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The effect of selenium on yield and primary terminal electron transport system activity in two cultivars of bean plants *Phaseolus vulgaris*

Vpliv selena na pridelek in dihalni potencial pri fižolu *Phaseolus vulgaris*

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Abstract: The effect of soaking the seeds in solution of selenium (Se) and foliarly spraying with Se on *Phaseolus vulgaris* cv. Stanko and Topolovec were studied. The flows of electrons in the photosynthetic apparatus and in the respiratory chain were measured in control plants and in plants developed from selenium treated seeds and in once and twice foliarly treated plants. Yield of control and treated plants was measured at the end of experiment. The respiratory potential of *Phaseolus vulgaris*, measured by electron transport system (ETS) activity in cv. Stanko, significantly increased in selenium treated plants. The potential and effective photochemical efficiency of photosystem II were similar comparing treated and untreated plants. The addition of selenium induced yield in twice Se foliarly treated plants in both cultivars growing in greenhouse.

Keywords: *Phaseolus vulgaris,* selenium, yield

Izvleček: Ugotavljali smo vpliv dodanega selena na fiziološke lastnosti in pridelek pri dveh kultivarjih fižola *Phaseolus vulgaris* cv. Stanko in Topolovec. Semena smo namakali v raztopini selenata v prvem poskusu in v drugem smo rastline dvakrat listno škropili z raztopino selenata. Merili smo fotokemično učinkovitost fotosistema II in respiratorni potencial. Respiratorni potencial, merjen s pomočjo aktivnosti terminalnega elektronskega transportnega sistema, je bil višji pri mladih rastlinah. Respiratorni potencial je bil pri cv. Stanko višji pri rastlinah, obravnavanih s selenom. Fotokemična učinkovitost je bila podobna pri kontrolnih rastlinah in rastlinah, obravnavanih s selenom. Dodatek selena je povečal pridelek pri rastlinah, ki so bile dvakrat listno obravnavane s selenom pri obeh kultivarjih, gojenih v rastlinjaku.

Ključne besede: *Phaseolus vulgaris,* selen, pridelek

Introduction

Selenium (Se) is an essential nutrient for humans and animals, but also an environmental toxicant. The boundary between the two is narrow and depends on its chemical form, concentration, and other environmentally regulating variables (Fan et al. 2002, Shardendu et al. 2003).

The essentiality of Se to plants remains unclear (Terry et al. 2003). Seppänen et al. (2003) reported that according to current thinking, higher plants do not require Se. Many studies confirmed antioxidative role of Se in plants at low concentration (Kuznetsov et al. 2003, Germ et al. 2005, Smrkolj et al. 2006). Studies by Pennanen et al. (2002) have indicated that plant growth was promoted by Se resulting from increased starch accumulation in chloroplasts. Se can increase the tolerance of plants to UV-induced oxidative stress (Valkama et al. 2003). Applied selenium has a high impact on the activity of oxidoreductase enzymes in wheat plants (Nowak et al. 2004). Selenium concentration 0.05 mmol/kg soil positively affected antioxidant defence in wheat plants, but higher concentrations provoked stress responses. When organisms are exposed to stress and demand more energy, ATP production and O_2 consumption are increased in the mitochondria (Packard 1985, Bartoli et al. 2005). The respiratory potential of different organisms can be measured *via* the terminal electron transport system activity in mitochondria (Breznik et al. 2005).

Its wide availability and nutritional value gives the species *Phaseolus vulgaris* a strategic value as food sown in many countries (Granito et al. 2009). The amount of proteins is two to three times higher as in cereals it is also reach source of vitamins, especially group B, pholic acid and certain elements like Fe, Zn and Ca. It contains polysaharids (amids and fibres) that have positive role in digestion (Ranalli et al. 2001).

We aimed to find out the impact of applied Se on yield and the vitality of the plants of *Phaseolus vulgaris* cv. Stanko and Topolovec.

Materials and methods

Growth conditions

Se solution was used for soaking of seeds (12 h) before sowing in water with addition of Na_2SeO_4 in the concentration 2.5; 5.0 and 10.0 mg Se/L. Seeds were then dried and sown the next year in greenhouse in glinopor substrate.

Foliar treatment was performed in two different setups:

A. Bean plants were foliarly treated with Se solution Na_2SeO_4 in the concentration 2.5; 5.0 and 10.0 mg Se/L in the beginning of flowering period. After 10 days we repeated the procedure with the same concentrations, so plants were treated foliarly twice. Experiment was performed in soil in the open area. We have sown 20 plants per m².

B. The same experiment was performed in the greenhouse in Glinopor substrate to provide semi-controlled conditions. We have sown 16 plants per m².

Foliarly treatments with Se were done in three replicates and in two subsequent years. The plant yield was measured in both years. Physiological parameters of plants were measured in the second year only.

Analyses were performed on the plants with similar state of development.

Respiratory potential

Terminal electron transport system (ETS) activity was measured using the assay originally proposed by Packard (1971), and modified by Kenner and Ahmed, (1975). A known fresh weight of leaves was crushed in a mortar in 4 ml final volume of ice-cold 0.1 M sodium phosphate buffer (pH = 8.4) containing 0.15% (w/v) polyvinyl pyrrolidone, 75 µM $MgSO_4$, and 0.2% (v/v) Triton-X-100, followed by an ultrasonic homogenizer (4710; Cole-Parmer, Vernon Hills, IL, USA) for 20 sec at 40 W. The homogenates were centrifuged in refrigerated ultracentrifuge for 4 min at 0 ºC at 10,000 rpm (2K15, Sigma). Within 10 min, 0.5 ml of supernatant (in triplicate) was incubated in 1.5 ml substrate solution (0.1 M sodium phosphate buffer (pH = 8.4), 1.7 mM NADH, 0.25 mM NADPH, 0.2% (v/v) Triton-X-100) and 0.5 ml of INT (20 mg 2-p-iodo-phenyl 3-p-nitrophenyl 5-phenyl tetrazolium chloride in 10 ml of bidistilled water), for 40 min at standard (20 °C) temperature. The formazan production was determined spectrophotometrically (Lambda 12, Perkin-Elmer) by measuring absorption at 490 nm against the blank. ETS activity was measured as the rate of tetrazolium dye reduction, and converted to equivalent oxygen as described by Kenner and Ahmed (1975).

Fluorescence measurements

Fluorescence measurements were taken on the first fully expanded leaf of randomly selected plants using the OS-500 (Opti-Sciences, Tyngsboro, MA, USA) fluorometer. Prior to measurements samples were dark adapted for 20 min. Measurements of minimal (F_0) and maximal (F_m) chlorophyll fluorescence were provided by dark-adaptation clips. Fluorescence was excited with a saturating beam of "white light" (PPFD = 8000 μmol m^{-2} s^{-1}, 0.8 s). The difference between F_m and F_0 is called the variable fluorescence (F_v). The effective quantum yield of photosystem II (PSII) was measured under saturating irradiance (1 800 μmol m^{-2} s^{-1}) at the prevailing ambient temperature by providing a saturating pulse of "white light" (PPFD = 9 000 μmol m^{-2} s^{-1}, 0.8 s) using a standard 60° angle clip. The effective quantum yield of PSII provides an estimate of the actual efficiency of energy conversion in PSII and is defined as $(F_m'-F)/F_m' = \Delta F/F_m'$. F_m' is the maximal fluorescence of an illuminated sample and F is the steady state fluorescence (Schreiber et al. 1995).

Seeds of bean plants were soaked in Se solution 10.0 mg Se/L, afterwards dried and stored, and then sown in greenhouse in glinopor next year. On the plants, grew from these seeds, respiratory potential and fluorescence measurements were performed. Respiratory potential and fluorescence measurements were done also on the young, once and twice foliarly treated bean plants cultured in the greenhouse in glinopor and in soil with concentration 10.0 mg Se/L. Both analyses were done on the same plants.

Respiratory potential and fluorescence measurements were done on the first, fully developed leaves.

Statistical Analysis

The data were evaluated by ANOVA (Statgraphics Version 4) and significance accepted at $p < 0.05$.

Results

Yield of bean plants cultivated in the soil after foliar spraying

Yield of bean plants cultivated in the soil in open area after one foliar spraying did not differ between treated and untreated plants (Table 1). However in the case of two foliar spraying in the same experimental conditions, bean yield significantly increased with increase Se treatment concentrations in both cultivars only in the first year (Table 1). When plants were treated with Se twice in the second year, there was a trend of increasing mean bean yield with increasing Se concentrations in cv. Stanko. Bean yield was lower in the second year in both treatment (one and two foliarly spraying) because of the heat stress in the period of plant growth.

Table 1: Yield of bean plants cultivated in the soil in open area after one and two foliar spraying. Mean values are presented (n = 3). Columns marked with different letters are significantly different for each cv. A - cultivation in the first year, B - cultivation in the second year.

Tabela 1: Pridelek fižola, gojenega na poskusnem polju, ki je bil enkrat in dvakrat listno škropljen. Predstavljene so povprečne vrednosti (n = 3). Stolpci, označeni z različnimi črkami, se med seboj statistično značilno razlikujejo znotraj cv. A - gojenje rastline v prvem letu, B- gojene rastline v drugem letu.

Cultivar	Conc. of Se (mg Se/L)	One foliar treatment				Two foliar treatment			
		A		B		A		B	
		g/m²	g/plant	g/m²	g/plant	g/m²	g/plant	g/m²	g/plant
Stanko	0	147 a	7.3	137 a	6.9	150 a	7.5	145 a	7.3
	2.5	148 a	7.4	136 a	6.8	149 a	7.5	145 a	7.3
	5.0	148 a	7.4	139 a	7.0	161 b	8.1	150 a	7.5
	10.0	148 a	7.4	139 a	7.0	160 b	8.0	150 a	7.5
Topolovec	0	146 a	7.3	137 a	6.9	148 a	7.4	134 a	6.7
	2.5	144 a	7.2	136 a	6.8	153 ab	7.7	138 a	6.9
	5.0	147 a	7.3	135 a	6.8	158 b	7.9	132 a	6.6
	10.0	148 a	7.4	138 a	6.9	167 c	8.4	135 a	6.8

Yield of bean plants cultivated in the glinopor after foliar spraying

There was a trend of increasing yield in bean plants, cultivated in glinopor in greenhouse in cv. Topolovec once foliarly treated with Se (10.0 mg Se/L) (Table 2) in the first year. Additionally, plants twice foliarly treated with selenium (10.0 mg Se/L) have statistically significant higher yield ($p<0.05$) in cv. Topolovec in both years. Yield was similar in control and treated plants in cv. Stanko regarding year and number of foliar treatments. The exception was higher bean yield in twice foliarly treated plants in the second year. In this case yield was the highest in plants, foliarly treated with 5 mg Se/L.

Respiratory potential

ETS activity of plants cv. Stanko, grown from the seeds, soaked in Se was higher in comparison to the control (Fig. 1). ETS activity enhanced with Se application also in young bean plants in the same cultivar grown in the greenhouse in glinopor, once foliarly treated with Se (Fig. 2a). ETS activity in cv. Stanko was unaffected by Se in mature plants, twice foliarly treated with Se grown in glinopor in greenhouse and soil in open area (Fig. 2b). ETS activity in the cv. Topolovec was similar in plants grown from seeds soaked in the solution of comparing to control. In addition ETS activity in the same cultivar was similar in once and twice foliarly treated plants grown in the glinopor in greenhouse. ETS activity in the cv. Topolovec decreased in the once and twice foliarly treated plants grown in the soil in open area. ETS activity of both cultivars was higher in young plants comparing to mature plants (Fig. 2a, 2b).

Table 2: Yield of bean plants cultivated in the glinopor in the greenhouse after one and two foliar spraying. Mean values are presented (n = 3). Columns marked with different letters are significantly different for each cv. A - cultivation in the first year, B - cultivation in the second year

Tabela 2: Pridelek fižola, gojenega v rastlinjaku , ki je bil enkrat in dvakrat listno škropljen. Predstavljene so povprečne vrednosti (n = 3). Stolpci, označeni z različnimi črkami, se med seboj statistično značilno razlikujejo znotraj cv. A - gojenje rastline v prvem letu, B- gojene rastline v drugem letu

Cultivar	Concentration of Se (mg Se/L)	One foliar treatment				Two foliar treatments			
		A		B		A		B	
		g/m²	g/plant	g/m²	g/plant	g/m²	g/plant	g/m²	g/plant
Stanko	0	99 a	6.2	98 a	6.1	100 a	6.3	95 a	5.9
	2.5	99 a	6.2	100 ab	6.3	103 a	6.4	98 a	6.1
	5.0	97 a	6.1	104 b	6.5	101 a	6.3	99 a	6.2
	10.0	99 a	6.2	100 a	6.3	105 a	6.6	98 a	6.1
Topolovec	0	96 a	6.0	102 a	6.4	97 a	6.1	92 a	5.8
	2.5	96 a	6.0	102 a	6.4	99 a	6.2	94 ab	5.9
	5.0	96 a	6.0	106 a	6.6	97 a	6.1	96 b	6.0
	10.0	100 a	6.3	104 a	6.5	105 b	6.6	97 b	6.1

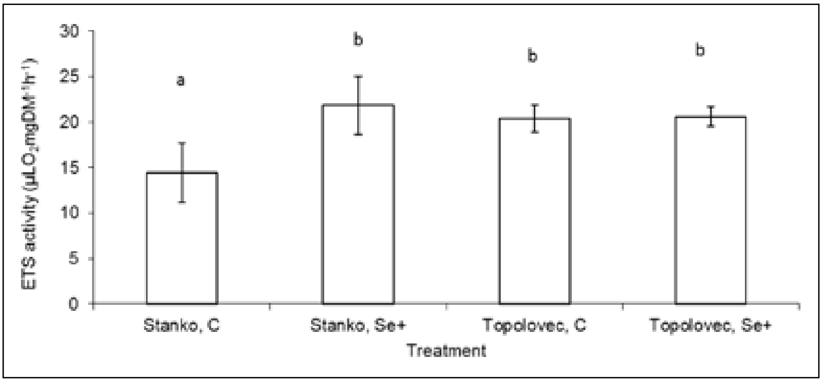

Figure 1: ETS activity in cv. Stanko and Toplovec. Mean values±SD are presented (n = 3). Columns marked with different letters are significantly different. C - control, Se+ - selenium treated plants

Slika 1: Aktivnost ETS pri kultivarjih Stanko and Toplovec. Predstavljene so povprečne vrednosti ±SD (n = 3). Stolpci, označeni z različnimi črkami, se med seboj statistično značilno razlikujejo. C - kontrola, Se+ - s selenom obravnavane rastline

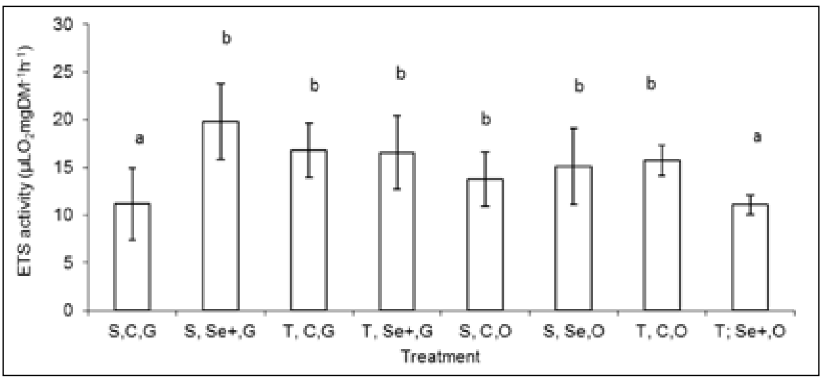

Figure 2a: ETS activity in cv. Stanko and Toplovec once foliarly treated with solution of selenate. Mean values ±SD are presented (n = 3). Columns marked with different letters are significantly different. S - cv. Stanko, T - cv. Topolovec, C - control, Se+ - selenium treated plants, G - greenhouse, O - open area

Slika 2a: Aktivnost ETS pri kultivarjih Stanko in Topolovec, ki sta bila enkrat listno škropljena z raztopino selenata. Predstavljene so povprečne vrednosti ±SD (n = 3). Stolpci, označeni z različnimi črkami, se med seboj statistično značilno razlikujejo. S - cv. Stanko, T - cv. Topolovec, C - kontrola, Se+ - s Se obravnavane rastline, G - rastlinjak, O - poskusno polje

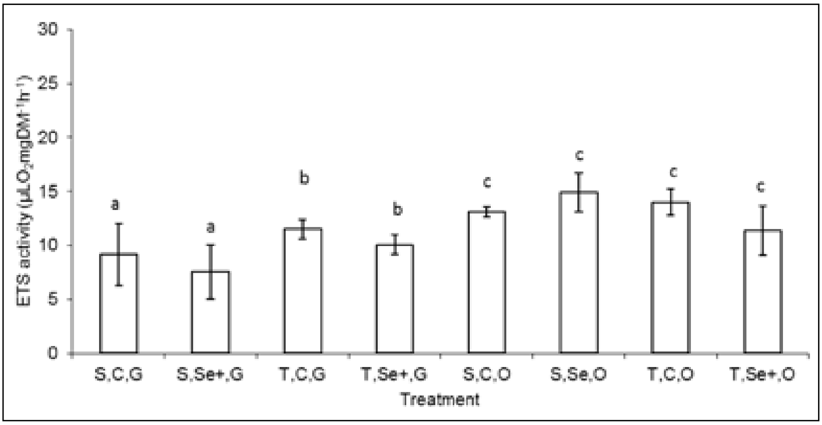

Figure 2b: ETS activity in cv. Stanko and Toplovec twice foliarly treated with solution of selenate. Mean values ±SD are presented (n = 3). Mean±SD (n=3). Columns marked with different letters are significantly different. S - cv. Stanko, T - cv. Topolovec, C - control, Se+ - selenium treated plants, G - greenhouse, O - open area

Slika 2b: Aktivnost ETS pri kultivarjih Stanko in Topolovec, ki sta bila dvakrat listno škropljena z raztopino selenata. Predstavljene so povprečne vrednosti ±SD (n = 3).. Stolpci, označeni z različnimi črkami, se med seboj statistično značilno razlikujejo. S - cv. Stanko, T - cv. Topolovec, C - kontrola, Se+ - s Se obravnavane rastline, G - rastlinjak, O - poskusno polje

Chlorophyll fluorescence

Se did not influence potential - F_v/F_m and effective - $\Delta F/F_m'$ photochemical efficiencyof PSII in any treatment (Table 3).

Table 3: Potential (Fv/Fm) and effective ($\Delta F/F_m'$) photochemical efficiency of PS II in control and Se treated plants. Mean values ±SD are presented (n = 5)

Tabela 3: Potencialna (Fv/Fm) in dejanska ($\Delta F/F_m'$) fotokemična učinkovitost FS II pri kontrolnih in obravnavanih rastlinah. Predstavljene so povprečne vrednosti ±SD (n = 5).

Treatment	Cultivar	Substrate		F_v/F_m	$\Delta F/F_m'$
Soaked	Cv. Stanko	Control		0.80 (0.01)	0.67 (0.06)
		Treated		0.82 (0.01)	0.72 (0.02)
	Cv. Topolovec	Control		0.80 (0.01)	0.72 (0.06)
		Treated		0.81 (0.01)	0.70 (0.04)
One foliarly treatment	Cv. Stanko	Glinopor	Control	0.78 (0.02)	0.20 (0.09)
			Treated	0.81 (0.01)	0.32 (0.08)
		Soil	Control	0.75 (0.04)	0.32 (0.10)
			Treated	0.77(0.02)	0.26 (0.06)
	Cv. Topolovec	Glinopor	Control	0.76 (0.02)	0.21 (0.07)
			Treated	0.81 (0.02)	0.22 (0.05)
		Soil	Control	0.77 (0.02)	0.26 (0.09)
			Treated	0.77 (0.04)	0.32 (0.10)

Two foliarly treatments	Cv. Stanko	Glinopor	Control	0.79 (0.02)	0.21 (0.05)
			Treated	0.78 (0.04)	0.22 (0.01)
		Soil	Control	0.76 (0.03)	0.22 (0.05)
			Treated	0.75 (0.04)	0.34 (0.08)
	Cv. Topolovec	Glinopor	Control	0.75 (0.05)	0.22 (0.07)
			Treated	0.78 (0.03)	0.21 (0.08)
		Soil	Control	0.79 (0.04)	0.30 (0.08)
			Treated	0.76 (0.04)	0.31 (0.09)

Legend: Soaked - cultivation on glinopor in a greenhouse with the technique of soaking seeds prior to sowing in a solution of selenate; once and twice foliar spraying with solution of selenate. Glinopor - glinopor in the greenhouse, Soil - in the soil in the open area

Legenda: Namakanje - semena so bila namakana v raztopino selenata, rastline iz teh semen so bile gojenje na glinoporju v rastlinjaku; enkrat oz. dvakrat foliarno škropljene rastline z raztopino selenata. Glinopor - glinopor v rastlinjaku, Soil – tla na poskusnem polju

Discussion

Growth and development

Se induced higher yield in twice foliarly sprayed plants in both cultivars grown in greenhouse and in cv. Topolovec grown in the soil in open area (Tables 1, 2). Present results of increased bean yield, cultivated with application of Se are in line with Marschner (2002) who claimed that Se induce luxuriant growth and higher yield of plants. Xue et al. (2001) reported about stimulating effect of added Se on the growth on lettuce in lower concentration of Se (0.1 mg/kg).

Se treatment significantly enhanced pumpkins yield (Germ et al. 2005) and buckwheat plants (Tadina et al. 2007). Hartikainen *et al. (*2000) also observed the growth promoting effect of Se in ryegrass that was partly a consequence of anti-oxidative effects, which can counteract the senescence processes. Xue *et al.* (2001) reported about stimulatory effect of foliar application of Se on growth of lettuce. Se also enhanced growth of potato (Turakainen *et al.* 2004), and leaves of green tea (Hu *et al.* 2003).

Respiratory potential and photochemical efficiency of PS II

Young plants had higher respiratory potential comparing to mature plants. Plants demand more energy during intensive growth and development, in order to build structural components (Smrkolj et al. 2006). Higher respiratory potential in young plants was reported for an aquatic plant *Potamogeton crispus* (Mazej and Gaberščik 1999), for *Fagopyrum esculentum* and *F. tataricum* (Breznik et al. 2005) and for *Pisum sativum* (Smrkolj et al. 2006). ETS activity of plants, grew from the seeds, soaked in Se was higher comparing to the control in cv. Stanko (Fig. 1). In addition, ETS activity was highest in leaves of young bean plants once foliarly treated with Se in cv. Stanko grown in the greenhouse comparing to control (Fig. 2a). The results are in line with the results from Smrkolj et al. (2006) obtained from the study on *Pisum sativum*. Enhanced ETS activity due to added Se, as presented here, has been known from chicory, foliarly treated with selenium twice with an aqueous solution containing 1 mg Se L^{-1} in the form of sodium selenate (to give 2 µg Se per plant) (Germ et al. 2007) and for *Eruca sativa,* where seeds were soaked for 4 hours in a solution of Na selenate (10 mg Se/L) (Germ and Osvald 2005). On the contrary, when *Glycine max* plants were foliary sprayed with an aqueous solution containing 10 mg Se/L in the forum of Na selenate, ETS activity was lower in treated compared to untreated plants (Mechora and Germ 2010). Sreekala et al. (1999) reported that when Se was applied to *Trigonella foenum-graecum* seedlings, mitochondrial oxygen uptake increased with the enhanced mitochondrial SOD activity. In pumpkin plants and common buckwheat (Germ et al. 2005, Breznik et al. 2005) foliar treatment with Se had no effect on the ETS activity. Higher respiratory potential presented here may reflect increased GSH-Px activity in mitochondria. Xue and coworkers reported that Se exposure increased GSH-Px activity in ryegrass and lettuce (Xue and Hartikainen 2000, Hartikainen et al. 2000, Xue et al. 2001). Higher respiratory potential in Se treated plants can be also a respond to higher demand of energy needed to mitigate toxic effect of Se. This is in line with the fact that Se can mimic sulphur, forming Se analogues of S compounds like replacing S in methionine and cysteine. The conformation of proteins containing selenoaminoacids could be perturbed leading to disturbing of their catalytic activity (Brown and Shrift 1982). However, visual symptoms of Se toxicity did not appear on bean plants. In addition the high potential - F_v/F_m and effective - $\Delta F/F_m$' photochemical efficiency of PSII in the plants, grew from the seeds, soaked in Se and Se foliarly sprayed plants evidenced that Se did not induce damage to photosynthetic apparatus (Table 3). Similar results were obtained in the study on pumpkins (Germ et al. 2005) and chicory (Germ et al. 2007). Potential photochemical efficiency of PSII were close to the theoretical maximum of unstressed plants (0.8- 0.83), that indicated an undamaged antenna complex (Bischof et al. 1998, Schreiber et al. 1995). In research on common buckwheat results shown that Se did not affect potential quantum use efficiency of PSII (Breznik et al. 2005). However, Se treatment induced the increase of the quantum use efficiency of PSII in strawberry as well as in common buckwheat (Valkama et al. 2003, Breznik et al. 2005). Se exerted a positive role on the photochemistry of PSII in these species.

Conclusions

Se increased the yield in twice foliarly sprayed plants in both cultivars of bean plants. Present results indicated positive effect of Se on crop. Young plants had higher respiratory potential than mature plants. ETS activity was higher in Se treated plants in cv. Stanko. Photochemical efficiency was unaffected by addition of Se.

Povzetek

Selen je široko razširjen po zemeljski obli in na razpolago rastlinam vsaj v majhnih količinah. Gojenje rastlin, obogatenih s selenom, je učinkovit način dodajanja selena ljudem in izboljšanju zdravja. V znanstvenem svetu poteka debata, ali je selen potreben za rastline. Obstajajo pa dokazi, da selen pri rastlinah pospešuje antioksidacijsko aktivnost, zavira procese, povezane s staranjem in omili stres zaradi visoke svetlobe in tudi suše. Ugotavljali smo vpliv dodange selena na fiziološke lastnosti in pridelek pri dveh kultivarjih fižola *Phaseolus vulgaris* cv. Stanko in Topolovec.

Semena smo namakali v raztopini selenata, jih posušili in naslednje leto posejali v rastlinjak. Na rastlinah, zrastlih iz obravnavanih in neobravnavanih rastlin, smo v drugem letu izvedli fiziološke meritve. V dveh zaporednih letih smo rastline listno škorpili z raztopino selenata in ugotavljali, kakšen je bil pridelek rastlin. Na kontrolnih, enkrat in dvakrat obravnavanih rastlinah smo izvedli meritve. Respiratorni potencial, merjen s pomočjo aktivnosti terminalnega elektronskega sistema, je bil višji pri mladih rastlinah. Mlade rastline rabijo energijo za izgradnjo svoje biomase. Respiratorni potencial je bil pri cv. Stanko višji pri rastlinah, obravnavanih s selenom, kar je lahko posledica višje aktivnost GSH-Px v mitohondrijih. Lahko pa je višji respiratorni potencial odraz povečane potrebe po energiji, ki jo rastlina rabi, da popravi škodo, ki jo je povzročil dodatek selena. Fotokemična učinkovitost je bila podobna pri kontrolnih rastlinah in rastlinah, obravnavanih s selenom, kar kaže na odsotnost stresa zaradi dodanega Se. Dodatek selena je povečal pridelek pri rastlinah, ki so bile dvakrat listno škropljene s selenom pri obeh kultivarjih, ki sta bila gojena v rastlinjaku. Z raziskavo smo želeli ugotoviti, ali selen stimulativno vpliva na pridelek fižola, ki je široko razširjena kmetijska rastlina. Raziskava je zanimiva tudi zato, ker je znano, da je v Sloveniji v tleh malo selena.

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Broadleaf and conifer tree responses to long-term enhanced UV-B radiation in outdoor experiments: a review

Odziv listavcev in iglavcev na dogotrajno povečano obsevanje z UV-B v naravnih razmerah: pregledni članek

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Abstract: Trees as a perennial growth form require time to evolve the complex response to enhanced ultraviolet-B radiation (UV-B), and this might lead to slow but important changes in forest ecosystem structure and function. Long-term outdoor experiments on trees however are few in number. The available published results suggest that broadleaf and conifer trees may show different response strategies to enhanced UV-B radiation. The long-term outdoor experiments indicate species- and season-specific differential responses in tree secondary metabolism, photosynthesis, water relations, and growth and development.

Keywords: broadleaf tree, conifer tree, UV-B radiation, long-term outdoor experiment

Izvleček: Drevesa s svojo dolgoživo življenjsko obliko potrebujejo čas, da razvijajo kompleksen odziv na povečano ultravijolično-B (UV-B) sevanje, kar lahko vodi do počasnih a pomembnih sprememb v zgradbi in delovanju gozdnih ekosistemov. Kljub temu so dolgotrajni poskusi na drevesih izvedeni v naravnem okolju maloštevilni. Rezultati iz literature kažejo, da listavci in iglavci lahko uberejo različne strategije odzivanja na povečano sevanje UV-B. Dolgotrajni poskusi izvedeni v naravnih razmerah kažejo različne vrstno in sezonsko specifične odzive na nivoju sekundarnega metabolizma, fotosinteze, gospodarjenja z vodo ter rasti in razvoja dreves.

Ključne besede: listavci, iglavci, sevanje UV-B, večletni poskus, naravne razmere

Introduction

Increases in solar ultraviolet-B radiation (UV-B) due to the continuing depletion of stratospheric ozone have been well documented over the past several decades (Madronich et al. 1998). A report from the World Meteorological Organization (WMO) estimates that full recovery of stratospheric ozone on a global scale will not occur before 2050 – 2100 and will depend upon continued compliance with the Montreal Protocol. The magnitude, longevity and spatial variation of changes in ozone concentrations remain a matter of study. Additionally, a linkage between ozone depletion and global warming has been proposed because of stratospheric cooling which tends toward ozone depletion reactions (WMO 2010).

The response of plants to UV-B radiation has gained attention over the past four decades. Most of the studies have involved agricultural and annual species, with fewer studies on trees. In spite of the importance of those woody plants in ecosystem productivity, function and in economics (Julkunen-Tiitto et al.2005), studies on trees are relatively few.

Studies on the effects of UV-B radiation on plants have used artificial UV-B irradiation sources such as sunlamps. The spectral output of sunlamps varies moderately according to lamp type and supply function, which is either a square-wave or a modulated wave (Sullivan 2005). The dosage used for irradiation in different studies varied immensely (Aphalo et al. 2012). Some experiments have utilized filters to reduce natural solar UV-B radiation and studies utilizing filters are of interest because they do not modify the spectral balance between UV-B and UV-A and UV-B and PAR and do not account for the UV-C that must be filtered from artificial lamp systems. Such experiments are more practical in remote natural environments where electric power is unavailable. Readers interested in more detailed explanation of UV-B research treatments, lamps, and filters are referred to Aphalo and co-workers (2012) for the most current detailed analysis of UV-B methodology.

Due to logistical limitations, a majority of studies conducted on trees have been performed on seedlings, but some studies have also been conducted on large trees (Laakso and Huttunen 1998, Sullivan et al. 2003).

Our knowledge of UV-B effects on trees is based to some extent on short-term experiments, covering less than one season. The perennial growth form of trees and/or the presence of evergreen foliage in the conifers and evergreen broadleaf species require time to evolve the complex response which is observed over a longer period of UV-B irradiation. The presumed cumulative effect of long-term UV-B radiation will not be observable in short-time experiments (Mirecki and Teramura 1984, Laakso et al. 2000). Accordingly, the present review will focus on studies which were carried out through one season or longer (Table 1).

Some studies on the effects of UV-B radiation on plants have been carried out in growth chambers or greenhouses where the natural light was limited and the artificial light failed to match, even closely, the intensity or spectral composition of natural light, specifically the ratio of UV-B to both UV-A and PAR (Sullivan 2005). The UV-B effect on plants in diminished and modulated light conditions is usually much more detrimental than is observed in studies of trees grown outdoors, where natural light conditions contribute to an effective tree response to UV-B (Laakso and Huttunen 1998). As a consequence, studies conducted in light conditions far from the natural solar light spectrum will not be considered in this review.

UV-B effects on plants

In general, the responses of trees to UV-B radiation are assumed to be similar to those of other plants. The response of high altitude and southern latitude plants to UV-B radiation is often less pronounced than response of low altitude and northern latitude plants, which shows acclimation of first two plant groups to enhanced UV-B (Turunen and Latola 2005). Sensitivity of trees to UV-B is however species-specific (Rozema et al. 2002) and related to the perennial growth form of trees and/or evergreen foliage in conifers and evergreen species.

Early investigations considered UV-B radiation merely as an environmental stressor for plants causing cell damage (Björn et al. 1999), but more recently, UV-B radiation has come to be regarded also as a regulator of plant growth and development. UV-B radiation regulates the expression of numerous genes involved in diverse plant processes, including metabolism, photosynthesis, morphogenesis, and defence against pests and pathogens (Rozema et al. 2002, Brown et al. 2005). Many of these responses are triggered by UV-B, and this is consistent with the involvement of a UV-B photoreceptor, UVR8, which regulates a range of UV-B responses by controlling transcription of over a hundred genes (Favory et al. 2009, Rizzini et al. 2011).

Plants respond to harmful doses of UV-B radiation with defence and repair mechanisms, namely reflection by waxy surface structures and hairs, induction of secondary metabolites, and repair of biomolecules (Day et al. 1992, Day 1993, Karabourniotis et al. 1999, Keiller and Holmes 2001, Rozema et al. 2002). Secondary metabolites such as flavonoids and other phenolic compounds are important in plant tolerance of UV-B radiation as screens that reduce UV-B penetration into leaf tissue (UV-B absorbing compounds), and as antioxidants protecting the plants from damage by reactive oxygen species (Rozema et al. 2002, Sullivan 2005). Further studies will research interactions occurring at the threshold UV-B dose where regulation and stress-induced plant morphogenesis overlap (Robson et al. 2014).

UV-B effects on trees

The first studies demonstrating UV-B effects on trees began in the 1970s and were conducted in greenhouses and growth chambers (Basioumy and Biggs 1975, Bogenrieder and Klein 1982). Sullivan (2005) reports that over 30 coniferous and broadleaf tree species were screened at that time and one-half of the conifers and one-third of the broadleaf species examined showed negative effects of exposure to supplemental UV-B radiation (Sullivan 2005). Subsequently, various valuable molecular approaches to studies of plant UV-B responses were developed. Among these there were a few long-term field studies on trees, and these offered additional insight into the complex response of trees to UV-B radiation and a natural condition of interactive environmental stresses (Trošt Sedej and Gaberščik 2008, Nybakken et al. 2012, Trošt Sedej and Rupar 2013, Virjamo et al. 2014).

Comparison of the responses of trees to UV-B radiation conducted on the same species in the field and in a greenhouse revealed that the responses were dominated by differences in experimental conditions. This article attempts to avoid the largest experimental variations by limiting further discussion to only long-term outdoor experiments.

UV-B effects on broadleaf trees

Enhanced UV-B radiation has been shown to induce various responses in broadleaf trees (Table 1). These range from morphological alterations to changes in biomass accumulation, physiology and metabolism (Kostina et al. 2001, Lavola et al. 2003, Sullivan 2005, Julkunen-Tiitto et al. 2005).

The most diverse response of broadleaf trees to enhanced UV-B radiation in long-term outdoor studies was observed in the rate of photosynthesis. This was found to decline in leaves of *Fraxinus excelsior*, *Betula pendula*, *Tilia cordata*, *Quercus robur*, and *Acer pseudoplatanus* after five years exposure to enhanced UV-B radiation (Keiller and Holmes 2001) and in *Fagus sylvatica* after three years exposure (Šprtova et al. 2003). Decrease in photosynthesis at multiple sites was attributed to direct UV-B damage to the photosynthetic apparatus, the oxygen evolving complex, D1/D2 reaction centre proteins, other components of photosystem II and Rubisco enzyme, as well as indirect damage through production of reactive oxygen species, reduced gas exchange by UV-B induction of stomatal closure and altered light environment within the leaf due to the changes of leaf anatomy (Nogues et al. 1999, Kataria et al. 2014). On the other hand, the lack of damage to photosynthesis in some broadleaf trees may be related to low penetration of UV-B through the epidermis (Day et al. 1992, Sullivan et al. 1996). The rate of photosynthesis was unaffected by exposure to enhanced UV-B radiation in *Quercus robur* after UV-B exposure for one year (Antonelli et al. 1998) and in *Liriodendron tulipifera* and *Liquidambar styraciflua* after three years (Sullivan et al. 2003). The potential photochemical efficiency was unchanged in *Fagus sylvatica* after three years of UV-B irradiation (Trošt Sedej and Rupar 2013). In *Acer rubrum* (Sullivan et al. 2003) the assimilation rate increased after three years of enhanced UV-B irradiation. It has been suggested that photosynthesis enhancement in *Acer rubrum* is due to increased levels of hydroxycinnamates in the mesophyll that could enhance blue light fluorescence induced by UV-B and contribute to carbon assimilation. Other researchers explained photosynthesis enhancement in terms of an increase in photosynthetic pigments (Warren et al. 2002).

In long-term outdoor studies, elevated UV-B radiation is found consistently to reduce a second, well-studied physiological parameter, transpiration. Transpiration was reduced in *Fraxinus excelsior*, *Betula pendula*, *Tilia cordata*, *Quercus robur*, and *Acer pseudoplatanus* after five years exposure to enhanced UV-B (Keiller and Holmes 2001) and in *Quercus rubrum*, *Liriodendron tulipifera* and *Liquidambar styraciflua* after three years of UV-B exposure (Sullivan et al. 2003). UV-B radiation induces stomatal closure directly by inhibiting K^+ accumulation in the guard cell plasmalemma, so that the transpiration through stomata decreases (Nogués et al. 1999). Besides, stomatal density may increase whereas the aperture of stomata may decrease under enhanced UV-B radiation (Zu et al. 2010). In Mediterranean plants cuticle thickening was observed under enhanced UV-B radiation, which reduced cuticle transpiration during the dry Mediterranean summer (Manetas et al. 1997).

Studies suggested that under enhanced UV-B radiation, trees may suffer an overall negative effect in total biomass. Furthermore, UV-B affected biomass accumulation in trees can be expected to be observed over several growing seasons as the defence costs accumulate (Keski-Saari and Julkunen-Tiitto 2003, Trošt Sedej and Gaberščik 2008). Decreased biomass may be a result of a decrease in chlorophyll content, photosynthesis or leaf area under enhanced UV-B radiation, which has been well established in crop plants but not in trees (Laakso et al. 1996, Li et al. 2010). Enhanced UV-B radiation reduces growth, even when there is no decrease of photosynthesis, and thus may be a consequence of altered carbon allocation or changes in the canopy structure (Nogués et al. 1998) or perhaps altered phytohormones (Robson et al. 2014). A long-term UV-B study on broadleaf tree detected no effect on biomass accumulation in *Salix myrsinifloia* after two years irradiation (Nybakken et al. 2012).

Long-term outdoor studies on the morphological parameters of broadleaf trees commonly demonstrated alteration in leaf thickness and leaf area. The increase in leaf thickness decreases the penetration of UV-B radiation to the photosynthetic leaf tissue. Leaf thickness increased with elevated UV-B radiation in *Quercus robur* (Antonelli et al. 1998) and in *Betula pendula* (Kostina et al. 2001), and leaf area was found to decrease in *Fraxinus excelsior*, *Betula pendula*, *Tilia cordata*, *Quercus robur* and *Acer pseudoplatanus* after five years exposure to enhanced UV-B radiation (Keiller and Holmes 2001), and in *Acer rubrum* and *Liliodendron tulipifera* after three years exposure (Sullivan et al. 2003). In contrast, leaf area in *Betula pendula* after one year of enhanced UV-B irradiation was not affected (Kostina et al. 2001) and was even increased in *Liquidambar styraciflua* after three years of enhanced UV-B irradiation (Sullivan et al. 2003). The mechanisms of leaf area reduction were not clarified but it appears that they are not necessarily the result of damage to the photosynthetic mechanism, because they do not always correlate with decrease in photosynthesis.

Trees contain a wide variety of different phenolic compounds, the bioactive components which are species-, genotype- and tissue-specific. They are present in leaves, stems, buds, flowers and roots, although their quantity and composition change in the course of plant development (Rozema et al. 2002). UV-B radiation has been shown to increase the accumulation of several secondary phenolic compounds under certain environmental conditions in some species. Flavonoids in particular and other phenolic compounds absorb strongly in the UV-B range (UV-B absorbing compounds) and also act as antioxidants scavenging reactive oxygen species (Tevini and Teramura 1989, Teramura and Sullivan 1994). The accumulation of UV-B absorbing compounds in the epidermis was shown to reduce UV-B radiation transmittance to deeper photosynthetic leaf tissues thus protecting sensitive targets (Caldwell et al. 1998).

The total UV-B absorbing compounds increased in *Quercus robur* after one year of UV-B exposure (Antonelli et al. 1998), in *Liriodendron tulipifera* after three years of UV-B exposure (Sullivan et al. 2003) and in *Salix myrsinifolia* after two years of UV-B exposure (Nybakken et al. 2012). Other studies demonstrated unchanged levels of total UV-B absorbing compounds in broadleaf trees after long-term UV-B irradiation, such as in *Quercus rubra* (Warren et al. 2002), *Acer rubrum* and *Liquidambar styraciflua* (Sullivan et al. 2003) and *Fagus sylvatica* (Trošt Sedej and Rupar 2013). The accumulation of UV-B absorbing compounds is dependent upon many environmental factors and in addition to UV-B protection, it may also impact plant-insect interactions in a variety of ways (Sullivan 2005).

UV-B effects on conifer trees

The most studied response of conifer trees to enhanced UV-B concerns phenolic compounds in their role as UV-B absorbing compounds (Table 2). Many studies have demonstrated that the epidermis of fully grown needles of conifers contains high amounts of phenolic compounds which can prevent the penetration of potentially detrimental UV-B radiation to the photosynthetic tissue below (Day et al. 1992, DeLucia et al. 1992). UV-B absorbing compounds are located both in vacuoles and within epidermal cell walls (Fischbach et al. 1999; Hoque

Table 1: Long-term (1year or more) field UV-B studies on broadleaf trees. Significant tree response is marked with: + (positive), - (negative), o (insignificant)

Tabela1: Dolgotrajni UV-B poskusi na listavcih v naravnih razmerah. Statistično značilen odziv drevesa je označen kot: + (pozitiven), - (negativen), o (neznačilen)

Broadleaf tree	**Exposure (years)**	Treatment	**Plant response**		**Authors**
Quercus robur	1	UV-B	Photosynthesis	o	Antonelli et al. 1998
			UV-B absorbing compounds	+	
			Leaf thickness	+	
Betula pendula	1	UV-B	Leaf area	o	Kostina et al. 2001
			Leaf thickness	+	
Acer pseudoplatanus	5	UV-B	**Photosynthesis**	-	Keiller and Holmes 2001
Betula pendula			**Transpiration**	-	
Fraxinus excelsior			**Leaf area**	-	
Quercus robur					
Tilia cordata					
Quercus rubra	3	UV-B	UV-B absorbing compounds	o	Warren et al. 2002
Acer rubrum	3	UV-B	**Photosynthesis**	+	Sullivan et al. 2003
			Transpiration	-	
			UV-B absorbing compounds	o	
			Leaf area	-	
Liquidambar styraciflua	3	UV-B	**Photosynthesis**	o	Sullivan et al. 2003
			Transpiration	-	
			UV-B absorbing compounds	o	
			Leaf area	+	
Liriodendron tulipifera	3	UV-B	Photosynthesis	o	Sullivan et al. 2003
			Transpiration	-	
			UV-B absorbing compounds	+	
			Leaf area	-	
Fagus sylvatica	3	UV-B	**Photosynthesis**	-	Šprtová et al. 2003
Fagus sylvatica	3	UV-B	Fv/Fm	o	Trošt Sedej and Rupar 2013
			Chlorophylls	o	
			UV-B absorbing compounds	o	
Salix myrsinifolia	2	UV-B	Biomass	o	Nybaken et al. 2012
			UV-B absorbing compounds	+	
		↑T	**Biomass**	+	
			UV-B absorbing compounds	-	
		UV-B x ↑T	**Biomass**	++	
			UV-B absorbing compounds	+	

and Remus 1999; Rozema et al. 2002, Turtola et al. 2006). The effective UV-B screening mechanism is an acclimation response to UV-B radiation and a significant part of a protective strategy of plants (Day et al. 1992, DeLucia et al. 1992, Trošt Sedej and Gaberščik 2008). Important component of the defence systems against enhanced UV-B is reflectance of UV light (Hoque and Remus 1999), special anatomical characteristics of the epidermis (Hoque and Remus 1999; Chalker-Scott and Scot 2004), and large amounts of different secondary compounds which, acting as antioxidants scavenge reactive oxygen species (Turtola et al. 2006).

Even though the accumulation of UV-B absorbing compounds is an acclimation response to solar UV-B that varies over spatial (latitude, altitude, canopy) and temporal (season, day) scales, phenolic compounds in conifers mostly do not respond to enhanced UV-B radiation in long-term outdoor experiments. Thus, UV-B absorbing compounds failed to respond to enhanced UV-B in *Pinus pinea* and *Pinus halepensis* after one year of irradiation and drought (Petropoulou et al. 1995), in *Pinus ponderosa* and *Pseudotsuga menziesii* after three years of irradiation (Warren et al. 2002) and in *Picea abies* after five and one years of irradiation (Trošt Sedej and Gaberščik 2008, Virjamo et al. 2014). These last two studies also demonstrated that UV-B absorbing compounds increased in *Picea abies* when UV-B radiation was combined with

Table 2: Long-term (1year or more) field UV-B studies on conifer trees. Significant tree response is marked with: + (positive), - (negative), o (insignificant)

Tabela 2: Dolgotrajni UV-B poskusi na iglavcih v naravnih razmerah. Statistično značilen odziv drevesa je označen kot: + (pozitiven), - (negativen), o (neznačilen)

Conifer tree	Exposure (years)	Treatment	Plant response		Authors
Pinus taeda	3	UV-B	**Biomass**	–	Sullivan and Teramura 1992
Pinus taeda	3	UV-B	**Fv/Fm**	–	Naidu et al. 1993
			Biomass	–	
Pinus halepensis	1	UV-B x drought	**Fv/Fm**	+	Petropoulou et al. 1995
Pinus pinea			**Photosynthesis**	+	
			Chlorophylls	o	
			UV-B absorbing compounds	o	
Pinus taeda	1	UV-B	**Biomass**	–	Sullivan et al. 1996
Pinus pinea	1	UV-B	Fv/Fm	o	Manetas et al. 1997
			Biomass	o	
	1	UV-B x drought	**Fv/Fm**	+	
			Biomass	+	
Picea abies	2	UV-B	**Photosynthesis**	–	Šprtova et al. 1999
			Chlorophylls	–	
Pinus sylvestris	3	UV-B	**UV-B absorbing compounds**	–	Kinnunen et al. 2001
Pinus ponderosa	3	UV-B	UV-B absorbing compounds	o	Warren et al. 2002
Pseudotsuga menziesii					
Picea abies	1	UV-B	Chlorophylls	–	Kirchgessner et al. 2003
Picea abies	5	UV-B	Fv/Fm	o	Trošt Sedej and Gaberščik 2008
			Photosynthesis	o	
			Chlorophylls	o	
			UV-B absorbing compounds	o	
			Biomass	o	
			Branch diameter	–	
			Fv/Fm	+	
	5	UV-B x drought	Photosynthesis	o	
			Chlorophylls	o	
			UV-B absorbing compounds	o	
			Biomass	o	
Picea abies	1	UV-B	UV-B absorbing compounds	o	Virjamo et al. 2014
			Biomass	o	
	1	UV-B x ↑T	**UV-B absorbing compounds**	+	
			Biomass	o	
	1	UV-B x ↑T x fertilization	**UV-B absorbing compounds**	+	
			Biomass	o	

other environmental stresses such as drought, high temperature and fertilizer. The UV-B absorbing compounds synthesis partly correlated with the needle age class (Trošt and Gaberščik 2008). The amount of UV-B absorbing compounds in *Pinus sylvestris* decreased after three years of enhanced UV-B radiation, which may indicate an inadequacy of the protective mechanisms when faced with an accumulated UV-B dose (Kinnunen et al. 2001).

High accumulation of UV-B absorbing compounds in the epidermis of many conifer trees results in low penetration of UV-B through the epidermis and absence of damage to the photosynthesis (Day et al. 1992, Sullivan et al. 1996, Fischbach et al. 1999; Hoque and Remus 1999, Turtola et al. 2006). This may also be the case in *Pinus pinea* after one year of enhanced UV-B irradiation and *Picea abies* after one/five years (Manetas et al. 1997, Trošt Sedej and Gaberščik 2008). The last two conifer species, *Pinus halepensis* and *Pinus pinea* (Petropoulou et al. 1995) even demonstrated an increase in photochemical efficiency under the interactive effect of enhanced UV-B combined with drought. This may be a result of an increase in leaf thickness and consequently, a decrease of UV-B penetration into mesophyll as well as a restriction in cuticular transpiration (Manetas et al. 1997). Decline in photosynthesis, which may be due to UV-B induced damage to the photosynthetic apparatus or to UV-B induction of stomatal closure or to altered light environment within the leaf, was observed in *Pinus teada* and *Picea abies* after three and two years of enhanced UV-B radiation, respectively. In *Picea abies* the decrease in photosynthesis could be correlated with the decrease in chlorophyll content (Naidu et al. 1993, Šprtova et al. 1999). Studies have reported negative, neutral and occasionally, positive effects of enhanced UV-B radiation on chlorophyll content (Bassman et al. 2003, Kirchgessner et al. 2003, Lavola et al. 2003, Trošt Sedej and Gaberščik 2008, Láposi et al. 2009). It was shown that UV-B radiation can not only inhibit chlorophyll synthesis or cause photo-oxidation of chlorophyll (Bornman 1989, Middleton and Teramura 1993), as was observed in *Picea abies* (Šprtova et al 1999, Kirchgessner et al. 2003) but under favourable irradiation conditions, it can also increase the biosynthesis of photosynthetic pigments (Middleton and Teramura 1993, Jordan 1996) although this has not been confirmed in any long-term field experiment. There was no significant impact on chlorophyll content in *Pinus halepensis* and *Pinus pinea* after one year or in *Picea abies* after five years of enhanced UV-B radiation (Petropoulou et al. 1995, Trošt Sedej and Gaberščik 2008).

The UV-B susceptibility of biomass accumulation in conifer species proved to be in *Pinus taeda*, where biomass decreased after one year (Sullivan et al. 1996) as well as after three years of enhanced UV-B irradiation (Sullivan and Teramura 1992, Naidu et al. 1993). In *Picea abies* no effect on biomass was detectable after either one and five years of enhanced UV-B irradiation or after interaction of UV-B, drought, high temperature and fertilization exposure (Trošt Sedej and Gaberščik 2008, Virjamo et al. 2014). Meanwhile, in *Pinus pinea* biomass accumulation was not affected by UV-B, while an alleviating effect of enhanced UV-B to biomass was observed during drought (Manetas et al. 1997).

Conclusion

The diversity of results in different studies reflects the complex response of trees to UV-B radiation as a function of a wide spectrum of environmental factors and their multilevel interactions. The levels of UV-B absorbing compounds in broadleaf trees increase frequently after enhanced UV-B radiation which is not the case in conifer trees which however may benefit more of constitutive flavonoid synthesis. Even though the responses are species-specific; there is no clear general agreement concerning the response of broadleaf and conifer trees to enhanced UV-B radiation.

Summary

The response of plants to UV-B radiation has attracted attention over the past four decades. Early investigations considered UV-B radiation to be merely an environmental stressor causing cell damage in plants, but more recently, UV-B radiation has also been regarded as a possible regulator of plant growth and development. In this review, in order to avoid the largest experimental variations, we discuss long-term UV-B studies conducted on

broadleaf and conifer trees in natural light conditions, with the aim of gaining some insight into the complex cumulative response of perennial plants.

In long-term outdoor studies in broadleaf trees, elevated UV-B radiation rather diversely affects photosynthetic rate, showing no effect on biomass accumulation but increasing leaf thickness in some cases. The UV-B absorbing compounds in the broadleaf tree either increased or were unchanged.

In conifer trees the most studied response to enhanced UV-B radiation was synthesis of UV-B absorbing compounds, which generally did not react to UV-B irradiation in long-term outdoor experiments. High accumulation of UV-B absorbing compounds in the epidermis of many conifer trees results in low penetration of UV-B through the epidermis and lack of damage to the photosynthetic mechanisms. The UV-B susceptible biomass accumulation in conifer species was observed in loblolly pine, but in other conifers, no effect on biomass was detected. In some conifers an alleviating effect of enhanced UV-B to biomass was observed during drought.

Povzetek

Raziskave učinkov povečanega sevanja UV-B na rastline so se razmahnile tekom zadnjih štirih desetletij. V prvih raziskavah so sevanje UV-B smatrali predvsem kot stresni dejavnik, ki povzroča poškodbe celic, kasnejše raziskave sevanje UV-B obravnavajo tudi kot pomemben regulator rasti in razvoja rastlin. V tem preglednem članku povzemamo samo rezultate dolgotrajnih študij sevanja UV-B na listavcih in iglavcih izvedenih v naravnem okolju, z namenom izogniti se velikim eksperimentalnim razlikam in s ciljem poglobiti vpogled kompleksni odziv dolgoživih rastlin na povečano sevanje UV-B.

Pri listavcih je povečano sevanje UV-B precej različno vplivalo na intenziteto fotosinteze, medtem ko učinka na kopičenje biomase ni bilo, vendar pa je pogosto prišlo do povečane debeline listov. UV-B absorbirajoče snovi so se povečale ali ostale nespremenjene.

Pri iglavcih so bile najbolj raziskane UV-B absorbirajoče snovi, ki se večinoma niso odzvale na povečano sevanje UV-B. Velike količine konstitutivnih UV-B absorbirajočih snovi so bile prisotne v povrhnjici mnogih iglavcev, kar je preprečilo prodiranje UV-B do fotosintezno aktivnih tkiv lista in s tem tudi poškodb fotosinteznih procesov. Biomasa se pod vplivom povečanega sevanja UV-B ni spremenila, razen pri eni vrsti. Pri nekaterih iglavcih je ob sočasnem delovanju povečanega sevanja UV-B in suše prišlo do blaženja negativnih posledic sevanja.

Raznolikost rezultatov v študijah potrjuje kompleksnost kot tudi vrstno specifičnost odziva listnatih in iglastih dreves na povečano UV-B sevanje v odvisnosti od celotnega spektra okoljskih dejavnikov in njihovih interakcij.

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Influence of red deer (*Cervus elaphus* L.) grazing on yield reduction and changes in the chemical composition of grassland forage: experiences from an organic farm at Stari Breg in the Kočevje Region

Vpliv paše jelenjadi (*Cervus elaphus* L.) na zmanjšanje pridelka in spremembe hranilne vrednosti krme trajnega travinja: izkušnje iz ekološke kmetije v Starem Bregu na Kočevskem

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Abstract: In 2013 and 2014 the effect of red deer grazing (*Cervus elaphus*) on yield decrease and its changes in forage quality was investigated on the permanent grassland of an organic cattle farm at Stari Breg in the Kočevje region. We performed the standard method of yield loss determination (iron cages) in the period from June to October in three cuts. In the first year of research we determined a 56% yield loss (4.0 t/ha of dry matter), and in the second year a 75% yield loss (5.0 t/ha of dry matter). In 2014 the content of crude proteins in the forage was always larger in an unprotected (control) treatment than in a protected treatment, and we annotate this to permanent vegetative sward regrowth due to severe and uncontrolled red deer grazing. On the contrary, the content of crude fiber was higher in the forage that was enclosed in cages (treatment protected). Due to large yield loss at all cuts we also observed a significantly smaller yield of crude proteins, metabolized energy, and NEL in the control treatment. We established that in the studied location that red deer were an important biotic factor that limited productivity on the permanent grassland. This is why it is necessary to find solutions in the future that enable the co-existence of humans (farmers) and wildlife.

Keywords: permanent grassland, yield reduction, cutting, feeding value, red deer, Kočevje region

Izvleček: V letih 2013 in 2014 smo na trajnem travinju ekološke govedorejske kmetije v Starem Bregu na Kočevskem preučevali vpliv paše jelenjadi (*Cervus elaphus*) na zmanjšanje pridelka in njegove hranilne vrednosti. S standardno metodo določanja zmanjšanja pridelka zaradi paše (železne kletke) smo v obdobju od junija do oktobra izvedli tri košnje in v prvem letu raziskave ugotovili za 56 % manjši pridelek suhe snovi (4 t suhe snovi/ha), v drugem letu pa celo za 75 % manjši pridelek (5 t suhe snovi/ha). Vsebnost surovih beljakovin je bila v letu 2014 na nezavarovani površini vedno večja kot na zavarovani površini trajnega travinja, kar pripisujemo stalnemu pomlajevanju travne ruše kot posledico obtrgavanja jelenjadi. Nasprotno je bila vsebnost surove vlaknine v povprečju večja v zelinju na zavarovanih mestih. Zaradi velikega zmanjšanja pridelka smo pri vseh treh košnjah na nezavarovanih parcelah ugotovili manjši pridelek surovih beljakovin (P < 0,05), presnovljive energije (P < 0,05) in NEL (P < 0,05). Ugotavljamo, da je jelenjad na preučevani lokaciji pomemben biotični dejavnik omejevanja produktivnosti trajnega travinja, zato bo potrebno v prihodnjih letih poiskati rešitve, ki bodo omogočile sobivanje človeka (kmeta) in divjih živali.

Ključne besede: trajno travinje, zmanjšanje pridelka, košnja, hranilna vrednost, jelenjad, Kočevska

Introduction

Wild ungulates are in Slovenia, which is the third most forest abudant (more than 60% of forest cover) European country, an imortant biotic factor which decreases the productivity of cultivated plants. In Slovenia, economically speaking, the most harmful species of game by far is the wild boar (*Sus scrofa*), which causes damage especially by rooting the grass sward, eating plants, and destroying the maize and other cereal fields. Also, red deer (*Cervus elaphus*), whose noxiousness is defined through grazing on grassland – as the grass feed represents arround 50% of their nutrition (Trdan and Vidrih 2008, Bleier et al. 2012, Verbič et al. 2013, Laznik and Trdan 2014). Already Adamič (1990) and afterwards Jerina (2006) and later also Adamič and Jerina (2010) report about red deer grazing activity on forest edges, pastures, and meadows through the entire year due to a lack of feed in the forest and its clearings, and in such a way cause direct yield reduction from grassland (Milner et al. 2006, Mysterud 2006, Lande et al. 2014). This issue is not encompassed by researchers in such experiments. We were the first group of agricultural experts in the area of southern and central Europe who already drew attention to the aforementioned situation at the beginning of the millennium (Trdan et al. 2000). Both wildlife species cause large difficulties to cattle and small ruminat producers in the Kočevje region, because they need to buy additional forage due to the yield loss of voluminous feed. Also, grassland restoration and prevention measures are expensive, due to the large density of the previously mentioned species because no type of prevention works efficiently. For damage on grassland caused by wild boar, which is reported to competent authorities, farmers can expect corresponding compensation due to the yield loss. Meanwhile farmers find it harder to receive compensation for the reduction of grass yield due to red deer grazing. In this research we wanted a standard and also internationally recognised methods of evaluating yield loss of voluminous feed, and to study the differences in the chemical composition of herbage from a protected plot and plots heavily grazed by red deer. The paper consists of experimental results from years 2013 and 2014, gained on permanent grassland in Stari Breg in the Kočevje region, and where red deer represent an important biotic factor of yield reduction in voluminous feed.

Materials and methods

We conducted research in years 2013 and 2014 on the permanent grassland of the Zemljič organic cattle farm in the area of village Stari Breg (45°41'7.12" N, 14°55"16.33" E) in the Kočevje region. We chose this farm due to the fact that they face substantial yield reduction of voluminous feed on permanent grassland due to red deer grazing. The level of forage production on meadows where we conducted the experiment is extensive to moderately intensive. They performed a late first cut, and afterwards the cattle graze till the end of the growing season. Meadows are only fertilized with stable manure and slurry, and animal excreta from grazing animals must be added as well. According to the typology of habitats in Slovenia (Jogan et al. 2004), meadows and pastures of the studied area belong to a habitat of manured mesotrophic and eutrophic slightly moist grasslands on relatively dry soils and inclined positions with prevailing tall oat-grass (Physis code 38.221), and which have less biomass and which is cut up to two times per year.

Experimental design

We started the experiment in 2013 on 10th of May, and on 9th of May 2014 when we first placed cages on selected plots (they were surrounded by forest, and red deer are present throughout the whole calendar year) in Stari Breg. The outer sizes of the cages were 1 x 1 x 0.5 m, and so even one time larger than we used in previous research (Trdan et al. 2003, Trdan and Vidrih 2008). In 2013 we placed five cages on each of three locations, and in 2014 we conducted the experiment only on one location with five cages. We placed cages in an area that was from 100 to 200 m away from the forest edge, and the distance between cages varied from 15 to 20 m. In order so the red deer could not move the cages, we anchored them with a 15cm long peg into the soil on both short sides of the cage.

In both years the position of the previously mentioned 15 cages was not changed from their placement until we took a sample of herbage mass (Tab. 1), as such plots (area of 1m^2) represented a protected surface (treatment "protected" or "PROT") with optimal yield. Only in 2013 we placed an additional three cages approximately two to three weeks before each of three sampling dates (cuts) in all three locations (treatment "regeneration" or "REG") to protect grass sward which was already grazed (defoliated) by red deer. When sampling the herbage we determined the grass sward capability of regeneration (renewal) or yield loss which appears in the sward that is previously (in early spring time) grazed by red deer – but two to three weeks before the appointed cutting time protected against grazing. The most direct yield loss or yield reduction (treatment "control" or "CONT") due to the aforementioned wild ungulates on grassland we measured on sampling dates when we were on each of the three locations (2013). Also, one location (2014) we placed three cages and in places that were most heavily grazed.

Weather conditions in the research area

To follow the growth and development pattern of grass sward, and also the time and type of utilization we need to know the weather conditions of the studied area. Both growing seasons were something special, according to long-term rainfall and air temperature averages. In Slovenia due to the warm and moist weather in winter of 2013 there was no real growth interruption of grasses, legumes, and herbs. Favourable weather conditions even accelerated early spring growth when grasses developed and transferred from the previous vegetative developmental phase to the generative phase and developed leaves on stems and inflorescence. May 2013 and June and July 2014 were relatively hot, and with significant rainfall (Fig. 1) which made difficulties for hay conservation and even silage making. If, however, farmers managed to conserve the latter forage from the grassland, we cannot confirm this for the most expanded form of conserved feed in Slovenia as the hay or fodder of the first cut on meadows was only utilized in the first 10 days of June, and this did not predict good quality of hay (Verbič and Žnidaršič 2013).

Table 1: Time line of activities on grassland experiment in Stari Breg in 2013 and 2014

Tabela 1: Časovni potek opravil pri poskusu v Starem Bregu v letu 2013 in 2014

Year	Placement date of cages	Date of shifting the cage (regeneration)	Date of cut
2013	10.5	27.5	18.6
	4.7	31.7	27.8
	27.8	-	14.10
2014	9.5	-	6.6
	6.7	-	13.8
	13.8	-	3.10

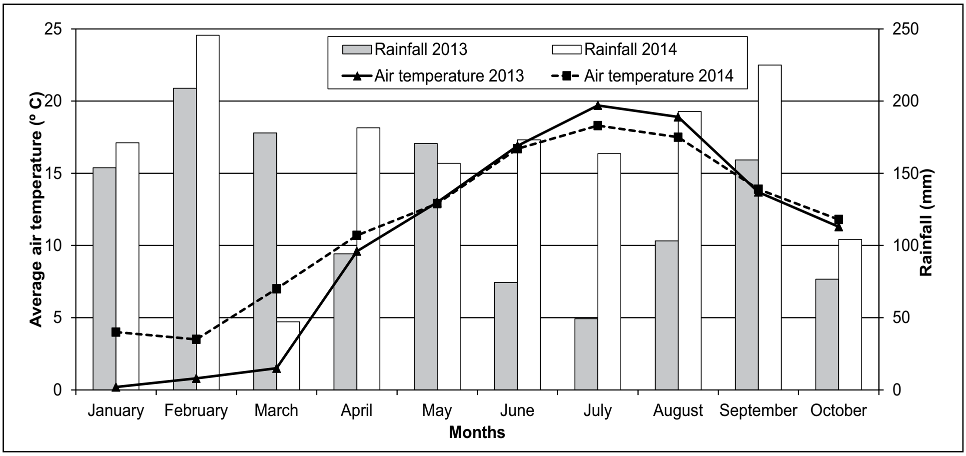

Figure 1: Average monthly air temperature and rainfall at Kočevje in years 2013 and 2014

Slika 1: Povprečna temperatura zraka in količina padavin po mesecih v Kočevju v letih 2013 in 2014

Herbage sample analysis

Herbage samples were hot air dried in a dryer at 45 °C to a constant weight at the Biotechnical Faculty, Agronomy Department in Ljubljana. After drying the samples we determined the air dry matter of the yield for each of the plots in the experiment. In 2014 we milled all samples for the further chemical analysis through a 1mm sieve (mill Brabender, no. 880804, Brabender, Duisburg, Germany). In the laboratory at the Institute for Hygiene and Pathology of Animal Nutrition of the Veterinary Faculty, a Weende analysis was conducted to determine the content of dry matter, moisture, crude protein, crude fiber, crude fat, and ash. The values of metabolic energy (ME) and net energy for lactation (NEL) were calculated according to the Univertität Hohenheim (1997). We selected measured parameters to help us calculate the yield of crude protein and net energy for lactation. Measured and calculated values of the chemical composition in the herbage ranged in selected classes of quality (excellent, good, undesirable) according to Verbič and co-workers (2011). Herbage samples from 18 experimental plots in Stari Breg from 2014 were included in the chemical analysis, nine from treatment PROT and nine from treatment CONT.

We evaluated the results of the experiments with the statistical software Statgraphics Centurion XVI (Statgraphics 2009). Using an analysis of variance (ANOVA) and the Newman-Keuls multiple test ($P<0.05$), we assessed the differences between treatments (PROT, REG, CONT). The results are graphically presented as an average yield of herbage dry matter (± SE), converted into t/ha in two (three – when considering the results of regeneration) treatments in Stari Breg at a particular cut. On the basis of such presented results we calculated the average reduction of grass yield due to red deer grazing in the Kočevje region and described it in percentages in figures.

Results

With the data analysis collected at the first cut, on 18 June 2013 we confirmed in two locations and in treatment PROT the expected and significantly highest yield (4.8-7 t/ha) of dry matter. The yield loss on treatment CONT (unprotected plots) ranged from 40 to 68%, while sward regeneration capability we determined only on 1 (52%) and 3 location (13%) (Fig. 2).

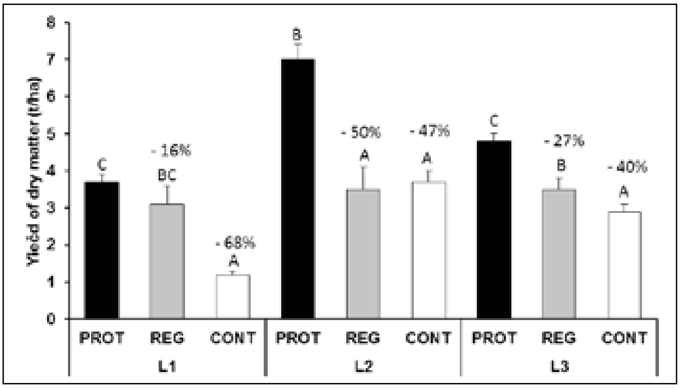

Figure 2: The yield of dry matter (t/ha) at the 1st cut in three locations (L1, L2, L3) and in three treatments (PROT - protected, REG - regeneration, CONT - control) in Stari Breg in 2013. The number above the columns indicates yield loss (%) due to deer grazing. The different letters denote values which represent statistically significant differences (Newman-Keuls test) at the 0.05 significance level

Slika 2: Pridelek sušine (t/ha) ob 1. košnji na treh lokacijah (L1, L2, L3) in v treh obravnavanjih (PROT, REG, CONT) v Starem Bregu v 2013. Številke nad stolpcem predstavljajo zmanjšanje pridelka (%) kot posledica paše jelenjadi. Različne črke označujejo vrednosti, ki se statistično razlikujejo (Newman-Keuls-ov preizkus) pri stopnji tveganja 0,05

At the second cut in Stari Breg, 27 August 2013, we established in two locations in treatment PROT the significantly highest yield of herbage dry matter (1.3-1.4 t/ha) and yield reduction in CONT plots from 77 to 86%. We do not explain the results of the third location, as cows trod on the experimental surface. At the first location we determined a 14% regeneration capability of grazed sward in the last three weeks before the cut took place (Fig. 3).

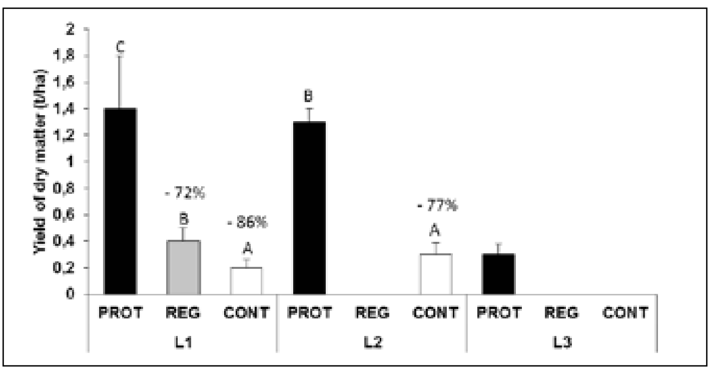

Figure 3: The yield of dry matter (t/ha) at the 2nd cut in three locations (L1, L2, L3) and in three treatments (PROT - protected, REG - regeneration, CONT - control) in Stari Breg in 2013. The number above the columns indicates yield loss (%) due to deer grazing. The different letters denote values which represent statistically significant differences (Newman-Keuls test) at the 0.05 significance level

Slika 3: Povprečni pridelek zračno suhega zelinja (t/ha) ob 2. košnji na treh lokacijah (L1, L2 in L3) in v treh obravnavanjih (PROT, REG, CONT) v Starem Bregu v 2013. Številke nad stolpcem predstavljajo zmanjšanje pridelka (%) kot posledica paše jelenjadi. Različne črke označujejo vrednosti, ki se statistično razlikujejo (Newman-Keuls-ov preizkus) pri stopnji tveganja 0,05

At the third cut in Stari Breg on 14 October 2013 (Fig. 4), we determined in all three locations in treatment PROT the significantly highest yield of dry matter (0.8-1.5 t/ha). The yield reduction on unprotected plots ranged from 71 to 94% or from only 50 to 280kg/ha. Also, the renegerative capacity was very low (1%), and which was determined due to the lack of water at the first location (Fig. 4).

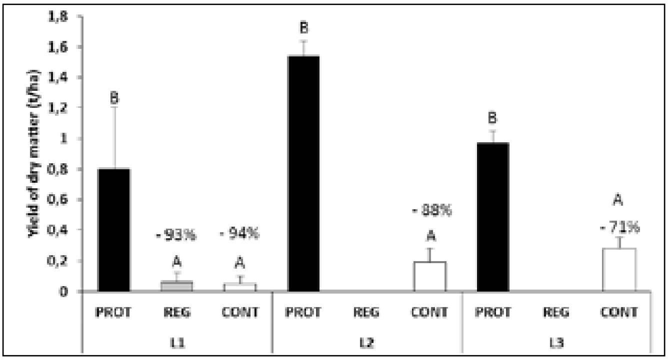

Figure 4: The yield of dry matter (t/ha) at the 3rd cut in three locations (L1, L2, L3) and in three treatments (PROT - protected, REG - regeneration, CONT - control) in Stari Breg in 2013. The number above the columns indicates the yield loss (%) due to deer grazing. The different letters denote values which represent statistically significant differences (Newman-Keuls test) at the 0.05 significance level

Slika 4: Povprečni pridelek zračno suhega zelinja (t/ha) ob 3. košnji na treh lokacijah (L1, L2 in L3) in v treh obravnavanjih (PROT, REG, CONT) v Starem Bregu v 2013. Številke nad stolpcem predstavljajo zmanjšanje pridelka (%) kot posledica paše jelenjadi. Različne črke označujejo vrednosti, ki se statistično razlikujejo (Newman-Keuls-ov preizkus) pri stopnji tveganja 0,05

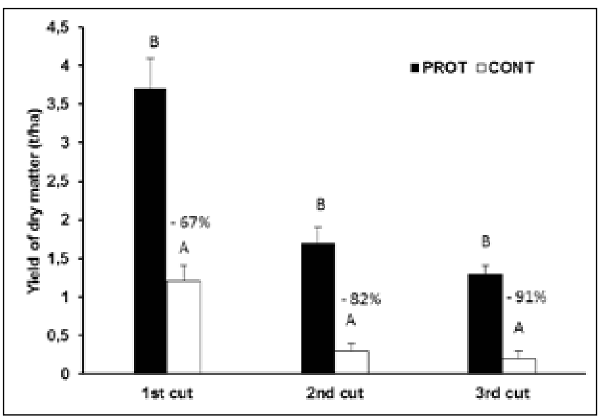

Figure 5: The yield of dry matter (t/ha) of all three cuts in Stari Breg in 2014. The number above the columns indicates the yield loss due to deer grazing. The different letters denote values which represent statistically significant differences (Newman-Keuls test) at the 0.05 significance level

Slika 5: Povprečni pridelek zračno suhega zelinja ob vsaki košnji v Starem Bregu v 2014 v obravnavanjih ZAV in KON. Številke nad stolpcem predstavljajo zmanjšanje pridelka kot posledica paše jelenjadi. Različne črke označujejo vrednosti, ki se statistično razlikujejo (Newman-Keuls-ov preizkus) pri stopnji tveganja 0,05.

In the year 2014 we conducted the first cut 12 days earlier than in year 2013. That led to a lower yield compared to the previous year and what we got in treatment PROT (3.7 t/ha) 1.5 t/ha lower yield. In the total yield the first cut represented 55% of the whole yield. The yield loss due to red deer grazing increased through the season and cuts, and we measured 67, 82, and 91% of yield loss and was always a statistically significant lower amount (Fig. 5).

Table 2: The chemical composition (crude protein - SB, crude fibre - SVI, metabolize energy - ME, net energy for lactation - NEL) (±SE) of forage in Stari Breg at all three cuts in two treatments in 2014. The different letters within the cuts denote values which represent statistically significant differences (Newman-Keuls test) at the 0.05 significant level

Tabela 2: Vsebnost surovih beljakovin (CP), surove vlaknine (CF), presnovljive energije (ME) in neto energije za laktacijo (NEL) (±SN) zelinja v Starem Bregu ob vseh treh košnjah in v obeh obravnavanjih v letu 2014. Različne črke znotraj košnje označujejo vrednosti, ki se statistično razlikujejo (Newman-Keulsov preizkus) pri stopnji tveganja 0,05

Cut	Treatment	CP (g/kg DM)	SVI (g/kg DM)	ME (MJ/kg DM)	NEL (MJ/kg DM)
1.	PROT	98.93±8.25a	274.63±11.52b	8.02±0.04a	4.57±0.02a
	CONT	104.17±1.99a	218.11±22.01a	7.99±0.08a	4.57±0.05a
2.	PROT	158.53±6.29a	236.13±13.26a	8.45±0.09a	4.86±0.05a
	CONT	175.20±7.71b	250.68±19.86a	8.36±0.05a	4.81±0.03a
3.	PROT	156.03±3.97a	189.52±12.25a	8.38±0.05a	4.83±0.03a
	CONT	157.07±9.03a	178.89±5.81a	8.39±0.05a	4.84±0.03a

We determined the difference in the content of crude protein between treatments PROT and CONT only at the second cut and that it was statistically significantly higher in favour of the yield on unprotected plots (treatment CONT -175.20 g/kg DM). When considering this nutritional parameter of feed quality the value of hay (first cut) on protected plots was of undesirable quality and on unprotected plots was of good quality (Verbič et al. 2011). Nutritional value of yield from second and third cut in both treatments was of excellent quality. When comparing data of the crude fibre content in herbage between treatments PROT and CONT we confirmed significant difference (in favour of crude fibre on protected plot) only at the hay cut meanwhile the dry matter yield of second and third cut showed no significant differences in the content of crude fibre between treatments. When taking this parameter into account of herbage quality the dry matter yield of all three cut was of excellent quality (Verbič et al. 2011). Comparison of ME and NEL content between treatments showed no significant differences at neither cuts and it ranged from 7.99 to 8.45 and 4.57 to 4.86 MJ/kg DM, respectively. When comparing ME and NEL values from experiment to appointed classes (Verbič et al. 2011) we got on protected and unprotected plots the lowest values in the first cut and that the following two cuts gave higher energy values (Tab. 2).

Table 3: The yield of dry matter (DM), crude protein (SB), and net energy for lactation (NEL) (±SE) in Stari Breg at all three cuts and and two treatments in 2014. The different letters within the cuts denote values which represent statistically significant differences (Newman-Keuls test) at the 0.05 significant level

Tabela 3: Pridelek sušine zelinja (DM), surovih beljakovin (CP), neto energije za laktacijo (NEL) (±SN) v Starem Bregu ob vseh treh košnjah in v obeh obranavanjih v letu 2014. Različne črke znotraj košenj označujejo vrednosti, ki se statistično razlikujejo (Newman-Keuls-ov preizkus) pri stopnji tveganja 0,05.

Cut	Treatment	Yield of DM (t/ha)	Yield of CP (kg/ha)	Yield of NEL (GJ/ha)
1.	PROT	3.7±0.4b	366.04±3.30b	16.91±0.00b
	CONT	1.2±0.2a	125.00±0.40a	5.48±0.01a
2.	PROT	1.7±0.2b	269.50±1.26b	8.26±0.01b
	CONT	0.3±0.1a	52.56±0.77a	1.44±0.00a
3.	PROT	1.3±0.1b	202.84±0.40b	6.28±0.00b
	CONT	0.2±0.1a	31.41±0.90a	0.97±0.00a

If red deer grazing did not have major influence on nutritional value of conserved forage, the yield results of selected parameters (Tab. 3) yet changed differently. In treatment CONT the yields of dry matter, crude protein and NEL were at the each of the three cuts statistically significantly lower as in treatment PROT. Only at the first cut we determined in treatment PROT higher yield than 3 t/haof dry matter, meanwhile in all other treatments through the season we harvested less than 1.8 t/ha of dry matter. Also crude protein yield only exceeded the value of 300 kg/ha at first cut in treatment PROT and later on the yield of crude protein only decreased in following two cuts. Only the first cut gave the yield of NEL higher than 15 GJ/ha and it happened merely in treatment PROT (Tab. 3).

Discussion

With more than 60% of forest cover, Slovenia is the third most forest abundant European country. The consequence of this fact is that a large portion of agricultural land borders forests – and animals living in the forest search for feed on arable land, meadows, pastures, and farm facilities (damaging round bale silage, flat silage silo) of better quality more intensively than they do in their native living environment. When looking for feed and then also consuming that forage, the game can cause considerable damage (Lande et al. 2014). The fact is that grassland occupies around two thirds of agriculturally utilized land, and that voluminous feed from semi-natural and sown grassland with no regard to what future strategy for fodder production the state will have, the grass fodder will always present an important component of cattle or small ruminant nutrition (Verbič at al. 2011). Also, the most importance in animal ruminant nutrion ratio the grassland forage will have – as it has had until now exactly in forested areas of Slovenia (Kočevje, Ribnica, Notranjska regions, and defined areas of Novo mesto and Gorenjska region) and also grassland land use for the purpose of livestock feed – represents a measure against land abandonment and shrub encroachment (Trdan et al. 2000). On previously mentioned areas red deer grazing on grassland has more significance than on areas with more intensive crop (arable) production (Prekmurje region) (Gönter et al. 2007), as in such regions despite the known fact of unwanted activity – they do not pay much attention to it.

On the basis of the results of a two-year study of grass sward productivity on permanent grassland in the vicinity of forests in Stari Breg in the Kočevje region, where red deer grazing exhibits an important biotic factor of yield reduction, especially on cut meadows, we conclude the following:

- The total yield of all three cuts in year 2013 in treatment PROT gave 7.2 t/ha and was statistically and significant higher than the yield of dry matter in treatment CONT (2.9 t/ha). Yield loss accounted for 4.0 t/ha or 56%.
- When considering results of all three cuts in year 2014 we established a yield loss of 75% (5.0 t/ha).
- With chemical analysis of sampled herbage in an experiment we determined that the content of crude protein in treatment CONT was always higher than in treatment PROT. This is due to red deer grazing which rejuvenate grass sward with progressive defoliation and removal of herbage and force grasses to form new leaves which also hold the most important part of fodder quality.
- On the contrary happened to crude fibre, which content was the higest in herbage in treatment PROT. Nutritional value of conserved feed at all locations was low as it not reached 5 MJ/kg dry matter even at the first cut. This we attribute to poor floristical composition of studied grassland (Trdan et al. 2014).
- Viewpoint, which is not included generally in similar research as this one, is that red deer does not reduce the yield on the grassland only with the direct defoliation of grass but also because of seasonally early and permanent and uncontrolled grazing. The latter namely restricts the intensive growth of grass sward on sown meadows and lessens the growing vitality of grassland plants and makes floristic composition poorer (Trdan et al. 2014) and this leads to long term negative consequences for forage production (Verbič et al. 2013).
- Management of big game (harvest quotas, oriented grazing) must be significantly changed in the near future in the Kočevje region if we expect farmers to cultivate the land in a manner of sustainable coexistance with wildlife.

Povzetek

Z več kot 60 % pokritostjo z gozdovi spada Slovenija s Finsko in Švedsko med tri najbolj gozdnate države v Evropski uniji. Posledično je divjad v Sloveniji pomemben biotični dejavnik zmanjševanja produktivnosti rastlinske pridelave. Gospodarsko najbolj škodljivi vrsti sta divji prašič (*Sus scrofa*), ki povzroča škodo zlasti z ritjem po travnikih in objedanjem in tlačenjem koruze ter jelenjad (*Cervus elaphus*), ki je kot prežvekovalec, ki mu voluminozna krma predstavlja približno 50 % hrane, škodljiv zlasti s pašo na travinju . Obe vrsti divjadi povzročata velike težave govedorejcem in rejcem drobnice na Kočevskem, ki morajo zaradi izpada voluminozne krme le to kupovati, sanacija zemljišč in njihovo varovanje pred divjadjo pa je drago in predvsem – tudi zaradi velikega staleža divjadi na omenjenem območju – več ne zagotavlja učinkovitosti. Za škodo, ki jo pozroči divji prašič na travinju, ki jo kmetje prijavijo, lahko pričakujejo ustrezno nadomestilo zaradi izpada pridelka, medtem ko je odškodnino za izpad pridelka zaradi paše jelenjadi od pristojnih organizacij težje pridobiti. V pričujoči raziskavi smo želeli s standardnimi in tudi v tujini uveljavljenimi postopki ovrednotiti izpad pridelka (voluminozne krme) in zmanjšanje hranilne vrednosti krme zaradi paše jelenjadi na travinju na Kočevskem. V prispevku predstavljamo rezultate dveletnega (2013 in 2014) poskusa na trajnem travniku v Starem Bregu, kjer predstavlja jelenjad že vrsto let pomemben biotični dejavnik izpada pridelka voluminozne krme. V poskusu smo z varovalnimi železnimi kletkami ugotavljali zmanjšanje pridelka zaradi paše jelenjadi ob vsaki košnji, in sicer v letu 2013 na treh lokacijah in v letu 2014 na eni lokaciji v Starem Bregu. Poskus v letu 2013 je obsegal tri obravnavanja: zavarovano, regeneracija in nezavarovano, v letu 2014 pa samo obravavanji zavarovano in nezavarovano. V letu 2014 smo odvzeli tudi 18 vzorcev zelinja za določevanje hranilne vrednosti. Zmanjšanje oz. izpad pridelka smo določili s košnjo in tehtanjem zelinja ter izračunom razlike med pridelki sušine na parcelicah, ki so bile zavarovane z železnimi kletkami od začetka poskusa in pridelki sušine na nezavarovanih parcelicah. Povprečni izpad pridelka mrve v letu 2013 je bil 56 %, v letu 2014 pa je znašal 75 %. Vsebnost surovih beljakovin je bila v letu 2014 na nezavarovani površini vedno večja kot na zavarovani površini trajnega travinja, kar pripisujemo stalnemu pomlajevanju travne ruše kot posledico obtrgavanja jelenjadi. Nasprotno je bila vsebnost surove vlaknine v povprečju večja v zelinju na zavarovanih mestih. Zaradi velikega

zmanjšanja pridelka smo pri vseh treh košnjah na nezavarovanih parcelah ugotovili manjši pridelek surovih beljakovin ($P < 0,05$), presnovljive energije ($P < 0,05$) in NEL ($P < 0,05$). V pričujoči raziskavi je bil preučevan samo izpad pridelka na travinju, ki nastane zaradi neposredne paše jelenjadi 3 do 4 tedne pred začetkom košnje. Ne smemo pa pozabiti tudi na posredni vpliv paše te velike parkljaste divjadi na travno rušo v poznem zimskem in zgodnjem spomladanskem času, s čimer prav tako zmanjšuje proizvodni potencial travinja.

Acknowledments

We would like to thank the Zemljič family from Stari Breg 2, Kočevje for letting us use their grassland for experimental purposes, and which they use for fodder production, as well as for the construction of the iron cages that we used in the research. The research work in both years was financially supported by the municipality of Kočevje.

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Kapusov goseničar (*Cotesia glomerata* (L.), Hymenoptera, Braconidae) v Sloveniji

Parasitoid wasp *Cotesia glomerata* (L.) (Hymenoptera, Braconidae) in Slovenia

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Izvleček: V prispevku predstavljamo parazitoidno oso kapusovega goseničarja (*Cotesia glomerata*) in njegov pomen v biotičnem varstvu rastlin. Vrsta je znan naravni sovražnik kapusovega belina (*Pieris brassicae*), ki je napadom kapusovega goseničarja izpostavljen zlasti v obdobju pojavljanja od prve do tretje larvalne stopnje. Palearktična vrsta, ki je po načinu razvoja endoparazitoid, je bila na območju današnje Slovenije prvič omenjena že leta 1870, vendar podrobnejših zapisov o njenem pojavljanju pri nas ni na voljo. Zastopanost kapusovega goseničarja smo v letu 2014 potrdili na dveh lokacijah v Sloveniji. Namen pričujočega prispevka je predstavitev kapusovega goseničarja z namenom njegove intenzivnejše prihodnje uporabe v varovalnem biotičnem varstvu rastlin.

Ključne besede: kapusov goseničar, *Cotesia glomerata, Apanteles glomeratus,* kapusov belin, *Pieris brassicae,* kapusnice, zelje, *Brassica oleracea*, varovalno biotično varstvo rastlin

Abstract: Paper represents parasitoid wasp of Cabbage Butterfly (*Cotesia glomerata*) and its importance for biological control. Species is known as a natural enemy of Cabbage Butterfly (*Pieris brassicae*) which is exposed to parasitoid wasp attacks particularly in the period between the first and third larval stage. Palaeartic species, which has a developmental characteristics of endoparasite, was in the area of Slovenia mentioned for the first already in 1870 but not enough detail records about its occurrence are available. We confirmed the presence of *Cotesia glomerata* in 2014 at two locations in Slovenia. The purpose of present contribution is the introduction of parasitoid wasp of Cabbage Butterfly with the intention of its future more intensive use in conservation biological control.

Keywords: parasitoid wasp of cabbage butterfly, *Cotesia glomerata*, *Apanteles glomeratus*, cabbage butterfly, *Pieris brassicae*, Cole crops, cabbage, *Brassica oleracea*, conservation biological control

Uvod

V red metuljev (Lepidoptera), ki je druga vrstno naštevilčnejša skupina žuželk, uvrščamo številne škodljivce gojenih in samoniklih rastlin. Med naravnimi sovražniki (biotičnimi agensi), s katerimi lahko vplivamo na manjšo številčnost populacij škodljivih vrst metuljev, strokovna literatura najpogosteje navaja parazitoidne ose. Med družinami os najezdnikov je najpogosteje omenjena družina Braconidae (Michel-Salzat in Whitfield 2004). Ugotovljeno je bilo, da parazitoidne ose iz omenjene družine uspešno zmanjšujejo številčnost gosenic vsaj 53 različnih vrst metuljev. Parazitoidne ose, ki najpogosteje napadajo različne vrste gosenic, uvrščamo v poddružino Microgastrinae. Med vrstno najštevilčnejšimi rodovi te poddružine je rod *Cotesia* Cameron (Hymenoptera, Braconidae, Microgastrinae), v katerega uvrščamo približno 1000 vrst, ki se pojavljajo na vseh celinah. Shaw in Huddleston (1991) opisujeta 400 različnih vrst. V Severni Ameriki je bilo doslej opisanih 84 vrst (Michel-Salzat in Whitfield 2004), v Evropi oziroma na območju Palearktika pa 93 različnih vrst (Fauna Europea 2013).

Rod *Cotesia* predstavlja skupina primarnih parazitoidov, ki se najpogosteje omenjajo kot naravni sovražniki gosenic iz družin sovk (Noctuidae), gobarjev (Lymantriidae), belinov (Pieridae), pedicev (Geometridae), veščcev ali somračnikov (Sphingidae) in medvedkov (Arctiidae) (Kankare in Shaw 2004, Michel-Salzat in Whitfield 2004). Kompleksnost odnosov med gostitelji in parazitoidi je bila preučevana prav s sodelovanjem žuželk iz rodu *Cotesia*, na primer vrste *C. congregata* (Say), *C. glomerata* (L.), *C. kariyai* (Watanabe) *in C. rubecula* (Marshall). Z namenom preučevanja vedenja parazitoidnih os se tako zelo pogosto uporablja vrsta *C. rubecula*, medtem ko vrsta *C. melitaearum* (Wilkinson) služi kot objekt za genetske raziskave (Michel-Salzat in Whitfield 2004).

Rod *Cotesia* je v 19. stoletju prvič opisal Cameron, vendar se je do ponovne klasifikacije družine Braconidae (Mason 1981) omenjeno rodovno ime uporabljalo kot sinonim rodovnemu imenu *Apanteles* Förster. V podobni povezavi so do leta 1865 uporabljali tudi rodovno ime *Microgaster*. Kljub temu, da ima rod *Cotesia* zelo velik pomen v znanstveno-raziskovalnem delu, pa je še vedno relativno malo znanega o njegovi sistematiki. Papp (1988) razvršča evropske predstavnike iz rodu *Apanteles* v 12 različnih skupin, in sicer *Apanteles* Förster, *Choeras* Mason, *Cotesia* Cameron, *Deuterixys* Mason, *Distatrix* Mason, *Dolichogenidea* Viereck, *Glyptapanteles* Ashmead, *Iconella* Mason, *Illidops* Mason, *Pholetesor* Mason, *Protapanteles* Ashmead in *Sathon* Mason. Njegova razdelitev temelji na morfologiji odraslih osebkov in se ne ujema s poznejšo razdelitvijo Michel-Salzatove in Whitfield-a (2004), ki sta s filogenetsko raziskavo potrdila obstoj 4 različnih skupin, in sicer *melanoscela* (kamor spadajo vrste *C. melanoscela* [Ratzeburg], *C. flavipes* (Cameron) in *C. ruficrus* [Haliday]), skupina *kariyai* (*C. kariyai*, *C. kazak* [Telenga], C. *cyaniridis* [Riley], *C. flaviconchae* [Riley], *C. anisotae* [Muesebeck] in *C. griffini* [Viereck]), skupina *rubecula* (*C. congregata* [Riley], *C. electrae* [Viereck], *C. euchaetis* [Ashmead], *C. marginiventris* [Cresson], *C. obsuricornis* [Viereck], *C. schizurae* [Ashmead]), skupina *glomerata* (*C. glomerata*, *C. melitaearum*, *C. plutellae* [Kurdjumov] in dodatno *C. hyphantriae* [Riley], *C. diacrisiae* [Gahan] in *C. empretiae* [Viereck]) (Michel-Salzat in Whitfield 2004).

Geografska razširjenost

Vrsti *C. glomerata* in *C. rubecula* sta palearktični in sta bili z namenom zatiranja repnega belina (*Pieris rapae* [L.]) načrtno vneseni v Severno Ameriko (Cox 2004). Kapusov gosenčar je zastopan v tropskih območjih Afrike, Avstraliji, območjih Nearktika in Neotropov. V Evropi je bila vrsta doslej ugotovljena na območju Nizozemske, Slovaške, Švedske, Poljske, Norveške, Irske, Danske, Češke, Kanarskih otokov, Cipra, Azorov, Finske in v Švici, Ukrajini, Rusiji, Španiji, Romuniji, Bolgariji, Veliki Britaniji, Belgiji, Franciji, Nemčiji, Turčiji, Litvi, Latviji in Italiji (Fauna Europea 2013).

Omenjeni vrsti parazitoidnih os sta se v Severno Ameriko širili tudi po naravni poti (le Masurier in Waage 1993, Van Driesche et al. 2003). Možno je tudi, da se je vrsta *C. glomerata* v Severni Ameriki pojavljala že pred repnim belinom, vendar je bila v tem primeru populacija najverjetneje zelo maloštevilna.

Medtem ko parazitoid *C. rubecula* svoj razvojni krog zaključi samo na gosenicah repnega belina (Brodeur et al. 1998), se kapusov gosenčar lahko prehranjuje na različnih gosenicah iz rodu *Pieris* (*P. brassicae* [L.], *P. rapae*, *P. protodice* Boisduval & Leconte, *P. napi* [L.] in *P. melete* [Ménétriès]) in še na nekaterih drugih vrstah, na primer *Colias lesbia* (Fabricus) in *Aporia crataegi* [L.] (van Driesche et al. 2003). V laboratorijskih razmerah lahko kapusov gosenčar razvojni krog zaključi tudi na vrsti *P. virginiensis* [Edwards] (van Driesche et al. 2003).

Na območju današnje Slovenije je najezdnika gosenic »zeljnega belina« (ki ga danes poznamo pod imenom kapusov belin) že leta 1870 opisal Viljem Schleicher, ime kapusov goseničar je prvi uporabil zaslužni profesor Franc Janežič (1951), ki pa podrobnejšega opisa vrste in njegove razširjenosti v Sloveniji ne navaja. Vrabl (1992) kapusovega gosenočarja omenja kot učinkovitega naravnega sovražnika kapusovega belina, pozneje (1999) pa je tega naravnega sovražnika kot vrsto, ki pri nas še ni preučena, omenjala Milevoj-eva.

Opis in razvojni krog kapusovega gosenočarja

Odrasli osebki kapusovega gosenočarja (slika 1) so črni in merijo od 2,6 do 3,5 mm. Iz bub prilezejo po približno 8 dneh (Le Masurier 1991, Milevoj 1999). Vse vrste iz rodu *Cotesia* so koinobionti, za katere je značilen endoparazitoizem. Za parazitoidne ose, ki so po svoji naravi koinobionti, velja, da imajo zelo ozek krog gostiteljev (Askew in Shaw 1986, Kankare in Shaw 2004). V strokovni literaturi zasledimo podatke, da so od kapusovega gosenočarja lahko napadene gosenice prve in druge (Brodeur in Vet 1996, Coleman et al. 1997), tretje (Hasan in Ansari 2010) ali celo pete razvojne stopnje (Laing in Levin 1982). Učinkovitost parazitiranja slednjih je najmanj verjetna, saj lahko gosenice s sunkovitim premikanjem telesa (obrambna reakcija) otežijo ali celo preprečijo ovipozicijo (Brodeur et al. 1995, Hasan in Ansari 2010).

Slika 1: Odrasel osebek kapusovega gosenočarja (foto: J. Rupnik)

Figure 2: An adult specimen of the wasp of Cabbage Butterfly (Photo: J. Rupnik)

V laboratorijskih razmerah lahko samica kapusovega gosenočarja v posamezno gosenico odloži od 20 do 40 jajčec (Gu et al. 2003), medtem ko lahko na prostem v gosenici najdemo okoli 60 jajčec (Tagawa 2000, Gu et al. 2003). Ličinke omenjene parazitoidne ose se približno 25 dni hranijo v notranjost gosenic s hemolimfo, medtem ko se gostitelj prehranjuje na gostiteljski rastlini. Ko ličinke kapusovega gosenočarja dosežejo drugo razvojno stopnjo, zapustijo gostiteljsko gosenico, ki je takrat navadno v peti razvojni stopnji. Na površju gosenic ali v njihovi bližini se nato ličinke zabubijo (slika 2). Najprej se iz bub (kokonov) izležejo samci. Feromoni, ki so značilni za omenjene bube, privlačijo samce iz drugih kokonov. Samice se lahko parijo takoj po preobrazbi iz bube (Tagawa in Kitano 1981). Lain in Levin (1982) ter Tagawa in Kitano (1981) navajajo, da se odrasli osebki parijo že 5 minut po prihodu iz bube. Po parjenju začnejo samice aktivno iskati gostitelje (Laing in Levin 1982).

Na prostem lahko samica kapusovega gosenočarja preživi od 3 do 5 dni (Geervliet et al. 1997). V tem času mora samica najti več gostiteljev (gosenic), v katere odloži od 500 do 2200 jajčec (Vos in Vet 2004). Samice privlačijo poškodovani listi rastlin, bodisi umetno oziroma zaradi prehranjevanja žuželk (Geervliet et al. 1998). Kapusov gosenočar prezimi v diapavzi v razvojnem stadiju predbube zunaj gostitelja (Shapiro 1976, Tagawa et al. 1984). Na jugu Anglije ima kapusov gosenočar navadno 3 rodove na leto, enako pa poročajo iz ameriške zvezne države Massachusetts (Le Masurier 1991).

Domače najdbe kapusovega gosenočarja v letu 2014

V letu 2014 smo parazitirane gosenice kapusovega belina (*Pieris brassicae*) našli na dveh različnih lokacijah, na Zgornji Lipnici (46°19′ N, 14°10′ E, nadmorska višina 511 m) in na Laboratorijskem polju Biotehniške fakultete v Ljubljani (46°04′ N, 14°31′ E, nadmorska višina 299 m). Na Zgornji Lipnici smo parazitirane gosenice

Slika 2: Gosenica kapusovega belina in bube kapusovega gosenièarja na listu zelja (foto: T. Bohinc)

Figure 2: The caterpillar of Cabbage Butterfly and pupae of wasp of Cabbage Butterfly (Photo: T. Bohinc)

škodljivca našli na zgodnjem zelju (*Brassica oleracea* L. var *alba*) 15. julija in 1. avgusta. Na Laboratorijskem polju Biotehniške fakultete v Ljubljani smo parazitirane gosenice kapusovega belina našli na listih kodrolistnega ohrovta (*Brassica oleracea* L. var *sabellica*) 1. oktobra 2014. Parazitirane gosenice kapusovega belina so bile v večini primerov v tretji in četrti razvojni stopnji.

Parazitirane gosenice kapusovega belina smo shranili v steklene insektarije v Laboratoriju za entomologijo na Katedri za fitomedicino, kmetijsko tehniko, poljedelstvo, pašništvo in travništvo (Oddelek za agronomijo Biotehniške fakultete v Ljubljani). Nabrane gosenice smo dopolnilno hranili z listi kapusnic, predvsem zelja. Po približno od 7 do 10 dneh so nekatere gosenice pokazale znake parazitiranosti. Na površju omenjenih gosenic so se pojavile bube rumene barve. Po nekaj dneh so iz bub začele izletavati parazitoidne ose. Izlegle odrasle osebke smo shranili v 70 % etanol in jih poslali v Edinburgh (Natural Museums of Scotland), kjer jim je vrsto določil dr. Mark R. Shaw. Osebke je identificiral kot vrsto *Cotesia glomerata*.

Pomen kapusovega gosenièarja v varstvu rastlin in sklepi

Kapusov gosenièar je bil doslej med drugim ugotovljen v Italiji, na Madžarskem in v Avstriji (Fauna Europea 2013). Po nam znanih podatkih je na območju današnje Slovenije kapusovega gosenièarja kot »gosenčnega najezdnika« prvi opisal Viljem Schleicher (1870). V besedilu, ki je za današnjega bralca zelo atraktivno, je med drugim zapisal: *»Gotovo je marsikdo zapazil, da je bilo med skoro popolnoma doraščenimi gosenicami (zeljnega belina, op.p.) mnogo bolnih in medlih, pa da koderkoli so se po stenah, plotovih itd. plazile, niso se mogle zabubiti. Kmalu potem se je prikazalo prav veliko majhnih, jajčastih in rumenih bačvic, ki so bile podobne zapredkom (kokonom) sviloprejke, to se ve, da neskončno manje, in v vsakej bačvici bila je po ena buba. Ako take kokončke, kterih se iz ene same gosenice večkrat čez 20 naredi, v zaprto posodo postavimo, ne bo nam treba dolgo čakati in videli bomo, da so se bačvice odprle, in da iz vsake en majhen najezdnik prileze. Kdor tega ne ve, ta si bo lahko mislil, da so bačvice, v katerih so bube najezdnika zaprte, in se jih po vrtnih plotovih in drugih enakih krajih dostikrat v prav velikem številu najde, škodljive, ali si bo pa mislil, da so to jajčica zeljnega belina, ter je bo na vse kriplje pokončaval, s tem pa dosegel le to, da se bodo drugo leto zeljni belini v toliko večem številu zaplodili«.*

Od omenjenega zapisa je do danes v Sloveniji o kapusovem gosenièarju pisala le še Milevoj-eva (1999), Janežič (1951) in Vrabl (1992) sta temu najezdniku namenila le nekaj besed. Milevojeva kapusovega gosenièarja omenja kot zelo znano in splošno razširjeno vrsto, ki pa v naših podnebnih in agroekoloških razmerah ni preučena. Omenjena entomologinja, sicer začetnica sistematičnega raziskovalnega in strokovnega dela na področju biotičnega varstva rastlin v Sloveniji (Milevoj 2011), je tako pred 15 leti izpostavila potrebo po preučitvi razširjenosti in učinkovitosti kapusovega gosenièarja v naših zelnikih, tudi z namenom zavarovanja vrste pred neustreznimi agrotehničnimi posegi in zaradi spodbujanja biotičnega varstva rastlin. Želimo, da je pričujoči prispevek razumljen kot odgovor na pobudo Milevojeve.

Pretirana raba fitofarmacevtskih sredstev (FFS) je v preteklosti povzročila veliko negativnih vplivov na okolje (Hallmann e tal. 2014). Omenimo naj predvsem številne pojave rezistence škodljivih organizmov na FFS in veliko smrtnost naravnih sovražnikov in ostalih koristnih organizmov (npr. opraševalcev) (Balmer et al. 2014). Na drugi strani pa imajo pozitiven vpliv na zastopanost in številčnost naravnih sovražnikov tudi cvetoče samonikle rastlinske vrste, kjer naravni sovražniki najdejo zavetje in alternativno hrano (Balmer et al. 2014). Na lokaciji Zgornja Lipnica, ki predstavlja eno od dveh lokacij, na katerih smo v letu 2014 našli kapusovega goseničarja na gosenicah kapusovega belina, za zatiranje škodljivih organizmov na zelju ne uporabljajo sintetičnih FFS, kar potrjuje hipotezo Balmer et al. (2014).

Za zatiranje kapusovega belina je v Sloveniji registriranih več sintetičnih insekticidov na podlagi sedmih aktivnih snovi (Seznam registriranih … 2014). Med njimi so doslej potrdili sprejemljiv vpliv piretroida lambda-cihalotrin na kapusovega goseničarja (Araya et al. 2005). Novejše raziskave v svetu so bile usmerjene predvsem v preučevanje delovanja azadirahtina na populacije kapusovega goseničarja, pri čemer negativnega delovanja tega rastlinskega insekticida na naravnega sovražnika niso ugotovili (Wawrzyniak in Wrzesinska 2000).

Na podlagi rezultatov našega strokovnega in raziskovalnega dela na področju biotičnega varstva rastlin in informacij o kronologiji in pomenu kapusovega goseničarja v Sloveniji in širše, menimo, da ima omenjeni parazitoid pri nas potencial za omejevanje škodljivosti kapusovega belina in nekaterih drugih vrst škodljivih gosenic. Njegovo širšo prihodnjo uporabo vidimo zlasti v okviru varovalnega biotičnega varstva rastlin, ki je lahko pomemben tehnološki ukrep v okviru integrirane ali ekološke pridelave živeža. Z nadaljevanjem zmanjševanja števila registriranih FFS, med katerimi imajo mnoga neželeno neciljno delovanje na naravne sovražnike, oziroma z intenzivnejšo uporabo okoljsko sprejemljivejših fitofarmacevtskih pripravkov, se bo v naravnem okolju povečevalo število naravnih sovražnikov, s tem pa tudi njihov pomen v agroekosistemih. V tej zvezi vidimo tudi svetlejšo prihodnost biotičnega varstva rastlin in v njegovem okviru tudi vrste, ki jo predstavljamo v tem prispevku.

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Summary

The paper for the first time in Slovenian professional literature presents in detail the insect species *Cotesia glomerata* (L.), the Palaearctic parasitoid wasp from the family Braconidae. The white butterfly parasite is today present in tropical regions of Africa, Australia, Nearctic and Neotropical regions. The species, known as a natural enemy of the cabbage butterfly (*Pieris brassicae*), has thus far been found in at least 25 European countries, also in Slovenia. The cabbage butterfly parasite was in the region of the present-day Slovenia for the first time described probably already in 1870, when Schleicher presented quite in detail the parasitic relationship between caterpillars of »Kohhveissling« and »ichneumon fly«; the pest was most probably the cabbage butterfly, while the natural enemy was the cabbage butterfly parasite. Later the cabbage butterfly parasite was in Slovenian expert literature (Janežič, Vrabl, Milevoj) mentioned several times, yet without any concrete data on its appearance in Slovenia. In 2014 we picked in Zgornja Lipnica (early cabbage) and Ljubljana (curly kale) the cabbage butterfly caterpillars in the third and the fourth developmental stage. The caterpillars, which were *in vitro* additionally fed with brassica leaves, after 7-10 days displayed signs of parasitism. The adult specimens, which developed from pupae on the surface of parasited caterpillars, were determined to be the species *Cotesia glomerata*. The species, which can conclude its developmental cycle also on caterpillars of some other species from the genus *Pieris* (P. *rapae, P. protodice, P. napi, P. melite*), has in our view the potential to limit the harmfulness of the caterpillars from the genus *Pieris*. Its more extensive application in future is envisioned primarily within concervation biological control,

which can be an important technological measure in integrated or organic production of Brassicas. By continuing reduction of the number of registered phytopharmaceuticals, many of which have unwanted effects on natural enemies, or by more intensive application of environmentally more acceptable phytopharmaceuticals, the natural environment will produce more natural enemies and thus augment their significance in agroecosystems. In this context we also see brighter future of biological control and the pertaining indigenous European species *Cotesia glomerata*.

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Cordycepin production by *Cordyceps militaris* cultivation on spent brewery grains

Proizvodnja kordicepina z gojenjem glive *Cordyceps militaris* na pivovarskih tropinah

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Abstract: This is a first report on *C. militaris* mycelia and fruiting bodies cultivation on solid-state containing spent brewery grains (SBG). Five different strains of *C. militaris* were cultivated on substrates containing rye grains and 0 to 60% SBG. Stromata formation on SBG containing substrates was noticed with two *C. militaris* strains. All strains failed to grow on substrates containing SBG amounts higher than 50%. Highest (10.42 mg/g) cordycepin concentration in cultivating substrate was determined with strain CM2 on 50% SBG. One gram of CM11 strain fungal biomass was able to produce 787.11 mg/g of cordycepin. SBG as a byproduct represent a readily available, low price substrate for cordycepin solid-state production. Obtained concentrations of cordycepin are so far the highest reported concentrations obtained on solid-state substrates therefore we can talk about cordycepin hyperproduction.

Keywords: *Cordyceps militaris*, spent brewery grains, cordycepin, cultivation, medicinal mushrooms

Izvleček: Trosnjake in podgobje glive *Cordyceps militaris* smo gojili na trdih substratih, ki so vsebovali pivovarske tropine. Pet različnih sevov glive *C. militaris* je preraščalo na substratih sestavljenih iz različnih razmerij rži in pivovarskih tropin. Trosnjaki so zrasli pri dveh sevih *C. militaris*. Noben od sevov ni preraščal substrata z vsebnostjo pivovarskih tropin večjo od 50%. Najvišjo koncentracijo kordicepina (10,42 mg/g) v substratu smo določili pri sevu CM2 na substratu s 50% pivovarskih tropin. En gram glivne biomase seva CM11 je proizvedel 787,11 mg/g kordicepina. Pivovarske tropine kot stranski produkt predstavljajo lahko dostopen in poceni substrat primeren za proizvodnjo kordicepina. Dosežene koncentracije kordicepina so po dosedaj znanih podatkih najvišje, zato lahko upravičeno govorimo o hiperprodukciji kordicepina.

Ključne besede: *Cordyceps militaris*, pivovarske tropine, kordicepin, gojenje, zdravilne gobe

Introduction

Cordyceps militaris

C. militaris belonging to *Ascomycota*, is a parasite of insects larval stage, forming fruiting bodies expanding outside the insect larvae or pupae (Buenz et al. 2005). *C. militaris* was traditionally used as a tonic and traditional folk medicine, especially in East Asia (Ying et al. 1987, Holliday and Cleaver 2008, Bhandari et al. 2010). This specie grows wild also in Slovenia (Ogris 2013) and in some other European countries, but its medicinal use in Europe has never been reported.

C. militaris polysaccharides show significant antitumor activities against cervical and liver cancer cells *in vitro* (Yang et al. 2014), extracts of its fruiting bodies show antioxidant, antibacterial, antifungal, antihuman tumor cell lines (Rao et al. 2010, Reis et al. 2013, Yang et al. 2014), anti-inflammatory (Won and Park 2005, Rao et al. 2010), anti-fibrotic (Nan et al. 2001), anti-angiogenetic (Yoo et al. 2004) and insulin secreting (Choi et al. 2004) activities. One of its main medicinal compounds this fungus is cultivated for is cordycepin (3′-deoxyadenosine), a nucleoside analogue with anti-tumour, anti-proliferative, anti-metastatic, insecticidal and anti-bacterial activities (Song et al. 1998).

In recent years *C. militaris* is extensively cultivated in liquid as well as solid media (Das et al. 2010) and is the most successfully cultivated *Cordyceps* specie (Kobayashi 1941, Sung 1996). In solid media different supplemented grain types and seeds are used, such as millet, rye, rice, brown rice, bean powder, corn grains, cotton seed hulls, sorghum, corn cobs, jowar, wheat, sunflower floral discs (Chen and Wu 1990, Zhang and Liu 1997, Li 2002, Holliday et al. 2004, Li et al. 2004, Zhao et al. 2006, Gao and Wang 2008, Wei and Huang 2009, Chen et al. 2011, Shrestha et al. 2012, Wen et al. 2014, Yi et al. 2014). SBG so far have not being reported as a substrate component. Wu and coworkers (2013) reported on successful *C. militaris* cultivation and cordycepin production on levan fermentation leftovers. Ni and coworkers (2009) extracted cordycepin from the spent *C. militaris* substrate, concluding it as appropriate source of cordycepin with concentrations ranging from 0.1 to 1 mg/g.

Spent brewery grains

Spent brewery grains (SBG) are a byproduct of the brewing industry which remain as the outer pericarp-seed coat layers from the original malted barley (*Hordeum vulgare*) grain after barley hot water extraction at 65–70°C (Mussatto et al. 2006). SBG are readily available, high volume and low cost byproducts and remain a potentially more valuable resource for industrial exploitation. Currently they are used as an animal feed. Indeed, value-added products are increasingly being sought for SBG (Robertson et al. 2010).

Besides potential uses of SBG for energy via intermediate pyrolysis (Mahmooda et al. 2013), as a potential material for coal production through wet hydrothermal carbonization (Poerschmanna et al. 2014) or a potential candidate for phenolic compounds extraction (Barbosa-Pereira et al. 2014), SBG have been successfully used as a cultivating substrate for *Pleurotus ostreatus* (Gregori et al. 2008), for immobilization of kefir and *Lactobacillus casei* for sourdough wheat bread making (Plessas et al. 2007), cultivation of *Lactobacillus plantarum* (Gupta et al. 2013), biomass and xylitol production of *Debaryomyces hansenii* (Carvalheiro et al. 2005). Till now no reports of SBG usage in *C. militaris* cultivation and cordycepin production was reported.

Materials and methods

Cultures cultivation and inoculum preparation

C. militaris cultures CM11, CM14 and CM15 were obtained from Edible Fungi Institute, Shanghai Academy of Agricultural Sciences culture collection, CM2 culture was kindly donated by prof. Wu Wei from Plant Protection Institute, Shanghai Academy of Agricultural Sciences and CM5 culture was obtained from culture collection of Mycomedica d.o.o., Podkoren, Slovenia. All cultures were transferred to Potato Dextrose Agar (Difco, USA) and incubated at 24°C in complete darkness. After mycelium overgrew the agar media, it was homogenized in a blender with 100 ml of sterile water (Wahring, USA). Liquified inoculum was further used for inoculation of cultivation substrates.

Substrates preparation and culturing

Rye (Rebernak, Šmartno na Pohorju, Slovenia) and spent brewery grains (Union d. d., Ljubljana, Slovenia) were mixed in different proportions (9:1, 8:2, 7:3, 6:4, 5:5, 4:6) and filled into 720 ml glass jars. Water was added to the mixture to achieve 65% moisture content and 100 g of substrate weight. Substrates were prepared in triplicates. Jars were covered with metallic lids having 14 mm hole in the middle, covered with HEPA class 14 membrane sterilized for 30 minutes at 121°C and cooled under the flow of sterile air. During inoculation liquid inoculum was constantly mixed on a magnetic stirrer with 5 ml transferred into the substrate jars. Jars were closed and incubated at 24°C under cool white fluorescent light. After incubation the substrate and fruiting bodies were dried for 48 hours at 60°C and milled in a coffee type grinder.

Figure 1: *Cordyceps militaris* (CM2 strain) forming stromata on substrates containing 20% spent brewery grains and 80% rye

Slika 1: *Cordyceps militaris* (sev CM2) tvori podgobje na substratu, ki vsebuje 20% pivovarskih tropin in 80% rži

Cordycepin analysis

10 ml of 20% ethanol was added to 200 mg of powdered sample, and extracted for 2 hours in ultrasonic water bath. The supernatant was centrifuged at 14 000g for 10 minutes and filtered through 0.22 µm membrane filter (Macherey Nagel).

The system consisted of Waters 2695 HPLC system equipped with UV detector. The working

Figure 2. Sterile strain (CM11) of *Cordyceps militaris* not being able to form stromata on substrate containing 20% spent brewery grains and 80% rye

Slika 2: Sterilni sev (CM11) *Cordyceps militaris* ni zmožen tvoriti podgobja na substratu, ki vsebuje 20% pivovarskih tropin in 80% rži

conditions were: YMC - polyamine column (5 µm, 250 mm × 4.6 mm); solvent A - acetonitrile; solvent B - double distilled water; linear gradient - acetonitrile : water (v : v) - (90:10) 15 minutes → (86.5:13.5) 20 minutes → (75:25) 30 minutes → (70:30) 35 minutes; flow rate – 1 ml/minutes; temperature - 30ºC; detective wavelength – 259 nm; injection volume – 10 µl.

Quantification of cordycepin produced by fungal biomass

For determination of ergosterol concentration in control sample 0.2 g of dry *C. militaris* mycelia cultivated on PDA media was extracted in 5 ml of cold absolute ethanol following a modified protocol by Martin and coworkers (1990). For test samples biomass determination one gram of grinded material was extracted in absolute ethanol (10 ml) for 30 minutes at 4ºC, centrifuged at 10 000 g for 10 minutes and filtered through a 0.22 µm membrane filter (Macherey Nagel).

Analysis was performed on a Waters HPLC system equipped with PDA 996 detector, 2690 Separation Module and Nucleosil C18, 250 × 4.6 mm, 5 µm column. Ergosterol was eluted at isocratic conditions of 50% methanol and 50% acetonitrile at a flow rate of 1.5 ml/min and identified with help of standard retention time and the specific absorption triple peak characteristic for ergosterol between 260 nm and 300 nm. For quantification a calibration curve was employed using purified (Nylund et al. 1992) ergosterol standard (Sigma, Germany). Ergosterol content was calculated using calibration curve for fungal mycelia and ergosterol.

Two parameters were calculated for determination of cultivation process effectiveness – cordycepin content in substrate (CCS) and fungal biomass cordycepin production (FBCP). CCS was calculated per substrate weight and shows the end concentration of cordycepin in the substrate (w/w). FBCP shows cordycepin production ability of certain fungal biomass/mycelia quantity (w/w).

Results

C. militaris mycelia overgrew all the tested substrate mixtures but failed to grow on substrates containing SBG amounts of 60% and higher. When transferred onto SBG containing substrates all strains except CM11 and CM14 formed mycelia with very strong rhyzomorphic primordia forming characteristics. Stromata formation was noticed with strain CM2 (0, 10, 20, 30, 40 and 50% SBG) and CM5 (10, 20, 30, and 50% SBG) (Figure 1).

With all strains except CM11 increase in CCS is noticed with increasing proportions of SBG in the cultivation substrates. Only with strain CM5 CCS decrease from 8,90 to 6,64 mg/g was observed on 50% SBG substrate. Maximum CCS (10,42 mg/g) was obtained with strain CM2 cultivated on 50% SBG substrate (Figure 3).

Highest FBCP (787.11 mg/g) was observed with strain CM11 cultivated on 0% SBG drastically reduced (to 305.75 mg/g) with addition of SBG to the substrate (Figure 4). The same FBCP reduction trend at SBG addition was noticed with CM5 strain. FBCP stayed the highest at all SBG concentrations compared to other strains, the second in FBCP was CM5 strain, followed by CM2, CM14 and CM15. The lowest FBCP was obtained with CM15 strain on average (Figure 2).

Different *C. militaris* strains react differently on SBG addition to the substrate, with CM2 being the strongest CCS producer and third strongest FBCP producer. CM11 was the strongest FBCP producer (787.11 mg/g), meaning that 1 g of CM11 biomass can produce up to 787.11 mg of intra and extracellular cordycepin (Figure 4).

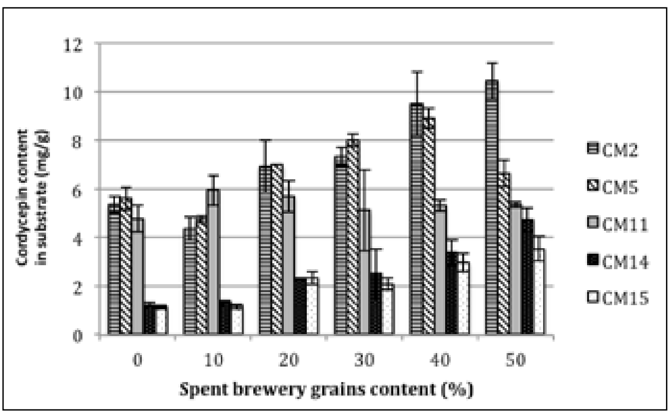

Figure 3: Average cordycepin content in substrates (CCS) containing rye and spent brewery grains overgrown with *Cordyceps militaris*

Slika 3: Povprečna vsebnost kordicepina v substratih (CCS), ki vsebuje rž in pivovarske tropine preraščene z glivo *Cordyceps militaris*

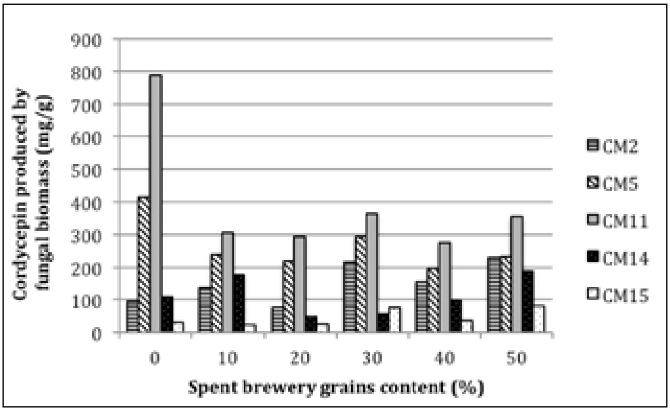

Figure 4. Average *Cordyceps militaris* fungal biomass for cordycepin production (FBCP) on substrates containing spent brewery grains

Slika 4: Povprečna biomasa glive *Cordyceps militaris* za proizvodnjo kordicepina (FBCP) na substratih (CCS), ki vsebuje pivovarske tropine

Discussion

According to our results SBG addition into *C. militaris* cultivation substrate very effectively increased CCS and at the same time decreased FBCP. CCS hyperproduction in SBG containing substrates could be caused by higher concentrations of low molecular compounds in SBG (simple sugars and other fermentation products produced through the brewing process), compared to unfermented rye grains.

Many different chemically defined substrate supplements are used in commercial *C. militaris* cultivation, with some researchers (Xie et al. 2009) reporting natural substrate components such as brown rice, malt and soybean being better sources of nutrition for *C. militaris* in comparison to chemically defined media. This suggests high cordycepin concentrations in SBG containing substrates could be achieved because SBG is a complex material composed of only natural components.

C. militaris characteristics (white color without stromata forming ability) noticed with CM11 strain were reported by Sreshtha et al. (2012) and is by this author linked to strain degeneration (Figure 2). This could mean that CM11 is a degenerated *C. militaris* strain, capable of producing high FBCP on rye substrate only. At the same time CM11 is the only strain of which CCS is not drastically influenced by addition of SBG to the substrate.

Holliday and coworkers (2004) reported 2.25 mg/g CCS in commercial *C. sinensis* products obtained through solid-state cultivation and 0.65 mg/g cordycepin in wild collected *C. sinensis* stromata. Ni and coworkers (2009) reported 0.1 to 1 mg/g CCS content in spent *C. militaris* cultivating substrates, Wen and coworkers (2014) optimized solid-state composition for *C. militaris* cultivation and achieved CCS of 9.17 mg/g. All reported concentrations are lower compared to results (10.42 mg/g) obtained in our research, showing SBG are a superior, readily available and low cost substrate for cordycepin production through *C. militaris* cultivation.

Why all strains failed to grow on substrates containing SBG amounts of 60% and higher is still unknown. This phenomenon could be linked with higher nitrogen content reported by Gao and coworkers (2000) to suppress *C. militaris* growth.

Conclusion

SBG represent a readily available, low price substrate for cordycepin solid-state production. Here reported concentrations of cordycepin are so far the highest reported concentrations (10.42 mg/g) obtained on solid-state substrates.

Use of SBG for cordycepin production by *C. militaris* is shown here as a very effective technique for producing high value food additive or medicated animal feed - with just drying SBG processed through *C. militaris* cultivation.

Further research are needed to determine the exact components and/or physical properties causing cordycepin hyperproduction in SBG containing substrates and for optimization of cultivation parameters such as temperature, incubation time, light and aeration.

Here described technique of SBG usage is already in the process of optimization and commercialization focusing on high cordycepin content in food and feed production.

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Povzetek

Pivovarske tropine predstavljajo lahko dosegljiv, cenen substrat za proizvodnjo kordicepina na trdih substratih. Proizvedene koncentracije kordicepina v substratu so najvišje (10,42 mg/g) koncentracije znane iz objav do sedaj.

Uporaba pivovarskih tropin za produkcijo kordicepina z gojenjem glive *C. militaris* je v članku predstavljena kot enostavna metoda za proizvodnjo hrane, prehranskih dopolnil ali krme z visoko vsebnostjo kordicepina.

Za hiperprodukcijo kordicepina so potrebne nadaljnje raziskave za določitev ključnih karakteristik pivovarksih tropin ter optimizacijo gojitvenih parametrov kot so temperatura, svetloba, trajanje inkubacije in prezračevanje.

Opisane tehnike gojenja *C. militaris* so že v procesu optimizacije in komercializacije za namene proizvodnje prehranskih dopolnil in medicinirane krme z visoko vsebnostjo kordicepina.

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Ultravijolično sevanje – nov pripomoček v vrtnarski industriji

Ultraviolet-light as a novel tool for the horticultural industry

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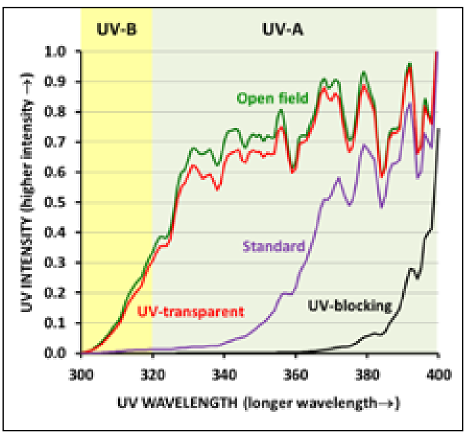
Uvod

Sodobna vrtnarska proizvodnja temelji na uporabi številnih znanstvenih dosežkov na področju rastlin in biologije tal. Razvoj sodobnih substratov in gnojil temelji na razumevanju fizikalnih in kemijskih lastnosti substratnih materialov ter razumevanju dostopnosti in izrabe hranil. Tehnike namakanja upoštevajo nova znanja s poročja uravnavaja vodne balance pri rastlinah. Pomanjkljiva pa so znanja o sevanju kot ključnem viru za rast rastlin. Pri poljščinah, ki rastejo na njivah, je težko "upravljati s svetlobo", v rastlinjakih pa je, z uporabo različnih materialov ali umetnim osvetljevanjem,



Slika 1: UV delež sončne svetlobe na prostem in v tipičnih komercialnih razmerah z uporabo standardne plastike ter UV-prepustnih in UV-zaščitnih folij. Opazimo, da je kakovost sončne svetlobe, na prostem podobna svetlobi pod UV-prepustno folijo. Standardne plastične obloge prestrezajo vse UV-B in večino UV-A sevanja, medtem ko UV-nepropustne folije prepuščajo le manjšo količino dolgovalovnega UV-A sevanja

Figure 1: The UV component of sunlight, in the open field, and under typical commercial examples of a standard plastic, UV-transparent plastic and UV-blocking plastic. Note that sunlight under UV-transparent is very close to that in the field. Standard plastic blocks all of the UV-B and much of the UV-A, while UV-opaque films typically transmit just a small amount of longer wavelength UV-A

to mogoče. Uravnavanje kakovosti in količine svetlobe lahko izboljša fotosintezno aktivnost, vendar je povezano s povečano porabo energije in večjimi stroški. Rastline pa ne uporabljajo svetlobe le za fotosintezo. Svetloba je ključnega pomena tudi pri nadzoru rasti in razvoja rastlin. To vedenje se danes delno komercialno že v uporablja, na primer z nočnim prekinjanjem osvetljevanja. Razmeroma novo pa je odkritje, da lahko rastline zaznavajo in se odzivajo na ultravijolično ali UV-sevanje (glej sliko 1). Še desetletje nazaj se o UV-sevanju kot sredstvu za zaščito pridelka še ni govorilo, saj so znanstveniki sevanje UV- sevanje obravnavali bolj z vidika potencialnega vira poškodb. V zadnjih nekaj letih je prišlo do preobrata osnovnega razumevanja rastlinskih odzivov na UV-sevanje. To razumevanje danes, skupaj z napredkom na področju tehnologij za manipulacijo UV- sevanja, ustvarja novo priložnost za zavarovanje pridelka. Evropski znanstveniki so na tem področju vodilni na svetu in združujejo temeljne raziskave ter njihovo uporabo. Raziskave potekajo v okviru številnih nacionalnih projektov ter v okviru EU programa COST UV4growth, ki vzpostavljanja sodelovanje med državami in med raziskovalci ter končnimi uporabniki (glej sliko 2). Pričujoči prispevek povzema trenutni napredek in obete, ki temeljijo na raziskavah raziskovalnih skupin vključenih v UV4growth omrežje in izkušnjah evropskih pridelovalcev, ki v praksi že izkoriščajo potencial UV sevanja.

Slika 2: Omrežje UV4Growth je združilo okoli 180 raziskovalcev iz 25 držav Evrope in drugih delov sveta. Omrežje je financiral COST, medvladni okvirni program za evropsko sodelovanje na področju znanosti in tehnologije ter združuje raziskovalce iz akademskih krogov, raziskovalnih inštitutov in industrijo. Cilj omrežja UV4Growth je prenos raziskav v hortikulturno prakso. To se nanaša na izsledke o spremembah rastlinskih metabolitov pod vplivom UV sevanja (kar je osnova okusa ali barve pridelka ali dela pridelka), o zatiranju škodljivcev in odpornosti na bolezni ter o vplivih na morfologijo rastlin (t.j. močnejših, bolj robustnih rastlin). Vodja projekta UV4growth, dr. Marcel Jansen iz Univerze v Corku (Irska) je dejal: "Večinoma raziskovalci imajo malo stikov s pridelovalci, zato imamo sodelavci projekta UV4growth pomembno nalogo graditi mostove med UV znanostjo in njenimi potencialnimi uporabniki. Zavedamo se, da lahko znatno prispevamo k trajnostnim pristopom v hortikulturi in boljši kakovosti pridelkov

Figure 2: UV4Growth is a network that brought together some 180 researchers from 25 countries in Europe and the rest of the world. The network was funded through COST, an **intergovernmental framework for European Cooperation in Science and Technology, and brings together researchers from academia, research institutes and industry.** An important aim of UV4Growth is also to translate the exciting research findings of the last decade, into relevant information for growers. This concerns data on UV-induced changes in plant metabolites (i.e. flavour or colour), pest and disease tolerance, and plant morphology (i.e. sturdier, more robust plants). The UV4growth chair, Dr Marcel Jansen from University College Cork, Ireland said "Many researchers have little contact with growers, so UV4growth has been an exciting and unusual opportunity and it's great to see it begin to build bridges between the UV science and the application". Within UV4Growth there is a real sense that we can contribute to both a more sustainable horticulture, as well as to better quality products

Zgodba o UV-B sevanju

Raziskave UV-B sevanja je spodbudila zaskrbljenost zaradi tanjšanja ozonske plasti v stratosferi, ki povečuje količino UV-B sevanja, ki doseže Zemljino površino. Danes vemo, da UV-B sevanje, ki je del sončevega sevanja, v naravnih razmerah navadno ne poškoduje rastlin, temveč deluje kot pomemben regulator rasti rastlin. Zato je pomembno, da poskrbimo, da je UV-sevanje prisotno tudi v rastlinjakih. Raziskave so pokazale, da je pri posevkih v rastlinjakih to mogoče z folijami, ki prepuščajo UV-sevanje in/ali dodajanem UV-B sevanja s svetili, kar ugodno vpliva na rast, izboljšanje kakovosti sadik, na spremembe v kemijski zgradbi rastlin, ki vplivajo na okus in vonj, povečano vsebnost olj v zeliščih in ugoden vpliv na zatiranje škodljivcev in bolezni. Na primer, G. Bean (Crystal Heart Salad Company, UK) poroča, da so sadike solate, ki jih gojijo pod za UV sevanje prosojno folijo, kompaktnejše in bolj intenzivne barve. G. Bilgehan (Fide Fethiye, Turčija) ugotavlja, da UV prepustna folija izboljša kakovost sadik paradižnika v primerjavi s standardno folijo. Tudi G. Öztürk (Imoz Tarim, Turčija) je ugotovil, da je uporaba UV prepustne folije vplivala na povečano število plodov, njihovo obarvanost in trdnost. Takšni učinki pa niso omejeni le na sončno sredozemsko okolje, ampak o njih poroča tudi g. Gaffney iz raziskovalnega centra Teagasc iz Kinsealya (Irska), ki se ukvarja z okrasnimi rastlinami. Številne vrste okrasnih rastlin, vključno s predstavniki rodov *Hebe*, *Cotinus*, *Prunus* in *Escallonia*, ki so jih gojili pod UV prepustno folijo so bile nižje rasti in kompaktnejše. Tudi barva listja ruja in barva cvetov sivke je bila intenzivnejša, v primerjavi z rastlinami, ki so jih gojili v rastlinjakih, kjer ni bilo UV sevanja. Dr. Gaffney pa poroča tudi o učinkih UV sevanja na obnašanje škodljivcev, kar kaže na to, da uporaba ustreznih folij v rastlinjakih lahko močno zmanjša napade škodljivcev in pojavljanje bolezni.

Zmogljivo UV-C sevanje

V sončevi svetlobi, ki pride na Zemljino površino, UV-C sevanja ni, zato so edini vir UV-C sevanja umetni viri (svetilke). UV-C sevanje ima že pri nizki jakosti velike učinke na mikrobe, rastline in ljudi. Danes so na voljo komercialni sistemi UV-C svetilk, ki omogočajo nadzor pepelaste plesni pri rastlinah v rastlinjakih. Svetila, ki oddajajo UV-C sevanje, moramo uporabljati z natančnim upoštevanjem previdnostnih ukrepov, saj lahko nasprotnem primeru pride do poškodb uporabnikov in rastlin.

UV-A sevanje

Za razliko ljudi, lahko številne živali vidijo UV-A svetlobo. Ker so med njimi so tudi žuželke, lahko z uravnavanjem jakosti in kakovosti UV-A sevanja nadziramo škodljivce. Brez UV-A sevanja se nekatere skupine, kot je na primer bela muha, ne razmnožujejo. Tudi glive, ki povzročajo hude bolezni pridelkov, na primer *Botrytis cinerea*, zaznavajo UV-A in ga uporabljajo za regulacijo proizvodnje spor, saj je proizvodnja spor upočasnjena, če UV-A sevanja ni. Zato so razvili folije, ki prestrezajo UV-A sevanje in tako omogočajo nadzor škodljivcev in bolezni. Takšno folijo na primer pogosto uporabljajo v Turčiji in Egiptu. Le redko pa se taka folija uporablja v zahodni in severni Evropi, čeprav raziskave kažejo, da bi z njo lahko nadzorovali sivo plesen in peronosporo.

Pogled naprej

Številni raziskovalci na področju učinkov UV sevanja, vključno s tistimi, ki jih je financirala EU v okviru projekta COST UV4growth (glej sliko 1), želijo svoje ugotovitve in izsledke uporabiti v proizvodnih sistemih. V Veliki Britaniji je Razvojna vrtnarska družba (Horticultural Development Company (HDC)) med leti 1991 in 1994 financirala obsežen program raziskav plastičnih folij. Rezultati raziskave so potrdili, da so lahko UV prepustne folije uporabne za izboljšanje rasti in kakovosti sadik različnih pridelkov ter za zatiranje škodljivcev. Razvoj plastičnih materialov, UV-prepustnih in odbojnih, skupaj z novimi tehnologijami osvetljevanja, omogoča učinkovito manipulacijo ravni in kakovosti UV sevanja. Trenutno so UV-LED diode še predrage za širšo uporabo, zato raziskave temeljijo na uporabi fluorescenčnih cevastih UV žarnic.

Zaključek

Danes imamo dovoj znanstvenih izsledkov, ki omogočajo razumevanje odzivov rastlin na UV sevanje, ter različne tehnološke pristope za izvajanje predlaganih praks. Tako je mogoče UV sevanje v pridelavo rastlin, ki rastejo pod steklom ali v rastlinjakih z uporabo UV prepustnih folij ter ustreznimi svetili, kar omogoča manipulacijo oblike, kakovosti in barve sadik ter nadzor nad škodljivci in boleznimi.

INSTRUCTIONS FOR AUTHORS

1. Types of Articles

SCIENTIFIC ARTICLES are comprehensive descriptions of original research and include a theoretical survey of the topic, a detailed presentation of results with discussion and conclusion, and a bibliography according to the IMRAD outline (Introduction, Methods, Results, and Discussion). In this category ABS also publishes methodological articles, in so far as they present an original method, which was not previously published elsewhere, or they present a new and original usage of an established method. The originality is judged by the editorial board if necessary after a consultation with the referees. The recommended length of an article including tables, graphs, and illustrations is up to fifteen (15) pages; lines must be double-spaced. Scientific articles shall be subject to peer review by two experts in the field.

REVIEW ARTICLES will be published in the journal after consultation between the editorial board and the author. Review articles may be longer than fifteen (15) pages.

BRIEF NOTES are original articles from various biological fields (systematics, biochemistry, genetics, physiology, microbiology, ecology, etc.) that do not include a detailed theoretical discussion. Their aim is to acquaint readers with preliminary or partial results of research. They should not be longer than five (5) pages. Brief note articles shall be subject to peer review by one expert in the field.

CONGRESS NEWS acquaints readers with the content and conclusions of important congresses and seminars at home and abroad.

ASSOCIATION NEWS reports on the work of Slovene biology associations.

2. Originality of Articles

Manuscripts submitted for publication in *Acta Biologica Slovenica* should not contain previously published material and should not be under consideration for publication elsewhere.

3. Language

Articles and notes should be submitted in English, or as an exception in Slovene if the topic is very local. As a rule, congress and association news will appear in Slovene.

4. Titles of Articles

Title must be short, informative, and understandable. It must be written in English and in Slovene language. The title should be followed by the name and full address of the authors (and if possible, fax number and/or e-mail address). The affiliation and address of each author should be clearly marked as well as who is the corresponding author.

5. Abstract

The abstract must give concise information about the objective, the methods used, the results obtained, and the conclusions. The suitable length for scientific articles is up to 250 words, and for brief note articles, 100 words. Article must have an abstract in both English and Slovene.

6. Keywords

There should be no more than ten (10) keywords; they must reflect the field of research covered in the article. Authors must add keywords in English to articles written in Slovene.

7. Running title

This is a shorter version of the title that should contain no more than 60 characters with spaces.

8. Introduction
The introduction must refer only to topics presented in the article or brief note.

9. Illustrations and Tables
Articles should not contain more than ten (10) illustrations (graphs, dendrograms, pictures, photos etc.) and tables, and their positions in the article should be clearly indicated. All illustrative material should be provided in electronic form. Tables should be submitted on separate pages (only horizontal lines should be used in tables). Titles of tables and illustrations and their legends should be in both Slovene and English. Tables and illustrations should be cited shortly in the text (Tab. 1 or Tabs. 1-2, Fig. 1 or Figs. 1-2; Tab. 1 and Sl. 1). A full name is used in the legend title (e.g. Figure 1, Table 2 etc.), written bold, followed by a short title of the figure or table, also in bold. Subpanels of a figure have to be unambiguously indicated with capital letters (A, B, …). Explanations associated with subpanels are given alphabetically, each starting with bold capital letter (**A**), a hyphen and followed by the text.

10. The quality of graphic material
All the figures have to be submitted in the electronic form. The ABS publishes figures either in pure black and white or in halftones. Authors are kindly asked to prepare their figures in the correct form to avoid unnecessary delays in preparation for print, especially due to problems with insufficient contrast and resolution. Clarity and resolution of the information presented in graphical form is the responsibility of the author. Editors reserve the right to reject unclear and poorly readable pictures and graphical depictions. The resolution should be 300 d.p.i. minimum for halftones and 600 d.p.i. for pure black and white. The smallest numbers and lettering on the figure should not be smaller than 8 points (2 mm height). The thickness of lines should not be smaller than 0.5 points. The permitted font families are Times, Times New Roman, Helvetica and Arial, whereby all figures in the same article should have the same font type. The figures should be prepared in TIFF, EPS or PDF format, whereby TIFF (ending *.tif) is the preferred type. When saving figures in TIFF format we recommend the use of LZW or ZIP compression in order to reduce the file sizes. The photographs can be submitted in JPEG format (ending *.jpg) with low compression ratio. Editors reserve the right to reject the photos of poor quality. Before submitting a figure in EPS format make sure first, that all the characters are rendered correctly (e.g. by opening the file first in the programs Ghostview or GSview – depending on the operation system or in Adobe Photoshop). With PDF format make sure that lossless compression (LZW or ZIP) was used in the creation of the *.pdf file (JPEG, the default setting, is not suitable). Figures created in Microsoft Word, Excel, PowerPoint etc. will not be accepted without the conversion into one of the before mentioned formats. The same goes for graphics from other graphical programs (CorelDraw, Adobe Illustrator, etc.). The figures should be prepared in final size, published in the magazine. The dimensions are 12.5 cm maximum width and 19 cm maximum height (width and height of the text on a page).

11. Conclusions
Articles shall end with a summary of the main findings which may be written in point form.

12. Summary
Articles written in Slovene must contain a more extensive English summary. The reverse also applies.

13. Literature
References shall be cited in the text. If a reference work by one author is cited, we write Allan (1995) or (Allan 1995); if a work by two authors is cited, (Trinajstić and Franjić 1994); if a work by three or more authors is cited, (Pullin et al. 1995); and if the reference appears in several works, (Honsig-Erlenburg et al. 1992, Ward 1994a, Allan 1995, Pullin et al. 1995). If several works by the same author

published in the same year are cited, the individual works are indicated with the added letters a, b, c, etc.: (Ward 1994a,b). If direct quotations are used, the page numbers should be included: Toman (1992: 5) or (Toman 1992: 5–6).The bibliography shall be arranged in alphabetical order beginning with the surname of the first author, comma, the initials of the name(s) and continued in the same way with the rest of the authors, separated by commas. The names are followed by the year of publication, the title of the article, the international abbreviation for the journal (periodical), the volume, the number in parenthesis (optional), and the pages. Example:

Mielke, M.S., Almeida, A.A.F., Gomes, F.P., Aguilar, M.A.G., Mangabeira, P.A.O., 2003. Leaf gas exchange, chlorophyll fluorescence and growth responses of Genipa americana seedlings to soil flooding. Experimental Botany, 50 (1), 221–231.

Books, chapters from books, reports, and congress anthologies use the following forms:

Allan, J.D., 1995. Stream Ecology. Structure and Function of Running Waters, 1st ed. Chapman & Hall, London, 388 pp.

Pullin, A.S., McLean, I.F.G., Webb, M.R., 1995. Ecology and Conservation of *Lycaena dispar*: British and European Perspectives. In: Pullin A. S. (ed.): Ecology and Conservation of Butterflies, 1st ed. Chapman & Hall, London, pp. 150-164.

Toman, M.J., 1992. Mikrobiološke značilnosti bioloških čistilnih naprav. Zbornik referatov s posvetovanja DZVS, Gozd Martuljek, pp. 1-7.

14. Format and Form of Articles

The manuscripts should be sent exclusively in electronic form. The format should be Microsoft Word (*.doc) or Rich text format (*.rtf) using Times New Roman 12 font with double spacing, align left only and margins of 3 cm on all sides on A4 pages. Paragraphs should be separated by an empty line. The title and chapters should be written bold in font size 14, also Times New Roman. Possible sub-chapter titles should be written in italic. All scientific names must be properly italicized. Used nomenclature source should be cited in the Methods section. The text and graphic material should be sent to the editor-in-chief as an e-mail attachment. For the purpose of review the main *.doc or *.rtf file should contain figures and tables included (each on its own page). However, when submitting the manuscript the figures also have to be sent as separate attached files in the form described under paragraph 10. All the pages (including tables and figures) have to be numbered. All articles must be proofread for professional and language errors before submission.

A manuscript element checklist (For a manuscript in Slovene language the same checklist is appropriately applied with a mirroring sequence of Slovene and English parts):
English title – (Times New Roman 14, bold)
Slovene title – (Times New Roman 14, bold)

Names of authors with clearly indicated addresses, affiliations and the name of the corresponding author – (Times New Roman 12)
Author(s) address(es) / institutional addresses – (Times New Roman 12)
Fax and/or e-mail of the corresponding author – (Times New Roman 12)
Keywords in English – (Times New Roman 12)
Keywords in Slovene – (Times New Roman 12)
Running title – (Times New Roman 12)
Abstract in English (Times New Roman 12, title – Times New Roman 14 bold)

Abstract in Slovene – (Times New Roman 12, title – Times New Roman 14 bold)
Introduction – (Times New Roman 12, title – Times New Roman 14 bold)
Material and methods – (Times New Roman 12, title – Times New Roman 14 bold)
Results – (Times New Roman 12, title – Times New Roman 14 bold)
Discussion – (Times New Roman 12, title – Times New Roman 14 bold)
Summary in Slovene – (Times New Roman 12, title – Times New Roman 14 bold)
Figure legends; each in English and in Slovene – (Times New Roman 12, title – Times New Roman 14 bold, figure designation and figure title – Times New Roman 12 bold)
Table legends; each in English and in Slovene – (Times New Roman 12, title – Times New Roman 14 bold, table designation and table title – Times New Roman 12 bold)
Acknowledgements – (Times New Roman 12, title – Times New Roman 14 bold)
Literature – (Times New Roman 12, title – Times New Roman 14 bold)
Figures, one per page; figure designation indicated top left – (Times New Roman 12 bold)
Tables, one per page; table designation indicated top left – (Times New Roman 12 bold)
Page numbering – bottom right – (Times New Roman 12)

15. Peer Review

All Scientific Articles shall be subject to peer review by two experts in the field (one Slovene and one foreign) and Brief Note articles by one Slovene expert in the field. With articles written in Slovene and dealing with a very local topic, both reviewers will be Slovene. In the compulsory accompanying letter to the editor the authors must nominate one foreign and one Slovene reviewer. However, the final choice of referees is at the discretion of the Editorial Board. The referees will remain anonymous to the author. The possible outcomes of the review are: 1. Fully acceptable in its present form, 2. Basically acceptable, but requires minor revision, 3. Basically acceptable, but requires important revision, 4. May be acceptable, but only after major revision, 5. Unacceptable in anything like its present form. In the case of marks 3 and 4 the reviewers that have requested revisions have to accept the suitability of the corrections made. In case of rejection the corresponding author will receive a written negative decision of the editor-in-chief. The original material will be erased from the ABS archives and can be returned to the submitting author on special request. After publication the corresponding author will receive the *.pdf version of the paper.