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 *Musca domestica*

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, and two more farms were used as controls without use of any chemical control measures against the houseflies. The control effect of the treatments was monitored by an estimate of the number of houseflies once a week. The resistance to fipronil was determined by standard feeding test 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 0.1 g a.i fipronil per 100 m^2 base area of the unit. On one of the farms, T3, only one application was made and on the two other farms, T1 and T2, a second treatment was made 4 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 stayed below nuisance level during the whole season. A comparison with the control farms indicated that the housefly infestation on farm T3 would not have become much higher than the nuisance level even without any 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.

There were 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, J. B. Jespersen and M. Kristensen

6.1.2 Field and laboratory evaluation of thiamethoxam-based water-soluble granular bait against *Musca domestica*

The efficacy of a thiamethoxam-based water-soluble granular bait containing 10% active ingredient was evaluated under field conditions as paint-on for control of houseflies. The objective was to determine the effect of the thiamethoxam bait on the control of the fly populations in closed farm units. Furthermore susceptibility in the treated housefly populations to thiamethoxam was evaluated to assess the risk of resistance development.
The bait was tested in a field trial on five Danish cattle farms including eleven livestock units. One of the farms was used as untreated control. The trial started in late June and lasted until mid-autumn. The effec-

tiveness was evaluated by weekly estimations of the number of flies in each trial unit by the use of a direct observation method, the DPIL Fly Index method. Adult flies for resistance testing were collected on the five trial farms before the first treatment and again at the end of the housefly season. A standard feeding bioassay with thiamethoxam was used for laboratory tests of susceptibility or resistance in the housefly populations.

250 g of thiamethoxam bait per 100 m^2 floor surface were applied as paint-on in the respective units. The paint-on suspension was applied in narrow stripes in order to make it as attractive to the flies as possible due to a maximum boundary between treated and untreated surface. The bait was applied optimally in this way on the surfaces in question when 75-100 ml water were used per 250 g test product for the preparation of the paint-on suspension. The bait showed no tendency to disappear or lose efficacy during four weeks after application. Reapplication of bait was made either because of high density of houseflies or because of depletion of the bait. When repeated application was made due to loss of bait, it took in most cases place after six weeks.

The efficacy of the paint-on bait was demonstrated on all four farms. In all treated units a high mortality of adult flies was shown. The effectiveness in reducing the infestation below the DPIL-defined nuisance level varied between the units, probably depending on the fly population level before bait application and the conditions for high fly reproduction due to differences in environmental parameters of the trial units. In six of the nine treated livestock units the applied bait resulted in infestation levels below the pre-defined nuisance level throughout the season. In two other units that had high numbers of flies before the initial application and a very intensive fly production, the bait was effective in killing flies, but it could not reduce the number of flies to below the pre-defined nuisance level. The fly infestation of one unit was too low to give significant results.

The susceptibility of flies to thiamethoxam-impregnated sugar tested in a standard feeding bioassay was slightly reduced in the field-collected strains compared to previously determined susceptible reference levels. The slightly to moderately higher tolerance level in the field strains did not change during the trial and hence there was no indication of induced resistance against thiamethoxam when the thiamethoxam bait was used in the field. A general conclusion on the potential of resistance development against thiamethoxam cannot be made because of the limited selection scheme covering only a few housefly generations.

M. Knorr, M. Kristensen and J. B. Jespersen

6.2 Insecticide resistance in *Musca domestica*

6.2.1 Susceptibility to thiamethoxam in Danish field populations of *Musca domestica*

A survey was made on samples of houseflies collected from 20 Danish livestock farms to assess the susceptibility/resistance to thiamethoxam in field populations of houseflies and to evaluate the potential of thiamethoxam for cross-resistance to traditional insecticides. The toxicity of the stomach poison effect of thiamethoxam and the carbamate methomyl and the organophosphate azamethiphos was measured by feeding tests. The toxicity level of the contact effect of the organophosphate dimethoate and the pyrethroid bioresmethrin synergized by PBO was measured by topical application tests. The resistance factors RF_{50} and RF_{95} were used for the evaluation of indications of cross-resistance.

The resistance to the four traditional insecticides was low to moderate in most of the populations, but among the studied strains the bioassays also revealed the existence of higher resistance in some of the populations to one or more of the compounds. At the LD_{50} level the resistance factors varied from 6 to 34 against dimethoate, from 4 to 27 against azamethiphos, from 4 to 13 against methomyl and from 3 to 38 against bioresmethrin/PBO at the endpoints of the bioassays.

The level of tolerance to the neonicotinoid thiamethoxam at the endpoint (72 hours) of the bioassay varied between the strains studied with resistance factors from 2 to 9 at LC_{50}. At LC_{95} the resistance factors varied from 2 to 9, with the exception of three strains with LC_{95} values at 19, 23 and 31.

In general, the survey strains demonstrated susceptibility or low resistance to thiamethoxam in the housefly populations. The three strains noticeable for higher LC_{95} could be characterized as multi-resistant field strains. It is probable that these strains have a higher capacity of general detoxification as the major component in their resistance mechanisms, and the existence of a neonicotinoid-specific resistance mechanism cannot be excluded completely.

The bioassay results of the survey strains did not show signs of any correlation between resistance factors for thiamethoxam and any of the four traditional insecticides tested. Thus there were no indications of specific cross-resistance to the feeding poison effect of thiamethoxam from resistance to the contact effect of bioresmethrin or dimethoate, respectively. And there were no indications of specific cross-resistance to the feeding poison effect of thiamethoxam from resistance to the feeding poison effect of methomyl or azamethiphos, respectively.

M. Kristensen, M. Knorr and J. B. Jespersen

6.2.3 Laboratory strains kept in 2001

At the end of 2001, DPIL kept 20 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, M. Kristensen and M. Knorr

Table 6a. Laboratory strains of *Musca domestica* maintained during 2001

	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*
	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
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 resistance 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

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

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

6.3.1 Biological control of nuisance flies (housefly and stable fly) indoors on organic dairy cattle farms with special reference to confinements with calves on deep bedding

Two fly species of veterinary importance in Denmark are the housefly, *Musca domestica* (L), and the stable fly, *Stomoxys calcitrans* (L), which are common pests on livestock facilities with porkers or dairy cattle. In particular organic farms with dairy cattle have severe problems with the biting stable fly because its high population densities in the late summer months may lead to reduced milk or beef production of the animals.

Timely sanitation procedures combined with glue traps or light traps will in most cases reduce the fly pressure to an acceptable level, but for farms where calves are kept indoors the organic matter that accumulates reaches a considerable thickness before it is removed. Such areas are excellent breeding sites for the housefly and the stable fly where zillions of flies will be developed throughout the summer and autumn months. Because the organic mat is not removed for 3-4 months, such indoor areas are well qualified for biological control with releases of pupal parasitoids (Hymenoptera: Pteromalidae) against the flies.

Based on previous studies (see Annual Report 2000) one parasitoid species, *Spalangia cameroni* (2-3 mm in size) was found a suitable candidate.

The main purpose for fly season 2001 was to evaluate whether biweekly releases *of S. cameroni* could suppress the populations of the housefly and stable fly to below nuisance level as release studies in 1999 and 2000 had produced good results, based on weekly releases. However, the farmers do not find weekly releases of *S. cameroni* economically and practically attractive.

Spalangia cameroni was released from mid April to October on two dairy cattle farms, and two farms were kept as controls. Every week, fly number (DPIL fly index) and the activity of released parasitoids were measured by exposing of laboratory-reared housefly pupae for seven days.

In line with previous results the housefly was suppressed to acceptable, low levels compared with the controls whereas early in the autumn the stable fly reached unacceptable levels. However, in spite of this the farm operators on both the release farms were more than satisfied indicating that although the stable fly population soared beyond control in late season, the period of fly annoyance had been shortened and the overall fly pressure reduced by the release of *S. cameroni*.

Based on the activity (parasitism of exposed pupae) releases of *S. cameroni*, biweekly and likely also every third week intervals seem appropriate for delaying the population increase and minimizing maximum abundance of especially the housefly and to some degree also the stable fly. Therefore, if indoor releases of *S. cameroni* is combined with control methods, like glue-traps which have in the U.S.A. proven to be very effective in reducing adult numbers of stable flies, an efficient reduction of both fly species could be expected.

H. Skovgård

6.3.2 Hyphomyceteous fungi

Field releases of the pupal parasitoid *Spalangia cameroni* have shown considerable potential for suppression of filth fly populations. However, pupal parasitoids cannot be expected to control filth flies in all types of farms and at all times of year, unless supplemented with other (bio)control agents. Different species of hyphomyceteous fungi have been tested against adults and larvae of houseflies (*M. domestica*), and are known to be able to infect both life stages although adults seem to be more susceptible to infection than larvae. Almost no attention has previously been given to the susceptibility of stable flies (*S. calcitrans*) to fungal pathogens, and nothing is known concerning the susceptibility of the larval instars.

An M.Sc. project was initiated in order to study: 1) the susceptibility of larvae of stable flies and houseflies to two species of hyphomyceteous fungi (*M. anisopliae* and *P. fumosoroseus*) and 2) the compatibility of these fungi with the pupal parasitoid *Spalangia cameroni*. In dose-response experiments with fly larvae (L3) and female parasitoids the insects were treated with aqueous conidial suspensions at concentrations ranging from $1 x 10^4$ to $1 x 10^8$ conidia/ml. *M. anisopliae* was the most virulent to stable fly larvae, causing 94% mortality at $1 x 10^8$ spores/ml, whereas housefly larvae were most susceptible to *P. fumosoroseus* (66% mortality at 1×10^8 spores/ml). *M. anisopliae* proved to be virulent to the parasitoids (mean mortality: 71% at 1×10^8 spores; *P. fumosoroseus* was not tested). Further studies will focus on the effect of *M. anisopliae* on parasitoid fecundity and development of progeny.

C. Nielsen, T. Steenberg and H. Skovgård

6.3.3 Laboratory evaluation of sticky traps for control of the housefly

The capture efficiency of two different glue traps was compared. Both traps were large sheets and they were differing in the design of the patterns and colours of the sticky surface.

The trial was made under laboratory conditions with the housefly *Musca domestica* and the traps were evaluated at full indoor light intensity as well as under lowered light intensity conditions. The capture efficiency tests were conducted in large regulated test chambers and they were designed as non-choice trials with only one type of trap placed in the chambers at a time.

In the tests conducted at full indoor light intensity as well as in the tests conducted under lowered light conditions, the laboratory investigation revealed no statistically significant difference in the capture efficiency of the two types of glue sheets.

M. Knorr and J. B. Jespersen

6.3.4 Comparison of the capture efficiency of glue boards for UV light traps

A trial was made to compare the duration of the initial capture efficiency for flying insects of two products of glue boards for UV light traps. The glue boards were differing in the composition of the adhesive ingredients. The capture efficiency of the boards was compared in a one-replicate trial as requested by a company and the results are therefore only indicative. The comparison was made on boards constantly placed in UV light traps located at two different room temperatures, 20°C or 40°C and 70% relative humidity. The capture efficiency was recorded twice a week throughout a 10-week trial period.

The capture efficiency of the boards was recorded by making female houseflies, *Musca domestica*, land on the glue surface. A fly was recorded as captured if it was still caught on the glue board exactly five minutes from the time of landing whereas it was recorded as not-captured if it was able to escape from the board within five minutes.

The trial indicated that one of the two glue board products maintained initial capture efficiency throughout the 10-week trial period at both room temperatures. The other product maintained initial capture efficiency at the low room temperature but lost the capture efficiency after few weeks when placed at the high room temperature.

M. Knorr and J. B. Jespersen

6.3.5 *Entomophthora muscae* in houseflies

On December 17, Vibeke Kalsbeek defended her Ph.D. thesis entitled ”The entomopathogenic fungi *Entomophthora muscae* and *E. schizophorae* infecting the housefly *Musca domestica*: redescription, distribution, persistence and behaviour”. Four of the five manuscripts included in the thesis have been published in refereed journals.

T. Steenberg

6.4 Flies and other arthropods and salmonella

A collaborative research project involving researchers from the Danish Veterinary Institute, Zoological Museum and DPIL entitled “Wildlife as a source of salmonella infection in food-animal production” was initiated in 2000. Farms with pig, cattle or poultry have been selected as study farms. As part of the project flies and other arthropods have been collected, for the purpose of being analysed for the presence of salmonella. The project will continue in 2002, and results will be published elsewhere.

J. B. Jespersen