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Scientific paper

Screening Test for Detection of *Leptinotarsa decemlineata* (Say) Sensitivity to Insecticides

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SUMMARY

In 2009, the sensitivity of 15 field populations of Colorado potato beetle (*Leptinotarsa decemlineata* Say.) - CPB was assessed to chlorpyrifos, cypermethrin, thiamethoxam and fipronil, four insecticides which are mostly used for its control in Serbia. Screening test that allows rapid assessment of sensitivity of overwintered adults to insecticides was performed. Insecticides were applied at label rates, and two, five and 10 fold higher rates by soaking method (5 sec). Mortality was assessed after 72h. From 15 monitored populations of CPB, 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. The application of insecticides at higher rates (2, 5 and 10 fold), that is justified only in bioassays, provided a rapid insight into sensitivity of field populations of CPB to insecticides.

Keywords: *Leptinotarsa decemlineata*-CPB; Insecticides; Sensitivity; Resistance; Monitoring

INTRODUCTION

To ensure the expected yield in Serbia potato crops are treated with insecticides two to four times during vegetation (Zabel et al., 2000). The most harmful species in potato production is certainly *Leptinotarsa decemlineata* (Say) (Insecta: Coleoptera: Chrysomelidae), commonly known as Colorado Potato Beetle (CPB). The negative effect of pesticide application, besides on human health and the environment, is also reflected in occurrence of insect resistance to certain insecticides. 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 noted 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).

Heim et al. (1990) stated that there were differences in CPB sensitivity to insecticides even among populations originating from the same area under potato production, but as quoted by Inđić (1997) also between life stages and generations. In practice, the problem of resistance in our region is resolved in accordance with principles of integrated pest management. Until 2008 there was no organized CPB resistance monitoring in Serbia. Only after the Ministry of Agriculture of Serbia recognized and accepted the screening test, the sensitivity of 41 CPB populations to five insecticides (belonging to five chemical groups) was evaluated. In six populations 1-6% of overwintered adults survived the label rate of thiamethoxam; in 29 populations 1-50% of adults survived cypermethrin, 2-55% of adults survived chlorpyrifos in 35 populations, in 27 populations 2-41% adults survived fipronil applied at the mentioned rate, and only 3% of adults survived abamectin in one population (Inđić et al., 2009). Chlorpyrifos has been in use for nearly 40 years and cypermethrin about 30 in our country. Commercially available products on the basis of chlorpyriphos in Serbia are Pyrinex 48-EC and Kozma (480 g a.i./l), however for the same purpose four products which contain chlorpyriphos (500 g a.i./l)+cypermethrin (50 g a.i./l) and one product which contain chlorpyriphos (18 g a.i./l)+cypermethrin (2 g a.i./l) are also registered. On the basis of cypermethrin itself three products with 200 g a.i./l are available. Thiamethoxam (product Actara 25-WG; 250 g a.i./kg) and fipronil (product Regent 800-WG; 250 g a.i./kg) has been in use for more than 10 years, considering that recently thiamethoxam became the most frequently used insecticide in CPB control.

The aim of the study was the creation of simple and rapid test for detection of sensitivity levels of CPB to insecticides and confirmation of resistance, as well as providing simplified presentation of results. The method is based on assessment of sensitivity of overwintered adults to insecticides. It allows, on the one hand, quick formation (24-72h) of information network related to production regions and on these bases further establishment of strategy for rational use of insecticides in CPB control. On the other hand, the test provides an initial inventory and an insight in sensitivity of populations, so that further expensive, sophisticated (lc-p lines, LC_{50}, LC_{95}, LC_{99}, CR, tests at the molecular level), time consuming tests are only employed for high risk populations.

MATERIAL AND METHODS

The site selection

For monitoring of CPB sensitivity to insecticides 15 sites on the territory of Serbia were selected based on the advice of experts from Agricultural extension service, and were marked with GPS coordinates. The first ten sites (Stepanovićevo, Gložane, Žabalj, Sirig Šajkaš, Ljutovo, Šimuze, Biserno Ostrvo, Novo Miloševo, Kikinda) are located in the northern region of Serbia, where potato is produced on large areas but also in crofts. The remaining five sites (Pečenog, Vitkovac, Stapari, Makovište and Makovište II) are located in central Serbia at higher altitude compared to previous 10, in the region of intensive production of seed and mercantile potato.

Insecticides applied

Insecticides (Table 1) belonging to four chemical groups were used in this assay: organophosphates (chlorpyrifos), pyrethroids (cypermethrin), neonicotinoids (thiamethoxam) and pyrazoles (fipronil), which differ in history of the application.

Test insects

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

Table 1. Insecticides and rates applied in tests

Active ingredient (chemical group)	Product	Applied rate*
chlorpyrifos		1.5 l/ha – label rate
(480 g/l)	Pyrinex 48-EC	7.5 l/ha – 5 x higher
(organophosphate)		15 l/ha – 10 x higher
cypermethrin		0.3 l/ha – label rate
(200 g/l)	Cipkord 20-EC	1.5 l/ha – 5 x higher
(pyrethroid)		3 l/ha – 10 x higher
thiamethoxam		60 g/ha – label rate
(250 g/kg)	Actara 25-WG	70 g/ha – label rate
(neonicotinoid)		140 g/ha – 2 x higher
fipronil		25 g/ha – label rate
(800 g/kg)	Regent 800-WG	50 g/ha – 2 x higher
(pyrazole)		125 g/ha – 5 x higher

* Insecticides were applied in 400 l/ha

Toxicological experiment

Experiment was carried out during 2009 in laboratory conditions. Screening test was based on the assumption that reduced sensitivity to insecticides is already present in CPB populations i.e. that overwintered adults will respond with lower sensitivity to insecticide label rate which is defined as rate determined in field experiments during the registration process and found to cause 100% mortality. Therefore, application of label rates aimed to dissemble effects realized in the field, while the application of higher rates aimed to point to shares of population with reduced sensitivity to tested insecticides. The bioassay is based on the evaluation of the response of overwintered adults of CPB populations to insecticide label rates and higher rates (Inđić et al., 2006). Chlorpyrifos and cypermethrin, which have a long history of use, were applied at label rates, five and 10 fold higher rates, and thiamethoxam and fipronil at label rates and two and/or five fold higher rates. Insecticides were applied by insects soaking for 5 sec. The experiment was set up in four replicates with 30 adults per replication (sex ratio 1:1). The sex was determined according to Tribelj and Korol (2001) instructions. After insect soaking in spray liquid, the excess liquid was removed by transferring insects on filter paper. Subsequently, the insects were placed in polyethylene containers (V=1.4 l) with filter paper on the bottom and covered with perforated lid. During the trial, insects were kept at 23±2°C and regular photoperiod (16/8h), with no additional feeding. Insecticide effects were determined 24, 48 and 72h after application of insecticides. 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). Results were corrected for mortality in the control (Schneider-Orelli, 1947) and expressed as the efficacy (E%) achieved only after 72h. 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 AND DISCUSSION

In Serbia CPB is considered to be a synonym for the occurrence of resistance. Biology of CPB is thoroughly studied in our agro-ecological conditions, and regardless of global climate changes, according to present available data there are two generations per year. Overwintered adults and first generation of larvae damage potato plants from sprouting to flowering, while adults of first generation and second generation larvae damage plants from tuber formation phase until green leaves are present. For CPB control in Serbia there are 43 registered products, based on 20 active ingredients, out of which 18 are of chemical origin, divided into 10 groups (organophosphates, carbamates, pyrethroids, neonicotinoids, pyrazoles, benzoilfenilureas, benzoilureas, macrolides, semikarbazons, antaranil diamides) and two bioinsecticides.

Analyzing the efficacy of recommended application (label) rates of insecticides, we tended to simulate effects that persist in 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. The abovementioned indicate which part of CPB population is not sensitive, tolerates or demonstrates reduced sensitivity to the label rates, or indicates that individuals have developed resistance. 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.

The sensitivity of CPB populations to chlorpyrifos, cypermethrin, fipronil and thiamethoxam was classified (Table 2 and 3) based on the insecticide efficacy achieved in screening test (72h of exposure) and slightly modified scale for classification of insecticide sensitivity (Anonymous, 2009).

Out of 15 surveyed CPB populations, only two (Stapari and Makovište II) were sensitive to label rate of chlorpyrifos, while all others slightly to highly resistant. Namely, only one population from Makovište I was slightly resistant, while 11 were resistant, as well as one population from Stepanovićevo that showed high resistance to this insecticide. It is interesting that populations originating from the same locality (Makovište I and II), but from sites at different altitudes, expressed different sensitivity. Population from Makovište I was slightly resistant, which was confirmed by the efficacy

achieved with five fold higher rates of chlorpyrifos, while the population from Makovište II expressed sensitivity to this insecticide. Three populations (Ljutovo, B. Ostrvo and N. Miloševo) of 11 that were classified as resistant remained in the same category even when treated with higher rates of this insecticide. In four populations (Gložan, Žabalj, Šajkaš and Šimuže), a slight increase in sensitivity was registered when rates of chlorpyrifos were increased, thus these populations were reclassified from resistant to slightly resistant. Four populations (Sirig, Kikinda, Pečenog and Vitkovac) demonstrated increase in sensitivity ranging from resistant to highly sensitive, when higher rates of this insecticide were applied. Given indicates distinct heterogeneity in sensitivity of these populations to chlorpyrifos.

The most interesting population was the one originating from Stepanovićevo which expressed high resistance to label rates of chlorpyrifos, and resistance to five and 10 fold higher rates. Classifying the efficacy according to the abovementioned scale, susceptibility status is very visible within one CPB population.

According to Wegorek et al. (2011) sensitivity of CPB adults to chlorpyrifos, for three populations from the territory of Poland, monitored in three consecutive years (2008-2010), and classified according to resistance coefficient (RC), varies within the same population. 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.

Two (Pečenog and Stapari) of 15 CPB populations were sensitive, while 13 populations were classified as slightly to highly resistant to cypermethrin label rates. Two populations originating from Vitkovac and Makovište II were slightly resistant, five (Sirig, Ljutovo, Šimuže, Kikinda and Makovište I) were resistant and six populations (Stepanovićevo, Gložane, Žabalj, Šajkaš, B. Ostrvo and N. Miloševo) were highly resistant to cypermethrin. One population (Ljutovo) of 13 that were classified as resistant remained in the same category even after cypermethrin was applied at 5 and 10 fold higher rates. Adults from populations (Stepanovićevo, B. Ostrvo and N. Miloševo) expressed slight increase in sensitivity when cypermethrin was applied at higher rates, therefore, regardless the applied rate, they were classified as resistant to highly resistant. Adults of two populations originating from Gložane and Žabalj, compared to the previous three, showed slightly higher heterogeneity in sensitivity, thus depending on the applied rate were classified as resistant to slightly resistant. Seven populations (Sirig, Šajkaš, Šimuže, Kikinda, Vitkovac, Makovište I and II) also expressed heterogeneity in sensitivity among adults, depending on the amount of cypermethrin, and were classified as resistant to highly sensitive.

The history of cypermethrin use in Serbia is shorter than of chlorpyrifos that has been in use for about 40 years. Nevertheless, more CPB populations developed resistance to cypermethrin, mostly in the northern region, compared to central parts of Serbia. A number of authors present information about the differences in sensitivity (LC_{50}) of different stages of CPB to insecticides (Zehnder and Gelernter, 1989; Zhao et al., 2000). According to Inđić (1994, 1997), the 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 the replacement of pyrethroids with neonicotinoid compounds had very good effect in terms of increased sensitivity to pyrethroids, which is a good example for antiresistance strategy.

CPB sensitivity to fipronil and thiamethoxam is shown in Table 3. These two insecticides are, unlike the previous once, in use for shorter period, over 10 years, and were introduced almost at the same time. Given the mentioned facts, and the results of a survey conducted in pesticides markets (Klokočar et al., 2006), we used thiamethoxam at label rates (0.06 and 0.07 kg/ha) and two fold higher (0.14 kg/ha), while the product based on fipronil was also applied at label rate (0.025 kg/ha), two and five fold higher (0.05 and 0.125 kg/ha), assuming that CPB sensitivity to these compounds has not yet been compromised.

Ten CPB populations of 15 demonstrated high sensitivity to recommended application (label) rate of thiamethoxam. Efficacy of thiametoxam was 97.4-99.1% in four populations originating from Ljutovo, Pečenog, B. Ostrvo and N. Miloševo, and they were evaluated as sensitive, while only one population (Stepanovićevo) could be considered sensitive to slightly resistant having in mind that achieved efficacy was 90.5-98.2% regardless of the pesticide rates.

CPB population from Stepanovićevo originated from area under intensive potato production, and continuous chemical control. Hence, one should consider the possible application of other neonicotinoid compounds that might have contributed to slight decrease in sensitivity of this population compared to others.

Table 2. Sensitivity of overwintered CPB adults to chlorpyrifos and cypermethrin, scale 1-5

Sites	Kg;	Efficacy (%) of chlorpyrifos					Kg;	Efficacy (%) of cypermethrin				
	l/ha	1	2	3	4	5	l/ha	1	2	3	4	5
Stepanovićevo	1.5	-	-	-	-	41.7	0.3	-	-	-	-	34.7
	7.5	-	-	-	75.6	-	1.5	-	-	-	72.1	-
	15	-	-	-	77.4	-	3.0	-	-	-	83.4	-
Gložane	1.5	-	-	-	82.8	-	0.3	-	-	-	-	43.2
	7.5	-	-	91.9	-	-	1.5	-	-	-	69.3	-
	15	-	-	93.7	-	-	3.0	-	-	93.6	-	-
Žabalj	1.5	-	-	-	71.6	-	0.3	-	-	-	-	33.1
	7.5	-	-	92.5	-	-	1.5	-	-	-	56.6	-
	15	-	-	91.6	-	-	3.0	-	-	91.5	-	-
Sirig	1.5	-	-	-	88.5	-	0.3	-	-	-	61.4	-
	7.5	-	-	93.8	-	-	1.5	-	-	92.6	-	-
	15	-	96.8	-	-	-	3,0	100	-	-	-	-
Šajkaš	1.5	-	-	-	84.3	-	0.3	-	-	-	-	39.9
	7.5	-	-	-	87.2	-	1.5	-	-	-	65.2	-
	15	-	-	92.1	-	-	3.0	-	97.3	-	-	-
Ljutovo	1.5	-	-	-	52.5	-	0.3	-	-	-	57.5	-
	7.5	-	-	-	82.5	-	1.5	-	-	-	78.3	-
	15	-	-	-	79.2	-	3.0	-	-	-	89.2	-
Šimuze	1.5	-	-	-	85.1	-	0.3	-	-	-	58.5	-
	7.5	-	-	90.4	-	-	1.5	-	-	-	86.2	-
	15	-	-	94.7	-	-	3.0	-	98.9	-	-	-
Biserno Ostrvo	1.5	-	-	-	54.7	-	0.3	-	-	-	-	37.3
	7.5	-	-	-	73.9	-	1.5	-	-	-	54.7	-
	15	-	-	-	81.7	-	3.0	-	-	-	84.3	-
Novo Miloševo	1.5	-	-	-	50.9	-	0.3	-	-	-	-	39.7
	7.5	-	-	-	81.9	-	1.5	-	-	-	72.4	-
	15	-	-	-	80.2	-	3.0	-	-	-	79.3	-
Kikinda	1.5	-	-	-	88.3	-	0.3	-	-	-	61.7	-
	7.5	-	97.7	-	-	-	1.5	-	-	-	89.2	-
	15	-	98.8	-	-	-	3.0	-	98.8	-	-	-
Pečenog	1.5	-	-	-	87.4	-	0.3	-	95.3	-	-	-
	7.5	-	-	91.6	-	-	1.5	-	98.3	-	-	-
	15	100	-	-	-	-	3.0	100	-	-	-	-
Vitkovac	1.5	-	-	-	88.3	-	0.3	-	-	91.6	-	-
	7.5	100	-	-	-	-	1.5	100	-	-	-	-
	15	100	-	-	-	-	3.0	100	-	-	-	-
Stapari	1.5	-	98.3	-	-	-	0.3	-	96.6	-	-	-
	7.5	-	98.3	-	-	-	1.5	-	99.1	-	-	-
	15	100	-	-	-	-	3.0	100	-	-	-	-
Makovište I	1.5	-	-	93.3	-	-	0.3	-	-	-	78.3	-
	7.5	-	-	92.4	-	-	1.5	-	99.1	-	-	-
	15	-	98.3	-	-	-	3.0	100	-	-	-	-
Makovište II	1.5	-	95.8	-	-	-	0.3	-	-	94.9	-	-
	7.5	-	99.1	-	-	-	1.5	-	96.6	-	-	-
	15	-	97.4	-	-	-	3.0	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%)

Table 3. Sensitivity of overwintered CPB adults to thiamethoxam and fipronil, scale 1-5

Locality	Kg; l/ha	Efficacy (%) of thiamethoxam					Kg; l/ha	Efficacy (%) of fipronil				
		1	2	3	4	5		1	2	3	4	5
Stepanovićevo	0.06	-	98.2	-	-	-	0.025	-	-	-	87.8	-
	0.07	-	-	90.5	-	-	0.05	-	-	93.0	-	-
	0.14	-	98.2	-	-	-	0.125	-	98.2	-	-	-
Gložane	0.06	100	-	-	-	-	0.025	-	99.1	-	-	-
	0.07	100	-	-	-	-	0.05	100	-	-	-	-
	0.14	100	-	-	-	-	0.125	100	-	-	-	-
Žabalj	0.06	100	-	-	-	-	0.025	-	-	92.5	-	-
	0.07	100	-	-	-	-	0.05	-	98.3	-	-	-
	0.14	100	-	-	-	-	0.125	100	-	-	-	-
Sirig	0.06	100	-	-	-	-	0.025	-	-	-	82.1	-
	0.07	100	-	-	-	-	0.05	-	96.9	-	-	-
	0.14	100	-	-	-	-	0.125	100	-	-	-	-
Šajkaš	0.06	100	-	-	-	-	0.025	-	96.4	-	-	-
	0.07	100	-	-	-	-	0.05	-	99.1	-	-	-
	0.14	100	-	-	-	-	0.125	100	-	-	-	-
Ljutovo	0.06	100	-	-	-	-	0.025	-	-	-	85.0	-
	0.07	-	98.3	-	-	-	0.05	-	95.8	-	-	-
	0.14	-	98.3	-	-	-	0.125	-	96.7	-	-	-
Šimuže	0.06	100	-	-	-	-	0.025	-	95.7	-	-	-
	0.07	100	-	-	-	-	0.05	-	-	95.0	-	-
	0.14	100	-	-	-	-	0.125	100	-	-	-	-
Biserno Ostrvo	0.06	-	99.1	-	-	-	0.025	-	-	-	83.5	-
	0.07	-	98.2	-	-	-	0.05	-	-	93.9	-	-
	0.14	100	-	-	-	-	0.125	-	-	-	82.6	-
Novo Miloševo	0.06	-	99.1	-	-	-	0.025	-	-	-	87.9	-
	0.07	-	97.4	-	-	-	0.05	-	-	90.5	-	-
	0.14	100	-	-	-	-	0.125	-	-	93.9	-	-
Kikinda	0.06	100	-	-	-	-	0.025	-	95.7	-	-	-
	0.07	100	-	-	-	-	0.05	100	-	-	-	-
	0.14	100	-	-	-	-	0.125	100	-	-	-	-
Pečenog	0.06	100	-	-	-	-	0.025	-	96.6	-	-	-
	0.07	-	98.3	-	-	-	0.05	-	98.3	-	-	-
	0.14	100	-	-	-	-	0.125	100	-	-	-	-
Vitkovac	0.06	100	-	-	-	-	0.025	100	-	-	-	-
	0.07	100	-	-	-	-	0.05	100	-	-	-	-
	0.14	100	-	-	-	-	0.125	100	-	-	-	-
Stapari	0.06	100	-	-	-	-	0.025	-	98.3	-	-	-
	0.07	100	-	-	-	-	0.05	100	-	-	-	-
	0.14	100	-	-	-	-	0.125	100	-	-	-	-
Makovište I	0.06	100	-	-	-	-	0.025	-	-	-	88.3	-
	0.07	100	-	-	-	-	0.05	-	96.6	-	-	-
	0.14	100	-	-	-	-	0.125	100	-	-	-	-
Makovište II	0.06	100	-	-	-	-	0.025	100	-	-	-	-
	0.07	100	-	-	-	-	0.05	100	-	-	-	-
	0.14	100	-	-	-	-	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)

Whalon and Ferro (1998) state, 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 the commercial potato crops in several U.S. states shortly after its introduction (Alyokhin et al., 2006; Mota-Sanchez et al., 2006). Alyokhin et al. (2007) noted a significant variability in neonicotinoid resistance between CPB populations. These authors recorded 37 fold higher resistance to imidacloprid, and 10 fold higher to thiamethoxam compared to sensitive, laboratory populations. LC_{50} values were significantly higher for imidacloprid, indicating higher toxicity of thiamethoxam to CPB. In production, especially in the northern region of Serbia, efficacy of thiametoxam, is so far satisfactory, although neonicotinoid based products are used for tubers treatment before planting (imidacloprid) and for foliar application, but there are certain restrictions in accordance with IPM.

Similar to chlorpyrifos and cypermethin, the presence of different levels of resistance between 2nd stage larvae and adults was also reported for imidacloprid by Zhao et al. (2000). Hence, the sensitivity of one insect life stage does not necessarily reflect the sensitivity level of the whole population, nor can predict the effectiveness of insecticides in the field (Pourmirza, 2005).

When fipronil was applied at label rate, two (Vitkovac and Makovište II) from 15 populations of CPB were highly sensitive, and regardless of the applied rates the efficacy was 100%. Six populations (Gložan, Šajkaš, Šimuže, Kikinda, Pečenog and Stapari) were sensitive, while one population (Žabalj) was slightly resistant and six resistant (Stepanovićevo, Sirig, Ljutovo, B. Ostrvo, N. Miloševo and Makovište I). Highly resistant populations to fipronil were not registered. In two populations (B. ostrvo and N. Miloševo) at higher application rates of fipronil, a slight increase in sensitivity was registered, and regardless of the applied rate, those populations were classified from resistant to slightly resistant. Five populations (Stepanovićevo, Žabalj, Sirig, Ljutovo and Makovište I), expressed high heterogeneity in sensitivity, depending on the application rates of fipronil and they were classified from resistant to highly sensitive.

The results obtained in screening test, based on the sensitivity of overwintered adults of CPB to insecticides are useful in rational selection of products to be used against CPB larvae which are about to occur in the vegetation, and for excluding insecticides that adults are less susceptible / resistant to, and choosing the most effective once. This test can be performed in scant experimental conditions, with no special requirements for specific equipment. Handling procedures are very simple and the results achieved are evident and do not require particular statistical analysis, aside from precise classification on the scale from 1 to 5. Overwintered adults are for a number of reasons chosen as test insect in the assessment of sensitivity to insecticides. First, it is familiar that they were not in direct contact with insecticides, and are easily maintained before inclusion in the test, they do not have to be fed, and the effects of insecticides (mortality, paralysis, vitality) are easily visible and measurable.

Varying sensitivity of CPB populations can be observed from several directions. "Horizontal" variation represents different sensitivity among populations, while „vertical" oscillations are in a function of time (between years and observation periods), and are crucial for rational strategy of insecticide application, and this is where the presented test could be employed. The mentioned test could also be a useful tool in detection of differences between sensitivity to insecticides of larval stages of CPB which presents the third "biological aspect" of CPB sensitivity.

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Skrining test u detekciji osetljivosti krompirove zlatice na insekticide

REZIME

Tokom 2009. godine ispitana je osetljivost 15 poljskih populacija krompirove zlatice (*Leptinotarsa decemlineata* Say.) - CPB na insekticide. Ispitivanje je izvedeno screening testom koji je omogućio brzu procenu osetljivosti prezimelih imaga na insekticide. Ocenjena je osetljivost na četiri insekticida (hlorpirifos, cipermetrin, tiametoksam i fipronil), koji se najčešće primenjuju u suzbijanju CPB u Srbiji. Insekticidi su primenjeni u preporučenoj, dva, pet i 10 puta većim količinama od preporučene, metodom potapanja (5 sec). Smrtnost insekata je određena posle 72h. Od 15 ispitanih populacija CPB, na količinu za praktičnu primenu hlorpirifosa samo su dve osetljive, jedna je blago rezistentna, 11 je rezistentno i jedna je visokorezistentna. Dve populacije su osetljive, dve blago rezistentne, pet je rezistentno i šest visokorezistentno na cipermetrin. Visokoosetljivo je 12 populacija na količinu za praktičnu primenu tiametoksama, a tri su osetljive. Na fipronil, dve su visokoosetljive populacije, šest je osetljivo, jedna blago rezistentna i šest je rezistentno. Primenom povećanih količina insekticida (2, 5 i 10 puta) od preporučenih, što opravdava samo metoda biotesta, došlo se brzo do saznanja o osetljivosti poljskih populacija CPB na insekticide.

Ključne reči: *Leptinotarsa decemlineata* – CPB; insekticidi; osetljivost; otpornost; praćenje