

Scientific and technical work

6. Flies

6.1 Chemical control of *Musca domestica*

6.1.1 Laboratory evaluation of NAF granular formulations for control of *Musca domestica*

Three NAF formulations with 0.5%, 1.0% or 2.0% spinosad active ingredient were evaluated under laboratory conditions for efficacy against the housefly *Musca domestica*. A reference granular bait with 1.0% azamethiphos and an untreated non-toxic control were included in the evaluation.

Adult flies of a susceptible *Musca domestica* laboratory strain were allowed to feed on one of the three NAF baits during 48 hours after release into a large test chamber, in which they had access to the specific bait. The mortality (flies knocked down and dead) was recorded after 0.5, 1, 2, 4, 7, 24 and 48 hours.

There was no significant difference in the killing effect between the three NAF formulations. The flies were killed faster by the reference bait, however, by the end of the 48-hour trial period the three NAF formulations and the reference bait had all killed 99-100% of the flies. The three NAF formulations killed fewer female flies than male flies during the first 24 hours of the trials, whereas the reference bait killed as many female flies as male flies during the whole 48-hour period of the trial.

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6.1.2 Laboratory evaluation of outdoor protein-bait flytrap for control of *Musca domestica*

The performance of an outdoor flytrap, Green Planet Flytrap, with a protein/water mixture as bait was evaluated against adult flies of the housefly *Musca domestica* in a laboratory test chamber in a non-choice situation. In this non-choice test flies had access to water only besides the Flytrap. The flytrap is sold for outdoor trapping of houseflies and other flies. In such situations it is hung to e.g. a tree not higher than 1.5 metre above the ground and 5-10 metres from buildings. The trap is placed in direct sunlight for 3-5 days to ensure that the bait is activated quickly.

In this laboratory test the fly trap was tested in a test chamber at 28°C after the protein/water mixture had been activated for 6-8 days at 32°C. For each of five tests 2000 houseflies were released into the chamber. The performance was measured as the number of flies trapped after four hours. For comparison three control trials were made in the same way using the flytrap without the protein component of the bait, only loaded with water. The Green Planet Flytrap trapped between 11% and 44% of the flies in the test chamber during a four-hour period, with a mean of 25.1%. In the three control experiments, where no protein bait was used, only 1 fly was trapped. This difference is statistically significant at a 5% level.

It was concluded that the flytrap is able to attract, trap and kill houseflies when loaded with the protein source and activated according to the instructions for use. In the present experiment the trap caught 25% of the released experimental houseflies during a four-hour experiment. As the trap is exclusively for outdoor use, the results of the laboratory test cannot be used to foresee the efficacy in a field situation. Likewise, the efficacy of the trap in relation to number of days in use, density and species of flies, and climatic conditions etc. have not been demonstrated by this investigation.

J. B. Jespersen and M. Knorr

6.1.3 Field and laboratory evaluation of spinosad granular fly bait against *Musca domestica*

The efficacy of spinosad granular fly bait, containing 1% active ingredient, was evaluated under field conditions for control of the housefly. The bait was evaluated in large-scale field trials in Denmark in the housefly populations of 17 livestock units on six farms with pig-producing facilities. The trials were conducted from July until mid-autumn. The objective was to evaluate the fly control effect of spinosad granular fly bait when applied either on hang-boards or directly as paint-on bait and to make a preliminary assessment on the risk of development of spinosad resistance in the treated housefly populations.

On each trial location the infestation level was assessed during a 10-week trial period including a 2-week pre-treatment period, a 6-week period during treatment and a 2-week post-treatment period. Two of the farms were used as untreated controls and three isolated livestock units on a spinosad treated farm were used as treated controls with applications of either an azamethiphos 1% granular fly bait or a methomyl 1% granular fly bait. Flies for resistance testing in the laboratory were collected during the pre-treatment and the post-treatment periods.

The quantity of bait applied for one complete treatment of an animal unit was 250 g bait per 100 m² floor space of the unit. The hang-boards were made of white cardboard (67 cm × 30 cm) with 20 g of bait granules glued to one side. The hang-boards were placed in the units at sites as attractive to the flies as possible, but out of reach of the livestock animals and where they were not inconvenient for the farm working routines. The paint-on formulation was painted in narrow stripes in order to obtain a maximum bait/surface boundary attractive to the flies. A mixture with a 4:1 granule-to-water ratio was found to be most appropriate for application to the relevant surfaces without dripping and running off.

Spinosad granular fly bait applied to hang boards was in general only effective for housefly control in low-ceilinged units and only if the hang-boards could be placed close to places where most flies gathered. However, in the more high-ceilinged animal units where the houseflies tended to stay on or very close to the farm animals, it was not possible to obtain a satisfactory control of the flies by hang-boards. At first the hang-boards hung from the ceiling, suspended 1½-m above the feeding troughs. Moving them into the pens, still out of reach of the production animals, did not solve the problem.

Spinosad granular fly bait applied directly as paint-on bait controlled the housefly populations satisfactorily in all units. The most important reason for the paint-on bait being more effective than hang-boards was that the paint-on bait could be applied closer to the animals and other sites where flies congregated. In contrast it was difficult to place the relatively large hang-boards (67 cm x 30 cm) at sites attractive to the flies without placing them too close to the livestock animals or interfering with the farm working routines.

For comparison with Spinosad granular fly bait hang-boards, treatments with azamethiphos and methomyl applied to the same type of hang-boards were carried out. The efficacy of the hang-board application method used with the control fly-baits was influenced by the same type of problems as the spinosad granular fly bait treated hang-boards. The control of houseflies became effective in the treated control units when the hang-board treatment was later replaced by a paint-on bait treatment.

The level of insecticide resistance in the housefly populations in the trial farms to commonly used insecticides was moderate to high, and typical for Danish conditions. The resistance to spinosad was in all trial farms low to moderate, and we found no indication of induced resistance development in the spinosad treated units – being unlikely to happen as well with a selection scheme of covering only a few housefly generations.

6.2 Insecticide resistance in *Musca domestica*

6.2.1 Speed of action of thiamethoxam and azamethiphos in *Musca domestica*

The speed of action of thiamethoxam and azamethiphos was tested by a new method developed at DPIL: Female houseflies, starved for 16 hours prior to testing, were tested individually in a petri-dish with a glass microscope slide with a centrally placed drop of insecticide. Three parameters were determined a) time from initiation of experiment until the fly starts eating, b) time from the fly starts eating until knockdown, and c) time from knockdown of the fly until death. The speed of action of thiamethoxam and azamethiphos were tested at two different concentrations in the susceptible housefly strain WHO. Approximately 100 flies were used for each product.

The fastest acting formulations contained 10% azamethiphos and 0.05% z-9-tricosene and 10% thiamethoxam and 0.05% z-9-tricosene; which showed 66 ± 8.8 sec. and $80 \text{ s} \pm 10$ from the fly started eating and until death. There was no significant difference between both 10% formulations.

The knock-down effect of the 1% formulations was significantly delayed to 137 ± 16 sec. for 1% thiamethoxam and 0.02% z-9-tricosene until death compared to $105 \text{ s} + 15$ for 1% azamethiphos and 0.02% z-9-tricosene. The baits with either 10% thiamethoxam or 10% azamethiphos and both with 0.05% z-9-tricosene led to the most rapid killing of houseflies. In addition most of the flies only ate once before paralysis and subsequent death.

M. Kristensen and J. B. Jespersen

6.2.2 Resistance to fipronil by insecticide selected strains and field populations of the housefly

The toxicity of fipronil against susceptible houseflies and the cross-resistance potential of fipronil were determined in seven laboratory housefly strains by topical application and feeding bioassay. The insecticide-resistant strains represented different mechanisms of resistance and different patterns of cross-resistance to pyrethroids, organophosphates, carbamates and organochlorines. The insecticide-resistant laboratory strains were susceptible to fipronil or showed a low level of cross-resistance to fipronil, with the exception of the highly γ -HCH resistant strain 17e. The 17e strain was >500-fold resistant to fipronil in the topical application bioassay and 27-fold resistant in the feeding bioassay. We also tested the toxicity of fipronil in feeding and γ -HCH in topical application bioassay on thirteen housefly field strains. One of the field strains were moderately resistant to fipronil and γ -HCH, as well as pyrethrin, dimethoate, azamethiphos and methomyl. A strong correlation between fipronil and γ -HCH toxicity was found in the field strains. The fipronil resistance observed in laboratory and field strains might be caused by elevated general detoxification or be the result of a target-site resistance mechanism with cross-resistance to γ -HCH.

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6.2.3 Laboratory strains kept in 2000

At the end of 2000, DPIL kept 21 strains representing a wide variety of resistance mechanisms and origins for use in testing and research work. A list of the strains and their origins is given in Table 6a. In all these strains, the resistance originated in the field. In several strains, selection with one (or two) insecticide(s) is carried out between one and four times a year in order to maintain the particular resistance. As has been the case since the beginning of our investigation of resistance in houseflies in 1948, all our strains are available to laboratories that wish to use them for research, development of new insecticides, etc. This has assisted international research on insecticide resistance and given us useful feedback on our resistance problems.

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Table 6a. Laboratory strains of *Musca domestica* maintained during 2000

Strain	Origin	Year	Remarks	Lab pressure
<i>1. Strains subjected to periodic insecticidal pressure (adult dipping, exposure to vapour, or feeding with treated sugar) from a compound to which at least part of the population showed clear resistance at the time of collection</i>				
17 e	DK	1950		lindane
150 b	DK	1955		diazinon*
39 m ₂ b	DK	1969		tetrachlorvinphos*
49 r ₂ b	DK	1970		dimethoate*
381 zb	DK	1978		permethrin and dimethoate*
690 ab	DK	1984		methomyl feeding*
594 vb	DK	1988		azamethiphos feeding*
213 ab	Sweden	1957	Pyr-R	pyrethrins/pbo*
571 ab	Japan	1980	High OP-R	fenitrothion
698 ab	Burma	1985	(not kdr)	DDT
790 bb	DK	1997		diflubenzuron
802 ab	DK	1997		cyromazine
807 ab	DK	1997		diflubenzuron
<i>2. Originally resistant field strains kept without insecticidal pressure</i>				
7	DK	1948	Reverted DDT-R	None
772 a	DK	1989	Common lab. test strain	None
791 a	DK	1997	Multi-R	None
<i>3. Susceptible strains</i>				
BPM	Leiden	1955		None
WHO Ij ₂	Pavia	1988		None
NAIDM	Texas	1991		None
<i>4. Strains with resistance mechanisms isolated</i>				
A ₂ bb	DK	1982	Super-kdr Chr. 1, 2 and 3 with marker genes	None
LPR	USA	1995	Pyr-R kdr, P450 monooxygenase	None

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Some resistance to various (other) OP compounds and to DDT

6.3 Biological control of *Musca domestica* and *Stomoxys calcitrans*

6.3.1 Parasitoids

The housefly, *Musca domestica* L. and the stable fly, *Stomoxys calcitrans* (L) are common pests in most livestock facilities in Denmark.

The aim of this project is to evaluate the possibility of using pteromalid pupal parasitoids (2-3 mm in size) as control organisms against populations of houseflies and stable flies in dairy cattle and swine facilities. Here, the final results based on two years of releases will be summarized.

One parasitoid species, *Spalangia cameroni*, was selected as potential candidate for mass-release into stables against the houseflies and stable flies. From April and to the end of September in 1999 and 2000 *S. cameroni* was released weekly on one dairy cattle farm and two swine farms. In both years control of the housefly was acceptable and below nuisance level whereas the density of stable flies was lowered, but not to a satisfactory level in two farms.

As control for activity of the released parasitoid, laboratory-reared housefly puparia were exposed to parasitism in the stables seven days. Based on the sentinel pupae, parasitism by *S. cameroni* was between 40 and 50% per week during the release period which was a significantly higher level than observed on control farms and in the former year (1998) on the release farms. However, during July-August a high percentage of the exposed pupae were parasitized by *Muscidifurax raptor*, a naturally occurring parasitoid in the environment. Although the exposed sentinel pupal bags reflected the true parasitism level of *S. cameroni* poorly (mainly because many bags seldom could be placed in areas of direct fly development), the main conclusion is that *S. cameroni* seems a prospective candidate for control of the two nuisance flies. However, a few factors still need to be evaluated under field conditions, such as the minimum release interval without loss of effectiveness of the parasitoid, and whether combining two parasitoid species (*M. raptor* or *Urolepis rufipes*) could supplement the effect of *S. cameroni* in the stable environments.

Furthermore, *S. cameroni* and *M. raptor* were released on several organic farms with confined dairy cattle on deep bedding for a study of their horizontal and vertical distribution in the bedding materials. Distribution was measured by the placing of sentinel pupal bags in the bedding in order that parasitism (i.e. activity) of released wasps from points of releases could be recorded.

Although approximately the same numbers of both parasitoid species were released, *Spalangia cameroni* predominated in the pupal bags. *S. cameroni* dominated especially in pupal bags placed deep (20-25 cm) in the organic substrate, whereas both *M. raptor* and *S. cameroni* were recorded in the upper 5 cm.

In some cases, *M. raptor* emerged from a pupa that had already been parasitized by *S. cameroni*. If this is a general phenomenon, the addition of a second parasitoid might render the overall effect of biological control less efficient than releasing *S. cameroni* alone.

In conclusion, this first study period showed that *S. cameroni* and *M. raptor* - when released - could act synergetic against the flies, mainly because *S. cameroni* forages in the lower parts of the bedding whereas *M. raptor* stays in the upper layers.

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6.3.2 Hyphomyceteous fungi

In the last year of this project, the effect of three species of hyphomyceteous fungi on the fecundity of houseflies was studied. The fecundity decreased in flies infected with *Beauveria bassiana*, *Metarhizium anisopliae* or *Paecilomyces fumosoroseus*. Observations on the egg-laying behaviour of treated and untreated flies did not reveal any differences between the two groups, nor did dissections of the ovaries reveal significant differences in the development stage or number of follicles. At present, the reduction in fly fecundity caused by fungus infection therefore remains unexplained. Another study showed that if flies were placed under a suboptimal light regime, untreated flies withheld their eggs for up to five days, whereas fungus-treated flies deposited eggs almost irrespective of the changed light conditions. Under these conditions, which are unlikely to be common in the field, fungus-treated flies produced more offspring than did untreated flies. The mechanisms underlying this effect still remain to be explored.

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