Luring houseflies (*Musca domestica* Diptera: Muscidae) to traps: Do cuticular hydrocarbons and visual cues increase catch?

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Running Title: Olfactory and visual lures for houseflies

Abstract

Houseflies (*Musca domestica* L.) are a major pest species of livestock units and landfill sites used for the disposal of domestic waste. Of the many methods used to limit housefly populations, the most common are chemical control and lure-and-kill trap systems. Insecticide resistance has seen increased emphasis on lure-and-kill, but the success of this method relies on effective attraction of houseflies using olfactory or visual stimuli. Here we examine the efficacy of olfactory (cuticular hydrocarbons) or visual (colours and groups of flies) attractants in a poultry unit. Despite simulating the cuticular hydrocarbon profiles of male and female houseflies, we found no significant increase in the number of individuals lured to traps, or any sex-specific responses. Similarly the use of target colours selected to match the three peaks in housefly visual spectral sensitivity yielded no improvement in catch rate. We also demonstrate that male and female flies have significantly different spectral reflectance (males are brighter between 320-470nm; females are brighter between 470-670nm). An experiment incorporating groups of recently killed flies from which cuticular hydrocarbons were either removed by solvent or left in-tact also failed to show any evidence of olfactory or visual attraction for houseflies of either sex. Thus variations on the most commonly applied methods of luring houseflies to traps in commercial livestock units failed to significantly increase capture rates. Our results support commonly observed inconsistencies associated with using olfactory or visual stimuli in lure-and-kill systems, possibly because field conditions lessen the attractant properties observed in laboratory experiments.

Key words: pest control, pheromones, poultry units, sexual dichromatism, spectral reflectance

Introduction

The housefly (*Musca domestica* L. (Diptera: Muscidae)) is widely regarded as an important pest species (Busvine, 1980; Chapman *et al.*, 1998a; Howard, 2001). Its habit of feeding on decaying matter, human waste and food, and concomitant close association with humans, has implicated *M. domestica* with the spread of numerous diseases including salmonella, diphtheria, tuberculosis, hepatitis and amoebic dysentery (Greenberg, 1973; Crosskey & Lane, 1993; Tan *et al.*, 1997). Problems with excessive housefly populations are generally connected with livestock units and landfill sites used for domestic waste (Goulson *et al.*, 1999; Howard, 2001; Winpisinger *et al.*, 2005) and populations seem set to increase with the projected warming of Earth's climate (Goulson *et al.*, 2005). The public health risks and annoyance associated with large housefly populations are therefore substantial and efforts to exert control over the species have been the focus of considerable research for several decades (Wiesmann, 1962; Mitchell *et al.*, 1975; Carlson & Leibold, 1981; Chapman *et al.*, 1999; Hanley *et al.*, 2004).

For many years effective control was achieved using the application of contact insecticides via spraying. However this approach has resulted in the widespread development of insecticidal resistance (Chapman *et al.*, 1993; Keiding, 1999; Shono *et al.*, 2004). More recently the use of toxic baits for control of *M. domestica* has become commonplace, particularly within enclosed areas such as livestock units. These lure-and-kill systems rely on attracting houseflies to targets baited with a poison, which is then ingested by the fly on contact with the target (Chapman *et al.*, 1998a,b). For this system to be effective, the targets must attract large numbers of flies of both sexes, and two different, but often interactive, approaches have been employed for this purpose. The principal technique has been to impregnate targets with (*Z*)-9-tricosene, one of many hydrocarbons associated with the cuticular layers of the housefly (Carlson *et al*., 1971; Nelson *et al.*, 1981; Noorman & Den

Otter, 2001; Dunn, unpublished data). Initial experimental inconsistencies and difficulties in understanding the role of this compound in housefly biology are reviewed by Howard & Blomquist (1982) and recent work has further cast doubt over the efficacy of (*Z*)-9-tricosene as an attractant, particularly over long distances and at the concentrations normally produced by wild houseflies (Chapman *et al.*, 1998b; Kelling *et al.*, 2003; Hanley *et al.*, 2004). Problems with this focused view of (*Z*)-9-tricosene as a sex attractant are illustrated by a field survey which failed to detect any (*Z*)-9-tricosene in many populations of housefly (Darbro et al 2005) and others finding evidence that (*Z*)-9-tricosene production is a response to environmental stress (Noorman & Den Otter, 2001 and 2002). Given that blends rather than single compounds are usual in insect communication systems and hydrocarbon blends of low volatility can evoke strong behavioural responses over the short-medium range (Howard & Blomquist, 1982; Schiestl et al., 1999 and 2000), the attractant properties of other cuticular hydrocarbons from houseflies, particularly when presented in realistic ratios needs to be assessed.

The second approach has been to attract houseflies to baited targets using visual cues. Early laboratory studies on the response of *Musca domestica* to colour suggested that they preferentially settle on black or red surfaces, and avoid blue or white surfaces (Waterhouse, 1948; Pospíšil, 1962; Hecht, 1963), although work conducted in the poorly illuminated conditions of livestock units indicated that paler colours such as yellow and white may be more attractive (Mitchell *et al.*, 1975). Other studies have suggested that the degree of contrast might be far more important than colour in evoking a response (Hecht, 1970; Howard & Wall, 1998), and in particular the use of black dots on a white background. Although Richter *et al.*, (1976) reported increased housefly attraction using targets painted with a regular spacing of black spots, Chapman *et al.*, (1999) found that clustered groups of black spots were more effective still. Clustered spots may be effective because they mimic the localised feeding behaviour of houseflies; Wiesmann (1962) first suggested that clustered groups of individuals were the principal optical cue involved in the location of food resources for houseflies.

Despite the many experimental trials of different visual attractants, there remains a great deal of uncertainty about which combinations of colour and pattern are most effective at luring houseflies to traps. This uncertainty stems in part from the contradictory results emerging from studies conducted in comparatively well illuminated laboratory conditions, and those performed in generally poorly-lit livestock units. However none of these earlier studies considered the importance of housefly vision and spectral sensitivity in relation to target design. This is surprising given that a great deal is known about the visual system of *M. domestica*, and in particular the way in which they capture and processes light. The optical sensitivity of the housefly lies between 310 and 700 nm (Strother & Casella, 1972). Moreover, in his review of the functional organisation of *M. domestica* vision, Hardie (1986) describes how the photoreceptors in a housefly's compound eye have three absorbance peaks, one at 490 nm (blue/green), and a second at 570 nm (yellow). The third (double peak) lies within the UV band. Houseflies are highly receptive to UV light and specialised UV receptors in the eye are particularly sensitive to wavelengths between 330 and 350 nm (Hardie 1984). However to-date there has been no attempt to determine how houseflies react to colours of these specific wavelengths.

In this paper we document the results of three separate field experiments in which we investigate the efficacy of olfactory and visual cues in housefly attraction. In the first experiment we examine the attractant properties of a combination of nine cuticular hydrocarbons identified from wild houseflies where the experiment was to be conducted (Dunn, unpublished data). The second study examines the role of colour, using three different paints selected to match the peaks in *M. domestica* spectral sensitivity (Hardie,

1986) to determine whether the species is more receptive to these colours than to a plain white target. Finally we examine the response of *M. domestica* to clusters of other houseflies. Having first measured the spectral reflectance of wild, male and female houseflies in the laboratory, we arranged groups of recently killed male or female individuals on traps in the field. In order to separate potential interactions between olfactory and visual cues on the number of housefly landings recorded, we removed cuticular hydrocarbons from half of the flies prior to the start of the experiment by solvent washing. The combination of field studies documented here therefore examines the principal methods (i.e. olfactory and visual cues) reputed to attract houseflies to commercial lure-and-kill traps, and provides the first experimental review of how colours approximating to the absorbance peaks in housefly vision influence trap success.

Materials and Methods

Field site

All experiments were conducted in a deep-pit caged layer poultry unit in southern England. The unit was 100-m long × 150-m wide and contained 40,000 chickens, housed in 11 rows of tiered cages running the length of the building. The birds were housed on the upper level, with a manure pit on the lower level below. Lighting was provided by 12 fluorescent strip lights positioned at 5 m intervals along each row, with a target temperature maintained close to 21^{0}C. Apart from the traps used in the experiments, no other housefly controls were employed during the duration of the trials.

Experiment 1 - Attractant properties of cuticular hydrocarbons

In this experiment we simulated the profile of principal hydrocarbons from wild male and female *M. domestica* to determine whether houseflies in the field respond to the olfactory cues provided by other flies. To prepare the experimental hydrocarbon solutions, we used

GC and GC-MS determined ratios and quantities of the individual hydrocarbons recorded on wild *M. domestica* from the experimental locality (Dunn, unpublished data). Using stock solutions, tricosane, tetracosane, pentacosane, hexacosane, heptacosane, nonacosane, (*Z*)-9-tricosene [Sigma-Aldrich Ltd, Poole, UK], (*Z*)-9-heptacosene, and (*Z*)-9-nonacosene [Denka International BV, Barneveld, The Netherlands]) were mixed and diluted to a concentration of 10 or 100 fly equivalents per 100 µl aliquot.

A circular 25 mm diameter filter paper (Whatman GF/B; Whatman International Ltd, Maidstone, Kent, UK) was fixed to the centre of a 400 × 245 mm yellow sticky trap (Agrisense-BCS Ltd, Pontypridd, UK). The use of such traps is a standard technique for studying housefly abundance (Black & Krasfur, 1985; Goulson *et al.*, 1999, 2005). A 100-µl aliquot of mixed hydrocarbon solution was applied to the centre of the filter papers using a glass pipette. The traps were then suspended 5 m apart by string from a wire running across the length of each row, with the centre of each trap at about 2.5 m high and immediately beneath one of the florescent tubes illuminating the unit casting 0.062±0.005 Lux at trap height. There were six rows of traps, each row having one randomly assigned, replicate sample of each of the test hydrocarbon solutions and the hexane control. Edge effects were avoided by suspending blank sticky traps at the ends of each row. The experiment was conducted over seven days until the traps were removed. However we quantified total fly numbers one day into the experiment, before quantifying the total number of flies and their sex ratio on day seven.

Experiment 2 - Attractant properties of colours

We examined the role of colour in attracting houseflies by using three paints mixed to provide spectral reflectance peaks approximating to the most sensitive regions of *Musca* spectral absorption (Hardie, 1986). Two of the paints were formulated from commercially available mixes (Crown Decorative Products Ltd, Preston, Lancashire, UK). A blue/green paint (Crown mixture code – 3060-B40G), and a yellow paint (code – S1050-G50Y) yielded spectral reflectance peaks at 490 nm and 565 nm respectively (Fig 1). A third paint (Yellow Glow B29, Plasti-kote Ltd, Cambridge, UK) provided a mixture with two reflectance peaks at 345 nm (UV) and 512 nm (yellow). The fourth mixture was a white paint (Code BS 00 E 55, Crown Decorative Products, Ltd.) with a continual peak in spectral reflectance between 400 and 700 nm.

Two coats of paint were applied to 400 × 245 mm pieces of cardboard, such that there were ten replicate targets of each colour. Once the paint had thoroughly dried, the targets were covered with a 1 mm thick layer of adhesive (OecoTAK A5; Oecos, Kimpton, UK) in order to trap houseflies. Spectral reflectance was checked after the application of glue to ensure that the adhesive did not significantly affect the paint’s spectral properties. The traps were then suspended in five rows in the poultry unit as described above, with two replicates of each colour randomly assigned to each row. Traps remained in the unit for seven days. Total fly numbers were scored on days one and seven and sex ratio scored on day seven.

Experiment 3 - Attractant properties of housefly groups

In order to test the hypothesis that stationary (~ feeding) groups of flies attract other individuals, we fixed ten newly freeze-killed *Musca* to the centre of a 400 × 245 mm yellow stick trap (Agrisense-BCS Ltd.). Prior to being fixed to the traps, the flies were sorted into male and female groups, and half of each sex group was washed for 60 min in hexane to remove cuticular hydrocarbons. The flies were arranged in a hexagonal array; each separated by 20 mm, and aligned so that their legs and ventral portion adhered to the trap surface. In addition to blank control traps, there were four treatment groups: 1 – washed male flies, 2 – washed female flies, 3 – unwashed male flies, 4 – unwashed female flies. These treatments allowed us to investigate the role of sexual dimorphism in visual and olfactory cues in attracting other houseflies. Once the flies were fixed onto the traps, they were suspended in five rows in the poultry unit as described above, with two replicates of each treatment and control group randomly assigned to each row. Traps remained in the unit for seven days. Total fly numbers were scored on days one and seven and sex ratio scored on day seven.

Prior to the experiment, we measured the spectral reflectance of 30 male and 30 female houseflies to determine any visual differences between sexes. The flies were collected from the same poultry unit used in the experimental trials, and were killed the same day by freezing for 30 min, prior to measurement of their thoracic spectral reflectance. Measurements were made using a bifurcated fibre optic reflectance probe (Ocean Optics R200-7, Ocean Optics B.V., Duiven, The Netherlands) connected to deuterium-halogen lamp (Ocean Optics DH2000), an Ocean Optics SD2000 dual channel spectroradiometer, and a notebook computer running Ocean Optics OOIBASE32 software. Each spectrum, comprising 1125 data points (reflectance from 300-700nm at 0.36nm intervals), was standardised for brightness differences by subtracting its mean reflectance across all wavelengths. Principal components analysis (PCA) was used to transform a large number of correlated variables (in this case, reflectance at 0.36nm intervals) into few orthogonal variables representing spectral shape (Endler & Mielke, 2005). Analysis of variance was used to test for differences between male and female reflectance spectra in terms of their PC1 and PC2 scores (Cuthill *et al.*, 1999).

Results

Experiment 1 - Attractant properties of cuticular hydrocarbons

There were no significant differences (ANOVA Day 1 $F_{4,25}$ = 0.04, P = 0.996; Day 7 F = $0.08_{4,25}$ P = 0.989) between the mean numbers of houseflies caught by the hexane control traps and by the four cuticular hydrocarbon mixtures (Fig 2). Although there was a great deal of variability within treatments, there was remarkably little difference between the mean numbers of flies caught on the most successful (*e.g*. day 7 'Female × 100' = 44.2 flies per trap) and the least successful traps ('Male × 100' = 36.2 flies per trap). Moreover there were no significant differences in the proportions of male and female flies recorded for each treatment on day 7 (X^2_4 = 0.634, P = 0.959).

Experiment 2 - Attractant properties of colours

Due to there being two replicate treatments within each row we used a nested ANOVA to examine differences in housefly catch rates between different colour treatments and to test for differences in catch rates between rows. However neither of these factors had a significant effect on housefly catches at day 1 (Nested ANOVA Colour treatment - $F_{3,20}$ = 0.52, P = 0.68: Row effects - $F_{16,20}$ = 2.01, P = 0.07), or day 7 (Nested ANOVA Colour treatment - $F_{3,20}$ = 0.90, P = 0.46: Row effects - $F_{16,20}$ = 1.09, P = 0.42). Moreover, we found no significant differences (X^2_3 = 0.679, P = 0.878) in the proportion of male and female houseflies caught by different colour treatments on day 7 of the experiment (Fig 3).

Experiment 3 - Attractant properties of housefly groups

There was a significant difference between male and female fly spectra in terms of PC1 (ANOVA - $F_{1,58}$ = 68.45, P < 0.001) and PC2 (ANOVA - $F_{1,58}$ = 6.87, P < 0.011) (Fig. 4). In PCA of natural spectra, PC1 represents variation in mean reflection (i.e. brightness)

(Cuthill *et al.*, 1999). Although there are significant differences in mean reflectance between male and female houseflies, there was also a significant difference between spectral shape (colour) represented by PC2. Specifically female housefly spectra have more negative PC1 coefficient values and therefore reflect relatively more light (are brighter) between 470-670nm (Figs. 5 and 6). Conversely male houseflies are brighter between 320-470nm. In terms of PC2, males reflect significantly more light between 570-720nm whereas females reflect more light below 570nm (Figs. 5 and 6). Therefore male houseflies are more ‘red’ in colour whereas female houseflies reflect more short-wavelength light.

Nested ANOVA revealed that groups of houseflies had no significant effect on housefly catches at day 1 (Nested ANOVA Target treatment - $F_{4,25}$ = 0.62, P = 0.665: Row effects - $F_{20,25}$ = 1.59, P = 0.136), or day 7 (Nested ANOVA Target treatment - $F_{4,25}$ = 1.04, P = 0.407: Row effects - $F_{20,25}$ = 1.02, P = 0.472). Moreover, we found no significant differences (X^2_4 = 4.314, P = 0.365) in the proportion of male and female houseflies caught by different target treatments on day 7 of the experiment (Fig 7).

Discussion

Considerable doubt has recently been cast over the role of (*Z*)-9-tricosene as a sex-attractant in *M. domestica* (Noorman & den Otter 2001; Kelling *et al.*, 2003; Darbro *et al.*, 2005) and thus its use as a means of luring houseflies to traps in field situations (Hanley *et al.*, 2004). Cuticular hydrocarbons such as (*Z*)-9-tricosene are of low volatility and can only influence insect behaviour at short-medium range (Noorman & den Otter 2001; Schiestl *et al.*, 1999 and 2000; Kelling *et al.*, 2003). By contrast to cuticular hydrocarbons, it is widely believed that variation in trap colour and the use of strong contrast patterns to mimic aggregations of feeding houseflies significantly enhances catch rates (Mitchell *et al*., 1975; Chapman *et al.,* 1999). It is surprising therefore that neither the use of trap colours employed to specifically match the peaks in housefly spectral sensitivity, nor groups of recently killed houseflies affixed to traps, resulted in significantly improved housefly attraction. Similarly, we were unable to detect any variation in male and female catch rates based on sex-specific olfactory stimuli. Although differences in cuticular hydrocarbon profiles between male and female houseflies are well known (Nelson *et al*, 1981; Noorman & Den Otter, 2001; Dunn, unpublished data), spectral variation associated with sex has not previously been demonstrated. Here we establish a clear sexual dichromatism between male and female houseflies. Nevertheless, and despite the significant differences in cuticular hydrocarbon profile and spectral reflectance shown by male and female houseflies, neither factor had any significant influence on the relative proportion of male and female flies caught in this experiment. Thus overall, none of the visual or olfactory cues we employed to lure houseflies to our traps resulted in improved catch rates of either sex above those caught on untreated control traps.

The majority of studies showing strong visual (Hecht, 1963; Richter *et al.*, 1976; Howard & Wall, 1998) or olfactory (Silhacek *et al.*, 1972; Carlson *et al.*, 1974; Cosse & Baker, 1996) stimulation in houseflies have been conducted in laboratory conditions where light and air quality are high and easily controlled. Trials conducted in field conditions have by contrast, often yielded results contradictory to laboratory experiments (Howard & Wall, 1998; Chapman *et al.*, 1999; Hanley *et al.*, 2004). The relatively poorly aerated and dimly lit conditions associated with commercial livestock units would foster competing odour sources and may be particularly effective at reducing the attractant properties of otherwise strong olfactory or visual stimuli, although in some cases pale coloured targets (white or yellow) impregnated with (*Z*)-9-tricosene have proved to be highly effective lures for houseflies in poultry units (Mitchell *et al.*, 1975; Burg & Axtell, 1984; Chapman *et al.*, 1998b). The use of male and female cuticular hydrocarbons (Experiment 1), and relatively pale visual cues (yellow, florescent yellow, or white targets in experiment 2) had no significant effect on *M. domestica* catch rates in this study, suggesting that when these olfactory and visual cues are used in isolation, they are ineffective. However it must be remembered that the hydrocarbons used in experiment 1 were applied to yellow-coloured traps, with no clear synergistic effect. Moreover experiment 3 combined the widely reported visual stimulation provided by clustered individuals (Richter *et al.,* 1976; Chapman *et al.,* 1999), with the potential olfactory stimulation arising from groups of freshly killed male or female houseflies, without yielding increased rates of fly capture.

The lack of observed differences in housefly catch rates for treated traps might also be ascribed to the generally high *M. domestica* populations present in the poultry unit during our experiments. After only 7-d, most traps had caught well in excess of 50 individuals. Moreover the random capture of flies on any given trap might in itself lead to increased landings simply because of the attractant effect that groups of houseflies appear to exert on other individuals (Richter *et al.,* 1976; Chapman *et al.,* 1999) (although no such effect was apparent in Experiment 3). Such high rates of capture could potentially mask the attractant effects of visual or olfactory stimuli examined in this study. However we did not find any effect of visual or olfactory stimulation on houseflies after only 1 day, presumably while olfactory cues were still most contrasting before the targets became heavily loaded with flies, suggesting that the high *M. domestica* density in the poultry unit had little influence over our lack of significant differences between treatments. Also, effective control is most needed when fly populations are high, and our data suggest that these approaches are not effective at attracting flies under these conditions.

Given the projected increases in housefly populations and incidence of vector-borne disease associated with global warming (Goulson *et al.,* 2005, Haines *et al.,* 2006), the need for effective housefly control may become increasingly pressing. From this point of view, our failure to lure houseflies to traps using hydrocarbon olfactory or visual cues suggests that lure and trap systems do not represent the most effective control measure for this pest species. Other options, relying on the use of biological control with fungal pathogens (Kaufman *et al.*, 2005; Lecuona *et al.*, 2005), or parasitoids (Skovgard & Nachman, 2004), or by using auto sterilisation (Howard & Wall, 1996a,b) may prove to be more effective control options for houseflies over coming decades.

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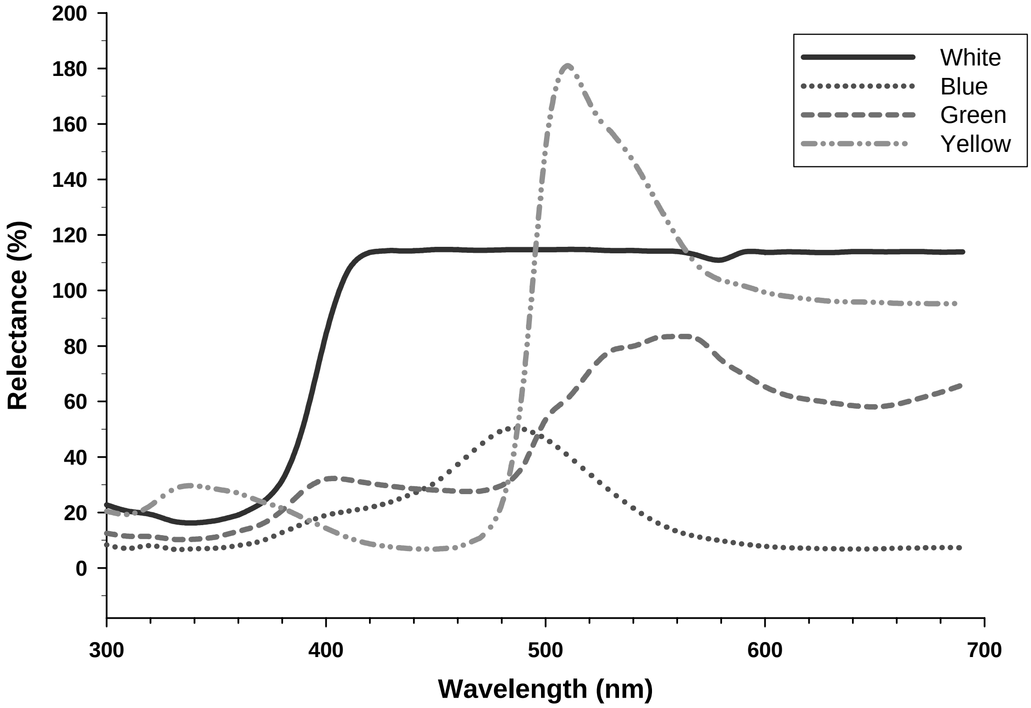

Figure 1: The spectral reflectance of four different paints used to examine the effect of trap colour on housefly catch rates in a domestic poultry unit in southern England.

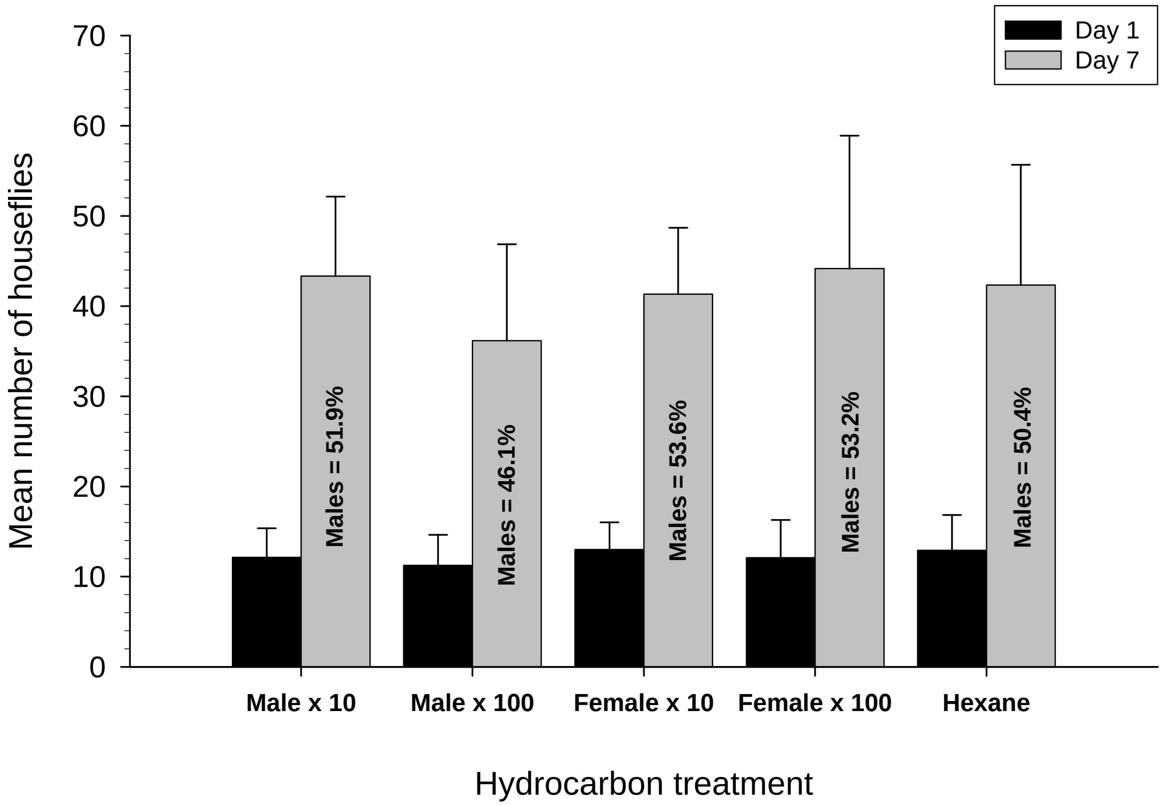

Figure 2. The effect of hydrocarbons on mean (± SE) housefly (*Musca domestica*) catch rates in a domestic poultry unit in southern England. Groups of hydrocarbons were mixed to simulate the cuticular hydrocarbon profile of male and female houseflies at 10 and 100 times the concentrations established in laboratory trials. The proportion of the total housefly catch at 7-d comprised of male flies is given.

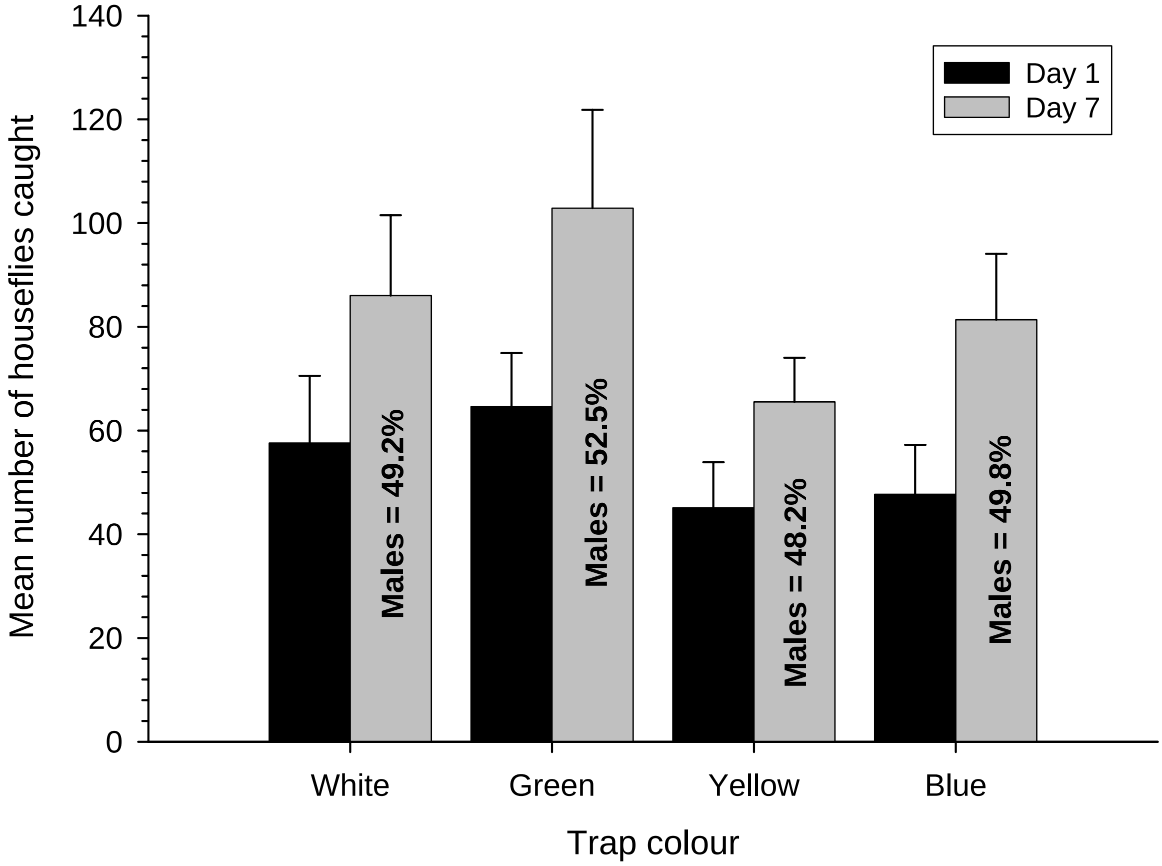

Figure 3. The effect of trap colour on mean (± SE) housefly (*Musca domestica*) catch rates in a domestic poultry unit in southern England. The proportion of the total housefly catch at 7-d comprised of male flies is given.

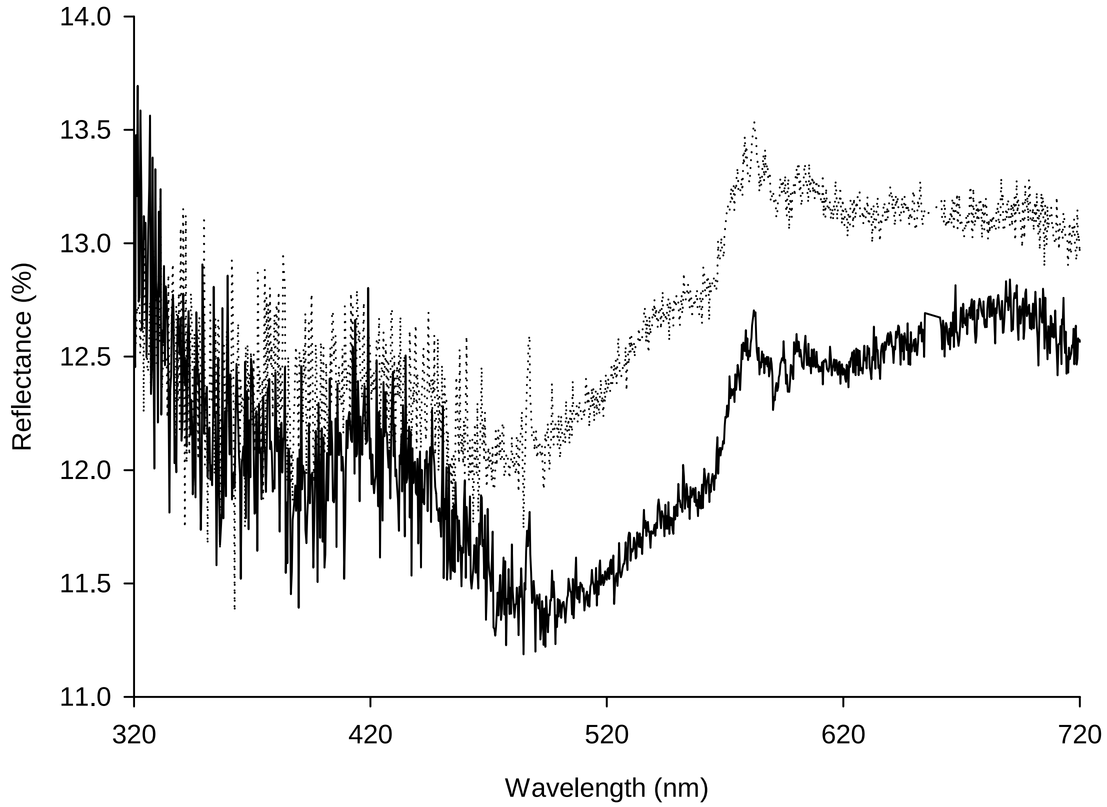

Figure 4: Comparison of thoracic spectral reflectance for male (black, *n*=30) and female (grey, *n*=30) houseflies (*Musca domestica*).

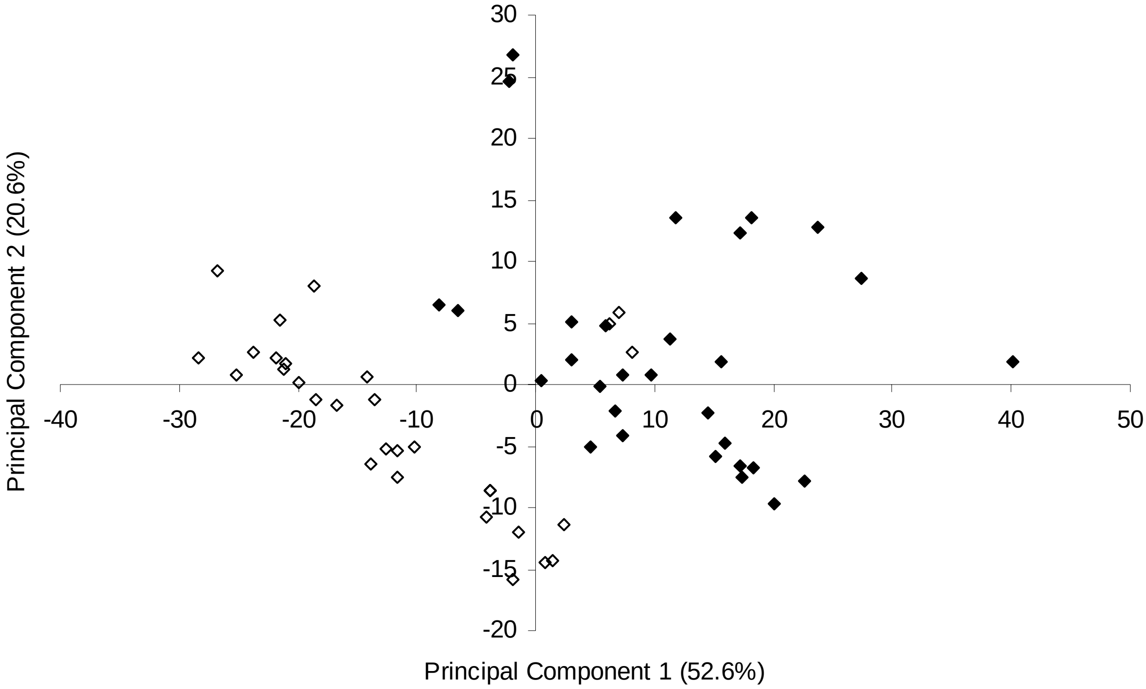

Figure 5: Biplot summarising the principal component components of male (closed diamonds) and female (open diamonds) housefly (*Musca domestica*) spectral reflectance.

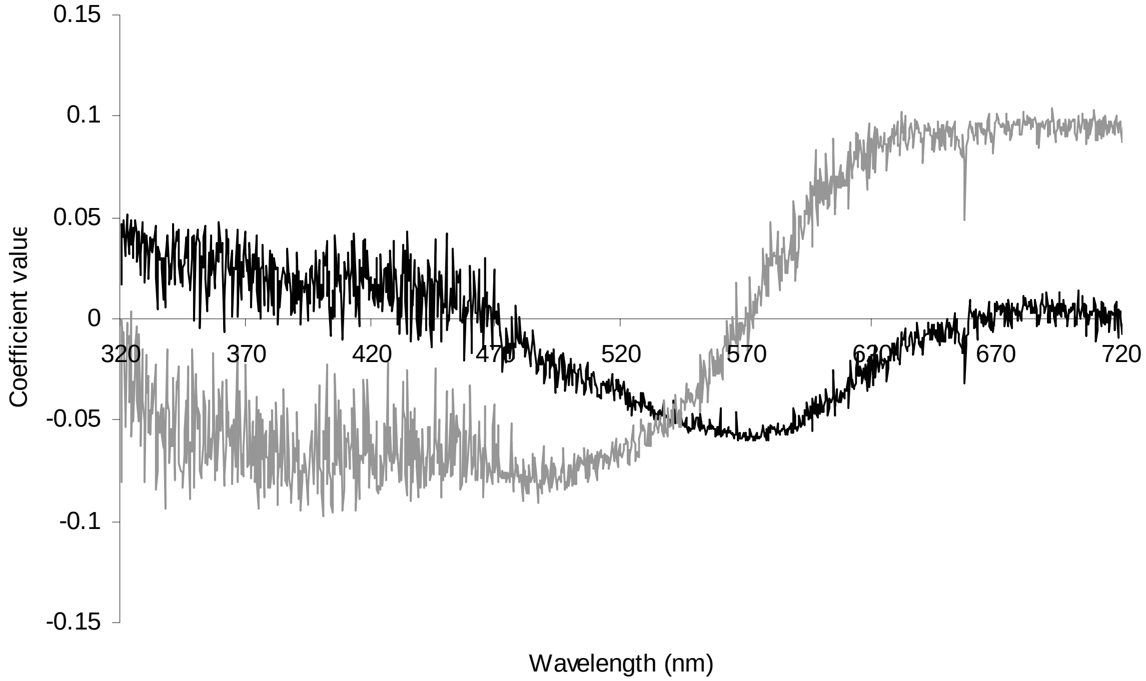

Figure 6: Coefficient values for the principal components 1 (black line) and 2 (grey line) of housefly (*Musca domestica*) spectral reflectance.

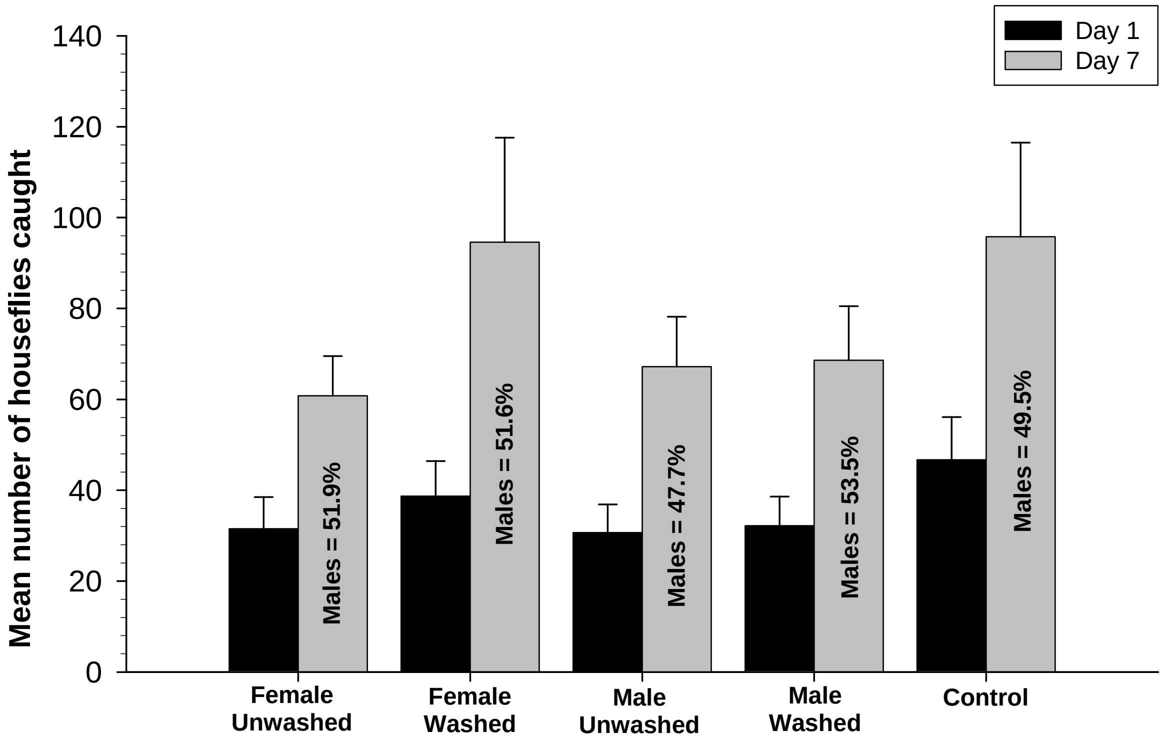

Figure 7: The effect of clusters of male and female houseflies on mean (± SE) housefly (*Musca domestica*) catch rates in a domestic poultry unit in southern England. Flies were either washed in hexane to remove their cuticular hydrocarbons, or presented unwashed. The proportion of the total housefly catch at 7-d comprised of male flies is given.