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Comparative Efficacy of Classical and Biorational Insecticides on Sugar Beet Weevil, *Bothynoderes punctiventris* Germar (Coleoptera: Curculionidae)

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Abstract

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The contact and ingestion activity and the potential of the insecticide spinosad for the control of sugar beet weevil were evaluated and compared with commercially used insecticides lambda-cyhalothrin and chlorpyriphos + cypermethrin. Results of three laboratory trials proved very good efficacy of spinosad applied at the dose of 72 g a.i./ha. Its efficacy was similar to chlorpyriphos + cypermethrin and significantly higher than that of lambda-cyhalothrin. Due to its favourable eco-toxicological properties and good ingestion activity, spinosad is a good candidate to be introduced in the integrated pest management strategy against beet weevil.

Keywords: *Bothynoderes punctiventris*; biorational insecticides; contact activity; ingestion activity; spinosad; sugar beet

Sugar beet (*Beta vulgaris* var. *saccharifera* Alef.) cultivation in Croatia has increased from 21 000 to 27 000 ha in the past five years (Statistical Yearbook of Republic of Croatia 2012). In Croatia, sugar beet has been sown since 1905. It is a profitable but highly demanded field crop (Pospišil 2010). Sugar beet plants are the most sensitive to pests at early emergence stage and when developing the first 3–4 pairs of leaves. If we prevent damage by any kind of pests in this period, we would ensure significantly higher yield and sugar content compared to fields in which the damage has not been prevented (Čamprag 1973; Bažok 2010; Pospišil 2010). Adult weevils are chewing sugar beet plants in early spring and may cause the complete destruction of plants. Čamprag (1973) listed 47 species of weevils attacking sugar beet plants. However, the most harmful are sugar beet weevil (*Bothynoderes punctiventris* Germar, 1824), black sugar beet weevil (*Psallidium maxillosum* [F.]), alfalfa snout beetle (or weevil) (*Otiorhynchus ligustici* L.) and maize leaf weevil (*Tanymechus dilaticollis* Gyll.). The first mass attack of sugar beet weevil in Croatia was recorded in Osijek, Vukovar and Vinkovci in 1922 (Kovacevic 1929), and a high occurrence of pests was recorded from 1925 to 1931. From 1965 through the early 2000s, the beet weevil was an important pest in the Vojvodina region but not in Croatia. In eastern Croatia, the population of the pests was below the economic threshold until 2008 (Bažok *et al.* 2012). In the last seven years the sugar beet weevil population has been regularly very high causing serious damage. One cause is global climate change, high temperatures, which have also been suggested for Ukraine by Fedorenko (2006) and for Vojvodina (Vuković *et al.* 2014). Bažok *et al.* (2012) concluded that besides the changes in climatic conditions, the high population density of weevils is a result of the change in the pest control practices. Compared to insecticides used in the 1980s, new insecticides used for the control of soil pests and

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for the control of flea beetle have very weak side effects on sugar beet weevils. Additionally, an increase in the cultivation area of sugar beet contributes to creating the preconditions for sugar beet weevil becoming the most important pest of sugar beet, as it is the case in neighbouring countries (Čamprag 1983; Vuković *et al.* 2014).

Pest control is mainly based on the use of insecticides (Sekulić *et al.* 1997). Chlorinated hydrocarbons (Čamprag 1986), organic phosphorus (OP) insecticides (Radin 1983), and pyrethroids (P) in combination with organophosphorus insecticides (Bažok *et al.* 2012) have been used with varying degrees of success. Due to the implementation of EU pesticide legislation, a number of active ingredients allowed for the sugar beet weevil control in Croatia has been reduced in the last ten years. Currently, three insecticides based on four active ingredients are allowed for the sugar beet weevil control: lambda-cyhalothrin (Karate Zeon, Syngenta), combination of chlorpyriphos and cypermethrin (Chromorel D, Agriphar), and acetamiprid (Mospilan, Nippon) (Bažok 2015). Allowed active ingredients belong to the group of OP insecticides (chlorpyriphos), pyrethroids (lambda-cyhalothrin and cypermethrin), and neonicotinoids (acetamiprid). There is an intention in the European Union to limit the use of all these insecticides in the future.

Due to the specific morphological structure of weevils, their large feeding capacity and the small leaf area of plants at the time of insecticide application, even allowed insecticides often give very poor results and require repeated treatment (Bažok *et al.* 2012), which is not in accordance with the principles of Integrated Pest Management (IPM) nor with the rational use of pesticides in modern agriculture.

Spinosad is a novel mode-of-action selective biological insecticide (Saunders & Bret 1997) with proved efficacy for controlling a wide range of pests including Lepidoptera, Diptera, Thysanoptera, Coleoptera, Orthoptera, and Hymenoptera (Sparks *et al.* 1995). This pesticide is obtained from the soil-borne bacteria, *Saccharopolyspora spinosa* (Mertz & Yao 1990), by fermentation and contains two active spinosoids: spinosyn A and spinosyn D (at an approximate ratio of 17 : 3) (Mertz & Yao 1990). Spinosad exerts its toxic effects on insects by affecting their nicotinic acetylcholine (nAChRs) and gamma-aminobutyric acid (GABA) receptors (Salgado 1997) and also inhibits acetylcholinesterase (Rabea *et al.* 2010). It has been used for the control of various agricultural and veterinary pests in many countries. Spinosad has low toxicity to mammals and fish, and it has been reported to exert relatively minimal effects on beneficial insects (Cleveland *et al.* 2002; Thomas & Mangam 2005; Ruiz *et al.* 2008; Mangan & Moreno 2009; Urbaneja *et al.* 2009). This makes it suitable for application in urban areas and integrated pest management programs (Cleveland 2007).

Thus, the objective of the study was to evaluate the contact and digestive toxicity of the insecticide spinosad on sugar beet weevil and to evaluate its potential for the control of this pest in comparison with currently used insecticides.

MATERIAL AND METHODS

Laboratory trials were set up in 2014 and 2015 with weevils collected in old sugar beet fields in the vicinity of Tovarnik (east Croatia, latitude 45°11'33.5''N, longitude 19°07'21.2''E and altitude 89 m a.s.l.). Collected adults were kept in entomological cages for five days until they were used in the test, without additional feeding during storage and without previous contact with insecticides. Sugar beet plants were grown in a laboratory from untreated seeds of the Artus variety (Strube International).

Application of insecticides. In three experiments (two in 2014 and one in 2015) the contact, digestive, and combined efficacy of three insecticides was compared. Widely used pyrethroid insecticides (lambda-cyhalothrin, Karate Zeon, Syngenta) and combination of pyrethroid and organophosphorus insecticides (chlorpyriphos and cypermethrin, Chromorel D, Agriphar) were compared with the biorational insecticide spinosad (Laser 240 SC). Investigated insecticides and doses are shown in Table 1. Each insecticide was evaluated for contact, digestive, and combined action separately.

Plastic cups (ø 11 cm, 500 ml in volume) were dipped in insecticide solutions in order to achieve contact action. After the treatment, cups were let to dry. Treated cups were used for the treatment in which contact and combined action was investigated. Sugar beet plants were dipped in the insecticide solutions as described by the modified IRAC N° 7 (Frac/Irac Newsletter, N° 5, 1990) method for the treatment in which digestive and combined action was investigated.

Contact action was evaluated by applying insecticides to the plastic cups in which weevils were set up without plants. For the untreated control weevils were placed into plastic cups treated with water and

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Table 1. Insecticide treatments used in trials

Treatment	Dose applied (g a.i./ha)	Action investigated	Trial (year)		
			No. 1 (2014)	No. 2 (2014)	No. 3 (2015)
Spinosad	36	digestive	+		
		contact	+		
		combined	+		
Spinosad	96	digestive			+
		contact			+
		combined			+
Lambdacychalothrin	7.5	digestive		+	+
		contact		+	+
		combined		+	+
Chlorpyriphos + cypermethrin	1000 + 100	digestive		+	
		contact		+	
		combined		+	
Untreated	without food		+	+	+
	with food		+	+	+

without plants. Digestive action was evaluated by placing weevils into untreated plastic cups in which treated sugar beet plants were placed. Combined action was evaluated by placing weevils into treated plastic cups in which treated sugar beet plants were placed. The untreated control for these two treatments includes a treatment in which weevils were placed into plastic cups treated with water in which untreated sugar beet plants were placed. One plastic cup represented one replicate and each application rate and the investigated action of tested insecticides was set in four replicates.

Efficacy assessment and data analysis. The number of dead weevils in each plastic cup was determined every 24 h during a period of 4 days in trials Nos 1 and 2, and 5 days in trial No. 3.

Based on the number of dead weevils found out in the treatment and in the untreated control the efficacy of the insecticides was determined according to Abbott's formula (Abbott 1925).

Results were analysed by analysis of variance procedures using the ARM 9® software (Gylling Data Management 2014) with means separation estimated using Duncan's multiple range test. Where appropriate, data were $\sqrt{(x + 0.5)}$ transformed.

RESULTS

In trial No. 1 conducted in 2014, the efficacy of the spinosad dose, which is allowed for other pests, in the control of sugar beet weevil adults was determined. Results obtained (Table 2) showed that this insecticide has certain activity against sugar beet weevils and that the main activity is achieved when weevils take the insecticide by ingestion. Similar results were obtained when the combined action was evaluated. However, the obtained efficacy four days after the treatment was only 45.45% and did not reach the level expected

Table 2. Efficacy of insecticides (in %) in the trial No. 1 (2014)

Treatment	Dose applied (g a.i./ha)	Action investigated	Days after the treatment			
			1	2	3	4
Spinosad	36	digestive	0.00[b]	5.55[b]	19.43[b]	36.36[a]
		contact	0.00[b]	0.00[c]	0.00[c]	0.00[b]
		combined	33.33[a*]	38.39[a]	44.44[a]	45.45[a]
LSD P = 5%			5.918	6.408	12.405	14.403

*means followed by same letter are not significantly different according to Duncans' multiple range test ($P = 0.05$)

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Table 3. Efficacy of insecticides (in %) in the trial No. 2 (2014)

Treatment	Dose applied (g a.i./ha)	Action investigated	Days after the treatment				
			1	2	3	4	5
Lambdacychalothrin	7.5	digestive	1.3	7.5^{b*}	17.5^{c}	27.5^{c}	53.85^{bc}
		contact	6.3	10.0^{b}	5.56^{c}	13.88^{c}	65.71^{ab}
		combined	2.26	7.5^{b}	15.0^{c}	20.0^{c}	30.77^{c}
Chlorpyriphos + cypermethrin	1000 + 100	digestive	5.97	27.5^{ab}	50.0^{ab}	57.5^{b}	66.67^{ab}
		contact	14.59	25.0^{ab}	33.3^{bc}	55.52^{b}	81.04^{ab}
		combined	1.3	37.5^{a}	65.0^{a}	87.5^{a}	92.37^{a}
LSD P = 5%			ns	19.26	28,06	25.72	27.09

*means followed by same letter are not significantly different according to Duncans‘ multiple range test (P = 0.05)

in the field conditions. Thus we concluded that the dose shall be increased in the following trials.

In trial No. 2 the efficacy of the widely applied insecticides, lambda-cyhalothrin and combination of chlorpyriphos and cypermethrin, was evaluated. Obtained results (Table 3) show that there are certain differences between insecticides. Lambda-cyhalothrin expressed very weak efficacy, especially if it was only ingested by weevils. Surprisingly, after applying the insecticide to both the cup surface and the plant (combined action), the efficacy was even somewhat lower. The combination of chlorpyriphos and cypermethrin gave significantly better results compared to those achieved by lambda-cyhalothrin, especially when applied to both the cup surface and the plant (combined action).

In trial No. 3 the dose of spinosad was doubled and the obtained efficacy (Table 4) was much higher. The only exception was when only the contact action was investigated. Again, lambda-cyhalothrin expressed very low efficacy.

DISCUSSION

In the trials we investigated the efficacy of lambda-cyhalothrin (Karate Zeon, Syngenta) and combination of chlorpyriphos and cypermethrin (Chromorel D, Agriphar) at the doses registered for the sugar beet weevil control in Croatia (Bažok 2015). For Chromorel D producers suggest to apply the higher dose of the product, 2.0 l/ha (i.e. 1000 + 100 g a.i./ha), which is much higher compared to the doses allowed for other pests (0.8–1.0 l/ha). We applied spinosad (Laser 240 SC, Dow AgroSciences) at two doses, 36 and 72 g a.i./ha (i.e. 0.15 and 0.3 l/ha).

Spinosad has been tested extensively on a global basis since 1990 (Kerns 1996; Carson & Trumble 1997; Palumbo 1997; Schuster 1997; Walgenbach & Palmer 1997; Fouche *et al.* 1998; Linduska *et al.* 1998; McLeod 1998; Riley 1998; Stansly & Connor 1998; Webb 1998; Campos *et al.* 2014). It is widely used for the control of various lepidopteran pests, thrips and coleopteran pests [Colorado potato

Table 4. Efficacy of insecticides (in %) in the trial No. 3 (2015)

Treatment	Dose applied (g a.i./ha)	Action investi-gated	Days after the treatment				
			1	2	3	4	5
Spinosad	96	digestive	16.58^{a}	26.15 a	42.07^{a}	87.5^{a}	94.88^{a}
		contact	1.28^{b}	1.76 b	6.95^{bc}	30.55^{b}	38.89^{b}
		combined	10.0^{ab}	14.02^{ab}	23.03^{ab}	85.0^{a}	92.31^{a}
Lambdacychalothrin	7.5	digestive	3.4^{b}	4.77^{b}	4.77^{c}	15.0^{b}	23.08^{bc}
		contact	1.17^{b}	2.93^{b}	3.1 c	25.0^{b}	33.33^{bc}
		combined	6.3^{ab}	6.3^{b}	12.19^{bc}	20.0^{b}	20.51^{c}
LSD P = 5%			1808t**	2171t	2034t	16.61	15.18

*means followed by same letter are not significantly different according to Duncans' multiple range test (P = 0.05). Data were transformed by $\sqrt{(x + 0.5)}$; **mean descriptions are reported in transformed data units and are not de-transformed

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beetle *Leptinotarsa decemlineata* (Say)] at doses between 15 and 100 g a. i./ha. Spinosad has also been used in fruit fly control programs in several countries (Chueca *et al.* 2007; Piñero *et al.* 2011; Gazit *et al.* 2013; Manrakhan *et al.* 2013). It has also been tested against certain stored product insect pests (Athanassiou & Kavallieratos 2014) and against fleas [*Ctenocephalides felis* (Bouché)] and commonly found intestinal nematodes of dogs in Europe if applied in combination with milbemycin oxime (Hayes *et al.* 2015). In Croatia, spinosad is available in a concentrated suspension formulation (Laser 240 SC, with 240 g a.i./l of spinosad) and as a ready-to-use toxic bait (Success Bait, which contains 0.24 g a.i./l of spinosad) (Bažok 2015). Laser 240 SC is allowed in Croatia for controlling various lepidopteran and dipteran pests at the doses of 32 g/ha to 60 g/ha. It is also allowed to control one coleopteran pest, Colorado potato beetle (CPB) at the dose of 32 g/ha (Bažok 2015). It is not allowed for the sugar beet weevil control either in Croatia or in any other country. Thus, this is the first investigation in which spinosad is tested against sugar beet weevil.

The applied doses in the first trial are doses used for the control of CPB. Igrc Barčić *et al.* (2006) proved that even a reduced dose of spinosad resulted in significant efficacy on CPB larvae. The control of CPB is mainly targeting larvae. Since the sugar beet weevil damages sugar beets as adult, the adult stage has been tested in our investigation. It is evident that the application of 36 g/ha did not reach efficacy needed for the successful control (Table 2) of adults. Therefore in trial No. 3, we doubled the dose, which resulted in satisfactory efficacy (Table 4). The dose used in trial No. 3 was still below the doses used for some other pests (Thompson *et al.* 2015). Although spinosad induces rapid contact and ingestion activity in insects (Thompson *et al.* 2015), in our trials the ingestion activity was stronger. When insecticides are applied for the control of sugar beet weevil, sugar beet plants are very small. Thus insecticides are mainly applied to the soil instead to the plants. When insecticides are applied to the soil, they often behave differently than if they are applied onto the plants. They often evaporate, degrade or leach or are adsorbed by the soil (Laznik *et al.* 2014). Sugar beet weevils migrate from overwintering places (old sugar beet fields) to sugar beet fields in which sugar beet plants start to emerge. Thus the contact of weevils with insecticides is limited to the contact with soil particles containing insecticides and to the movement of weevils on very small plants. The ingestion activity of insecticides is possible when weevils feed on the plant tissue. But due to the large feeding capacity and very small plant size only the strong digestive action will result in a satisfactory effect. Otherwise, sugar beet weevils will destroy the whole plants before the insecticide ingestion activity starts. For good efficacy it is very important that insecticides will evoke very good ingestion activity, which is the case of spinosad.

The regular dose of the combination of chlorpyriphos and cypermethrin in our trial (Table 3) showed good activity although it was somewhat lower compared to the results obtained by Vuković *et al.* (2014). In their trials the same combination resulted in 100% efficacy after 48 h only, while in our trials insecticide treatments reached satisfactory effects four to five days after treatment.

The regular dose of lambda-cyhalothrin did not reach good efficacy in any trial (Tables 3 and 4). The lambda-cyhalothrin based insecticides are often used for the sugar beet weevil control in Croatia with poor results. Producers do not suggest any increase of the applied dose of lambda-cyhalothrin if the sugar beet weevil is controlled, although it is common practice with other insecticides. The increase of the recommended dose could result in higher efficacy and therefore producers should consider this fact in order to avoid poor results and the need for repeated applications.

Due to the low efficacy and small number of available active ingredients for the sugar beet weevil control, this pest could become a limiting factor for the production of sugar beet in Croatia. These facts imposed a need for the elaboration of a system of measures which would ensure optimal crop protection according to the principles of integrated pest management. The pest management system involves cultural, mechanical, biological, biotechnical and chemical measures. Some of them shall be aimed at the long-term population suppression. All of them shall be primarily based on long- and short-term forecasts for this pest. Cultural and mechanical control measures shall involve spatial isolation and good organisation of the production. Biological control could be effectuated by the use of the nematodes *Steinernema* and *Heterorhabditis* (together with symbiotic bacteria *Xenorhabdus* and *Photorhabdus*) for the suppression of the weevil population (Trdan *et al.* 2006; Susurluk 2008; Hassan 2010). However, the commercial use of products based on the aforementioned organisms

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has not been reported. The discovery and employment of aggregation attractant for the beet weevil enables development and implementation of new methods for the population abundance monitoring, and also for the control by adult mass trapping as a means of the overall population suppression (Toth *et al.* 2002; Sivčev *et al.* 2006; Tomasev *et al.* 2007; Bažok *et al.* 2013a,b; Drmić *et al.* 2014). However, chemical control is the main control measure (Inđić *et al.* 1998; Vuković *et al.* 2014) and it will probably remain this way in the near future. Thus, it is very important to find new insecticide compounds which could be used for the beet weevil control. This study proved that spinosad is a good candidate and should be introduced in the sugar beet weevil control. In laboratory trials good efficacy was obtained with the dose of 72 g a.i./ha, but for determining the dose further field trials are needed. The use of spinosad against beet weevil would be in line with IPM principles because spinosad has a unique mode of action and low toxicity to non-target organisms (including many beneficial arthropods), which makes spinosad an excellent tool for the management of various insect pests (Thompson *et al.* 2015). Many authors mentioned binary mixtures of insecticides as a strategic measure in the sugar beet weevil control (Inđić *et al.* 1997, 1998; Vuković *et al.* 2014). Spinosad may be a very good candidate for the use in mixtures and this possibility will be further investigated. Based on our result and results of the studies conducted by Vuković *et al.* (2014) the good candidates for mixtures could be chlorpyriphos or cypermethrin.

Piri *et al.* (2014) proved that the exposure of females of the lesser mulberry pyralid to sublethal doses of spinosad may reduce the female fecundity. The reduction of female fertility in the multicoloured Asian lady beetle was reported by Galvan *et al.* (2005). Some studies (Boiteau & Noronha 2007; Mahmoudvand *et al.* 2011) proved that spinosad has ovicidal activity as well. It would be good to test the possibility of applying spinosad at the time of oviposition in order to reduce the female fecundity and the number of viable eggs and to achieve the overall beet weevil population suppression.

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