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Chapter 3

The attractiveness of flickering and non-flickering ultraviolet light to the housefly (*Musca domestica* L.)

<u>SUMMARY</u>

Electrocuting traps with fluorescent lamps emitting light in the ultraviolet are considered to be promising devices to reduce fly populations to acceptable levels. The possibility to increase the attractiveness of ultraviolet light to mature female and male houseflies (Musca domestica L.) by manipulating the flicker frequency of the light (without effecting its radiance) was examined. Therefore, 15 different flicker frequencies were tested in 1- and 2-choice experiments in a dark room. No significant differences between males and females were found. The results revealed that 'flickering' light (flicker frequency below the flicker fusion frequency of houseflies; 270 Hz) is equally attractive as or less attractive than 'non-flickering' light, although lamps flickering at 175 Hz caught males faster than lamps flickering at the other frequencies tested. The attractiveness was also independent of the timecourse of the light output. Switching on an ultraviolet lamp for short periods (1 minute alternated with 14 minutes of darkness) during an experiment of 21/2 hours attracted as many houseflies as when the lamp was burning continuously.

INTRODUCTION

Electrocutor light traps used for indoor control of houseflies (*Musca domestica* L.) are usually provided with fluorescent lamps emitting light in the ultraviolet range. In most cases the efficiency of these traps is low (Bowden, 1982; Pickens and Thimijan, 1986; Muirhead-Thomson, 1991), which was confirmed by our results described in Chapter 2.

The electricity supplies in Europe and America provide 50 and 60 cycles per second alternating currents, respectively. Hence, the lamps used in light traps and driven by these sources produce periodic light intensity fluctuations at frequencies of 100 and 120 Hz. The frequency of light an eye cannot longer distinguish as discontinuous is called the flicker fusion frequency or critical fusion frequency. Above this frequency the light is perceived as constant. Several studies on the optomotor and the electroretinogram responses of insects showed that insects like locusts, stick-insects, cockroaches, and moths (classified as 'slow-eved' insects; Autrum, 1950) have flicker fusion frequencies of 40 to 60 Hz, whereas the receptor potentials of photoreceptor cells of diurnal, fast-flying insects like honeybees, dragonflies, and houseflies do not fuse before 200 to 300 light flashes per second (classified as 'fast-eyed' insects; Autrum, 1950; Ruck, 1961; Mazokhin-Porshnyakov, 1969; Miall, 1978). These high flicker fusion frequencies enable the latter insects to see fast-moving objects (Autrum, 1950). It also implies that these insects see the flickering of lamps which are driven by the usual alternating current, and this may affect their behaviour. For example, Van Praagh (1972) observed that when the light-ripple of fluorescent tubes in a bee flight room was changed from 300 to 100 Hz, flight behaviour of honeybees (Apis mellifera L.) changed: at 100 Hz flight was slower, more often interrupted, and more 'hesitating' than at 300 Hz. The wing-beat frequency increased from 230 at 300 Hz to 255 at 100 Hz. In 2-choice experiments Syms and Goodman (1987) found that traps with 'flickering' ultraviolet lamps (100 Hz) caught more houseflies than traps with 'non-flickering' ultraviolet lamps (33000 Hz), even when the intensity of the flickering lamps was lower than the intensity of the 'non-flickering' lamps. We, however, did not find differences in attractiveness between two white fluorescent lamps with flicker frequencies of 100 Hz and 40000 Hz, respectively (Chapter 2). In a pilot study (unpublished data) we found that, in a dark flight chamber, mature female houseflies (≥3 days old) landed more frequently on an ultraviolet lamp with a flicker frequency of 4 Hz than on an ultraviolet lamp with a flicker frequency of 40000 Hz. No differences in the number of landings were found between the 40000 Hz ultraviolet lamp and ultraviolet lamps with frequencies of 40, 50, 100, 400, 4000 and 40000 Hz, respectively. All these lamps emitted the same spectrum of light with an ultraviolet peak at 335 nm (Sylvania CFS 11W BL350; UV3; see Table 1, Chapter 2). These results seem to be consistent with the finding that movement detecting neurons in the third optic ganglion of the visual system of the blowfly *Calliphora vicina* Meig. respond maximally at a contrast frequency of about 4 Hz (Zaagman *et al.*, 1978; Mastebroek *et al.*, 1980). Moreover, a flickering sine wave light stimulus yields a response of retinula cells of this fly up to 240 Hz, with a peak between 4 and 20 Hz (Leutscher-Hazelhoff, 1973).

In view of the results of these electrophysiological experiments and the pilot study, we considered it important and promising to examine the attractiveness of flickering ultraviolet light to houseflies more carefully. Hoping to find a frequency attracting higher numbers of flies than were found in previous experiments (Chapter 2) we tested 15 different flicker frequencies. The results of these studies are described in this chapter.

MATERIALS AND METHODS

Insects

In the experiments described in this chapter only 'Pesse' flies (*Musca domestica* L.) of 5-20 days old were used. See Chapter 2 for details.

Test room and test lamps

Catching experiments took place at the same temperature (24°C) and R.H. (60-80%) and in the same white-walled room as described in Chapter 2, either in the dark or with the room illuminated by a 36 Watt white fluorescent lamp (Philips TLD36W/33, 100 Hz) suspending from the centre of the ceiling. In each experiment 25 well-fed males or females of the same age were released in the room.

Two ultraviolet secondary light sources, UV5 (see Table 2, Chapter 2), with emission peaks at 350 nm were standing at a height of 160 cm at a distance of 275 cm from each other against a long side of the room. Before each light source an electrocutor trap (Insectron type I-70, Berson Milieutechniek B.V., Nuenen, The Netherlands), from which the lamp was removed, was present. The UV-C tube (Philips PL-S 9W UV-C) of one of the secondary light sources was connected to a mains adapter to achieve a current with a 40 kHz flicker frequency. Because the flicker fusion frequency of the housefly lies around 270 Hz (Vogel, 1956), this lamp is called the 'non-flickering' control lamp. The UV-C tube of the other light source, the test lamp, was connected with a function generator (Wavetek 11 MHz Stabilized Function Generator, model 21) with which the light flicker frequency could be varied. Both 2- and 1-choice experiments were done with either both or only one light source burning.

Measurements with an optical power meter (see Chapter 2) revealed that changing the light flicker frequency did not affect the mean radiance of the test lamp. When the lamp was flickering at 40 kHz it emitted a similar amount of energy in the ultraviolet (330-385 nm) and visible light region (400-1000 nm) as the control lamp: approximately 5 and 14 μ Watt/cm², respectively. The

radiance of the test lamp was slightly lower at the lower frequencies: approximately 4 in the ultraviolet and 9 μ Watt/cm² in the visible region.

A personal computer controlled on and off switching of the lamps and the traps. The computer also recorded when an electrocuting grid was hit by a fly, using the computer programme 'DUAL VLIEGENVAL N293' (S.J. Greven, University of Groningen, The Netherlands). In order to prevent flies which had not been killed by the electrocuting grids from escaping, the catch trays at the base of each trap were filled with a shallow layer of soap water.

The experiments lasted $2\frac{1}{2}$ hours and were done between 8 a.m. and 6 p.m.

Experiments

To examine possible positional effects within the room, 2-choice experiments were done with both the control lamp and the test lamp at 40 kHz. The two light sources changed place after each experiment. The experiments were done in the dark with female or male flies. Possible variations in catches during the day were examined by carrying out 1-choice experiments in a dark room during different periods on the same day: from 08:00 - 10:30 a.m., 10:30 a.m. - 01:00 p.m., 01:00 - 03:30 p.m., and from 03:30 - 06:00 p.m. During these experiments only the control lamp was burning, either at one or the other end of a long side of the room.

Subsequently, flicker frequencies of 4, 10, 20, 30, 40, 50, 75, 100, 150, 175, 200, 250, 300, 350, and 40000 Hz were tested in the dark for their attractiveness to mature houseflies. These experiments were done with light sources with the standard, sinusoid output. Each frequency was tested four times in 2-choice experiments, during which the test lamp stood twice at one end and twice at the other end of the long side of the room. In addition, 1-choice experiments were done in which each frequency was tested twice. Frequencies were applied in random order.

To examine the effect of ambient illumination on catches, the attractiveness of the control and the test lamps was tested in 1-choice experiments both when the room was dark and when the room was illuminated by the white fluorescent tube.

To examine the effect of the waveform of the light output, the attractiveness of 'flickering' ultraviolet secondary light sources (UV5) with a sinusoid and 'block-shaped' output, respectively, was tested in 2-choice experiments in a dark room. In these experiments the flies had to choose between a lamp emitting 100 Hz sinusoid waves (Philips PL-S 9W UV-C) and a lamp with 40 Hz (when females were tested) or 50 Hz (when males were tested) sinusoid or block-shaped output. The latter two frequencies were chosen because they seemed to be most attractive to females and males, respectively (see 'Results').

Finally, hoping to increase the catches, 1-choice experiments were done in the dark during which one frequency was alternated with periods of darkness

or during which two frequencies were alternated: a lamp with a frequency of 40 kHz was switched on for 1 minute alternated with 14 minutes of darkness or it was burning for 14 minutes alternated with 1 minute periods during which the lamp was flickering at 40 Hz (in experiments with females) or 50 Hz (in experiments with males). The results were compared with those of experiments during which a lamp was burning continuously at a frequency of 40 or 50 Hz, or 40 kHz.

Statistics

A Generalized Linear Model (GLM) was used to investigate possible relations between the catches in the 1-choice experiments and the various factors (e.g., time of day, sex, frequency). Two-sided t-probabilities were calculated to test pairwise differences of means. Effects were considered to be significant at P<0.05.

The mean numbers of flies caught by the two electrocutor traps during 2choice experiments were subjected to the Fisher's exact test of independence (2-tailed) for each light frequency. This test was used to analyse whether the distribution of catches was significantly different from a 1:1 distribution which may be expected when the flies do not distinguish between the two test frequencies. A P-value less than 0.05 was assumed to indicate attractiveness of the frequency causing the highest number of trapped flies (Oude Voshaar, 1994; Sokal and Rohlf, 1998).

<u>RESULTS</u>

Effects of lamp position and time of the day

Control experiments were done to examine possible effects on the attractiveness of the test lamps of their position in the room and of the period of the day during which experiments were performed.

No significant differences in catches of females or males in different corners of the room were found in both 2-choice and 1-choice experiments. Therefore, it was not necessary to correct the catches for positional effects.

Over the whole day significantly less males (57%) than females (82%) were attracted to the 40 kHz lamp. The mean percentages of flies caught during different periods of the day are shown in Figure 1. For male flies, no differences in light responses over the day were found. Females were more responsive at the end of the morning and in the afternoon than during the first period of the day. Therefore, all experiments with females described below were done after 10:30 a.m.

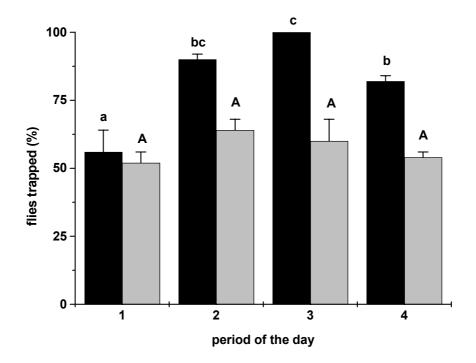


Figure 1. Percentages of houseflies attracted to a 40 kHz light source at four different periods of the day in a dark room. Each column represents the mean of two experiments each with 25 flies. Vertical lines show standard errors of the mean. For each sex (females: dark bars; males: grey bars), columns marked with different letters are significantly different (least significant difference method, P<0.05). 1: 08:00 - 10:30 a.m.; 2: 10:30 a.m. - 01:00 p.m.; 3: 01:00 - 03:30 p.m.; 4: 03:30 - 06:00 p.m.

1-Choice experiments

Fifteen different light flicker frequencies were tested for their attractiveness to both male and female houseflies to reveal which frequency is the most attractive to them. Figure 2 shows the percentages of females and males which were trapped with an ultraviolet light source burning at various frequencies. A mean of 73 ± 25 percent of the flies was caught. GLM analysis showed that the light response was significantly affected by frequency, and it revealed an interaction between frequency and sex. A frequency of 150 Hz attracted less females than males. The other frequencies attracted about equal numbers of both sexes. Averaged over the sexes, the numbers of flies caught were lower when frequencies of 4, 10, 20, 200 or 40000 Hz were tested, whereas 40, 175, 250 and 350 Hz attracted significantly more flies than 30, 50, 75, 100, 150 and 300 Hz.

Two examples of catches in the course of time during a 1-choice experiment are given in Figure 3. These 40 and 175 Hz lamps were the fastest catchers of females and males, respectively. Within the first 15 minutes of the experiment they caught around half of the flies.

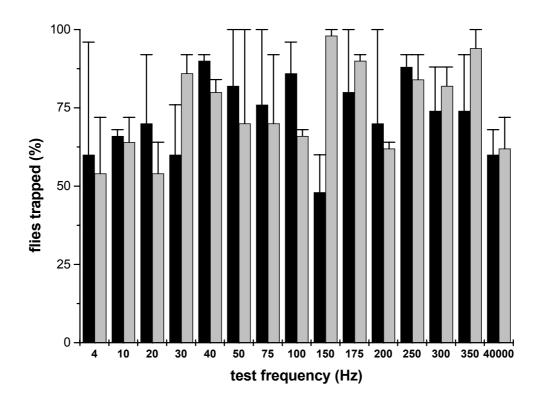


Figure 2. Attractiveness of one lamp flickering at various frequencies in a dark room to female (dark bars) and male (grey bars) houseflies. Each column represents the mean of two experiments each with 25 flies. Vertical lines show standard errors of the mean.

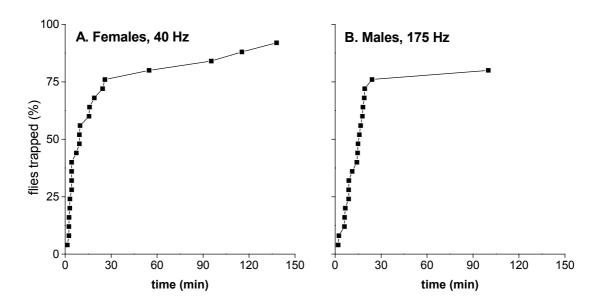


Figure 3. Examples of houseflies caught in the course of time during a 1-choice experiment with 25 flies in a dark room. A. Females; test lamp with frequency 40 Hz; B. Males; test lamp with frequency 175 Hz.

2-Choice experiments

The attractiveness of the same 15 frequencies was examined when in competition with a 'non-flickering' lamp (the control lamp flickering at 40000 Hz). Figure 4 shows the mean percentages of flies caught by the traps during the 2-choice experiments with the test lamp flickering at different frequencies. No significant differences were found in attractiveness between the test and control lamp, except when the former flickered at 10 Hz; then this lamp was significantly less attractive to both males and females than the control lamp. A similar, but not significant, difference was found at 4 Hz.

In Figure 5 two examples are given of catches in the course of time during two different 2-choice experiments. It appeared that the catch rate varied considerably when the same frequency was tested in different experiments. This renders it difficult to determine a frequency which attracts females or males the most rapidly when in competition with the control lamp. However, when the test lamp was flickering at a frequency of 40 and 175 Hz the two traps together caught 50 percent of the released females and males, respectively, within 40 to 60 minutes, whereas, on the average, it took longer when the other frequencies were tested.

Effect of ambient illumination

The light response of houseflies to different frequencies was examined when in competition with room illumination to reveal whether flickering light attractants should be used in illuminated or in dark rooms. It was found that less flies were caught in an illuminated room than in a dark room (Figure 6). No differences between females and males were found. The tested frequencies were equally attractive to both males and females under the same light conditions.

Effect of the waveform of the light output

Lamps that are usually used in electrocutor traps have a sinusoid light-output. We examined whether a 'block-shaped' light-output would increase the attractiveness of an ultraviolet lamp. However, equal numbers of both females and males were attracted to the test lamp and to the standard UV5 lamp irrespective of the shape (sinusoid or block-shaped) of the light output of the test lamp (Figure 7).

Alternating two frequencies in time or one frequency with periods of darkness

Another approach was taken with the idea that it may increase the attractiveness of an ultraviolet lamp to houseflies. Instead of having a lamp burning continuously at one and the same frequency, one frequency was alternated with periods of darkness or two frequencies were alternated in time.

Compared to a continuously burning 'flickering' or 'non-flickering' light source, alternating a 'non-flickering' with a 'flickering' light source or varying darkness with short periods of light, did not have an effect on the number of females caught, whereas it slightly decreased the number of males attracted to the light sources. On the average, 69% of the females and males was attracted by the 'non-flickering' and 'flickering' light source.

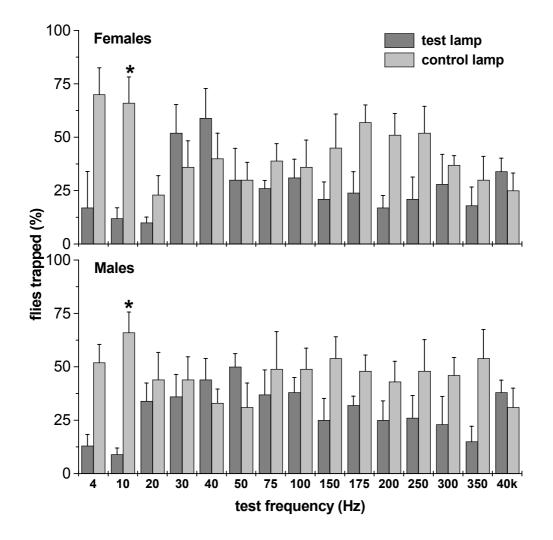


Figure 4. Attractiveness of a 40 kHz lamp (grey bars) and a test lamp (dark bars) flickering at 15 different frequencies to female and male houseflies. Each column represents the mean of four 2-choice experiments in a dark room each with 25 flies. Vertical lines show standard errors of the mean. Asterisks indicate a significant difference in attractiveness between the two light sources (Fisher's exact test, 2-tailed, P<0.05).

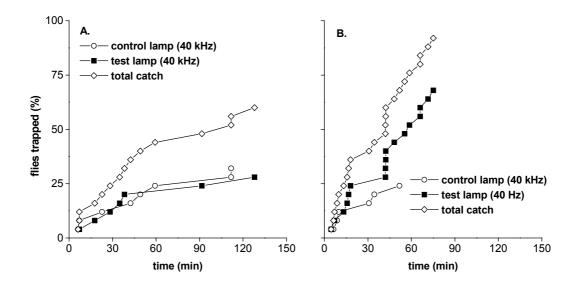


Figure 5. Examples of female houseflies caught in the course of time during a 2-choice experiment with 25 flies in a dark room. A. Test lamp and control lamp both with frequency 40 kHz; B. Test lamp with frequency 40 Hz; control lamp at 40 kHz.

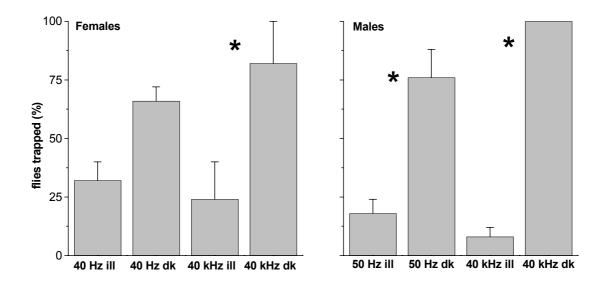


Figure 6. Attractiveness of a 'flickering' (40 or 50 Hz) or 'non-flickering' (40 kHz) test lamp in a dark (dk) and in an illuminated (ill) room (1-choice experiments) to female and male houseflies. Each column represents the mean of two experiments each with 25 flies. Vertical lines show standard errors of the mean. Asterisks indicate a significant difference in attractiveness of one frequency under the two light conditions (least significant difference method, P<0.05).

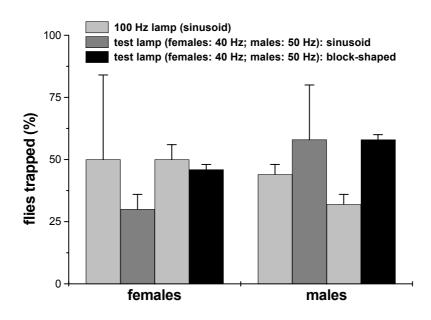


Figure 7. Percentages of female and male houseflies attracted to a 100 Hz lamp with sinusoidally modulated light-output and the test lamp with sinusoid-or block-shaped light-output in 2-choice tests in a dark room. The test lamp had a flicker frequency of either 40 (females) or 50 Hz (males). Each column represents the mean of two experiments each with 25 flies. Vertical lines show standard errors of the mean.

DISCUSSION

Male flies are known to chase moving conspecifics (West, 1951; Vogel, 1956; Land and Collet, 1974; Wehrhahn *et al.*, 1982). Presumably, light modulations are associated with wing movements of conspecifics and induce chasing behaviour. Voss (1913: see West, 1951) found that the wing beat frequency of the housefly varied between 180 and 197 Hz, whereas Marey (1901: see West, 1951) and Weldon (1946: see West, 1951) counted 330 strokes of the wings per second. The rate of vibrations estimated from the sound that is produced by the vibrations of the wings of the flies in our laboratory is 175 Hz (F.J. Kelling, pers. comm.). Indeed, in both 1- and 2-choice experiments we found that the test lamps when flickering at 175 Hz caught males faster than lamps flickering at the other frequencies. Since this frequency attracted between 60 and 100 percent of both males and females during 1-choice experiments, it may be worthwhile to apply lamps with this frequency in light traps.

For both sexes, with one exception (see below), no distinct differences in attractiveness between 'flickering' and 'non-flickering' (i.e. below and above

270 Hz, respectively; Vogel, 1956) ultraviolet lamps were found in 1- and 2choice experiments. These results are in agreement with the results described in Chapter 2 where no difference in attractiveness was found between two white fluorescent lamps with flicker frequencies of 100 Hz and 40 kHz. Hence, the results obtained by Syms and Goodman (1987) are not confirmed. These authors found that in both a dark and an illuminated room ultraviolet lamps flickering at 100 Hz attract more houseflies than 'non-flickering' ultraviolet lamps of 33 kHz during 2-choice experiments. The fact that males and females are equally sensitive to the various frequencies agrees with the absence of differences in electroretinogram responses to flickering stimuli between the sexes in *Musca* (see Syms and Goodman, 1987).

In 2-choice experiments, we found at only one test frequency a significant difference between the numbers of males and females attracted to the 'flickering' and the 'non-flickering' lamp. When competing with the 'nonflickering' light source the lamp with a flicker frequency of 10 Hz seemed to cause an escape response in both males and females towards the 'nonflickering' light source. A similar reaction, although not significant, was found at 4 Hz (Figure 4). This reaction to low frequencies may be explained by the fact that the sensitivity of the photoreceptors in the retina and the movement detecting neurons of houseflies is high to low frequencies, as was found for the blowfly Calliphora vicina (Leutscher-Hazelhoff, 1973; Zaagman et al., 1978; Mastebroek et al., 1980). In addition, reduction of the light intensity, which was noticeable at low frequencies, is supposed to induce an escape response, probably because it mimics the attack of a predator (Holmquist and Srinivasan 1991; Trimarchi and Schneiderman, 1995). Normally, the escape response will be directed towards the sky (Mazokhin-Porshnyakov, 1969; Menzel, 1979), the natural source of 'non-flickering' ultraviolet light, which was probably mimicked by the control lamp. This phenomenon may be used in a 'push-pull system' in order to drive houseflies outdoors or towards a 'nonflickering' ultraviolet lamp in a trap.

However, care should be taken with application of flickering lamps. The maximum flicker rate humans can distinguish is 50-60 Hz. Because ultraviolet light is not perceptible to the human eye and can damage human skin and eyes, blue phosphors are usually added to the light source of a trap to indicate to man that the light is on. However, long-term exposure to flickering light may give rise to emotional stress in humans. When the use of frequencies below the human critical fusion frequency is required, other solutions, like an indication lamp, should be found to show that the ultraviolet lamps are switched on.

Light traps with flickering lamps may also affect behaviour and reproduction of cattle or poultry. For example, Nuboer *et al.* (1992) showed that 105 Hz is the critical fusion frequency of chicken. And in contrast to humans, chickens can see ultraviolet light; the chicken retina is sensitive to wavelengths down to 300 nm (Wortel, 1987).

Taking these problems into account it is advisable to use 'non-flickering' lamps in light traps in areas where humans or animals are present. However, the push-pull system may appear to be a good control method for houseflies and can be applied in other areas.

The results described in this chapter and in Chapter 2 show that during daytime light traps are especially effective in dark rooms; more flies are caught in the dark (Chapter 2 and 3) and they are caught quicker in the dark than in illuminated rooms (Chapter 2). We also found that the attractiveness of ultraviolet lamps is not improved by changing the shape of its light output. In addition, our results showed that a light trap does not have to be switched on all day in order to catch a large part of an indoor fly population. This will reduce the costs of fly control.

In Chapter 2 we showed that only flies older than 2 days are attracted to light. Around this age, the houseflies become sexually mature. In order to be able to also catch younger, immature flies additional attractive stimuli like odours may be necessary. In addition, better attractants than light are required in illuminated rooms. In the next chapters experiments are described in which the attractiveness of odours and their effects on the attractiveness of light sources are studied.

OLFACTION