Scientific and technical work

6. Flies

6.1 Chemical control of *Musca domestica*

6.1.1 Field evaluation of Fipronil Fly-bait Gel for control of the housefly *Musca domestica*

At the request of the manufacturer, the efficacy of a paint-on bait (a.i. 0.1% fipronil) for control of the housefly, *Musca domestica,* was evaluated in a field trial. The objective was to evaluate the efficacy of the bait formulation in animal houses and to evaluate the risk of a rapid development of resistance to fipronil.

The bait was used for housefly control during the whole fly season on three animal farms. Two other farms were used as controls without use of any chemical control measures against houseflies. The control effect of the treatments was monitored by a weekly estimate of the number of houseflies. Resistance to fipronil was determined by standard feeding tests on strains collected immediately before the first treatment with fipronil in the animal units and again on strains collected once or twice late in the season. Moreover, the resistance to dimethoate and pyrethrin/PBO was determined by topical application tests.

The quantity of bait applied for one complete treatment of an animal unit was 0.1g fipronil (a.i.) per 100 m^2 base area of the unit. On one of the farms, T3, only one application was made, while on the two other farms, T1 and T2, a second treatment was made four weeks after the initial treatment.

On two of the treated farms, T1 and T2, the housefly density was above the nuisance level when the initial bait application was made; and in both, the housefly reproduction was intensive at the time. The bait restricted the growth of the housefly populations, but it could not reduce the number of flies to a level below the nuisance level on these two farms. On the third treated farm, T3, the initial fipronil application was made when the housefly population was still below the nuisance level. No further bait applications were made, and the number of houseflies remained below nuisance level during the whole season. The 1998 fly season was exceptionally cold, and as the air temperature in the animal units of the T3 farm was dependent on the outdoor temperature, the number of houseflies on this farm would probably not have increased very much above the nuisance level even in the absence of chemical control measures.

A small longevity trial, where paralyzed or dead flies were collected in receptacles below fipronil-treated plywood boards in the animal units, indicated that the efficacy of the bait was unchanged during a 6-week period.

The strains collected showed no indications of increased resistance to fipronil in the three housefly populations during the 9- to 16-week period of fipronil exposure in the animal units.

M. Knorr Lauridsen and J. Brøchner Jespersen

6.1.2 Laboratory evaluation of CGA 293 343 paint-on bait for control of the housefly *Musca domestica*

Three paint-on-bait formulations, WP 10, WP 5 and GB 1, with 10%, 5% and 1% CGA 293’343 active ingredient were evaluated under laboratory conditions for efficacy against the housefly *Musca domestica*. A reference paint-on-bait which contained 10% azamethiphos, a reference paint-on-bait containing 1.1% methomyl and an untreated control were 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 only access to water and bait, and (2) as *Choice trials,* in which the flies had access to water, milk powder, sugar and bait.

Mutually compared, the overall killing effect of the three CGA 293'343 formulations did not vary significantly. This was the case in both the Non-Choice and the Choice trials.

The three CGA 293'343 formulations were at least as effective as the two reference baits. In the Non-Choice trials the differences between the CGA 293'343 baits and the two reference baits were small and in general not significant. In the Choice trials in which the flies had access to alternative food sources in addition to the bait, the overall mortality to the three CGA 293'343 baits and to the methomyl reference was in general not significantly different (96-99% after 48 hours). Sensitivity to the azamethiphos reference (87% mortality after 48 hours) was, significantly, the lowest recorded among the toxic baits in the Choice trials.

In both the Non-Choice and the Choice trials, the proportion of immediately killed flies recorded in the receptacles was higher to the two reference baits than to the three CGA 293'343 baits. The proportion of killed flies recorded in the receptacles below the three CGA 293'343 baits was highest for WG 10, intermediate for WG 5, and lowest for GB 1. The mean mortality for WG 10 and WG 5 was not significantly different at any time. In the Choice trials, however, GB 1 had significantly lower mean mortality values in general than WG 10 and WG 5.

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6.2 Insecticide resistance in *Musca domestica*

6.2.1 Larvicidal efficacy of CGA 293'343 against susceptible strains of houseflies

The larvicidal efficacy of CGA 293'343 was tested in two insecticide susceptible strains of the housefly ($WHOij_2$ and BPM). The efficacy of CGA 293'343 was estimated in standard larvicide tests, where newly laid housefly eggs or third stage instar larvae were seeded on artificial media impregnated with the insecticide.

The lethal concentrations based on the larvicide tests with eggs or third stage instar larvae were estimated by probit analysis. The results showed that CGA 293'343 was not an efficient larvicide at low concentrations.

M. Kristensen and J. Brøchner Jespersen

6.2.2 Larvicidal efficacy of dicyclanil against susceptible strains of houseflies

The larvicidal efficacy of dicyclanil was tested in two insecticide susceptible strains of the housefly ($WHOij_2$ and BPM). The efficacy of dicyclanil was estimated in standard larvicide tests, where newly laid housefly eggs or third stage instar larvae were seeded on artificial media impregnated with the insecticide.

The lethal concentrations based on the larvicide tests with eggs or third instar larvae were estimated by probit analysis. The results showed that dicyclanil is an efficient larvicide against houseflies at low concentrations.

M. Kristensen and J. Brøchner Jespersen

6.2.3 Larvicidal efficacy of dicyclanil in tests with three resistant strains of houseflies

We evaluated the larvicidal efficacy of dicyclanil, cyromazine and diflubenzuron in *Musca domestica* laboratory tests using three strains of houseflies representing different patterns of insecticide resistance. The strains were tested in feeding tests with methomyl and azamethiphos, and in topical application tests with dimethoate and pyrethrin synergized by piperonyl butoxide (PBO) to assess the resistance level to traditionally used insecticides.

One of the three tested strains, 802ab, showed a low level of resistance to dicyclanil. It also showed a low level of resistance to cyromazine, whereas it was susceptible to diflubenzuron. The 802ab population has two components: a dicyclanil susceptible fraction and a dicyclanil resistant fraction. The strain 802ab was moderately resistant to pyrethrin/PBO, dimethoate, azamethiphos and methomyl.

The 381zb strain showed a moderate to low level of resistance to diflubenzuron, whereas it was susceptible to cyromazine and dicyclanil. It was moderately resistant to pyrethrin/PBO and highly resistant to dimethoate. The 807ab strain was almost susceptible to cyromazine and dicyclanil while it was highly resistant to diflubenzuron. It showed a moderate level of resistance to the topically applied pyrethrin/PBO and dimethoate.

The low level of cross-resistance to dicyclanil observed in the cyromazine selected and multi-resistant 802ab strain might be explained by an elevation of the general detoxification system involving glutathione S-transferases and/or P450 monooxygenases. It was not possible to elucidate whether the cross-resistance to dicyclanil observed in 802ab was linked to the low cyromazine resistance or to resistance to other insecticides including the four adulticides tested.

M. Kristensen and J. Brøchner Jespersen

6.2.4 Biochemical and toxicological analysis of CGA 293’343 in susceptible and resistant strains of the housefly *Musca domestica*

The mechanism(s) responsible for cross-resistance to the novel insecticidal compound CGA 293’343 in housefly strains were investigated by topical application of the synergists piperonyl butoxide (PBO) or S,S,S-tributyl phosphorotrithioate (DEF) prior to feeding bioassay on CGA 293’343 impregnated sugar.

The organophosphate, carbamate, and pyrethroid, multiresistant 381zb selected laboratory strain (selected by dimethoate and permethrin) was moderately/highly resistant to CGA 293’343. The resistance was partly synergized by PBO, while DEF had no effect.

The methomyl resistant 690ab selected laboratory strain (selected by methomyl) was low to moderately resistant to CGA 293’343. The resistance was partly synergized by PBO. DEF had no effect.

The organophosphate, carbamate, and pyrethroid, multiresistant 791a field strain was moderately to highly resistant to CGA 293’343. The resistance was partly synergized by PBO, whereas DEF had no effect.

The effect of CGA 293’343 on AChE activity was tested. In the dose range relevant for the use of CGA 293’343 there was no effect of the compound on AChE activity.

Glutathione S-transferase activities were measured with the 1-chloro-2,4-dinitrobenzene (CDNB) and 3,4-dichloronitrobenzene (DCNB) substrates. P450 monooxygenase activity was measured with the substrate ρnitroanisole (PNA). Within the wide range of activity levels measured in the housefly strains used, we did not observe a correlation with any of the activities and CGA 293´´343 toxicity.

PBO acts as a synergist of CGA 293'343 resistance in the resistant strains 381zb, 791a and 690ab. Thus, a P450 monooxygenase enzyme activity was expected to be responsible for this effect. However, full susceptibility was not achieved by adding PBO: resistance factors of between 5 and 10 remained. This low to moderate non-synergizable resistance did not correlate with any of the enzyme activities measured and could be due to natural variation of the CGA 293'343 target site.

M. Kristensen. A. Spencer and J. Brøchner Jespersen

6.2.5 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 the 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, but also propethamphos, 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.

In the 1997 survey we found the beginnings of resistance towards the benzoylurea diflubenzuron. This had to some extent been observed earlier. For the first time we found field strains resistant to cyromazine.

We found resistance to diflubenzuron in strain 790b and a tendency to resistance development in five other strains. We found resistance to cyromazine in strain 791a and a tendency to resistance in three other strains. No correlation between diflubenzuron and cyromazine tolerance was observed. In the laboratory we applied a selection pressure with either diflubenzuron or cyromazine to four strains. In strain 807ab we were able to select a high level of resistance to diflubenzuron, while and in strain 790b a moderate level of resistance to diflubenzuron was achieved. The selected strain 807bb now kept at DPIL is approximately 100-fold resistant to diflubenzuron at LC_{50}, but susceptible to cyromazine. We were not able to select for cyromazine resistance in 791a. The highest level of cyromazine resistance, 8-fold, was created by the selection of strain 802ab. We are currently trying to identify the larvicide resistance mechanisms in housefly larvae.

M. Kristensen and J. Brøchner Jespersen

6.2.6 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 ρ-nitrophenyl butyrate (ρNPB), and the P450 dependent monooxygenase substrate ρ-nitroanisol (ρNA). 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

6.2.7 Laboratory strains kept in 1998

At the end of 1998, the 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. Brøchner Jespersen and M.Kristensen

Table 6a. Laboratory strains of *Musca domestica* maintained during 1998

Strain	Origin	Year	Remarks	Lab pressure
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				
17 e	DK	1950		lindane
150 b	DK	1955		diazinon*
39 m_2b	DK	1969		tetrachlorvinphos*
49 r_2b	DK	1970		dimethoate*
381 zb	DK	1978		permethrin and dimethoate*
690 ab	DK	1984		methomyl feeding*
594 vb	DK	1988		azamethiphos feeding*
213 ab	Swe-den	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

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

Table 6a. continued

	Strain	Origin	Year	Remarks	Lab pressure
2.	*Originally resistant field strains kept without insecticidal pressure*				
	7	DK	1948	Reverted DDT-R	None
	772 a	DK	1989	Common lab. test strain	None
	791 a	DK	1997	Multi-R	None
3.	*Susceptible strains*				
	BPM	Leiden	1955		None
	WHO Ij_2	Pavia	1988		None
	NAIDM	Texas	1991		None
4.	*Strains with R mechanisms isolated*				
	A_2bb	DK	1982	Super-kdr. Chr. 1, 2 and 3 with marker genes	None
	LPR	USA	1995	Pyr-R kdr, P450 monooxygenase	None

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 important pests in most confined animal production units in Denmark. The flies are a nuisance to animals as well as to humans and are potential vectors of pathogens. Demands by farmers and the public for alternative or supplementary methods of insecticide control of these flies have increased the last ten years, mainly because of the risk of insecticide residues in animal products, the contamination of the environment and the fact that the flies develop resistance to some of the insecticides in use. One alternative method is biological control, where natural enemies are released to suppress the fly populations below nuisance levels.

The two first years in a project partially funded by the Ministry of Food, Agriculture and Fisheries to evaluate the possibility of the use of parasitic wasps in the control of houseflies and stable flies have resulted in a full description of the species composition and seasonal activity of parasite wasps on confined pig and cattle farms in this country. Furthermore, based on these studies two parasite species, *Spalangia cameroni* and *Muscidifurax raptor* have proven to be promising control candidates of the flies (DPIL, Annual report 1997).

This third year has concentrated mainly on dispersal activity of released parasitoids in a stable environment. The most dominant pupal parasitoid, *S. cameroni* at indoor sites was chosen as the study organism. At each experiment, about 10,000-15,000 individuals were released at the center of a stable on a traditional pig farm. Five experimental releases of *S. cameroni* were conducted from May to October on this farm. Laboratory-reared *M. domestica* puparia were laid out systematically in the stable environment as "traps" in order to make it possible to detect dispersal activity of the female wasps. With fixed intervals from the time of release, the pupal traps were examined for *S. cameroni* individuals. The results were consistent among experiments and showed that female *S. cameroni* within a day disperse little from the point of release whereas the opposite was observed for the males which moved towards the surrounding windows within an hour of release.

The results are important for understanding that if a species like *S. cameroni* is to be used in control of the flies, one important factor to consider is to release the wasp in a large number of locations in the stable environment. However, release efforts can be concentrated to include only the areas in the stable where the flies are commonly known to develop.

The dispersal experiment is to be continued in 1999 with the inclusion of *M. raptor*. Moreover, movement of the two parasite species will be evaluated in the stable environment for one week to see if the picture of dispersal will change.

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6.3.2 Entomophthora muscae

This work was conducted in co-operation with Professor Bradley A. Mullens, Department of Entomology, University of California, Riverside in the last year of V. Kalsbeek's Ph.D project.

Houseflies (*Musca domestica*) infected with the entomopathogenic fungi *Entomophthora muscae/E. schizophorae* are known to cure themselves of this infection by seeking high temperatures shortly after infection - a phenomenon called "behavioural fever". Two studies were carried out in August/September 1998 in Denmark to evaluate the occurrence and extent of behavioural fever under natural conditions. In the first study conducted on an open organic dairy farm, the pathogen present was *E. muscae* with an average of about 17 nuclei per conidium. In the second study, *E. schizophorae* with four to eight nuclei per conidium was used in the release of infected flies.

(1) At different times of the day on each of four consecutive days, flies were collected with a sweep net from cool indoor and warm outdoor surfaces. The flies were kept individually in the laboratory and, since the incubation time for *E. muscae* is quite constant at a particular temperature, flies dying of mycosis shortly after capture were regarded as being late in incubation and vice versa.

The preliminary results show that the infection level was consistently higher indoors, i.e. at cooler conditions (38%) than outdoors (27% on relatively warm dark surfaces and 15% on sun-exposed feed). Furthermore, the data indicate that a higher proportion of flies collected indoors died of mycosis within the first three days after capture, i.e. late in incubation.

This is in accordance with studies conducted elsewhere, showing that flies seek to cooler areas shortly before they die of *E. muscae.*

(2) To determine whether infected flies prefer warmer surfaces, approximately 3,000 fungus-inoculated (marked with yellow paint; 30% became infected) and 3,000 non-inoculated flies (marked with blue paint) were released on the second day in incubation in an enclosed swine farrowing barn. The flies were reared from wild parents to avoid the unnatural behaviour that may take place in laboratory strains of houseflies. The number of yellow and blue flies was recorded in different areas of the barn with different temperatures including heating lamps that provided resting surfaces with temperatures above 40^{o}C. Observations took place every second hour from 8 a.m. to 6 p.m. for 6 days.

Especially on the day of release, a higher total number of inoculated flies was found on the heating lamps compared to non-inoculated flies which might indicate that behavioural fever took place. Another factor that may indicate behavioural fever was the decrease in initial fungal prevalence from 30% to 5%, two days after release and to 0% four days after release. No yellow flies were observed in high numbers on the cooler surfaces late in incubation and finally only one yellow-marked cadaver was found when the barn was examined thoroughly.

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

6.3.3 Hyphomyceteous fungi

This project was initiated in 1998 and aims to evaluate fungi from the class Hyphomycetes as biological control agents against flies in stables. The natural occurrence of these fungi was studied in houseflies and stable flies collected from a large number of farms. The following species were isolated from houseflies: *Beauveria bassiana*, *Metarhizium anisopliae*, *Paecilomyces fumosoroseus*, *Verticillium lecanii* and *Verticillium fusisporum*. From stable flies we isolated *B. bassiana* and *V. lecanii*. The fungus prevalence was always low (below 5%) and there did not appear to be any correlation between fungus prevalence and time of year.

All fungi have been isolated *in vitro* and will be tested in the next year against the two species of flies in order to select the most suitable isolates for fly control in stables. Furthermore, houseflies were included in a study of the entomopathogenic potential of *Verticillium* and *Acremonium* species, the pathogenicity of which to insects needed clarification. *Bemisia tabaci* (the sweet-potato whitefly) was used as another insect host in this basic study. The *V. fusisporum* isolates originating from houseflies did not cause mortality in the homologous host, while different isolates of *V. lecanii*, *V. psalliotae* and *Acremonium* species caused high mortalities. However, none of the isolates had much effect within the first two weeks of incubation and thus do not seem to have potential for fly control, in contrast to some of the other species of Hyphomycetes that we have tested.

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