

Scientific and technical work

6. Flies

6.1 Chemical control of *Musca domestica*

6.1.1 Laboratory evaluation of Fipronil Fly-bait gel for control of the housefly *Musca domestica*

The efficacy of Fipronil Fly-bait gel, containing 0.1% active ingredient, was evaluated under laboratory conditions for control of houseflies. Two paint-on-baits containing 10% azamethiphos or 1.1% methomyl as active ingredients were used as references and furthermore an untreated control was included in the evaluation.

Adult flies of a susceptible *Musca domestica* laboratory strain were allowed to feed on bait during 48 hours after the release into a large test chamber, in which they had access to a plywood board treated with the test formulation. The mortality was recorded by counting of the number of knocked down and dead flies after ½, 1, 2, 4, 7, 24 and 48 hours of exposure to the bait. The mortality was recorded as “overall mortality” (all flies knocked down and dead at the specific time of recording) and as “immediate mortality” (the quantity of the knocked down and dead flies which were caught in a receptacle suspended closely beneath the painted board).

The baits were tested in two situations: 1) as *non-choice trials*, in which the flies had access to only water and bait, and 2) as *choice trials*, in which the flies had access to water, milk powder, sugar and bait.

Fipronil Fly-bait gel was at least as effective as the two reference-baits in both the non-choice trials and the choice trials.

During the initial 2 hours of exposure, the overall mortality to Fipronil Fly-bait gel was low compared to the methomyl reference bait but resembled the overall mortality to the azamethiphos reference bait. In the period of 2-4 hours after the initiation of bait exposure there was a marked increase in the mortality response to the Fipronil Fly-bait gel; and after 4 hours the differences between the overall mortality to methomyl and to Fipronil Fly-bait gel were small and no longer significant. This was the situation in both the non-choice trials and the choice trials. The overall mortality to the azamethiphos bait was generally smaller.

In the non-choice trials the overall mean mortality after 48 hours was above 95% to all three toxic baits. In the choice trial the overall mean mortality to the azamethiphos bait was 61% after 48 hours, whereas the mean mortality to the methomyl bait and Fipronil Fly-bait gel was 95% and 98%, respectively.

The flies were killed more slowly by Fipronil Fly-bait gel than by the reference baits. Approximately 20% of the flies killed by Fipronil Fly-bait gel were recorded close to the bait in the receptacles, whereas approximately 60% of the flies killed by the azamethiphos-bait or the methomyl-bait were recorded close to the bait.

M. Knorr and J. B. Jespersen

6.1.2 Field evaluation of triflumuron-impregnated targets for control of the housefly *Musca domestica* and the stable fly *Stomoxys calcitrans*

The efficacy of triflumuron-impregnated targets when exposed to adult flies was evaluated under field conditions. The targets were used in the animal holding facilities on four farms for control of the housefly, *Musca domestica*, and the stable fly, *Stomoxys calcitrans*. The trials went on for a whole housefly season in Denmark, running from May to the end of September. The control effect was evaluated by monitoring of the number of flies in the trial units, throughout the season. This was done once a week by estimation of the number of flies by direct observation, using the “DPIL Fly Index” method and by use of sticky traps.

The targets were impregnated with an average amount of triflumuron of 3 g a.i./m² target and with sugar. Two farms, H1 and H2, were treated with a high number of targets and two farms, L1 and L2, were treated with a low number of targets. The amount of impregnated target surface per 100 m² stable ground area was 26 × 0.15 m² on the H-farms and 13 × 0.15 m² on the L-farms. Two more farms, C1 and C2, were included as untreated controls. Application of the targets was made in mid May on all the farms and replacement of old targets with new targets later in the trial period was made on one farm (H1).

In the four livestock units of the two H-farms the fly pressure was high when the target treatment was initiated. In three of these units it was not possible to obtain a sufficient control, and in one unit the treatment may have resulted in part of the reduction in the number of flies. The 1998 fly season was exceptionally cold, and in the livestock units having indoor temperatures depending on the outdoor weather, the housefly populations did consequently not reach as high nuisance levels as usual. In the livestock units of the L-farms the targets were placed while the population size was still low after the winter. A common feature of the L-units was that the indoor temperature was quite depending on the outdoor temperature. The cold 1998 fly season was therefore an important factor in limiting the housefly pressure in the L-farm units during the trial season, but the temperature was probably not the only major reason. In two trial units with nursing sows, the infestation level would presumably have been higher without insecticide control.

The impregnated targets may limit reproduction in the housefly populations to a certain extent, but the method needs improvement in order to be satisfactorily effective. In heavily infested livestock units it was not possible to detect any effect of the targets even though a high number of targets was used. In livestock units where the fly production was limited due to low temperature, the targets may have been an important factor for obtaining satisfactory, low infestation levels. This was probably the case in some of the livestock units. But due to the cold summer the results in general were inconclusive as it was not possible to weigh the relative importance of cold weather effect and impregnated target effect.

The impregnated targets did not control the populations of stable flies, *Stomoxys calcitrans*, at all.

M. Knorr and J. B. Jespersen

6.1.3 Laboratory tests with fiprole RP782 against susceptible and resistant strains of the housefly *Musca domestica*

We evaluated the insecticidal effect of the fiprole RP782 on various laboratory strains of *Musca domestica* with topical application and feeding bioassay. The susceptible level of RP782 was determined in the susceptible reference strain WHO. The potential of cross-resistance was evaluated in five insecticide-selected laboratory strains with different patterns and levels of resistance to organochlorines, organophosphates, carbamates and pyrethroids.

Three of the highly insecticide-resistant strains (39m₂b, 381zb, 698ab) were susceptible to RP 782, one strain (690ab) had a low level of cross-resistance, and one strain (17e) was highly resistant to RP 782.

The lindane-resistant strain (17e) showed a high level of cross-resistance to topically and orally applied RP 782. The resistance mechanism involved was probably reduced sensitivity of the fiprole and lindane target site, the GABA-gated chloride channel and/or an increased metabolism of both structurally unrelated insecticides. A common resistance mechanism based on metabolism is not very likely since other resistant strains, which are characterized by increased general metabolism of xenobiotic compounds, are susceptible to RP 782. It is more likely that an increased general metabolism of the lindane-resistant strain has a multiplicative effect on a target site-based resistance mechanism.

M. Kristensen and J. B. Jespersen

6.2 Insecticide resistance in *Musca domestica*

6.2.1 Route of uptake of CGA-293 and azamethiphos in a susceptible and multi-resistant strain of the housefly *Musca domestica*

The purpose of this investigation was to evaluate the route of uptake of CGA-293'343 in houseflies. This was analysed by comparing of the toxicity of CGA-293'343 and azamethiphos by tarsal contact test, topical application bioassay, standard feeding bioassay and forced feeding bioassay in the susceptible strain WHO and the multi-resistant strain 381zb.

Azamethiphos performed better compared to CGA-293'343 when applied topically to both susceptible and resistant houseflies. When applied topically in susceptible strains, azamethiphos was about >10 times more effective to kill similar amounts of houseflies. CGA-293'343 was more toxic and faster acting than azamethiphos in the tarsal contact bioassay in both susceptible and multi-resistant houseflies. The efficacy in the standard feeding bioassay of the two insecticides were almost identical, with azamethiphos showing an advantage in the immediate knockdown. Azamethiphos was more toxic than CGA-293'343 in the forced feeding bioassay in both susceptible and multi-resistant houseflies.

The differences in toxicity of CGA-293'343 and azamethiphos in the tarsal contact and the forced feeding bioassays thus seem to equal each other out, when tested in the combined standard feeding bioassay. Taken into consideration, that both products have different modes of action, this underlines the potential of CGA-293'343 as an important part in resistance management programs.

M. Kristensen and J. B. Jespersen

6.2.2 Resistance tests in housefly populations on Danish farms

To improve the use of existing insecticides and delay the onset of resistance and treatment failures, it is important with regular surveys to establish the real extent of insecticide resistance, even for species with an extensive resistance history. Regular surveys of resistance to insecticides of interest in relation to housefly control in Denmark have been carried out for many years at DPIL by collection of houseflies on farms in various parts of the country and tests of resistance on their offspring. Aerosols or space sprays with either pyrethrum or bioresmethrin both synergized with piperonyl butoxide, commonly used for housefly control, are still effective on most farms in Denmark, but give only temporary control. Residual synthetic pyrethroids are not allowed for housefly control on farms in Denmark. More widely used are persistent insecticide treatments, which are performed by paint-on baits with organophosphates, mainly azamethiphos or stick-on baits with the carbamate methomyl. Residual sprays with dimethoate are still registered for housefly control in Denmark. Larvicides containing the insect development inhibitors diflubenzuron or cyromazine were effective where breeding places could be treated properly. Larvicide usage is increasing in Denmark.

M. Kristensen and J. B. Jespersen

6.2.3 Resistance mechanisms of houseflies

Sixty-three individuals from each of our laboratory strains were assessed for activity towards the glutathione *S*-transferase substrates 3,4-dichloronitrobenzene (DCNB) and 1-chloro-2,4-dinitrobenzene (CDNB), the general esterase substrate *p*-nitrophenyl butyrate (*p*NPB), and the P450 dependent monooxygenase substrate *p*-nitroanisole (*p*NA). Specific activity towards the AChE substrate ATCI was measured in 63 individuals from each strain. The effect of three inhibitors, azamethiphos, methomyl and omethoate was also measured on each fly tested. The results gained showed many different insecticide resistance phenotypes.

M. Kristensen and A. Spencer

6.2.4 Laboratory strains kept in 1999

At the end of 1999, 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.

J. B. Jespersen and M. Kristensen

Table 6a. Laboratory strains of *Musca domestica* maintained during 1999

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

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

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

6.3.1 Parasitic wasps

The housefly, *Musca domestica* L. and the stable fly, *Stomoxys calcitrans* (L) are common pests on most livestock facilities in Denmark. The flies are a nuisance to animals as well as to humans and are potential vectors of diseases. Demands by farmers and the public for alternative or supplementary methods to control flies instead of the use of insecticides have increased steadily within the last twenty years - mainly because of the risk of insecticide residues in the animal product, the contamination of the environment and the fact that the flies develop resistance to some (or maybe all) insecticides in use. One alternative method is biological control, where natural enemies are mass-released to suppress the fly populations below nuisance levels.

The results of the three first years of a project (funded partially by the Ministry of Food, Agriculture and Fisheries, 1996-2000) to evaluate the possibilities of using pupal parasitoids (2-3 mm in size) for control of houseflies and stable flies are summarized here:

1: A full description of the species composition and seasonal activity of pupal parasitoids on pig and cattle farms in the country.

2: Two promising parasitoids, *Spalangia cameroni* and *Muscidifurax raptor*, have been isolated on the basis of primarily their high, relative abundance and activity in the field. *Spalangia cameroni* is the dominant species at indoor sites and *M. raptor* outdoors.

3: Life-history characteristics, like fecundity, survival, sex-ratio have been described for *S. cameroni* at constant temperatures. Maximum fecundity for the parasitoid lies in the temperature regime 25-30°C and with lowest fecundity close to 15°C. Survival declines with increasing temperatures, approx. 80 days at 15°C and approx. 10 days at 35°C. Independent of temperature or age, sex-ratio is female biased with about 60-70% females to males.

4: Movements of *S. cameroni* following mass releases have been studied for two years. The general trend was that females moved little from the point of release (5-8 m). On one occasion when the temperature reached 27-28°C in the stable nearly all released females (males including) dispersed from the system. Therefore, releases should be concentrated near areas of fly development due to the restricted movement of *S. cameroni*. Furthermore, in periods of hot weather or in stable systems with high temperatures there is a high risk that the released *S. cameroni* will disappear from the system with a consequently little effect on the fly populations.

5: Cultures of the most common parasitoids are established at SSL.

This fourth year has concentrated on two major tasks:

Firstly, winter survival of *S. cameroni*, *M. raptor* and *Urolepis rufipes* in stable environments shows that *M. raptor* and *U. rufipes* are able to survive in parasitized fly pupae (only in late larval stage) up to 6 months and without any substantial mortality compared with the initial start population. On the other hand, only a few individuals of *S. cameroni* can survive the winter period on Danish farms. Temperature is of great importance to the number of survivors during a winter period and especially to *S. cameroni* where prolonged periods of low temperatures result in substantial mortality.

Therefore, one conclusion of the study would be that *S. cameroni* has to be released each year if this species is going to have any influence on the fly populations early in the fly season. In contrast if *M. raptor* or *U.*

rufipes are established in the stable system it is likely that only a few releases are needed as supplements to the emerging winter populations of the parasitoids.

Secondly, weekly *S. cameroni* has been mass released on two livestock farms (one with pigs and one with cattle) from April and to October to study the impact on the *M. domestica* and *S. calcitrans* populations. The released parasitoids delayed the population increase of *M. domestica*, and the fly population never reached the nuisance level as was observed on the farms the year before, with no releases of parasitoids. On the other hand, the release of *S. cameroni* had no visible effects on the population development of *S. calcitrans* or their numbers.

Releases with *S. cameroni* are to be continued in 2000 on the same two farms and with the inclusion of one more pig farm.

H. Skovgård Pedersen

6.3.2. *Entomophthora muscae* s.l. in houseflies

Entomophthora muscae s. str. and *E. schizophorae* have been studied in relation to houseflies in Vibeke Kalsbeek's Ph.D. project (see DPIL Annual Reports 1996, 1997 and 1998). The work has been compiled into four manuscripts that have been submitted to international journals. The Ph.D. thesis will be completed in 2000.

V. Kalsbeek, J. B. Jespersen and T. Steenberg

6.3.3. *Entomophthora muscae* s.l. in stable flies

Although stable flies (*S. calcitrans*) and houseflies often co-occur in stables, stable flies have not been reported to suffer infections from *E. muscae* s.l. In a survey of fungal pathogens in stable fly populations, adult flies were collected from four livestock farms in May, and during the peak fly season in August, September and October. Stable flies from all four farms proved to be infected by *E. muscae* s.str. in late summer, and flies from one farm were also infected with *E. schizophorae*. Infection levels ranged between 0.5% and 4%. In contrast, infection rates in houseflies sampled from the same stables reached up to 90%. Laboratory studies in the U.S. have shown *E. schizophorae* to be poorly adapted to stable flies. These field data indicate that this is also the case for *E. muscae* s.str. since 1) the fungus never causes epizootics in stable flies even when abundant inoculum is present due to epizootics in houseflies, 2) in contrast to houseflies fungus-infected stable flies are not attached firmly to the substrate via the proboscis, and infected flies are likely to fall to the ground before they can produce inoculum, and 3) many fungus-killed stable flies only produce scarce amounts of inoculum compared to houseflies.

T. Steenberg

6.3.4. Hyphomyceteous fungi

A number of fungal isolates were tested against adult houseflies in immersion tests. Three isolates were selected for further studies (*M. anisopliae*, *B. bassiana* and *P. fumosoroseus*). All caused 100% mortality within 6 days (mean lethal time 4 days). Similar tests with adult stable flies unfortunately had to be cancelled due to problems with unacceptable levels of mortality in controls.

Eight isolates were tested in bait experiments against adult houseflies. Conidia were mixed with sugar and supplied to flies that also had access to uncontaminated sugar as well as water. Six of the eight isolates caused mortality under these experimental conditions. The cumulated mortality only reached a maximum of 40%, but this will probably be increased if higher doses of spores are used and/or the period of time with access to the fungus bait is prolonged.

T. Steenberg and J. B. Jespersen