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THE EFFECT OF DIFFERENT ENTOMOPATHOGENS ON WHITE GRUBS (COLEOPTERA: SCARABAEIDAE) IN AN ORGANIC HAY-PRODUCING GRASSLAND

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Abstract - In 2011, a field block trial examined the biological control of white grubs of June beetle (*Amphimallon solstitialis*), margined vine chafer (*Anomala dubia*) and garden chafer (*Phyllopertha horticola*) on a permanent cut grassland in Gotenica (SE Slovenia). The efficacy of *Beauveria brongniartii*, *Beauveria bassiana*, *Bacillus thuringiensis* var. *kurstaki* and *Heterorhabditis bacteriophora* in the form of water suspension and infested grain was tested against a control treatment. The initial number of white grubs (April 12; 39 white grubs/m^2) was reduced with all tested entomopathogens up until the third evaluation (May 26; 32 white grubs/m^2). However, the studied treatments were not sufficient to reduce the white grub population in the soils below the economical threshold (20 individuals/m^2). The average number of white grubs was affected mostly by the treatment where the active ingredient was *B. thuringiensis* var. *kurstaki*. With one application in April, only the abundance of overwintered white grubs was reduced. To decrease the summer generation of white grubs, an application of biological agents is also required at a later time. The 8% higher dry matter yield at the first cut (June 10) compared to the second cut (September 6) provided evidence for the prior statement.

Key words: Grubs, *Beauveria*, *Bacillus*, *Heterorhabditis*, grassland

INTRODUCTION

White grubs are the root-feeding larvae of scarab beetles (Coleoptera: Scarabaeidae), and they are among the most destructive pests of turfgrass, pastures and horticultural plants in many parts of the world. Extensive damage to turfgrass can be caused by the large larvae under warm, dry conditions in late spring and through the summer (Jackson and Klein, 2006). In addition, vertebrate predators, such as birds, European badger (*Meles meles* L.) and wild boar (*Sus scrofa* L.), may damage the turf when foraging for the larvae, even at larval densities that in themselves would not cause damage (Genov, 1981).

Important scarab species include the common European cockchafer (*Melolontha melolontha* [L.]), June beetle (*Amphimallon solstitialis* [L.]), margined vine chafer (*Anomala dubia* [Scop.]) and garden chafer (*Phyllopertha horticola* [L.]) (Jackson and Klein, 2006). Most of these white grub species are also pests of nursery stock and various horticultural crops (Koppenhöfer and Fuzy, 2008a). In Slovenia, these species have the following life cycles: June beetle and margined vine chafer have a two-year life cycle; garden chafer has a one-year life cycle; and common European cockchafer has a three-year life cycle (Vrabl, 2011).

In previous years, reports on damage due to white grubs came from several countries in Europe (Wagner et al., 2002). Mass multiplication may be the result of climate warming in the last decade, as milder winters do not contribute to the natural reg-

ulation of soil pests (Bale et al., 2002). In Slovenia, the most evident mass multiplication of common European cockchafer took place in the Idrija Region, especially in the area of the Črnovrška Planota (Poženel, 2005 a,b). This event led to questions regarding what triggered the mass multiplication and how to conduct an effective and environmentally proper sanitation.

The control of adults has almost no meaning and generally is not necessary, but more attention devoted to white grubs is required. In Slovenia, only one product is currently registered (active ingredient [a.i.] tefluthrin) for controlling soil pests, but it is not to be used in grassland areas. Biological control options are available, but they are relatively unreliable and infrequently used (Laznik et al., 2010).

Entomopathogenic nematodes (Heterorhabditidae and Steinernematidae) may offer an environmentally safe and IPM-compatible option for curative white grub control (Grewal et al., 2005). Well-adapted nematode species/strains, such as the domestic Slovenian *H. bacteriophora* Poinar (Laznik et al., 2009), used under favourable conditions, can provide curative control of different scarab species equal or superior to that of standard insecticides (Koppenhöfer et al., 2004).

Several studies have shown that the efficacy of entomopathogenic nematodes in curatively controlling white grubs can be improved if they are integrated with other pathogens, but these reported combinations have limitations (Koppenhöfer and Kaya, 1997). For example, the combination of nematodes and *Bacillus popilliae* Dutky (Thurston et al., 1994) is feasible only for long-term control in high economic threshold situations, and the combination of nematodes and *Bacillus thuringiensis* Berliner Buibui strain (Koppenhöfer and Kaya, 1997) is feasible only for scarab species that are sufficiently susceptible to this bacterium.

The two fungal entomopathogenic species, *B. bassiana* (Balsamo-Crivelli) Vuillemin and *B. brongniartii* (Saccardo) Petch, were described for the first time approximately 170 and 110 years ago, respectively, (Zimmermann, 2007). Since that time, they have always been considered as fungi that can and should be used for the control of pest insects. In Europe, *B. brongniartii* mainly attacks *M. melolontha* and *M. hippocastani*. However, this fungus may also occur on other insects (Zimmermann, 2007; Shahid et al., 2012). *B. bassiana* is a ubiquitous entomopathogenic fungus that has been found and isolated from a wide variety of insects from different orders (MacLeod, 1954; Goettel et al., 1990). The virulence of this fungus against white grubs is well characterized (Zimmermann, 2007; Dhoj et al., 2008).

The improper use and fertilization of organic grassland could lead to a larger abundance of white grubs in soils. Due to the same or increased number of cuts with lower inputs of organic fertilizers in an organically utilized sward, the sward can become so scarce that conditions for the mass appearance of white grubs are feasible, which can be increased by intensive egg deposition into soils (Keller et al., 1997).

The purpose of this research was to determine the connection between wild boar damage on grassland and the number of white grubs in grassland soils. However, this linkage is not presented in detail in the present paper. Other aims of this study, which are discussed in detail, are as follows: 1) to test the efficacy of different biological control agents (*H. bacteriophora*, *B. brongniartii*, *B. bassiana* and *B. thuringiensis* var. *kurstaki*) on a different white grub species under field conditions; 2) to test the hypothesis that *B. thuringiensis* bacteria act as stressors and increase the efficacy of entomopathogenic nematodes added 2 weeks after the application of *Bt* (Koppenhöfer and Kaya, 1997); 3) to test different application methods (foliar application of a water suspension infected with *B. brongniartii* fungus or incorporation of sterilized barley seeds infected with *B. brongniartii* fungus) on the control of white grubs in grassland; and 4) to test if one application of the studied entomopathogens is sufficient to reduce the number of white grubs below the economic threshold (Horber, 1954) throughout the entire vegetation period.

MATERIALS AND METHODS

In 2011, a 5 block field experiment in controlling the white grubs of June beetle, margined vine chafer and garden chafer was conducted on an organically utilized meadow with dry forage conservation near Gotenica (Kočevska Region in southeastern Slovenia: 45°36′42.53″N and 14°44′49.72″E; 659 m a.s.l.). The choice of land for the experiment was based on the fact that sward damages were observed at the beginning of March due to the rooting activities of wild boars, which like white grubs and other members of soil fauna (earthworms etc.) and flora because they provide feed rich in protein (Baubet et al., 2003; Bueno et al., 2009). The analysis of the soil excavation (March 29, 2011) in the experimental land, which was in the vicinity of a mixed forest, determined that the critical number of white grubs in the soil was exceeded (Horber, 1954) and that their control was economically legitimate.

Identification of white grubs from the Scarabaeidae family was aided by knowing the developmental cycles of different species from this family in Slovenia (Vrabl, 1992) and by using an identification key previously reported by Krell (2004). The soil excavation analysis showed that the larvae of margined vine chafer (L_1 and L_3), June beetle (L_3) and garden chafer (L_3) were present in April 2011 but that larvae of common European cockchafer were not found in this location in 2011. The presence of white grubs in the excavated soils were monitored on April 12, April 29, May 26, June 21, July 26, September 23 and October 18. Application of a different biological control agent was performed on April 12. Entomopathogenic nematodes were applied 14 days after the application of *B. thuringiensis* var. *kurstaki*, which has been previously shown as the most effective way to control the white grubs, *Cyclocephala hirta* and *C. pasadenae* (Koppenhöfer and Kaya, 1997). In both terms, the application was performed in forenoon hours. At both application times, the weather was cloudy and rainy, and the sun did not appear. The day after application, rain fell with 23.7 mm of precipitation, and no precipitation occurred after this day until April 26 when 15 mm of precipitation was recorded until the end of April. The total amount of precipitation in April (50.5 mm) represented only 45% of the long-term average in this area (Bilten ARSO, 2011).

In addition to an untreated control (only water with no addition of entomopathogens), five experimental treatments of different biological control agents and combinations of agents were applied (Table 1). Each treatment (plot size 5 m x 5 m) was repeated in each block with one treatment per block. Melocont Pilzgerste (grain) was incorporated into the soils with a plot drill for direct sward seeding (Hunter Rotary Strip Seeder, UK). Using the drill before cultivation provides better conditions for the survival and activity of the biological agent on the barley grain. The biological agents, which were mixed and prepared in a water solution, were applied with a hand-held high pressure garden sprayer (SOLO SO 463, Solo Kleinmotoren GmbH, Sindelfingen, Germany) with a manometer and maximum pressure of 3 bars.

When setting up the field experiment, an iron cage (0.5 m^2) was placed on each of the 30 plots to prevent the grazing of reed deer (*Cervus elaphus* L.) and sward rooting of wild boar (*Sus scrofa* L.). Moreover, the area within the cage was used to compare the grass yield between the protected and unprotected sites. On two days (June 10 and September 6), biomass sampling was carried out on the unprotected and protected subplots with a 620 W Max motor mower (BCS S.p.A., Italy) with dry type fingers (width of 1.15 m) to determine the dry-matter yield in treatments protected against wild herbivores. As in a previous study by Trdan and Vidrih (2008), the attained values were expressed as kg/ha.

To determine the linkage between the presence of white grubs in the soils and the production characteristics of grassland in the experiment, the soils were sampled and analyzed to determine their fertility. The soil analysis on the trial location had the following results: pH 7, phosphorus (P_2O_5) content 57 mg/100 g of soil, potash (K_2O) content 16 mg/100 g of soil, and the organic matter content of 12.4%.

Table 1. Experimental treatments representing different biological controls.

Control agent*	Legend	Formulation	Active ingredient	Commercial name	Source	Rate
Fungal entomopathogen	Bbro	Grain	*Beauveria brongniartii* isolate IMBST 95.031	Melocont Pilzgerste	Manufacturer: Agrifutur, Brescia	50 kg ha^{-1}
Fungal entomopathogen	BbroL	Water suspension	*Beauveria brongniartii* isolate IMBST 95.031	Melocont Pilzgerste	Manufacturer: Agrifutur, Brescia	0.23 %
Fungal entomopathogen	Bbas	Water suspension	*Beauveria bassiana* isolate ATCC 74040	Naturalis	Manufacturer: INTRACHEM Bio Italia; distributed by Karsia, Dutovlje, d.o.o	3 l ha^{-1}
Bacterial pathogen	Btk	Water suspension	*Bacillus thuringiensis* var. kurstaki	Delfin	manufacturer: Agrisense, Great Britain; distributed by Karsia, Dutovlje, d.o.o	750 g ha^{-1}
Bacterial pathogen + entomopathogenic nematode	Btk+Hb	Water suspension	*Bacillus thuringiensis* var. kurstaki + *Heterorhabditis bacteriophora*	Delfin + Nemasys H	manufacturer: Agrisense, Great Britain; distributed by Karsia, Dutovlje, d.o.o + manufacturer Becker&Underwood, Great Britain; distributed by Metrob d.o.o., Slovenia	750 g ha^{-1}; 5.000.000.000 IJ ha^{-1}
Untreated control	C	-	water	-	-	

* Application of a different biological control agents was performed on 12 April. Entomopathogenic nematodes were added 14 days after application of Btk.

Table 2. ANOVA results of the mortality of different developmental stages of grubs.

Source	L_1			L_2			L_3			pupa			adults		
	F	df	P	F	df	P	F	df	P	F	df	P	F	df	P
block	0.88	4	0.4789	2.59	4	0.0390	8.33	4	<0.001	1.77	4	0.1387	1.02	4	0.3971
sampling time	21.44	6	<0.001	16.20	6	<0.001	23.66	6	<0.001	24.44	6	<0.001	3.41	6	0.0035
treatment	1.54	5	0.1798	1.57	5	0.1717	0.62	5	0.6881	0.57	5	0.7247	1.90	5	0.0980
sampling time × treatment	0.65	30	0.9138	1.55	30	0.0480	1.47	30	0.0720	0.58	30	0.9608	1.26	30	0.1872

Differences in the number of the different developmental stages of *A. solstitialis, A. dubia* and *P. horticola* (L_1, L_2, L_3, pupa and adults), in addition to yields of sward dry matter between individual treatments were analyzed with the use of ANOVA. Prior to analysis, each variable was tested for homogeneity of variance, and the data that showed to be non-homogenous were transformed to log(Y) before ANOVA. Significant differences ($P \leq 0.05$) between the mean values were identified using the Student-Newman-Keuls multiple range test. The difference in the number of white grubs/m^2 between the third and first evaluation dates and between the fourth and seventh evaluation dates was given as an index in change ($I=x_3/x_1$; x_7/x_4; where x is the number of white grubs/m^2 at a selected date of sampling). All statistical analyses were performed using Statgraphics Plus for Windows 4.0 (Statistical Graphics Corp., Manugistics, Inc., Rockville, MD, USA). The data were presented as untransformed means ± SE.

RESULTS

Seasonal dynamics of white grubs in the soil ANOVA of pooled results are presented in Table 2.

L_1 stage

Between April (1.6 larvae L_1/m^2) and June (2.1 larvae L_2/m^2), there were not many larvae belonging to the youngest larval stage (L_1) in the soil. The analysis of scarab beetles showed that the smallest amount of larvae found in the soil between April and May consisted of margined vine chafer (*A. dubia*) white grubs, which flew into the Gotenica area in 2010. Among the studied biological products, the number of margined vine chafer white grubs was reduced only when treated with grain covered with the *B. brongniartii* fungus (I=0.85) (Fig. 2), and the number of grubs increased with the other treatments during the same period (between April and June). In June (2.1 larvae/m^2) and July (9.6 larvae/m^2), garden chafer (*P. horticola*) larvae started to appear. Between September (35 larvae/m^2) and October (20 larvae/m^2), the number of the youngest larvae increased due to the successful eclosion of the June beetle and margined vine chafer, which flew into the Gotenica area in 2011.

L_2 stage

In April, the number of larvae in the second larval stage increased (L_2=6.3 larvae L_2/m^2). The developmental cycle studies of scarab beetles showed that June beetle (*A. solstitialis*) larvae were present that had flown into the area of Gotenica in 2010 (between June and July). Among the tested products, only Delfin (a.i. *B. thuringiensis*) (I=0.53) and the *B. brongniartii* fungus (liquid) (I=0.83) showed sufficient activity (Fig. 3). Renewed increase in the number of larvae of June beetle and garden chafer, which flew into the area in 2011, appeared in the soils in September (26 larvae/m^2) and October (29 larvae/m^2).

L_3 stage

Among all of the developmental stages of white grubs determined in April, the highest number was in the oldest larval stage (L_3=21 larvae L_3/m^2). The analysis of scarab beetles indicated the presence of the larvae of June beetle (*A. solstitialis*) and margined vine chafer (*A. dubia*), which flew into the Gotenica area in 2009, as well as the presence of larvae of garden chafer (*P. horticola*), which flew into the area in 2010. The above-mentioned white grubs did not belong to common European cockchafer because neither type M_0 nor type M_1 was represented in Slovenia, and these types did not overlay with the above-mentioned developmental stage in 2011 between April and May. Among the tested products, Delfin (a.i. *B. thuringiensis*) had satisfactory activity that decreased the number of white grubs in the soil by two-thirds (I=0.36), and the *B. thuringiensis* combined with the *H. bacteriophora* entomopathogenic nematode also decreased the number of white grubs (I=0.73) (Fig. 4). In June and July, the larvae population in the soil was reduced (1 larvae/m^2) due to pupation. In September and October (12 larvae/m^2), there was an increase in the number of larvae of June beetle and margined vine chafer, which flew into the Gotenica area in 2010, as well as garden chafer, which flew into the area in 2011.

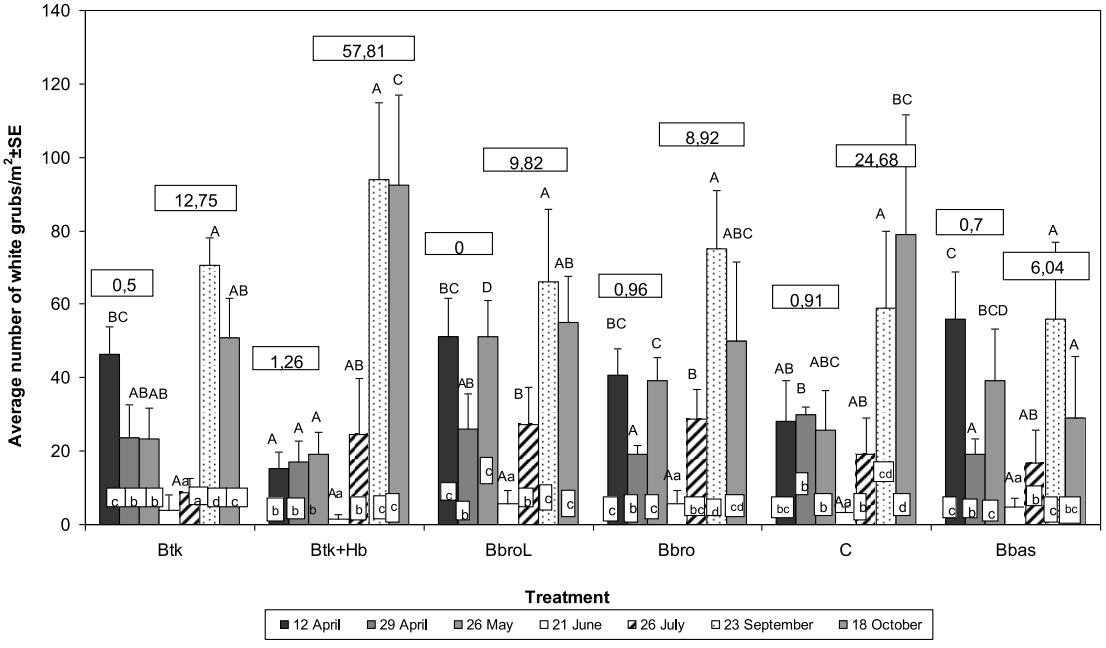

Fig. 1. Average number of white grubs per m^2 at different treatments and sampling dates. Capital letters above the bars indicate statistically significant differences within the sampling date but at different treatment. Small capital letters indicate statistically significant differences within the same treatment but at different sampling date. Differences in the numbers of white grubs per m^2 between the 3rd and 1st sampling and 4th and 7th sampling dates are referred to as the indices of change ($I=x_3/x_1$; x_7/x_4, x=number of white grubs /m^2 in selected sampling date). Values significantly different ($P \leq 0.05$) were determined by the Student-Newman-Keuls test.

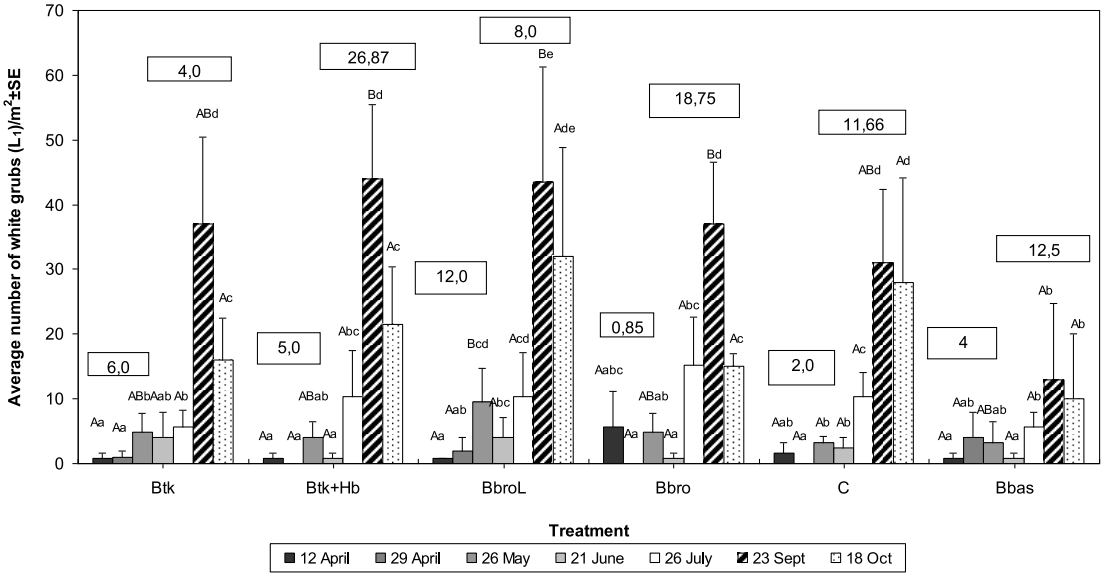

Fig. 2. Average number of white grubs of L_1 per m^2 at different treatments and sampling dates. Capital letters above the bars indicate statistically significant differences within the sampling date but at different treatments. Small capital letters indicate statistically significant differences within the same treatment but at different sampling dates. Differences in the numbers of white grubs per m^2 between the 3rd and 1st sampling and 4th and 7th sampling dates are referred to as the indices of change ($I=x_3/x_1$; x_7/x_4, x=number of white grubs /m^2 in selected sampling date). Values significantly different ($P \leq 0.05$) were determined by the Student-Newman-Keuls test.

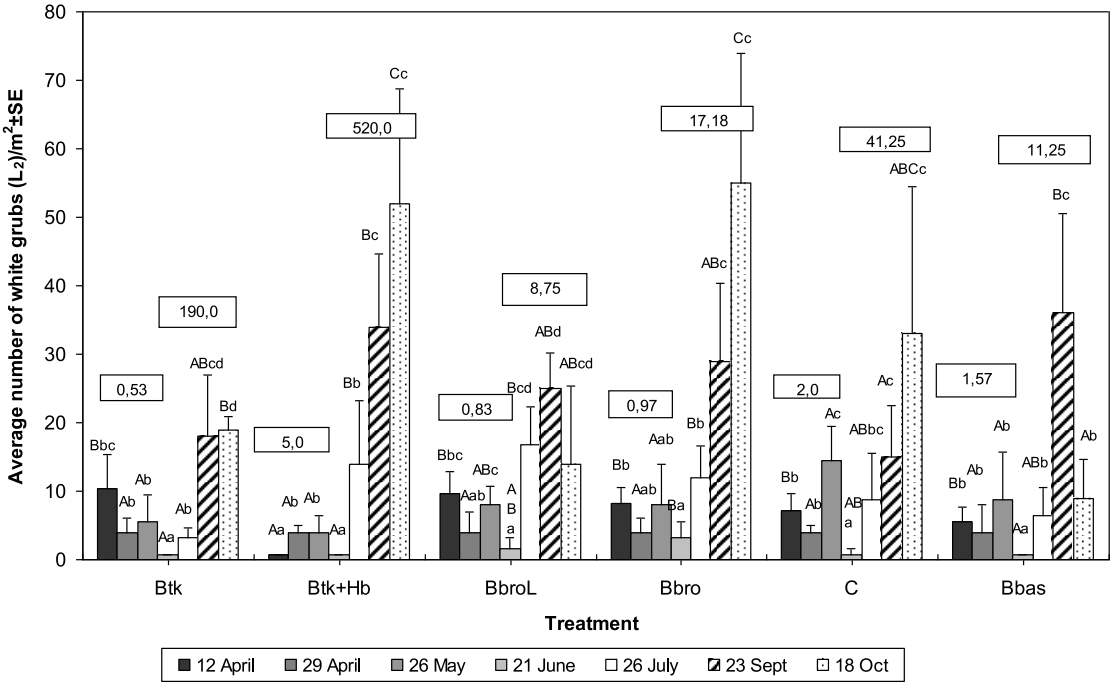

Fig. 3. Average number of white grubs of L_2 per m^2 at different treatments and sampling dates. Capital letters above the bars indicate statistically significant differences within the sampling dates but at different treatments. Small capital letters indicate statistically significant differences within the same treatment but at different sampling dates. Differences in the numbers of white grubs per m^2 between the 3rd and 1st sampling and 4th and 7th sampling dates are referred to as the indices of change ($I=x_3/x_1$; x_7/x_4, x=number of white grubs /m^2 in selected sampling date). Values significantly different ($P \leq 0.05$) were determined by the Student-Newman-Keuls test.

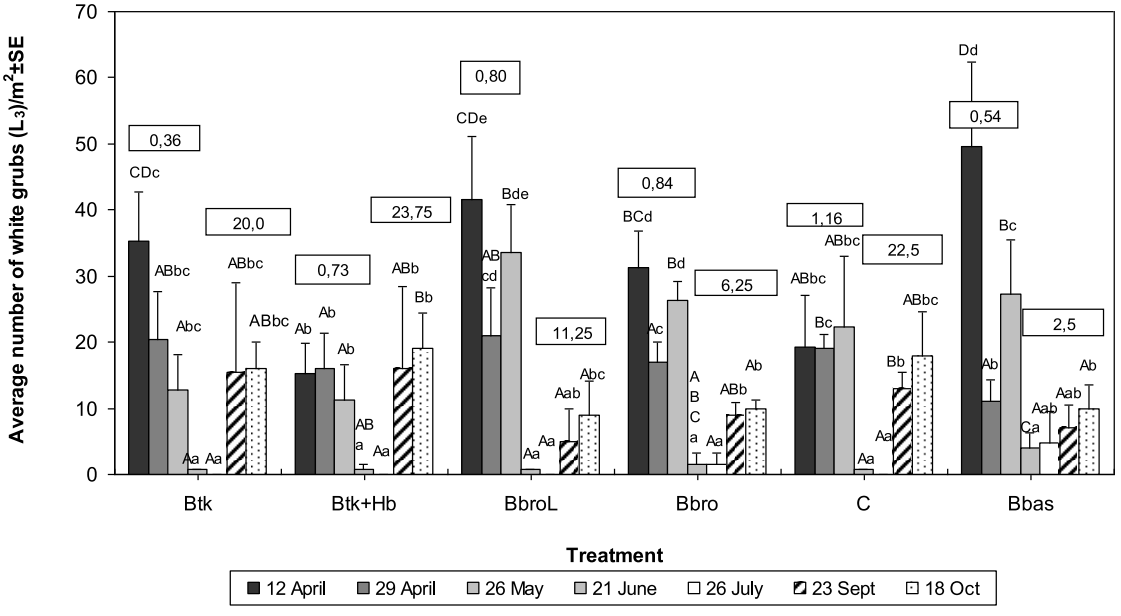

Fig. 4: Average number of white grubs of L_3 per m^2 at different treatments and sampling dates. Capital letters above the bars indicate statistically significant differences within the sampling dates but at different treatments. Small capital letters indicate statistically significant differences within the same treatment but at different sampling dates. Differences in the numbers of white grubs per m^2 between the 3rd and 1st sampling and 4th and 7th sampling dates are referred to as the indices of change ($I=x_3/x_1$; x_7/x_4, x=number of white grubs /m^2 in selected sampling date). Values significantly different ($P \leq 0.05$) were determined by the Student-Newman-Keuls test.

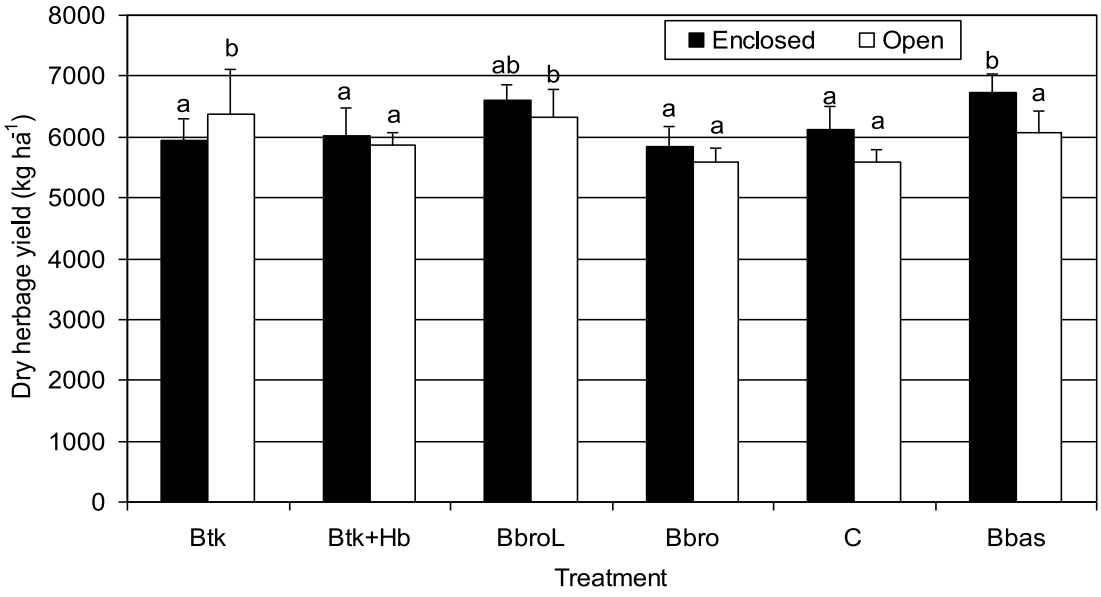

Fig. 5. Total (both cuts) average total herbage yield under cage (solid bar) and in the open (open bar) in the field experiment. Values significantly different (P≤ 0.05) by Student-Newman-Keuls test.

Pupal stage

Among all of the developmental stages of white grubs determined in June, the highest number was observed for the pupas (14 pupas/m^2) of June beetle and margined vine chafer, which flew into the area of Gotenica in 2009. None of the treatments in April had a direct affect on controlling the pupas (P=0.7247).

Adult stage

Between April and June, adults were not found in the soils. The first adults found were June beetle adults in July (0.66 individuals/m^2). Margined vine chafer and garden chafer adults were observed in September (0.16 individual/m^2) and the beginning of October (0.68 individual/m^2). None of the treatments in April had a direct affect on controlling the adults.

Productivity of grassland sward

The first cut of the plots where the different biological agents were tested was performed on June 10. Comparing the herbage yield between the area under the cage and the area in the open allowed the influence of wild ungulate grazing on sward regeneration before the first cut as well as the efficacy of controlling white grubs in soils regarding the quantity of grassland output to be determined. The average herbage dry-matter yield under the cage was 3220 kg/ha, and the average herbage dry-matter yield in the open was 2713 kg/ha. At the first cut, there was 20% more herbage yield for the protected plot than for the unprotected plot. For the first cut, the highest yield under the cage was observed in the control treatment (3404 kg/ha), and the lowest yield was observed in the BbroL treatment (3020 kg/ha).

At the second cut (September 6, 2011), the average yield was higher in the open area (3256 kg/ha) than under the cage (2985 kg/ha). At the second cut, an 8% increase in herbage yield was noted for the area under the cage (enclosed) compared to the unprotected area. On average, the yield under the cage was larger at the first cut (3220 kg/ha) than at the second cut (2985 kg/ha), and this trend was reversed for the unprotected plot. For the second cut, the highest yield of herbage dry matter under the cage was ob-

tained with the Bbas treatment (3316 kg/ha), and the lowest yield was obtained with the Bbro treatment (2730 kg/ha).

Analysis of total dry-matter yield (Fig. 4) showed that the largest statistically significant yield was obtained with the Bbas treatment (6648 kg/ha). The other treatments did not show any statistically significant differences, and their values ranged from 5792 kg/ha (Bbro) to 6364 kg/ha (Btk+Hb).

DISCUSSION

The suitable natural conditions in the Kočevje Region have led to the development of soil fauna that is an important part of the soil developmental stages of June beetle, margined vine chafer and garden chafer. Curry (1994) described the climate conditions, feed quality, natural enemies, disease inducers and competition as important factors that have a great influence on the abundance and species composition of grassland fauna, and suggested that intensive precipitation and temperatures below freezing point may cause extensive deaths of the garden chafer at different developmental stages. Frost in late autumn can be detrimental to larvae in the third larval developmental stage because the larvae at this stage still feed closer to the soil surface before they go deeper into the soil for winter. When other environmental factors (oxygen concentration, soil humidity and feed) are suitable, the larval growth in the 110-day period of feeding depends directly on soil temperature, which also determines pupa mass and, consequently, the fertility of adult females. However, intensive precipitation can lessen the portion of oxygen, which can slow down the development and fertility of adult females in the following generation (Curry, 1994). A previous study has determined that numerous common European cockchafer adults can be reduced by later spring snow and frost during the adult flight (Poženel, 2005a).

The present study demonstrated that the total number of white grubs with all tested products was reduced between the first (April 12; 39 white grubs/m^2) and third evaluation terms (May 26; 32 white grubs/m^2), but the studied methods were unable to decrease the number of white grubs in soils below the economical threshold of noxiousness (20 individuals/m^2), which was reported by Horber (1954) when studying the white grubs of common European cockchafer in grass swards. The tested products did not show sufficient efficacy in reducing the white grub populations, especially in the unsuitable weather conditions in the period after the application of active ingredients. The biological agents used in this study required large amounts of water for their activity. However, the weather conditions at the time of application were not adequate, so the activity of the biological products was worse than the activity reported in other studies (Koppenhöffer and Kaya, 1997; Koppenhöfer et al., 2004; Koppenhöfer and Fuzy, 2008ab) that investigated the efficacy of different biological agents to control white grubs in soils. Koppenhöfer and Fuzy (2008a) reported that controlling white grubs with *H. bacteriophora* EPNs offers a safe and highly IPM-compatible alternative for remedial white-grub control. Several studies have also shown that watering either by irrigation (Downing, 1994) or washing (Selvan et al., 1994) after application of biological control agents provides better control of white grubs than treatment without watering.

The reduction of the total number of white grubs in June (4 larvae/m^2) was attributed to the presence of many pupas in the soils (14 pupas/m^2), which developed from the L_3 white-grub stage. The number of white grubs increased in the soils in July (21 white grubs/m^2), September (72 white grubs/m^2) and October (61 white grubs/m^2) due to the appearance of June beetle, margined vine chafer and garden chafer, which flew in between May and August and laid eggs in the soil from which the larvae at the youngest larval stage developed in September (35 white grubs L_1/m^2) when the biological agents applied in April no longer had any effect. The second application of active ingredients between July and August in addition to sufficient moisture in the soils may have caused a reduction in the number of white grubs that came from eggs laid between May and June of the same year. Similar results were reported by Koppenhöfer

and Fuzy (2008a), who determined the optimum time of application of combined usage of neonicotinoids and entomopathogenic nematodes in controlling the grubs of the *Anomala orientalis* (Waterhouse) and *Popillia japonica* Newman species, and they showed that a combination of imidacloprid and *H. bacteriophora* provides a more consistent control of the studied larvae in field experiments when applied in late August.

The present study demonstrated that the average number of white grubs in all developmental stages was affected mainly by the product containing *B. thuringiensis* var. *kurstaki* bacteria. In practice, this product is mainly used for controlling butterfly caterpillars (Mostafa et al., 2005), but Koppenhöfer and Kaya (1997) also reported the efficacy of this strain in controlling white grubs in soils. In the present study, *B. thuringiensis* var. *kurstaki* bacteria reduced the white grub population by half by the third evaluation date. The product containing *B. bassiana* fungus also had a satisfactory efficacy in reducing the number of white grubs (I=0.7). In contrast to a similar experiment conducted by Koppenhöfer and Kaya (1997), the present results did not indicate a potential synergism between *B. thuringiensis* var. *kurstaki* bacteria and *H. bacteriophora* entomopathogenic nematodes; this was attributed to the inadequate weather conditions in the days after nematode application.

This study determined the productivity of a grassland sward with two cuts. Both the number and dates of cutting were adapted to actual grassland use in neighboring areas in which the experiment was conducted. The total dry-matter results from the protected area (under the cage) showed that the grass yield at the first cut was higher than at the second cut by 8%, which was attributed to the increased number of larvae (L_2 and L_3) of June beetle, margined vine chafer and garden chafer in August and September. Importantly, the productive potential of the grassland sward in the spring prior to the first cut cannot be neglected (Pontes et al., 2007). When the defined density of the third stage of white grubs is reached, they cause mass destruction of grass roots, and this destruction is indicated by the yellowing and weakening of the grass stand followed by the appearance of scattered, irregular dead patches. The total dry matter in the protected area (under the cage) was 12% higher than the unprotected (data not shown), which suggested that the red deer acted as a reduction factor in the production of fibrous feed. Similar conclusions were reported by Trdan and Vidrih (2008) when they confirmed that the grazing of red deer diminishes the yield on permanent grassland by 50 to 80% in an area near the forest in the Kočevje Region. White grubs represent an additional source of food for some organisms, such as the shrew mouse (Soricidae), European hedgehog (*Erinaceus europaeus* L.), European mole (*Talpa europaea* L.), European badger (*Meles meles* L.) and wild boar (*Sus scrofa*) (Bugg and Pickett, 1998; Trdan and Vidrih, 2008; Veličković et al., 2010), which poses a problem to the producers of fibrous feed on grassland due to these organisms rooting the grass swards (Cocca et al., 2007).

More intense management of grasslands by sowing palatable species and increasing fertility has provided greater energy resources for some herbivorous species. Typically, white grubs are most common in sandy or sandy loam soils rather than heavier soil types, but white grubs can also attack turf on clay soils. With favourable conditions in grassland soils, grub populations will increase in a predictable fashion (Jackson and Klein, 2006). The grassland soils in the present study in which a high number of white grubs appeared were Rendzic Leptosols and had relatively high pH (7.1), phosphorus (77 mg/100 g of soil) and organic matter (12.4%) levels. The linkage between soil texture and white-grub abundance has not yet been identified in the region of Europe. However, similar research has been conducted in Australia where the influence of soil type on the distribution of greyback canegrub (*Dermolepida albohirtum* [Waterhouse]) in sugarcane fields has been confirmed (Ward, 2003). At a regional or district level, the preference of greyback canegrub for sandy delta-type soils over soil with higher clay contents has been hypothesized to be the result of preferential oviposition and im-

proved larval survival in sandy soils over soils with a high clay content. The results reported by Schon et al. (2011), who studied the impact of phosphorus content in soils on the number of earthworms, confirmed the positive correlation reported in the present study. Organic matter, which was widely present in the soils used in the current study, may have a buffering effect on the plant damage caused by soil-dwelling scarab larvae (Villalobos et al., 1997), and this factor may be responsible for the relatively small reduction in grassland production due to white-grub attacks occurring in the soils of the present study.

Jackson and Klein (2006) reported that grub populations often collapse after attaining a high population density in a grass sward, which may be due to combat mortality after starvation following the destruction of grassland where larvae will bite and injure each other causing death by bacterial invasion. To achieve sustainable, long-term grassland systems, different grub-control methods should be used. Moreover, low cost systems that can provide a sustainable output of high quality products should be developed.

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