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Evaluation of (*Z*)-9-tricosene baited targets for control of the housefly (*Musca domestica*) in outdoor situations

M. E. Hanley, D. W. Dunn, S. R. Abolins and D. Goulson
School of Biological Sciences, University of Southampton, Bassett Crescent East, Southampton, SO16 7PX, UK

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Abstract: Houseflies (*Musca domestica* L.) are a major pest species in a variety of outdoor situations, notably on and around livestock farms and landfill used for the disposal of domestic waste. Currently no effective options are available for control of houseflies outdoors, because many populations exhibit at least some resistance to all available synthetic pesticides. (*Z*)-9-tricosene is the only commercially available pheromone for use in lure-and-kill approaches to housefly control, and it is widely used in indoor livestock units in combination with sugar/insecticide bait. Here we examine the potential of this approach for use outdoors, on a landfill site. We investigate the efficacy of toxic targets painted with a sugar/insecticide/(*Z*)-9-tricosene mix. The effects of target size and pheromone concentration were examined in two replicate trials, conducted in June and September 2003. As expected, catch consisted largely of males, was consistently higher on larger traps, and generally increased with (*Z*)-9-tricosene concentration even up to very high levels. However, in repeated trials, and despite mass release of marked flies, catch rates appeared to be insufficient to provide adequate control. We suggest that this is probably because (*Z*)-9-tricosene is primarily a short-range attractant, and fly populations in outdoor situations are generally distributed over a large area. Catch declined rapidly to zero within 2 weeks, indicating that improved formulation or target design is needed to slow the weathering of active ingredients on the targets. It seems unlikely that (*Z*)-9-tricosene is sufficiently attractive to houseflies to provide an effective and economic lure in outdoor situations.

Key words: fly control, landfill, muscalure, pest control, pheromone

1 Introduction

The housefly (*Musca domestica*) is widely regarded as an important pest species (Busvine, 1980; Chapman et al., 1998a; Howard, 2001), because of its close association with human settlements and habit of breeding in waste (Anderson and Poorbaugh, 1964). By feeding on decaying matter, human waste, and food, *M. domestica* has been implicated in the spread of numerous diseases including salmonella, diphtheria, tuberculosis, hepatitis and amoebic dysentery (Greenberg, 1973; Crosskey and Lane, 1993; Tan et al., 1997). The public health risks and annoyance associated with large housefly populations are thus considerable. Problems with excessive housefly populations are generally associated with livestock units and around landfill sites used for domestic waste, because of an abundance of decaying organic matter (Howard, 2001).

The potential scale of the housefly problem on or around landfill sites is enormous. Imai (1984) for instance found that up to 1500 flies emerged from only 1 m^2 of landfill waste within 1 month of its deposition. The abundance of food provided by the regular supply of organic waste, combined with above-ambient temperatures present immediately below the surface layers, help promote the rapid proliferation of many fly species throughout much of the year (Goulson et al., 1999; Howard, 2001). In the UK the problem is compounded by domestic waste often being held for up to 2 weeks by individual households before collection and further storage for up to 48 h at transfer stations. As household waste is favourable for larval development for about one month after disposal (Ikeda et al., 1972; Imai, 1985), this delay in processing allows decomposition to occur and provides ample opportunity for adult flies to locate the waste and lay eggs on it (Goulson et al., 1999). Consequently waste often arrives at landfill sites already heavily contaminated with fly eggs and larvae.

The most common method of housefly control is to spray the site with synthetic insecticide (Imai, 1985), but this is neither satisfactory nor particularly effective as it poses a health risk to humans and because houseflies often exhibit cross and multiple insecticide resistance (Chapman and Morgan, 1992; Chapman et al., 1993). Additionally, in outdoor situations, large volumes are needed and spray is likely to drift from the target site. On landfill sites, physical means of control are often practiced and are partially effective. Waste can be covered with a 15 cm layer of soil at the end of the working day to trap emerging flies (Goulson et al., 1999). Toyama (1988), however, reported that even a 25 cm layer of soil may not effectively prevent adult *M. domestica* from emerging and placing soil in landfill reduces space available for waste. Despite use of soil coverings and regular insecticidal sprays in the summer months, substantial housefly outbreaks still occur on landfill sites (Goulson et al., 1999) and an effective way of reducing the spread of emergent houseflies to surrounding residential areas is required.

Targets baited with toxic mixtures of the housefly sex pheromone (*Z*)-9-tricosene mixed with a sugar bait and insecticide have been successfully employed in enclosed environments like poultry units (Mitchell et al., 1975; Chapman et al., 1998a,b). They have several advantages over insecticide sprays; there is greatly reduced exposure to humans, and resistance is less likely if the insecticide is ingested compared with cuticular absorption (Keiding, 1975; Chapman et al., 1998b). To our knowledge, however, this form of housefly control has not been investigated in any outdoor situations.

In this study we investigate the efficacy of baited target-traps for the control of houseflies emerging from household waste on landfill. In addition to examining the effect of varying target size on the numbers of houseflies caught, we also determine how varying concentrations of (*Z*)-9-tricosene affect trap performance. In the second of two identical experiments conducted in June and September, we attempted to estimate the proportion of the total housefly population caught using a mark-recapture technique.

2 Materials and Methods

Design of toxic targets closely followed Chapman et al. (1998b, 1999). We used three sizes of target (small 25 × 50 cm, area = 1250 cm^2; medium 37× 67.5 cm, area ≈ 2500 cm^2; or large 100 × 50 cm, area = 5000 cm^2) all made from plywood board painted with two coats of white, water-soluble, gloss paint. White targets have been shown to be more attractive to houseflies than other colours tested (Mitchell et al., 1975). A toxic bait mixture was prepared by mixing 1 kg of the insecticide azamethiphos (Alfacron 10% active ingredient; Ciba-Geigy, UK), with 2 Kg of granulated sugar, and 400 ml of water to form a thick paste (Chapman et al., 1999). The toxic bait mixture was then subdivided into three 750 ml aliquots, ready to receive one of three pheromone treatments.

The synthetic female housefly sex pheromone, (*Z*)-9-tricosene (Agrisense BCS, Pontypridd, UK) [technical grade, 65% (*Z*)-9-tricosene, 15% (*E*)-9-tricosene, and 20% impurities] was used in our studies. One 750 ml aliquot was mixed with 86 ml of pheromone to produce a 6.8% concentration of the pheromone, similar to that used by Chapman et al. (1998b). The second 750 ml aliquot of alfracon was mixed with 258 ml of pheromone to produce a concentration three times greater. Three alfracon aliquots were then evenly applied to the targets using a paintbrush at the following rates: small targets 17.5 ml, medium 35 ml, large 70 ml. There were six replicates of each (*Z*)-9-tricosene/target size for each treatment (54 targets in total).

Table 1. *Costs of toxic target construction, including active ingredients, in Euros*

	1250 cm^2	2500 cm^2	5000 cm^2
Control (€)	3.22	5.04	7.28
Tricosene – low (€)	4.62	7.84	12.88
Tricosene – high (€)	7.42	13.44	24.08

The targets were erected on supporting posts at a landfill site in Lymington, Hampshire, England (UK national grid reference SZ 310 925). The targets were arranged in a randomized block design, 10 m apart, along the fence surrounding the active landfill cell. The base of each target was approximately 10 cm from ground level. At the base we attached a plastic trough (Plasticotto, Larciano, Italy) containing a sticky trap (Agrisense-BCS Ltd, Pontypridd, UK) adhered to the sides of the trough. Each sticky trap completely covered the inner surface of the trough in which it was placed. The length of each trough equated to the width of the board under which it was placed. Flies landing on the target above, having ingested enough of the toxic bait mixture to kill or incapacitate them, were collected on the sticky traps within the plastic trough. Approximate costs of the toxic targets are given in table 1.

The experiment was first conducted in June 2003, and was repeated in September 2003 (target boards were first washed before being re-treated with the same tricosene/bait mixtures used in the first experiment). We collected the sticky traps 4, 7, 11, and 14 day after the toxic bait mixture was painted onto the boards. Sticky traps were replaced on each occasion. All houseflies caught in this way were then sexed and counted in the laboratory. The total number of flies caught over the four sample intervals was calculated. To determine the effect of pheromone treatment and target size on mean catch rate per treatment-combination, we used GLIM (Generalised Linear Interactive Modelling) with Poisson errors (effectively similar to a two-way anova), with the error structure checked during the analysis (Crawley, 1993).

In the September trial we released approximately 1500 newly emerged laboratory-reared flies per week. These animals were descended from approximately 600 wild individuals that were caught in a poultry house (North Winchester Farm, Kings Worthy, Hampshire, UK), and were the third and fourth generation of captive-reared flies. To rear the flies in the laboratory, adults were kept in 40 × 40 × 40 cm wire cages covered with fine cotton netting. Approximately 300 adults were kept in each cage. Fresh water and food (mixed powdered milk, sugar and yeast; 20 : 10 : 1 by volume) were provided *ad libitum*. Eggs were collected daily from plastic containers containing cotton wool soaked in milk. Approximately 100 eggs (mixed from different laying cages to avoid inbreeding) were then transferred to 500 ml plastic beakers containing larval diet. The diet consisted of mixed bran, powdered milk and yeast (250 : 25 : 1 by volume), moistened with tap water. When the adults eclosed, approximately 300 were transferred to each cage so that the next generation of eggs could be collected. All laboratory rearing took place at 25°C and a 12 : 12 light/dark photoperiod. Before release, adult flies 3–7-days old were marked with a fluorescent powdered dust (Magenta 10, Sterling Industrial Colours Ltd, London, UK). Flies were loaded with powder by placing a 90 mm culture dish filled to a depth of 3–4 mm with powder in a fly cage for 12 h.

Flies were released onto the active cell of the landfill, approximately 150 m from the baited targets, 4 and 11 days after the targets were put in place. Numbers of these individuals caught in subsequent samples were noted in order to estimate the proportion of houseflies from the local population caught by our traps.

3 Results

In both trials, larger targets caught more houseflies ($\chi^2_2 = 11.1$, $P < 0.01$ and 18.5, $P < 0.001$ for June and September, respectively). Also in both trials, increasing concentration of (*Z*)-9-tricosene led to a greater catch of houseflies, although in the September trial this trend was not significant ($\chi^2_2 = 40.9$, $P < 0.001$ and $\chi^2_2 = 5.33$, n.s. for June and September, respectively). In neither trial was there a significant size × concentration interaction (fig. 1). Catch declined rapidly during the 2-week period of both field trials, and no houseflies were caught after 11 days in either trial (fig. 2). Weather during the trials was exceptionally hot and dry and there was no rainfall. Therefore we can conclude that the efficacy of the alfracon/sugar bait had not significantly reduced during the experiments and that the observed decline in fly catch was most likely because of a reduction in tricosene volatility.

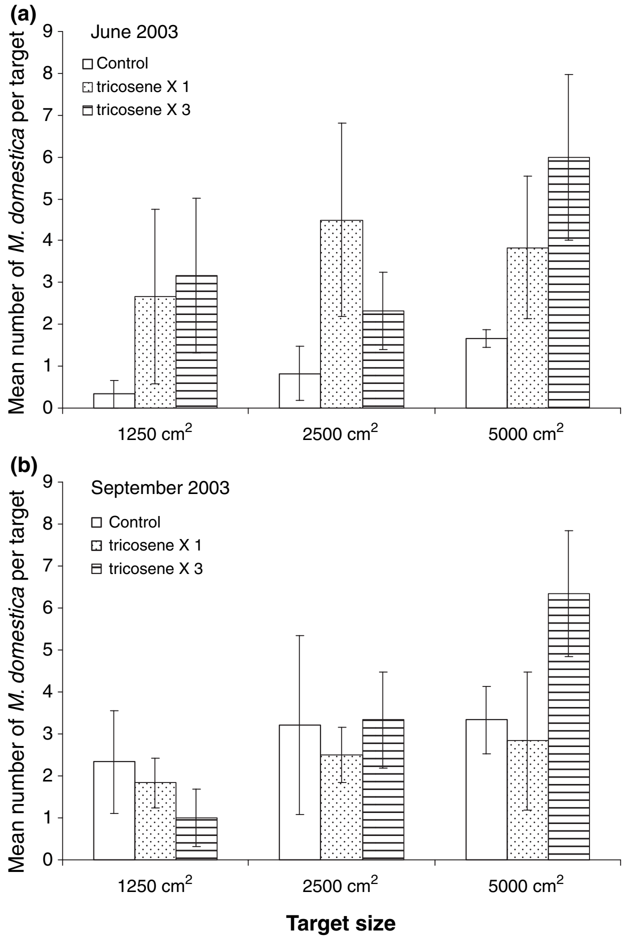

Fig. 1. *Mean numbers of* Musca domestica *caught per target (males and females), in separate field trials conducted in (a) June and (b) September 2003. Three sizes of target were compared, each treated with one of three levels of (Z)-9-tricosene*

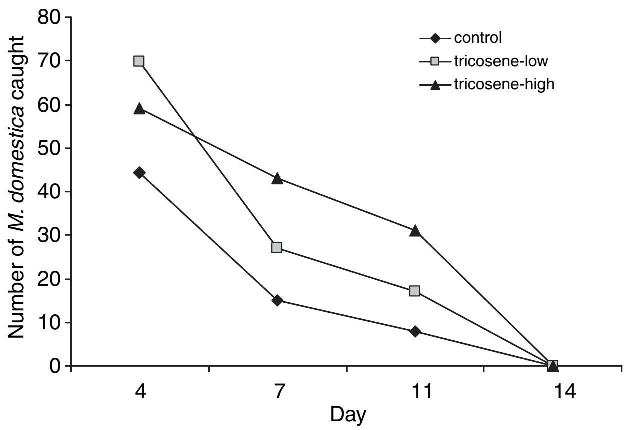

Fig. 2. *Total numbers of* Musca domestica *caught, combined across target sizes and the two field trials, at 4, 7, 11 and 14 days after the targets were first deployed*

The majority of the flies caught were male, even on control traps. Combining trap sizes and field trials, the percentage catch of males was 65.9, 80.7 and 81.2, for controls, low and high concentration of (*Z*)-9-tricosene, respectively. All three depart significantly from a ratio of 1 : 1 ($\chi^2_1 = 7.62$, 40.0 and 50.6, $P < 0.01$ for all). Overall, the catch of houseflies was low (152 in June and 157 in September). Despite releasing 1500 marked houseflies per week in the September trial, only three were recaptured (1.7% of houseflies caught).

4 Discussion

These two field trials demonstrate three main findings. (i) Larger targets attract more flies, (ii) (*Z*)-9-tricosene attracts mainly male flies, and (iii) higher concentrations of (*Z*)-9-tricosene increase the catch. (Z)-9-tricosene is present in the cuticular hydrocarbons of female houseflies, and has long been known to attract as a short-range attractant and copulatory stimulant for males (Rogoff et al., 1964; Mayer and James, 1971; Mansingh et al., 1972; Carlson et al., 1974; Richter et al., 1976). In field trials in poultry houses it also increases the catch of females (Mitchell et al., 1975; Chapman et al., 1998a). The highest concentrations of (Z)-9-tricosene previously tested and found to act as an attractant to male houseflies (Chapman et al., 1998b) was 5 g of technical grade (*Z*)-9-tricosene per toxic target (approximately 3600 cm^2), and our lower concentration was identical to this. This concentration, however, is far higher than is likely to occur in natural situations, and most sex pheromones operate at concentrations several orders of magnitude lower (Wilson and Bossert, 1963). Our trials demonstrate that when the concentration of (*Z*)-9-tricosene is increased still further (our highest concentration was 24 g of (*Z*)-9-tricosene per 5000 cm^2 target), it remains attractive to male flies.

Unlike the earlier studies of (Mitchell et al., 1975; Chapman et al., 1998a), few female flies were caught at either of the tricosene concentrations we employed. It has been suggested that the capture of male

M. domestica by tricosene-baited traps results in the attraction of female flies to the same traps (Wiesmann, 1962; Richter et al., 1976). Aggregation of male flies could result in increased concentration of male pheromones, which might attract females to traps. A second, and not mutually exclusive, possibility is that the optical cue of motionless feeding flies attracts other individuals into the area (Wiesmann, 1962; Richter et al., 1976). In the studies conducted by Mitchell et al. (1975) and Chapman et al. (1998a), when flies were caught they remained on view to other individuals. Our design prevented this possibility, reducing the potential for other flies to be lured onto our targets through visual stimulation. Moreover the generally low catch rate in this study would have also have minimized male pheromone release and any resulting attraction of female flies to our targets.

Our trials strongly suggest that the usefulness of (*Z*)-9-tricosene as an attractant for housefly control is greatly reduced in outdoor situations compared with indoor animal units. The early work of Carlson and co-workers found that tricosene-impregnated traps were highly successful at attracting houseflies in open poultry houses and sheep barns (Carlson and Beroza, 1973; Mitchell et al., 1975; Carlson and Leibold, 1981). Although partially open, these systems by their very nature provide shelter from the elements (including roofing and walls). Moreover, mobile houseflies are more highly concentrated in and around animal rearing units than they are on landfill, and it is not surprising that in these situations tricosene-impregnated traps are effective at attracting large numbers of flies. More recently a series of field trials in closed poultry units reported variable catches of 70–200 houseflies per day (Chapman et al., 1998a,b, 1999), underscoring the high density of fly populations in animal units and the efficiency of lure-and-kill traps in enclosed conditions. Chapman et al.'s targets also continued to attract flies for up to 3 months. In contrast, the highest average catches we obtained were 3.83 and 6.33 flies per trap, at our low and high (Z)-9 tricosene concentrations respectively, although in the second trial we augmented the wild fly population by releasing additional flies. Our traps attracted no houseflies after 2 weeks.

These differences in efficacy are probably mostly attributable to the difference in density of flies on landfill compared with poultry houses. As with most landfills, our study site covers several hectares, and the density of flies is much lower than in the confined space of an animal unit (D.G. pers. obs.). (*Z*)-9-tricosene has low volatility and has been suggested to act only as a short-range attractant (Wall and Howard, 1994; Chapman et al., 1998b). Kelling et al. (2003) found recently that the olfactory apparatus of *M. domestica* does not react to (Z)-9 tricosene, and suggested that (Z)-9 tricosene acts as a contact-sex pheromone perceived by taste (but see also Noorman (2001). Flies may simply pass close to a target at random, which will be more likely in a confined space than in a large open area. Mortality may be highest on baited traps if flies spend longer on or very close to baited compared to control traps. On a landfill site the fly population probably consists largely of newly emerging adults many of which probably disperse from the active cell of the landfill before reaching sexual maturity. The response of newly emerged flies to (*Z*)-9-tricosene is not a strong as that shown by older individuals (Silhacek et al., 1972). Finally, the more rapid decline in efficacy that we observed compared to Chapman et al. (1998a,b, 1999) may simply be because of exposure to the elements removing the active ingredients from the targets in outdoor situations.

Overall, our trials suggest that (*Z*)-9-tricosene may be of little use in outdoor situations. Improved target design and formulation could reduce the effects of outdoor exposure, but the efficacy of freshly painted targets was too low to be economically viable. Indeed, averaged across both trials the most cost effective combination of target size and (*Z*)-9-tricosene concentration was to use small boards with a low concentration of (*Z*)-9-tricosene, but even here the cost exceeded two Euros per fly caught. Clearly other approaches are needed to tackle housefly problems in outdoor situations.

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Author's address: Mick Hanley (corresponding author), School of Biological Sciences, University of Southampton, Bassett Crescent East, Southampton, SO16 7PX, UK. E-mail: m.e.hanley@soton.ac.uk