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ORIGINAL PAPER

Resistance of Colorado potato beetle (Coleoptera: Chrysomelidae) to neonicotinoids, pyrethroids and nereistoxins in Serbia

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Abstract

Colorado potato beetle (CPB, Leptinotarsa decemlineata Say) resistance is one of the limiting factors in potato production in some regions of Serbia. Imidacloprid is first introduced neonicotinoid insecticide in use for effective CPB control. Introduction of such insecticides with novel mode of action and physicochemical properties, demands investigations on resistance risks and management tactics. Primary candidates for cross resistance are insecticides acting on same target sites (such as bensultap, with CPB resistance reported), although other cross resistance patterns between apparently unrelated insecticides are not uncommon. Results of the investigations presented in this paper refers to the resistance of CPB adults to 3 insecticides - imidacloprid, bensultap and cypermethrin, most commonly used by farmers in recent years. First generation adults from nine field populations in first and four representative populations in second year of investigation were tested by topical application. Resistance ratios for bensultap ranged from 1.0 to 645.3 (LD_{50} from 0.04 – 25.81 μg/insect). Cypermethrin resistance ratios ranged from 1.0 to 60.0 ($_{LD50}$ from 0.02 – 2.05 μg/insect). Imidacloprid resistance ratio was from 1.0 to 82.9 (LD_{50} from 0.0039 – 0.0323 ng/insect). Based on our results there was no correlation between resistance levels for bensultap and imidacloprid. Although LD_{50} values for imidacloprid were very low, significant resistance ratios obtained in these investigations, together with threads of cross-resistance and importance of conserving field efficacy of neonicotinoids, strongly recommands systematic resistance monitoring as important resistance management tool.

Keywords: : Colorado potato beetle, resistance, toxicity, bensultap, cypemethrin, imidacloprid.

Introduction

Colorado potato beetle (CPB, *Leptinotarsa decemlineata* Say) (Coleoptera: Chrysomelidae) causes great losses in tuber yields by defoliation caused by larval feeding in 2 consecutive generations. Potato plants can tolerate light to moderate defoliation but without control major to complete crop loss is common. Management of CPB is mainly based on chemical control which influences on multiple spatial and temporal scales its colonization and invasion process [4].

The majority of the potato protection programmes requires the reduction of the CPB population at the beginning and in the middle of the growing season, while the pest higher abundance is tolerated at the end of the growing season. In Serbia, potato is treated by insecticides 3-4 times per year with the purpose of providing the expected yield [17].

Colorado potato beetle expresses extraordinary adaptability to effects of toxicants of different chemical groups by the development of various levels of resistance to all groups of insecticides applied in its control all over the range of its distribution in North America, Europe, Asia Minor, Iran, central Asian and western China [3, 10, 12, 13, 14, 16, 17, 19, 22, 26, 28,].

Developing of resistance to insecticides significantly increases the potato production costs, affects environmental contamination and disturbance of equilibrium in biocenosis. The development of a series of mechanisms of resistance - reduced permeability, increased metabolism of insecticides, changes in susceptibility of the sites of action and behaviour [3, 16, 22], make control of this pest difficult. Resistance levels vary greatly among different populations and between beetle life stages [5, 8, 15, 23]. Resistance mechanisms are sometimes highly diverse even within a relatively narrow geographical area. These findings can be effectively used in management programs and Resistance Potential prediction when new compounds are introduced [7]. After imidacloprid other neonicotinoids belonging to the same mode of action group were introduced and their rotation with each other is not recommended [2, 12].

Costs of resistance to imidacloprid are significant in the Colorado potato beetle. Reduced fitness among resistant versus susceptible individuals slows resistance evolution and makes it easier to manage. A loss of resistance costs could indicate novel adaptations or mutations contributing to resistance [5].

The first quantitative data on resistance to insecticides (DDT) in Serbia were obtained in 1969. Succeeding studies determined highly variable resistance to organophosphoric insecticides, carbamates and pyrethroids in many locations [15, 17, 23]. Significant variability in levels of susceptibility (or resistance) exists in geographically distinct CPB populations. In some populations resistance is attributed to cross or multi resistance to DDT and other organochlorine compounds. Certain CPB populations developed significant levels of resistance to all studied insecticides. Level of resistance was correlated with the history of the application of insecticides. Due to high reliance on chemical control and problems which are raised there is a need for alternative methods of CPB control as well as it is in other infested regions [6].

Due to faster development of resistant CPB populations resistance monitoring became more important [18]. Therefore, we wanted to establish baseline levels of resistance for monitoring of 9 geographically distinct CPB populations on 3 widely used insecticides a.i. but with different history of use in the field.

Materials and methods

Adults of both sexes of the first CPB generation were used in the study. Test insects collected in fields were transferred to a laboratory in which they were kept at the temperature of 5-8^{o}C. When the trial was set up, insects were kept in the micro climate chamber (Danfoss, EKH 20) under control conditions: t=26±1^{o}C, RH=60% and 16:8 (L:D) photoperiod. Test insects were collected in locations that cover the majority of the country (Dobanovci, Majur, Malošište, Rečka, Orid, Požarevac, Ruma, Toljevac and Vršac). As there was no normal susceptible strain, populations collected in the locations Orid and Toljevac showing exceptional susceptibility were considered as the referent populations. In the following year, four representative populations (Dobanovci, Majur, Rečka, and Toljevac) were observed.

Toxicity of bensultap, cypermethrin and imidacloprid belonging to the most important chemical groups (nereistoxins, pyrethroids and neonicotinoids, respectively) used in CPB control was studied. Commercial preparation used in our research were: Bancol 50

WP (with 49.89% a.i.); cypermethrin (technical grade with 95,39% a.i.), Confidor 200 SL (190.56 g L^{-1} a.i.).

Technical grades were provided by the Department of Phytopharmacy of the Institute for Plant Protection and Environment, Belgrade, and the determination of the a.i. content of the grades and commercial preparations was performed there.

Toxicity to CPB was assessed by a method of topical application. A 1-μl droplet of acetone solution was administered to the ventral sternum between coxae with a Socorex micro litre pipette. Ten insects each were treated and placed into Petri dishes. The precision of the micro litre pipette at the calibration was 0.1 μl. The check insects were treated with acetone.

The mortality was registered daily during 96 h, with food addition, and the data on dead insects were used for the further calculations. The observed mortality data were corrected for control mortality using Abbott's formula. Regression lines (ld - p), values of LD_{50} with 95% fiduccial limits/confidence intervals, were estimated by the probit analysis using the computer programme. Four-replicate experiments with the use of at least five concentrations of the insecticide were set up. The results were expressed in μg active substance per insect. Resistance ratios (RR) were determined by the comparison of LD_{50} values of each population with LD_{50} values of reference populations. Beside the comparison among LD_{50} values, the comparison of their intervals is also very important.

Results and Discussions

Values of LD_{50} for bensultap (Table 1.) show the significant difference in susceptibility of CPB adults over 9 studied locations.

Table 1. Bensultap toxicity to adults of *Leptinotarsa decemlineata* Say.

Rank (group)	Locality (Population)	Regression slope (b)	LD_{50} (μg / insect) (95% CL)	Resistance ratio (RR)
			1st year	
I group	Toljevac	0,68	**0,04** (0,01 – 0,08)	1,00
	Orid	1,02	**0,08** (0,05 - 0,12)	2,00
	Požarevac	0,81	**0,15** (0,08 – 0,27)	3,75
II group	Malošište	0,39	**0,28** (0,10 – 1,53)	7,00
	Majur	1,07	**0,46** (0,29 – 0,70)	11,50
	Dobanovci	0,85	**1,53** (0,91 – 2,61)	38,25
III group	Ruma	0,63	**6,37** (3,13 - 13,75)	159,25
	Rečka	0,78	**9,36** (5,45 - 17,35)	234,00
	Vršac	0,89	**25,81** (14,55 - 51,92)	645,25
			2nd year	
I group	Toljevac	0,61	**0,06** (0,02 - 0,12)	1,00
II group	Dobanovci	0,76	**0,61** (0,34 - 1,11)	10,17
	Majur	1,38	**0,71** (0,50 - 0,99)	11,83
III group	Rečka	0,94	**4,30** (2,26 - 7,05)	75,67

Based on the LD_{50} overlapping intervals the highest susceptibility was detected in the insects in the locations of Toljevac, Orid and Požarevac. The populations in Malošište and Majur showed a somewhat lower susceptibility. A significant degree of resistance to bensultap was characteristic for the population in Dobanovci, and especially for populations in Ruma, Rečka and Vršac.

The highest slope was detected in the populations Majur (1.07) an Orid (1.02), while the lowest one was found in the population Malošište (0.39).

The population Dobanovci is characterised by the existence of two plateaus - the first, i.e. the second plateau occurred at the 10- (0.0385 – 0.385 μg/insect), i.e. 5-fold (7,7061-38,5305 μg/insect) dose increase, respectively. Plateaus in the population Rečka occurred at the 5-fold (7,7061-38,5305 μg/insect) and 2-fold dose increase (0.0193 – 0.0385 μg/insect). On the other hand, plateau in the population Požarevac occurred at the 10-fold dose increase (3,8530 -38,5305 μg/insect).

During the second year of investigation, the significant differences in respect to susceptibility to bensultap were confirmed by the resistance ratios that ranged from 10.17 to 75.67. The population Toljevac showed the highest susceptibility and its LD_{50} interval did not overlap intervals of other populations.

The populations Dobanovci and Majur had 10-fold higher resistance and had mutually overlapping LD_{50} intervals, hence they can be classified into the same group. The population Rečka had the highest degree of resistance and significantly differed from others. The regression slopes significantly varied over observed populations. The populations Toljevac, Dobanovci and Rečka had a lower slope, while the population Majur had a higher slope (1.39). The lower slopes were a result of both, the existence of plateaus at 10 fold dose increase in the populations Toljevac (0.3853 – 3.8530 μg/insect, with corrected mortalities of 80.00% and 82.50%, respectively) and Dobanovci (0.0193 – 0.1927 μg/insect, with corrected mortalities of 22.50% and 25.00%, respectively), and the presence of the saddle-point in the population Rečka at 10-fold doses increase (0.7706 – 3.8530 – 7.7061 μg/insect, with corrected mortalities of 44.74%, 34.21% and 39.47%, respectively).

Regarding LD_{50} values, it could be possible to rank populations on possibility for ‘’in field’’ use of bensultap. Ranking is done based on 95% CI (confidence intervals) overlapping among populations. Bensultap could be effective in CPB control only in populations Toljevac, Orid, Malošište and Požarevac, eg. in populations with LD_{50} lower than 0.30 μg/insect.

Concerning susceptibility of CPB adults from different locations to cypermethrin (Table 2.), the clearly expressed differences among populations exist.

The populations Toljevac and Malošište were the most susceptible, while the susceptibility of the populations Orid and Požarevac was somewhat lower. LD_{50} intervals of these populations overlapped, furthermore confirming their similarity. The populations Ruma and Rečka had lower susceptibility, as well as, equal values of LD_{50} and almost identical intervals and slopes of regression. Populations Majur and Dobanovci, and especially Vršac, had a highest resistance to cypermethrin.

The LD_{50} intervals were narrow in all populations. Slopes did not differ much from one another, except in the population Majur in which the slope was significantly higher (2.30). The lowest slope was detected in the population Malošište. The slopes varied from 0.75 to 1.00 in all other populations and were very similar. A saddle-point (the 2-fold dose increase: 0,0189 - 0,0378 μg/insect) and a plateau (the 5-fold dose increase: 0,3775 - 1,8877 μg/insect) occurred in the population Dobanovci and Orid (the 10-fold dose increase: 0.0378-0,3775 μg/insect).

Moreover, toxicity of cypermethrin to CPB adults significantly differed among observed field populations during the subsequent year of investigation. The susceptibility of populations Toljevac and Rečka was the most pronounced, while their LD_{50} intervals overlapped to a great extent. Based on the comparison of LD_{50} intervals, the populations

Dobanovci and Majur were significantly more resistant (7.14 and 29.29, respectively). The upper limits of LD_{50} in these two populations did not significantly differed, while their lower limits of LD_{50} were almost identical, hence in respect to resistance to cypermethrin these populations could be classified into the same group.

Table 2. Cypermethrin toxicity to adults of *Leptinotarsa decemlineata* Say.

Rank (group)	Locality (Population)	Regression slope (b)	LD_{50} (μg / insect) (95% CL)	Resistance ratio (RR)
			1st year	
I group	Toljevac	0.75	**0.02** (0.01 – 0.04)	1.00
	Malošište	0.64	**0.02** (0.001 – 0.06)	1.00
	Orid	0.85	**0.04** (0.03 – 0.08)	2.00
	Požarevac	0.89	**0.09** (0.05 – 0.15)	4.50
II group	Rečka	0.94	**0.25** (0.15 – 0.42)	12.50
	Ruma	1.00	**0.25** (0.15 – 0.41)	12.50
III group	Majur	2.30	**0.80** (0.60 – 1.13)	40.00
	Dobanovci	0.87	**0.72** (0.43 – 1.22)	43.50
	Vršac	0.99	**1.20** (0.71 – 2.11)	60.00
			2nd year	
I group	Toljevac	0.96	**0.07** (0.04 – 0.12)	1.00
	Rečka	1.26	**0.11** (0.05 – 0.17)	1.57
III group	Dobanovci	1.29	**1.90** (1.35 – 2.63)	27.14
	Majur	1.29	**2.05** (1.36 – 3.21)	29.29

The regression slope was the lowest (0.96), i.e. highest (1.29) in the populations Toljevac and Rečka, respectively. The identical slopes (1.29) were found in the populations Dobanovci and Majur, while populations Dobanovci and Rečka were characterised by one plateau at the 5-fold dose increase (0.0378 – 0.1888 and 0.7550 – 3.7752 μg/insect, respectively).

If we use ranking of LD_{50} values for cypermethrin, there is wider possibility for its use, comparing to bensultap. Cypermethrin use will not be suitable for CPB control in populations with LD_{50} higher than 0.70 μg/insect. Out of nine investigated localities, cypermethin could be effective for use in six localities.

Very low values of LD_{50} indicated very high toxicity of imidacloprid to CPB (Table 3), hence values for this insecticide were expressed in μg per insect. The LD_{50} values themselves differed indicating that there was a certain degree of resistance to this insecticide.

The populations Orid, Toljevac, Požarevac and Rečka were the most susceptible, hence they could be classified into the same group due to overlapping of the LD_{50} intervals. These populations can be considered generally susceptible to imidacloprid, taking into account not only the LD_{50} values, but also relatively higher slopes and therefore they can be used as reference populations (particularly populations Požarevac and Orid) in establishing the initial susceptibility and monitoring the further development of resistance. The populations Ruma, Rečka and Majur were over 10-20-fold more resistant to imidacloprid than the population Orid. The highest resistance was detected in the populations Dobanovci and Vršac. Significantly higher LD_{50} values in populations which were less susceptible to other groups of insecticides suggest the presence of a mechanism of resistance to this insecticide. The lowest values were obtained in the population Malošište (0.58), and the highest values in the population Požarevac (2.32), that ranked second by its susceptibility. The more significant presence of saddle-points was characteristic for the population Majur at the 10-fold higher doses (0.0476 – 0.476 ng/insect), as well as, for the population Ruma (0.2382 – 2.3820 μg/insect). At such increases of doses, a plateau each was detected in the populations Mološište and Vršac.

Table 3. Imidacloprid toxicity to adults of *Leptinotarsa decemlineata* Say.

Rank (group)	Locality (Population)	Regression slope (b)	LD_{50} (μg / insect) (95% CL)	Resistance ratio (RR)
			1st year	
I group	Orid	1.46	**0.39** (0.14 – 0.66)	1.00
	Toljevac	0.93	**0.61** (0.31 – 1.02)	1.56
	Požarevac	2.32	**0.61** (0.44 – 0.83)	1.56
	Malošište	0.58	**0.63** (0.19 – 1.34)	1.62
II group	Ruma	1.16	**3.49** (2.33 – 5.48)	10.10
	Rečka	1.14	**5.52** (3.63 – 9.10)	14.15
	Majur	0.75	**8.28** (4.86 – 16.20)	21.23
III group	Dobanovci	0.89	**28.30** (16.20 – 65.30)	72.56
	Vršac	0.89	**32.33** (18.70 - 66.30)	82.90
			2nd year	
I group	Toljevac	0.81	**0.62** (0.34 – 1.08)	1.00
	Rečka	0.85	**0.88** (0.29 – 1.66)	1.42
III group	Majur	0.89	**16.40** (10.11 – 25.98)	26.45
	Dobanovci	0.88	**22.60** (14.39 – 38.62)	36.45

Toxicity of imidacloprid significantly varied over observed populations during the second year of investigation. CPB individuals of the population Toljevac were 26.45- and 36.45-fold more susceptible than individuals of the populations Majur and Dobanovci, respectively, which was in accordance with results gained in the previous year.

The regression slopes did not significantly differ and varied from 0.81 to 0.89 and were similar to values obtained in the previous year. The most resistant population Dobanovci was characterised by a saddle-point at the 20-fold dose increase (0.0476 – 0.9528 μg/insect). This was also characteristic for the population Majur (at the double dose increase, from 4.764 to 9.528 μg/insect) and Rečka (at the 4-fold dose increase: 11.91 – 47.64 μg/insect).

There are 3 separate groups of populations regarding LD_{50} values for imidacloprid, too. Use of this insecticide is very effective, but careful selection of rotating insecticides and lower number of applications with imidacloprid could be of importance for management of CPB populations Dobanovci, Majur and Vrsac.

In our paper obtained results point out to a significant level of resistance of the majority of observed populations (Vršac, Ruma, Dobanovci, Rečka, Malošište) to insecticides applied for a longer period of time (bensultap, cypermethrin) but also to resistance of some populations (Vršac, Dobanovci, Majur, Ruma) to imidacloprid, neonicotinoid of the first generation, a group of insecticides introduced into practice during late 90`s. The highest level of overall resistance was detected in the populations Vršac, Ruma, Dobanovci, Rečka and Majur, while the lowest level of resistance was found in the populations Toljevac, Orid, and in case of cypermethrin, in the population Malošište. Such results were expected as CPB resistance has been observed for a long period. Based on results of our earlier studies [17], we can now have a clear picture of CPB susceptability levels for nine investigated populations.

We noticed variations among RR between two investigated years. Our samples of CPB adults were taken from the same field, but within two years of investigations, variations in LD_{50} values were noticeable. It can be explained by mixing of populations with different susceptibility levels [4] due to a lot of small neighbouring potato fields with different history of insecticide use and therefore different resistance profiles. In addition, insecticide use during the season at investigated fields changed resistance levels within the populations.

Resistance of adults to bensultap

The following populations are singled out for their resistance to bensultap: Vršac (645.3 times), Rečka (234.0 times), Ruma (159.3 times) and Dobanovci (38.3 times), which was also confirmed in the subsequent year of investigation. The moderate resistance was determined in the populations Majur (11.3 times) and Malošište (7.0 times). The populations Toljevac (1.0 time), Orid (2.0 times) and Požarevac (3.8 times) showed extremely high susceptibility. In the subsequent year, resistance of the populations Rečka (75.7 times), Dobanovci (10.7 times) and Majur (12.5 times), as well as, susceptibility of the population Toljevac (1.0) were confirmed. The values of LD_{50} (μg per insect) ranged from 0.04 to 25.81.

Similar values for bensultap (0.02-14.51 μg per insect) were obtained by [15]. The same authors established the reduction of resistance in some populations, such as the populations Dobanovci (0.61-1.53 μg per insect) and Rečka (4.3-9.36 μg per insect) in this study. The regression slopes varied from 0.16 to 1.38, similar to results obtained by previously mentioned authors (0.62-2.02).

Resistance of adults to cypermethrin

According to its toxicity, cypermethrin, an active substance, ranking second in our study, has history of resistance in Serbia [15]. The populations Vršac, Dobanovci and Majur expressed high resistance (60.0, 43.5 and 40.0 times, respectively), while Rečka (12.5 times) and Ruma (12.5 times) showed moderate resistance. Susceptibility to this pyrethroid was expressed by the populations Požarevac (4.5 times), Orid (2.0 times) and especially Toljevac (1.0) and Malošište (1.0). The values of LD_{50} ranged from 0.02 – 1.20 μg per insect. A high level of resistance was determined in the populations Majur (29.3 times) and Dobanovci (27.1 times), while the population Rečka (1.6 times) was less resistant than the population Toljevac. The increase of LD_{50} (0.02-0.07 μg per insect) was established in the population Toljevac in the subsequent year of investigation.

The values of LD_{50} for CPB adults presented by [15] amounted to 0.12-10.22 μg per insect. According to the same authors, the regression slopes ranged from 0.38 to 1.51. The corresponding values in the present study amounted to 0.64-2.29 (1st year) and 0.96-1.29 (2nd year). Values gained in the two-year study are in accordance with the results obtained by other authors, in respect to both, toxicity of cypermethrin in relation to other active substances, and values of LD_{50}.

Resistance of adults to imidacloprid

First, among several neonicotinoids, introduced into CPB management in '90s, imidacloprid effecivelly controled pests resistant to conventional insecticides dominating the markets at that time [9]. Neonicotinoids greatly reduced the need to spray insecticides for Colorado Potato Beetle control [21].

Toxicity of imidacloprid, in this study, varied over studied populations. The differences in susceptibility of CPB populations were extremely pronounced. Individuals of the populations Vršac (RR 82.9 times) and Dobanovci (72.6 times) with overlapping LD_{50} intervals were the most resistant. Populations Majur (21.2 times), Rečka (14.2 times) and Ruma (10.1 times) were somewhat more susceptible, while the most susceptible group consisted of the following populations: Malošište, Požarevac, Toljevac and Orid (RR approximately 1). Slopes were very different and varied from 0.58 to 2.32, while LD_{50} ranged from 0.39 to 32.33 (μg per insect).

In the subsequent year, the individuals of the populations Dobanovci and Majur were significantly more resistant (RR 36.5 and 26.5, respectively) than the population Toljevac, while a certain reduction in resistance was detected in the population Rečka. Resistance of the population Dobanovci showed a particular stability: LD_{50} ranged from 28.3 μg per insect (1st year) to 22.6 μg per insect (2nd year), while the regression slope was insignificantly changed. A double increase of LD_{50} in the population Majur (8.28 in the 1st year and 16.40 μg per insect in the 2nd year), as well as, a slight increase of the regression slope were observed. Furthermore, the LD_{50} values were stable and the regression slope was slightly increased in the population Toljevac. It is interesting to mention that a certain increase, i.e. decrease of resistance in the populations Majur, i.e. Rečka, respectively, was observed, especially when it is considered that test insects for these two locations were collected in the same field during both years.

Differences in susceptibility of CPB populations to imidacloprid were not registered only in this study, but in the studies carried out by other authors [1, 2, 12], hence the occurrence and the development of resistance of field populations in Serbia can be discussed. Based on the comparison of results on RR obtained in our country (1.6-82.9 times in the 1st year and 1.4-36.5 times in the 2nd year) and in the state of Michigan, USA (RR 29 times, [14] and RR 100 times, [25] it can be concluded that the level of resistance was similar. Recent data obtained from the Long Island CPB population showed 309-fold resistance to imidacloprid compared to standard susceptible beetles [12].

Like the naturally occurring alkaloid -nicotine, all neonicotinoids act selectively on the insect central nervous system as agonists of the post-synaptic nicotinic acetylcholine receptors, their molecular target site [24]. Taking into account that imidacloprid is a newer compound in this region, it can be considered that some of already existing mechanisms of resistance were involved in resistance to the group of neonicotinoids. [25] have already proved the synergic effects of piperonyl butoxide (PBO) and S, S, S-tributyl phosphorotrithioate on adults and only PBO on larvae. Effects of these synergists directly point out to mechanisms of resistance of CPB to imidacloprid - metabolic oxidase- (PBO) and hydrolyse-based detoxification. Since synergists did not completely eliminate resistance, it is considered that there are some additional mechanisms of resistance. Imidacloprid-resistant beetles from Long Island, New York showed a reduction in resistance from 300-fold without PBO treatment to 108-fold with PBO treatment [12]. Besides that, nerve recording suggested that insensitivity at the target site may be one of the mechanisms of beetle resistance to imidacloprid [20]. In vivo distribution and metabolism studies using [^{14}C] imidacloprid suggested that the tolerance observed in a resistant population from Long Island, New York was primarily due to increased excretion of the parent compound [14]. Other authors [24] agree that some cases of resistance appeared to be unconnected with imidacloprid use, and was probably a consequence of cross-resistance from chemical classes of insecticides used earlier. Imidacloprid is systemic insecticide of high efficiency however in field conditions its uneven distribution within potato plants could result in differential concentrations, which may result in different mortality of CPB of varying susceptibility and can influence the resistance development [27].

In our study, Imidacloprid is the most toxic compound causing mortality of 50% in observed populations at the dosage rate of 0.39-32.33 μg a.i. per insect. Less toxic was cypermethrin with LD_{50} = 0.02 – 2.05 μg/insect and finaly, bensultap caused 50% mortalities in amounts of 0.04 – 25.81 μg/insect. Therefore based on results obtained following ranking could be set: imidacloprid > cypermethrin > endosulphan > bensultap > chlorpyrifos > carbosulfan.

Properties of populations in relation to adult resistance

Determined levels of resistance to bensultap clearly highlighted the difference among observed populations. Recent studies state that there is no cross resistance between bensultap and imidacloprid [11, 14]. Neither could these correlations be established in our country. It was recorded that the populations most susceptible to bensultap were also the most susceptible to imidacloprid. This cannot be applied for the populations resistant to bensultap, and especially to the population Rečka, which is extremely resistant to this insecticide (RR 234.0 time in the 1st year and 75.8 times in the 2nd year), and far less resistant to bensultap (RR 14.2, i.e. 9.1 times in relation to the population Toljevac in the 1st year and 1.4 times in the 2nd year).

If the increased oxidase-based detoxification is considered a key mechanism of resistance to pyrethroids and modified hydrolytic detoxification to organophosphates, it is interesting to compare differences in susceptibility of populations to cypermethrin and chlorpyrifos on one hand and imidacloprid on the other hand from the aspect of established synergism of PBO and S, S, S-tributyl phosphorotrithioate [25]. The sequence (descending) of populations significantly resistant to cypermethrin was as follows: Vršac, Dobanovci, Majur, Rečka and Ruma, then to chlorpyrifos were: Dobanovci, Vršac, Majur, Malošište, Ruma, and Negotin. The sequence (descending) of populations resistant to imidacloprid was as follows: Vršac, Dobanovci, Majur, Rečka and Ruma. The correlation in resistance of populations to these three insecticides can be observed from this sequences, then values of RR and classification into groups based on LD_{50} intervals. The detail comparison shows that the correlation in resistance was the highest between cypermethrin and imidacloprid and that this resistance, occurred as a result of modified oxidative metabolism, is a decisive for resistance to imidacloprid. The modification of hydrolytic metabolism in adults within a population affects, to a smaller extent, resistance to neonicotinoids.

Results on the level of resistance in individuals in the location Toljevac to chlorpyrifos and carbosulfan [17] indicate to the fact that beside the same mode of action, the mode of resistance to these compounds is different, which is not so rare.

Studies with adults show no cross-resistance between bensultap and imidacloprid according to the mode of action of the most related insecticides. Furthermore, results globally obtained were confirmed - there is a certain and significant level of resistance of field populations to imidacloprid, depending on the previous presence of resistance to pyrethroids (cypermethrin) to a greater extent and to organophosphates (chlorpyrifos) to a smaller extent.

Conclusions

Due to fast development of resistant CPB populations to applied insecticides, resistance monitoring is important. In this paper we established levels of resistance for 9 geographically distinct CPB populations on 3 widely used insecticides a.i. but with different history of use in the field.

Results of the investigations refer to the resistance of CPB adults to imidacloprid, bensultap and cypermethrin, most commonly used by farmers in recent years. Resistance ratios for bensultap ranged from 1.0 to 645.3 (LD_{50} from 0.04 – 25.81 μg/insect). Cypermethrin resistance ratios ranged from 1.0 to 60.0 (LD_{50} from 0.02 – 2.05 μg/insect). Imidacloprid resistance ratio was from 1.0 to 82.9 (LD_{50} from 0.0039 – 0.0323 μg/insect). Based on our results there was no correlation between resistance levels for bensultap and imidacloprid. Correlations of resistance among these populations for imidacloprid and cypermethrin strongly suggest the common mode of resistance – enhanced oxidative metabilism.

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