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MONITORING OF COLORADO POTATO BEETLE (*LEPTINOTARSA DECEMLINEATA* SAY) SENSITIVITY TO INSECTICIDES

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Abstract

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Sensitivity of two field populations of Colorado potato beetle (*Leptinotarsa decemlineata* Say.) - CPB (Vilovo and Čurug) adults to insecticides were determined in 2008 and 2012. Bioassay was performed using screening test that allows rapid assessment of sensitivity of over wintered adults to insecticides. Sensitivity to four insecticides commonly used for CPB control in Serbia (chlorpyrifos, cypermethrin, thiamethoxam and fipronil) was assessed. Insecticides were applied at label rates, and two, five and 10 fold higher rates by soaking method. Insect mortality was assessed after 72 h. Sensitivity was assessed using a modified IRAC method (2009) and 1-5 scale. In 2008, both tested CPB populations were resistant to chlorpyrifos label rate, as well as in 2012. Both tested populations were also resistant to cypermethrin in 2008, while in 2012, population from Vilovo was resistant, and population from Čurug was moderately resistant. Significant differences in CPB sensitivity to thiamethoxam between populations tested in 2008 and 2012 were recorded.

Key words: Leptinotarsa decemlineata, insecticides, monitoring, sensitivity

Abbreviations: CPB - Colorado potato beetle

Introduction

Besides many well-known advantages, use of pesticides in agricultural production gradually caused some serious problems, particularly regarding alternations or reduced sensitivity of controlled insect species or – resistance of insects to insecticides.

Accounting for share of potato in human nutrition, even among high-risk groups (pregnant women, babies, and children in intensive growth) as well as quantities and diversity of pesticides used for pest management in potato during vegetation and storage, quality of this aliment is of high importance. To ensure the expected yield in Serbia, potato crops are treated with insecticides two to four times during cropping season (Zabel et al., 2000). According to several authors (Stanković et al., 2004; Inđić et al., 2006), CPB resistance to carbamates and organophosphates, as well as to pyrethroids (Inđić, 1997; Perić et al., 1997) was registered in a number of populations in Serbia. Literature data indicate that CPB resistance was registered for 42 insecticides, belonging to different chemical groups (organophosphates, carbamates, pyrethroids). This fact justifies CPB ranking among 10 species that most rapidly develop resistance (Mota-Sanchez et al., 2006; Whalon et al., 2008).

In practice, the problem of resistance in our region is resolved in accordance with principles of integrated pest management using different measures (manual collection, less frequent use of biocides and growth regulators) which reduce insecticide selection pressure. However, major producers mainly use chemical measures within the strategy of delaying the mentioned problem that involve application of insecticide mixtures containing active ingredient with different modes of action. Although producers are familiar with other measures, such as use of predators and parasitoids, plant extracts, combined crops (Copping and Menn, 2000; Gokce et al., 2006), planting of resistant cultivars or spatial distance of potato fields, which directly or indirectly reduce insecticide selection pressure, they are not present in practice.

The aim of the paper was to compare sensitivity to insecticides of same CPB populations in years 2008 and 2012, using rapid and simple testing method.

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Materials and Methods

Site selection - For monitoring of CPB sensitivity to insecticides two sites on the territory of Serbia (Vilovo, Čurug) were selected based on the advice of experts from Agricultural extension service, and were marked with GPS coordinates in two years - 2008 and 2012.

Tested insecticides - Insecticides belonging to four chemical groups were used in this assay: organophosphates (chlorpyrifos - Pyrinex 48-EC /1.5 l/ha – label rate; 7.5 l/ha – 5 x higher rate; 15 l/ha – 10 x higher rate/), pyrethroids (cypermethrin - Cipkord 20-EC /0.3 l/ha - label rate; 1.5 l/ha – 5 x higher rate; 3 l/ha – 10 x higher rate/), neonicotinoids (thiamethoxam - Actara 25-WG /60 g/ha - label rate; 70 g/ha - label rate; 140 g/ha – 2 x higher rate) and pyrazoles (fipronil - Regent 800-WG /25 g/ha - label rate; 50 g/ha – 2 x higher rate; 125 g/ha – 5 x higher rate), which differ in history of application. Chlorpyrifos has been in use for nearly 40 years in our region, cypermethrin about 30 years, while thiamethoxam and fipronil for more than 10 years, considering that recently thiamethoxam has become the most frequently used in CPB control.

Test insect - Overwintered adults of CPB field population, which were not in direct contact with insecticides prior to testing, were used in bioassay. Insects were kept in laboratory conditions, without additional feeding, at temperature of 23±2°C and normal photoperiod (16/8h).

Toxicological experiment - Bioassay was based on the assumption that CPB populations have reduced sensitivity to insecticides, and on evaluation of the response of over wintered adults to insecticide label rates (the rate determined in field experiments during the registration process and found to cause 100% mortality) and higher rates. Chlorpyrifos and cypermethrin, which have a long history of use, were applied at five and 10 fold higher quantities than the label rate, and thiamethoxam and fipronil at two and/or five fold higher rates. Insects soaking for 5 s applied insecticides. The experiment was set up in four replicates with 30 adults per replication (sex ratio 1:1). Assessment of insecticide efficacy consisted of counting the number of dead (with no signs of vitality), paralyzed (uncoordinated movements and inability to move) and alive insects (normal mobility and vitality). The effect was determined 24, 48 and 72 h after insecticide application. Results were corrected for mortality in the control (Schneider Orelli, 1947) and expressed as the efficacy (E%) achieved only after 72 h. Sensitivity was evaluated on the scale 1-5, which was created as a slight modification of IRAC method No. 011 (Anonymous, 2009) that refers to pollen beetles (*Meligetes* spp.).

1 - highly sensitive populations (E = 100%)
2 - sensitive (100> E ≥ 95%)
3 - slightly resistant (95> E ≥ 90%)
4 - resistant (90> E ≥ 50%)
5 - highly resistant (E <50%)

Results

There are 43 registered products for CPB control in Serbia, based on 20 active ingredients, among which 18 are of chemical origin, divided into 10 groups (organophosphates, carbamates, pyrethroids, neonicotinoids, pyrazoles, benzoilfenilureas, benzoilureas, macrolides, semikarbazons, antaranil diamides) and two are bioinsecticides.

Analyzing the efficacy of recommended application (label) rates of insecticides, we tended to simulate conditions and effects that persist in a field. The application of two, five or 10 fold higher rates aimed to verify that individuals from the same population survive higher rates of insecticides. Given the fact that there was no correlation between increase in insecticide doses and mortality in the population, i.e. that survival rate was very high regardless of rates applied, but also that the population became resistant, sets the necessity for unconditional exclusion of these compounds from use in potato production, strategy compliance, and continuous mapping of these populations.

CPB sensitivity to chlorpyrifos, cypermethrin, thiamethoxam and fipronil was classified (Tables 1 and 2) based on insecticide efficacy achieved in screening test (72 h after exposure) and slightly modified scale for classification of insect sensitivity (Anonymous, 2009).

Both investigated CPB populations (Vilovo and Čurug) were resistant to chlorpyrifos label rate in 2008 and 2012. A minor increase in sensitivity was recorded for higher applied rates of chlorpyrifos. Results for efficacy of cypermethrin label rates show that both CPB populations were resistant in 2008, while in 2012 the population from Čurug was slightly resistant. Application of higher rates of cypermethrin resulted in increase in sensitivity of tested populations from resistant to sensitive, in both investigation years.

CPB sensitivity to thiamethoxam and fipronil is shown in Table 2.

Sensitivity of tested CPB populations to thiamethoxam has significantly changed since 2008, which is probably the consequence of frequent use of neonicotinoids for CPB control in recent years in production conditions of Serbia. In 2008, CPB populations (Vilovo and Čurug) were highly sensitive to thiamethoxam label rate, while in 2012 both tested populations were resistant. Population from Vilovo was slightly resistant to fipronil label rate in 2008 and population from Čurug was resistant. Higher application rates caused increase in sensitivity from resistant to highly sensitive. In 2012, both

Table 1
Sensitivity of overwintered CPB adults to chlorpyrifos and cypermethrin, scale 1-5

Sites	kg; l/ha	Efficacy (%) of chlorpyrifos					kg; l/ha	Efficacy (%) of cypermethrin				
		1	2	3	4	5		1	2	3	4	5
Year 2008												
Vilovo	1.5	-	-	-	78.3	-	0.3	-	-	-	74.8	-
	7.5	-	-	-	83.3	-	1.5	100	-	-	-	-
	15	-	-	90.7	-	-	3.0	100	-	-	-	-
Čurug	1.5	-	-	-	65.0	-	0.3	-	-	-	76.6	-
	7.5	-	-	-	85.8	-	1.5	-	97.5	-	-	-
	15	-	-	-	87.5	-	3.0	-	99.2	-	-	-
Year 2012												
Vilovo	1.5	-	-	-	72.5	-	0.3	-	-	-	77.5	-
	7.5	-	-	-	80.0	-	1.5	-	98.7	-	-	-
	15	-	-	91.2	-	-	3.0	-	98.7	-	-	-
Čurug	1.5	-	-	-	82.2	-	0.3	-	-	95.0	-	-
	7.5	-	-	93.3	-	-	1.5	-	98.3	-	-	-
	15	-	97.8	-	-	-	3.0	-	98.3	-	-	-

1 - highly sensitive population (E= 100%); **2** – sensitive (100 > E ≥95%);
3 - slightly resistant (95>E ≥90%); **4** - resistant (90>E ≥50%); **5** - highly resistant (E < 50%)

Table 2
Sensitivity of overwintered CPB adults to thiamethoxam and fipronil, scale 1-5

Sites	kg; l/ha	Efficacy (%) of thiamethoxam					kg; l/ha	Efficacy (%) of fipronil				
		1	2	3	4	5		1	2	3	4	5
Year 2008												
Vilovo	0.06	100	-	-	-	-	0.025	-	-	92.4	-	-
	0.07	100	-	-	-	-	0.05	-	98.2	-	-	-
	0.14	100	-	-	-	-	0.125	100	-	-	-	-
Čurug	0.06	100	-	-	-	-	0.025	-	-	-	86.7	-
	0.07	100	-	-	-	-	0.05	-	95.0	-	-	-
	0.14	100	-	-	-	-	0.125	100	-	-	-	-
Year 2012												
Vilovo	0.06	-	-	-	67.5	-	0.025	-	-	-	76.3	-
	0.07	-	-	-	76.3	-	0.05	-	-	90.2	-	-
	0.14	-	-	93.8	-	-	0.125	-	98.8	-	-	-
Čurug	0.06	-	-	-	75.0	-	0.025	-	-		90.0	-
	0.07	-	-	-	70.0	-	0.05	-	-	91.7	-	-
	0.14	-	98.3	-	-	-	0.125	100	-		-	-

1 - highly sensitive population (E= 100%); **2** – sensitive (100 > E ≥95%);
3 - slightly resistant (95>E ≥90%); **4** - resistant (90>E ≥50%); **5** - highly resistant (E < 50%)

populations were resistant to fipronil label rate, yet higher application rates still caused increase in sensitivity.

Discussion

Sensitivity of three populations of CPB adults from the territory of Poland to chlorpyrifos monitored during three years (2008-2010), and classified according to resistance coefficient (RC), varied within the same population as shown in studies conducted by Wegorek et al. (2011). Results of this study indicate that the same population (Krotoszyn) in 2008 and 2010, in respect to RC was not resistant to chlorpyrifos, while in 2009 it showed slight resistance or some variation in sensitivity.

A number of authors (Zehnder and Gelernter, 1989, Zhao et al., 2000) found differences in sensitivity (LC50) between different stages of CPB to insecticides. According to Inđić (1994, 1997), toxicity of chlorpyrifos and cypermethrin to overwintered adults, adults and larvae of first generation (stage III) originating from the same population (Zmajevo) differed. LC_{50} values for chlorpyrifos were 761, 69 and 972 mg a.i./l respectively, and for cypermethrin 44, 55 and 2 mg a.i./l respectively, indicating heterogeneity in sensitivity among these life stages of CPB.

Results of Zamojska et al. (2011) showed that use of neonicotinoid compounds instead of pyrethroids had very good effect in terms of increased sensitivity to pyrethroids, which is a good example for antiresistance strategy.

Whalon and Ferro (1998) stated, which is also our opinion, that the introduction of neonicotinoid based insecticides in 1997, brought a relief in production areas where CPB had become resistant to other insecticides. However, first cases of resistance to neonicotinoid compounds (imidacloprid) were registered in commercial potato crops in several U.S. shortly after its introduction (Mota-Sanchez et al., 2006; Alyokhin et al., 2006). Alyokhin et al. (2007) noted a significant variability in neonicotinoid resistance between CPB populations. The authors recorded 37 fold higher resistance to imidacloprid, and 10 fold higher to thiamethoxam compared to sensitive, laboratory populations. Results in this paper also indicated that in tested populations resistance to thiamethoxam has occurred.

Results of Inđić et al. (2012) showed that from 15 monitored populations of CPB in Serbia, two were sensitive to label rate of chlorpyrifos, one was slightly resistant, 11 were resistant and one population was highly resistant. Concerning cypermethrin, two populations were sensitive, two slightly resistant, five were resistant and six highly resistant. Highly sensitive to thiamethoxam label rate were 12 populations, while three were sensitive. In the case of fipronil applied at label rate, two populations were highly sensitive, six sensitive, one slightly resistant and six were resistant.

Conclusions

Based on the results of screening test for comparison of insecticides (label rates) sensitivity of two Colorado potato beetle populations in 2008 and 2012 the following conclusions can be drawn:

During 2008 and 2012, both populations were resistant to chlorpyrifos;

During 2008, both populations were resistant, while in 2012, population from Vilovo was resistant and population from Čurug was slightly resistant to cypermethrin;

Both populations in 2008 were highly sensitive to thiamethoxam, while in 2012 they were resistant;

In 2008, population from Vilovo was slightly resistant and from Čurug was resistant, to fipronil, while in 2012 both populations were resistant.

Using the described screening test, a rapid evaluation of Colorado potato beetle sensitivity to insecticides is possible, and the obtained results are of great importance for further research, sophisticated work, and for general practice.

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