

## POSSIBILITY OF QUICK DETECTION OF *Leptinotarsa decemlineata* (Say) SENSITIVITY TO INSECTICIDES

Dušanka INI<sup>1</sup>, Slavica VUKOVI<sup>2</sup>, Sonja GVOZDENAC<sup>3</sup>, Tatjana KERESI<sup>4</sup>,  
Snežana TANASKOVI<sup>5</sup>

<sup>1,2,3,4</sup> University of Novi Sad, Faculty of Agriculture, Novi Sad, Serbia

<sup>5</sup> Faculty of Agronomy, Čačak, Serbia

### ABSTRACT

The sensitivity of 10 field populations of Colorado potato beetle (*Leptinotarsa decemlineata* Say.) - CPB (Žednik, Vrbas, Miki evo, Ljutovo, Turin, Alibunar, Banatski Brestovac, Gloganj, Karesvo and Pan evo) was determined in laboratory conditions. Bioassay was performed using screening test that allows rapid assessment of sensitivity of overwintered adults to insecticides. Sensitivity towards four most commonly used insecticides in CPB control in Serbia (chlorpyrifos, cypermethrin, thiamethoxam and fipronil) was assessed. Insecticides were applied by soaking method at label rates, and two, five and 10 fold higher rates. Insect mortality was assessed after 72 h. Sensitivity of CPB adults was determined according to modified IRAC method (2009) using 1-5 scale (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%/). Out of 10 monitored populations of CPB, one was highly sensitive, four were sensitive, three slightly resistant and two resistant to chlorpyrifos applied at label rate. Three populations were highly sensitive, three sensitive and four slightly resistant to cypermethrin. To thiamethoxam applied label rate, six populations were highly sensitive, one was sensitive and two slightly resistant. Regarding fipronil, two populations were highly sensitive, five were sensitive, one was slightly resistant and two were resistant.

**Key words:** Colorado potato beetle, sensitivity, insecticides

### 1 INTRODUCTION

The most harmful pest in potato production is certainly *Leptinotarsa decemlineata* (Say), commonly known as Colorado Potato Beetle (CPB). To ensure the expected yield in Serbia potato crops are treated with insecticides two to four times during vegetation. However, the negative effect of pesticide application, besides on human health and the environment, is also reflected in occurrence of insect resistance to certain insecticides. Literature data indicate that CPB resistance was noted for 42 insecticides, belonging to different chemical groups (organophosphates, carbamates, pyrethroids). According to several authors (Stanković *et al.*, 2004; Inić *et al.*, 2006), CPB resistance to carbamates and organophosphates, as well as to pyrethroids (Inić, 1997; Perić *et al.*, 1997) was registered in a number of populations in Serbia.

<sup>1</sup> prof. dr., Trg Dositeja Obradovića 8, 21000 Novi Sad, Serbia

<sup>2</sup> assist. prof., *ibid.*; e-mail: vukovic@polj.uns.ac.rs

<sup>3</sup> mag., *ibid.*

<sup>4</sup> prof. dr., *ibid.*

<sup>5</sup> assist. prof., Cara Dušana 34, 32000 Čačak, Serbia

This fact justifies CPB ranking among 10 species that most rapidly develop resistance (Mota-Sanchez *et al.*, 2006; Whalon *et al.*, 2008). 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 a simplified presentation of the results. The method is based on the assessment of sensitivity of overwintered adults to insecticides. It allows quick formation (24-72 h) of information network related to CPB sensitivity in different production regions, and further establishment of strategy for rational use of insecticides in CPB control.

## **2 MATERIAL AND METHODS**

### **2.1 The site selection**

For monitoring of CPB sensitivity to insecticides 10 sites on the territory of Serbia (Žednik, Vrbas, Miki evo, Ljutovo, ur in, Alibunar, Banatski Brestovac, Gloganj, Ka arevo and Pan evo) were selected based on the advice of experts from Agricultural extension service, and were marked with GPS coordinates.

### **2.2 Applied 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).

### **2.3 Test insect**

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 usual photoperiod (16/8h).

### **2.4 Toxicological experiment**

Bioassay was based on the assumption that CPB populations have reduced sensitivity to insecticides, and on evaluation of the response of overwintered 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 5fold 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). Assessment of insecticide efficacy consisted of counting the number of dead, paralyzed and alive, 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 (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%/), which was created as a slight modification of IRAC method No. 011 (Anonymous, 2009) that refers to pollen beetles (*Meligethes* spp.).

### 3 RESULTS AND DISCUSSION

Analyzing the efficacy of recommended application (label) rates of insecticides, we tended to simulate conditions and effects that persist in the 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 sensitivity of CPB populations to chlorpyrifos, cypermethrin, thiamethoxam and fipronil was classified (Table 1 and 2) based on the insecticide efficacy achieved in screening test (72 h of exposure) and slightly modified scale for classification of insecticide sensitivity (Anonymous, 2009).

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
Žednik	1.5	-	-	-	66.9	-	0.3	-	-	87.1	-	-
	7.5	-	-	92.6	-	-	1.5	-	99.1	-	-	-
	15	-	-	-	88.9	-	3.0	100	-	-	-	-
Vrbas	1.5	-	-	90.2	-	-	0.3	-	97.3	-	-	-
	7.5	-	99.1	-	-	-	1.5	100	-	-	-	-
	15	100	-	-	-	-	3.0	100	-	-	-	-
Miki evo	1.5	-	-	92.9	-	-	0.3	-	95.6	-	-	-
	7.5	-	-	92.9	-	-	1.5	100	-	-	-	-
	15	-	98.2	-	-	-	3.0	100	-	-	-	-
Ljutovo	1.5	-	98.1	-	-	-	0.3	-	-	93.6	-	-
	7.5	100	-	-	-	-	1.5	-	97.2	-	-	-
	15	100	-	-	-	-	3.0	-	99.1	-	-	-
ur in	1.5	-	-	-	77.5	-	0.3	-	-	93.3	-	-
	7.5	-	-	-	90.0	-	1.5	-	99.2	-	-	-
	15	-	-	90.8	-	-	3.0	100	-	-	-	-
Alibunar	1.5	-	98.3	-	-	-	0.3	-	-	94.9	-	-
	7.5	100	-	-	-	-	1.5	100	-	-	-	-
	15	100	-	-	-	-	3.0	100	-	-	-	-
Banatski Brestovac	1.5	-	95.8	-	-	-	0.3	100	-	-	-	-
	7.5	100	-	-	-	-	1.5	100	-	-	-	-
	15	100	-	-	-	-	3.0	100	-	-	-	-
Gloganj	1.5	100	-	-	-	-	0.3	100	-	-	-	-
	7.5	100	-	-	-	-	1.5	100	-	-	-	-
	15	100	-	-	-	-	3.0	100	-	-	-	-
Ka arevo	1.5	-	-	94.1	-	-	0.3	-	99.0	-	-	-
	7.5	-	96.6	-	-	-	1.5	100	-	-	-	-
	15	-	99.1	-	-	-	3.0	100	-	-	-	-
Pan evo	1.5	-	96.6	-	-	-	0.3	100	-	-	-	-
	7.5	100	-	-	-	-	1.5	100	-	-	-	-
	15	100	-	-	-	-	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%)

Out of 10 surveyed CPB populations only one (Gloganj) was highly sensitive to label rate of chlorpyrifos, while four (Ljutovo, Alibunar, B. Brestovac, Pan evo) were sensitive, three (Vrbas, Miki evo, Ka arevo) were slightly resistant and two (Žednik, ur in) were resistant. Namely,

only three out of 10 populations (Žednik, Miki evo and ur in) that were classified as resistant remained in the same category even when treated with higher rates of this insecticide. According to Wegorek *et al.* (2011) the sensitivity of CPB adults to chlorpyrifos, monitored in three consecutive years varied within the same population, and the one from Krotoszyn (Poland) in 2008 and 2010 was not resistant to chlorpyrifos, while in 2009 it showed slight resistance. In this work, three (B. Brestovac, Gloganj, Pan evo) out of 10 CPB populations were highly sensitive to cypermethrin label rates. Three populations (Vrbas, Miki evo, Ka arevo) were sensitive, while four populations originating from Žednik, Ljutovo, ur in and Alibunar were slightly resistant to cypermethrin. All populations CPB expressed slight increase in sensitivity when cypermethrin was applied at higher rates, therefore they were classified as sensitive or highly sensitive.

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
Žednik	0.06	-	97.2	-	-	-	0.025	100	-	-	-	-
	0.07	100	-	-	-	-	0.05	100	-	-	-	-
	0.14	100	-	-	-	-	0.125	100	-	-	-	-
Vrbas	0.06	-	-	92.9	-	-	0.025	-	98.2	-	-	-
	0.07	100	-	-	-	-	0.05	-	98.2	-	-	-
	0.14	100	-	-	-	-	0.125	100	-	-	-	-
Miki evo	0.06	-	-	94.7	-	-	0.025	-	98.2	-	-	-
	0.07	100	-	-	-	-	0.05	100	-	-	-	-
	0.14	100	-	-	-	-	0.125	100	-	-	-	-
Ljutovo	0.06	100	-	-	-	-	0.025	-	99.1	-	-	-
	0.07	100	-	-	-	-	0.05	-	99.1	-	-	-
	0.14	100	-	-	-	-	0.125	100	-	-	-	-
ur in	0.06	-	-	91.7	-	-	0.025	-	-	92.5	-	-
	0.07	100	-	-	-	-	0.05	100	-	-	-	-
	0.14	100	-	-	-	-	0.125	100	-	-	-	-
Alibunar	0.06	100	-	-	-	-	0.025	-	-	-	87.4	-
	0.07	100	-	-	-	-	0.05	-	-	91.8	-	-
	0.14	100	-	-	-	-	0.125	100	-	-	-	-
Banatski Brestovac	0.06	100	-	-	-	-	0.025	-	-	-	86.5	-
	0.07	100	-	-	-	-	0.05	-	-	-	79.7	-
	0.14	100	-	-	-	-	0.125	100	-	-	-	-
Gloganj	0.06	100	-	-	-	-	0.025	-	98.0	-	-	-
	0.07	100	-	-	-	-	0.05	-	98.0	-	-	-
	0.14	100	-	-	-	-	0.125	100	-	-	-	-
Ka arevo	0.06	100	-	-	-	-	0.025	100	-	-	-	-
	0.07	100	-	-	-	-	0.05	-	99.1	-	-	-
	0.14	100	-	-	-	-	0.125	100	-	-	-	-
Pan evo	0.06	100	-	-	-	-	0.025	-	96.2	-	-	-
	0.07	100	-	-	-	-	0.05	-	97.1	-	-	-
	0.14	100	-	-	-	-	0.125	-	97.1	-	-	-

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%)

CPB sensitivity to thiamethoxam and fipronil is shown in Table 2. Six of 10 CPB populations demonstrated high sensitivity to recommended application (label) rate of thiamethoxam. One

population (Žednik) was classified as sensitive and three populations were slightly resistant to thiamethoxam. The increase in thiamethoxam application rate caused slight increase in sensitivity of all populations. When fipronil was applied at label rate, two (Vitkovac and Makovište II) out of 10 CPB populations were highly sensitive, and regardless on the applied rates the efficacy was 100%. Five populations were sensitive, while one population was slightly resistant and two were resistant (Alibunar, B. Brestovac). Highly resistant populations to fipronil were not registered. Three populations (ur in, Alibunar, B. Brestovac), expressed heterogeneity in sensitivity, depending on the application rates of fipronil and they were classified from resistant to highly sensitive.

#### 4 CONCLUSIONS

Based on the results of screening test on sensitivity of 10 CPB populations from Serbia to insecticides (label rates) it can be conclude that one population was highly sensitive, four were sensitive, three were slightly resistant and two were resistant to chlorpyrifos; Three populations were highly sensitive, three sensitive, and four were slightly resistant to cypermethrin; Six populations were highly sensitive, one was sensitive and three were slightly resistant to thiamethoxam; Two populations were highly sensitive, five were sensitive, one was slightly resistant and two were resistant to fipronil.

#### 5 ACKNOWLEDGEMENT

The research was conducted within the project III 46008, funded by the Ministry of Education and Science of the Republic of Serbia.

#### 6 REFERENCES

- Anonymous, 2009. IRAC Susceptibility Test Methods Series, Version 3, Method No. 011.
- Inić, D. 1997. Effect of joint action of insecticides on colorado potato beetle (*Leptinotarsa decemlineata* Say). Review of Research Work at the Faculty of Agriculture, Belgrade, 42 (1): 7-22.
- Inić, D., Vuković, S. and Klokošar-Šmit, Z. 2006. Rapid detection of Colorado potato beetle (*Leptinotarsa decemlineata* Say) sensitivity to insecticides. XVII Czech and Slovak Plant Protection Conference, Prague, Czech, 2006, pp. 459-464.
- Inić, D., Vuković, S., Tanasković, S., Grahovac, M., Kerešić, T., Gvozdenac, S. and Savić-Petrić, S. 2012. Screening Test for detection of *Leptinotarsa decemlineata* (Say) sensitivity to insecticides. Pesticides & Phytomedicine, 27 (1), 59-67.
- Mota-Sanchez D., Hollingworth, R.M., Grafius, E.J. and Moyer, D.D. 2006. Resistance and cross-resistance to neonicotinoid insecticides and spinosad in the Colorado potato beetle, *Leptinotarsa decemlineata* (Say) (Coleoptera: Chrysomelidae). Pest Management Science, 62: 30-37.
- Perić, I., Miloševski, N. and Kljajić, P. 1997. Insecticide susceptibility of the Colorado potato beetle in the vicinity of Belgrade, Yugoslavia. Acta Horticulturae, 462: 983-990.
- Schneider-Orelli, O. 1947. Entomologisches Praktikum. Aufl.- Aarau.
- Stanković, S., Zabel, A., Kostić, M., Manojlović, B. and Rajković, S. 2004. Colorado potato beetle (*Leptinotarsa decemlineata* Say) resistance to organophosphates and carbamates in Serbia. Journal of Pest Science, 77(1): 11-15.
- Whalon M.E., Hollingworth, R.M. and Mota-Sanchez, D. 2008. The MSU database of pesticide resistance. <http://www.pesticideresistance.org>.
- Wegorek P., Zamojska, J. and Mrowczyński, M. 2011. Susceptibility level of the colorado potato beetle (*Leptinotarsa decemlineata* Say) to chlorpyrifos, and acetamiprid in Poland and resistance mechanisms of the pest to chlorpyrifos. Journal of Plant Protection Research, 51 (3): 279-284.