

Scientific and technical work

6. Flies

6.1 Chemical control of *Musca domestica*

6.1.1 Efficacy of CGA-X against susceptible and resistance strains of houseflies

The effect of feeding adult male houseflies with CGA-X was bioassayed in laboratory tests with several laboratory strains of *Musca domestica*, i.e. a susceptible reference strain WHO-SRS and eight strains with different patterns and levels of resistance to organochlorines, organophosphorus (OP) compounds, carbamates and pyrethroids.

The CGA-X tolerance in the strains was measured in feeding tests by feeding of granular sugar impregnated with a range of doses of CGA-X to adult male flies. The effect was determined by observance of the mortality of the flies after 24 hours and after 48 or 72 hours. The lethal concentrations (LC-values) were estimated by probit analysis.

Topical application tests and a few feeding tests were made to measure the resistance of the fly strains to various conventional adulticides against which the respective resistance mechanisms of the various resistant strains are directed. The results of these tests confirmed that the resistance level was moderate-to-high or high: in strain A₂bb against pyrethroids, in strain 17e against lindane, in strain 381zb against dimethoate and permethrin, in strain 690ab against methomyl, in strain 698ab against DDT and in strain 594vb against azamethiphos.

The results of the feeding tests with CGA-X indicated a cross-resistance between some of the conventional insecticides and CGA-X as the two strains 381zb and 690ab were clearly more resistant to CGA-X than the susceptible reference strain. In strain 381zb, which is both resistant to OP-compounds and to pyrethroids, it is most likely the resistance mechanisms against dimethoate which are responsible for the relatively high cross-resistance to the feeding effect of CGA-X. In strain 690ab the resistance against methomyl is responsible for the low-moderate cross-resistance to the feeding effect of CGA-X.

6.1.2 Efficacy of two Fiproles against susceptible and resistant strains of houseflies

The insecticidal effect of two Fiproles was bioassayed in laboratory tests with various laboratory strains of *Musca domestica*, i.e. a susceptible reference strain WHO-SRS and four strains with different patterns and levels of resistance to organochlorines, organophosphorus compounds, carbamates and pyrethroids.

Topical application and feeding tests were made to measure the resistance of the four fly strains to various conventional adulticides against which the respective resistance mechanisms of the various resistant strains are directed. The results of these tests confirmed that the resistance level was moderate-to-high or high: in strain 17e against lindane, in strain 381zb against dimethoate and permethrin, in strain 690ab against methomyl and in strain 698ab against DDT.

The fiprole tolerance in the strains was measured in feeding and topical application tests with a range of doses of fiprole I and II to adult male and female flies, respectively. The effect was determined by observance of the mortality of the flies after 24 hours and after 48 and/or 72 hours. The lethal concentrations (dosages) were estimated by probit analysis.

The results of the feeding and topical application tests showed possible crossresistance between some of the conventional insecticides and the fiproles as some of the fly strains were clearly resistant to fiprole I and II when compared to the susceptible reference strain. The fiprole resistance is most likely caused by a reduced sensitivity of the GABA receptors, but this has to be confirmed. However, enhanced metabolism and penetration resistance may add to the effect, and other resistance mechanisms cannot be ruled out as resistance mechanisms involved.

J. B. Jespersen and M. K. Lauridsen

6.2 Insecticide resistance in *Musca domestica*

6.2.1 Resistance tests in fly 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 by tests of resistance in the 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 and propethamphos 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, and larvicide usage is increasing.

Samples of 21 *Musca domestica* field populations were collected from animal houses in Denmark in 1997. We determined the susceptibility of the first generation offspring to different types of insecticides: the pyrethroids bioresmethrin and pyrethrin both synergized with piperonyl butoxide; the organophosphates dimethoate, azamethiphos and propethamphos; the carbamate methomyl and the two insect growth regulators diflubenzuron and cyromazine. The results showed that pyrethroid resistance is increasing in Denmark. On 4 of the 21 farms more than a 100-fold resistance was observed. Resistance to azamethiphos was widespread and high and has increased since the 1980s. Four strains is hypothesized to have an inherited behavioural phenotype contributing to the azamethiphos resistance observed. Status of dimethoate resistance showed a trend of populations with either increasing susceptibility or populations with increasing resistance. Two strains with high methomyl and propetamphos resistance were observed. Methomyl and propetamphos resistance is not increasing.

Resistance risk assesment of larvicides has been performed from their introduction in the early 1980s without finding strong indications of resistance development. In the 1997 survey we found beginning develop-

ment of resistance towards the benzophenylurea diflubenzuron, which to some extent has been observed earlier, and for the first time we found field strains with increased tolerance to cyromazine.

M. Kristensen and J. B. Jespersen

6.2.2 Resistance mechanisms of Danish houseflies

The 21 populations of *M. domestica* collected in 1997 for resistance assessment were analysed *in vitro* for biochemical characteristics commonly associated with insecticide resistance. Sixty-two females from each strain 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 dependant monooxygenase substrate *p*-nitroanisole (*p*NA). Specific activity towards the AChE substrate ATCI was measured in 63 females from each strain. The effect of three inhibitors, azamethiphos, methomyl and omethoate was also measured on each fly tested. The results gained show a significant elevation in enzyme activity in many of the populations - particularly those showing high levels of resistance. It can also be concluded that the majority of flies from most of the populations maintains enzyme activities consistent with susceptibility. Insensitivity to methomyl and omethoate is widespread but of low frequency. Azamethiphos insensitivity, however, is rare.

We have together with Martin Williamson, IARC-Rothamsted, developed and implemented a molecular diagnostic method for detecting pyrethroid resistance caused by modification of the voltage dependent sodium channel protein in the housefly, in order to survey the mutation frequency of this mechanism in field populations. The method enables us to determine the genotype of individual flies. The method is simple, reproducible and applicable for determining genotype frequencies in laboratory-reared or field-collected populations.

M. Kristensen and A. Spencer

6.2.3 Laboratory strains kept in 1997

At the end of 1997, the DPIL kept 22 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

Table 6a. Laboratory strains of *Musca domestica* maintained during 1997

Strain	Origin	Year	Remarks	Lab pressure
<i>I. Strains subjected to periodic insecticidal pressure (adult dipping, exposure to vapour, or with feeding treated sugar) from a compound to which at least part of the population showed clear resistance at the time of collection</i>				
17 bb	DK	1950	Also some R to dieldrin and pyrethroids	DDT
17 e	DK	1950	Some R to OPs	lindane
150 b	DK	1955		diazinon*
39 m ₂ b	DK	1969	DDT-R due to oxidation (mfo)	tetra-chlorvinphos*
49r ₂ b	DK	1970		dimethoate*
381 zb	DK	1978		permethrin 1979+ and dimethoate 1983+*
690 ab	DK	1984	OP-R. Moderate R to methomyl	methomyl feeding*
594 vb	DK	1988		azamethiphos*
213 ab	S	1957	Py-R, Low OP-R. Collected near Stockholm	pyrethrins/ pbo*
571 ab	J	1980	Collected on Tokyo's city dump (by K.Ya-sutomi). Very high OP-R, susceptible to pyrethroids	fenitrothion
698 ab	Burma	1985	Collected at a market in Rangoon. R to DDT (no kdr), dieldrin, and malathion. Low-moderate R to other OPs	DDT

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

Table 6a. continued

Strain	Origin	Year	Remarks	Lab pressure
2. <i>Originally resistant field strains kept without insecticidal pressure</i>				
7	DK	1948	Heterogeneous R to dieldrin. DDT-R reverted	None
772 a	DK	1989	Moderate R to OPs and pyrethroids. Common laboratory test strain	None
3. <i>Susceptible strains</i>				
BPM	Leiden	1955		None
WHO Ij ₂	Pavia	1988, 1996		None
RAC	Wage- ningen	1982	Chr. 1, 2 and 3 with marker genes	None
NAIDM	Texas	1991		None
4. <i>Strains with R mechanisms isolated</i>				
A ₂ bb	DK	1982	Origin: Danish strain 381 z (1978) Chr. 3 with super-kdr isolated by Oppenoorth. Chr. 1, 2 and 3 with marker genes	None
LPR	USA	1995	Received from J. Scott (Cornell University) R to pyrethroids	None
Rutgers	USA	1995	Received from R. Feyereisen (University of Arizona) R to diazinon	None

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

6.3.1 Parasitic wasps

The house fly, *Musca domestica* L and the stable fly, *Stomoxys calcitrans* L are important pests in today's poultry, dairy cattle and pig production. The flies are a nuisance to animals as well as to humans and are potential vectors of pathogens. In traditional farming systems houseflies are controlled by means of an array of chemicals. This intensive control practice has led to widespread resistance to many of the chemicals used. Therefore, exploration for alternative or supplementary methods for fly regulation is needed. A promising method is biological control, where natural enemies are released into the pest habitat to bring down the pest population below injury or annoyance level.

One of the first steps in a biological control project should be a determination of the native enemy complex that interacts with the pest population. Thus, a nation-wide survey has been conducted in the period August to October, 1996 and 1997, where 84 pig and cattle farms were visited. Fly puparia were gathered on each farm to obtain information on activity and species composition of pupal parasitoids (*Hymenoptera: Pteromalidae*). In the survey there were registered ten species of pupal parasitoids being seven more than recorded in a previous study made about 30 years ago. The pre-dominant species in the survey were *Spalangia cameroni* Perkins and *Muscidifurax raptor* Girault & Sanders, respectively. A total of 69,880 puparia were collected, of which 12.9% of the puparia per farm were killed by the parasitoids indicating a relatively low activity by the parasites on Danish farms.

Parallel with the survey, the seasonal activity of the parasitoids has been studied on five farms scattered all over northern Sealand. Every second week starting in April and ending in December, sentinel bags with laboratory-reared *M. domestica* puparia were laid out in stables and dung heaps as "bait" for the wasps. After one week of exposure the puparia were collected and incubated for two months in the laboratory for the parasitoids to emerge. The species of parasitoids and their activity presented as percentage-parasitised puparia were then determined. Consistent with the above survey the predominant species of parasitoids were *Spalangia cameroni* and *Muscidifurax raptor*. Parasitoids that appeared in low numbers were *Spalangia nigra*, *Spalangia nigripes*, *Nasonia vitripennis*, *Urolepis rufipes*, and *Phygaedeon fumator*. The first records of parasitoids took place in May with a gradual increase in numbers and parasitism up to August which coincided with high ambient temperatures and peak abundance of flies.

Based on the survey and seasonal study it can be concluded: (i) the guild of pupal parasitoids was relatively large attacking both stable and housefly puparia at confined livestock in Denmark; (ii) the two dominating species on most farms in the country were *S. cameroni* and *M. raptor*; (iii) the native parasitoids combined attacked only a small fraction of the fly puparia available, but on a few farms the parasitisation reached 69% of the total puparia collected.

The studies above were the first step in a five-year-project partially funded by the Ministry of Food, Agriculture and Fisheries, with the aim of studying prospects of using parasitic wasps as control organisms against stable and houseflies in connection with confined animals in Denmark.

H. S. Pedersen and J. B. Jespersen

6.3.2 *Entomophthora muscae*

This study was conducted as part of a Ph.D. project. Previous studies have shown that although very high infection levels of the entomopathogenic fungus *Entomophthora muscae* have been observed during the summer, the fungus seems to have no obvious effect on the high number of houseflies in stables this time of year. The scope of this study was thus to evaluate the effect of the type of stable and time of year on the prevalence of *E. muscae*.

A survey was conducted including 33 farms with cattle and/or pigs (16, 9 and 8 farms with cattle, pig and both, respectively). Ten farms were visited in August, 10 in September, and 13 farms in October. On pig farms, it was noted if the unit used for fly collection was a farrowing unit. The use of heating lamps providing resting surfaces with temperatures higher than 40°C in these units might give the flies the opportunity to make "behavioural fever" and thus cure themselves from the disease. Houseflies were collected in various locations inside the barns with a sweep net. On the day of collection the flies were anaesthetized with CO₂ and sorted in species and sex, 50 males and 50 females from each stable were placed individually in 22 ml plastic cups. The houseflies were examined daily for 10 days for patent *E. muscae* infection.

The prevalence increased significantly from August until October and a significantly lower proportion of houseflies was infected on the pig farms.

These results indicate that “behavioural fever” may be carried out by houseflies in stables with heating lamps.

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