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# Development of Resistance in Field Housefly (*Musca Domestica*): Comparison of Effects of Classic Spray Regimes versus Integrated Control Methods

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**Abstract**

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The development of resistance in the housefly (*Musca domestica*) to azamethiphos, pirimiphos-methyl, bendiocarb, permethrin, cypermethrin and deltamethrin was investigated on pig farms over a 4-year period. The results obtained in laboratory tests were compared with those obtained under practical conditions in pig houses. An intensive use of insecticides induced resistance in the course of 2 to 3 seasons. The classic spray regimes of insecticides led to the development of high resistance after one or two seasons. Integrated control, based on rotational application – organophosphate, pyrethroid, carbamate, pyrethroid, organophosphate – retained the resistance at low to moderate levels. Because of the variability of resistance factor (RF) in the field populations observed, the monofactorial and rotational selective pressure of insecticides was investigated. The rotational application of azamethiphos and permethrin or cypermethrin having sufficient insecticidal effect retained the resistance at low to moderate levels over a 4-year period. Although the alternation of insecticides cannot prevent the development of resistance, it can extend several times the period of their successful application on farms. This knowledge can help to formulate the strategies for fly control programmes.

*Muscidae, nuisance flies, resistance, organophosphates, carbamates, pyrethroids, control*

Because of increasing awareness of risks of insects, great attention of veterinarians is paid not only to well-known blowfly species causing myiases of birds and mammals, but also to other non-biting and blood-sucking flies that affect animal welfare and health in a variety of ways, including nuisance, fly worry and dissemination of diseases (Fischer 1999 and 2000). The house fly, *Musca domestica* (L.) can be managed to some extent by sanitation measures that reduce accumulation of waste materials that serve as breeding sites. For the most part, however, fly control is most commonly achieved with insecticides, but unfortunately, house flies have shown a remarkable ability to develop resistance to these. On the other hand, knowledge about the immunosuppressive effects of insecticides and their possible interference with the genetic material of live organisms indicates that it is necessary to restrict gradually the extensive use of a broad spectrum of pesticides through accentuated application of scientifically justified agrotechnical procedures (Kačmár et al. 1999).

The control of flies in animal houses where they find both attractive substrates for feeding and breeding and an all-year-round suitable environmental temperature important for their rapid reproduction is not a simple task and is becoming a worldwide problem. The main reason is that they have high reproductive potential and acquire resistance to insecticides rather rapidly. European countries, including Slovakia, are no exception to this rule. After many years of the use of pyrethroids, organophosphates and carbamates we are confronted

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with the serious problem of resistance to these compounds (Kočišová 1998). Flies are so flexible that it is completely absurd to consider their total eradication. We recognize several important factors that have undoubtedly an unfavorable effect on the successful control of flies. One of the most important is the limited range of really potent insecticides due to the present state of resistance. Therefore, it is necessary to apply as extensively as possible the rules of integrated control of flies which means the use of combinations of the greatest possible variety of products, application methods and the ways of fly control.

In our study we present results of the comparison of effects of monofactorial and rotational and combinational insecticide selection pressure on the development of resistance. Investigations of resistance stability in field populations can help to formulate strategies for fly control programmes in practice.

## Materials and Methods

### Populations of flies tested

Wild populations of flies were collected on 9 farms for rearing of pigs. Flies were kept and prepared for individual tests in an insectary at 24–27 °C under standard conditions and methods (Rupeš and Rettich 1998) complying with the Slovak legislation dealing with scientific experiments on live organisms (Bugarský et al. 1999). The tests for determination of resistance level were carried out on female flies, 4- to- 7-day-old, of $F_1$-$F_3$ laboratory generations. The results were compared with those obtained for adult flies of the susceptible strain SRS/WHO - Standard Reference Strain/World Health Organization, kept under identical laboratory conditions as the wild populations.

### Factor of resistance and evaluation of the resistance

The resistance factor (RF) was calculated from the ratio of mean values of $LC_{50}$ (lethal concentration for 50% of tested flies) of the wild population tested and the respective values for the sensitive strain SRS/WHO. The resistance was evaluated on the basis of RF for $LC_{50}$ in four categories: low RF < 10; moderate RF = 11 - 40; high RF = 41 – 160 and very high RF > 160. A method of tarsal contact was used to determine the values of $LC_{50}$ (Rupeš et al. 1975). The impregnation was carried out with insecticides the doses of which were expressed as concentration of the active ingredient in mg per 63.5 $cm^2$ area of filtration paper (Table 1). A minimum of 6 different concentrations of preparations were tested each time. Fifteen females of the tested population of flies were exposed to the impregnated paper for 24 h. During this time good access of flies to water was ensured, but the impregnated filter paper remained dry. The mortality of flies was determined after 24 h. Controls were carried out simultaneously by exposing flies to a filter paper impregnated with drinking water dry at the time of test. In control groups with mortality ranging from 5 to 20%, the correction of experimental values was carried out according to the equation by Abbott (1925). The final values of $LC_{50}$ were calculated using the probit method (Roth et al. 1962). The obtained results were processed with the statistic program Prism 3.0.

Table 1
List of preparations used in the experiment

| Preparation | Active ingredient (a. i.) | The concentration used ($mg\cdot 63.5\cdot cm^{-2}$) |
|---|---|---|
| ACTELLIC 25 EC | pirimiphos-methyl ($250 g.l^{-1}$) | 6.0-0.0117 |
| ALFACRON 50 WP | azamethiphos ($500 g\cdot kg^{-1}$) | 7.5-0.0073 |
| FICAM 80 W | bendiocarb ($800\ g\cdot kg^{-1}$) | 1.44-0.0014 |
| COOPEX 25 WP | permethrin ($250\ g\cdot kg^{-1}$) | 0.38-0.0004 |
| KORDON 10 WP | cypermethrin ($100\ g.kg^{-1}$) | 0.6-0.0006 |
| K-OTHRINE 25 FLOW | deltamethrin ($25\ g\cdot l^{-1}$) | 0.03-0.0001 |

### Practical application of insecticides - observation of selection pressure

Practical application of insecticides was carried out in three selected animal houses for mating and pregnant sows, housed in group-pens with bedding. The treated surfaces were approximately 1200 $m^2$. The emulsions or suspensions for spraying were prepared by diluting the preparations with water to concentrations recommended by the manufacturer (Actellic 25 EC - 4%; Alfacron 50 WP - 2.5 %; Ficam 80 W - 0.3 %; Coopex 25 WP - 0.25 %; Kordon 10 WP – 1 %; K-Othrine 25 Flow – 0.5 %). A pressure sprayer Maruyama MS 055 S with a storage tank of 23 l in volume, maximum pump output of 2.5 MPa, and a nozzle with two control settings producing 250 and 400 µm size particles was employed.

## Results and Discussion

The study presents results obtained by classic and rotational regimes under practical conditions. The products Alfacron 50 WP, Actellic 25 EC, Ficam 80 W, Coopex 25 WP, Kordon 10 WP and K-Othrine 25 Flow were applied separately on individual farms in the course of 4 years. Intensive repeated application of a product containing the same active ingredient suggests the danger of rapid development of resistance in practice already during one or two seasons (Table 2). The monofactorial selection pressure of pirimiphos-methyl led to the development of high resistance within 10 weeks in 1999. The application of azamethiphos resulted in a 7-fold increase in the resistance of houseflies when expressed by the resistance factor (RF) value from low (RF = 7.3) to high (RF = 49). Similar results were obtained after intensive applications of carbamate bendiocarb and pyrethroids cypermethrin and deltamethrin, a high resistance developing after two seasons.

Table 2
Development of resistance in housefly populations after classic spray regimes (4-6 times during the season) (Mean ± SEM)

| Insecticide | Resistance factor (RF) for $LC_{50}$ during 4 years | | | | |
|---|---|---|---|---|---|
| | Before application | 1998 | 1999 | 2000 | 2001 |
| Azamethiphos | 7.3 ± 1.15 | 12 ± 2.3 | 22.8 ± 2.7 | 33 ± 4.1 | 49 ± 2.1 |
| Pirimiphos-methyl | 2.1 ± 0.7 | 27.6 ± 4.2 | 79.4 ± 15.9 | - | - |
| Bendiocarb | 9.2 ± 1.28 | 21.2 ± 5.09 | 84.6 ± 2.1 | 131.8 ± 2.9 | - |
| Permethrin | 5.6 ± 0.5 | 10.2 ± 1.65 | 22.2 ± 2.13 | 35.4 ± 2.2 | 64 ± 6.3 |
| Cypermethrin | 9.4 ± 0.92 | 11.8 ± 1.88 | 38.8 ± 4.39 | 107 ± 2.9 | - |
| Deltamethrin | 11.6 ± 0.92 | 26 ± 4.18 | 51.4 ± 3.95 | - | - |

$LC_{50}$ – Lethal concentration

On the other hand, the interruption of monofactorial selective pressure by rotational regimes based on different active ingredients (organophosphate – pyrethroid – carbamate –pyrethroid – organophosphate) results in the deceleration of the development of resistance (Table 3). The rotational application of azamethiphos, permethrin and cypermethrin having sufficient insecticidal effect retained resistance at low to moderate levels over a 4-year period. Although alternation of insecticides cannot prevent the development of resistance, it can extend several times the period of their successful application on farms.

Table 3
Development of resistance in housefly populations after integrated control – rotational regimes (2-3 times during the season)

| Insecticide | Resistance factor (RF) for $LC_{50}$ during 4 years | | | | |
|---|---|---|---|---|---|
| | Before application | 1998 | 1999 | 2000 | 2001 |
| Azamethiphos | 7.3 ± 1.15 | 12 ± 2.3 | 22.8 ± 2.7 | 33 ± 4.1 | 49 ± 2.1 |
| Azamethiphos | 8.2 ± 2.65 | 7.6 ± 0.67 | 11.4 ± 1.3 | 7 ± 1.2 | 7.4 ± 1.28 |
| Permethrin | 6.4 ± 1.5 | 9.4 ± 1.8 | 13.8 ± 1.56 | 10.4 ± 1.1 | 14 ± 1.14 |
| Cypermethrin | 10.6 ± 1.72 | 13 ± 3.1 | 21 ± 1.09 | 18.4 ± 2.63 | 28.8 ± 0.86 |

Studies of insecticide resistance should eventually result in preparation strategies that can prevent or slow down its development. Insecticides still remain the primary means of the control of flies in animal production. It is paradoxical that the development and spreading of resistance accelerates while the development of new preparations decelerates and it becomes more and more difficult to discover new, more effective insecticides with different modes of action. The development of new insecticides must be based on essential criteria related to the preservation of high effectiveness on noxious insects, low acute and chronic toxicity (Legáth 2000; Pistl et al. 2001), harmlessness to non-target organisms (Dudriková et al. 2000), low persistence in the environment and prevention of the development of additional resistance. The use of insecticides with long residual action against flies in closed facilities is counter-productive because such insecticides strongly affect selection for resistance. One cannot rely on migration of sensitive individuals from the environment, i.e. on the so-called "natural dilution" of resistant populations in animal houses. The efforts aimed at deceleration of the development of resistance in closed animal houses are counteracted by the fact that the environment in them is suitable for selection. Flies can multiply inside them rapidly in great numbers (Kočišová 2001) and their killing should be more intensive than anywhere else. Frequent and thorough application of insecticides guarantees very strong selection pressure. The result of such interventions is, as presented in our study, that insect control measures may fail as soon as after one or two seasons. This is evidenced by the one-sided action of azamethiphos, bendiocarb and deltamethrin. The frequency of resistance in housefly populations results mostly, as it has been already mentioned, from the selection pressure by an insecticide. The starting point of verification of the effectiveness of the developed strategy are the results of resistance before and after realization of individual management principles. Integrated fly control regimes have to be viewed as a challenge requiring a continued high level of organization and cooperation between the agrochemical industry, entomologists and advisers, a sound biological foundation based on resistance monitoring, cross-resistance studies, and versatility of response based upon feedback on the current state of the programme.


## Porovnanie účinku klasických postrekov a integrovanej ochrany na vývoj rezistencie terénnych populácií muchy domácej (*Musca domestica*)

Počas štyroch rokov bol sledovaný vývoj rezistencie populácií muchy domácej (*Musca domestica*) na azamethiphos, pirimiphos-methyl, bendiocarb, permethrin, cypermethrin a deltamethrin počas pôsobenia jednostranného a rotačného selekčného tlaku. Z dôvodov variability faktora rezistencie (RF) u sledovaných terénnych populácií múch sme na farmách sledovali pôsobenie jednostranného a striedavého selekčného tlaku insekticídov. Pri jednostrannom pôsobení insekticídov sa mierna až vysoká rezistencia vyvinula po jednej resp. dvoch sezónach. Pri striedavej aplikácii insekticídov v rotačnom kolotoči – organofosfát, pyrethroid, karbamát, pyrethroid, organofosfát – sa rezistencia počas štyroch rokov udržala na nízkej až miernej úrovni. Striedavou aplikáciou insekticídov nezabránime vývoju rezistencie, ale môžeme niekoľkonásobne predĺžiť dobu ich úspešného použitia v praxi. Prezentované výsledky sú pomôckou pre vytváranie jednotlivých stratégií boja s muchami.



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