# STERILISATION of ADULTS with INSECT GROWTH REGULATORS for the CONTROL of *MUSCA DOMESTICA* in an URBAN WASTE TREATMENT PLANT

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**Abstract** In the context of an integrated control strategy for house fly, *Musca domestica*, development within an urban solid waste treatment plant, tests were conducted to evaluate the sterilisation of *M. domestica* adults by feeding them with Insect growth regulator (IGR) products commonly used for larvae control. Products containing triflumuron, buprofezin, hexaflumuron, and diflubenzuron were evaluated in the laboratory using insects collected in a waste treatment plant. Each test was conducted on pairs of house fly adults aged 1, 2, 3, 4, and 7 days. Environmental conditions were recorded during the tests; the temperature was  $24^{\circ}C \pm 1^{\circ}C$  and the RH was  $65\% \pm 10\%$ . Each treatment was replicated three times and consisted of exposing ten pairs of house flies to contact with discs of filter paper treated with 0.1% or 0.75% of each active ingredient diluted in a water solution containing 40% sugar as an attractant. The pairs of house flies were exposed to the treated discs for 3 to 6 days in a cage of 4.67 1. The results show that some IGRs (diflubenzuron, hexaflumuron, and triflumuron) sterilise 100% of eggs laid by couples of *Musca domestica* adults aged 1 day. The sterilisation effect is similar in adults that come into contact with these IGRs for up to one week after their emergence.

Key Words House fly ovicidal activity buprofezin diflubenzuron hexaflumuron triflumuron

## INTRODUCTION

Urban solid waste has been transformed into compost in some cities of Italy for several years, in an attempt to find a solution to the problem of waste treatment.

It is possible to utilise different methods. In some cases the material, after a first rough mincing, is amassed and covered with debris of bark; in other cases, the waste is selected, fragmented, and stored in closed rooms. By turning over the mass weekly, progressive selection and blowing with hot air, which creates very high relative humidity and temperatures, a compost can be obtained after about 70 days.

Unfortunately, the waste is inevitably colonised by many insects, and *Musca domestica* (L.) is the most common and important pest. The problem has been observed all over the world, with different solutions being proposed.

Figueiredo (1979) suggested, in Rio de Janeiro, to cover a deposit of waste with 30 cm of consolidated soil to prevent the emergence of M. *domestica* adults, but observed that this technique cannot be utilised commercially due to the high cost.

As a result, Imai (1985), in Osaka, used only 15 cm of soil for covering every week, with good results. On the other hand, Saccà (1984) found fly larvae nesting 20 cm deep inside soil impregnated with liquid manure both in a Middle East shanty town and in a food industry facility in Italy, under a waste heap of fermenting artichoke leaves.

In the Milan area, the composting system is in a closed plant; the treatment includes collecting, fragmenting, and mixing undifferentiated waste and turning over the mass. Every day the plant works from 600 to 1200 tonnes of waste; the compost is produced after about 70 days, while the inorganic components are put into dumps or incinerators. The problem caused by the very high infestations of house flies in this plant has been studied since 1998. A specific technique of IPM has been developed where the control of this pest includes prevention, by the use of double doors both for the staff entrance and for the transit of vehicles, and monitoring, which is made outside by traps activated with bait and inside by periodic records of the flies to test if the *M. domestica* strain is becoming resistant to insecticides used in its direct control. The control is effected by different techniques; in particular, insecticide-treated nets, adulticides, larvicides, and black chromotropic panels, activated with sweet baits and with triflumuron, are used. This IGR causes sterilisation of house fly eggs when ingested or when contacted directly by the legs of the pest.

The first results of these strategies were reported in the past ICUP (Süss et al., 1999). This study was continued and, as a consequence, the real efficacy of some IGRs used against adults was evaluated. There are records in the scientific literature of several authors who have referred to the possibility of creating autosterilisation by feeding the insects with IGR (including triflumuron) treated sweet baits (Morgan et al., 1975; Hamman and Sirrenberg, 1980; Miller, 1982; Beck et al., 1983; Howard and Wall, 1995a,b; Howard and Wall, 1996a,b; Smith and Wall, 1998). Low doses of triflumuron cause a diminution of fertility and can interfere with post-embryonic development of *M. domestica*, resulting in a reduction of population in the next generation. Less knowledge is available on the effects of triflumuron and of other IGRs when the larvicides are swallowed, or penetrate the fly body in other ways, some days after emergence but not shortly afterwards. As a result of this work, a question arises of what happens if, under practical conditions, house fly females, already fertilised, feed on fermenting organic waste for some hours or days, before being attracted to panels treated with IGRs. In such a situation, it is possible to expect a reduction of efficacy of adult sterilisation caused by these products.

The overall aim of this study has been to estimate ovicidal activity against house fly Musca domestica (L.) of four chitin synthesis inhibitors, which are normally used for larvae control. The effects of three benzoylphenylureas — diflubenzuron, hexaflumuron, and triflumuron — and a thiadiazinone, buprofezin, were evaluated. These compounds have shown ovicidal or sterilising activity against some species of insects, but the inhibition of hatch of house fly eggs is documented in literature only for triflumuron and diflubenzuron. The larvicidal efficacy of diflubenzuron is well documented; consequently, this a.i. has been used commercially for several years, including in the waste plant of Milan for the control of *M. domestica* larvae. Laboratory tests at the University of Milan have attempted to verify if the ovicidal effect is maintained unchanged during the adult stage or if, as indicated by Howard and Wall (1995a) for triflumuron, younger adults are more susceptible to IGRs.

# **MATERIALS and METHODS**

# **Test Insects**

House fly adults, collected in an urban solid waste treatment plant situated in the periphery of the city of Milan, were used as the breeding material at the Institute of Agricultural Entomology. The flies were reared in cubical plastic cages ( $35 \times 35 \times 35$  cm) placed in a thermostatic chamber at  $25^{\circ}C \pm 1^{\circ}C$  and 65% RH $\pm 5\%$ , using milk powder as the food source. One plastic pot (diameter 10 cm; height 7 cm) containing the medium 24% bran, 71% water, 5% milk powder (Saccà, 1984) suitable for oviposition and larval development was placed in each cage every week. The pots were removed after three days and maintained in a thermostatic chamber until the emergence of the adults. The tests have been carried out from the second generation.

#### Treatments

Two solutions of every a.i., containing 40% sucrose (w/v) as bait, were prepared at concentrations of 0.75% and 0.1% (w/v). Filter paper discs (diameter 15 cm) were dipped into the two

solutions and allowed to dry. Every disc absorbed  $0.02 \text{ g/cm}^2$  of solution, corresponding respectively to the doses 1.5 g and 0.2 g a.i. /m<sup>2</sup>. The filter papers were stored separately at room temperature and in the dark. Some filter paper was dipped into a 40% sucrose solution only and used as control in the tests.

# Bioassays

The method of exposure of adult flies to treated surfaces was similar to that used by Howard and Wall (1995b). The tests were carried out in cylindrical cages (4.6 l), containing water and 10 g of milk powder as the food source. Batches of 20 newly emerged adult males and females (aged not more than 24 hours; sex ratio 1:1) were transferred into every container. The treated filter papers were suspended from the roof of the cages at 0, 1, 2, 3, and 7 days after the emergence of the adults. At the same time, a cylindrical plastic pot (diameter 6 cm; height 8 cm), containing the rearing medium described previously, was placed into each cage.

The pots were replaced every 3 days. The rearing medium, eventually with house fly eggs, was transferred in mesh-covered larger containers (diameter 10 cm; height 15 cm), adapted to allow larval development, into an incubator. The control cages were prepared with the filter paper dipped in 40% sucrose solution only. Three replicates of each treatment were carried out. The tests were conducted at  $24^{\circ}$ C  $\pm 1^{\circ}$ C, at  $65\% \pm 5\%$  RH and at a light intensity of 250 lux.

#### **Data Analysis**

The number of emerged adults was counted. Abbott's formula (1925) was used to correct the data for control mortality. The obtained data were transformed into an index using the equation: I (%) =  $100 - A/\underline{M}$  (%), where "A" is the number of emerged house fly adults F1 in each replicate, " $\underline{M}$ " the mean number of emerged house fly adults F1 in the controls, and "I" the mean percentage inhibition of development.

All values were subjected to angular arc-sin transformation. Data were then analysed by analysis of variance (ANOVA). Mean separations were made by Duncan's multiple range test (P<0.05) (SPSS 10.0 for Windows).

## **RESULTS and DISCUSSION**

Table 1 shows the mean percentage inhibition of development (I) achieved by exposing house fly adults of different ages to IGRs at the greater tested concentration (0.75% w/v). There are no significant differences amongst the results produced by diflubenzuron, hexaflumuron, and triflumuron. Buprofezin exhibited no ovicidal activity at this concentration.

The sterilisation effect of the three benzoylphenylureas was maintained unchanged in adults which came into contact with these IGRs up to one week after their emergence. The same result

Table 1. Mean percentage inhibition of	development (I)	obtained by	exposing house fly	adults to
IGRs at the concentration of 0.75%	for three days			

		Age of treated house fly adults (hours):									
	0-24		24-48		48-7	48-72		72-96		168-192	
A.I.	I(%)	SD	I(%)	SD	I(%)	SD	I(%)	SD	I(%)	SD	
buprofezin	8.6a	8.5	10.9a	9.9	7.5a	5.7	8.2a	11.3	6.2a	5.6	
diflubenzuron	100.0b	0.0	100.0b	0.0	100.0b	0.0	98.3b	2.9	100.0b	0.0	
hexaflumuron	100.0b	0.0	100.0b	0.0	100.0b	0.0	100.0b	0.0	100.0b	0.0	
triflumuron	100.0b	0.0	100.0b	0.0	95.7b	6.2	96.4b	3.6	96.5b	5.3	

The data not followed by the same letter are significantly different (P<0.05) as determined by Duncan's multiple range test.

has been obtained in tests against house fly adults 0-24 hours old at 0.1% w/v concentration, with hexaflumuron and triflumuron being the more effective active ingredients in the inhibition of egg hatch (Table 2).

Table 3 shows the mean number of house fly F1 adults per female obtained in the control groups. The age of the flies at 1st exposure did not affect the extent of the ovicidal effect induced, as indicated by Howard and Wall (1995b) and Knapp and Cilek (1988), but in our experiment house fly adults were exposed to IGRs for three days instead of one. The concentrations assessed were also different, being 0.75% and 0.1% instead of 10% and 3%.

Triflumuron and diflubenzuron confirm their potential for the control of house fly as larvicidal and ovicidal agents. Inhibition of egg hatch by exposure to these IGRs is recorded in many works (Grosscurt, 1976; Knapp and Cilek 1988; Wall and Howard 1994; Howard and Wall, 1995a,b; 1996a,b; Süss et al., 1999). Grosscurt (1976) concluded that the ovicidal effect of diflubenzuron was due to interference with chitin synthesis in the embryo, which prevents the fully developed larva from breaking out of the egg, and not to a sterilising action as such. Moreover, diflubenzuron had no contact effect on the house fly eggs.

The ovicidal activity of hexaflumuron against pest species other than *M. domestica* has been demonstrated by some other authors, e.g., *Blattella germanica* (L.) (DeMark and Bennet, 1990), *Cydia pomonella* L. (Charmillot et al., 2001), *Spodoptera littoralis* Boisd. (Emam and Degheele, 1993). References on the sterilising effect of buprofezin are scarce; at 0.05%, this a.i. produced strong inhibition of egg hatch in *Planococcus citri* (Risso), resulting in over 80% inhibi-

Table 2. Mean percentage inhibition of development (I) obtained by exposing house fly adults to IGRs at the concentration of 0.1% for three days

for three days			
_	Age of treated house fly adults (hours) 0-24		
A.I.	I(%)	SD	
buprofezin	14,6a	12,7	
diflubenzuron	96,4b	3,3	
hexaflumuron	100,0b	0,0	
triflumuron	100,0b	0,0	

The data not followed by the same letter are significantly different (P<0.05) as determined by Duncan's multiple range test.

Table 3. The mean number of house fly F1 adults per female obtained in the control groups

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Age of house fly adults (hours)	Oviposition time (days)	F1 Adults/ female	SD
0-24	$1^{st}$ - $3^{rl}$	45,4	7,1
	4 <sup>th</sup> -6 <sup>th</sup>	28,8	4,7
24-48	1 <sup>st</sup> -3 <sup>rl</sup>	47,3	8,8
	4th-6th	39,0	5,5
48-72	1 <sup>st</sup> -3 <sup>rd</sup>	30,6	2,4
72-96	1 <sup>st</sup> -3 <sup>rl</sup>	31,0	4,1
168-192	$1^{st}$ - $3^{rl}$	11,6	1,7

tion of egg hatch (Mendel et al., 1991). In the present study buprofezin showed no ovicidal activity against house fly.

The results achieved in the tests with the benzoylphenylureas suggest that the utilisation of sucrose-baited panels activated with a low concentration of these IGRs may be an important step in the development of autosterilising systems for house fly control in an urban waste treatment plant.

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