The OCCURRENCE, DEVELOPMENT, STABILITY, and REGRESSION of HOUSE FLY (DIPTERA: MUSCIDAE) RESISTANCE to INSECTICIDES in SLOVAKIA

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Abstract The resistance of the house fly to insecticides is a limiting factor for its control even in Slovakia, where more than 70% of flies are highly resistant to synthetic pyrethroids (permethrin, cypermethrin, deltamethrin, and lambdacyhalothrin), 66% of fly populations are highly resistant to bendiocarb, 40% to azamethiphos, 32% to dimethoate, and 25% to chlorpyriphos. On the other hand, no resistance to diflubenzuron and cyromazin has been detected. The stability of resistance to azamethiphos, bendiocarb ,and deltamethrin was investigated during 4 years (60 laboratory generations) under laboratory conditions in flies not exposed to the selection pressure by insecticides. The highest stability of resistance was observed for bendiocarb, which decreased considerably starting with the 35th laboratory generation. The decrease in resistance to deltamethrin occurred in four stages, characterized by alternative decrease and increase in resistance factor values. In the case of azamethiphos, a marked decrease in resistance was recorded starting from the fourth laboratory generation. 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 in dependence on the number of residual spray applications during the season, i.e., from May to September. After cessation of selection pressure, resistance to bendiocarb during 7 years and deltamethrin during 4 years remained at a high level. However, the decrease of resistance to moderate or low level did not solve the problem because a repeated use of insecticides resulted in development of high resistance in about one season.

Key Words *Musca domestica* resistance pyrethroid organophosphate carbamate

INTRODUCTION

Very early after the introduction of modern insecticides (chlorinated hydrocarbons, organophosphates, carbamates, and pyrethroids) into a wide practice, their unwanted effects began to appear in house fly. One of them is the development of resistance, which became a serious and complex problem hindering or even precluding the effective use of these compounds. The resistance is a worldwide problem affecting both the development and production of insecticides and the practical protection of farm animals against noxious insects and houseflies in particular. Resistance is a multifarious and difficult to define phenomenon. From the practical point of view, the most acceptable definition of resistance is that presented by WHO (1970) according to which resistance is the ability of arthropods to withstand the doses of insecticides that would be lethal to the majority of individuals in a normal population of the same species. In practice, it is manifested as a decreased efficacy of the treatments. Wiesmann (1947) recorded the first case of resistance of flies after three seasons of intensive use of DDT in Sweden. In subsequent years, resistance to organophosphates malathion, diazinon, and parathion developed in Denmark (Keiding, 1956). The first occurrence of flies resistant to DDT in Slovakia was recorded by Vostal (1960), who described four years later (1964) also resistance to malathion. Additional cases of resistance of flies to insecticides in the former Czechoslovakia were described relatively late, owing particularly to Greco (1975) and Rupeš (1983). We performed an intensive monitoring of the resistance in the field populations of house fly in the last 10 years.

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The present study gives the results of 10-year monitoring of resistance of the house fly (*Musca domestica)* and of the action of one-sided insecticide selection pressure on the development of resistance, stability of resistance after cessation of the pressure and after repeated use of the respective insecticide, and on regression of resistance. The investigations of resistance stability in field populations may give an answer to the question if an insecticide to which resistance has developed can be used effectively again. This knowledge can help to formulate the strategies for fly control programmes.

MATERIALS and METHODS

Populations of Flies Tested

The wild populations of flies were collected on 47 farms for rearing of pigs, calves, and sheep in 1991-2001. Flies were captured two-times during the season, before the fly control in months May and June, and at the end of the season in September. They 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 days old, of F_1-F_3 laboratory generations. The tests with chitin production inhibitors (diflubenzuron, cyromazin) were conducted on fly larvae of F_1-F_3 generations. The larvae were reared on a medium prepared from 20g agar, 100 g dried milk, 100 g dried yeast, and 1 l water. The results obtained were compared with those obtained for adult flies and larvae 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 Resistance

The resistance factor (RF) of the tested insecticide shows how many times resistance of the wild population exceeds that of the sensitive strain of the same insect species. It was calculated from the ratio of mean values of LC_{50} and LC_{90} 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} and LC_{90} (Rupeš et al., 1975). Discs of filter paper (Filtrak 388), 9 cm in diameter (63.5 cm^2), were impregnated drop-by-drop either with 0.6 ml (approximately 60 drops) water emulsion or with a suspension of insecticide preparation. They were dried for 24 h in a way which prevented the loss of insecticide. Individual concentrations were selected so that they caused mortality ranging from 0 to 100%. The impregnation and practical application 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 or, in case of larvicides, per 50 g of the multiplication medium (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 hours. 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 hours. Controls were carried out simultaneously by exposing flies to a filter paper impregnated with drinking water. The larvicides were tested using larvae of the 3rd developmental stage (100 larvae per one concentration tested) and the mortality was determined during 6 subsequent days in 24 h intervals. The control was obtained using larval medium supplemented with drinking water in the amount corresponding to the dose of the respective larvicide. 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 were calculated using the probit method (Roth et al., 1962).

Practical Application of Insecticides, Observation of Selection Pressure

Practical application of insecticides was carried out in three selected animal houses. Farm NL I. - house for mating and gravid sows, housing in group pens without bedding. The treated surface area (Alfacron 50 WP) was 420 m^2. Farm NL II. - delivery house, housing in pens with bedding. The treated surface area (Ficam 80 W) was 780 m^2. Farm Sel. - house for weaned piglets, two-deck cages. The treated surface area (K-Othrine 2.5 Flow) was 1200 m^2. The emulsions or suspensions for spraying were prepared by diluting the preparations with water to concentrations recommended by the manufacturer (Table 1). A pressure sprayer Maruyama MS 055 S with a storage tank of volume 23 l, maximum pump output of 2.5 Mpa, and a nozzle with two control settings producing particles of sizes 250 and 400 µm was employed.

RESULTS

The monitoring of resistance of flies to individual insecticides was carried out in correspondence with their use in practice. The results showing the ranges and mean values of resistance factors for LC_{50} are presented in Table 2. In the period from 1991 to 2001, we tested more than 100 various populations of flies. They exhibited the lowest resistance (87%) to pirimiphos-methyl and to diflubenzuron and cyromazin, for which the factors of resistance were low (2-5 and 4-7, respectively). However, we must admit that the monitoring of resistance to chitin inhibitors was initiated only in 2000, and only 8 fly populations have been tested up to this date. The evaluation of our results showed high resistance of flies in Slovakia. We observed that 40% of fly populations were highly resistant to azamethiphos, 32% to dimethoate, 25% to chlorpyriphos, 66% to bendiocarb, 38% to permethrin, 25% to cypermethrin, 69% to deltamethrin, and 75% to lambdacyhalothrin. Our additional experiments focused on the development, stability, and regression of resistance.

Azamethiphos

Resistance to this active ingredient persisted under laboratory conditions only up to 3 generations. In the period from generation 4 to 15 a marked decrease in the values of resistance factors was observed (Figure 1). In the subsequent period, between generations 20 and 60, the RF stabilized between 1.0 and 1.7. Under practical conditions (Figure 2), the flies with moderate resistance (RF=13) were subjected to an intensive selection pressure and developed high resistance within 4 years. In this stage, the selection pressure ceased and resistance of flies varied

Table 1. Formulations and insecticides and their concentrations used in the experiments

Insecticide	Active ingredient (a.i.)	Concentrations of a.i. in mg.63.5 cm^{-2} of filter paper or in mg.50 g^{1} of larval medium	Doses used in the experimental treatments in g of a.i. m^{-2}
Alfacron 50 WP	Azamethiphos	7.5-0.0073	2.5% / 50 ml.m^2
Bi-58 (% of a.i).	Dimethoate	6.7-0.0065	—
Actellic 25 EC	Pirimiphos-methyl	6.0-0.0117	—
Technical diazinon	Diazinon	0.3-0.001	—
Technical chlorpyriphos	Chlorpyriphos	0.08-0.007	—
Ficam 80 W	Bendiocarb	1.44-0.0014	0.3% / 40ml.m^2
Kordon 10 WP	Cypermethrin	0.6-0.0006	—
K-Othrine 2.5 Flow	Deltamethrin	0.03-0.0001	1% / 50ml.m^2
Coopex 25 WP	Permethrin	0.38-0.0004	—
Karate 5 EC	Lambdacyhalothrin	0.3-0,00183	—
Dimilin 25 WP	Diflubenzuron	1.0-0.00315	—
Neporex 2 SG	Cyromazin	1.25-0.391	—

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Table 2. Resistance of the housefly (*Musca domestica*) in Slovakia during 1991-2001, expressed as range (R) and mean value (N+M) of resistance factor (RF)

Insecticide	LC	LC SRS/WHO ($\mu g.cm^2$)	Factor of resistance (RF) for LC_{50} and LC_{90} 1991 R	1991 M	1992 R	1992 M	1993 R	1993 M	1994 R	1994 M	1995 R	1995 M	1996 R	1996 M	1997 R	1997 M	1998 R	1998 M	1999 R	1999 M	2000 R	2000 M	2001 R	2001 M
Azamethiphos	LC_{50}	0,604	1-13	5	1-18	7	1-32	10	1-35	13	5-76	18	2-106	37	14-230	78	28-390	89	24-290	70	28-280	57	18-140	41
	LC_{90}	2,445	1-20	8	2-23	9	1-166	24	4-139	26	3-847	84	24-790	102	19-813	93	63-1520	316	32-938	938	28-495	63	22-218	52
Dimethoate	LC_{50}	0,089	4-56	21	3-48	17	9-81	27	4-71	23	1-12	5	12-156	19	1-82	19	-	-	-	-	-	-	-	-
	LC_{90}	0,208	6-204	59	5-87	31	18-196	75	13-283	70	2-14	6	16-168	21	2-525	149								
Pirimiphos-methyl	LC_{50}	0,508	1-10	6	2-13	9	1-14	4	1-12	3	1-6	5	9-42	25	1-14	4	-	-	-	-	-	-	-	-
	LC_{90}	3,425	1-20	8	6-17	11	1-15	4	1-58	7	1-8	3	10-79	30	2-16	5								
Diazinon	LC_{50}	0,026	1-22	11	4-18	9	2-40	21	4-27	13	-	-	-	-	-	-	-	-	-	-	-	-	-	-
	LC_{90}	0,243	4-53	27	6-38	18	2-45	24	4-28	14														
Chlorpyriphos	LC_{50}	0,007	2-24	13	3-21	8	22-90	47	14-1041	137	-	-	-	-	-	-	-	-	-	-	-	-	-	-
	LC_{90}	0,016	6-48	20	4-35	16	77-515	164	40-1659	317														
Bendiocarb	LC_{50}	0,103	-	-	-	-	5-453	228	6-43	24	5-62	24	28-277	111	12-216	63	32-210	78	-	-	19-135	39	-	-
	LC_{90}	1,848					16-971	280	2-127	226	4-75	28	30-1000	430	12-1000	155	45-380	86			36-203	51		
Permethrin	LC_{50}	0,104	1-12	6	9-563	239	4-33	16	1-9	3	3-60	19	8-189	37	1-20	9	2-17	9	1-36	14	1-15	6	1-17	9
	LC_{90}	0,259	1-17	9	30-821	257	4-78	29	1-52	8	2-210	43	90-264	53	3-82	18	7-31	12	4-63	28	2-28	13	3-41	21
Cypermethrin	LC_{50}	0,011	-	-	-	-	5-139	35	79-925	483	13-134	52	2-52	17	3-28	8	2-41	23	3-30	18	1-22	11	1-32	9
	LC_{90}	0,035					1-1422	48	155-2580	1057	18-159	87	8-80	32	3-162	33	5-63	28	10-49	20	8-53	23	4-41	17
Deltamethrin	LC_{50}	0,006	-	-	25-1050	290	2-572	101	9-78	35	11-154	63	17-168	79	1-35	13	1-68	37	7-35	19	3-58	32	2-18	8
	LC_{90}	0,017			50-1157	330	3-627	174	9-170	84	54-1819	254	15-1080	145	3-63	20	9-103	46	10-63	29	7-101	48	7-51	12
Lambdacyhalothrin	LC_{50}	0,005	-	-	177-931	444	14-180	82	79-213	134	3-77	26	-	-	-	-	-	-	1-20	14	-	-	-	-
	LC_{90}	0,011			190-944	504	67-1548	372	120-561	287	5-401	74							3-38	19				
Diflubenzuron	LC_{50}	0,008	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	2-4	2	1-6	3
	LC_{90}	0,019																			3-8	4	2-8	5
Cyromazin	LC_{50}	0,011	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	1-9	6	1-8	4
	LC_{90}	0,098																			2-10	7	1-9	5

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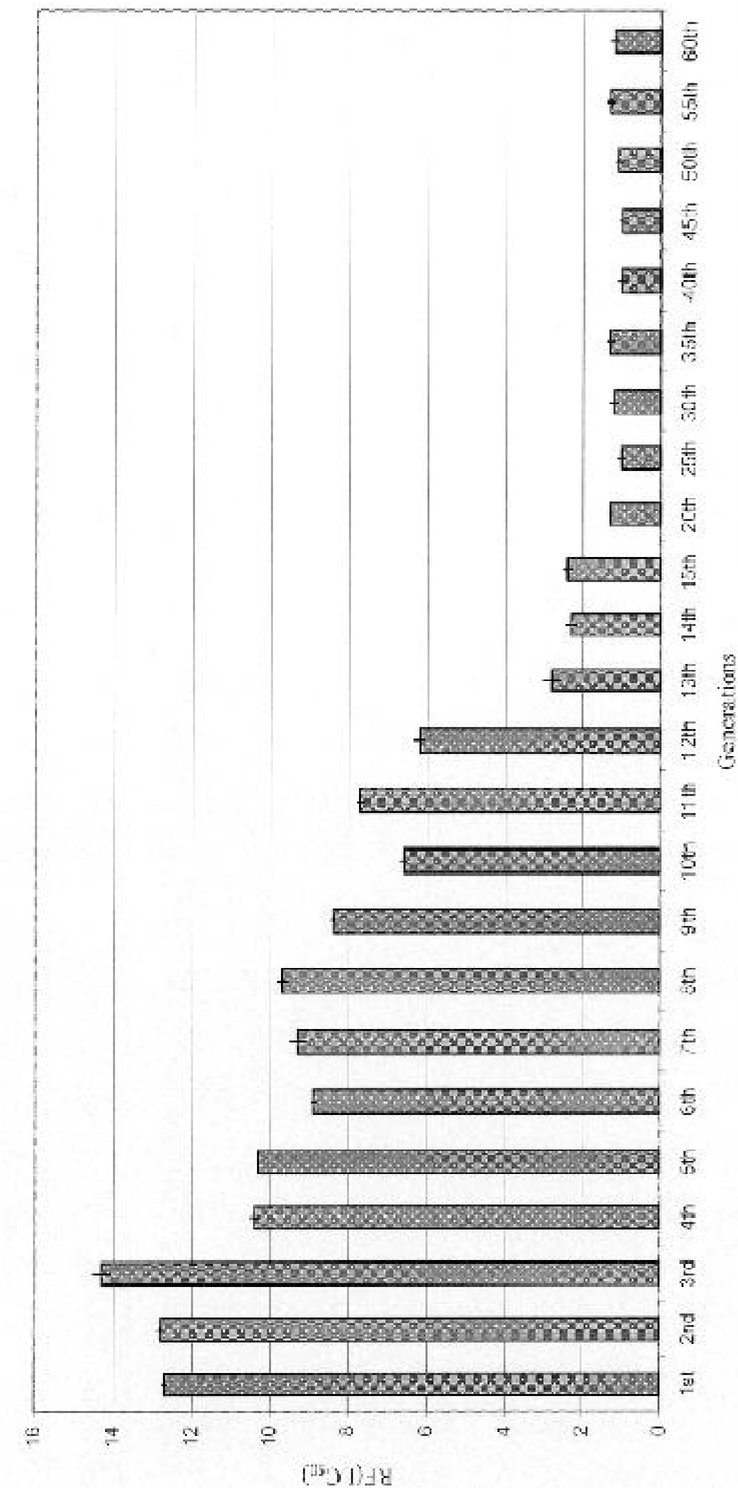

Figure 1. Stability of resistance to azamethiphos expressed as a decrease of RF (LC_{50}) during 60 generations after cessation of selection pressure under laboratory conditions.

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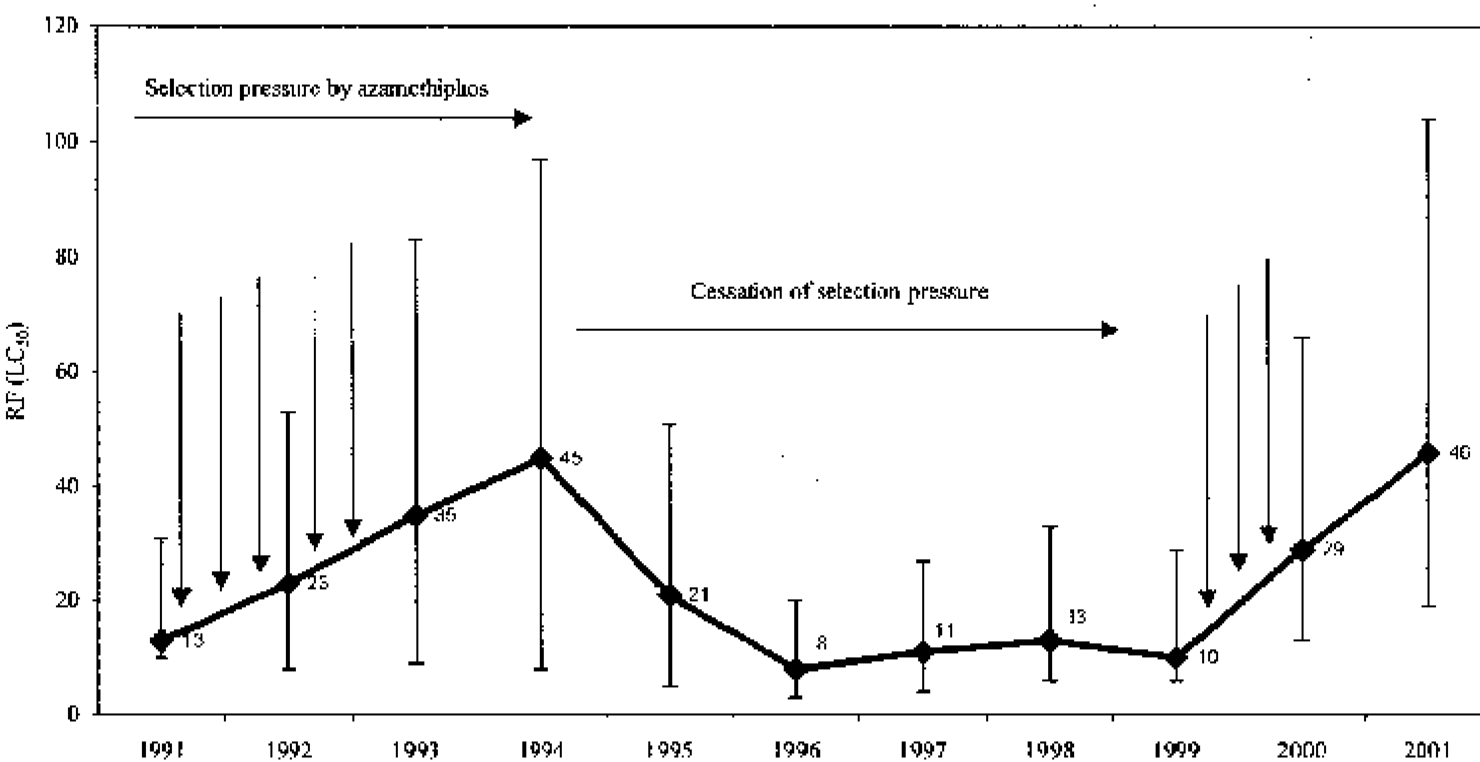

Figure 2. The development, stability, and regression of resistance to azamethiphos under practical conditions in pig rearing — farm NL I.

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between low and moderate in the subsequent years (1995-1999). The repeated use of azamethiphos in the form of residual spray for fly control caused a 3-fold increase in resistance within one season.

Bendiocarb

Resistance of the tested population to bendiocarb showed its high stability up to the 35th generation (Figure 3). Between generations 35 and 60, the resistance factors decreased by more than 21-fold compared to the starting values. Our tests performed under practical conditions (Figure 4) revealed high to very high levels of resistance after exposure to selection pressure for 2-3 years. Ficam 80 W has not been used in the respective animal house since 1994; however, the resistance factor values remained high for additional 7 years and decreased markedly only in 2001.

Deltamethrin

The laboratory investigations of resistance detected a decrease, which occurred in four stages (Figure 5). The first stage (F_1-F_6) peaked in the 3rd generation reaching RF=108. The second stage (F_7-F_{13}) culminated in the 9th generation (RF=118) and then a moderate decrease was observed. The third stage started with the 14th generation and showed a repeated increase in resistance, which culminated in the 20th generation (RF=93) and then decreased gradually up to the 40th generation. The last, fourth stage (F_{45}-F_{60}), could be characterized as a stage of gradual decrease in RF from 28 to 9. Resistance of flies to deltamethrin in the house for piglets increased to a high level within 2 seasons (Figure 6). Even after cessation of selection pressure resistance values continued to increase in the course of the subsequent two years. Resistance culminated in 1996 and then started to decrease; however, after repeated use of deltamethrin (three years later in 1999), it showed a 5-fold increase within one season.

DISCUSSION

Studies of insecticide resistance should eventually result in working out the strategies that can prevent or slow down its development. Insecticides still remain the primary means of 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. The use of insecticides with long residual action against flies in closed facilities is counter-productive because such insecticides affect strongly selection of resistance. One cannot rely on migration of sensitive individuals from the environment, i.e., on the so-called “natural dilution” of resistant population in animal houses. The efforts aimed at decelerating 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 (Kocišová, 2001) and their killing there should be more intensive than anywhere else.

Azamethiphos is considered an insecticide highly effective against adult flies. It has been used in Europe in the form of residual sprays or coats since the early 1980s. Our investigations showed that resistance to this substance ranged from low to very high. The wide range of RF values suggest considerable differences in the effect of azamethiphos in individual populations, which is closely related to different frequency of its use. When determining the degree of resistance, one should consider not only the season and climatic conditions at the time of fly catching but also whether or not the sanitation has already been performed.

Preparations based on dimethoate were applied frequently in the 1970s to control flies in animal production, but later, due to development of resistance, their application became limited. At the present, only two preparations based on dimethoate (Bi-58 EC new and Dandim 40 EC) are

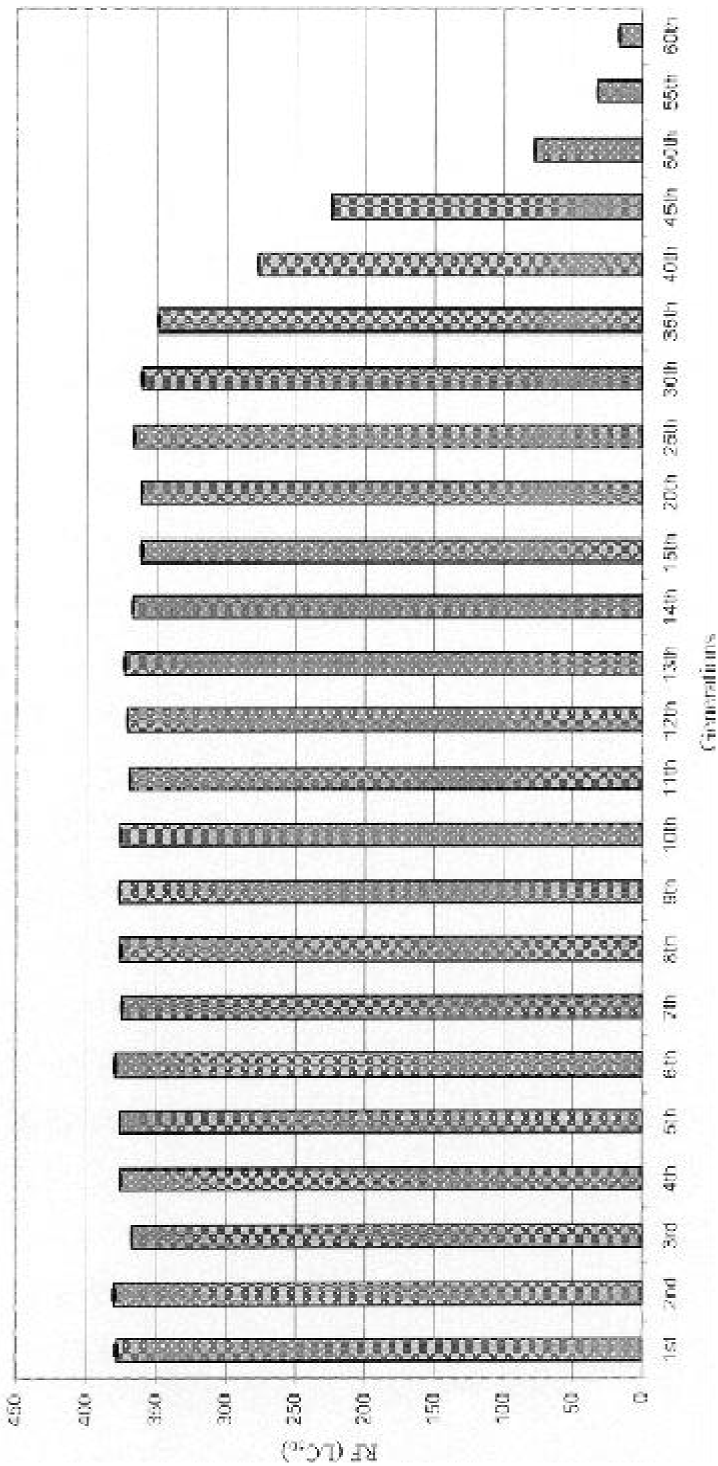

Figure 3. Stability of resistance to bendiocarb expressed as a decrease of RF (LC_{50}) during 60 generations after cessation of selection pressure under laboratory conditions.

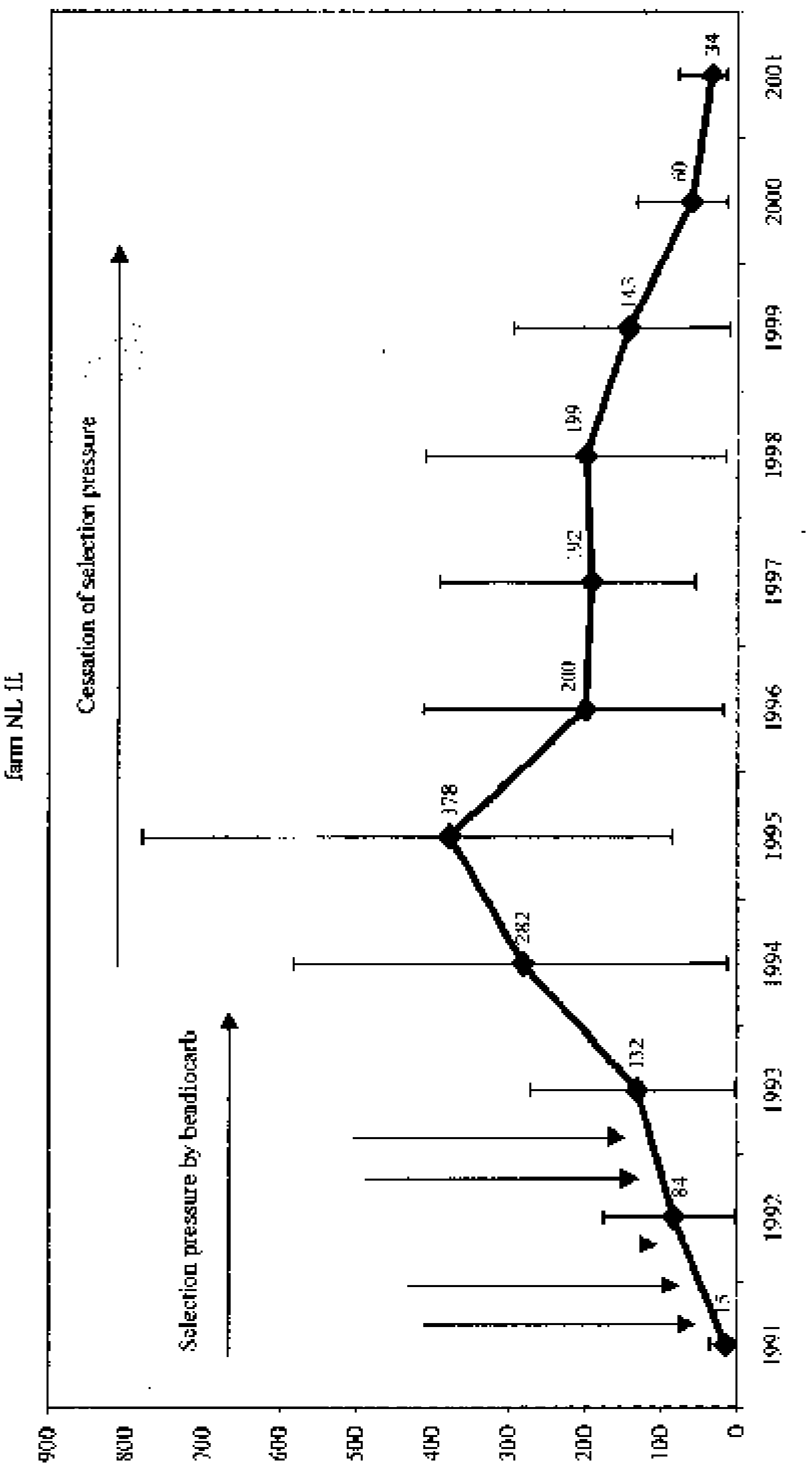

Figure 4. The development, stability, and regression of resistance to bendiocarb under practical conditions in pig rearing — farm NL II.

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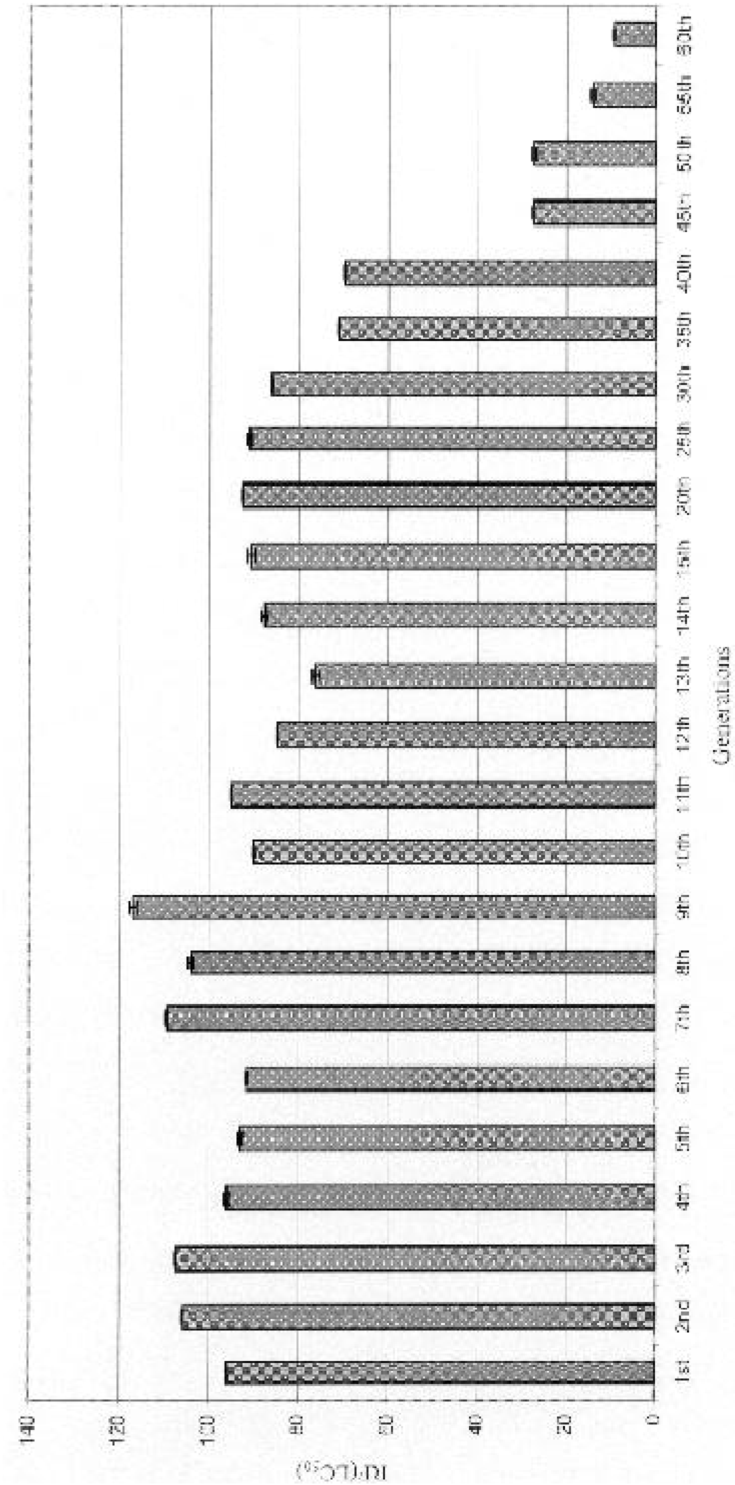

Figure 5. Stability of resistance to deltamethrin expressed as a decrease of RF (LC_{50}) during 60 generations after cessation of selection pressure under laboratory conditions.

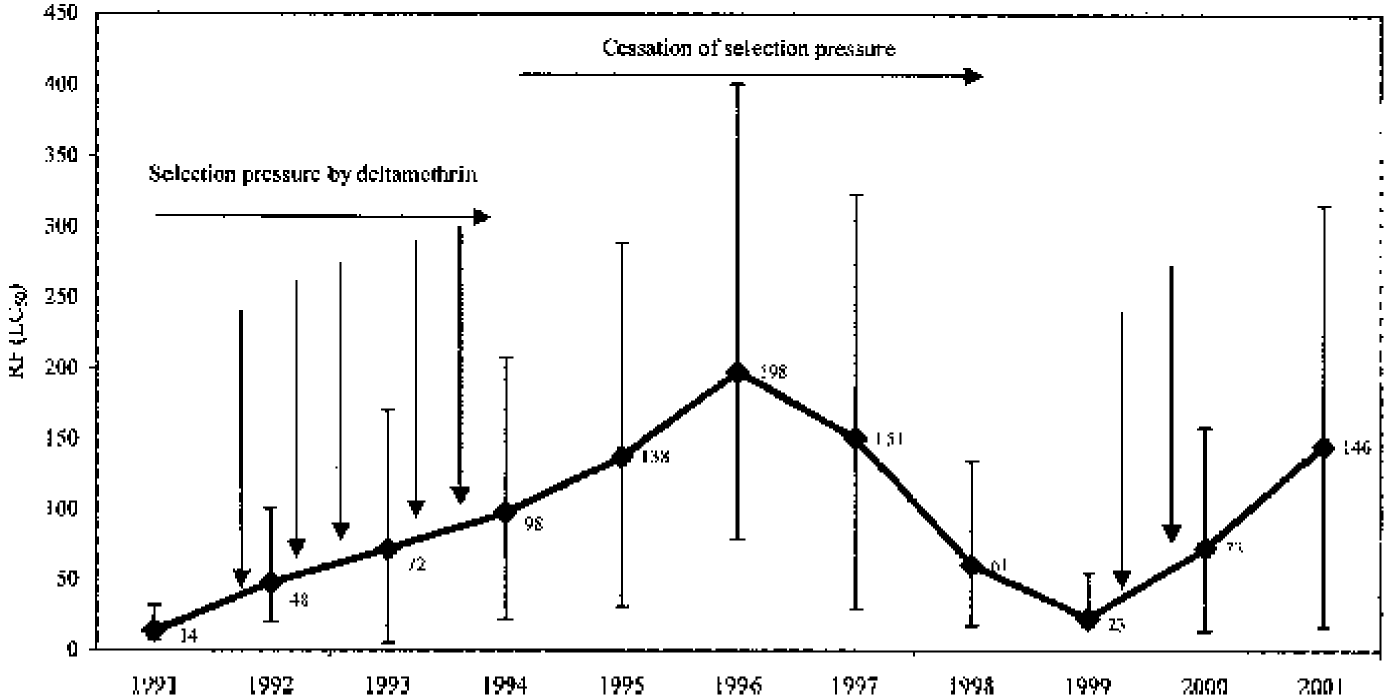

Figure 6. The development, stability, and regression of resistance to deltamethrin under practical conditions in pig rearing — farm SEL.

allowed in protection of plants against black flies. The presented review (Table 1, 2) shows that resistance to dimethoate was moderate in 28% and high in 32% of populations.

In the case of pirimiphos-methyl, uncommonly steady resistance was observed with only small exceptions. Low resistance was found in 87% and moderate in 13% of all cases. After 1990, the data about resistance of flies to this compound around the world are scarce. Our previous studies (Kocišová et al., 1994) dealing with the situation up to 1991 revealed high resistance in 96% of house fly populations. Up to the end of 1993, resistance declined to low levels in 90% of populations, a result that is closely related to the limited use of this compound in animal production.

The biological effect of carbamates, i.e., inhibition of enzyme cholinesterase, resembles that of organophosphate insecticides. The similarity of the toxic effect of carbamates and organophosphates raises concerns about development of cross-resistance between these compounds because resistance to bendiocarb was high in 66% of cases. However, according to our observations, this compound has not been commonly used to control populations in the houses observed in our study. The development of this high resistance is most likely related to cross-resistance not only to organophosphates but also to chlorinated hydrocarbons (Jespersen, 1992) that were used extensively in eastern Slovakia in the past (Vostal et al., 1963).

The investigation of pyrethroids provided RF values ranging from the lowest level to those exceeding 1000. This is related to both high frequency of their use and high stability of resistance to them which is maintained at the same level for 30-40 generations after termination of their selection pressure (Kocišová, 1996). As the mean number of house fly generations in one season in our climatic zone ranges from 10 to 12, high resistance to pyrethroids may be recorded under practical conditions 3 or more years after cessation of their selection pressure.

Frequent and thorough application of insecticides guarantees very strong selection pressure. The limited migration from the environment further increases selection pressure because dilution of the population treated is limited. 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. After stabilization of resistance and subsequent decrease in its values to low to moderate level we presumed that some preparations could be included again in the fly control programmes. However, after two applications, the values of resistance increased to a high level and the preparations became ineffective in practice by 10-15 weeks after their first application. It is probable that once the resistance genes became

Table 3. Percentage proportion of resistance level in housefly populations in Slovakia during 1991-2001

Insecticide	Number of populations tested	Level of resistance			
		Low (RF<10)	Moderate (RF10-40)	High (RF41-160)	Very high (RF>160)
Azamethiphos	102	41	19	36	4
Dimethoate	70	40	28	29	3
Diazinon	24	33	67	–	–
Chlorpyriphos	24	33	42	25	–
Pirimiphos-methyl	71	87	13	–	–
Bendiocarb	53	8	26	38	28
Permethrin	90	24	38	28	10
Cypermethrin	60	20	55	25	-
Deltamethrin	90	8	23	46	23
Lambda-cyhalothrin	48	6	19	27	48
Diflubenzuron	8	100	–	–	–
Cyromazin	8	100	–	–	–

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established in wild populations, they can persist for a long time. After elimination of the selective insecticide pressure, the frequency of specific alleles of resistance genes decreases which is reflected in the lowered phenotype values of resistance. The persistence of changed genetic background in house fly genomes causes, however that high resistance is induced in the population as soon as the respective substance is used repeatedly (Metcalf, 1989, and our experiments). This fact limits considerably the successful use of an insecticide in the case of its long-term repeated application even if fly populations appear fully sensitive.

The frequency of resistance in house fly populations results mostly, as was already mentioned, from the selection pressure by an insecticide. The strategies invented for management of resistance focus, therefore, on resistance reduction through new methods of insecticide application in ways that can prolong their effectiveness in practice, as witnessed by many various developed and recommended procedures (Georghiou, 1994; Maciver et al., 1998; Darja and Vinkovic, 1998). 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. The complexity of the successful management of resistance phenomenon in practice requires mutual collaboration of a number of research branches, breeders, and particularly sanitation personnel.

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