area where the R7 receptors are sensitive to the blue-green (identical to R1-6). The signals of these R7 cells are added to the signals of R1-6 cells (neural superposition principle). It is suggested that this improves spotting a potential mating partner as a contrasting dot against the sky (review Hardie, 1986).

The dorsal margin of the flies’ eyes contains large R7 and R8 photoreceptors which are ultraviolet and polarization sensitive. This area detects the polarization of sky light, which may be used for orientation (reviews Hardie, 1986; Stavenga and Wunderer, 1999).

The phenomenon that houseflies are positively anemotactic, i.e. tend to fly upwind (West, 1951), may be induced by airborne odours. Odours are used to find mating partners, food or oviposition substrates. Although the majority of the olfactory organs (olfactory sensilla) of the houseflies is situated on the funiculi of the antennae, they can also be found on the palpi (Kelling, 2001). The sensilla contain pores through which airborne odours can reach the chemoreceptors (Figure 4). Electrophysiological research of the olfactory receptor neurons can be used to show which odour complexes or odour components can be detected by the insects (visualized in electroantennograms (EAGs), electropalpograms (EPGs) or single-cell recordings). Behavioural research can reveal which odours are actually used by the insects. Although contradictory results are mentioned in literature, it was shown that in general *M. domestica* is attracted to putrefying, fermenting, and sugar-containing products (Brown *et al.*, 1961; Künast and Günzrodt, 1981).

**Transmission of pathogens**
Houseflies are not only a nuisance to humans and animals, but they may also transport disease-causing organisms. Their movements between human and animal food, organic wastes, garbage, faeces, manure, and other sources of filth on which they may feed and breed make them ideal transmitters of human and animal pathogens. About a hundred different pathogens have been found in and on houseflies. There are three different ways in which houseflies may transmit pathogens. The surface of their body, particularly the legs and proboscis, may be contaminated; and because a housefly sucks food after it has been liquefied in regurgitated saliva, pathogens may be deposited onto food with the vomit drop. Thirdly, pathogens may pass through the gut of the fly and be deposited with its faeces.

Pathogens that may be transmitted by houseflies are, for example, viruses causing diarrhoea, cholera bacteria, *Salmonella* species and *Escherichia coli* bacteria causing enteric infections, haemolytic streptococci, agents of typhoid, diphtheria, tuberculosis, leprosy and yaws. In addition, they may carry cysts of Protozoa, including those causing amoebic dysentery, and the eggs of nematodes. Finally, houseflies may be vectors and intermediate hosts of certain cestodes of poultry and horse nematodes (Hewitt, 1910, 1912;
Ostrolenk and Welch, 1942; West, 1951; Saccà, 1964; Kettle, 1995; Grübel et al., 1997; Tan et al., 1997; Kurahashi et al., 1998).

**Control methods**

Gauze screens before windows and doors can be used to keep houseflies outside. Indoors, sticky fly paper, electrocuting light traps and odour-baited traps may be used. Large sticky traps can be effective, but their use is limited by the rapid accumulation of dust on the sticky material (Kaufman et al., 2001). Odour-baited traps are not very popular because of their smell, which is often unpleasant to humans. In addition, the light and odour-baited traps may also kill harmless and beneficial insects. Moreover, in most cases only a negligible proportion of the fly population is caught by the traps because of competing environmental factors, such as ambient light conditions and odour sources (Bowden, 1982; Browne, 1990; Muirhead-Thomson, 1991).

Sanitation and removal of possible breeding sites using efficient garbage and sewage disposal systems are probably the most effective control methods for houseflies breeding in domestic wastes and waste materials from animals. Garbage containers should have tight-fitting lids and should be cleaned regularly. Manure, straw, and spilled feed should be removed at least twice a week. At waste disposal sites, the disposal should be covered with a layer of about 15 cm soil or other inorganic materials every week (Kettle, 1995).

Application of insecticides may initially be effective, but muscids readily develop resistance to persistent insecticides either because enzymes enable the flies to break down the insecticides or because behavioural adaptations enable the flies to avoid the insecticides. Also cross-resistance has been reported, for example to juvenile hormone mimics. Not only the increase of tolerance and resistance of flies to insecticides but also the increasing costs of the use of insecticides and their toxicity to other organisms make them less desirable for fly control. Besides, it appears hard to discover new insecticides and the costs of their development are high (Scott and Georghiou, 1985; Meyer et al., 1987; Pickens and Miller, 1987; Kettle, 1995; Pospischil et al., 1996; Keiding, 1999; Scott et al., 2000).

Houseflies have many natural enemies, like entomopathogenic fungi (e.g., *Entomophthora muscae*) and nematodes, parasitic wasps (e.g., various pteromalid species), predatory beetles (histerid and staphylinid species) and mites, and birds. Various authors examined these biological agents for control purposes. Only in a few cases successful control with natural enemies was achieved, mostly in combination with other control methods (integrated fly control) (Hewitt, 1910, 1912; West, 1951; Saccà, 1964; Geden et al., 1993; Glofcheskie and Surgeoner, 1993; Møller, 1993; Kettle, 1995; Skovgård and Jespersen, 1999).
Aim of the research described in this thesis

Because of its widespread distribution, high rate of development, abundant presence inside stables, houses, kitchens, restaurants, and food factories, the nuisance it causes, its filthy behaviour and because it is a potential vector of diseases, the motivation of controlling *M. domestica* is high. However, the present control methods have various disadvantages. Areas in which food is stored, prepared or consumed require hygienic control methods. Sticky and electrocuting traps have the potential to accumulate various species of insects, micro-organisms, and dirt which may fall on the food products. The use of insecticides is undesirable because of the risk for human and animal health, and the rapid development of resistance in houseflies. Also other control methods, like light and odour-baited traps, turned out to be less effective than desired or have a negative direct or indirect side effect on other organisms. Beside these problems, the European Union has recently decreed (Directive 93/43/EEC) that member states should adopt the main principles of the so-called Hazard Analysis of Critical Control Points (HACCP). HACCP is a total quality management system for food safety which, if implemented fully, would result in a minimal level of food contamination (Howard, 1999). This implies that it may no longer be sufficient to reduce the number of pest insects, but that it is necessary to eradicate them, because it is realized that even a single fly may carry pathogens. This, of course, requires very efficient control methods.

In the past century much research has been done on various fly species. Especially their biology, their visual and olfactory systems, and possibilities to control the flies have been investigated. These studies were conducted in the field as well as in the laboratory. Much information was collected about the optical characteristics of visual stimuli inducing landing or optomotor responses in flies and about the activity of the neurons involved, as well as about the spectral sensitivities of the eyes of several fly species and the molecular components involved in generating the light response (e.g., Mazokhin-Porshniakov, 1960; Burkhardt, 1962; Bishop *et al.*, 1968; Goldsmith and Fernandez, 1968; Fernandez Perez de Talens and Taddei Ferretti, 1970; Zaagman *et al.*, 1977; Agee and Patterson, 1983; Green and Cosens, 1983; Tinbergen and Abeln, 1983; Lenting *et al.*, 1984; Hardie, 1986; Schuling *et al.*, 1989; Bellingham and Anderson, 1993; Stavenga, 1995). In addition, various studies have been carried out on the attractiveness of lamps and odours and on the effectiveness of control devices (e.g., Cameron, 1938; Brown *et al.*, 1961; Deay and Taylor, 1962; Thimijan *et al.*, 1973; Künast and Günzrodt, 1981; Skovmand and Mourier, 1986; Lillie and Goddard, 1987; Roberts *et al.*, 1992). In many of these investigations tethered or wingless flies were used, which renders it difficult to extrapolate the results to free-flying insects in natural surroundings. Moreover, results from one fly species are not always valid for other species.
Traps provided with fluorescent lamps may be promising devices for indoor control of houseflies (Lillie and Goddard, 1987). However, the effectiveness of these traps still has to be improved (Bowden, 1982; Pickens and Thimijan, 1986; Muirhead-Thomson, 1991). These devices as well as odour-baited traps rely on one specific stimulus (light or odour) which affects one type of the flies’ behaviour and are therefore often only effective during certain periods of the flies’ life cycle. A combination of various stimuli may increase the effectiveness of traps.

In this thesis studies are described which were performed to investigate the attractiveness of visual and olfactory stimuli to *Musca domestica* L. adults. These behavioural studies were done in the laboratory with free-flying flies while several physiological and environmental factors were taken into account.

**Outline of this thesis (and main conclusions)**

When we started our research we hoped to find a combination of visual and olfactory stimuli that would attract houseflies to a trap, both males and females, immature and mature ones, and under all possible circumstances.

To control a pest insect, it is necessary to know which is the best period of the day to catch it. It is most likely to catch insects, using attractants, during the period that they are active under natural circumstances. Therefore, we started to examine the circadian activity rhythm of the housefly. We found that houseflies are only active during the day; they are diurnal insects.

The first part of this thesis mainly deals with visual stimuli. We wanted to know the appropriate light wavelength that should be used in light traps to be able to control housefly populations indoors. In other words, which wavelength or combination of wavelengths is, in general, the most attractive one to houseflies. Therefore, the attractiveness of lamps emitting different wavelengths to houseflies of different age, sex, and origin was investigated under controlled circumstances in the laboratory in a flight chamber of 210 cm long, 60 cm wide and 60 cm high (Chapter 2). It was shown that both physiological and environmental parameters (age, sex, origin, and locomotor activity of flies, energy output of light, ambient illumination) affect the number of houseflies attracted to a light source. Flies younger than 3 days were hardly or not attracted to the test lamps, whereas older flies were positively phototactic. Overall, ultraviolet light attracted the highest number of flies. Within the ultraviolet region no preferences were found. To investigate the results obtained in the flight chamber in closer relation to possible practical use, the attractiveness of lamps with different wavelengths was also studied during longer periods in a room of larger dimensions (310 cm long, 200 cm wide, 240 cm high). During these experiments, using females of different origin, results were found similar to those obtained in the flight chamber (Chapter 2). Next, the possibility to increase the attractiveness of an ultraviolet lamp to houseflies by manipulating its flicker frequency was examined in a dark room. Fifteen different light flicker frequencies were tested in the
experimental room. For both females and males, ‘flickering’ light (flicker frequency below the flicker fusion frequency of houseflies which lies around 270 Hz; Vogel, 1956) was found to be less or equally attractive as ‘non-flickering’ light (Chapter 3). Lamps with a frequency of 40 and 175 Hz attracted females and males, respectively, the most rapidly. A flicker frequency of 4 or 10 Hz seemed to cause an escape response in both males and females towards a 'non-flickering' (40 kHz) light source.

We also examined the possibility that our laboratory flies may differ from wild type flies in the amount of visual pigment in their eyes. In vivo measurements were made of eyes of wild-type flies, first generation laboratory flies and flies that had been reared in laboratories during several generations. The amount of visual pigments of the latter appeared to be considerably lower than that of the wild-type and first generation laboratory flies. This strongly suggests that the amount of visual pigment decreases during rearing in the laboratory which may have had effects on the light responses of the flies in our experiments.

The responses to olfactory stimuli are the main subject of the second part of the thesis. We hoped to find volatile compounds that may be used as odorous baits in fly traps. Several odours were tested in the flight chamber for their attractiveness to female and male houseflies, either immature or mature, well-fed or food-deprived (Chapters 4 and 5). The odours of chicken manure, tainted chicken and pig meat, fly food, and bread soaked in water or milk were attractive to well-fed as well as to food-deprived flies, both immature and mature. Males and females appeared to be attracted to different odours; males were attracted to soaked bread, whereas moist yeast and chicken manure were only attractive to females. Tainted meat attracted both sexes (Chapter 5). Only young flies were attracted to foul eggs of houseflies. Hardly any response was observed to single synthetic chemicals. A synthetic mixture of manure components showed attractiveness, but was less effective than natural (chicken) manure (Chapter 4). Of course, flies are never found in an environment without ambient odours. Therefore, we had to determine whether an olfactory bait will still attract flies in an environment loaded with other attractive volatiles. We tested several ‘natural’ products for their attractiveness in the presence of an attractive background odour. When the ambient air was already loaded with the odour of chicken manure, only food-deprived females could be lured, and only with the odour of tainted chicken meat (Chapter 4).

The objective of the experiments which are described in the third part of the thesis was to examine whether a combination of an attractive visual and olfactory stimulus is more attractive than these stimuli when tested separately. Unexpected, adding ultraviolet light suppressed the attractiveness of attractive odours (Chapter 5).

In Chapter 6, the results that are described in the other chapters are summarized and discussed with a view to possible practical use in control measures.
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J.W. Koenes and W.H. Venema (University of Groningen, The Netherlands) constructed the flight chamber. Dr N. Noorman of the Group Sense Organs and Behaviour (University of Groningen) took care of rearing the houseflies. The in vivo measurements of visual pigment in the eyes of flies were done in collaboration with Dr D.G. Stavenga and Dr J. Oberwinkler of the Department of Neurobiophysics of the University of Groningen. The actographs were developed by Dr J.N.C. van der Pers of VDP Laboratories. Dr F.W. Maes (University of Groningen) developed the software to record and analyse the flies' locomotor activities. The lamps used in the experiments were provided by Berson Milieutechniek B.V. and Philips Lighting B.V. The former firm also provided the electrocutor traps. Some of the chemicals that were used in the experiments were provided by Denka International B.V. The emission peaks of the test lamps were measured in collaboration with H. Leertouwer of the Department of Neurobiophysics. S.J. Greven (Department of Electronics, University of Groningen) wrote the software ‘DUAL VLIEGENVAL N293’ and provided technical support together with B.A. Pijpker (Department of Neurobiophysics). Ir W.J. de Boer and Mrs. Ir S.L.G.E. Burgers gave statistical advice for analysing the data. Ing J. Smallegange designed the cover of this thesis. Mrs. Ir T.C. Everaarts made the drawings that are shown in Chapter 1. F. Ialenti, G. Fuhler and F. Obbema performed some initial pilot studies as part of short student projects.
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