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The potential of some insect growth regulators in housefly (*Musca domestica*) control

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Diflubenzuron and cyromazine strongly affect the development of housefly larvae from the earliest stages. Eggs treated with diflubenzuron produced no adult flies while controls averaged 73.6 to 76.4%. Similarly 100% mortality was recorded in tests with 1st instar larvae in both housefly populations tested. Larvae treated in the 2nd and 3rd instar exhibited low hatchability that reached 0.6–2% in the susceptible strain SRS/WHO and 1.1–1% in the wild population of housefly REKe. Field experiments included the testing of preparations Dimilin 25 WP (a.i. diflubenzuron) and Neporex 2 SG (a.i. cyromazine) and their effect on the abundance of flies over 10 weeks. The first marked decrease (by 70–87%) in the number of flies in animal houses was observed 4–5 weeks after treatment with diflubenzuron. Cyromazine caused marked reduction (74–81%) in fly populations in weeks 5–6. Up to the 10th week of the experiment, flies were reduced by 71–82% with diflubenzuron and by 65–83% with cyromazin when compared with flies in the control houses. Methoprene affected most of the 3rd instar larvae ensuring 100% mortality in the susceptible strain SRS/WHO and 99.5% mortality in the wild population of housefly. The most pronounced effect of pyriproxyfen was recorded after its topical application to white pupae. Their mortality reached 87.2% on average in the susceptible strain SRS/WHO and 84.3% in the wild population of housefly REKe. In field conditions the most pronounced reduction in flies was observed in weeks 4 to 6. The number of flies was reduced by 45–61%. In the remaining weeks, only low reduction of flies (by 5–41%) was found after treatment by pyriproxyfen.

Key words: *Musca domestica*, growth regulator, insecticides, diflubenzuron, cyromazine, methoprene, pyriproxyfen, control.

Introduction

The environment in animal houses is an integrated dynamic system within which diverse physical, chemical, biological and social factors interact with each other. Animals are constantly exposed to a range of negative environmental factors, such as pesticides, heavy metals, exogenous sub-

stances, radiation, and others (FALIS et al., 2002; JACKOVÁ et al., 2002). A decisive role is played by those factors that directly affect the internal environment of animals. They include undoubtedly the biological quality of the environment expressed by the intensity of the occurrence of living organisms that adversely affect the health and welfare of the housed animals. An inevitable part of the monitoring and assessment of the biological quality of the environment in animal houses is the observation of the occurrence of noxious insects and the related entomological research. With regard to their life cycle, dipteran insects are involved in the dissemination of diseases including zoonoses. They transmit disease agents (bacteria, viruses, micromycetes, helminth eggs, protozoa) by contact with the surface of their bodies, and by regurgitation and the passage of excrements through the digestion tract. Infection occurs due to contamination of the skin, mucous membranes and wounds of hosts and also of food and the surrounding surfaces. It is therefore obvious that one of the basic goals of veterinary sanitation is the protection of farm animals against health-affecting and noxious insects. The intensification of agriculture disturbs natural biodiversity and many insect species, particularly dipteran insects, compel us to take sanitation measures to suppress and eliminate them. Many of these interventions take place in the presence of animals and therefore all insecticides, not only the newly developed ones, must fulfil the basic criteria related to high effectiveness, harmlessness to non-target animals, low persistence in the environment and prevention of resistance (JESPERSEN, 1997). From this point of view the biorational insecticides appear highly prospective. This refers to the substances that occur in nature, or are prepared artificially according to a natural model, and act at very low doses. In the majority of cases they are not poisonous neither to the noxious insects nor to non-target organisms, i.e. they do not have a direct toxic effect. Instead, they affect some vital functions, such as reproduction or behaviour of the pests and, in comparison with conventional pesticides, are safer to humans and animals (MARRIS et al., 1997). These insecticides, sometimes termed insecticides of the 3rd generation, find increasing practical applications, particularly the inhibitors of chitin production (diflubenzuron, cyromazine, and similar) and synthetic analogues of juvenile hormones (methoprene, fenoxycarb, pyriproxyfen, and others).

The insect growth regulators are advantageous because they do not persist long in the environment due to their rapid biodegradation and exhibit low toxicity. The development of resistance to these substances has not been proved as yet (FARKAS & PAP, 1991; KEIDING et al., 1991; SHEPPARD et al., 1992) and their effectiveness in practical applications has been considered sufficient (KOČIŠOVÁ et al., 2000). However, their production is costly and it takes some time until their final effect is observed. They do not exhibit the presumed narrow species specificity and can also eliminate natural enemies of pests.

The aim of the present study was to validate the effects of selected insect growth regulators under laboratory conditions and compare their effectiveness under practical conditions in the rearing of pigs and calves. The results can serve as a basis for the development of optimum and reliable technological procedures, which will improve their prospects and use in the control of flies within an integrated, fly protection programme.

Material and methods

The laboratory tests were carried out on larvae and pupae of the housefly (*Musca domestica* L., 1758) of the susceptible strain SRS/WHO (University Pavia, Italy) and a wild population of houseflies captured in animal houses and designated REKe. Flies were reared in the laboratory under standard conditions complying with the Slovak legislation dealing with scientific experiments on live organisms (BUGARSKÝ et al., 1999; KORIM et al., 2003).

The larval medium prepared from agar (20 g), dried milk (100 g), yeast (100 g) and water (1000 ml) was intended for keeping flies (KOČIŠOVÁ et al., 2000). A hundred millilitres of larval medium was dosed into plastic containers (100 ml/container) commonly used for this purpose. Test containers were polyethylene cups (dimensions $9 \times 13 \times 11$ cm) with the total treated surface 112.5 cm^2. Ten minutes before the initiation of tests larval media were treated with IGRs either as aqueous emulsion, suspension, or directly by granules. The application was made by pipette to the surface. The substances that were tested on white and dark pupae were diluted with acetone. Pupae were treated topically with a supernatant of the tested insecticides by a manual automatic applicator, applying 1 μl aliquots/pupa. The effectiveness of insecticide intervention depends on a number of factors, among them the concentration used, the dose applied, formulation of the preparations, properties of the treated surfaces, residual properties of the preparation and others (BEŇOVÁ et al., 2001), of which the formulation and the properties of the surface play a critical role with regard to insecticide activity. The surface of the larval medium was treated with the tested IGRs investigated at the selected basic dose recalculated per quantity of substrate in the plastic container. The doses of indi-

Table 1. Application and doses of insecticides used.

Growth regulator (a.i.)	The dose applied to the reproduction substrate	Concentration (in %) at topical application 1 μl/pupa
Diflubenzuron (Dimilin 25 WP)	5.6 mg/container (0.5 g m^{-2})	0.1 (0.25 μg a.i./1 μl)
Cyromazine (Neporex 2 SG)	5.6 mg/container (0.5 g m^{-2})	–
Pyriproxyfen (Sumilarv 0.5 G)	1.1 mg/container (0.1 g m^{-2})	0.1 (0.05 μg a.i./1 μl)
Methoprene (Altosid)	33.4 mg/container (3 g m^{-2})	1 (0.05 μg a.i./1 μl)

vidual growth regulators used in our experiments are summarized in Table 1.

The following developmental stages were placed on the surface of the larval medium: eggs laid within 12 h – 0.5 g quantity; 1st instar larvae (24 h); 2nd instar larvae (60 h); 3rd instar larvae (3–5 days old). One hundred larvae of each instar were used. Eggs or larvae were covered by a cotton wool dipped in water to ensure sufficient humidity. Wood shavings were placed on the top before pupation. The effectiveness of growth regulators was checked once every 48 h and the live adults were counted after 7–12 days dependent on the instar treated. Additional eggs and larvae were placed in control containers in parallel. The number of live adults in the control was considered 100% and was compared with the number of flies hatched in individual tests. Each test was repeated 5 times, always with different group of flies. The results are reported as means of live adults together with standard deviations. They were processed by means of the computer program Prism 3.0.

Field tests on flies were conducted in pig and calf houses under almost identical conditions at small independent farms, in an environment abundant in flies before intervention. Practical application of insecticides was carried out in two selected animal houses (treatment by Dimilin 25 WP and Neporex 2 SG) for mating and pregnant sows, housed in group pens with bedding. The treated surfaces were approximately 1200 m^2. The experiment with Sumilarv was carried out in the house for calves during the period of milk nutrition. This was a one-floor building of dimensions 10 x 50 m with calves housed in group pens on a concrete floor using straw bedding. The treated surface area was 780 m^2. Individual tests were carried out in summer (June – August). The mean density of flies in the houses was determined one week before intervention using sticky traps according to the standard method of state sanitation institutes (Venglovský, 1992). The infestation with flies was determined by means of flypapers hanging along the diagonal axis of the house at a 5 m distance and 1 m above the housed animals. It was determined as a statistical mean of flies caught during 24 h on one flypaper. Control houses were at a 12–15 km distance from the experimental houses. Insecticides were applied to the litter manually by watering cans, using the doses shown in Table 1. After intervention, the flies were counted at weekly intervals in approximately the same locations of the individual houses. The effectiveness of insecticides was evaluated as the reduction in adult flies populations in experimental houses compared to the control (during the experiment no insecticides were used in the control houses), expressed as a percentage.

Results

The effect of diflubenzuron (Dimilin 25 WP) and cyromazine (Neporex 2 SG) on the developmental cycle of flies under laboratory conditions (Tabs 2, 3) was very similar when added to the reproduction substrate. Both substances strongly affected the development of fly larvae from the earliest stages in both the susceptible strain SRS/WHO and wild population REKe originating from pig rearing. After placing eggs on the substrate treated with diflubenzuron no adult flies hatched from them while the mean hatchability in the controls ranged from 73.6 to 76.4% ($\pm 2.9 - 5.1$). The moulting process stopped, the larvae usually lost fluid, gradually blackened and finally died. The surviving larvae were abnormal, with decreased locomotion, atypical shapes and thickened central parts, various distortions, elongated puparia and larvae died or developed abnormal pupae. Similarly 100% mortality was recorded in the tests with 1st instar larvae in both fly populations tested. The treated larvae of the 2nd and 3rd instar exhibited low hatchability that reached 0.6–2% in the strain SRS/WHO and 1.1–1% in the population REKe. Topical treatment of white and dark pupae with diflubenzuron resulted in high hatchability of flies, exceeding 80% with white pupae and 90% with dark pupae of both populations. Unfortunately, topical application of cyromazine to pupae was not carried out due to failure of the automatic dosing device.

Table 2. Mean numbers of hatched adults of *Musca domestica* after treatment of substrate with diflubenzuron at a dose of 0.5 g m^{-2} and its topical application to pupae.

Treatment	SRS/WHO strain				"REKe" wild population			
	Experiment		Control		Experiment		Control	
	Mean	SD	Mean	SD	Mean	SD	Mean	SD
Eggs	0	–	76.4	±5.1	0	–	73.6	±2.9
1st instar larvae	0	–	82.8	±3.4	0	–	75.4	±3.2
2nd instar larvae	0.6	±0.9	83.0	±4.4	1.4	±1.1	79.0	±7.7
3rd instar larvae (5 days old)	2.0	±2.8	83.8	±7.3	2.4	±3.1	78.8	±7.6
White pupae	85.2	±3.2	93.6	±2.9	81.4	±6.3	93.0	±2.3
Dark pupae	91.2	±4.4	93.2	±6.0	93.2	±6.0	88.0	±3.2

Table 3. Mean numbers of hatched adults of *Musca domestica* after treatment of substrate with cyromazin at a dose of 0.5 g m^{-2}.

Treatment	SRS/WHO strain				"REKe" wild population			
	Experiment		Control		Experiment		Control	
	Mean	SD	Mean	SD	Mean	SD	Mean	SD
Eggs	0.4	±0.5	75.0	±4.3	1.0	±1.0	74.2	±4.6
1st instar larvae	0.6	±1.3	79.6	±2.9	0.4	±0.5	83.0	±4.3
2nd instar larvae	1.0	±1.0	82.4	±4.0	1.4	±1.1	84.6	±2.4
3rd instar larvae (5 days old)	1.6	±2.1	82.4	±5.9	2.8	±1.9	76.8	±7.9

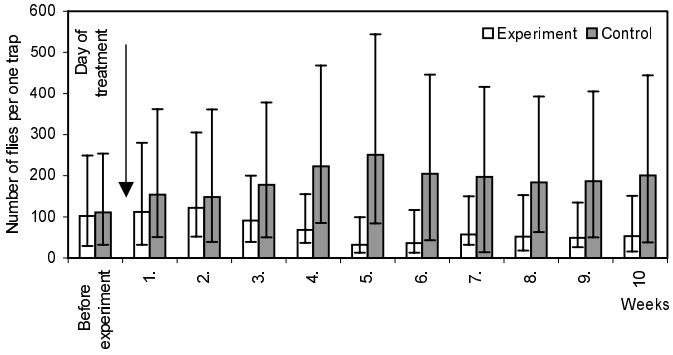

Fig. 1. Mean, maximal and minimal number of adult *Musca domestica* in pig delivery house after treatment by Dimilin 25 WP.

Field experiments included the testing of Dimilin 25 WP (a.i. diflubenzuron) and Neporex 2 SG (a.i. cyromazine) formulations and their effect on the abundance of flies over 10 weeks. Individual formulations were applied twice to the locations where the flies reproduced, i.e. to the litter, during the first and second week of the experiment, observing the procedures recommended by manufacturers of these preparations. The first marked decrease (70–87%) in the number of flies in animal houses was observed 4–5 weeks after the treatment with diflubenzuron (Fig. 1). Cyromazin (Fig. 2) caused a marked reduction (74–81%) in fly populations in weeks 5–6. Up to the 10th week of the experiment, the flies were reduced by 71–82% with diflubenzuron and by 65–83% with cyromazin when compared with flies in the control houses.

Observation of the effect of methoprene (Tab. 4) on the development of flies showed that this compound affected most of the 3rd instar larvae ensuring 100% mortality in the susceptible strain SRS/WHO and 99.5% mortality in the

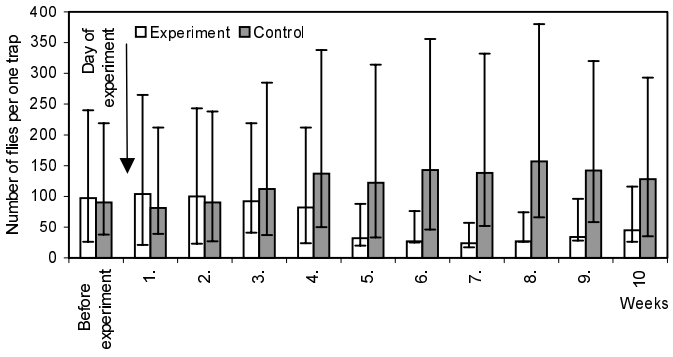

Fig. 2. Mean, maximal and minimal number of adult *Musca domestica* in pig delivery house after treatment by Neporex 2 SG.

Table 4. Mean numbers of hatched adults of *Musca domestica* after treatment of with methoprene at a dose of 3 g m^{-2} and its topical application to pupae.

Treatment	SRS/WHO strain				"REKe" wild population			
	Experiment		Control		Experiment		Control	
	Mean	SD	Mean	SD	Mean	SD	Mean	SD
Eggs	32.6	±3.8	76.2	±4.2	35.0	±4.8	75.2	±3.8
1st instar larvae	23.6	±4.3	82.2	±6.8	22.0	±3.2	81.8	±2.8
2nd instar larvae	20.4	±8.7	83.6	±3.8	21.6	±8.6	84.2	±4.4
3rd instar larvae (5 days old)	0	–	85.4	±1.5	0.4	±0.5	79.8	±4.7
White pupae	15.6	±5.0	92.4	±2.9	29.4	±1.7	92.8	±2.7
Dark pupae	25.0	±6.3	91.8	±2.2	24.8	±6.6	90.8	±4.5

Table 5. Mean numbers of hatched adults of *Musca domestica* after treatment of substrate with pyriproxyfen at a dose of 0.1 g m^{-2} and its topical application to pupae.

Treatment	SRS/WHO strain				"REKe" wild population			
	Experiment		Control		Experiment		Control	
	Mean	SD	Mean	SD	Mean	SD	Mean	SD
Eggs	47.2	±3.7	70.2	±4.9	52.0	±5.5	75.4	±4.6
1st instar larvae	54.0	±4.6	78.0	±4.4	51.6	±2.4	81.2	±6.4
2nd instar larvae	57.8	±3.7	80.8	±3.6	64.2	±4.7	80.0	±3.1
3rd instar larvae (5 days old)	65.2	±5.2	90.0	±2.2	66.6	±2.7	82.2	±2.9
White pupae	11.6	±3.4	90.4	±3.1	13.6	±3.6	86.6	±5.5
Dark pupae	28.8	±5.6	92.2	±3.3	23.2	±4.1	92.0	±3.2

wild population REKe. When applied to lower larval stages, it ensured 71.3–75.6% mortality in the susceptible strain SRS/WHO and 71–74.3% mortality in the wild population REKe. High mortality was also observed in treated pupae, reaching 72.8–8.1% in the susceptible strain SRS/WHO and 68.3–73.7% in wild population REKe.

The most pronounced effect of pyriproxyfen was recorded after its topical application to white pupae (Tab. 5). Their mortality reached 87.2% on average in the susceptible strain SRS/WHO and 84.3% in the wild population REKe. Relatively high mortality (68.8–74.8%) was observed after its application to dark pupae. The treatment of eggs and larvae resulted in mortality ranging from 27.6 to 32.8% in the susceptible strain SRS/WHO and from 18.9 to 36.5% in the wild population REKe. Formulation Sumilarv (a.i. pyriproxyfen)

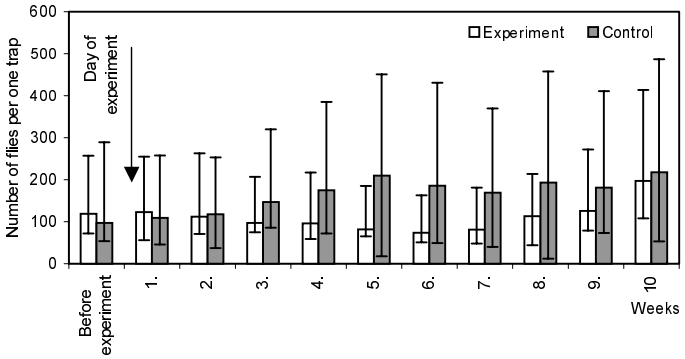

Fig. 3. Mean, maximal and minimal number of adult *Musca domestica* in calf house after treatment by Sumilarv.

in a granulated form was used under field conditions treating litter in a calf house. The most pronounced reduction in fly populations was observed in weeks 4 to 6 (Fig. 3). The number of flies was reduced by 45–61%. In the remaining weeks, only a low reduction of flies (by 5–41%) was observed.

Discussion

Resistance of insects and particularly of flies is one of the factors that limit the use of insecticides in practice and motivate the search for suitable preparations, technologies and working procedures to be used in animal production. This is a worldwide challenge for both the field of development and production of insecticides and practical control. The integrated control of flies focuses on the primary task, the environmental protection. This involves combinations of suitable technological procedures and preparations that may decrease the populations of flies in a way that is both economical and environmentally friendly. The integrated control of flies relies on the reduced use of insecticides. Individual strategies or principles of fly control should focus on lengthening the shelf life of the insecticide and decreasing its negative impact on the environment (Cilek & Knapp, 1991). The development of resistance to these substances has not been detected yet. A risk of its development, pointed by Keiding et al. (1992) and Sauphanor et al. (2000) exist mainly as a result of intensive and long-term selection pressure supported by both the multiple treatment of locations and feeding of diflubenzurone and cyromazin to animals (Alam & Motoyama, 2000). Insecticides are still the primary means of fly control in animal production. It is a paradox that the development and spreading of resistance accelerates while the development of new preparations decelerates. It becomes invariably more difficult to discover new substances that can act more intensively than the existing ones, or substances with different mode of action. Many chemicals highly effective against some pests have not been introduced into practice because their production is costly and the costs incurred cannot be recouped. The range of chemicals potentially usable in fighting the resistance is therefore rather narrow.

With regard to marked differences in the development of insects and vertebrates, the growth and development regulators (IGRs) fulfil, to a considerable degree, the requirements of high selectivity and low toxicity (Graf, 1993; Ginarte & Dorta, 1996). Diflubenzuron and cyromazine induce changes associated with larvae shedding, from the earliest developmental stages (Schmidtmann et al., 1989; Miller et al., 1991). Larvae cannot shed their old skin and move to the following developmental stage. Those of them that survive have anabnormal shape and some produce deformed pupae. Diflubenzuron administered to adult flies in feed had no effect on egg laying but caused irreversible changes in hatchability (Kočišová, et al., 2000). Under practical conditions, the first marked reduction in the number of flies was recorded 4–5 weeks following the first application, which limits partially their single use. However, according to our previous observations (Kočišová et al., 1999), these insecticides are highly prospective in the integrated control of flies, particularly in combination with aerosols or insecticide traps.

Investigations into the effect of methoprene showed that the 3rd instar larvae and partially also white and dark pupae are most sensitive to this substance. Similar high effectiveness to white and dark pupae was observed with pyriproxyfen. From a practical point of view, only a small reduction in flies was achieved which probably resulted from its specific effectiveness. The promising laboratory results obtained in our study allow us to assume that these substances can be used in practice for

the control of flies as they are suitable from the toxicological point of view and place little load on the environment (Bull & Meola, 1993; Zhang & Shono, 1997). Because of this, further research into their use and of production of the respective insecticides is desirable.

The regulators of growth and development of insects can be used successfully in the control of flies provided that we can affect the population of the target species in the susceptible stage. Another precondition of sufficiently high effectiveness is the synchronous occurrence of the susceptible insect stage in a time-acceptable interval (from the point of view of the persistence of the active ingredient in the environment). On this assumption the biorational insecticides can successfully reduce the pest population while other components of insect entomocoenosis that are not in the susceptible stage at the time of intervention remain unaffected. However, the insect regulators usable in practice are not so far as selective on the level of organisms as many authors have assumed (Sláma, 1999).

In the future we can expect additional development and more advanced final adjustment of the insecticides and application techniques and obtaining new knowledge about physical-chemical properties that determine their destiny in the environment and in biological systems. It should be stressed that there are no safe insecticides, there are only safe methods of their use.

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