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The natural occurrence of toxigenic moulds and mycotoxins in Slovenian primary grain production

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ABSTRACT

The aim of the present study was to determine the contamination of grains, grown in Slovenia and used for animal diets by Slovene farmers in year 2009. A total of 66 samples was examined on toxigenic moulds from genera *Fusarium, Penicillium, Aspergillus, Alternaria* and on 8 different mycotoxins. The leading contaminants among moulds were from *Fusarium spp.*, detected in 51 samples, mostly in barley (19). The average number of *Fusarium spp.* colony forming units (cfu) in different grains was from 5.5-23.3 x 10^3/g, whereas the contamination of barley with *Penicillium, Aspergillus* and *Alternaria spp.* was 4.5, 19.3 and 5 x 10^3 cfu/g. Using liquid (HPLC) and gas chromatography (GC) methods, the presence of various mycotoxins (expressed for 12% of moisture content) was proved in 57.6% of all samples; mostly deoxynivalenol (DON, 54.5%) in concentrations of 130-2860 µg/kg, followed by zearalenone (ZON, 15.1%, 70-800 µg/kg), fumonisin B1 (3%, 120-210 µg/kg), while fumonisin B2, ochratoxin A, diacetoxyscirpenol (DAS), HT-2 and T-2 toxins were not detected. The results indicate that further control of toxigenic moulds and mycotoxins in Slovenian primary grain production is thus required and justified.

Keywords: animal feed, grains, grains-microbiology, mycotoxins-analysis, Slovenia

IZVLEČEK

NARAVNA KONTAMINACIJA ŽIT IZ PRIMARNE PROIZVODNJE V SLOVENIJI S TOKSIGENIMI PLESNIMI IN MIKOTOKSINI

Z raziskavo smo želeli dobiti vpogled v kontaminacijo žit, ki so jih kmetje v Sloveniji pridelali in uporabili za prehrano živali v letu 2009. Na toksigene plesni iz rodu *Fusarium, Penicillium, Aspergillus, Alternaria* in 8 različnih mikotoksinov smo preiskali skupno 66 vzorcev. Najbolj razširjene so bile plesni iz rodu *Fusarium*. Izolirali smo jih iz 51 vzorcev, najpogosteje iz ječmena (19). Njihovo povprečno število kolonij (cfu) je bilo v različnih žitih od 5,5-23,3 x 10^3/g, v ječmenu pa je bilo 4,5, 19,3 in 5 x 10^3 cfu/g kolonij plesni iz rodu *Penicillium, Aspergillus* in *Alternaria.* S tekočinsko (HPLC) in plinsko kromatografijo (GC) smo dokazali različne mikotoksine (rezultati so izraženi pri 12% vsebnosti vlage vzorca) v 57,6% vseh preiskanih vzorcev. Največ vzorcev je vsebovalo deoksinivalenol (DON, 54,5%) v koncentraciji od 130-2860 µg/kg, sledijo zearalenon (ZON, 15,1%, 70-800 µg/kg) in fumonizin B_1 (3%, 120-210 µg/kg). Fumonizinov B_2, ohratoksina A, diacetoksiscirpenola (DAS), HT-2 in T-2 toksina nismo dokazali v nobenem vzorcu. Rezultati kažejo, da je nadaljnja kontrola toksigenih plesni in mikotoksinov v krmi iz primarne pridelave v Sloveniji vsekakor potrebna in upravičena.

Ključne besede: krma, žita-mikrobiologija, mikotoksini-analize, Slovenija

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1 INTRODUCTION

Ubiquitous microorganisms can be found in the air, water, soil and crops, on animals and human beings and in animal feed. Animal feed is thus being contaminated with microorganisms during its production, modification, storage and consumption (Marquardt, 1996; Maciorowski et al., 2007). Certain microbial groups (collective term: field flora of primary flora) can be found on plants predominantly at the time of harvesting. They are described also as product-typical microflora, including saprophytic bacteria (yellow pigmented bacteria, *Pseudomonas spp.)* and moulds (*Fusarium spp., Cladosporium spp., Verticillium spp., Acremonium spp.*). During storage, a change in microflora can occur due to the reduction of the content of product-typical microorganisms or due to the reproduction of the spoilage-indicating microflora, adapted to the storage conditions. The main spoilage-indicating moulds are *Penicillium spp., Aspergillus spp.*, *Scopulariopsis spp.*, and *Mucorales spp.* Microorganisms causing spoilage are also all species of yeasts (VDLUFA, 2007 d). The growth of moulds is greatly influenced by the water content of the substrate. At low moisture content (usually less than 14-16%) most storage fungi do not grow or grow very slowly (Smith and Yarrow, 1996).

Some species of mould are able to produce highly toxic compounds called mycotoxins. Different moulds can produce different mycotoxins on the same commodity. These toxins can adversely affect animal health and production and can have harmful effects on humans if transmitted to foods (Marquardt, 1996). However, it has been noted that mycoflora can colonize crops and produce mycotoxins without visible damage. Conversely, signs of fungal infestations do not necessarily correlate with the presence of mycotoxins (Patino et al., 2004).

Until now more than 300 mycotoxins have been identified. In grains, most important mycotoxins, affecting animals' health, are: aflatoxins, ochratoxins, fumonisins, zearalenone (ZON), deoxynivalenol (DON), T-2 toxin and other trichothecenes and ergot alkaloids (Kumar et al., 2008). The most commonly known mycotoxins are aflatoxins due to the fact that they represent one of the most potential carcinogenic substance known so far. Aflatoxins are produced by the moulds of genus *Aspergillus*. Trichothecenes, most important among them are DON, T-2 toxin, HT-2 toxin and DAS, are in general potent inhibitors of eukaryotic protein synthesis (Bennett and Klich, 2003). Although DON is one of the least acutely toxic trichothecenes, it should be treated as an important food safety issue because of very common contamination of grains. Other trichothecens, T-2 and HT-2 toxins may cause haematological changes and immune suppression, reduced feed intake and skin irritation as well as diarrhoea and haemorrhages of internal tissues. Trichothecenes are mainly produced by the moulds of genus *Fusarium,* which produce also ZON. ZON is a mycotoxin with strong hyper-estrogenic effects, resulting in impaired fertility, stillbirths of females offsprings and a reduced sperm quality in male animals. Ochratoxin A, which is produced by a number of *Aspergillus* and *Penicillium* species, has been listed as possibly carcinogenic to humans. It causes renal toxicity, nephropathy and immune suppression in several animal species, resulting in reduced animal production performance parameters. The most recently described mycotoxins with relevance in human and animal nutrition are fumonisins. They cause severe animal diseases such as equine leukoencephalomalacia (ELEM) in horses, and porcine pulmonary oedema (PPE) in pigs. Fumonisins are produced by a number species from genus *Fusarium*, as well as *Alternaria* (Binder, 2007). Recently, the mycotoxin analyses were performed on a total of 2798 samples sourced from Europe, Mediterranean and Asian-pacific region. More than a half of samples of grains and feeds from Europe were contaminated at levels above the limit of quantification of methods applied with DON, ZON and T-2 toxin (Binder et al., 2007).

In Slovenia, there is relatively little information related to the natural occurrence of fungi and mycotoxins in grains. In this context, the aim of this work was to investigate the contamination of grains on toxigenic moulds (*Fusarium, Penicillium, Aspergillus* and *Alternaria*) and mycotoxins: ochratoxin A, ZON, DON, DAS, T-2 toxin, HT-2 toxin as well as fumonisins B1 and B2.

2 MATERIALS AND METHODS

2.1 Sample collection

The collecting of 66 samples of different grains, grown on Slovene farms, took place in the period between June and August 2009. Samples of raw material were taken directly at the farms. Several incremental samples were taken randomly from the whole lot and combined to the aggregate sample. After homogenisation and grinding of the aggregate sample in the lab, a laboratory sample of 1.5 kg was taken for the examination. A total of 100 g was randomly selected for the analysis of mycoflora, which was done immediately while the other part of the sample was stored at 8 ^{o}C for mycotoxicological analyses.

2.2 Isolation and identification of moulds and yeasts

To examine the contamination of the samples on saprophytic moulds and yeasts, a slightly modified method of Verband Deutscher Landwirtschaftlicher Untersuchungs-und Forschungsanstalten (VDLUFA) was used (VDLUFA, 2007a; b; c; d). To 20 g of each sample, 180 ml of 0.5% peptone water was added. The mixture was homogenised using linear shaker for 20 min and than diluted to final concentration of 10^{-2}, 10^{-3} and 10^{-4} (VDLUFA, 2007a). 1 ml aliquots of each dilution were spread (in duplicates) on the surface of solid medium, described by Schmidt et al. (1981). The composition of the medium was (per litre) as follows: 40 g malt extract, 2 g glucose, 2 g yeast extract, 1 ml Marlophen 810®, 60 mg Bengal Rose, 60 mg oxytetracycline-HCl (OTC), 12 g agar, 1000 ml distilled water. Plates were incubated for 3 days at 25 ^{o}C in the dark and in the normal atmosphere. Afterwards, plates were stored at room temperature for 2-3 days. Finally, the colonies were counted and the results were expressed as average colony forming units in thousands per gram of sample (10^3 CFU/g) using the following formula (VDLUFA, 2007c):

$$N = \frac{\sum C}{V \times n \times d}$$

N = number of colony-forming units per gram of sample (CFU/g)
ΣC = sum of all colonies of the count plates
V = volume of the dilutions pipetted in the count plates in ml
n = number of count plates that can be evaluated
d = dilution factor

Taxonomic identifications of different genera of moulds were made visually and where applicable, by means of a magnifying glass or a stereomicroscope. Closer characterisation was possible using a light-optical microscope. In this case, selected parts of colonies were transferred onto a slide using adhesive film (Smith and Yarrow, 1996).

2.3. Mycotoxins analysis

2.3.1 Apparatus

Linear shaker IKA HS 501 digital (IKA Labortechnik, Germany) was used for the extraction. For liquid chromatography, the system Waters Alliance 2690 with the fluorescence detector Waters 474 (Waters, MA, USA) equipped with columns Prodigy 5µ ODS(2) and Synergy 4µ Hydro-RP 80A (Phenomenex, CA, USA) in the case of aflatoxin B_1 and fumonisins, respectively and with Hypersil ODS 5 µm (Thermo Scientific, MA, USA) in the case of ochratoxin A and ZON was used. For the post column derivatisation, either Kobra cell (Rhône diagnostics, Scotland) or post-column reactor RXN 1000 (Waters) was mounted between chromatographic column and fluorescence detector. Trichothecenes (DON, DAS, T-2, HT-2) were determined by gas chromatography with mass spectrometry (GC-MS), using system HP 6890 Series (Hewlett Packard, CA, USA) with mass selective detector 5975B inert XL (Agilent Technologies, CA, USA). The column was HP-5MS, 30 m, 0.25 mm I.D., 0.25 µm (Agilent Technologies) and helium with a flow rate of 1 ml/min was used as the carrier gas.

2.3.2 Chemicals and reagents

Standard solutions of aflatoxin B_1, ochratoxin A, ZON and fumonisins B_1 and B_2 were purchased from Biopure (Tulln, Austria) and a standard solution of trichothecenes (including DON, DAS, HT-2, and T-2) was purchased from R-Biopharm Rhône (Glasgow, Scotland). For sample clean-up, immunoaffinity columns (R-Biopharm Rhône) and Mycosep 227 Trich+ (Romer Labs, MO, USA) were used. Reagents purchased at Merck (Darmstadt, Germany) and Supelco (PA, USA) were of analytical or chromatography grade purity.

2.3.3 Analytical procedure

Aflatoxin B1, ochratoxin A, zearalenone, and fumonisins were determined following the instruction for use enclosed to immunoaffinity columns and a standard (R-Biopharm Rhône, 2003a, b, c; 2005; European Committee for Standardization, 2004). For the determination of trichothecenes, the procedure based on analytical methods described by Radová et al. (1998), Langseth and Rundberget (1998), Tanaka et al. (2000), Melchert and Pabel (2004), and Schothorst et al. (2005) was used.

Mycotoxins were extracted from samples with appropriate solvents. After sample clean-up, mycotoxins were determined by liquid chromatography or GC-MS. Before the detection, aflatoxin B_1 was derivatised with bromine in Kobra cell and fumonisins were derivatised with o-phtaldialdehyde and N-acetyl-L-cystein in the post-column reactor.

The limit of detection (LOD) and the limit of quantification (LOQ) for aflatoxin B_1 were 0.2 and 0.6 µg/kg, respectively. LOD and LOQ for ochratoxin A were 10 and 30 µg/kg, for ZON 20 and 50 µg/kg, for fumonisins B_1 and B_2 30 and 100 µg/kg, and for DON, DAS, T-2 and HT-2 50 and 100 µg/kg. To enable the comparison between samples and their estimation regarding recommended European guidance values (European Commission, 2006) the results of mycotoxicological analysis were recalculated to a sample moisture content of 12%. Samples with the concentration of mycotoxins higher than LOD were designated as positive.

2.4 Statistical analysis

The statistical analysis was performed using the statistical program SPSS (Statistical Package for Social Sciencies, Version 12, November 2003). The number of positive samples, arithmetic mean and median of positive samples as well as the maximum level were calculated.

3 RESULTS

3.1 The contamination of grain samples with toxigenic moulds

The microorganisms, isolated from all 66 samples are shown in Table 1. Because of a small number of triticale (3), fodder pea (2), oats (1) and rye (1), these samples were presented together under the label "other". The leading contaminant among moulds was *Fusarium spp.*, detected in 51 of 66 samples (77.2%). Maize had the highest average number of growth colonies of *Fusarium spp.* and *Penicillium spp.* The samples of barley

contained the highest average number of *Aspergillus spp.* and *Alternaria spp*. colonies.

Table 1: The presence of toxigenic moulds in grain samples.

	Samples of grain				
	Barley	Maize	Wheat	Other	Total
Number of samples	23	18	18	7	66
Micro-organisms	(num. positive)[a] mean/median[b]; max.level (x 10^3 cfu/g)[c]				(num. positive)[d] %
Fusarium	(19) 5.5/5.0; 17.0	(12) 23.3/8.0; 128.0	(15) 5.2/6.0; 12.0	(5) 6.6/8.0; 11.0	(51) 77.2
Penicillium	(11) 4.5/2.0; 12.0	(6) 8.8/5.5; 30.0	(9) 4.4/4.0; 11.0	(1) 8.0	(27) 40.9
Aspergillus	(11) 19.3/4.0; 100	(9) 10.4/7.0; 38.0	(5) 8.6/9.0; 16.0	(3) 11.3/13.0; 20.0	(28) 42.2
Alternaria	(14) 5.0/2.0; 26.0	(1) 5.0	(10) 1.5/1.5; 3.0	(4) 2.0/2.0; 3.0	(29) 43.9

[a] Number of positive samples.
[b] Arithmetic mean/median of positive samples in x 10^3 cfu/g.
[c] Maximum level detected in x 10^3 cfu/g.
[d] Total number of positive samples regarding one microorganism.

3.2. The contamination with mycotoxins

A total of 66 grain samples was analysed, resulting in 528 analyses. Table 2 gives an overview of contamination grade of the tested samples, stating the total number of each sample tested, the number of positives, arithmetic mean and median of positive samples as well as the maximum level. In cases, where one sample was positive, the value is presented with one number. The samples of maize were most frequently contaminated and contained DON, ZON and fumonisin B1. Generally, in most samples, DON was detected (54.5%). It was proven in 15 out of 18 samples of wheat, as well as in all other grain samples. DON was closely followed by ZON, which was detected in all grain samples. Fumonisin B1 was detected only in 2 maize samples.

Ochratoxin A, DAS, T-2 toxin and HT-2 were under the detection limit in all analysed samples.

Table 2: Occurrence of mycotoxin contamination in grain samples (results expressed for 12% moisture content).

	Samples of grain				
	Barley	Maize	Wheat	Other	Total
Number of tested samples	23	18	18	7	66
Number of positive samples	9	12	15	2	38 (57.6%)
	(num. positive)[a] mean/median[b]; max. level[c]				(num. positive)[d] %
Ochratoxin A	n.d	n.d	n.d	n.d	n.d
Fumonisin B1	n.d	(2) 180/180; 210	n.d	n.d	(2) 3.0
Fumonisin B2	n.d	n.d	n.d	n.d	n.d
Fumonisin B1 + B2	n.d	(2) 180/180; 210	n.d.	n.d	(2) 3.0
Zearalenon	(2) 260/260; 390	(4) 310/170; 800	(3) 190/120; 370	(1) 400	(10) 15.1
Deoxynivalenol	(9) 310/260; 600	(10) 440/340; 920	(15) 580/290; 2860	(2) 845/845; 890	(36) 54.5
DAS	n.d	n.d	n.d	n.d	n.d
T-2	n.d	n.d	n.d	n.d	n.d
HT-2	n.d	n.d	n.d	n.d	n.d

n.d.= not detected
[a] Number of positive samples regarding individual mycotoxin.
[b] Arithmetic mean/median of positive samples in µg/kg.
[c] Maximum level detected in µg/kg.
[e] Estimated value (LOD).
[d] Total number of positive samples regarding individual mycotoxin.

4 DISCUSSION

Mycrobiological examination proved that 77.2%, 40.9%, 42.2% and 43.9% of the samples of different grains contained moulds of genera *Fusarium, Penicillium*, *Aspergillus* and *Alternaria,* respectively. As a result of this contamination, the 57.6% of samples were positive to at least one mycotoxin.

Slovenia, like all other EU countries, has no written legislative limits regarding the mycrobiological status of animal feed. There are no official methods for detection of the microbial contamination. With traditional culturing methods, used also in our study, it takes 5-7 days to get results. Molecular methods hold promise as a viable technology to reach these results, but will require considerable optimisation and standardisation before this becomes routine in the animal feed industry (Maciorowski et al., 2007). Most of the countries tend to set general standards in the field of microbiological examinations, but agreement and setting of international regulatory standards is very difficult, as not only potential health benefits but also political and economic issues have to be considered.

The average contamination with colonies of *Fusarium spp*. in maize were lower than in previous years, when the average contamination was 250 x 10^3 cfu/g and for *Penicillium* and *Aspergillus* 744 and 7 x 10^3 cfu/g (Jakovac-Strajn et al., 2008). The moulds from genus *Alternaria* had the highest average number in barley. Based on the number of positive samples of all grains (43.9%), the potential presence of toxins such as

tenuazonic acid, alternariol and alternariol monomethyl ether may pose a contamination risk.

It is well known, that environmental conditions have great influence on the development and spread of moulds and consequently on the production of mycotoxins. Water stress, temperature stress and insect damage of a host plant are, under field conditions, the major determining factors of mould infestation and toxin production. With stored grain, factors which are likely to affect mycotoxin formation include moisture content and the composition of the substrate, environmental temperature, exposure time, damage to seeds, oxygen availability, carbon dioxide concentrations, fungal abundance, prevalence of toxic strains, spore loads, microbial interaction and invertebrate vectors, particularly insects. Spoilage, fungal growth and mycotoxin formation result from the complex interactions of these factors (Abramson et al., 1980; Santin, 2005).

There are also great differences in sensibility to mycotoxins among individual animal species. Factors such as breed, sex, environment, nutritional status, as well as other toxic entities can affect the symptoms of intoxication and may contribute to the significance of mycotoxin damage on economic output and animal health (Binder, 2007). Many countries have, therefore, established measures to control the contamination of these toxins in foodstuffs and animal feed. However, Slovenia and EU have, besides a Commission Recommendation on the presence of DON, ZON, ochratoxin A, T-2 and HT-2 and fumonisins (European Commission, 2006), placed legislative limits only for aflatoxin B1 in feed for different species and categories of livestock. Aflatoxin B1 concentrations in animal feed, grains being among them, may thus range up to 0.02 mg/kg of feed (European Commission, 2003a), but this mycotoxin was not the aim of our study. For DON, ZON, ochratoxin A and for the sum of fumonisins B1 and B2, the recommended highest concentration per kg in cereals and cereal products is 8 mg, 2 mg, 0.25 mg and 60 mg, respectively. Recommended maximum levels for T-2 and HT-2 toxin have not been set in Europe yet.

The levels of mycotoxins in our research did not reach the recommended levels, which is very satisfying. In general, our results are similar to those reported from around the world. DON is most widely spread mycotoxin, which is confirmed also by the investigation including samples from eleven European countries examined on trichothecenes contents. 57% of the samples were DON positive (0.003-3.7 mg/kg), mostly maize, and 20% contained T-2 toxin and HT-2 toxin (European Commission, 2003b; JECFA, 2001). Maize is known to be a good substrate for mould infection and production of potentially dangerous mycotoxins harmful to both humans and animals (Kumar et al., 2008), but in our investigation wheat was the most contaminated grain with DON, which has happened for the first time. Up to the present, also in Slovenia maize was the most contaminated grain (Jakovac-Strajn et al., 2008).

In neighbouring Croatia, a seven year long investigation of grains or animal feed samples (1998-2004) revealed 41% of DON, 16.8% of T-2 toxin and 27.6% of DAS (Sokolović and Šimpraga, 2006). In former investigations (Domijan et al., 2005), maize samples were tested on ochratoxin A, ZON and fumonisins B1 and B2. Most frequently fumonisin B1 was found (100%), followed by ZON (84%) and ochratoxin A (39%), while fumonisin B2 was found only in three samples. The concentrations (mean ± SD) of fumonisin B1, ZON and ochratoxin A in positive samples were 459.3 ± 310.7, 3.84 ± 6.68 and 1.47 ± 0.38 μg/kg, respectively. In 2008, (Jajić et al., 2008) the results of the first examinations on DON in 139 samples of various grains were published. DON was found in 44.7% of maize samples, 37.5 % of wheat and 25% of barley samples. In positive samples, DON was found in concentration range between 40-2460 μg/kg, but in our research in concentration at 130-2860 μg/kg.

Taken together, the presence of toxigenic moulds in different grains grown in Slovenia showed and confirmed the presence of different mycotoxins, which were consumed by livestock. Although concentrations were not above the recommended levels, a relatively high percent of positive samples (57.6%) require a further control in Slovenian primary grain production.

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Testing the efficacy of different substances against *Arion* slugs (Arionidae) under laboratory conditions

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ABSTRACT

In 2008 and 2009 we studied molluscicidal activity of 26 substances in 89 different treatments under laboratory conditions. The experiments in which slugs (*Arion* spp.) were a part took place in two series: 1) with the injection of active substances in slug intestines; and 2) with the application of pellets. After giving the injection we observed 100% mortality of slugs in treatments with *Bacillus thuringiensis* var. *kurstaki* (0.25 ml in 10% concentration/individual), caffeine (0.25 ml in 10% concentration/individual), sodium dodecyl sulphate (0.25 ml in 10% concentration/individual, 0.125 ml in 10% concentration/individual, 0.125 ml in 5% concentration/individual, 0.0625 ml in 10% concentration/individual), and pirimicarb (0.25 ml in 10% concentration/individual, 0.125 ml in 10% concentration/individual, 0.125 ml in 5% concentration/individual, 0.0625 ml in 10% concentration/individual). Meanwhile, the application of pellets resulted in the highest (100%) slug mortality when sodium dodecyl sulphate in 0.5% concentration with caraway as a supplement was used.

Keywords: laboratory experiment, *Bacillus thuringiensis* var. *kurstaki*, caffeine, sodium dodecyl sulphate, pirimicarb, slugs, *Arion* spp., molluscicides, efficacy

IZVLEČEK

PREIZKUŠANJE UČINKOVITOSTI RAZLIČNIH SNOVI ZA ZATIRANJE LAZARJEV (*Arion* spp., Arionidae) V LABORATORIJSKIH RAZMERAH

V letih 2008 in 2009 smo v laboratorijskih razmerah preizkušali limacidno delovanje 26 snovi v 89 različnih obravnavanjih. Poskusi, v katere smo vključili polže lazarje (*Arion* spp.), so potekali v dveh serijah, in sicer z injiciranjem aktivne snovi v prebavilo polžev in z uporabo pelet. Pri injiciranju smo 100 % smrtnost polžev ugotovili v obravnavanju z bakterijo *Bacillus thuringiensis* var. *kurstaki* (0,25 ml v 10 % koncentraciji/osebek), kofeinom (0,25 ml v 10 % koncentraciji/osebek), natrijevim dodecil sulfatom (0,25 ml v 10 % koncentraciji/osebek; 0,125 ml v 10 % koncentraciji/osebek; 0,125 ml v 5 % koncentraciji/osebek; 0,0625 ml v 10 % koncentraciji/osebek), in pirimikarbom (0,25 ml v 10 % koncentraciji/osebek; 0,125 ml v 10 % koncentraciji/osebek; 0,125 ml v 5 % koncentraciji/osebek; 0,0625 ml v 10 % koncentraciji/osebek), medtem ko smo največjo (100 %) smrtnost polžev pri uporabi pelet dosegli z natrijevim dodecil sulfatom v 0,5 % koncentraciji z dodatkom kumine.

Ključne besede: laboratorijski poskus, *Bacillus thuringiensis* var. *kurstaki*, kofein, natrijev dodecil sulfat, pirimikarb, lazarji, *Arion* spp., limacidi, učinkovitost

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1 INTRODUCTION

Terrestrial slugs and snails are destructive agricultural pests that cause economic damage to a wide variety of plants including vegetables, forage crops, tree fruits, shrubs, flowers, ground green cover, and newly sown lawngrasses. Morover, they play an important role in transmitting and spreading disease to cultivated plants (Ohlendorf, 1999). Thus, the use of specific molluscicides is considered one of the most effective measures for terrestrial molluscs control (Barker, 2001).

Carbamates proved to be the most potent class of molluscicides (Miller *et al*., 1988; Radwan *et al*., 1992). Growers and farmers often experience difficulty controlling these pests with conventional bait pellets containing molluscicides such as methiocarb and metaldehyde. For example, in wet conditions the efficacy of these pellets can be very low (Hata *et al*., 1997), and lead to unsatisfactory control levels. Furthermore, poison baits can be toxic to other non-target soil invertebrates, as well as birds and mammals such as shrews and field mice (Hagin and Bobnick, 1991; Purvis, 1996). Clearly, additional molluscicides are needed that are highly selective and toxic for slugs and/or snails with minimal effect on other species.

The development of effective alternatives to conventional molluscicides, particularly those which could be used in an integrated control strategy, would reduce plant losses, improve quality, and offer a sustainable strategy for controlling slug and snail pests with reduced molluscicide input. The development of alternative snail and slug control methods compatible with Integrated Pest Management (IPM) strategies used to control other pests would help satisfy increasing market demands for ornamental plants and edible crops grown with environmentally responsible production methods (Schüder *et al*., 2003).

In biological control the usage of the parasitic nematode *Phasmarhabditis hermaphrodita* (Schneider) has already been confirmed to be efficacious (Wilson and Grewal, 2005). Since the researching of parasitic nematodes hasn't been done in Slovenia yet, the aforementioned species is still on the list of exotic agents and its application is therefore limited to laboratory work. At the moment there are seven molluscicide products on the Slovenian market. There are two products in a catalogue of permitted products in organic agriculture (Ozimič *et al*., 2007), but each of them has some deficiencies which have led researchers, industry, and producers to introduce a better and environmentally-friendly product. The product Carakol (active ingredient metaldehyde, 5%) is environmentally unsafe (toxic to warm-blooded organisms), but it is on the list because there are no more suitable molluscicides on our market. The product Feramol (active ingredient iron [III] phosphate), the second of two previously mentioned products, is environmentally safe but its efficacy is slow and often unsatisfactory.

The scope of our research was: (1) to study the efficacy of different substances according to their molluscicidal activity with injection; and (2) to study the efficacy of different substances in the form of pellets. Verification of satisfactory activity with injection would lead to test substance activity in the form of a pellet. This would further lead to the future testing of selected substances outdoors, and the potential implementation of such a product into the systems of food production in conditions when slugs represent an economically important group of pest organisms.

2 MATERIALS AND METHODS

The investigation was carried out during 2008 and 2009 at the Laboratory of Entomology (University of Ljubljana, Biotechnical Faculty, Department of Agronomy, Chair of Phytomedicine, Agricultural Engineering, Crop Production, Grassland and Pasture Management) in Ljubljana, Slovenia. To begin, 890 *Arion* spp. slugs were collected at the Experimental Field of Biotechnical Faculty in Ljubljana, Slovenia (46°04'N, 14°31'E, 299 m alt.). The slugs used in the experiments were of different in size and age, and of different species of genus *Arion* – as we wanted to draw near (not sure what 'draw near' means) the conditions in the open.

We included 26 different substances in a trial and tested their potential molluscicidal activity in 89 different treatments (Table 1). All treatments were repeated 10 times, with five replicates where a treated slug had accessible feed (cabbage or salad leaf), and five replicates without feed (only pellets). In each plastic petri dish (150 x 20 mm; producer: Kemomed d.o.o., Kranj, Slovenia) we put one slug and a paper tampon (35 x 11 mm; Tosama d.d., Vir pri Domžalah, Slovenia) which was soaked in water prior to the start of the experiment. We put in each Petri dish around 4 g of pellets. The petri dishes were put in a rearing chamber (type: RK-900 CH, producer: Kambič Laboratory equipment, Semič, Slovenia) without light at 20 ºC and at a relative humidity of 85%. We observed slug mortality and their potential feeding on 1, 2, 3, 4, 5, 6, and 7 DAT (days after treatment). The tampon in the petri dish was wetted daily with distilled water. If mould appeared on pellets they were replaced with fresh material.

In order to determine the toxicity of the substances tested against *Arion* slugs, they were injected on the dorsal side introducing a needle no more than 4 mm and at a 30º angle in relation to the slug's skin to reduce the risk of damaging vital organs (Aguiar and Wink, 2005). For injection we used pen needles (0.36 x 13 mm; B-D Micro-fine IV, Becton Dickinson, U.S.A.), meanwhile pellets (composition: 18% maize meal, 36% wheat meal, 15% wheat flour, 4.6% soybean meal, 4.6% notilac, 0.16% mycosorb, 1.8% lignobond, and 9.5% starch) with selected active ingredients and concentrations were produced by the company Unichem d.o.o. The results of the experiments are shown in Tables from 2 to 15 as a % of mortality in selected treatments for each day after treatment (DAT).

Fig. 1: Tested substances with potential molluscicidal activity

Substance and treatment	ml of liquid /slug (injection) or g pellets/slug (pellets)	Form of application	Experiment
Bacillus thuringiensis var. *kurstaki* (Delfin 10%)	0.25; 0.125; 0.0625	I	8; 9; 11
Bacillus thuringiensis var. *kurstaki* (Delfin 5%)	0.125 4	I, P	10 6
Bacillus thuringiensis var. *kurstaki* (Delfin 1%)	4	P	7
Bromadiolone 10%	4	P	5
Diatomaceous earth 10%	4	P	1; 2; 3
Diatomaceous earth 5%	4	P	1; 2; 3
Diatomaceous earth 1%	4	P	1; 2; 3
Glyphosate (Boom efekt 10%)	0.25	I	8
Glyphosate (Boom efekt 4%)	4	P	5; 6
Glyphosate (Boom efekt 1%)	4	P	7
HDK 2.5%	0.125	I,	14
HDK 1.25%	0.125	I	14
Carboxylic acid 0.2%	4	P	1; 2; 3
Caffeine 10%	0.25; 0.125; 0.0625	I	8; 9; 11
Caffeine 5%	0.125 4	I, P	10 6
Caffeine 1%	4	P	7
Control	0.25; 0.125; 0.0625	I, P	8; 9; 11; 14 1; 2; 3; 4; 5; 6; 7; 10; 12; 13
Caraway (milled seed) 10%	0.25; 0.125; 0.0625 4	I, P	8; 9; 11 2; 3
Caraway (milled seed) 5%	0.125 4	I, P	14 2; 3
Lactose 10%	0.125	I	14
Lactose 5%	0.125	I	14
Limonene 10%	0.125 4	I, P	14 1; 2; 3
Limonene 5%	0.125 4	I, P	14 1; 2; 3
Natren 10%	0.125	I	14
Natren 5%	0.125	I	14
Niclosamide 10%	0.25	I	8
Niclosamide 5%	0.125 4	I, P	14 6
Niclosamide 1%	4	P	7
Pirimicarb (Pirimor 10%)	0.25; 0.125; 0.0625	I	8; 9; 11
Pirimicarb (Pirimor 5%)	0.125 4	I, P	10 6
Pirimicarb (Pirimor 1%)	4	P	7
Pirimicarb (Pirimor 0.25%)	4	P	12
Pirimicarb (Pirimor 0.125%)	4	P	12
Pirimicarb (Pirimor 0.25%) + caraway	4	P	12
Pirimicarb (Pirimor 0.125%) + caraway	4	P	12
Pirimicarb (Pirimor 0.25%) + malt	4	P	12
Pirimicarb (Pirimor 0.125%) + malt	4	P	12

Quackgrass 12.4 mg /250 g dry matter	4	P	1; 2; 3
Quackgrass 40 mg /250 g dry matter	4	p	1; 2; 3
Castor oil plant oil 10%	0.125	I	14
Castor oil plant oil 5%	0.125	I	14
Sodium dodecyl sulphate 10%	0.25; 0.125; 0.0625	I	8; 9; 11
Sodium dodecyl sulphate 5%	0.125 4	I, P	10 6
Sodium dodecyl sulphate 1%	4	P	7
Sodium dodecyl sulphate 0.25%	4	P	12
Sodium dodecyl sulphate 0.125%	4	P	12
Sodium dodecyl sulphate 0.25% +Caraway (milled seed)	4	P	12
Sodium dodecyl sulphate 0.125% + caraway (milled seed)	4	P	12
Sodium dodecyl sulphate 0.5% + caraway (milled seed)	4	P	13
Sodium dodecyl sulphate 0.5% + malt	4	P	13
Sodium dodecyl sulphate 0.5% + caraway (milled seed) + bran	4	P	13
Sodium dodecyl sulphate 0.5% + malt + bran	4	P	13
Sodium dodecyl sulphate 0.25% + malt	4	P	13
Sodium dodecyl sulphate 0.125% +malt	4	P	13
Salt 10%	0.25 4	I P	8 1; 2; 3
Coated salt 5%	4	P	1; 2; 3; 6
Coated salt 1%	4	P	1; 2; 3; 7
Thymol 10%	0.25	I	8
Thymol 5%	4	P	6
Thymol 1%	4	P	7
Yew (milled needles) 10%	0.25	I	8
Yew (milled needles) 5%	4	P	5; 6
Yew (milled needles) 1%	4	P	7
Ureaformaldehyde 10%	0.125	I	14
Ureaformaldehyde 5%	0.125	I	14

Legend: I- injection; P-pellets

3 RESULTS

In the first experiment (beginning of the trial: June 17, 2008) we studied molluscicidal activity of eight different substances included in 17 treatments in pellets. The highest slug mortality (100%) was determined in commercial products Mesurol (active ingredient methiocarb) and Terminator (a.i. metaldehyde) already by the second day after treatment. From the rest of the substances (caraway 1%, 5%, and 10%; limonene 1%, 5%, and 10%; diatomaceous earth [DE] 1%, 5%, and 10%; salt 1%, 5%, and 10%, quackgrass extract [12.4 mg/ 250 g dry matter, 40 mg / 250 d dry matter]), 0.2% carboxylic acid) we observed only 20% mortality on the 6th day with the latter. In the second experiment (Table 2), which lasted for four days and in which we tested the efficacy of nine substances included in 19 treatments, the fastest activity (100% mortality in 24 hours after the exposure) and highest mortality rate of slugs was observed in the application of pellets Arion (a.i. metaldehyde), followed by carboxylic acid with 40% slug mortality after the first day after treatment (DAT) and with caraway 1% on third and fourth DAT with 60% slug mortality.

In the third experiment (beginning of the trial: July 14, 2008) treatments from the second experiment were repeated and we concluded that mortality was attained with the commercial product Arion + (a. i. metaldehyde), while carboxylic acid was not effective. Caraway with 1% caused only a 20% slug mortality only by the four DAT.

In the fourth experiment (beginning of the trial: August 19, 2008) we decided for a combination of seven active ingredients in 15 different treatments: 1) DE and limonene, 2) DE and carawy, 3) DE and boletus, 4) DE and starch, 5) salt and caraway, 6) salt and boletus, 7) salt and starch, 8) salt, DE and caraway, 9) salt, DE, caraway and boletus, 10) salt, DE, caraway and starch, 11) caraway and malt, 12) salt and malt, 13) DE and malt, 14) caraway and starch, and 15) caraway and

boletus), where we noted 20% slug mortality only at four DAT in treatments with caraway and starch as an additive in pellets.

In the fifth experiment (Table 3) we tested the activity of nine active ingredients. On the fourth DAT we determined 60% mortality only in the treatment glyphosate, while the highest mortality (100%) was registered on the seventh DAT in the same treatment. Fruits of yew gave only 60% slug mortality. We also found out that slugs fed on additional cabbage in the majority of the treatments, except in treatments when pellets contained yew (milled needles), salt+caraway+diatomaceus earth and salt+caraway.

In the sixth experiment (Table 4) we studied the efficacy of nine active ingredients in 18 different treatments. After seven days 20% slug mortality was determined in treatments pirimicarb (pirimor 5%) and sodium dodecyl sulphate 5%, while other treatments did not result in death to the slugs.

In the seventh experiment (beginning of the trial: July 7, 2009) we repeated the treatments from the sixth experiment, only we lowered the concentration of active ingredients in the pellets (Boom-effect 1%, coated salt 1%, Delfin 1%, sodium dodecyl sulfate 1%, niclosamide 1%, Pirimor 1%, thymol 1%, yew meal 1%, caffeine 1%; all treatments with and without cabbage leaf as an additional food source). None of the treaments killed slugs although slugs fed on pellets and the enclosed cabbage.

In the eighth experiment (beginning of the trial: July 10, 2009) we injected 0.25 ml of nine active ingredients in 10% solution into the slugs. The highest mortality we determined already on the first day in treatments *Bacillus thuringiensis* var. *kurstaki* (product Delfin), pirimicarb (product Pirimor), caffeine and sodium dodecyl sulphate, while the 33% slug mortality in pellets with added salt was determined only on the second day. In the rest of the treaments (Boom-effect [a.i. glyphostae], niclosamide, thymol, and yew meal) slugs fed with the enclosed cabbage, and their death was not confirmed until the end of the experiment.

In the ninth experiment (Table 5) we employed substances which proved to be effective in the previous experiment, only we now injected half of the dose into slugs (0.125 ml of 10% suspension) of the active ingredient. Pirimicarb as well as sodium dodecyl sulphate provoke the death of all the slugs already in the first few days, while bacteria and caffeine were less efficient.

The 10th experiment (Table 6) was a repetition of the ninth, only we injected 0.125 ml of 5% suspension of active ingredients into each slug. Pirimicarb and sodium dodecyl sulphate acted with the same efficacy as in the preceding experiment. In the meantime, the activity of *Bacillus thuringiensis* var. *kurstaki* and caffeine was somewhat worse.

In the 11th experiment (Table 7) we again used active ingredients from the ninth and 10th experiments, only this time we injected 0.0625 ml of suspension in 10% solution. Pirimicarb and sodium dodecyl sulfate again demonstrated the best molluscicidal activity. Meanwhile, the other two substances were only 33% effective after seven days.

In the 12th experiment (beginning of the trial: August 4, 2009) we tested the activity of four active ingredients in 24 different treatments. Despite feeding on pellets, all slugs survived. Therefore, we redoubled the concentrations in pellets in the 13th experiment (Table 8), and in combination with sodium dodecyl sulphate in 0.5% and caraway, and attained 100% mortality of slugs already after the first day of the experiment. Lower molluscicidal activity also showed a combination of sodium dodecyl sulphate and malt, but only on the fifth day of the beginning of the experiment. In the rest of treatment slugs survived, even though they fed on pellets.

In the 14th experiment (Table 9) we injected seven different active ingredients in 14 treatments into slugs. Sufficient molluscicide activity was determined only at 5% concentration of limonene and caraway, while slug mortality in the rest of the treatments was distinctively lower.

Table 2: Mortality (%) of *Arion* slugs after pellet treatment (beginning of the trial: July 7 2008).

Treatment	Days after treatment			
	1	2	3	4
Control	0	0	20	20
Carboxylic acid 0. 2%	40	40	40	40
Coated Salt 5%	0	0	40	40
Arion	0	0	40	80
Salt 10%	0	0	0	0
Salt 5%	0	0	0	0
Salt 1%	0	0	20	20
Coated salt – new 1%	0	0	20	20
Caraway 10%	20	20	40	40
Caraway 5%	0	0	0	20
Caraway 1%	0	40	60	60
Arion +	100	100	100	100
Limonene 10%	0	20	20	20
Limonene 5%	20	20	20	20
Limonene 1%	0	0	0	0
Diatomaceus earth 10%	0	20	20	20
Diatomaceus earth 5%	0	0	40	40
Diatomaceus earth 1%	20	20	20	60
Quickgrass extract 40 mg	0	0	0	0
Quickgrass extract 12.4 mg	20	20	40	40

Table 3: Mortality (%) of *Arion* slugs after pellet treatment (beginning of the trial: October 8 2008).

Treatment	Days after treatment						
	1	2	3	4	5	6	7
Control	0 (100)*	0 (100)	0 (100)	0 (100)	0 (100)	0 (100)	0 (100)
Yew – milled needles 1%	0	0	0	0	0	40	40
Yew – fruits 1%	0	0	0 (20)	20 (25)	20 (50)	20 (50)	60 (100)
Starch + caraway + coated salt 10%	0	0	20	20	20 (25)	20 (25)	20 (25)
Bromadiolone 10% + starch	0	0	0	0 (20)	0 (20)	0 (20)	20 (25)
Salt 10% + caraway 10%	0	0	20	20 (25)	40 (33)	40 (66)	40 (66)
Salt 10% + caraway + diatomaceus earth 10%	0	0	0	0	0	0	0
Salt 10% + caraway 5%	0	0	40	40	40	40	100
Boom-efect (glyphosate) 4%	0	0	20 (75)	60 (100)	60 (100)	80 (100)	100

* Number in parenthesis means % of slugs, eating additional food

Table 4: Mortality (%) of *Arion* slugs after pellet treatment (beginning of the trial: May 18 2009).

Treatment	Days after treatment						
	1	2	3	4	5	6	7
Control	0 (100)	0 (100)	0 (100)	0 (100)	0 (100)	0 (100)	0 (100)
Boom-efect 4% (Glyphosate)	0 (80)	0 (100)	0 (100)	0 (100)	0 (100)	0 (100)	0 (100)
Boom-efect 4% (Glyphosate) (cabbage)	0 (100, 100)	0 (100, 100)	0 (100, 100)	0 (100, 100)	0 (100, 100)	0 (100, 100)	0 (100, 100)
Coated salt 5%	0 (100)	0 (100)	0 (100)	0 (100)	0 (100)	0 (100)	0 (100)
Coated salt 5% (cabbage)	0 (100, 80)	0 (100, 100)	0 (100, 100)	0 (100, 100)	0 (100, 100)	0 (100, 100)	0 (100, 100)
Delfin 5% (*Bacillus thuringiensis* var. *kurstaki*)	0 (100)	0 (100)	0 (100)	0 (100)	0 (100)	0 (100)	0 (100)
Delfin 5% (*Bacillus thuringiensis* var. *kurstaki*) (cabbage)	0 (80, 80)	0 (100, 100)	0 (100, 100)	0 (100, 100)	0 (100, 100)	0 (100, 100)	0 (100, 100)
Sodium dodecyl sulfat 5%	0 (80)	0 (100)	0 (100)	0 (100)	0 (100)	0 (100)	0 (100)
Sodium dodecyl sulfat 5% (cabbage)	0 (100, 60)	0 (100, 80)	0 (100, 100)	0 (100, 100)	20 (100, 100)	20 (100, 100)	20 (100, 100)
Niclosamide 5%	0 (80)	0 (100)	0 (100)	0 (100)	0 (100)	0 (100)	0 (100)
Niclosamide 5% (cabbage)	0 (80, 100)	0 (100, 100)	0 (100, 100)	0 (100, 100)	0 (100, 100)	0 (100, 100)	0 (100, 100)
Pirimor 5% (pirimicarb)	0 (100)	0 (100)	0 (100)	0 (100)	0 (100)	0 (100)	0 (100)
Pirimor 5% (pirimicarb) (cabbage)	0 (100, 80)	20 (100, 75)	20 (100, 75)	20 (100, 75)	20 (100, 75)	20 (100, 100)	20 (100, 100)
Thymol 5%	0 (60)	0 (100)	0 (100)	0 (100)	0 (100)	0 (100)	0 (100)
Thymol 5% (cabbage)	0 (100, 40)	0 (100, 60)	0 (100, 80)	0 (100, 80)	0 (100, 80)	0 (100, 80)	0 (100, 80)
Yew 5%	0 (100)	0 (100)	0 (100)	0 (100)	0 (100)	0 (100)	0 (100)
Yew 5% (cabbage)	0 (60, 60)	0 (60, 100)	0 (100, 100)	0 (100, 100)	0 (100, 100)	0 (100, 100)	0 (100, 100)
Caffeine 5%	0 (100)	0 (100)	0 (100)	0 (100)	0 (100)	0 (100)	0 (100)
Caffeine 5 % (cabbage)	0 (80, 100)	0 (100, 100)	0 (100, 100)	0 (100, 100)	0 (100, 100)	0 (100, 100)	0 (100, 100)

* First number in parenthesis means % of slugs, eating additional food, second number reffers to % of slugs, eating pellets.

Table 5: Mortality (%) of *Arion* slugs after inject treatment (beginning of the trial: July 13 2009).

Treatment	Days after treatment						
	1	2	3	4	5	6	7
Control	0 (100)	0 (100)	0 (100)	0 (100)	0 (100)	0 (100)	0 (100)
Delfin (*Bacillus thuringiensis* var. *kurstaki*)	66	66	66	66	66	66	66
Pirimor (pirimicarb)	100	100	100	100	100	100	100
Caffeine	66	66	66	66	66	66	66
Sodium dodecyl sulfate	100	100	100	100	100	100	100

* 0.125 ml of 10% suspension was injected in every slug. The number in parenthesis means % of slugs, eating additional food.

Table 6: Mortality (%) of *Arion* slugs after inject treatment (beginning of the trial: July 13 2009).

Treatment	Days after treatment						
	1	2	3	4	5	6	7
Control	0 (100)	0 (100)	0 (100)	0 (100)	0 (100)	0 (100)	0 (100)
Delfin (*Bacillus thuringiensis* var. *kurstaki*)	33 (50)	33 (50)	33 (50)	33 (50)	33 (50)	33 (50)	33 (50)
Pirimor (pirimicarb)	100	100	100	100	100	100	100
Caffeine	33 (50)	66 (100)	66 (100)	33 (50)	33 (50)	66 (100)	66 (100)
Sodium dodecyl sulfate	100	100	100	100	100	100	100

* 0.125 ml of 5% suspension was injected in every slug. The number in parenthesis means % of slugs, eating additional food.

Table 7: Mortality (%) of *Arion* slugs after inject treatment (beginning of the trial: July 15 2009).

Treatement	Days after treatment						
	1	2	3	4	5	6	7
Control	0 (100)	0 (100)	0 (100)	0 (100)	0 (100)	0 (100)	0 (100)
Delfin (*Bacillus thuringiensis* var. *kurstaki*)	33 (50)	33 (50)	33 (50)	33 (50)	33 (50)	33 (50)	33 (50)
Pirimor (pirimicarb)	100	100	100	100	100	100	100
Caffeine	0	33 (50)	0	33 (50)	0	33 (50)	0
Sodium dodecyl sulfate	100	100	100	100	100	100	100

* 0.0625 ml of 10 % suspension was injected in every slug. The number in parenthesis means % of slugs, eating additional food.

Table 8: Mortality (%) of *Arion* slugs after pellet treatment (beginning of the trial: August 25 2009).

Treatment	Days after treatment						
	1	2	3	4	5	6	7
Controla	0 (100)	0 (100)	0 (100)	0 (100)	0 (100)	0 (100)	0 (100)
Pirimor 0.5% (pirimicarb) + malt	0 (20)	0 (80)	0 (80)	0 (100)	0 (100)	0 (100)	0 (100)
Pirimor 0.5% (pirimicarb) + malt (cabbage)	0 (20, 40)	0 (100, 80)	0 (100, 100)	0 (100, 100)	0 (100, 100)	0 (100, 100)	0 (100, 100)
Pirimor 0.5% (pirimicarb) + caraway	0 (60)	0 (100)	0 (100)	0 (100)	0 (100)	0 (100)	0 (100)
Pirimor 0.5% (pirimicarb) + caraway (cabbage)	0 (60, 20)	0 (80, 60)	0 (100, 100)	0 (100, 100)	0 (100, 100)	0 (100, 100)	0 (100, 100)
Sodium dodecyl sulfate 0.5% + malt	0 (100)	0 (100)	0 (100)	0 (100)	0 (100)	0 (100)	0 (100)
Sodium dodecyl sulfate 0.5% + malt (cabbage)	0 (20, 80)	0 (40, 100)	0 (60, 100)	0 (60, 100)	20 (75, 100)	20 (75, 100)	20 (75, 100)
Sodium dodecyl sulfate 0.5% + caraway	100 (100)	100 (100)	100 (100)	100 (100)	100 (100)	100 (100)	100 (100)
Sodium dodecyl sulfate 0.5% + caraway (cabbage)	0 (80, 80)	0 (100, 100)	0 (100, 100)	0 (100, 100)	0 (100, 100)	0 (100, 100)	0 (100, 100)
Sodium dodecyl sulfate 0.5% + malt + bran	0 (80)	0 (100)	0 (100)	0 (100)	0 (100)	0 (100)	0 (100)
Sodium dodecyl sulfate 0.5% + malt + bran (cabbage)	0 (60, 100)	0 (100, 100)	0 (100, 100)	0 (100, 100)	0 (100, 100)	0 (100, 100)	0 (100, 100)
Sodium dodecyl sulfate 0.5% + caraway + bran	0 (80)	0 (100)	0 (100)	0 (100)	0 (100)	0 (100)	0 (100)
Sodium dodecyl sulfate 0.5% + caraway + bran (cabbage)	0 (80, 80)	0 (100, 100)	0 (100, 100)	0 (100, 100)	0 (100, 100)	0 (100, 100)	0 (100, 100)

* First number in parenthesis means % of slugs, eating aditional food, second number reffers to % of slugs, eating pellets.

Table 9: Mortality (%) of *Arion* slugs after injection treatment (beginning of the trial: September 30 2009). We injected 0.125 ml of active ingredient of related concentrations into slugs.

Treatment	Days after treatment						
	1	2	3	4	5	6	7
Control	0 (80)	0 (100)	0 (100)	0 (100)	0 (100)	40 (100)	40 (100)
HDK 2.5%	0 (20)	0 (40)	100	100	100	100	100
HDK 1.25%	0	0 (20)	20 (50)	20 (50)	20 (75)	20 (75)	40 (66)
Caraway 10%	0 (80)	0 (100)	0 (100)	0 (100)	0 (100)	20 (100)	20 (100)
Caraway 5%	20 (75)	20 (100)	60 (100)	60 (100)	60 (100)	60 (100)	60 (100)
Lactose 10%	0 (80)	0 (100)	0 (100)	0 (100)	0 (100)	0 (100)	0 (100)
Lactose 5%	0 (100)	0 (100)	0 (100)	0 (100)	0 (100)	20 (100)	20 (100)
Castor oil plant oil 10%	0 (100)	0 (100)	20 (100)	20 (100)	20 (100)	20 (100)	20 (100)
Castor oil plant oil 5%	0 (80)	0 (100)	20 (100)	20 (100)	20 (100)	40 (100)	40 (100)
Ureaformaldehyde 10%	0 (40)	0 (80)	0 (100)	0 (100)	0 (100)	20 (100)	20 (100)
Ureaformaldehyde 5%	20 (100)	20 (100)	20 (100)	20 (100)	20 (100)	20 (100)	20 (100)
Natren 10%	0 (100)	0 (100)	0 (100)	0 (100)	0 (100)	0 (100)	0 (100)
Natren 5%	0 (80)	0 (100)	0 (100)	0 (100)	0 (100)	0 (100)	0 (100)
Limonene 10%	0 (100)	0 (100)	20 (100)	20 (100)	20 (100)	20 (100)	20 (100)
Limonene 5%	80	80 (100)	80 (100)	80 (100)	80 (100)	80 (100)	100

* First number in parenthesis means % of slugs, eating additional food, second number reffers to % of slugs, eating pellets.

4 DISCUSSION

In our research we included substances that have been used in the research of other authors as potentially or actually efficient when controlling different species of slugs. But no aditional information was acquired about their molluscicide activity on slugs from the genus *Arion*, which tend to be the most noxious group of these animals on vegetables in Slovenia (Laznik and Trdan, 2009).

A quackgrass (*Agropyron repens* L. Beauv.) extract fraction containing phenolic glycosides showed in some previous research both dermal and gastrointestinal toxicity toward two slug species, *Deroceras reticulatum* (Müller) and *Deroceras leave* (Müller) (Hagin, 1989; Hagin and Bobnick, 1991). However, testing this substances in our experiment as pellet treatment with different concentrations of the active ingridient (*Agropyron* extract, carboxylic acid) did not show any molluscicidal effect on the *Arion* slugs.

The study of Kumar and Singh (2006) indicated that dried seed powder of *Carum carvi* L. is an important source of botanical molluscicides. It has been also reported that limonene found in *C. carvi* seed is metabolized into more toxic carvone and causes death of the snail *Lymnaea acuminata* Lamarck after 24 hours of exposure with very low LC_{50} value. However, in our experiment these substances with different concentrations of the active ingridients (powder of the *C. carvi* seeds, limonen) as a pellet treatment did not show any molluscicidal effect on *Arion* slugs.

It is well known that the 'salt effect,' causes slug and snails to emit copious amounts of slime, which leads to dehydration of the animals (Hagin and Bobnick, 1991; Ester and Molendijk, 2003). The method is limited by the requirement to spray the material directly on the slugs. In our experiment we wanted to bring salt to the slug and to determine if salt can act also in the stomach. We did not get any slug mortality using this method. Similarly, as salt and also diatomaceous earth act abrasively, and when in contact with a slug it harms its exoskeleton (Sibley and Thompson, 2004). However, the results in our experiment did not show any molluscicidal effect after trying to feed the *Arion* slugs with diatomaceous earth pellets. Mushroom, starch, and molasses extracts were used as attractants in pellets. These extracts are also known to attract slugs (Keller and Snell, 2002).

Yew extracts have hormonal activity in insects and mammals (Reddy *et al*., 2001), meanwhile researchers as yet have not determined any molluscicide activity of yew. Our research demonstrated that neither extracts from milled yew needles nor from fruits have satisfatory molluscicidal activity on slug from the genus *Arion*. Pirimicarb is a carbamate insecticide used to control aphids on vegetable, cereal, and orchard crops by inhibiting acetylcholinesterase activity (McGregor, 2006). In our experiment, when injected into slugs, pirimicarb also showed molluscicidal activity, while its molluscicidal activity in the continuation with pellets was not confirmed.

Sodium dodecyl sulphate (SDS) is a surfactant, which is widely used as an emulsifier in agricultural chemicals. Tseng *et al*. (unpublished) found that sodium dodecyl sulphate (SDS) was an effective molluscicide, when used on its own at a concentration of 100 ppm, for the semi-aquatic golden apple snail, *Pomacea canaliculata* (Lamarck). SDS was used in this case as an aqueous solution applied on the water surface. These researchers believed that the molluscicidal activity of SDS appeared to be due to "dermal" absorption, rather than as an "oral" (stomach) poison. Also in our experiment, when conducting the injection of SDS, we determined molluscicidal activity. When added to pellets, SDS showed molluscicidal activity only with 0.5% caraway as an additive. Niclosamide is a chlorinated salicylanilide pesticide principally used against aquatic vertebrates and crustaceans. In the research of Dai *et al.* (2008) molluscicidal activity of niclosamide against adult slugs of *Oncomelania hupensis* (Gredler) was confirmed, and at the same time we concluced that niclosamide does not have molluscicidal activity on slugs from the genus *Arion*.

Ester and Nijënstein (1995) reported that *Bacillus thuringiensis* significantly reduced the attack by slug *Deroceras reticulatum* (Müller) in winter wheat after two days, but after seven days this substance was no longer effective. In our experiment the product Delfin (a.i. *Bacillus thuringiensis* var. *kurstaki*) in treatment with pellets showed no molluscicidal activity, while an injection treatment with the highest concentration of suspension managed to kill slugs 100% of the time. In related research Hollingsworth *et al*. (2003) tested the activity of caffeine and discovered molluscicidal activity to some species of slugs. But our results confirmed such caffeine attribute only when injected into slugs, similar to bacteria *B. thuringiensis* var. *kurstaki*. When slugs fed on pellets treated with caffeine we could not make similar conclusions.

In contrast to our research, in which we conclude that thymol does not result in molluscicidal activity against slugs from the genus *Arion,* El-Zemity (2006) in his research establised that thymol has a potential molluscicidal activity in controlling snails from the species *Helix aspersa* Müller. Some preceding research have shown that some herbicides have molluscicidal activity too (El-Fiki and Mohamed, 1978; Zidan *et al*., 1998), but this was not confirmed in our example when testing the active ingredient glyphosate.

The results of our research indicate that the highest molluscicidal potential had sodium dodecyl sulfate in combination with extract of caraway, but future work will be needed to optimize the use of this substance in practical use as a pellet treatment. In several injection treatments we attained sufficient results, yet in the future we have to pay more attention to the production of appropriate pellets which can enable higher consumption rate by slugs from the genus *Arion*.

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Activity of entomopathogenic nematodes (Rhabditida) against cereal leaf beetle (*Oulema melanopus* [L.], Coleoptera, Chrysomelidae) adults under laboratory conditions

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ABSTRACT

In 2009, three Slovenian strains of entomopathogenic nematodes) and commercial product Entonem (active ingredient *S. feltiae*), were tested under laboratory conditions for their activity against adult cereal leaf beetles (*Oulema melanopus*). The nematode strains were tested at four different doses (250, 500, 1000, and 2000 infective juveniles/adult) and at three temperatures (15, 20, and 25 ºC). *Steinernema carpocapsae* strain C101 was the most effective and showed itself to be a good alternative to chemical insecticides, and appears to have the highest potential for controlling overwintered cereal leaf beetles under field conditions. In our bioassay the temperature had the greatest influence on the efficacy of the entomopathogenic nematode strains; both *S. feltiae* treatments (strain B30 and Entonem) proved to work better at the lowest temperature, however the strain *H. bacteriophora* D54 had its best efficacy at the highest temperature in the experiment. Several species *(S. feltiae* and *S. carpocapsae*) have been efficient at lower suspension concentrations, which enables their economical usage against the cereal leaf beetle in integrated cereal production in the future.

Key words: entomopathogenic nematodes, *Oulema melanopus*, biological control, laboratory experiment

IZVLEČEK

DELOVANJE ENTOMOPATOGENIH OGORČIC (Rhabditida) NA ODRASLE OSEBKE RDEČEGA ŽITNEGA STRGAČA (*Oulema melanopus* [L.], Coleoptera, Chrysomelidae) V LABORATORIJSKIH RAZMERAH

V letu 2009 smo v laboratorijskem poskusu preizkušali učinkovitost treh domačih ras entomopatogenih ogorčic in komercialnega pripravka Entonem (aktivna snov *S. feltiae*) zoper odrasle osebke rdečega žitnega strgača (*Oulema melanopus*). Delovanje entomopatogenih ogorčic smo preizkušali pri štirih različnih koncentracijah (250, 500, 1000 in 2000 infektivnih ličink/osebek) in treh različnih temperaturah (15, 20 in 25 ºC). Rasa C101 vrste *Steinernema carpocapsae* je bila najbolj učinkovita in bi lahko predstavljala dobro alternativo kemičnim insekticidom pri zatiranju prezimljenih odraslih osebkov rdečega žitnega strgača na prostem. V našem poskusu je imela največji vpliv na delovanje entomopatogenih ogorčic temperatura; obe obravnavanji z vrsto *S. feltiae* (rasa B30 in Entonem) sta bili učinkoviti tudi pri nižjih temperaturah, rasa D54 vrste *H. bacteriophora* pa je najbolje delovala pri najvišji temperaturi v poskusu. Ogorčici *S. feltiae* in *S. carpocapsae* sta zadovoljivo učinkovali tudi pri nižji koncentraciji suspenzije ogorčic, kar omogoča večjo gospodarnost rabo njihove uporabe pri zatiranju odraslih osebkov rdečega žitnega strgača v integrirani pridelavi žit v prihodnje.

Key words: entomopatogene ogorčice, *Oulema melanopus*, biotično varstvo, laboratorijski poskus

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1 INTRODUCTION

In Central Europe seven chrysomelid beetles belong to the genus *Oulema* (Chrysomelidae family). Two of them, the cereal leaf beetle (CLB), *Oulema melanopus* (L.) and *O. gallaeciana* (Heyden), are pests of various cereals (Ulrich *et al*. 2004). The CLB is spread across Europe, the Middle East and Asia and in North America (Haynes and Gage, 1981; Olfert *et al*. 2004). The life history and biology of CLB is well known (Casagrande *et al*., 1977). The adults hibernate gregariously in the soil, such as in field debris, in the crevices of tree bark, or inside rolled leaves (Casagrande *et al*., 1977). The adults become active in the spring, when temperature reaches 10 ºC, and feed initially on wild grasses. Oviposition begins about 14 days after adults resume activity in the spring. During the following two months each female lays several hundred eggs. The larvae pass through four instars, each lasting two to three days. Pupation occurs in the soil up to five cm beneath the surface. The species are univoltine. Adults feed but become less and less active during the summer and early autumn. The larvae and adults feed on the upper layer of green mesophyll cells, down to the cuticle, staying between the leaf veins (Ulrich *et al*., 2004). This feeding pattern is characteristic of the CLB and is one way of detecting its presence (Campbell *et al*., 1989).

The economic impact of the CLB can be significant. Heyer (1977) estimated that a single larva reduces assimilation by about 10 %. A massive attack of larvae reduces total assimilation by up to 80 % (Grala *et al*., 1991) causing losses of about one tonne of grain per ha. Previous studies recommended an economic threshold of one larva per stem (Haynes and Gage, 1981), but this infestation level often results in unacceptably high levels of defoliation (Buntin *et al*., 2004). More recent studies have suggested a much lower economic threshold (Herbert and van Duyn, 1999). Pre-harvest efforts to control CLB are primarily based on the release of natural enemies; the most successful have been the egg parasite *Anaphes flavipes* (Foerster) (Maltby *et al*., 1971) and the larval parasitoid *Tetrastichus julis* (Walker) (Haeselbarth, 1989).

The application of entomopathogenic nematodes (EPNs) as biological control agents in protected environments is well documented (Kaya and Gaugler, 1993). EPNs carry species specific symbiotic bacteria which, after nematodes infect insect hosts, are released into the hemolymph of the host (Gaugler, 2002). Only infective juveniles (IJs) are able to infect the insect host (Kaya, 2000). Research has demonstrated that EPNs at high concentrations, together with favourable abiotic factors (high humidity, optimal temperature) can be effective biological control agents of adult chrysomelids (Journey and Ostlie, 2000; Trdan *et al*., 2008). Recent research has confirmed their efficacy in controlling adult western corn rootworm (*Diabrotica virgifera virgifera* LeConte) (van der Burgt *et al*., 1998; Toepfer *et al.,* 2005), flea beetles (*Phyllotreta* spp.) (Trdan *et al*., 2008), and the Colorado potato beetle *Leptinotarsa decemlineata* (Say) (Campos-Herrera and Gutiérrez 2009; Trdan *et al*., 2009). Since adult CLB are found in protected areas we hypothesize that they should be particularly susceptible to EPN infections.

The aim of our research was to study the activity of entomopathogenic nematodes against the CLB adults, to determine which species of EPN (*S. feltiae*, *S. carpocapsae*, *Heterorhabditis bacteriophora*) is the most effective, and to investigate how the effectiveness of EPNs is related to temperature and the nematode concentration. In practice, farmers usually do not control the adult stages of CLB, although it was established in one research study that the most effective treatments were low rates of lambda cyhalothrin when applied early while adults were still laying eggs and before or near 50% egg hatch (Buntin *et al*., 2004). With the potential efficacy of entomopathogenic nematodes with regard to CLB adults, we would acquire the results necessary for replacing insecticides with the biological control agents mentioned. The most efficient strain shown by our research would then be suggested for incorporation in a sustainable strategy of cereals production. In this way we would contribute to more environmentally friendly production of cereals.

2 MATERIALS AND METHODS

2.1 Entomopathogenic nematodes and the cereal leaf beetle

The investigation was carried out during 2009 in Ljubljana (Biotechnical Faculty, Dept. of Agronomy), Slovenia. The commercial preparation (Entonem) was obtained from Koppert B.V., the Netherlands. The EPNs in this preparation is *Steinernema feltiae* (Filipjev). Once received, the nematode preparation was stored in the dark in a refrigerator (2-4 ºC). Three Slovenian isolates of EPNs were also included in the experiment. All three strains were isolated from the soil (Laznik *et al*., 2008; Laznik *et al*., 2009abc). Two Slovenian species (*S. carpocapsae* C101 and *H. bacteriophora* D54) were tested for the first time in this experiment, while *Steinernema feltiae* strain B30 has been proven to very effective in a field experiment against the Colorado potato beetle (Laznik *et al*., 2009d) and in a laboratory assay against rice weevil (Laznik *et al*., 2010). All EPN strains were reared

using late instar larvae of *Galleria mellonella* (L.) (Bedding and Akhurst, 1975). We used only infective juveniles which were less than 2 weeks old. During the experiment we stored the infective juveniles in a water suspension at 4 ºC in the refrigerator.

CLB adults were collected from a test plot of winter wheat being grown by members of the Biotechnical Faculty in Ljubljana. The CLB were caught in sweep nets in late morning, after the dew had dried. We stored the beetles after catching them in ventilated plastic containers (Trdan *et al*., 2008) and transported to the laboratory, where they were used for experimental purposes no later than 5 hours later. The adults were of different ages, replicating conditions in practice.

2.2 Laboratory bioassay

We tested the efficacy of the EPNs in controlling adults of the CLB by exposing individuals to either 0, 250, 500, 1000, or 2000 IJ/adult. We determined the number of infective juveniles in a previously prepared unknown concentration of nematode suspension by counting the number of such in droplets (5 µl x 5) and by diluting (adding tap water solution) or by concentrating (reduction to an adequate volume with the assistance of centrifugation). In this manner we obtained the selected concentrations of nematode suspensions (0, 2500, 5000, 10000, and 20000 IJ/ml).

We used the procedure described of Trdan *et al.* (2008). We placed 10 adult CLBs on a filter paper in a glass Petri dish (diameter = 9 cm) with a fresh leaf of wheat. Each treatment was repeated 10 times for a total of 100 CLB/nematode concentration. The following procedure was performed with a time interval repeated three times. One ml of each nematode concentration was added to the Petri dish which was then sealed with parafilm to prevent the beetles from escaping. The Petri dishes were put in a rearing chamber (type: RK-900 CH, producer: Kambič Laboratory equipment, Semič, Slovenia) with out light at temperatures of 15, 20, and 25 ºC at a relative humidity of 70 %.

The number of dead adult *O. melanopus* was determined 2, 4, and 6 days after treatment (DAT). The dead individuals were dissected to determine if the nematodes were present. In such a manner we wanted to prove that the insects died due to EPN activity.

2.3 Statistical analysis

A multifactor analysis of variance (ANOVA) was conducted to determine the differences in mortality rates (%) between the adults of *O. melanopus* reared in 48 different treatments (four strains of EPNs – each with four different concentrations at three different temperatures). Before the analysis, the mean mortality was tested for the homogeneity of treatment variances. Mortality rate data were corrected for control mortality, using Abbott's formula (Abbott, 1925). The arcsine square-root was transformed before this analysis. A Student-Newman-Keuls multiple range test ($P \leq 0.05$) was used to separate mean differences among the parameters in all the treatments. For the 6 days after treatment (DAT) the values of LC_{50} and LC_{90} (the numbers of IJs/adult causing 50% and 90% mortality) were estimated, and the overall efficacy of the tested nematodes was determined from this estimates (Trdan *et al*., 2008) All statistical analyses were performed using Statgraphics Plus for Windows 4.0 (Manugistics, Rockville, MD, USA) and the figures were created with MS Office Excel 2003. The data are presented as untransformed means ± SE.

3 RESULTS

Data on analysis of the pooled results are presented in table 1.

At 15 ºC, the natural (control) mortality of the CLB adults was 0.0±0.0 % (2 DAT), 0.64±0.35 % (4 DAT) and 9.50±2.92 % (6 DAT). At 20 ºC, the comparable values were 4.82±2.16 % (2 DAT), 5.66±2.22 % (4 DAT), and 9.7±3.97 % (6 DAT). At 25 ºC, the natural mortality of the CLB adults was 2.42±1.51 % (2 DAT), 6.06±2.55 % (4 DAT) and 35.59±10.86 % (6 DAT). In all nematode treatments, mortality was greater than in the control treatments and so all of the treatments values could be corrected for the natural mortality.

Two days after treatment, the highest mortality (100 %) was recorded with the *S. carpocapsae* strain C101 kept at 20 and 25 ºC (Table 2), and the lowest with the *H. bacteriophora* strain D54 kept at 15 and 20 ºC, where the mortality at all concentrations of the nematode suspension was less than 4% (Table 2). When only *S. feltiae* strains are taken into consideration, at all three temperatures and concentrations of the nematode suspension Entonem (51 %) was more efficient than the Slovenian native strain B30 (34 %). When all strains are considered, the *S. carpocapsae* strain C101 has the highest efficacy (68 %) at the lowest temperature and at all concentrations of the nematode suspension, while only Entonem, at a concentration of 500 IJs/adult, performed equally well (62 %) (Table 2).

Table 1: ANOVA results for corrected mortality of adults of the cereal leaf beetle

Source	Adults F	df	P
DAT	1204.29	2	<0.0001*
Nematode concentration	346.56	3	<0.0001*
EPN strain	1398.56	3	<0.0001*
Temperature	233.60	2	<0.0001*
Replication in time	2.17	9	0.0703
Replication in space	0.28	2	0.7590
DAT × nematode concentration	5.96	6	0.0051
DAT × EPN strain	86.05	6	<0.0001*
DAT × temperature	72.62	4	<0.0001*
Nematode concentration × EPN strain	50.56	9	<0.0001*
Nematode concentration × temperature	58.57	6	<0.0001*
EPN strain × temperature	352.40	6	<0.0001*
DAT × nematode concentration × EPN strain	8.22	18	<0.0001*
DAT × nematode concentration × temperature	8.53	12	<0.0001*
DAT × EPN strain × temperature	25.94	12	<0.0001*
Nematode concentration × EPN strain × temperature	54.37	18	<0.0001*
DAT × nematode concentration × EPN strain × temperature	5.0	36	<0.0001*

* Source of variation significant at α=0.05

Four days after treatment, the highest mortality was recorded with Entonem (25 ºC; 2000 IJs/adult) and the *S. carpocapsae* strain C101; the latter was the most efficient at all temperatures and concentrations of the nematode suspension (Table 1). The lowest efficacy was observed with the *H. bacteriophora* strain D54 at 15 ºC, with less than 20 % efficacy on average (Table 2). Among the *S. feltiae* strains, at all three temperatures and concentrations of the nematode suspension, the efficacy of Entonem (71 %) was higher than that of the Slovenian native strain B30 (61 %). At the lowest temperature the *S. carpocapsae* strain C101 had the highest efficacy (99 %) of all the observed strains at all concentrations of the nematode suspension. At concentrations of 500 and 2000 IJs/adult at the lowest temperature, the Entonem and the Slovenian strain B30 achieved more than 80 % efficacy (Table 2).

Six days after treatment, the highest mortality was caused with the use of Entonem (25 ºC, 2000 IJs/adult), the *H. bacteriophora* strain D54 (25 ºC, all concentrations of the nematode suspension) and the *S. carpocapsae* strain C101, which caused the death of 100% of the adults of the CLB at all temperatures and concentrations of the nematode suspension (Table 2). The lowest mortality was observed with the B30 strain, which on average caused the death of 69 % of the beetles. At 15 ºC, a mortality rate of more than 90 % was achieved with the *S. carpocapsae* strain C101 and Entonem, at all suspension concentrations, and with the *S. feltiae* strain B30 at 250, 500, and 2000 IJs/adult, respectively. At the lowest temperature, the commercial and native *S. feltiae* strains performed better than at the higher two temperatures in our laboratory assay (Table 2), on the other hand, the *H. bacteriophora* strain D54 performed its best at the highest temperature (100 %).

Overall, the nematode treatments were generally more effective at 25 ºC (74 %) than at 15 ºC (64 %), and 20 ºC (60 %). Among the observed strains, C101 performed better (95 %) than the other strains included in the laboratory assay (Entonem 67 %, B30 54 %, D54 49 %). Both *S. feltiae* strains performed better at the lowest temperature (over 70 %) than at the higher temperatures, where their efficacy was only 54 %. At the highest temperature, the strain *H. bacteriophora* D54 performed better (83 %) than at the lower two temperatures (39 % and 24 %, respectively). At 2000 IJs/adult, all four nematode strains killed over 75 % of the CLB adults. Lower doses caused only from 53 % to 65 % mortality. All concentrations of the nematode suspension performed better at 25 ºC (74 %) than at 15 ºC (64 %) and 20 ºC (60 %). At 6 DAT the mortality of the CLB adults was higher (81 %) than for the other two observed days (4 and 2 DAT), where mortality was only 71 % and 48 %, respectively.

In our research we also determined LC_{50} and LC_{90} values for all four studied strains and at all three temperatures for 6 DAT, all of which are summarized in Table 3. The results showed that strain C101 had the lowest LC_{50} (2 DAT at 15 ºC: 561 IJs/adult) and LC_{90} (2 DAT at 15 ºC: 1398 IJs/adult) values at all three temperatures. The commercial product Entonem had the lowest LC_{50} and LC_{90} values at 15 ºC (422 IJs/adult and 884 IJs/adult), while strain D54 reached the lowest values of LC_{50} (4 DAT: 375 IJs/adult) and LC_{90} (4 DAT: 875 IJs/adult) at the higher temperatures (Table 3).

Table 2: Mean adult mortality (± SE) of *Oulema melanopus* adults after being treated with four different doses of four strains of EPNs and kept at 15, 20, and 25 °C. The mortality data, corrected for the control mortality, are shown for two, four, and six days after treatment.

		15 °C				20 °C				25 °C			
		Nematode concentration (IJs/adult)											
EPN strain	DAT	250	500	1000	2000	250	500	1000	2000	250	500	1000	2000
Entonem	2	46.0 ±8.7b	62.0 ±4.9c	42.0 ±3.7b	50.0 ±6.3b	29.0 ±3.2c	17.9 ±14.0b	60.5 ±0.0c	63.2 ±4.9b	3.3 ±3.3a	66.7 ±14.3b	80.6 ±7.1b	88.9 ±5.2b
	4	86.0 ±5.1b	82.0 ±7.4b	88.0 ±3.7c	94.0 ±2.5b	40.5 ±5.4b	29.7 ±11.6a	81.0 ±3.3b	78.4 ±9.2a	2.4 ±2.4a	76.5 ±10.0b	91.2 ±5.9b	100.0 ±0.0b
	6	97.3 ± 2.7bc	91.9 ±3.3b	91.9 ±3.3b	97.3 ±2.7bc	52.8 ±5.6b	52.8 ±11.3a	91.7 ±3.4b	88.9 ±6.8a	12.4 ±4.2a	85.3 ±9.3b	94.1 ±5.9b	100.0 ±0.0b
S. feltiae B30	2	6.0 ±4.0a	38.0 ±8.6b	40.0 ±4.5b	46.0 ±8.1b	4.4 ±2.6b	24.4 ±7.1b	41.5 ±4.6b	68.3 ±10.6b	35.9 ±15.5b	32.4 ±5.9a	35.3 ±5.9a	38.8 ±14.6a
	4	78.7 ±6.7b	85.1 ±2.6b	70.2 ±8.5b	87.2 ±6.2b	17.5 ±5.0a	35.0 ±4.7a	47.5 ±8.3a	85.0 ±10.0a	54.5 ±13.6b	48.5 ±3.7a	57.6 ±8.8a	63.6 ±13.2a
	6	90.5 ±6.9b	92.9 ±2.9b	83.8 ±7.1b	92.8 ±4.8b	30.6 ±6.2a	58.3 ±6.2a	61.1 ±12.0a	97.2 ±2.8ab	37.1 ±18.3b	47.6 ±4.8a	71.4 ±13.9a	71.4 ±13.8a
S. carpocapsae C101	2	34.0 ±4.0b	72.0 ±5.8d	80.0 ±4.5c	86.0 ±4.0c	87.5 ±2.1d	100.0 ±0.0c	100.0 ±0.0d	100.0 ±0.0c	100.0 ±0.0c	100.0 ±0.0c	100.0 ±0.0c	100.0 ±0.0c
	4	98.0 ±2.0c	100.0 ±0.0c	98.0 ±2.0d	100.0 ±0.0c	100.0 ±0.0c	100.0 ±0.0b	100.0 ±0.0c	100.0 ±0.0b	100.0 ±0.0c	100.0 ±0.0d	100.0 ±0.0c	100.0 ±0.0b
	6	100.0 ±0.0c	100.0 ±0.0c	100.0 ±0.0c	100.0 ±0.0c	100.0 ±0.0c	100.0 ±0.0b	100.0 ±0.0c	100.0 ±0.0b	100.0 ±0.0c	100.0 ±0.0c	100.0 ±0.0b	100.0 ±0.0b
H. bacteriophora D54	2	2.0 ±2.0a	0.0 ±0.0a	2.0 ±2.0a	0.0 ±0.0a	0.0 ±0.0a	1.6 ±1.6a	1.6 ±1.6a	3.3 ±2.0a	51.0 ±7.5b	63.3 ±8.9b	69.4 ±10.7b	40.8 ±3.8a
	4	14.0 ±5.1a	26.0 ±7.5a	12.0 ±5.8a	18.0 ±5.8a	34.1 ±6.2b	26.8 ±6.7a	51.2 ±6.7a	78.0 ±8.1a	97.1 ±2.9c	91.4 ±3.5c	94.3 ±3.5b	97.1 ±2.9b
	6	48.8 ±7.0a	60.4 ±2.9a	41.9 ±8.2a	69.8 ±10.8a	27.3 ±11.1a	72.7 ±11.1a	77.3 ±7.2a	95.4 ±4.6ab	100.0 ±0.0c	100.0 ±0.0c	100.0 ±0.0b	100.0 ±0.0a

Table 3: The calculated numbers of the nematodes needed to kill 50 % (LC_{50}) and 90 % (LC_{90}) of *Oulema melanopus* adults, six days after treatment, at three different temperatures.

	Temp. (ºC)	Strain of entomopathogenic nematodes			
		Entonem	B30	C101	D54
LC_{50}[z] (95 % CL[y])	15	422 (0-2668)	866 (0-2011)	561 (254-868)[(2)]	876 (551-1201)
	20	551 (207-896)	711 (497-924)	-[(2)]	671 (386-956)
	25	664 (372-955)	877 (566-1187)	-[(2)]	375 (0-2573)[(4)]
LC_{90}[z] (95 % CL[y])	15	884 (438-1286)	938 (606-1269)	1398 (1062-1733)[(2)]	1347 (689-2004)
	20	1269 (946-1592)	1479 (1200-1758)	499 (70-927)[(2)]	1257 (957-1558)
	25	1141 (868-1414)	1228 (786-1671)	-[(2)]	875 (467-1283)[(4)]

[z] LC_{50} and LC_{90} expressed as the number of IJs per adult.
[y] Confidence limits, CL, are given in parentheses
[(2)] 100% mortality at 2 DAT
[(4)] 100% mortality at 4 DAT

4 DISCUSSION

The results of the present research have demonstrated that the mortality of CLB adults is mostly affected by temperature in connection with the concentration of the nematode suspension, different strains, and DAT. All four studied strains (B30, C101, D54, and Entonem) caused the highest mortality of CLB adults 6 days after treatment (81 %) and the highest concentration of the nematode suspension (78 %). Among the studied strains, *S. carpocapsae* C101 showed the best performance, causing a mortality rate of almost 96 % of CLB adults. On the other hand, the only *Heterorhabditis* nematode in our laboratory bioassay caused an insect mortality of only 49 %. In a comparison between *S. feltiae* nematodes, the commercial product Entonem performed better than the native strain B30 (67 % and 54 %, respectively).

At 15 and 20 ºC, lower mortality was recorded than at 25 ºC, thus supporting the results of our previous research studies (Trdan *et al*., 2006; Trdan *et al*., 2009) and the research of other groups (Belair *et al*., 2003; Yang *et al*., 2003). However both *S. feltiae* strains performed their best at the lowest temperature, which corresponds to some previous research (Williams and MacDonald, 1995; Trdan *et al*., 2009), while, on the other hand, the *H. bacteriophora* strain D54 caused the highest mortality at the highest temperature, which was also found in the results of the research of Trdan *et al*. (2008).

S. carpocapsae C101 performed the best at all three temperatures. Controlling insect pests with foliar application is becoming a more widely-used practice (Broadbent and Olthof, 1995). If this method is required for the control of the first (overwintered) adults of the CLB, the application of *S. feltiae* or *S. carpocapsae* suspensions is recommended, as our research demonstrated that this species showed the highest efficacy in controlling adults at 15 ºC. The first adults in the central and south parts of Europe usually appear in the first half of April, when the nights are still relatively fresh in the area in which our research was carried out (Stamenković, 2004).

The higher concentrations proved to be more efficient in our experiment, however all steinernematid species in the present research demonstrated sufficient efficacy also at lower concentration doses. Based on our current findings, we conclude that the activity of EPNs is influenced more by temperature than by the numbers of nematodes applied, but this tends to be species-specific (Arthurs *et al*., 2004). The minor role of the nematode concentration can be explained by the fact that only a few invasive nematodes need to penetrate an insect host in order to kill it (Bednarek and Nowicki, 1986). Our finding that several species of EPNs demonstrated the same results at lower concentrations as with higher concentrations, gives these biocontrol agents in integrated agriculture better prospects from an economical point of usage, as the cost of plant protection is closely connected to the quantity of the applied EPNs.

However, it is also important to note that results from laboratory tests are not always comparable to field testing (Cantelo and Nickle, 1992) as the functioning of EPNs in the open is influenced by an extensive list of factors. In one relevant study, the 100 % efficacy rate of *S. carpocapsae* in controlling Colorado potato beetle adults, pupae, and larvae in the laboratory manifested as only a 31 % reduction rate in this pest population when the test was repeated outdoors (Stewart *et al*., 1998). Some further results from studies on the activity of EPNs on other species of beetles (Toepfer *et al*., 2005; Trdan *et al*., 2006) have also shown that these agents could be an effective alternative to insecticides. Some

research has also shown that with proper application techniques and right timing as regards the insect developmental stage, we can reach almost the same results as with the use of insecticides (Schroer *et al*., 2005). Our current aim is to continue the present research under field conditions as soon as possible. Now that the use of *S. feltiae*, *S. carpocapsae*, *S. kraussei*, and *H. bacteriophora* is allowed in Slovenia – namely, due to the fact that recently all of them became an indigenous species in our country (Laznik *et al*., 2008; Laznik *et al*., 2009abc) – there are no longer any legal obstacles to carrying out field experiments with these biological control agents.

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Cabbage moth (*Mamestra brassicae* [L.]) and bright-line brown-eyes moth (*Mamestra oleracea* [L.]) – presentation of the species, their monitoring and control measures

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ABSTRACT

The paper describes polyphagous pests, the cabbage moth (*Mamestra brassicae*) and bright-line brown-eyes moth (*Mamestra oleracea*), which were not systematically investigated up to now in Slovenia. The cabbage moth, which is more abundant, preferably attacks *Brassica* plants, and its caterpillars are especially harmful in the cabbage. The paper deals with the morphology, distribution and methods of monitoring the pest populations and discuss on their control. The protection of vegetables from both pests is primarily based on the use of chemical insecticides. However, the use of natural enemies and various agro-technical measures can also be very important in diminishing the populations of the pests. With interlacing of all of these approaches, healthy and quality food can be produced even in the growing seasons with high attack of the pests mentioned.

Key words: cabbage moth, *Mamestra brassicae*, bright-line brown-eyes moth, *Mamestra oleracea,* presentation, distribution, damage, monitoring, control

IZVLEČEK

KAPUSOVA SOVKA (*Mamestra brassicae* [L.]) IN ZELENJADNA SOVKA (*Mamestra oleracea* [L.]) – PREDSTAVITEV VRST IN UKREPOV ZA NJIHOVO SPREMLJANJE IN ZATIRANJE

V prispevku sta predstavljena polifagna škodljivca, kapusova sovka (*Mamestra brassicae*) in zelenjadna sovka (*Mamestra oleracea*), ki v Sloveniji doslej nista bila načrtneje preučevana. Kapusova sovka, ki se pri nas pojavlja bolj številčno, najraje napada kapusnice, njene gosenice pa se najraje hranijo na zelju. V prispevku predstavljamo morfologijo, razširjenost, način spremljanja sezonske dinamike vrst ter njuno zatiranje. Varstvo vrtnin pred kapusovo sovko in zelenjadno sovko še vedno temelji zlasti na uporabi kemičnih insekticidov, čeprav je številčnost populacij omenjenih škodljivcev mogoče zmanjšati tudi z naravnimi sovražniki in ustreznimi agrotehničnimi ukrepi. S prepletanjem omenjenih ukrepov je namreč mogoče tudi ob močnem napadu pridelati zdrav in kakovosten živež.

Ključne besede: kapusova sovka, *Mamestra brassicae*, zelenjadna sovka, *Mamestra oleracea,* predstavitev, razširjenost, škodljivost, spremljanje, zatiranje

1 INTRODUCTION

Cabbage moth (*Mamestra brassicae* [L.]) and bright-line brown-eyes moth (*Mamestra/Lacanobia oleracea* [L.]) are classified into family Noctuidae (owlet moths and underwings) and order Lepidoptera (butterflies, moths, and skippers). Both pests are polyphagous, their larvae feed with aboveground parts of plants in night and morning hours. During the day caterpillars are hiden under the leaves and in the aboveground plant parts near to the soil surface. Damage is visible on leaves and flowers of vegetables and occasionally also

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on fruits of horticultural plants. Cabbage moth is treated as one of the most important *Brassica* pest, while bright-line brown-eyes moth rather attacks tomato and lettuce. From time to time caterpillars from both species cause larger damage also on tobacco plants. Pests prefer specially grounds where weeds are grown or no herbicides are used (Sannino, 2005). In last period when rainy and not too hot summers prevailed, we observe also in Slovenia larger appearance of caterpillars from genus *Mamestra*. With the purpose of studying their bionomics and representative ratio ob both species we placed pheromone traps on two locations in the period from 2008 to 2009. The results of male catches will be used in preparing their control strategy and in present paper we present both pest species and measures for their monitoring and control if eventual outbreak in the near by future appears.

2 CABBAGE MOTH (*Mamestra brassicae* [L.])

2.1 Distribution and damage

Cabbage moth is distributed in Europe and in greater part of Asia (Pollini, 2006). It feeds mainly on *Brassica* plants, leaves of sugar beet, tobacco, sunflower and cereals. Beside these plants it makes damage to spinach, tomato, potato, mangold, lettuce and pepper. Metspalu *et al.* (2004) report that larvae of above mentioned pest most likely feed with leaves of white cabbage (*Brassica oleracea* convar. *capitata* var. *alba*) (Figure 1) and red cabbage (*Brassica oleracea* var. *capitata* var. *rubra*). As regards the susceptibility borecole (*Brassica oleracea* convar. *acephala* var. sabellica) follows the cabbage and the *Brassica* species, which attracts caterpillars less, is oilseed rape (*Brassica napus* L. subsp. *napus*).

Feeding of insects depends on the period between separate meals and the quantity of consumed energy in this time interval (Shimizu in Yagi, 1983). More than one thousand substances are known, which are emitted by the plants into the environment with the aim to attrack other organisms (Ulland, 2007). Volatile components affect directly on organisms in a way that they lure them to oviposit or they have an indirect role as an attractant for natural enemies of the pests.

Sannino and Espinoza (1998) report about the noxiousness of cabbage moth on peach fruits, while Pollini (2006) report about the same on pears. Caterpillars reach the branches which are closer to the ground and cause round bores in fruits (Corvi and Nardi, 1998). In a laboratory experiments, conducted by Sannino in Espinoza (1998), moths fed also on meadow plants such as ribwort plantain (*Plantago lanceolata* L.) and common sowthistle (*Sonchus oleraceus* L.). Caterpillars feed during the night time and are most noxious in autumn when they eat mainly leaves of vegetables.

Beside the mechanical damages, caterpillars lessen the quality of crops also through their excrements on flowers and leaves (Pelosini, 1999). Their indirect influence can be observed through the transmission of pathogenic fungi and bacteria into attacked plants (Corvi in Nardi, 1998).

Figure 1: Damage caused by *Mamestra* caterpillars on the exterior leaves of cabbage plant (left) and cabbage head (right) (photos: S. Trdan)

2.2 Morphology

The forewings are brown and mottled with a prominent white-edged stigma and a broken white subterminal line. The hindwings are grey, darker towards the termen. The species varies considerably in size, with a wingspan of 34-50 mm. The prominent spur on the tibia of the foreleg is a diagnostic feature (Pollini, 2006).

Figure 2: Different caterpillar instars of the cabbage moth (*Mamestra brassicae* [L.]) (photo: S. Trdan)

Eggs are slightly oblong and ridged lengthwise. A red-brown marking is in the middle of egg. An egg has 1.2 mm in diameter. First instar caterpillar is yellow-green and its three pairs of legs on thorax, a pair of appendages (anal prolegs) at the posterior end, and one to four pairs of abdominal prolegs in between are black. The caterpillars of first five instars have copper like head and abdomen is lightly green with white stripe which goes above stigmata. The sixth instar caterpillar is brown on dorsal side and yellow on ventral one and is 40 mm in length. Head stays copper like coloured (Figure 2). Pupa is 20 mm long and red-brown (Pollini, 2006).

2.3 Bionomics

First generation adults appear in Italy from the end of April till the beginning of June. Butterflies fly at night and look for cover between the plants during the day. After the copulation female lays up to 2500 eggs on lower surface of leaves in clusters from 25 to 350 eggs. Incubation period depends from the environmental factors. At 25 °C the incubation period is 5 days and at lower temperatures it lasts from 10 to 12 days. Larvae have up to six stages. First generation of pest develops in 20 to 30 days at temperature from 20 to 25 °C and the second one develops in autumn. It lasts from 40 to 60 days at 12 to 15 °C. Adult caterpillars pupate in the ground, at the depth from 2 to 4 cm. Butterflies come in sight again in July and fly till the first half of October. Species is most abundant between middle of September and middle of October. Butterflies lay eggs from which larvae of the second generation develops and after that overwinter as pupae (Pollini, 2006).

Cabbage moth has up to two or even three generations annually in Middle and South Europe, meanwhile Johansen (1996) from Norway reports only about one generation. It is characteristic for pupae of cabbage moth that they have diapause, which is a consequence of environmental temperature, photoperiod and food quality. Pupae diapause of first generation lasts till 80 days and in winter time for six months (Sannino in Espinosa, 1998).

3 BRIGHT-LINE BROWN-EYES MOTH (*Mamestra/Lacanobia oleracea* [L.])

3.1 Distribution and damage

Bright-line brown-eyes moth is distributed in the area of EuroAsia and North Africa. It's a polyphagous insect and most likely feeds with vegetables such as tomato, lettuce, cabbage, root and petiole celery and mangold. It feeds also on soybean, tobacco, sugar beet and even with trees like willow and elm tree. Pest attacks also fruit trees, mostly apple tree and peach tree (Pollini, 2006).

In the beginning caterpillars cause minor damages on lower surface of leaves and in later stages, when their feeding is more formed they can easily eat greedily the whole leaf mass. Damage made by caterpillars can be observed also on apples and peaches, particularly in extensive plantation where less plant protection products is used (Pollini, 2006).

3.2 Morphology

The forewings are brown and mottled with a prominent white-edged stigma and a broken white subterminal line. When forewings are stretched they amount from 30 to 50 mm. On wings there are two yellow spots and two spots of brown colour. Hind wings are grey. Thorax is red-brown and abdomen is lightly brown. Hind tibia is lacking a hooked terminal spur (INRA, 2008).

Eggs of bright-line brown-eyes moth are bright green, hemispherical and flattened on the substratum. They are 0.7 mm in length (Pollini, 2006). Caterpillars are dark green with a light brown head and dark and yellowish white light stripes along the body. These stripes are less visible when larvae are close to pupation. They measure 35 to 40 mm in length when fully grown (INRA, 2008). They go through five instars to pupate. Young caterpillars are often found in groups feeding near the egg mass. Older caterpillars disperse moving from plant to plant. Caterpillars actively feed for 10 to 18 days, descending into the soil to pupate. They have 16 legs and false legs together (Vacchi in Cioni, 2006). The pupa is yellowish green when formed, turns dark brown later and measures from 16 to 19 mm in length (Pollini, 2006). Pupation takes place in a loose silken cocoon 2 to 6 cm below soil surface. Complete developmental cycle lasts for 30 days (Vacchi in Cioni, 2006).

3.3 Bionomics

In the second half of April butterflies appear and still fly in May and June and stay active during the night time. Females of bright-line brown-eyes moth lay eggs similar as females of cabbage moth on the underside surface of the leaves in clusters of 200 to 800 eggs. Embrional development brings to an end after five to ten days. Young caterpillars leave some days together and then separate. After the end of development caterpillars pupate into the ground, at the depth of 10 cm. Adults of second generation fly from the end of July to August. Often they fly also in September and in the beginning of October. Caterpillars of second generation mature in second half of October and overwinter in diapause (Vacchi in Cioni, 2006).

4 MONITORING MOTHS FROM GENUS *Mamestra*

Moths (Noctuidae) control is based upon the application of chemical insecticides. To gain more reasonable and effective usage, plants should be treated in time when caterpillars are younger and feed only on outer leaves and at least 10 to 15 % of leaf area is damage. Convenient time for treating the attacked plants is evening or morning when caterpillars are more active (Vacchi and Cioni, 2006).

To achieve optimal efficiency of insecticides the caterpillars of cabbage moth must be smaller than 12 mm (Johansen, 1996). Older and larger caterpillars hide between plant leaves and are better protected against insecticides. Suchlike example is iceberg salad which offers due to the rosette compactness a good hideout to bright-line brown-eyes moth (Gengotti, 2008).

Because the development of cabbage moth and bright-line brown-eyes moth larvae depends mostly from the environmental temperature it is very uncertain to predict accurate time of treatment on a predefined area. That is why constant monitoring of butterfly seasonal dynamic is needed. Monitoring of adults can be done in different ways. Among more known and prosperous detection methods is usage of pheromone traps. With this method we can allure males and prevent copulation. The trap also helps to determine the most proper time to apply insecticides. Pop *et al*. (1999) refer that pheromone traps, which are used to control and monitor butterflies, can be improved with a supplement of ethers what makes such traps much cheaper. While pheromone traps help us to monitor the population dynamic of the pest, the expected damage assessment must be done through determination of oviposition and pertinent egg development (Corvi and Nardi, 1998).

Insect light traps with mercury bulbs with wavelength till 400 nm can be used to monitor moths. But their disadvantage is unselectivity that is why such traps are used in abundance research of different harmful, beneficial and indifferent species in the environment (Dodok, 2003). Following the results of the experiment in which many butterflies was determined with pheromone traps in the period of three years, Johansen (1996) developed a mathematical model for predicting of cabbage moth with consideration of daily temperature in Norway.

Butterfly catch of cabbage and bright-line brown-eyes moths is likely low with regard to the extent of the damage, which can be caused by caterpillars. Campagna (2005) quoted that this could be due to the polyphagous characteristic of these pests.

5 CONTROL OF CATERPILLARS FROM GENUS *Mamestra*

5.1 Chemical control

In controlling leaf moths still mostly are used organic phosphorus esters. In this group we classify active compounds such as chlorine pirifos-methil, phenitrotion and acephate (Pelosini, 1999). Sufficient efficacy in this relation we can attain also with pyrethroids (cypermethrin, deltamethrin, lambda-cyhalothrin, beta-cyfluthrin and tefluthrin). In Slovenia registered products for controlling cabbage moth are from a group of pyrethroids, a product on the basis of pyrethrin, a product which corresponds to oxadiazine and one from the group of insect development inhibitors (IRI). Pyrethroids which are registered in Slovenia are Fastac 10 % SC (alfa-cypermethrin) and Karate Zeon 5 CS (lambda-cyhalothrin). Latter is the only registered insecticides for controlling bright-line brown-eyes moth.

Two products are also used when controlling cabbage moth, namely pyrethrin (Spruzit powder) and indoxacarb (Steward). Active ingredient indoxacarb refers to the group of oxadiazines which is also advanced one. Insecticides from the oxadiazines group block Na-channels in nerve fibers. Target insects stop feeding, stay paralyzed and die soon. Product Steward is suitable for integrated production.

Chitinase inhibitors display minor danger for human being and are suitable specially for controling eggs and young larvae (Corvi in Nardi, 1998). Among inhibitors of insect development we assign active ingredients such as teflubenzuron, esaflumuron and lufenuron (Pelosini, 1999). The last one is registered in Slovenia and represents an active ingredient of product Match 050 EC.

If there are caterpillars of various developmental stages on the ground, Corvi and Nardi (1998) recommend the application of pyretroids or carbamates. Both groups of insecticides belong to neurotoxins and act as a contact or stomach insecticides. In case if we want controlling also other pest species on plants, the authors recommend the usage of organic phosphorus esters which acts through the respiratory system.

In case of cabbage moth control on cauliflower (*Brassica oleracea* var. *botrytis*) in autumn, Corvi and Nardi (1998) advise double treatment with synthetic insecticides (pyretroids, carbamates, organic phosphorus esters and growth regulators) and at least spraying with microbiological products on the basis of *Bacillus thuringiensis* var. *kurstaki*.

5.2 Crop protection with natural products

Beside the insecticides with chemical components, in integrated and biological production the products of natural origin are more and more used (Gengotti in Censi, 2004). Along effective natural products recon bacterium *Baccilus thuringiensis* var. *kurstaki*, which replaces in some places considerable amount of chemical products. Azadirachtin, rotenone and natural pyrethrin showed in some experiments good results when controlling cabbage moth and bright-line brown-eyes moth as well.

Azadirachtin is a natural insecticide present in the seeds of tropical plant *Azadirachta indica* A. Juss. Its characteristic is low toxicity to mammals. The product acts systemically while it is absorbed through the roots and leaves. From there it is transported to other parts of the plant. Azadirachtin has a wide spectrum of control, however it does not cause instant death of an insect but alters the life-processing behavior in such a manner that the insect can no longer feed, breed or undergo metamorphosis. Products from azadirachtin have short withholding period and are intended for preventive treatments (Gengotti in Censi, 2004).

Bacillus thuringiensis is an aerobic bacterium which produces toxin. This toxin activates in target organism after the consumption. Caterpillars which eat up treated parts of the plants immediately stops feeding and dies in few days. Bacterial subspecies *kurstaki* and *aizawai* are specially appropriate in controlling larvae from order Lepidoptera, while subspecies *tenebrionis* and *israelensis* have suitable insecticidal control of organisms from orders Coleoptera and Diptera. Benefit

of products with active ingredient *Bacillus thuringiensis* var. *kurstaki* when compared to other products which are also used against cabbage moth and bright-line brown-eyes moth is the fact that product is nontoxic for vertebrates and does not harm beneficial insects. Due to the leaching and photolability spraying with abovementioned insecticidal product is needed to be repeated frequent.

Pyrethrins are compounds which are gained with maceration of flowers from plant *Chrysanthemum cinerariaefolium* Vis. and which are not toxic to mammals. They have a broad control spectrum but the problem causes their non selective control and weak persistence on plants which leads to reappeared presence of pest on plants in a short time (Gengotti in Censi, 2004). To prolong the persistence of products natural or synthetic compounds like for example piperonil butoxide are added (PBO).

Rotenon is obtained from tropical legume *Derris elliptica* (Wallich) Benth. Insecticides which contain aforementioned compound are very toxic for mammals and beneficial insects. Its characteristic is fast control to pest organisms and has longer withholding period as it comes up to ten days (Gengotti in Censi, 2004).

5.3 Biological control

Natural enemies of cabbage moth and bright-line brown-eyes moth are bacteria, birds, lizards and insects. The latter are the most important, particularly members of Diptera and Hymenoptera families. It is well known that natural enemies attack specially individuals of last generation, namely at the end of summer and in autumn (Vacchi, 2006).

Tramblay (1993) acknowledges that cabbage moth could have more than 50 different natural enemies. One of the most efficient is parasitoid *Trichogramma evanescens* Westwood which feeds with moth eggs and can reduce the pest population also up to 80 %. Similar efficiency can be observed also with *Trichogramma dendrolimi* Matsumura which can destroy 60 to 80 % of cabbage moth eggs. Beside the effective egg control this hymenopteran species is appropriate also for its simple host breeding. Takada *et al*. (2000) tried to breed *Trichogramma dendrolimi* exclusively on Mediterranean flour moth (*Ephestia kuehniella* Zeller) as a host species. After the twelve generation of parasitoid which bred on above mentioned host, the females of parasitoid *Trichogramma dendrolimi* still rather choose specimens of *Mamestra brassicae*. They also report that eggs found in cabbage moth cadavers were bigger from those found in Mediterranean flour moth cadavers and that female also laid two times more eggs in cabbage moth (Takada *et al*., 2000).

Beside aforementioned organisms, also some important parasitoids of moth caterpillars exist, such as *Meteorus gyrator* (Thunberg), *Exorista larvarum* (L.), *Exorista fasciata* (Fallén), *Nemoraea pellucida* (Meigen) and *Compsilura concinnata* (Meigen). Very important parasitoids *Amblyteles armatorius* (Förster) and *Pimpla instigator* F. are from family Ichneumonidae; their larvae feed with caterpillars of cabbage moth and bright-line brown-eyes moth (Sannino in Espinosa, 1998).

Sannino (1998) references on effective biological control of *Protapanteles praecipuus* (Papp) from family Braconidae in a laboratory experiment. This parasitoid lays more than ten eggs into the caterpillar which afterwards develop into the larvae. Moth caterpillar dies in few days and parasitoid larvae pupate outside the prey's body.

Meteorus gyrator appears in the area of North Europe, Great Britain, Asia and North Africa (Smethurst *et al*., 2004). This endoparasitoid has a wide spectrum of hosts, among which the most frequent from order Lepidoptera are owlet moths (Noctuidae), geometrid moths (Geometridae) and Lymantriidae (tussock moths). Only one egg is laid by the wasp in victim's body. Despite the general opinion that *Meteorus gyrator* is a superior adapted parasitoid (superparasitoid), Smethurst *et al*. (2004) ascertained that sometime in victim's body from which larva already came out, later laid egg or larva of wasp can still be found. Introduced phenomenon at this host indicates to incapability of separating between parasited and unparasited hosts. Among moths the wasp parasites mostly caterpillars of bright-line brown-eyes moth. A slow growth of wasp infected organisms compared to normal one is characteristic trait and after the end of parasitizing the host is exploited. Differences exist also in time of parasitoid development, which depends from the development of host organism. It was discovered that this wasp has the longest development in caterpillars of cabbage moth (Smethurst in sod., 2004).

Exorista larvarum appears infrequently and oviposites eggs on the surface of victim's body. Caterpillars which contain eggs are identified after the dark spot, which lies in the place where endoparasite entered the victim's body (Sannino in Espinosa, 1998).

5.4 Interseeding and intercropping

With the aim to restrain the use of chemical products for plant protection and to lessen the number of pest organisms, the application of intercrops and mixed crops of two or more plants is used on farm holdings. Diverse ecosystem enable the presence of higher

number of natural enemies which helps to control pest organisms.

Theunissen *et al.* (1995) reported about the findings in which white clover (*Trifolium repens* L.) as an intercrop can reduce the number of different pests on cole crops. Pests which are mentioned are cabbage aphid (*Brevicoryne brassicae* L.), flea beetles (*Phyllotreta* spp.), onion thrips (*Thrips tabbaci* Lindeman) and cabbage moth.

According to Wiech and Kalmuka (2004) white clover acts as the best intercrop against moth larvae. Larvae which move between the white clover plants during the search for the food are exposed to natural enemies such as beetles from the family Carabidae.

6 CONCLUSIONS

Representative members of owlet moths (Noctuidae), specially cabbage moth and bright-line brown-eyes moth, can cause serious troubles to cole crops and vegetable growers. To restrict their attacks beside the use of chemical insecticides also natural products which are environmentally friendly in larger extent are used. As important measure to take into consideration in vegetable production beside new insecticides is also soil cultivation. Among latter worth to mention are deep autumn cultivation with the aim to destroy overwintered pupae, use of interseeding, intercropping and cover crops.

Next to above mentioned measures, more significance is given to natural enemies of pest organisms. They harm or totally destroy eggs and moth caterpillars. Their application is recommended particularly for growing vegetables in greenhouses (hydroponics growing) where their efficacy is expected to be much higher. Some natural enemies of moths as parasitoid *Trichogramma evanescens*, represent remarkable potential in plant protection in the future and at the same time enable lower environmental burden. This is why more attention should be given in searching species from genus *Trichogramma* on the territory of Slovenia as momentarily we do not have any information on their abundance. If their domestic status will be confirmed, they could be introduced into food production systems.

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Evaluation of buckwheat sprouts as microgreens

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ABSTRACT

Microgreens from common and tartary buckwheat genotypes were evaluated for total flavonoid content (rutin, quercetine and kaempferol separately), bound phenolic acids content, carotenoids and α-tocopherol content and antioxidant activity. The results have shown that in common and tartary buckwheat microgreens antioxidant activity was found. High level of flavonoids, carotenoids, and α-tocopherol was detected as well. Higher amount of flavonoids was found out in tartary buckwheat microgreens. No significant differences were detected between common and tartary buckwheat microgreens in content of phenolic acids. Microgreens of both common and tartary buckwheat represent potential nutritional sources for alternative vegetable in the Czech Republic.

Key words: buckwheat, microgreens, flavonoids, rutin, phenolic acids, carotenoids, antioxidant activity

IZVLEČEK

PREHRANSKE LASTNOSTI MLADIH RASTLIN AJDE

Vsebnost celokupnih flavonoidov (posebej rutina, kvercetina in kempferola), vezanih fenolnih kislin, karotenoidov, α-tokoferola in antioksidantna aktivnost so bili raziskani pri mladih rastlinah navadne in tatarske ajde. Ugotovljena je antioksidativna aktivnost izvlečkov mladih rastlin navadne in tatarske ajde ter visoka vsebnost flavonoidov, karotenoidov in α-tokoferola. Posebej visoka vsebnost flavonoidov je bila ugotovljena pri tatarski ajdi, medtem ko glede na vsebnost fenolnih snovi ni bilo razlike med mladimi rastlinami navadne in tatarske ajde. Mlade rastline tako navadne kot tatarske ajde so možen alternativni vir zelenjave v Češki republiki

Ključne besede: ajda, mlade rastline, flavonoidi, fenolne kisline, karotenoidi, antioksidativna aktivnost

1 INTRODUCTION

Antioxidants help organisms to deal with oxidative stress, caused by free radical damage. Free radicals are chemical elements, which contains one or more unpaired electrons due to which they are highly unstable and cause damage to other molecules by extracting electrons from them in order to attain stability (Ali *et al.,* 2008). Some of these molecules can be physiologically useful, but they can also cause damage under certain circumstances. The most notorious among these damages being neurodegenerative conditions like Alzheimer's and Parkinson's disease. Other neurodegenerative diseases significantly associated with oxidative stress include multiple sclerosis, Creutzfeldt–Jacob disease, and meningoencephalitis (Darley-Usmar and Halliwell, 1996; Ali *et al.,* 2008). Reparative processes of organism are not sufficient enough to eliminate all damages in organism caused by free radicals. One of the possibilities how protect organism against free radicals is supplement of antioxidants. The main sources of antioxidants in the human nutrition are fruits and vegetables (Traka and Mithen, 2009).

Microgreens are very specific type of vegetable. They are very similar to sprouts but grow several days longer making them larger leaved, and greener, they are the crop being grown hydroponically and organically. Microgreens are considered to be in the group of what are newly referred to as "functional foods" which are food products that possess particular health promoting or disease preventing properties that are additional to their normal nutritional values. Demand for these products is growing rapidly. Microgreens have been found to contain higher levels of concentrated active compounds than found in mature plants or seeds.

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Microgreens are filled with vitamins, minerals, and antioxidants (MicroGreensUSA, 2009; Brentlinger, 2007). Common microgreens are grown mainly from cabbage, beet, kale, kohlrabi, mizuna, mustard, radish, Swiss chard, and amaranth.

Common buckwheat is traditional crop in the Czech Republic territory. It is very important for low-input agricultural system where it is one of most often growing crops (Michalová, 2003). Tartary buckwheat is not traditional in the Czech Republic, but in the last few years, there has been a demand for this crop, mainly as a medicinal plant, because of its high content of rutin and other polyphenols and suitability for the production of nutraceutical products and functional foods (Michalová, 2000).

The aim of this study was to evaluate common and tartary buckwheat as microgreens and to compare flavonoids, carotenoids, and selected phenolic acids content.

2 MATERIALS AND METHODS

Plant materials and growth conditions

The common and tartary buckwheat genotypes were obtained from the buckwheat collection of the Czech Gene Bank, CRI and from Institute of Biotechnology, Shanxi University, Taiyuan, China. The passport data about the samples origin are listed in Table 1.

Table 1 Passport data about the buckwheat samples

No.	ECN*	Name of variety	Origin
	common buckwheat (Fagopyrum esculentum)		
1	01Z5000072	Sudtirol Nr. 3	unknown
2	01Z5000123	Kara-Dag	Ukraine
3	01Z5000127	Jana	Ukraine
	tartary buckwheat (Fagopyrum tataricum)		
4	01Z5100001	unnamed	Bhutan
5	01Z5100010	Lifago	Germany
6		Jianzui	China
7		Liu	China
8		Jiujing	China

*National accession number

Common and tartary buckwheat seeds were soaked with distilled water for 24 h and shaken frequently. After that time, seeds were rinsed by running distilled water. Then they were put into holes of germination equipment and grown hydroponically. Microgreens were grown at daylight for 10 days until the first true leaves appeared. Plants were washed every day by distilled water. After cultivation, all samples were frozen in -25°C and then lyophilized and milled for later extraction.

Determination of DPPH activity

Free radical scavenging capacity was evaluated according to the previously reported procedure using the stable DPPH radical according to Şensoy et al. (2006). This method measures radical scavenging capacity and results are expressed as gallic acid equivalents per g of dry matter (mg GAE.g^{-1} dm). Antioxidant activities were determined by reacting 1 mL of methanolic extract of grains with 100 µL 200 µM DPPH. Absorbance of the samples at 515 nm was measured after 4 min reaction at room temperature in dark.

Determination of total flavonoids, bound phenolic acids and carotenoids

Total flavonoids were analysed spectrophotometrically after 1 hour extraction in mixture methanol: H_2O: acetic acid (100:100:2) using non-specific reaction with $AlCl_3$ and expressed as equivalents of rutin according to Van Hung et al. (2008). Three flavonoids – rutin, quercetine, and kaempferol were analyzed by HPLC method with UV detection according to Kreft et al. (2006) slightly modified. Comparing of retention time in sample and analytical standard was used for compound identification. Flavonoids were quantified using external calibration.

Bound phenolic acids were determined using HPLC with UV detection after the alkaline hydrolyzation according to Kim et al. (2006). Selected cinnamic acid derivatives (caffeic, p-coumaric, and ferulic acid) and benzoic acid derivatives (vanillic and syringic) were monitored at 320 and 280 nm, respectively. Carotenoids were extracted with water-saturated butanol according to Hidalgo et al. (2006) and chromatographic separation was performed on C18 stationary phase with mixture of methanol, acetonitrile, and dichloromethane as mobile phase and spectrophotometric detection using in house method. Identification and quantification of all compounds was performed using external analytical standards.

3 RESULTS AND DISCUSSION

DPPH activity

The DPPH activity (Fig. 1) depended on species and genotypes. Microgreens of both species possessed higher antioxidant activity than seeds. Microgreens of tartary buckwheat showed higher antioxidant activity than those of common buckwheat, contrary to Kim *et al.* (2008); they published similar results of antioxidant activity in common and tartary buckwheat sprouts grown for 10 days. Only common buckwheat genotype ´Sudtirol 3´ had higher activity than genotype ´01Z5100001´of tartary buckwheat. The tartary buckwheat varieties 'Jianzui' and ´Liu´ originated in China showed the highest antioxidant activity.

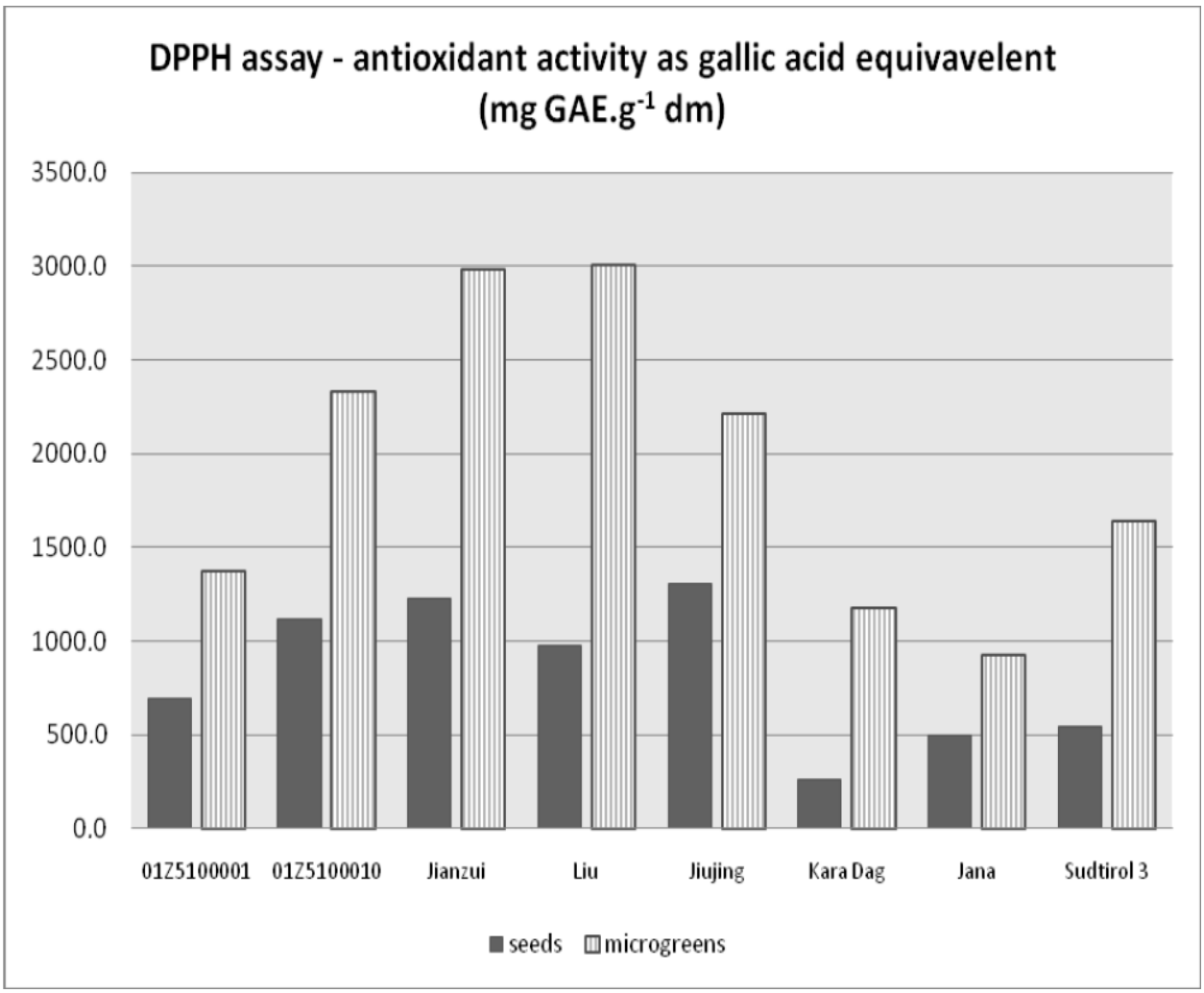

Fig. 1. DPPH assay of common and tartary buckwheat microgreens

Total flavonoids, phenolic acids and carotenoids

The quantities of phenolics such as flavonoids (including rutin, kaemferol, and quercetine), and phenolic acids (such as cinnamic acid derivatives caffeic, p-coumaric and ferulic acid and benzoic acid derivatives vanillic and syringic) and α-tocopherol in microgreens from common and tartary buckwheat are shown in Fig. 2 and Fig. 3 respectively. The higher content of flavonoids was in tartary buckwheat microgreens. It corresponds with results of many authors; they published comparison of flavonoids content in seeds of tartary and common buckwheat (Fabjan *et al.,* 2003; Kim *et al.*, 2008). The main flavonoid in common and tartary buckwheat is rutin as published Liu *et al.* (2008). . Its higher content was detected in tartary buckwheat microgreens, which corresponded with Kim *et al.* (2008) who determined higher rutin content in tartary buckwheat sprouts.

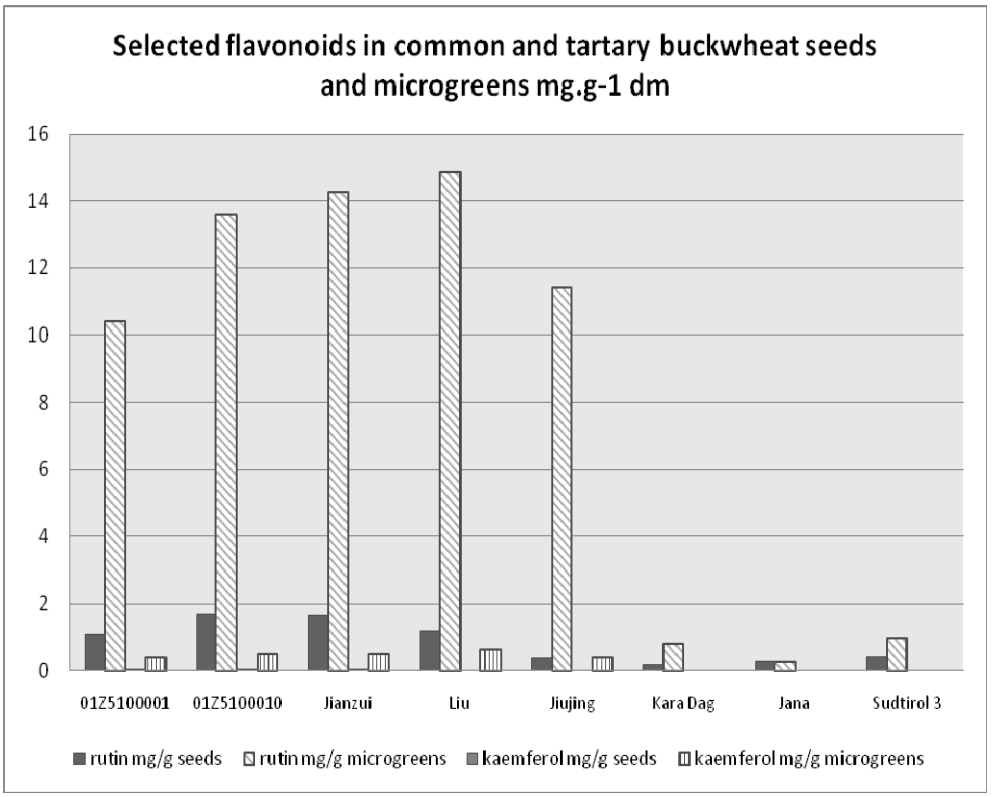

Fig. 2. Flavonoids in common and tartary buckwheat seeds and microgreens

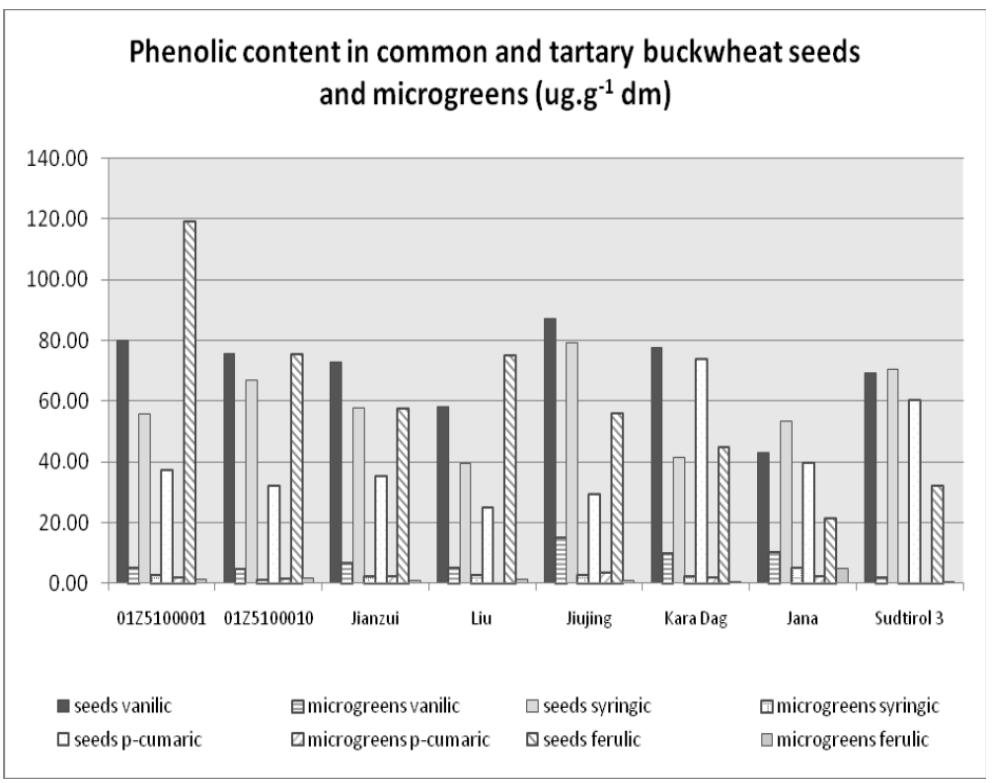

Fig. 3. Selected phenolic acids in common and tartary buckwheat seeds and microgreens

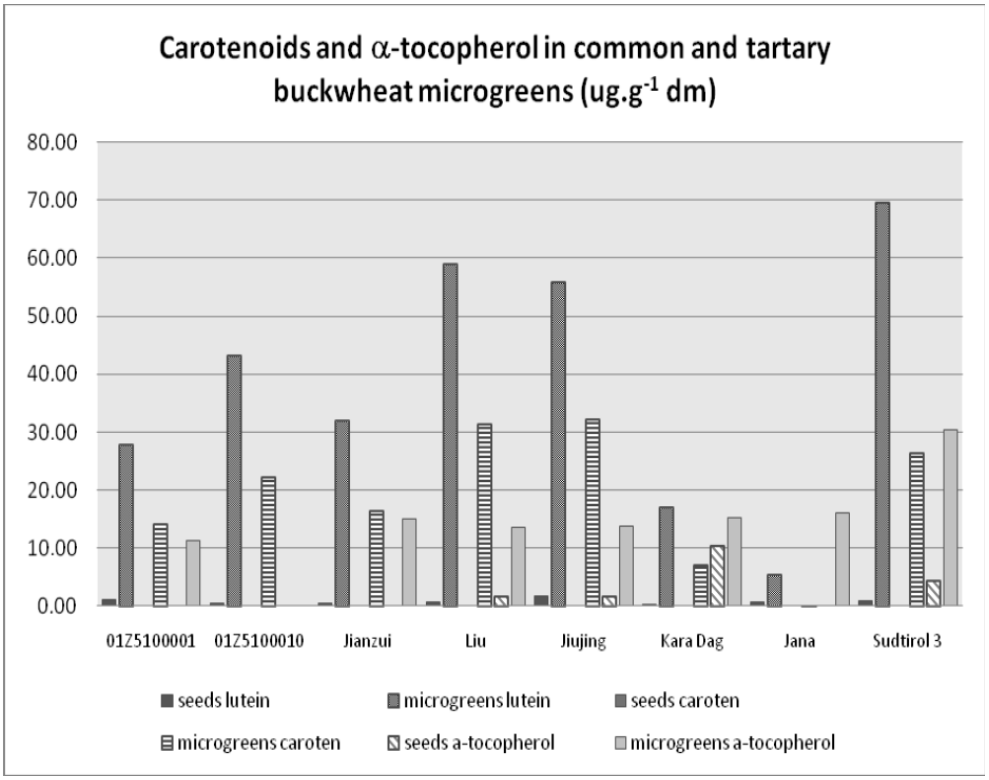

Fig.4. Selected carotenoids and α-tocopherol in common and tartary buckwheat seeds and microgreens

No significant differences were detected in content of phenolic acids between common and tartary genotypes. On the other hand, the differences between seeds and microgreens in phenolic acids content were statistically significant. Generally, the amount of phenolic acids was higher in seeds than in microgreens of both species. Similar results published Alvarez-Jubete *et al.* (2010) in common buckwheat seeds and sprouts. In case of vanillic acid in microgreens, the highest amount was detected in tartary buckwheat genotype ´Jiujing´ and the second highest level in common buckwheat variety ´Jana´. The caffeic acid was determined only in common buckwheat genotype ´Sudtirol 3´. Contrary to phenolic acids content, there was higher content of carotenoids and α-tocopherol in microgreens than in seeds of both species. Caroten was under quantification level in seeds, which was 0.3 $ug.g^{-1}$ dm. . In microgreens of ´Jiujing´ genotype, the caroten level was more than 100times higher than in seeds Very similar results were obtained in the case of lutein and α-tocopherol content in all genotypes. The highest content of lutein and α-tocopherol was determined in microgreens of genotype ´Sudtirol 3´.

4 CONCLUSION

Our results have shown that antioxidant activity was found both in common and tartary buckwheat microgreens. High levels of flavonoids, carotenoids, and α-tocopherol were detected as well. Higher amount of flavonoids was detected in tartary buckwheat microgreens. No significant differences were detected between common and tartary buckwheat microgreens in content of phenolic acids. Microgreens of both common and tartary buckwheat represent potential nutritional sources of alternative vegetable in the Czech Republic.

5 ACKNOWLEDGEMENTS

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Agris category code: F60,Q04

Impacts of gibberellin (GA_3) on sensorial quality and storability of table grape (*Vitis vinifera* L.)

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ABSTRACT

The eventual impacts of the gibberellin (GA_3) application on grapevine (*Vitis vinifera* L.) varieties 'Cardinal' and 'Michele Palieri', on grape quality and their storage potential were studied. The grape quality was determined as individual and total carbohydrates and organic acids, but also the external skin colouration was measured. The measurement of polyphenol oxidases (PPO) activity was contributed to clearly understand storage potential. During the grape maturation the statistical differences in grape quality were observed according to the treatments. The treatments with GA_3 showed similar and related impacts on grape quality at both varieties only at harvest, where the statistically highest total sugar concentration (223-226 g kg^{-1}) was determined at 50 ppm, followed by 20 ppm (216-223 g kg^{-1}) and the lowest 201-220 g kg^{-1} at control. The organic acid concentrations in the grape of both varieties did not show any similar correlation according to treatments. The PPO activities were slightly higher at mature grape of 'Cardinal' (2.5-3.0 $\Delta A\ min^{-1}g^{-1}$) compared to 'Michele Palieri' (1.0-1.2 $\Delta A\ min^{-1}g^{-1}$), and the activities at both varieties during storage decreased. At harvest the *CIRG* indices of 'Cardinal' (5.5-6.1) and of 'Michele Palieri' (6.6-6.9) did not statistically differ among treatments as at the end of storage, where the highest indices were calculated at treatment 50 ppm at both varieties. The grape quality of table grape responded differently to GA_3 applications, especially the different impacts were observed according to varieties.

Key words: carbohydrate, organic acid, gibberellin, PPO, quality

IZVLEČEK

VPLIV GIBERELINOV (GA_3) NA SENZORIČNO KAKOVOST IN SKLADIŠČENJE NAMIZNEGA GROZDJA (*Vitis vinifera* L.)

V poskusu smo vrednotili morebitne vplive uporabe giberelinov (GA_3) pri pridelavi žlahtnih vinskih trt (*Vitis vinifera* L.) na kakovost namiznega grozdja sort 'Cardinal' in 'Michele Palieri' in njun potencial za skladiščenje. Kakovost grozdja smo opisali z vsebnostjo posameznih in skupnih ogljikovih hidratov in organskih kislin, kot tudi z barvo kožice jagod. Potencial skladiščenja smo določili z merjenjem aktivnosti polifenol oksidaz (PPO). Med zorenjem grozdja so se v kakovosti grozdja med obravnavanji pokazale statistično značilne razlike. Tretiranja z GA_3 imajo enak vpliv na kakovost grozdja obeh sort, vendar samo ob trgatvi, ko so se statistično značilne največje vsebnosti skupnih sladkorjev (223-226 g kg^{-1}) pokazale pri 50 ppm, sledita še 20 ppm (216-223 g kg^{-1}) in najmanjša vsebnost pri kontroli (201-220 g kg^{-1}). Vsebnosti organskih kislin se niso odzvale na uporabo GA_3. Aktivnost PPO je bila nekoliko večja pri sorti 'Cardinal' (2.5-3.0 $\Delta A\ min^{-1}g^{-1}$), kot pa pri sorti 'Michele Palieri' (1.0-1.2 $\Delta A\ min^{-1}g^{-1}$), vendar se je le-ta pri obeh sortah med skladiščenjem precej zmanjšala. Povprečni *CIRG* indeks kožice jagod je bil pri sorti 'Cardinal' 5,5-6,1, medtem ko pri sorti 'Michele Palieri' 6,6-6,9 in statistično neznačilen med obravnavanji, čeprav se je povprečno največji indeks pri obeh sortah pokazal pri 50 ppm. Kakovost grozdja se pri različnih sortah različno odziva na uporabo giberelinov GA_3.

Ključne besede: ogljikovi hidrati, organske kisline, giberelini, PPO, kakovost

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1 INTRODUCTION

Table grapes (*Vitis vinifera* L.) are an important crop traditionally produced in the Mediterranean and in the last decades also all over the World, where total table grape production has increased, especially in Asia (China and India (O.I.V., 2007).

Grape quality is determined by primary metabolites as carbohydrate and organic acid concentrations, by secondary metabolites (phenols and aromatic compounds) and their ratios in grape berries, but also by morpho-physical parameters (colour, size) (Amerine *et al.* 1965; Shiraishi 1993, 1995); however, grapes are also an important source of minerals, vitamins and amino acids, and are therefore used not only for fresh consumption, but also in the food, pharmaceutical and cosmetic industries (Winkler *et al.,* 1974; Adams, 2006; Kennedy *et al.,* 2006).

Glucose, fructose and sucrose are the predominant sugars in grapes, which concentration depends of variety, cultivation practice, ecological condition and vintage (Winkler *et al*., 1974). The 90% concentration of total organic acids is consisted by tartaric and malic acids, which have important effects on the characteristics of grape quality, such as colour and microbiological stabilization and mouth-feel. High acidity also has a negative influence on the palatability of table grape.

The colouration of grape has been associated with the presence of phenol compounds, especially with anthocyannins in berry skins (Kennedy *et al.,* 2006; Rusjan *et al.*, 2008). In fruit, colouration is the basic quality parameter (visual appreciation) and according to the numerical evaluation the *CIRG* index of external skin colour and the CIE L*a*b* system can be evaluated. The *CIRG* index ((180-*h*)/(L*+C*)) has been proposed, which is based on the parameters L* (lightness), *h* (hue angle) and C* (chroma). Using this index, skin colour can be classified into five groups (Carreño *et al.,* 1997). Table grape is regularly stored in freezer chambers to prolong its quality, where different chemical and physical processes were observed (Winkler *et al.*, 1974). Polyphenol oxidases (PPO) are known to catalyse the oxidative reactions, what involve phenols, amino acids, quinons and other oxidizable substances (Wissemann and Lee, 1980; Hooper *et al.*, 1985). The enzymatic oxidation (browning) in grape is caused primarily by polyphenol oxidases (EC 1.10.3.1.; cateholasa) which decrease grape quality (Carvajal-Millán *et al.*, 2001).

Use of gibberellic acids (GA_3) is quite common in table grape production, where the impacts on grape quality are mentioned. Gibberellins influence berry size and weight (Perez, 1994), colour of berry surface (Bianchi *et al.*, 1991) and a yield per vine (Winklet *et al.*, 1974).

Postharvest grape deterioration effects morpho-physical and chemical factors, especially berry dehydration because of vapour pressure deficit (Nelson, 1985), skin browning (Vial *et al.*, 2005), changes in carbohydrates and phenols concentrations (Zoffoli *et al.*, 2009). Skin browning and consequent changes in skin colour are frequently caused by enzymes such are polyphenol oxidases (Ryan *et al*., 1982).

The GA_3 impacts on table grape quality were involved in many studies, but not at 'Cardinal' and 'Michele Palieri' varieties. The main focus of this study was to present the potential of table grape quality according to two varieties below of different GA_3 treatments during grape maturation and after in storage chamber.

2 MATERIALS AND METHODS

2.1 Plant materials

The study was carried out on two red table grape varieties (*Vitis vinifera* L.) from the Ampelographic vineyard owned by the University of Ljubljana, Biotechnical Faculty, situated in a sub-Mediterranean winegrowing region in Slovenia. The grapes from 10 plants of each grape variety 'Cardinal' ('Flame Tokay' x 'Ribier') and 'Michele Palieri' ('Ribier' x 'Red Malaga') were sampled according to the treatment in the year 2008.

2.2 Treatments

The 3 x 3 block experiment was done where ten vines in three rows were treated at full blossom with three different aqueous gibberellins concentrations of GA_3: 0 ppm (control; no gibberellins application), 20 ppm (20 mg GA_3 kg^{-1}) and 50 ppm (50 mg GA_3 kg^{-1}).

The vines were grown under the same agricultural practices and geographical and climatic conditions, while the guidelines of integrated pest management (IPM) in viticulture were considered.

2.3 Sampling

Bunches per each grapevine variety were randomly harvested at different ripe stages ('Cardinal' on 8th, 14th, 28th Aug., and 'Michele Palieri' on 8th, 14th, 21st, 28th Aug., 5th Sept.), where 100 berries per each variety and sampling were included additionally in the analysis. After picking, the grape samples were frozen and stored as rapidly as possible in PE bags in the

dark at –20 °C for further analysis. After harvest the main part of the grape was put separately per variety into boxes and into the storage chamber under controlled storage conditions (4 °C and 96% r. h.). During the storage bunches per variety were sampled ('Cardinal' on 1st, 8th 15th and 22nd Sept.; and 'Michele Palieri' on 11th, 18th, 22nd, 29th Sept., 5th Oct.) and stored in freezer until analysis.

2.5 Chemicals

The following standards prepared in aqueous solutions were used for the determination and quantification of quality parameters; fructose and tartaric acid from Fluka Chemie GmbH [Buchs, Switzerland], glucose, malic acid and H_2SO_4 from Sigma Chemical Co. [St. Louis, MO]. The water was additionally purified using Milli-Q water purification system [Millipore; Bedford, MA].

2.6 Extraction and analysis of carbohydrates and organic acids

The separate carbohydrates and organic acids were extracted according to Šturm *et al.* (1999) and analysed using a Thermo Separation Product with the HPLC system and a UV detector set at 210 nm for organic acids and with a differential RI detector for detection of carbohydrates. The grapes were pressed by hand, and 1 mL of grape juice per sample was diluted with MilliQ purified water (grape juice : water = 1 : 10 (*v/v*)). The mixtures were centrifuged for 7 min at 4200 rpm at room temperature (Eppendorf 5810 R, Hamburg, Germany). The supernatant was used and filtered through a 0.45 µm syringe filter (Chromafil A-25/25, Macherey-Nagel) and stored at –20 °C prior to injection. A Phenomenex (Rezex RCM-Monosaccharid Ca^+; 300 x 7.80 mm) column for carbohydrates and a Phenomenex (Rezex ROA-Organic acid H^+; 300 x 7.8 mm) column for acids were used and operated at 65 °C. The mobile phase for organic acids was an aqueous solution of H_2SO_4 (4 mM) and MilliQ purified water for carbohydrates. The volume of injection was 20 µL and the flow rate was 0.6 mL min^{-1}.

2.7 Identification and quantification of carbohydrates and organic acids

The carbohydrates (glucose and fructose) and organic acids (malic and tartaric) were quantified using standard solutions with known concentrations additionally combined with retention times as well as by the addition of external standard solutions in samples. The carbohydrate and organic acid concentrations were expressed as g per kg of fresh weight (g kg^{-1} FW).

2.8 Fruit Colour Determination

As soon as possible the colouration of grape skin was determined on fresh samples according to variety and treatment. On the 20 fresh berries, five colour measures per berry (around berry) and per variety were done. Surface colour of grape berries was recorded with a Minolta CR-300 Chroma portable colourimeter (Minolta Co, Osaka, Japan), where the colour was expressed in L*, C* and *h* and by calculating the *CIRG* index defined as *CIRG* = (180-*h*)/(L*+C*), where L* is lightness and represents the dark-light axis (0% = black; 100% = white), h is the hue angle, and $C^*=(a^{*2}+b^{*2})^{0.5}$ is the chroma and represents colour intensity. Before use the colourimeter was calibrated with a standard white calibration plate (Carreño *et al.* 1997).

2.9 Preparation of crude PPO extraction

The polyphenol oxidases were extracted from grape pomace samples according to the methods described by Donko (2001) with some modification. The grape was grinded in liquid nitrogen and 200 µL of of pomace was placed in 2.5 ml test tube (eppendorf) and homogenized for 15 min in 1 mL of ice-cold 0.1 M phosphate buffer (pH 7.3) containing 0.1 M Tris X-100, 1 mM PMFS, 3 g PVPP/100 mL and 1.5 g Tris X-100/100 mL. The samples were centrifuged for 15 min at 4 °C and 20,000 x *g*. Supernatant I (800 µL) was added to 600 µL Triton X-100 solution (8 g/100 mL) and heated for 10 min at 35 °C; sediment was rejected. The mixture was centrifuged again for 15 min at 20,000 x *g* and 4 °C. The obtained supernatant II was used as enzyme source.

2.10 Polyphenoloxidase assay

The assay procedure of PPO activities of cateholase was measured according to Sanchez *et al.*, (1988) and Valero *et al.*, (1989) with some modifications. The cateholase activity was measured by using 4-MC (methyl catechol) solution (64 mg/250 mL 0.1 M phosphate buffer; pH 7.3). Enzyme extract (100 µL) was added to 2.9 mL of 0.1 M phosphate buffer (pH 7.3) mixed and an aliquot was transferred immediately to a 1.0 cm path-length cuvette. Absorbance at 420 nm was measured using spectrophotometer UV/VIS Lambda Bio 20, against a same solution, but without enzyme extraction. The change of absorbance into 10 min at 420 nm was recorded. The activity was expressed as change in absorbance per minute per gram ($\Delta A\ min^{-1}\ g^{-1}$).

2.11 Statistical Analyses

Data are presented as means with standard errors (milligrams or grams per kilogram of fresh material). The one-way analysis of variance (ANOVA) to test the significance of the observed differences was performed using the Statgraphic plus 4.0 software. The differences in quantified concentrations were evaluated using LSD test at $P < 0.05$ and were considered to be statistically significant.

3 RESULTS AND DISCUSSION

3.1 Concentrations of carbohydrates

Carbohydrate concentrations in grapes according to variety, date of sampling and treatment are given in Table 1. The differences in carbohydrate concentrations were expected, especially between varieties, where 'Michele Palieri' is known as later mature variety (VCR, 2007). The statistical differences in carbohydrates concentrations among treatments were observed at both varieties. At the first grape samples the statistically highest fructose and glucose concentrations were determined at treatment with 50 ppm, followed by 20 ppm and the lowest at control. More frequent

differences in concentration were observed at fructose compared to glucose, at both varieties.

At harvest of 'Cardinal' the statistically highest fructose concentrations (116 g kg^{-1}) were determined in grape treated with 50 ppm and control, the lowest (111 g kg^{-1}) at treatment 20 ppm. 'Michele Palieri' was harvested one week later and the statistically highest fructose (110-113 g kg^{-1}) concentrations were determined at treatments with GA_3. Similar results were observed in glucose concentrations (113 g kg^{-1}). Comparing the total sugar contents at harvest, we can conclude that the GA_3 influenced the sugar contents, where treatment with 50 ppm gave the statistically highest concentration (223-226 g kg^{-1}), followed by treatment with 20 ppm (216 – 223 g kg^{-1}) and control (201-220 g kg^{-1}) at least, what has been already mentioned by Winkler *et al.* (1974) for other varieties. The average sugar concentration at harvest of both varieties can be compared to the concentrations already mentioned by Huai-Feng *et al.* (2006), but Rusjan *et al.* (2008) noticed quite lower concentrations in previous study.

During the storage in chamber the sugar concentrations increased, because of water losses from grape berries, what was expected according to Zoffoli *et al.* (2009) and to Carvajal-Millán *et al.* (2001). After a month of grape storage the statistically highest fructose (144 g kg^{-1}) and glucose (132 g kg^{-1}) were determined in 'Cardinal' grape treated with 20 ppm GA_3, while the lowest concentrations at control samples. The average concentrations at 20 ppm increased for 27 g kg^{-1} after a month of storage. At variety 'Michele Palieri' some differences in carbohydrates compared to 'Cardinal' were observed, where the highest concentrations were determined at control grape samples and the lowest at 20 ppm GA_3 treatment. The highest increase in sugar concentrations were at control samples, approximately around 31 g kg^{-1}. At the end of grape storage the similar effects of GA_3 applications on total sugar concentration in grape of both varieties were not confirmed (Table 1).

3.2 Concentrations of organic acids

The most important organic acids in table grape are tartaric and malic acids. The concentrations of mentioned acids in grape were screened during grape maturation and storage according to treatments and the results are presented in figures 1 and 2. At the first sampling the highest malic concentrations 8.8 g kg^{-1} at 20 ppm treatment of 'Cardinal' and 9.1 g kg^{-1} at 50 ppm treatment of 'Michele Palieri' were determined. The tartaric concentrations were quite lower, around 3.6 g kg^{-1} at 'Cardinal', and 4.2 g kg^{-1} at 'Michele Palieri'. As expected the organic acid concentrations decreased during grape maturation, especially malic acid (Zoffoli *et al.* 2009). The statistical differences in malic acid concentrations were observed at all samplings between control and treated grape with hormone. The statistically lowest malic concentration at harvest was observed at control grape of 'Cardinal', while the statistically highest at 50 ppm treatment of grape 'Michele Palieri'. The differences in tartaric acid concentrations among treatments at the same sampling were not observed. During the storage the organic acid concentrations drastically decreased after the first week and later stabilised to the end of storage. At the end of the storage the malic acid concentration around 2.0 g kg^{-1} at 'Cardinal' and around 1.0 g kg^{-1} were determined. The average concentration of tartaric acid was similar between varieties and ranged from 1.2 to 2.8 g kg^{-1} (figures 1, 2).

According to the results we can conclude that treatments with GA_3 hormone influence the malic acid concentration in grape during maturation, but differently according to the varieties. The influence of GA_3 applications on tartaric acid concentration in grape was minimal according to treatments and to varieties.

3.3 Evaluation of external skin colour

The average values of the *CIRG* index according to grape varieties and treatments have been studied and the results are presented in figure 3. At the first sampling the statistical differences in berry surface colour were shown only at variety 'Michele Palieri', where treatment with 20 ppm had the most colouration (5.0). At the harvesting at both varieties statistical differences in colouration were determined. The most coloured grape of 'Cardinal' was that treated with 20 ppm GA_3, while at 'Michele Palieri' the control and with 20 ppm GA_3 treated grape. Therefore we can conclude the treatments with GA_3 have a different influence on grape colouration during maturation among varieties. According to Carreño *et al.* (1996) the variety 'Cardinal' was classified as a red variety, while 'Michele Palieri' as a dark-blue coloured variety.

In the end of storage only differences in grape colouration were observed only at 'Cardinal' grape. The highest *CIRG* index 7.3 was calculated at grape treated with 50 ppm GA_3, whereas the lowest at 20 ppm. At 'Michele Palieri' the differences at the end of storage were not shown therefore we can conclude that treatments with GA_3 do not show the same influences on grape colouration at different varieties. Rusjan *et al.* (2008) cited the average *CIRG* index for non treated variety 'Michele Palieri' around 6.05, but quite lower 5.84 for 'Cardinal', what could be explained by vintage (clime conditions etc.) and cultivation practices.

Table 1: Carbohydrate concentrations (g kg^{-1}) in grape pomace of table grape varieties 'Cardinal' and 'Michele Palieri' according to treatment and sampling in year 2007. The means and standard errors are presented. The different letters indicate statistically significant difference at $P < 0.05$ (LSD test).

			Sampling								
Variety	Sugar	Treatment	8thAug	14thAug	21stAug	28thAug	1stSep	8thSep	15thSep	22ndSep	
'Cardinal'	Fructose	0 ppm	90 ± 3 c	97 ± 3 b	111 ± 4	114 ± 1 a	115 ± 1 b	116 ± 1 c	119 ± 1 c	123 ± 5 b	
		20 ppm	96 ± 3 b	106 ± 7 a	107 ± 4	111 ± 1 b	123 ± 5 a	135 ± 5 a	140 ± 6 a	144 ± 6 a	
		50 ppm	106 ± 1 a	112 ± 4 a	114 ± 6	116 ± 1 a	118 ± 5 ab	120 ± 1 b	129 ± 5 b	138 ± 2 a	
	Glucose	0 ppm	93 ± 1 b	97 ± 5	97 ± 6	106 ± 3	106 ± 3 b	107 ± 2 b	112 ± 1 b	119 ± 5 b	
		20 ppm	97 ± 4 b	99 ± 1	102 ± 1	112 ± 5	120 ± 2 a	120 ± 2 a	126 ± 2 a	132 ± 3 a	
		50 ppm	105 ± 2 a	104 ± 2	107 ± 6	110 ± 1	112 ± 4 b	119 ± 1 a	124 ± 4 a	134 ± 2 a	
			8thAug	14thAug	21stAug	28thAug	5thSep	11thSep	18thSep	22ndSep	29thSep	5thOct
'Michele Palieri'	Fructose	0 ppm	66 ± 1 c	75 ± 2 b	98 ± 4 b	98 ± 4 b	99 ± 3 b	108 ± 6	111 ± 1 b	128 ± 7	121 ± 6 b	142 ± 4 a
		20 ppm	76 ± 0 b	90 ± 3 a	109 ± 1 a	109 ± 5 a	113 ± 6 a	114 ± 4	118 ± 4 a	125 ± 4	124 ± 3 b	129 ± 2 b
		50 ppm	79 ± 1 a	89 ± 3 a	99 ± 2 b	107 ± 6 ab	110 ± 2 a	111 ± 6	114 ± 1 ab	125 ± 2	134 ± 5 a	141 ± 6 a
	Glucose	0 ppm	74 ± 1 c	78 ± 1 b	83 ± 5 b	92 ± 4	102 ± 5 b	102 ± 4 b	105 ± 1 b	109 ± 7 ab	114 ± 7 b	131 ± 5 a
		20 ppm	79 ± 1 b	91 ± 2 a	92 ± 1 a	102 ± 6	103 ± 2 b	109 ± 7 ab	109 ± 4 b	111 ± 1 b	112 ± 2 b	118 ± 6 b
		50 ppm	85 ± 1 a	87 ± 5 a	91 ± 1 a	101 ± 1	113 ± 3 a	116 ± 2 a	121 ± 5 a	123 ± 5 a	128 ± 4 a	130 ± 8 a

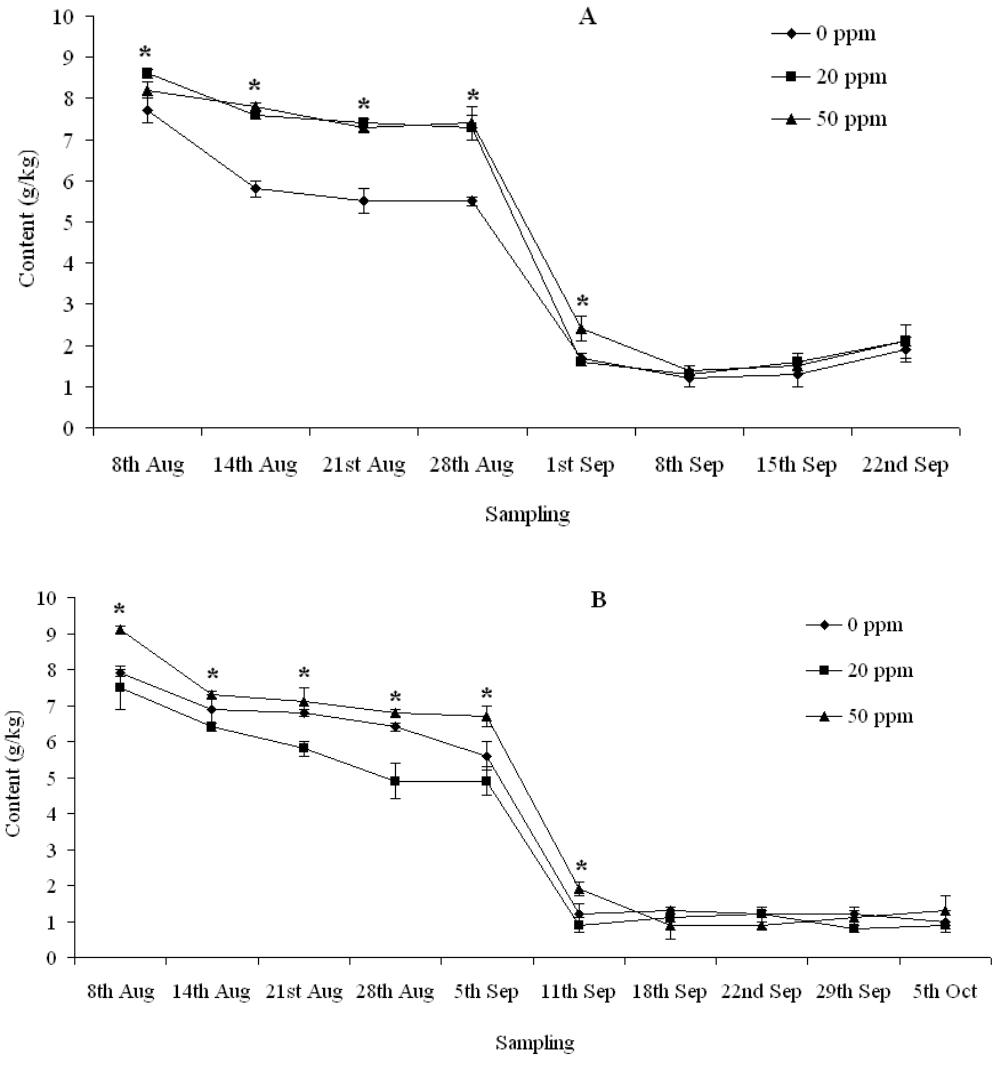

Figure 1: The concentrations (g kg^{-1}) of malic acid in grape pomace of table grape varieties 'Cardinal' (A) and 'Michele Palieri' (B) according to sampling and treatment. The means and standard errors are presented. The sign '*' indicates statistically significant difference at $P < 0.05$ (LSD test).

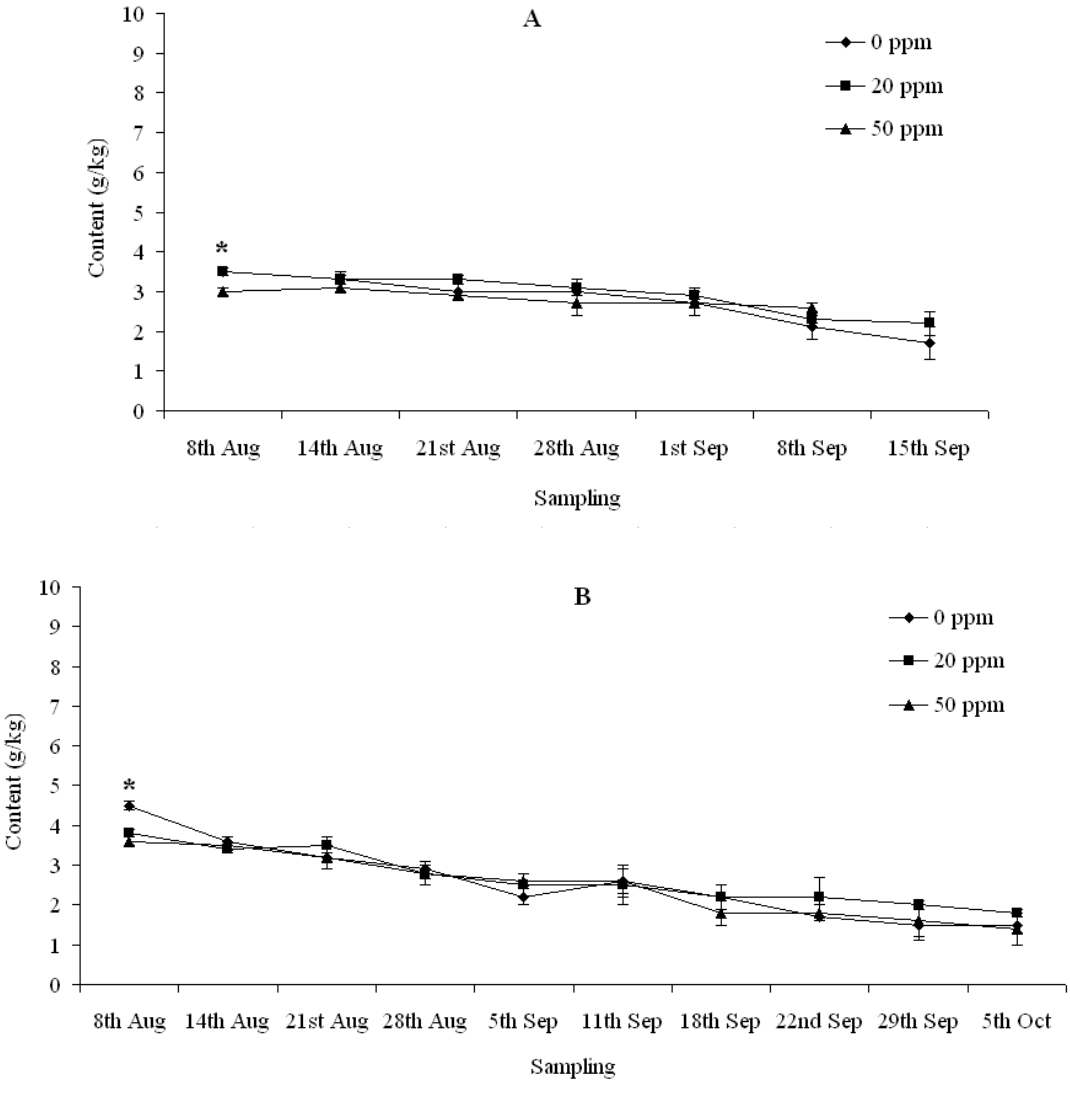

Figure 2: The concentrations (g kg^{-1}) of tartaric acid in grape pomace of table grape varieties 'Cardinal' (A) and 'Michele Palieri' (B) according to sampling and treatment. The means and standard errors are presented. The sign '*' indicates statistically significant difference at $P < 0.05$ (LSD test).

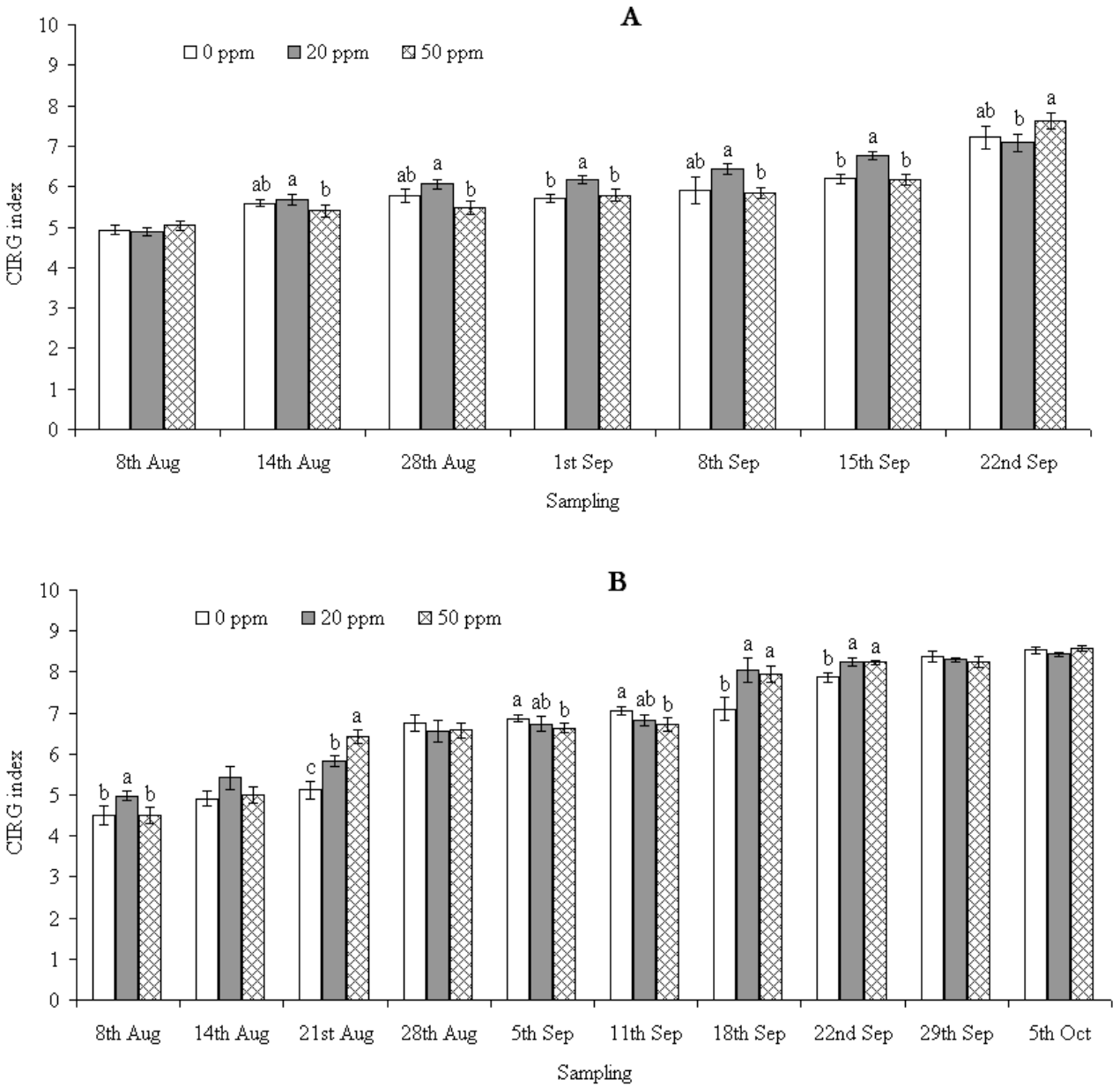

Figure 3: Average *CIRG* indices of external skin colour of table grape varieties 'Cardinal' (A) and 'Michele Palieri' (B) according to sampling and treatment. The means and standard errors are presented. The different letters indicate statistically significant difference at $P < 0.05$ (LSD test).

3.4 PPO activity

PPO activity according to treatments and variety was given in figure 4. The PPO activities were screened during grape maturation and storage to check theeventual effects of GA_3 on their activity. At the first grape sampling some similarities according to treatments and varieties were observed. Statistically highest PPO activity 1.2-2.3 $\Delta A\ min^{-1}\ g^{-1}$ was determined in grape treated with 50 ppm GA_3, followed by treatment with 20 ppm and the lowest activity 0.6-1.0 $\Delta A\ min^{-1}\ g^{-1}$ at the control. Generally during the grape maturation or subsequent samplings according to variety the similarities in PPO activities were not observed. The statistically highest activity of PPO at harvest in grape 'Cardinal' was determined at control, followed by 50 ppm and the lowest at treatment 20 ppm GA_3. But at harvest of 'Michele Palieri' grape the statistical differences in PPO activities followed from highest at 50 ppm, 20 ppm, to the lowest at control.

During the storage the average PPO activities slightly increased at both varieties, what was expected according

to storage conditions. The highest PPO activity was determined at 'Cardinal' grape compared to 'Michele Palieri'. The statistically highest activity of 'Cardinal' was determined at treatment with 50 ppm followed by control grape. At 'Michele Palieri' just the opposite PPO activities were shown, meaning the statistically lowest at treatment 50 ppm GA_3.

According to the results of the experiment we can conclude that direct influences of GA_3 on PPO activity were not determined, however we also have to stress the differences between the varieties.

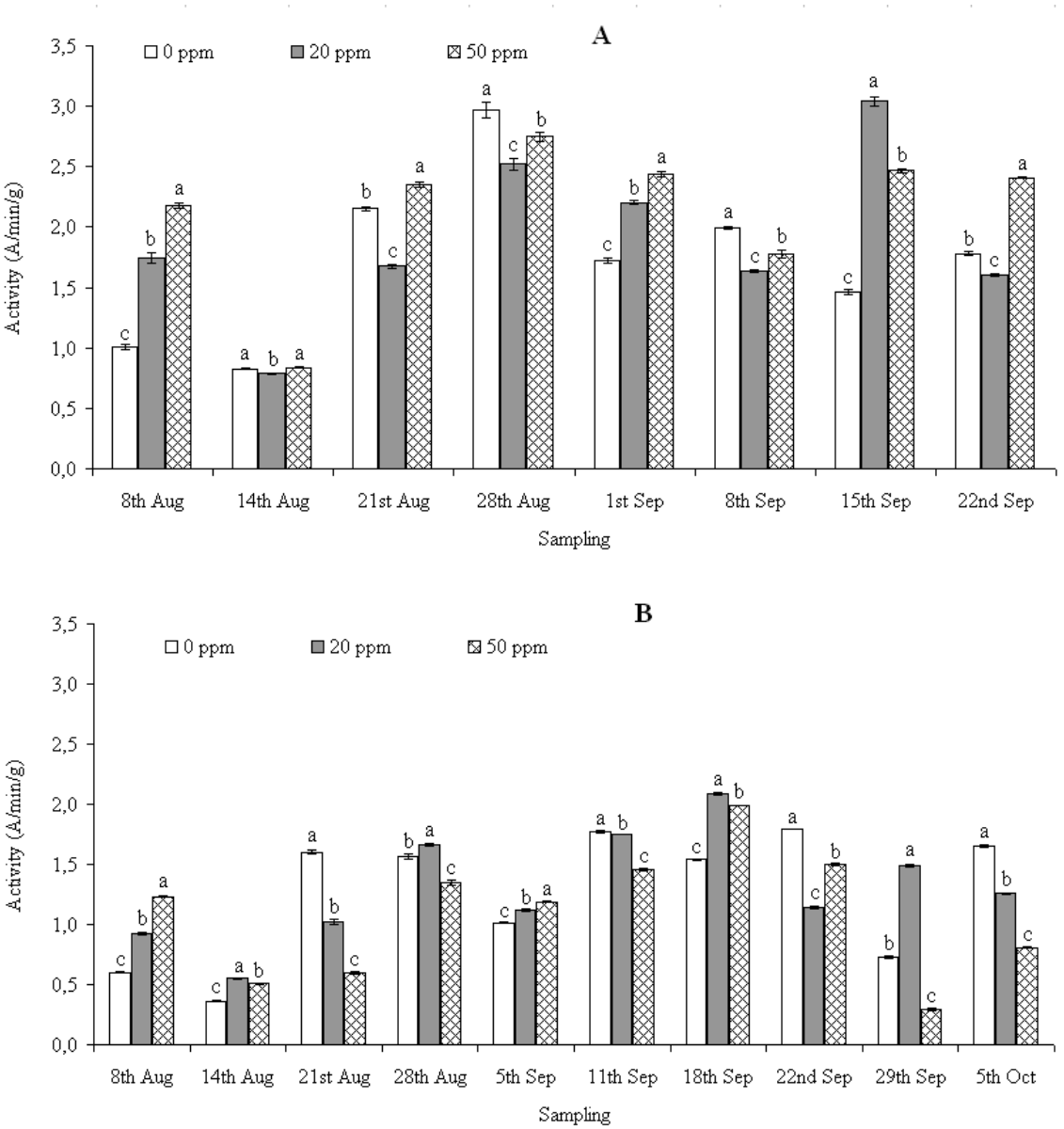

Figure 4: Average POP activity (ΔA/min/g) in grape pomace of table grape varieties 'Cardinal' (A) and 'Michele Palieri' (B) according to sampling and treatment. The means and standard errors are presented. The different letters indicate statistically significant difference at $P < 0.05$ (LSD test).

4 CONCLUSIONS

The application of GA_3 on grape is a quite frequent practice at table grape production, especially to increase the berry size of seedless varieties, but also their quality. The usage of GA_3 affects grape quality among table

grape varieties differently, what was also confirmed in our study. The higher concentrations of GA_3 applied on grape increased its sugar contents, compared to control, what was observed at both varieties. The separate organic acids did not show any response to GA_3 application, not during grape maturation, even less during storage. As organic acid also PPO activities were not linked to the use of GA_3, therefore we can conclude the grape browning was not influenced by GA_3, especially during storage. The grape colouration evaluated as CIRG index showed statistical differences among treatments at both varieties, but only at the end of storage. The most coloured grapes were those treated with 50 ppm of GA_3.

The results of the study confirmed the potential impacts of GA_3 application on grape quality. However, the use of GA_3 has to be adjusted according to each table grape variety, because they show different responses during grape maturation and its storage.

5 ACKNOWLEDGEMENTS

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Agris category code: F01, N20, H01

Potato yield and tuber quality in 75 cm and 90 cm wide ridges

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ABSTRACT

In 2002, 2003, and 2004, a field trial was carried out in three locations in Slovenia, i.e., Pšata, Brnik, and Brežice with two different inter-row widths (IRWs) of 75 cm and 90 cm using three different potato cultivars (i.e., Agria, Bright, and Carlingford). The aim of this trial was to determine the influence of inter-row width (75 cm or 90 cm) with three potato cultivars on the market yield, the yield of green tubers, the cross-sectional area of the ridge, and the percentage of tubers with a hollow heart or blackspot. The trial was designed in the form of split plots with five repetitions. In the years with abundant rainfall during the period of growth (2002 and 2004), when plants completely covered the ground, the market yield at the 90 cm IRW was higher than at the 75 cm IRW. In 2003, however, the market yield was higher at the 75 cm IRW. The yield of green tubers was lower at the 90 cm IRW than at the 75 cm IRW, due to a larger cross-sectional area of the ridge (> 1000 cm^2). Thus, the soil covering of tubers in the ridge was better. The hollow heart and blackspot disorders affecting the tubers depend more on the susceptibility of a particular cultivar and the meteorological conditions during the period of growth than the IRW. The findings showed that the Agria cultivar was more susceptible to the hollow heart disorder, particularly in the years with a higher yield with thicker tubers (2002 and 2004). The Bright cultivar was affected by blackspot in 2003, the year with a lack of rainfall.

Keywords: potato, ridge, cultivator, ridger, inter-row width, cultivar

IZVLEČEK

PRIDELEK TER KAKOVOST GOMOLJEV PRI 75 IN 90 CM ŠIROKIH GREBENIH

V letih 2002, 2003 in 2004 smo na Pšati, Brniku in v Brežicah izvajali poljski poskus z dvema medvrstnima razdaljama (MVR) 75 in 90 cm ter s tremi sortami krompirja Agria, Bright in Carlingford. Namen poskusa je bil ugotoviti vpliv MVR 75 in 90 cm pri treh sortah krompirja na tržni pridelek gomoljev, na pridelek zelenih gomoljev, na površino prečnega preseka grebena in na odstotek gomoljev z votlim srcem in rjavo pegavostjo. Poskus je bil zasnovan v obliki deljenih blokov s petimi ponovitvami. V letih z dovolj padavinami v rastnem obdobju (leto 2002 in 2004), ko so rastline popolnoma prekrile tla, je bil tržni pridelek pri MVR 90 cm višji kot pri MVR 75 cm. Ravno nasprotno je bilo v letu 2003, ko je bil tržni pridelek višji pri MVR 75 cm. Pridelek zelenih gomoljev je bil pri MVR 90 cm nižji kot pri MVR 75 cm zaradi večje površine prečnega preseka grebena, ki je znašala več kot 1000 cm^2. To je pomenilo boljšo pokritost gomoljev z zemljo v grebenu. Votlo srce in rjava pegavost na gomoljih sta bolj odvisni od občutljivosti sorte in vremenskih razmer v rastnem obdobju kot od MVR. Izkazalo se je, da je sorta Agria bolj občutljiva na votlo srce predvsem v letih z visokimi pridelki in debelimi gomolji (leto 2002 in 2004). Pri sorti Bright se je rjava pegavost pojavila v letu 2003, ko je bilo pomanjkanje padavin.

Ključne besede: krompir, greben, okopalnik, osipalnik, medvrstna razdalja, sorta

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1 INTRODUCTION

Constant amelioration of potato cultivars in terms of fertility enhancement constrains potato producers from increasing the ridge volume. One option is to apply a wider inter-row width (IRW). The latter is, however, largely dependent on tractor's wheel spacing, as well as the machinery used in potato production. In the Netherlands, the predominant IRW is 75 cm. However, some potato producers have, in recent years, started to cultivate at the 90 cm IRW (Spiess and Heusser, 1995). A wider IRW allows more soil to be used in order to shape sufficiently high ridges, while the lateral sides of the ridge suffer less pressure from tractor's wheels. At harvest time, the higher soil volume additionally protects tubers from being damaged. Furthermore, less time is spent for the cultivation and harvesting of a particular area (Bouman, 1998; van der Zaag, 1992).

The better the conditions for potato growth, the higher the inter-row width may be (Professional ..., 2005; Seed ..., 2001). Field trials performed in the Netherlands since the 1970s show that market yields are higher at the 90 cm IRW than at the 75 cm IRW (Kouwehoven and Perdok, 2000). Moreover, the secondary growth of tubers is not as strong; there is a smaller yield of non-market tubers (< 50 mm) and a 20% higher yield of market tubers (> 50 mm). Beukema and van der Zaag (1990) state that, if an undisturbed growth of haulm is ensured, the yield at the 90 cm IRW will not be decreased. If the growth of haulm is, however, disturbed, the soil will not be completely covered by leaves, which will cause a greater evaporation of water from the soil and consequently result in a lower yield.

The yield of green tubers not suitable for sale and further processing also influences the tuber quality. Kouwenhoven *et al.* (2003) determined that the yield of green tubers was smaller at the 90 cm IRW than at the 75 cm IRW. This was due to a larger cross-sectional area of the ridge, and a better soil covering of tubers.

When subjected to stress, potato tubers can develop physiological disorders, such as a change of shape, their usefulness, or appearance (Dolničar *et al.*, 2004). These disorders diminish tuber quality. The hollow heart disorder occurs on thick tubers of only certain potato cultivars. It causes the centre of a tuber to become hollow, while the colour of the medulla surrounding it remains unchanged. This disorder is influenced by a quick growth of tubers during the rainfall following a dry spell, by low soil temperatures during the formation of tubers, and the increased soil moisture, which is connected with an intensity of tuber growth (Dolničar, 1997; Dolničar *et al.*, 2004; Kus, 1994; Bugarčić, 2000). During very hot summers, thick tubers of some potato cultivars (especially those planted on sandy soil) develop small brown-coloured spots. These spots are in fact groups of damaged and dead cells in tubers' medulla. The disorder is called blackspot and can be increased by high temperatures associated with drought stress (Dolničar, 1997; Dolničar *et al.*, 2004; Kus, 1994).

The aim of the trial was to determine the influence of IRW (75 cm or 90 cm) and potato cultivar on the market yield, the yield of green tubers, the cross-sectional area of the ridge, and the percentage of tubers with the hollow heart or blackspot disorders. In Slovenia, potatoes are still produced at IRWs that do not exceed 70 cm. The 75 cm IRW is currently in use by bigger, more specialized potato producers who use modern tractors with the standard 150 cm wheel spacing and more state-of-the-art potato-production machinery. The 90 cm IRW, however, is not in use at all. Furthermore, not much research has been performed on the influence of IRW on the occurrence of the hollow heart or blackspot disorders.

2 MATERIALS AND METHODS

The trial included two IRWs (75 cm and 90 cm) and three potato cultivars (i.e., Agria, Bright, and Carlingford). It was carried out in three different locations (Brnik, Pšata in the vicinity of Ljubljana, and Brežice) in three consecutive years (2002, 2003, 2004). It was designed in the form of split plots with five repetitions. Each plot included two randomly positioned IRWs (75 cm and 90 cm) representing the two main plots. Within each IRW, the three potato cultivars were randomly positioned in order to form subplots. The main plots measured 15m in length, while subplots were 5m long. On each main plot, four rows of potato were planted. All measurements were carried out in the inner two rows.

One of the factors determining the choice of these three cultivars was the fact that all of them are able to produce high yields in favourable meteorological conditions. The occurrence of green tubers is, however, possible, especially at the smaller IRW. The Agria cultivar produces a smaller quantity of very thick tubers. The Bright cultivar produces a medium quantity of thick tubers, while Carlingford grows a large quantity of medium-thick tubers. Moreover, all three cultivars may be subject to physiological disorders if exposed to unfavourable meteorological conditions. The Agria cultivar is sensitive to the hollow heart disorder. The Bright cultivar is sensitive to the blackspot disorder. When subjected to stress, the Carlingford cultivar produces tubers that are not

sufficiently big. Meteorological conditions varied throughout the trial years. In 2003, the average air temperature in the period from April to September was 3°C higher than the long-term average in the years 1961-1990. In that year, the rainfall total in the period between April and September was substantially lower. In 2002 and 2004, variations from the long-term average were much smaller (Table 1).

Table 1: The average air temperature (°C) and the rainfall total (mm) in the period between April and September in 2002, 2003, and 2004 compared to the long-term average in the years 1961-1990 in Brnik, Bežigrad, and Bizeljsko (°C) (Mekinda - Majaron, 1995; Meteorološki letopisi, 2005)

Year	Average temperature (°C)			Rainfall total (mm)		
	Bežigrad	Brnik	Bizeljsko	Bežigrad	Brnik	Bizeljsko
2002	17.5	15.6	17.4	799	746	612
2003	19.0	17.1	19.0	536	430	261
2004	16.8	14.8	16.6	861	866	629
1961-1990	16.1	14.7	16.0	784	768	607

The planting density was 45,000 tubers/ha. There was a 29.6 cm inter-tuber width in the row with a 75 cm IRW, and a 24.7 cm inter-tuber width with the 90 cm IRW. A modified 4-row IRW-forming machine was used to create planting furrows. Those were then prepared to be manually planted with all three potato cultivars. The planting depth was equal in both IRWs, and it allowed seed tuber tops to be levelled with the soil. The 75 cm and 90 cm ridges were shaped with a PTO-driven potato cultivator/ridger (University of Ljubljana, Biotechnical Faculty, Department of Agronomy, Chair of Agricultural Engineering) (Figure 1). This machine is, in fact, a rotary tiller used for the cultivation of the inter-row space. Attached to its shaft are four rotors with blades. In order to set the IRW, rotors must be shifted along the shaft and placed into provided holes (Godeša, 2002). In the rear, a ridger forming 75 cm and 90 cm trapezoid-shaped ridges is attached (Figure 2). The rotation speed of the PTO shaft was 540 rpm, while the operating speed amounted to 1.5-3 km/h. At both IRWs, cultivation was carried out at a depth of 15 cm. Cultivation and ridging was performed a few days before the potato emergence. All further work concerning agricultural technology was carried out in accordance with good agricultural practice.

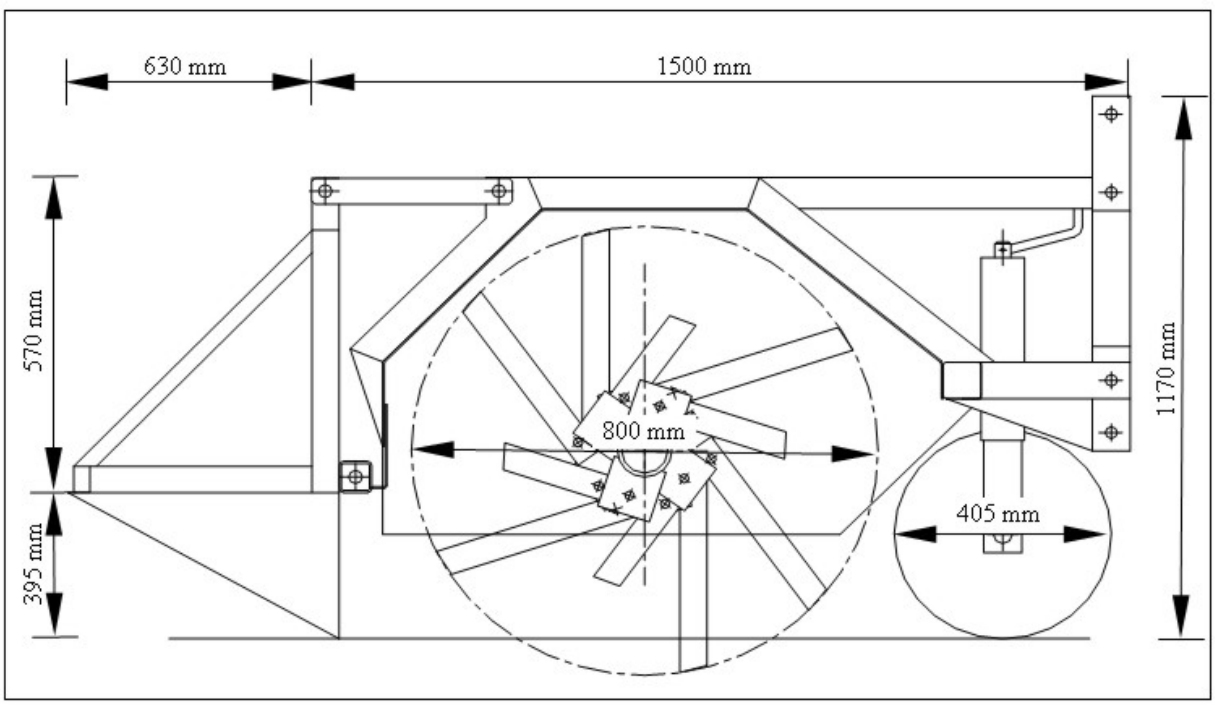

Figure 1: PTO-driven cultivator/ridger used for ridge shaping

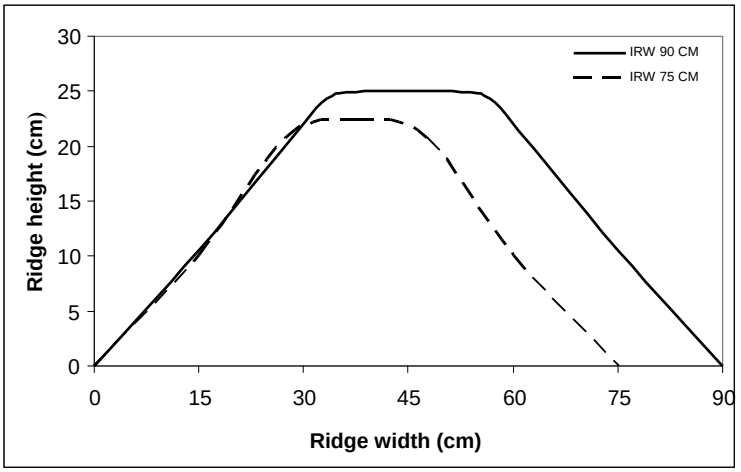

Figure 2: Ridge shape at the 75 cm and the 90 cm IRWs

On each subplot, the yield was harvested from the inner two rows in order to eliminate the impact of the marginal area. Tuber samples were then analysed at the Agricultural Institute of Slovenia. Each sample was placed into a selection device made of a frame, onto which nets were vertically placed. The screen meshes had diameters of 65, 55, 45, 35, and 25 mm. Only tubers smaller than 25 mm remained at the bottom of the device. The remaining tubers left on each net were then counted and weighed. Based on this data, the market-tuber yield (> 35 mm) was calculated. Furthermore, green tubers thicker than 35 mm were counted and weighed, and the yield of green tubers was calculated.

Physiological disorders occurring on tubers were determined by taking 10 tubers thicker than 45 mm from each sample and slicing them in half. Then, the number of tubers with hollow heart or blackspot disorder was determined. This data allowed us to calculate the percentage of tubers with the hollow heart and blackspot disorders.

Before the harvest, coordinates of the ridge were measured with a three-dimensional coordinate measuring device (University of Ljubljana, Biotechnical Faculty, Department of Agronomy, Chair of Agricultural Engineering). Based on these, the cross-sectional area of the ridge was calculated with the LabView program.

The basic data processing was carried out in Microsoft Excel. A statistical analysis was made using the Statgraph 4.0 program, while an analysis of variance was carried out pursuant to the procedure valid for the split-plot trial. Furthermore, a Duncan Multiple Range Test (α=0.05) was performed. When the interaction was statistically significant for the outcome, standard errors of the mean value difference were calculated.

3 RESULTS AND DISCUSSION

In all trial years and at all three locations, statistically significant differences occurred in the market yield of tubers according to the IRW (Table 2). In 2002 and 2004, market yields at the 90 cm IRW were higher than at the 75 cm IRW, amounting to 3.2-8.1 t/ha. In both years, the rainfall exceeded its long-term average (1961-1990) in all three locations. Plants were thus provided with an optimal water supply throughout the entire period of growth (April 1 – September 30). In 2003, market yields at the 75 cm IRW were higher than at the 90 cm IRW in all three locations. The differences between market yields oscillated from 3.2 t/ha to 5.8 t/ha. In that year, the rainfall was 248 mm, 338 mm, and 346 mm lower (in Pšata, Brnik, and Brežice, respectively) during the period of growth lasting from April 1 to September 30 when compared with the long-term average between 1961-1990 (Table 1). Rows in the plot with 90 cm IRW did not join, and so we assume that larger evaporation, lower water content, and higher temperature occurred in ridges. These results match the findings of Spiess *et al.* (2005), determining that market yields at the 90 cm IRW are larger than at the 75 cm IRW only when the soil has been optimally supplied with water. Beukema and van der Zaag (1990) offer similar findings. The results are only partially compatible with those gained by Kouwenhoven and Perdok (2000), who determined higher yields at the 90 cm IRW.

Table 2: Influence of IRW on the market yield of tubers (> 35 mm) in all three locations in 2002-2004 (t/ha) (Duncan's Test α=0.05)

Year	IRW (cm)	PŠATA	BRNIK	BREŽICE
2002	75	45.2[a†]	-*	53.1[a]
	90	53.3[b]	-	56.4[b]
2003	75	42.6[a]	23.2[a]	25.1[a]
	90	36.8[b]	20.0[b]	21.5[b]
2004	75	49.9[a]	44.8[a]	50.2[a]
	90	53.1[b]	51.7[b]	54.1[b]

† The same letters within a column in the same year and the same location are not statistically different.
* Tuber samples taken from the Brnik location in 2002 were mixed up and could not be analysed.

Statistically significant differences occurred according to the cultivar as well. They were, however, not as pronounced as with IRW (Table 3). In 2002, market yield of the Carlingford cultivar in Pšata was higher than with the other two cultivars. This was expected, as this cultivar is known to produce high yields during the years with an abundant rainfall. In 2003 the Bright cultivar had a higher market yield than other the two cultivars in the Pšata and Brežice locations. This year was extremely dry (the driest in 200 years), though this fact does not allow us to make any actual conclusions. In 2004, the market yield by the Agria cultivar in Brnik was significantly lower than with the other two cultivars. Furthermore, the market yield with the same cultivar was significantly higher than with the Bright cultivar in Brežice.

Table 3: Influence of the cultivar on the market yield of tubers (> 35 mm) in all three locations in 2002-2004 (t/ha) (Duncan's Test α=0.05)

Year	Cultivar	PŠATA	BRNIK	BREŽICE
2002	Agria	46.6[a†]	-*	52.8[a]
	Bright	40.0[a]	-	47.1[b]
	Carlingford	52.0[b]	-	55.1[a]
2003	Agria	38.2[a]	22.8[a]	24.2[a]
	Bright	42.8[b]	21.9[a]	28.3[b]
	Carlingford	36.1[a]	19.2[a]	21.9[a]
2004	Agria	49.7[a]	41.9[a]	52.9[a]
	Bright	53.7[a]	50.2[b]	47.2[b]
	Carlingford	50.7[a]	51.3[b]	51.3[ab]

† The same letters within a column in the same year and location are not statistically different.
* Tuber samples taken from the Brnik location in 2002 were mixed up and could not be analysed.

The yield of green-tubers at the 90 cm IRW was lower than at the 75 cm IRW in Brežice in 2002, in Pšata and Brnik in 2003, and in Pšata and Brežice in 2004 (Table 4). There were no green tubers at the 90 cm IRW in 2003, due to very low market yields. We assume that the low yield of green-tubers at the 90 cm IRW is interconnected with a larger cross-sectional area of the ridge. At the aforementioned IRW, it amounts more than 1000 cm^2 before the harvest, while at the 75 cm IRW it ranges from 752 to 874 cm^2 (Table 4). Such a cross-sectional area of the ridge at the 90 cm IRW ensures a better soil covering of tubers. Kouwenhoven *et al.* (2003) state that cultivars with tubers that are longer and have a wider horizontal span require ridges with a cross-sectional area exceeding 900 cm^2. Furthermore, the authors state that this can be achieved by enlarging the IRW from 75 cm to 90 cm. The obtained results correspond to the results gained by Kouwenhoven and Perdok (2000), Kouwenhoven *et al.* (2003), and Spiess *et al.* (2005), all of whom concluded that, at the 90 cm IRW, the percentage of green tubers was smaller than at the 75 cm IRW. There were no statistically significant differences in the yield of green tubers according to the cultivar.

Table 4: Influence of IRW on the yield of green tubers (> 35 mm) (t/ha) and the cross-sectional area of the ridge before the harvest (cm^2) in all three locations in 2002-2004 (Duncan's Test α=0.05)

Year	IRW	Green tubers (t/ha)			Cross-sectional area (cm^2)		
	(cm)	PŠATA	BRNIK	BREŽICE	PŠATA	BRNIK	BREŽICE
2002	75	$1.2^{a\dagger}$	$-^{*}$	8.9^{a}	799^{a}	862^{a}	815^{a}
	90	0^{a}	-	4.3^{b}	1046^{b}	1070^{b}	1038^{b}
2003	75	2.0^{a}	1.5^{a}	0.3^{a}	812^{a}	756^{a}	874^{a}
	90	0^{b}	0.1^{b}	0^{a}	1100^{b}	1065^{b}	1151^{b}
2004	75	2.3^{a}	1.5^{a}	2.0^{a}	752^{a}	776^{a}	800^{a}
	90	0.8^{b}	0.6^{a}	0^{b}	1035^{b}	1076^{b}	1066^{b}

†The same letters within a column in the same year and location are not statistically different.
* Tuber samples taken from the Brnik location in 2002 were mixed up and could not be analysed.

The choice of IRW did not affect the percentage of tubers with the hollow heart or the blackspot disorders. Both physiological disorders are, in fact, more dependent on the cultivar and meteorological conditions within an individual year than the IRW. In 2003 when the average air temperature during the period of growth was very high and soil water content was low hollow heart in tubers did not occur. This disorder generally occurs on thicker tubers in the years with higher yields (e.g. 2002 and 2004), while its occurrence is not very common in dry years when tubers are not as thick (e.g. in 2003). Hollow heart occurred predominantly with the Agria cultivar in 2002 and 2004, which was not entirely unexpected, since this cultivar is particularly susceptible to this disorder (Table 5). In Pšata and Brnik (2004), the percentage of tubers with the hollow heart disorder was statistically higher with Agria than with the other two cultivars. These results are in accordance with the statements of Dolničar (1997) and Kus (1994). A more serious occurrence of the blackspot disorder happened in 2003, a year with very high temperatures and lacking rainfall, which correspond with the findings of Dolničar (1997) stating that the occurrence of blackspot can be increased by high temperatures associated with drought stress. Particularly susceptible to this disorder is the Bright cultivar. In 2003, the latter had a statistically higher percentage of tubers with blackspot in all three locations in comparison with the other two cultivars. In 2002 and 2004, the occurrence of blackspot was less frequent because there was optimal precipitation, and air temperature was lower than compared to 2003. We also assume in these two years that there was a lower average soil temperature and higher water content in the ridges.

Table 5: Influence of the cultivar on the percentage of tubers with the hollow heart and blackspot disorders in all three locations in 2002-2004 (%) (Duncan's Test α=0.05)

Year	Cultivar	Hollow heart (%)			Blackspot (%)		
		PŠATA	BRNIK	BREŽICE	PŠATA	BRNIK	BREŽICE
2002	Agria	$3.3^{a\dagger}$	$-^{*}$	3.3^{a}	0^{a}	-	6.7^{a}
	Bright	0^{a}	-	5.3^{a}	4.7^{a}	-	2.7^{a}
	Carlingford	0^{a}	-	4.0^{a}	1.3^{a}	-	6.0^{a}
2003	Agria	0^{a}	0.7^{a}	0^{a}	0^{a}	0.7^{a}	4^{a}
	Bright	0^{a}	0^{a}	0^{a}	26^{b}	10^{b}	14.7^{b}
	Carlingford	0^{a}	0^{a}	0^{a}	0^{a}	1.3^{a}	4^{a}
2004	Agria	9.3^{a}	8.7^{a}	0.7^{a}	3.3^{a}	0^{a}	0^{a}
	Bright	2.0^{b}	1.0^{b}	0.7^{a}	4.0^{a}	0.7^{a}	4.7^{b}
	Carlingford	0^{b}	0^{b}	0^{a}	3.3^{a}	0^{a}	0^{a}

† The same letters within a column in the same year and location are not statistically different.
* Tuber samples taken from the Brnik location in 2002 were mixed up and could not be analysed.

4 DISCUSSION

We determined that, at the 90 cm IRW, market yields are higher in the years with sufficient rainfall during the period of growth. When these conditions are met, plants completely cover the inter-row space. Year 2003 was the driest in the last 200 years, which is why we can not make any realistic conclusions on market yield at both IRW in the drought period. The cross-sectional area of the ridge exceeding 1000 cm^2 at the 90 cm IRW allows

a good soil covering of tubers in the ridge. This is why, at this IRW, the yield of green tubers is lower than at the 75 cm IRW. The hollow heart and blackspot disorders affecting the tubers are more dependent on the cultivar and the meteorological conditions during the period of growth than the IRW. Particularly sensitive to hollow heart are thick tubers like the Agria cultivar, especially in the years with abundant rainfall. Blackspot occurs predominantly in the Bright cultivar, in the years of high average temperatures and a lack of rainfall. In Slovenia, other medium-late and late potato cultivars are also in use. These are planted predominantly at the 66 cm and the 70 cm IRWs, which is why a long-term trial involving the cultivation of those cultivars at the 75 cm and the 90 cm IRWs should be carried out. The field trial would provide even clearer insight on the subject.

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Mikrosatelitski markerji uporabni za identifikacijo kultivarjev vinske trte (*Vitis vinifera* L.)

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IZVLEČEK

Identifikacija kultivarjev vinske trte z metodami, ki temeljijo na morfoloških razlikah med rastlinami, je lahko zaradi vpliva ekoloških dejavnikov nepravilna, zato so bile razvite metode, ki omogočajo analizo kultivarjev na nivoju genotipa. V zadnjih dvajsetih letih so se uveljavile različne tehnike za karakterizacijo kultivarjev, od izoencimskih analiz do analiz na nivoju DNA (RFLP, RAPD, AFLP, SCAR in SSR markerji). Med omenjenimi so se najbolj uveljavili mikrosateliti, ki kažejo največjo informacijsko vrednostjo polimorfizma in so večinoma zelo variabilni. Sestavljeni so iz kratkih, tandemsko ponovljivih motivov DNA, ki so prisotni pri večini organizmov. Glavni vzrok polimorfizma med posamezniki so spremembe v številu ponovitev osnovnega motiva mikrosatelita. Za izdelavo začetnih oligonukleotidov mikrosatelitskih lokusov so v začetnih raziskavah uporabili zaporedja obrobnih regij že znanih mikrosatelitov, ki so bila shranjena v podatkovnih bazah DNA. Kasneje pa se je strategija razvijala v smeri nastanka genomskih knjižnic obogatenih z mikrosateliti. Pri vinski trti je bilo razvitih več kot 500 mikrosatelitskih markerjev in njihov izredni potencial in uporabnost pri določanju kultivarjev vinske trte in podlag je bil dokazan v številnih raziskavah. Za genotipizacijo se večinoma uporablja set šestih oz. devetih mikrosatelitskih markerjev (označevalcev), ki so zelo polimorfni in najbolj primerni za ugotavljanje genetske variabilnosti med evropskimi kultivarji vinske trte. Uporaba enotnega seta markerjev ter vključevanje referenčnih kultivarjev v analize genotipizacije omogoča primerjave genotipov med različnimi raziskovalnimi skupinami, in tako lahko rešujemo številne dilem o sinonimih, homonimih ali o izvoru sort vinske trte.

Ključne besede: mikrosateliti, SSR markerji (označevalci), vinska trta, *Vitis vinifera* L.

ABSTRACT

MICROSATLLITE MARKERS FOR CULTIVAR IDENTIFICATION IN GRAPEVINE (*Vitis vinifera* L.)

Identification of grapevine cultivars using methods based on morphological differences between plants may be incorrect due to the influence of environmental factors. For these reasons, alternative methods for cultivar identification, which better illustrate differences at the genotype level, were developed. The diverse techniques for the molecular characterization (isoenzyme, RFLP, RAPD, AFLP, SCAR and SSR markers) developed in the past twenty years have been also applied to the grapevines. Among them, microsatellites show the best value of polymorphism information and are generally very variable. Microsatellites consist of short, tandem repeated DNA motifs and are ubiquitous in most organisms. The polymorphism among individuals is due to changes in the number of repeat units. In the early studies primers for the PCR amplification of the microsatellite loci were designed on the basis of the microsatellite sequences already existing in the Gene Bank databases. Subsequently a strategy was developed towards the construction of a genomic libraries enriched in microsatellites. A large number of microsatellite markers (more than 500) have been developed in grapevines and their extraordinary potential in determining grapevine cultivars and rootstocks was confirmed by numerous studies. The set of six or nine microsatellite markers, which are highly polymorphic and the most appropriate to determine the genetic variability among the European grapevine cultivars, is mainly used in the genotyping analysis. Using a definite set of markers and the integration of reference cultivars in the genotyping analysis allows comparison of genotypes between different research groups, and thus allows solving many dilemmas of synonyms, homonyms and origin or grape varieties.

Key words: microsatellites, SSR markers, grapevines, *Vitis vinifera* L.

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1 UVOD

Identifikacija kultivarjev vinske trte s tradicionalnimi metodami, kot sta ampelografija in ampelometrija, ki temeljita na morfoloških razlikah med sortami, je otežena zaradi vpliva ekoloških dejavnikov, razvojnega stadija rastline, zdravstvenega stanja in načina pridelave. Zato je bilo v preteklosti smiselno poiskati metode, s pomočjo katerih lahko analiziramo razlike med kultivarji na nivoju genoma. Prednost tovrstne identifikacije je v tem, da je neodvisna od zgoraj naštetih dejavnikov, ki vplivajo na morfologijo rastline. Najprej so bili za ta namen razviti izoencimski markerji, na osnovi katerih so karakterizirali in klasificirali vinsko trto (Stavrakakis in Loukas, 1983; Benin in sod., 1988; Calo in sod., 1989). Naslednji korak pri razvoju molekularnih markerjev so bile analize na nivoju DNA. RFLP (polimorfizem dolžin restrikcijskih fragmentov) je bil prvi razvit markerski sistem, ki je omogočil odkrivanje polimorfizma na nivoju DNA. Tehnika, ki temelji na razrezu DNA z restrikcijskimi encimi in zaznavanju specifičnih fragmentov DNA s hibridizacijo z radioaktivno označeno sondo je bila pri analizah vinske trte uspešno uporabljena (Striem in sod., 1990; Bourquin in sod., 1991; 1993), vendar pa je izvajanje metode dolgotrajno in drago. Bolj enostavna in poceni je RAPD tehnika (naključno namnožena polimorfna DNA), ki temelji na namnoževanju neznanih predelov DNA z uporabo začetnega oligonukleotida s poljubnim nukleotidnim zaporedjem v verižni reakciji s polimerazo (PCR) in je bila zelo pogosto uporabljena za določanje genetskih razlik med sortami vinske trte (Tschammer in Zyprian, 1994; Moreno in sod., 1995; Stavrakakis in Biniari, 1998; This in sod., 1997). Največja slabost te metode je zahtevna standardizacija postopka oz. slaba ponovljivost rezultatov. Zato so bili iz RAPD markerjev razviti SCAR markerji (Sequence characterized amplified region), ki predstavljajo pretvorbo RAPD markerja v tip eno-lokusnega (kodominantnega) markerja. Razvoj SCAR markerjev za potrebe identifikacije genotipov vinske trte se ni razširil (Lahogue in sod., 1998), zaradi sočasnega razvoja in večje uporabnosti mikrosatelitskih markerjev. Mikrosateliti ali enostavne ponovljive sekvence (SSR - simple sequence repeats) pa so se izkazali za najbolj učinkovite markerje za genotipizacijo vinske trte (Thomas and Scott 1993, Cipriani in sod., 1994; Sefc in sod., 1998; 2000; Sanchez-Escribano in sod., 1999). Ti polimorfni elementi jedrnega genoma so sestavljeni iz kratkih, tandemsko ponovljivih motivov DNA, ki so prisotni pri večini organizmov. Številni mikrosateliti so zelo variabilni tako znotraj vrst, kot med vrstami. Do polimorfizma med posamezniki prihaja večinoma zaradi sprememb v številu ponovitev osnovnega motiva (Eisen, 1999). Velika variabilnost mikrosatelitov pa je povezana tudi z dejstvom, da je v genomu evkariontov naključno razporejenih od 10^4 do 10^5 mikrosatelitskih lokusov, kar pomeni veliko število polimorfnih mest, ki jih lahko uporabimo za genetske markerje. Prav zaradi visoke mutacijske stopnje predstavljajo mikrosateliti visoko informativne molekulske markerje z največjo informacijsko vrednostjo polimorfizma in kot taki so se uveljavili za identifikacijo kultivarjev vinske trte. Thomas in sod. (1993) so prvi uporabili mikrosatelite za identifikacijo kultivarjev vinske trte in dokazali, da so mikrosatelitske sekvence pogosto zastopane v genomu vinske trte in zelo informativne za identifikacijo *V. vinifera* kultivarjev. Odkrivanje mikrosatelitskega polimorfizma s PCR tehniko namnoževanja je hitro, nezahtevno in učinkovito že pri zelo majhni količini DNA, kar pomeni da v primeru vinske trte namesto rastlinskega tkiva lahko za DNA analizo uporabimo tudi produkte kot sta mošt in vino. Poleg identifikacije kultivarjev so mikrosateliti tudi zelo uporabni za reševanje dilem o sinonimih, homonimih ali o izvoru sort. Zaradi opisanih lastnosti so se mikrosateliti uveljavili kot molekulski markerji za genotipizacijo, v filogenetskih študijah sorodnosti, v populacijski genetiki, za identifikaciji klonov, v selekciji s pomočjo markerjev, idr.

2 MIKROSATELITI

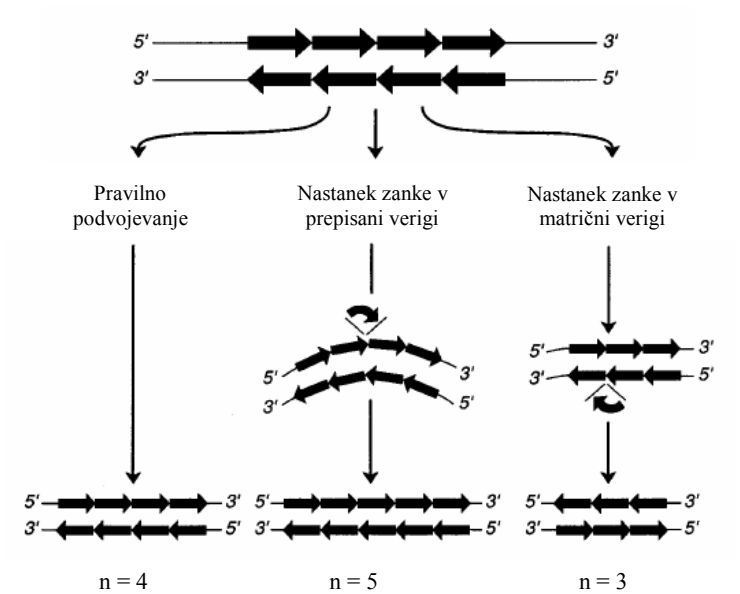
Satelitna DNA je sestavljena iz tandemsko ponavljajočih se zaporedij DNA, ki so v številnih kopijah prisotna v genomu višjih organizmov. Na osnovi dolžine ponovitve so ta zaporedja razdeljena v tri razrede in sicer na satelite (enota ponovitve dolga nekaj tisoč baznih parov), minisatelite (enota ponovitve daljša od 10 bp) in mikrosatelite (enota ponovitve dolga od 2-8 bp) (Armour in sod., 1999).

V zgodovini je termin mikrosatelit označeval samo ponovitve dinukleotidnega motiva CA/ GT (Weber in May, 1989), številna druga imena, kot npr. enostavna zaporedja (SSRs- short sequence repeats) in kratke tandemske ponovitve (STRs- short tandem repeats) pa so bila v uporabi za opisovanje ostalih ponovljivih zaporedij (Jeffreys in sod., 1985). Nakamura in sod. (1987) pa so od preostalega ločili še termin VNTRs (- Variable Number of Tandem Repeats), kar pomeni spremenljivo število tandemskih ponovitev in označuje variabilnost v dolžini ponovitev med posamezniki na enem lokusu.

Mikrosatelite delimo na popolne (sestavljeni samo iz enega motiva osnovne ponovitve, ki se ponavlja brez prekinitve, npr.: ctctctctctctctctctct), nepopolne (ena ali več ponovitev vsebuje bazo, ki ne odgovarja osnovnemu motivu ponovitve, npr.: ctctctct**g**tctctct), prekinjene (vsebujejo krajšo insercijo baznih parov, ki se ne ujemajo z osnovnim motivom ponovitve, npr.: ctctctctct**ggg**ctctctct) in sestavljene (vključujejo dva ali več mikrosatelitov, ki pa se med seboj razlikujejo po tipu ali motivu ponovitve, npr.: ctctctctctct**gatgatgatgat**).

Mikrosateliti se tvorijo iz regij prikritih zaporednih ponovitev (angl. cryptic simplicity), to so predeli, kjer so različni motivi enostavnih, ponavljajočih se DNA zaporedij prisotni v večji meri (Tautz in sod., 1986). Do variabilnost pri mikrosatelitih v večini primerov pride zaradi zdrsa DNA polimeraze in posledično nepravilnega parjenja med replikacijo (SSM- Slipped-Strand Mispairing) (Levinson in Gutman, 1987) ter neučinkovitega DNA replikacijskega popravljalnega mehanizma (Strand in sod., 1993). Z *in vitro* raziskavami je bilo dokazano, da je takšen zdrs DNA polimeraze zelo pogost pojav (Schlötterer in Tautz, 1992), vendar pa se je izkazalo, da je mutacijska stopnja mikrosatelitov v *in vitro* pogojih veliko večja od dejanske mutacije *in vivo* in vzrok temu je *in vivo* delovanje replikacijskega popravljalnega mehanizma. Le ta naj bi namreč zmanjšal stopnjo mutacije mikrosatelitov za 100 – 1000 krat (Strand in sod., 1993). Mutacijska stopnja mikrosatelitov na posamezno generacijo se giblje od 10^{-5} do 10^{-2} (Weber in Wong, 1993) in je veliko višja kot stopnja baznih substitucij. Prevladuje mnenje, da mikrosateliti večinoma niso podvrženi selekciji in prav zato proces mutacije igra toliko večjo vlogo pri določanju dolžine in razporeditve mikrosatelitov (Schlötterer, 2000).

Nepravilno parjenje vijačnice med DNA sintezo zaradi zdrsa DNA polimeraze se pri mikrosatelitu kaže v pridobitvi ali izgubi ene ali več ponovitev. Ali se bo mikrosatelit daljšal ali krajšal pa je odvisno od tega ali nastane zanka v na novo sintetizirani DNA veriga ali v matrični verigi (Slika 1)



Slika 1: Shematski prikaz mehanizma SSM med podvajanjem, katerega rezultat je podaljšanje ali skrajšanje mikrosatelitne ponovitve. Posamezne enote ponovitev so označene s puščicami; nastajanje zank je posledica ostanka neparne baze, ki prekine dvojno vijačnico DNA. Nastanek zanke v prepisani verigi vodi do večjega števila ponovitev; v matrični verigi pa do manjšega števila ponovitev. Med replikacijo lahko pride do nastanka zank na obeh delih verige in efekt insercije ali delecije je tako nevtraliziran. Število ponovitev se lahko poveča ali zmanjša za več enot, v primeru da nastane več zank na eni verigi (van Belkum in sod., 1998).

Figure 1: Schematic representation of the mechanism of SSM during replication, which results in shortening or lengthening of SSRs. Individual repeat units are identified by arrows; bulging is the presence of non-base-pair base residues interrupting a regular 2-strand DNA helix. Bulging in the nascent strand leads to a larger number of repeat units; bulging in the template strand results in a smaller numbers of units. During replication, bulges can occur in both strands, and the effect of insertion or deletion can be neutralized by occurrence of the adverse event. The number of repeat units can decrease or increase by multiple repeats once multiple bulging in one strand has occurred (van Belkum in sod., 1998).

Za mikrosatelite je znano, da je mutacijska stopnja med posameznimi lokusi različna (Di Rienzo in sod., 1998) in obstaja kar nekaj potencialnih faktorjev, ki prispevajo k dognani raznolikosti dinamike razvoja mikrosatelitskih sekvenc: število ponovitev, nukleotidno zaporedje motiva ponovitve, dolžina ponovljive enote, obrobna regija, prekinitve v mikrosatelitu, stopnja rekombinacije, stopnja transkripcije, itd. Tako mnogi raziskovalci na osnovi številnih raziskav predvidevajo, da mutacijska stopnja narašča s številom ponovitev (Weber in Wong, 1993; Brinkmann in sod., 1998; Wierdl in sod., 1997, Rajora in sod., 2001), vendar pa obstaja premalo dokazov, da bi lahko to dejstvo upoštevali kot dejanski faktor razvoja mikrosatelitov.

Nestabilnost ponovitev je v večini primerov torej povezana z zdrsom DNA polimeraze med procesom podvojevanja, alternativno pa je variabilnost v številu ponovitev in degeneraciji nukleotidnega zaporedja lahko povezana tudi z DNA rekombinacijo med lokusi, ki so sestavljeni iz homolognih ponovitev.

Nastanek novega mikrosatelita se nanaša na kombinacijo dveh mutacij. Pri prvi mutaciji se mikrosatelit začne razvijati, kar pomeni da nastanejo ponovitve, ki so dovolj dolge, da lahko na njihovi osnovi med replikacijo pride do nepravilnega parjenja zaradi zdrsa DNA polimeraze. Drugo mutacijo pa povzroči nepravilno parjenje zaradi zdrsa DNA polimeraze in tako pride do dolžinskega polimorfizma. Prav tako pa je propad mikrosatelita povezan s kombinacijo dveh mutacij. Prva mutacija prekine popoln mikrosatelit oz. dolgo mikrosatelitsko ponovitev in tako prepreči nepravilno parjenje zdrsnjene vijačnice ter stabilizira ponovitev. Pri drugi mutaciji pa pride do delecije večjega odseka ponovitve. Končen rezultat je neprepoznavna homologna sekvenca DNA, ki vsebuje le majhen del motiva osnovne ponovitve in zato lahko takšen proces imenujemo 'smrt' mikrosatelita (Taylor in sod., 1999).

2.1 Ničti aleli

V obrobnih regijah mikrosatelitskih lokusov pogosto nastajajo mutacije (Orti in sod., 1997) in posledica tega je lahko nastanek ničtih alelov. Lažna smrt mikrosatelita se lahko pojavi kadarkoli in je posledica nukleotidnih substitucij, insercij in delecij, ki nastanejo v obrobnih regijah, zaradi česar začetni oligonukleotidi ne prepoznajo mest prileganja. Rezultat takšnega procesa so ničti aleli (Callan in sod., 1993), ki se lahko ustalijo v populaciji. Posledica lažne smrti mikrosatelita so številni na videz neuspeli poskusi namnoževanja lokusov med vrstami. Obstoj ničtih alelov lahko dokažemo tako, da naredimo nove začetne oligonukleotide, ki se prilegajo na drugem mestu obrobne regije mikrosatelita, ki ni bil podvržen mutaciji ali pa ga lahko odkrijemo z analizo segregacije križancev. Ni pa vedno nujno, da ničti alel odkrijemo, saj je lahko zamaskiran z alelom homolognega kromosoma. V populaciji je navzočnost teh alelov glavi razlog za veliko odstopanje med dejansko in pričakovano heterozigotnostjo, pri potomcih križancev pa za odstopanje od pričakovanih segregacijskih razmerij (Callan in sod., 1993).

2.2 Homoplazija

Mikrosateliti so običajno na osnovi različne strukture in tudi zgodovine nastanka različno dolgi, vendar pa obstajajo tudi aleli enake dolžine, ki so strukturno in evolucijsko popolnoma različni. In ker običajno variabilnost mikrosatelitov vrednotimo na osnovi elektroforetskih profilov PCR produktov, torej glede na dolžino namnoženega produkta in ne na osnovi nukleotidnega zaporedja, lahko takšen pojav, ki ga imenujemo dolžinska homoplazija privede do napačnega interpretiranja rezultatov. Na genskem nivoju lahko rečemo, da med dvema aleloma obstaja homoplazija, če sta identična po svoji pojavni obliki (dolžini), ne pa tudi po izvoru (Estoup in Cornuet, 1999). Glede na to, da je za mikrosatelitske lokuse značilna visoka mutacijska stopnja (Weber in Wong, 1993) in da se pod pritiskom selekcije ohranja le določena dolžina alelov in se tako zmanjšuje število možnih alelnih oblik, je pričakovana stopnja homoplazije pri mikrosatelitih velika (Nauta in Weissing, 1996). Torej so potencialno mikrosatelitski aleli enake dolžine lahko sestavljeni iz homolognih in homoplastičnih alelov. Dolžinsko homoplazijo znotraj vrste predstavljata dva možna vira variabilnosti, ki temeljita na dejstvu, da mikrosateliti niso vedno sestavljeni iz enega samega motiva. Poznamo tudi prekinjene in nepopolne mikrosatelite in prekinitve predstavljajo še en nivo polimorfizma, saj se aleli enake dolžine lahko razlikujejo po številu in/ali lokaciji prekinitev (Viard in sod., 1998). Drug nivo polimorfizma in odkrivanja dolžinske homoplazije mikrosatelitskih alelov pa predstavlja variabilnost v obrobnih regijah mikrosatelita, ki je bila bolj pogosto odkrita med vrstami in je zelo redka znotraj vrste (Blanquer-Maumont in Crouau-Roy, 1995).

3 IZOLACIJA MIKROSATELITOV

Mikrosatelite lahko izoliramo iz genoma s pomočjo PCR amplifikacije znanih MS lokusov. Za izdelavo začetnih oligonukleotidov se uporabijo zaporedja obrobnih regij že znanih mikrosatelitov, ki so shranjena v podatkovnih bazah DNA ali iz genomskih knjižnic obogatenih z mikrosateliti. Zadnji pristop se je uveljavil pri številnih vrstah, posebno pri tistih, kjer ni na voljo podatkovnih baz o nukleotidnih zaporedjih. V začetku so mikrosatelite izolirali s pregledom velikega števila klonov genomskih knjižnic, novejše tehnike obogatitve genomske knjižnice pa temeljijo na povečanju deleža DNA fragmentov z mikrosatelitskimi ponovitvami (Jakše in Javornik, 2001). Na osnovi obogatitvenega postopka izolacije mikrosatelitov je mogoče dobiti tudi do 40-krat več uporabnih začetnih oligonukleotidov za namnoževanje mikrosatelitov, kot pa pri tradicionalnih metodah izolacije, ki vključujejo hibridizacijsko preverjanje rekombinantnih klonov (Brondani in sod., 1998). Težava, ki se pojavi pri izolaciji večjega števila mikrosatelitov na osnovi obogatitvene knjižnice je presežek posameznih mikrosatelitov, kar pomeni, da se določeni kloni v knjižnici pojavijo večkrat in tako zmanjšajo učinkovitost izolacije. Temu problemu se lahko delno izognemo tako, da pri izdelavi knjižnice uporabimo različne restrikcijske encime, ki povečajo delež neodvisnih DNA fragmentov (Chen in sod., 1997; Jakše in Javornik 2001). S pomočjo sekvenciranja lahko določimo nukleotidno zaporedje obrobne regije mikrosatelita in izdelamo lokusno specifične začetne oligonukleotide. Na osnovi komplementarnosti med začetnimi oligonukleotidi in mikrosatelitskimi obrobnimi regijami pa lahko v procesu verižne reakcije s pomočjo DNA polimeraze, pri osebkih namnožujemo produkte različnih dolžin. Odkrivanje mikrosatelitskega polimorfizma s PCR tehniko namnoževanja je hitro, nezahtevno in učinkovito že pri zelo majhni količini DNA.

4 VREDNOTENJE MIKROSATELITSKEGA POLIMORFIZMA

Metode za vizualizacijo in vrednotenje PCR produktov so se v zadnjih 20 letih zelo izboljšale. Najprej je bila uveljavljena detekcija namnoženih fragmentov s pomočjo radioaktivno označenih začetnih oligonukleotidov ter barvanje PCR produktov s srebrom ali etidijevim bromidom in ročno vrednotenje dobljenih fragmentov. Kasneje so se v večji meri začeli uporabljati fluorescentno označeni začetni oligonukleotidi, fragmente oz. namnožene alele pa je bilo možno vrednotiti avtomatsko, s pomočjo priloženih programskih aplikacij. PCR vzorce mikrosatelitskih lokusov lahko ločujemo v poliakrilamidnem denaturacijskem gelu na različnih sekvenčnih napravah (npr. ALFII Express sekvenator -Amersham Bioscineces, ABI sekvenator - PE Applied Biosystems). Zaradi različnih sistemov elektroforeze pa prihaja do razlik med dolžinami detektiranih alelov in če želimo dobljene rezultate primerjati med različnimi laboratoriji, jih je potrebno standardizirati. Dolžine alelov lahko spremenimo/standardiziramo tako, da v analizo genotipizacije vključimo nekaj referenčnih vzorcev, na osnovi katerih lahko ostalim 'neznanim' vzorcem popravimo dolžine alelov. Znotraj posameznega lokusa so razlike v dolžini alelov vedno enake, zato lahko na osnovi parih vzorcev vključenih v gelsko analizo standardiziramo cel gel oz. vse vzorce (This in sod., 2004). Tako lahko podatke o alelnih dolžinah, ki so bili pridobljeni v različnih laboratorijih, primerjamo in združimo v skupno podatkovno zbirko. Standardizacija podatkov na osnovi referenčnih vzorcev nam s pomočjo podatkovnih zbirk, ki vključujejo rezultate genotipizacije kultivarjev neke vrste omogoča identifikacijo nepoznanih genotipov. Za natančno določanje dolžine mikrosatelitskih alelov uporabimo dolžinski (eksterni) standard (npr. ALFexpress Sizer, 50, 100, 150, 200, 250, 300, 350, 400, 450 500 bp Amersham Biosciences), notranje (interne) standarde, pa pri omenjenem sistemu uporabimo za poravnavo fragmentov enake dolžine, saj med elektroforezo pride do odklona potovanja v različnih delih gela. Če želimo, da so fragmenti na gelu poravnani po vsej širini čim bolj natančno, potem pri elektroforezi posameznega mikrosatelitskega lokusa vedno uporabimo 2 interna standarda (krajšega in daljšega od pričakovane dolžine mikrosatelitskih alelov). Vendar pa tudi pri mikrosatelitskih markerjih obstajajo nekatere omejitve, kot je npr. ta, da pri PCR namnoževanju lahko namesto enega ali dveh pričakovanih fragmentov (alelov) dobimo skupino fragmentov, ki se med seboj razlikujejo le za 2 bp. Dodatni fragmenti, imenovani tudi sekundarni fragmenti (angl. stutter bands), so običajno posledica zdrsa med amplifikacijo s *Taq* polimerazo in zato je določanje dolžin alelov lahko oteženo, še posebej če se dva alela razlikujeta samo za dva bp in je potrebno ločevati homo- in heterozigotno obliko.

5 KARAKTERIZACIJA MIKROSATELITSKIH MARKERJEV

V zadnjih letih je bilo pri vinski trti razvitih več kot 500 mikrosatelitskih markerjev, ki so javno dostopni (Bowers in sod., 1996, 1999, Sefc in sod., 1999, Lefort in sod., 2001, Arroyo-Garciab in sod., 2004, Adam-Blondon in sod., 2004, Di Gaspero in sod., 2005, Merdinoglu in sod., 2005, Goto-Yamamoto in sod., 2006, VMC - Vitis Microsatellite Consortium, 1997-2004). Njihov izredni potencial in uporabnost pri določanju kultivarjev vinske trte in podlag je bil dokazan v številnih raziskavah in narejena je bila identifikacija kultivarjev vinske trte v večini evropskih vinorodnih dežel. V raziskavah genotipizacije vinske trte se večinoma uporablja set šestih (projekt Genres081) oz. devetih (projekt GrapeGen06) mikrosatelitskih markerjev, ki so zelo polimorfni in najbolj primerni za ugotavljanje genetske variabilnosti med evropskimi kultivarji vinske trte (Sefc in sod., 2001).

Mikrosatelitske markerje vrednotimo na osnovi različnih parametrov variabilnosti: dejanska heterozigotnost (Ho) predstavlja delež heterozigotnih posameznikov v analiziranem vzorcu; pričakovana heterozigotnost (He) ali genetska raznolikost predstavlja delež populacije, ki bi bila heterozigotna, v primeru, da bi med posamezniki prišlo do naključnega križanja, hkrati pa predstavlja zmožnost markerja za ločevanje med genotipi; informacijska vrednostjo polimorfizma (PIC – Polymorphic Information Content) predstavlja informativnost posameznega markerja oz. stopnjo pri kateri z markerjem nedvoumno določimo genetsko identiteto posameznika in vključuje tako število alelov odkritih na posameznem lokusu, kot tudi frekvence posameznih alelov; verjetnostjo enakosti genotipov (PI – Probability of Identity) pa predstavlja verjetnost, da imata katerakoli dva naključno izbrana posameznika dva enaka alela na proučevanem lokusu in višja vrednost PI kaže na nizko informativnost, kar je ponavadi posledica majhnega števila alelov ali pa visoke frekvence enega izmed alelov.

V preglednici 1 so predstavljeni parametri variabilnosti za 10 SSR lokusov, ki so bili analizirani na osnovi 54 genotipov vinske trte s slovenskega Primorja. V povprečju se je pri analiziranih sortah namnožilo 9,8 alelov na lokus, kar je podobno rezultatom analiz drugih nacionalnih kolekcij vinske trte (Sefc s sod., 2000; Lefort and Roubelakis-Angelakis 2001; Lopes s sod., 1999; Ibáñez s sod., 2003). Pri slovenskih *Vitis vinifera* L. sortah sta bila najbolj informativna lokusa SsrVrZAG79 in VVMD5 s PIC vrednostjo 0,83. Zanimivo je dejstvo, da je dobljeni rezultat za lokus SsrVrZAG79 v nasprotju z rezultatom, dobljenim za portugalske sorte vinske trte (Lopes s sod., 1999), pri katerih je bil ta lokus ocenjen kot najmanj informativen. S tem primerom lahko potrdimo navedbe Sefc in sod. (2000), da je informacijska vrednost posameznega markerja odvisna od niza vzorcev, ki jih analiziramo, kar je povezano z dejstvom, da v različnih regijah, kjer raste vinska trta, prevladujejo različni aleli. Vrednosti dejanske heterozigotnosti so pri analiziranih slovenskih sortah vinske trte na 6 lokusih nižje od vrednosti pričakovane heterozigotnosti. Največja razlika med omenjenima vrednostma je bila odkrita pri lokusu VVMD36, kar je lahko posledica prisotnosti ničtih alelov na tem lokusu. Prisotnost ničtih alelov na omenjenem lokusu je v preteklosti že bila potrjena (Vouillamoz s sod., 2004) in delno je potrjena tudi z našo analizo, saj pri nekaj vzorcih ni bilo PCR amplifikacije, kar bi lahko pomenilo, da gre za prisotnost homozigotnih ničtih genotipov. Na osnovi nizkih vrednosti za verjetnost enakosti genotipov (0,07–0,19) na posameznih proučevanih lokusih lahko rečemo, da so aleli enakomerno razporejeni med analiziranimi vzorci. Pri treh od desetih markerjev (SsrVrZAG79, VVMD5, VVMD32) so bile PI vrednosti manjše od 0,10, kar pomeni, da so ti lokusi zelo informativni. Največja verjetnost enakosti genotipov (PI) pa je bila, kljub velikemu številu namnoženih alelov (10 alelov), ugotovljena pri lokusu VVMD7, kar je posledica neenakomerne razporeditve frekvenc alelov v analiziranem vzorcu.

Preglednica 1: Parametri variabilnosti po posameznih lokusih izraženi z dejansko heterozigotnostjo (Ho), pričakovano heterozigotnostjo (He), informacijsko vrednostjo polimorfizma (PIC), in verjetnostjo enakosti genotipov (PI).

Table 1: The parameters of variability including observed heterozygosity (Ho), expected heterozygosity (He), polymorphic information content (PIC) and probability of identity (PI) calculated for different loci.

Lokus	**Št. alelov**	**Ho**	**He**	**PIC**	**PI**
SsrVrZAG79	13	0,815	0,859	0,834	0,072
VVMD5	9	0,755	0,853	0,828	0,075
VVMD32	11	0,868	0,814	0,788	0,098
SsrVrZAG62	9	0,870	0,817	0,783	0,115
VVS2	10	0,808	0,790	0,756	0,125
SsrVrZAG21	8	0,852	0,787	0,754	0,127
VVMD25	9	0,759	0,790	0,751	0,140
VVMD36	11	0,596	0,762	0,728	0,140
SsrVrZAG47	8	0,759	0,786	0,745	0,150
VVMD7	10	0,741	0,745	0,697	0,191
Total	106				[a]**$8.32x10^{-11}$**
Average	**9,8**	**0,782**	**0,8**	**0,766**	

[a] Zmnožek vrednosti vseh lokusov.

6 GENOTIPIZACIJA VINSKE TRTE

Skupen cilj evropskih projektov, ki se nanašajo na vinsko trto je postavitev javno dostopne mikrosatelitske podatkovne zbirke, ki bi obsegala genotipe vinske trte iz vseh vinorodnih evropskih držav. Trenutno pa mednarodna mikrosatelitna zbirka (VIVC - Vitis International Variety Catalogue), ki je bila narejena v okviru evropskega projekta Genres081, omogoča identifikacijo 46-ih evropskih kultivarjev z uporabo 6-ih mikrosatelitskih markerjev. Nekatere vinorodne dežele imajo javno dostopne mikrosatelitske podatkovne zbirke, ki vključujejo genotipe domačih sort vinske trte in tujih kultivarjev, ki jih tudi gojijo na določenem območju. Tako npr. švicarska mikrosatelitska podatkovna zbirka (SVMD - Swiss Vitis Microsatellite Database) vključuje genotipe za 170 sort vinske trte analizirane na šestih mikrosatelitskih lokusih (VVMD5, VVMD7, VVMD27, VVS2, VrZAG62 and VrZAG79), ki rastejo na območju Švice. Grška zbirka (Greek Vitis Microsatellite Database) obsega vse možne podatke o trtah, ki rastejo v Grčiji in je kombinacija dveh starejših ampelografskih podatkovnih baz, dopolnjena z mikrosatelitskimi podatki (298 sort in podlag). Italijanska podatkovna zbirka (GMC - Grape Microsatellite Collection) pa omogoča celoten pregled mikrosatelitskih analiz vinske trte narejenih v različnih laboratorijih/državah in tako vključuje tudi podatke o avtorjih ter metodi dela. Hrvaška mikrosatelitska podatkovna zbirka vključuje genotipe 34 kultivarjev z ampelografskimi opisi in v glavnem gre za stare sorte, zgodovinsko povezane s hrvaškim teritorijem (Maletić in sod., 1999). Obstajajo pa še nekatere mikrosatelitske podatkovne zbirke (kot npr. zbirka Univerze v Kaliforniji, INRA – Francija, idr.), ki niso javno dostopne.

6.1 Genotipizacija slovenskih sort vinske trte

Slovenija ima dolgo vinogradniško tradicijo. Kompleksnost tipov klime in tal, pomemben zgodovinski in geografski prostor, vse to je vodilo k raznolikosti trtnih sort, tipov, sinonimov in homonimov, ki jih je potrebno urediti in evidentirati. Napad trtne uši (*Viteus vitifoliae* Fitch) v začetku 20. stoletja, dve svetovni vojni, propad kmetij v času socializacije kmetijstva, so okrnile trsni izbor. Včasih so vinogradniki gojili predvsem lokalne, manj poznane sorte, ki pa so v času intenzivne obnove vinogradov izginile in nadomestile so jih nove, razširjene sorte, ki jih gojijo tudi drugod po Evropi (Korošec- Koruza, 1992). Po letu 1970 se je začela svetovna akcija zbiranja, ohranjanja in vrednotenja starih kultivarjev in klonov ter organizacija kolekcijskih nasadov, katere osnovni namen je zbiranje genskega materiala vinske trte za nadaljnje žlahtnjenje.

Uporaba mikrosatelitskih markerjev nam omogoča tudi identifikacijo in določanje sorodnosti pri starih sortah vinske trte, ki so ohranjene v kolekcijskih nasadih ali pa jih najdemo samo še v nekaterih vinogradih, kjer jih ponavadi gojijo v manjšem obsegu. Glede na to, da so

opisi teh sort in z njimi povezani razpoložljivi podatki zelo nepopolni oz. okrnjeni, je potrebno nekatere kultivarje tudi identificirati oz. razrešiti njihovo poimenovanje. Večkrat za neko sorto obstaja več sinonimov, kar pomeni, da se posamezne sorte različno imenujejo, čeprav so genotipsko enake, kar lahko dokažemo z analizo mikrosatelitov. V nekaterih primerih pa poznamo tudi skupine oz. pare sort, ki imajo enako ali zelo podobno poimenovanje, v svoji genetski zasnovi pa so različne in take sorte imenujemo homonimi.

V Sloveniji so stari kultivarji ohranjeni v kolekcijskih nasadih na štirih lokacijah, in sicer v Ložah pri Vipavi, v Novi Gorici, na Dobrovem-Goriška Brda in v Ormožu in v nekaterih starih vinogradih. Vendar pa so razpoložljivi podatki za omenjene sorte pogosto nepopolni ali netočni. Težava, ki se pojavlja pri identifikaciji starih sort je tudi njihovo poimenovanje, saj je pestro zgodovinsko dogajanje in s tem povezano večjezično območje, prispevalo k različnemu poimenovanju starih sort, ki jih danes odkrivamo pod različnimi sinonimi. Poleg tega pa so populacije *Vitis vinifera* L. pogosto zelo heterogene oz. so trsi posameznega klona pogosto zelo neizenačeni, kar tudi ovira identifikacijo na morfološkem nivoju. Zato smo v preteklosti z mikrosatelitskimi markerji genotipizirali 33 slovenskih sort, ki rastejo v kolekcijskih nasadih v Vipavski dolini, Novi Gorici in Goriških Brdih (Štajner in sod., 2008) ter 38 starih slovenski sort, ki so bile pridobljene iz različnih vinogradov Vipavske doline in Slovenske Istre (Štajner in sod., *v tisku*). V analizi podobnosti smo identificirali 11 skupin sinonimov in nekaj homonimov, kot npr. sorte z imenom Rebula (Rebula, Stara Rebula in Rebula-100let), ki na osnovi genetske analize kažejo veliko raznolikost. Poleg tega smo potrdili nekatere domneve o identičnosti sort, ki so bile narejene na podlagi morfoloških znakov rastlin in tako smo npr. s pomočjo analize mikrosatelitov ugotovili, da je sorta Ferjanščkova sinonim za Merlot, ter da je Grganc sinonim za Rebulo, kar pomeni, da se je starodavno poimenovanje očitno ohranilo na nekaterih področjih v Slovenski Istri. Med skupinami sinonimov, ki smo jih dobili z analizami mikrosatelitov izstopa skupina petih sinonimov (Glera = Prosecco = Briška Glera = Števerjana = Beli teran). Nekateri izmed njih so bili v preteklosti že opisani, medtem ko je npr. Števerjana povsem nov sinonim za omenjene sorte. Velika raznolikost na osnovi mikrosatelitskih lokusov pa je bila odkrita med Glero/Briško Glero in Belo Glero, kar bi lahko razložili z dejstvom, da se je v preteklosti ime Glera uporabljalo za različne bele sorte vinske trte, ki so rasle v submediteranskem delu Slovenije. Pri primerjavi nekaterih slovenskih sort s hrvaškimi, ki so jih genotipizirali Maletić in sod. (1999) smo tudi odkrili tako sinonime kot homonime; tako je bila npr. na osnovi 7 mikrosatelitskih lokusov potrjena identičnost med sortama Muškat Ruža Porečki (hrvaška) in Cipro (slovenska) ter med sortama Ranfol bijeli (hrvaška) in Belina Pleterje (slovenska). Homonim je bil odkrit med hrvaško sorto Plavina (Calo in sod., 2008) in slovensko sorto z enakim imenom, saj je njuna podobnost na osnovi SSR analize le 20%. Prav tako se med seboj zelo razlikujejo slovenska, hrvaška (Calo s sod., 2008) in italijanska (Muganu in sod., 2009) sorta z imenom Pagadebiti. Italijanska beseda pagadebito pomeni 'plačevanje davkov' in to poimenovanje se je včasih uporabljalo za različne zelo produktivne sorte, katerih pridelek so imeli za plačevanje davkov in tako je pričakovati, da se pod tem imenom skriva več različnih sort oz. homonimov.

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Kemična komunikacija med parazitoidi in organizmi z drugih trofičnih nivojev

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IZVLEČEK

Vsi organizmi v ekosistemu so biokemično povezani. Semiokemikalije v receptorjih dražljajev vzbudijo vedenjske ali fiziološke odzive, kar privede do interakcije med dajalcem in prejemnikom dražljajev. Medvrstne semiokemikalije, ki jih imenujemo alelokemikalije, povezujejo organizme z različnih trofičnih nivojev in omogočajo naravnim sovražnikom najti gostitelje ali plen in jih prepoznati kot ustrezne. Tudi gostiteljske rastline v tem sistemu niso nevtralne in tako se lahko rastlinojedi ter njihovi naravni sovražniki odzovejo na njihove dražljaje. Številni parazitoidi se odzivajo na vonj rastlin in lahko ločijo med nepoškodovanimi rastlinami, napadenimi rastlinami in celo poškodovanimi rastlinami, na katerih škodljivec ni več prisoten. V prispevku predstavljamo semiokemikalije, ki so vključene v prehranjevalno verigo parazitoidov listnih uši.

Ključne besede: semiokemikalije, kemični dražljaji, interakcije gostiteljska rastlina – škodljivec - naravni sovražniki, parazitoidi

ABSTRACT

CHEMICAL COMMUNICATION BETWEEN PARASITOIDS AND ORGANISMS FROM OTHER TROPHIC LEVELS

All the organisms in an ecosystem are biochemically linked. Semiochemicals elicit behavioral and physiological responses in the receiver, which results in the interaction between the donor and the receiver. Interspecific semiochemicals, called allelochemicals, connect all trophic levels and so help natural enemies to locate and recognize their hosts or prey. Also the host plants of herbivores are not neutral substrates, so herbivores and their natural enemies can respond to elicited cues. Many parasitoids respond to plant odors and many can also distinguish undamaged from host-infected or previously host-damaged plants. In this paper we present semiochemicals involved in food chain of aphid parasitoids.

Key words: semiochemicals, chemical cues, host-plant – herbivore – natural enemy interactions, parasitoids

1 UVOD

Hlapljive snovi imajo pomembno vlogo v tritrofičnem sistemu, ki vključuje gostiteljsko rastlino, rastlinojeda in parazitoida (ali plenilca). Tako lahko specifično lastnost rastlin, da privabljajo naravne sovražnike herbivorov, imenujemo kar posredna obramba rastlin pred škodljivci (Thompson, 1996). Rastline lahko posredno ali neposredno vplivajo na parazitoide ali plenilce (predatorje), tako z morfološkimi lastnostmi (velikost rastline, oblika celotne rastline in posameznih organov, barva rastlin, razlike v fenologiji in površinskih lastnostih, kot sta dlakavost ali voščeni poprh), kot tudi s semiokemikalijami, kemičnimi snovmi, ki so vpletene v interakcije med organizmi in delujejo kot signalne kemikalije, ki neposredno vplivajo na naravne sovražnike (Price, 1984; Hare, 2002).

Hlapljive semiokemikalije so pogosto atraktanti, ne le za herbivore, ampak tudi za naravne sovražnike. Nekatere od teh snovi nastanejo v rastlinah, ki so poškodovane, in tudi v nepoškodovanih rastlinah. Druge snovi pa se izločajo ob mehanskih poškodbah ali le ob prehranjevanju točno določene vrste herbivora.

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[2] mag. agr. znan., mlada raziskovalka, ibid.

[3] izr. prof., dr. agr. znan., ibid.

Alelokemikalije rastlin, ki jih sprejmejo herbivori, so lahko neugodne za naravne sovražnike. To se lahko zgodi, ko se herbivor prilagodi rastlinskim toksinom, parazitoid pa ne, ali pa posredno, ko rastlinski toksini zmanjšajo prehranjevanje herbivorov; takšni gostitelji parazitoidov so manjši in manj kakovostni. Po drugi strani pa lahko toksini oslabijo obrambne sposobnosti gostiteljev in tako povečajo njihovo občutljivost, kar je ugodno tako za naravne sovražnike, kot tudi posredno za rastline. Na njih vpliva tudi rastlinska diverziteta in gostota rastlin v prostoru. Neposredno pa lahko rastline na naravne sovražnike vplivajo tako, da izločajo semiokemikalije kot posledico napada herbivora, da vplivajo na kakovost gostitelja zaradi prehrambenih lastnosti in odpornosti rastlin in da herbivori prevzamejo rastlinske toksine (Hare, 2002).

2 SEMIOKEMIKALIJE

Semiokemikalije delimo na intraspecifične feromone, ki služijo le komunikaciji organizmov znotraj iste vrste in na interspecifične alelokemikalije, ki služi komunikaciji med osebki različnih vrst ter tudi med različnimi nivoji prehranjevalne verige (Dicke in Sabelis, 1988, cit po Minks in Harrewijn, 1988).

Feromoni so snovi, ki jih kot kemične signale izloča en osebek, sprejemajo pa jih osebki iste vrste in se nanje odzivajo na značilen način. Feromoni sprožijo posebne vzorce vedenja ali pa posebne fiziološke oziroma razvojne procese. Pri mnogih vrstah živali omogočajo feromoni pravo kemično govorico. Pri različnih vrstah lahko najdemo iste feromone, vendar je njihovo razmerje v mešanici vonjav vrstno značilno (Gogala, 1983; Price, 1984).

Uveljavljena delitev feromonov, ki jo je podal Shorey (1977), pozneje pa so jo večkrat izpopolnili, zajema spolne feromone, feromone zbiranja (agregacije), alarmne feromone, feromone razpršitve (disperzije) in sledovne feromone. Spolni feromoni so kemične snovi, ki služijo medsebojnemu privabljanju predstavnikov različnih spolov, da bi med njima prišlo do parjenja. Lahko jih oddajajo samci ali samice, kar je odvisno od vrste žuželke, prevladujejo pa feromoni samic. Mnogokrat so različne sestavine feromonov odgovorne za različne vzorce obnašanja pri parjenju (izbira kraja parjenja, načina dvorjenja idr.) in so najbolj preučene pri metuljih. Feromoni zbiranja (agregacije) so kemične snovi, ki vplivajo na zbiranje osebkov v bližini feromona. Feromoni preplaha (alarmni feromoni) so snovi, ki povzročijo, da osebki neke vrste zbežijo od izvira feromona. So snovi z nizko molekulsko maso in so hitro hlapljive, zato se hitro razpršijo, delujejo pa kratek čas. Feromoni razpršitve ali disperzije (epideiktični feromoni) povzročijo pri žuželkah obnašanje, ki ima za posledico razpršitev osebkov in zmanjšanje konkurence med osebki iste vrste. Takšni feromoni so lahko zelo koristni tedaj, ko je na primer nek vir hrane prenaseljen. Sledovni feromoni so znani zlasti pri socialnih žuželkah (še posebno pri mravljah in termitih) in povzročijo priseljevanje v kolonije, kjer so novi viri hrane.

Zelo velik pomen pri določanju vedenjskega vzorca parazitoidov in plenilcev ima kemična komunikacija med žuželkami, ki pripadajo različnih vrstam ter med žuželkami in rastlinami. Takšna komunikacija poteka z alelokemikalijami. Vsaka informacija pri interakciji med dvema individuuma ima kemično osnovo.

Alelokemikalije delimo na alomone, kairomone, sinomone in apneumone. Alomoni v prejemniku (receptor) vzbudijo odziv, ki je adaptivno ugoden le za dajalca; kairomoni v prejemniku vzbudijo odziv, ki je adaptivno ugoden le za prejemnika, ne pa tudi za dajalca; sinomoni povzročijo odziv prejemnikov, ki je adaptivno ugoden za oba, prejemnika in dajalca (Dicke in Sabelis, 1988, cit. po Minks in Harrewijn, 1988); pri apneumonih pa kemične dražljaje izloča neživa snov in ti so ugodni za prejemnika, ne pa za organizem, ki je na tej snovi (fermentacija sadja, ki gnije, privabi parazitoida vinske mušice) (Price, 1984).

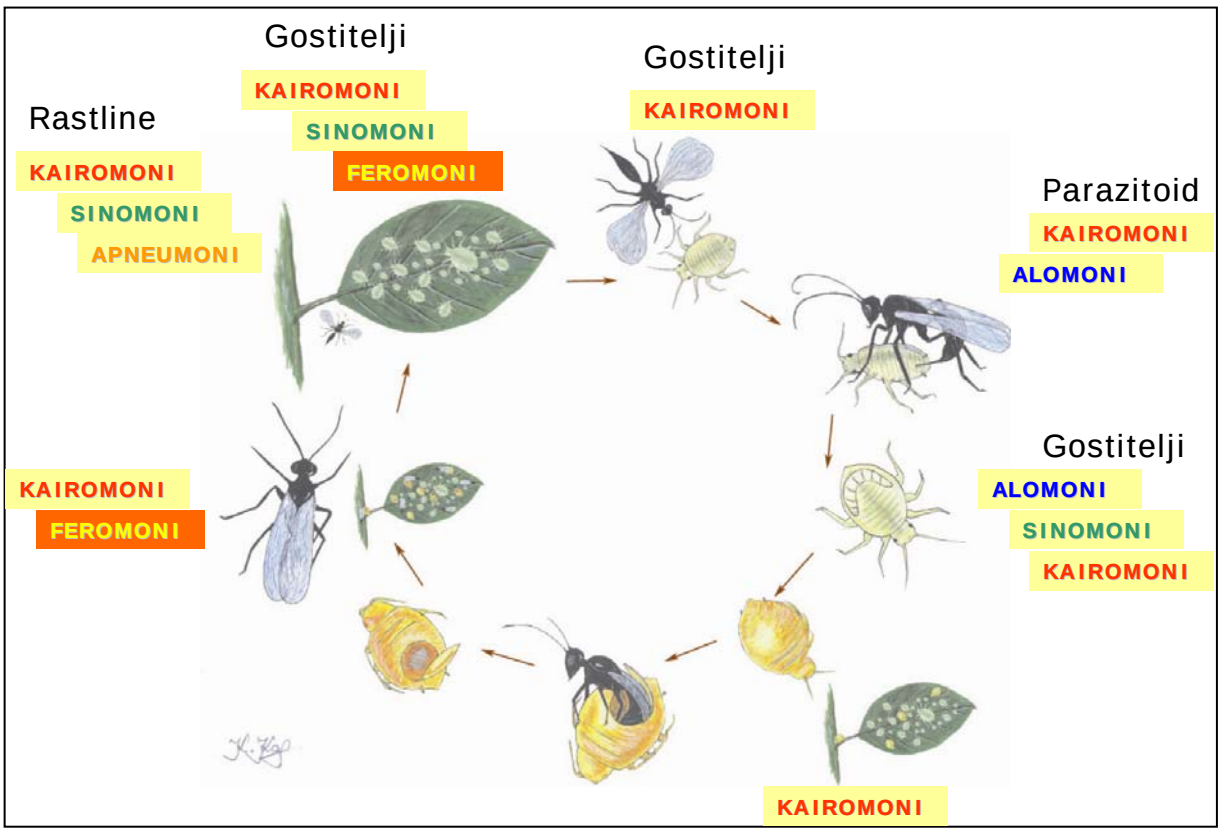
Te kemikalije izvirajo iz gostiteljev samih, v tem primeru so te kemikalije za parazitoida kairomoni; iz rastlin, na kateri se gostitelj hrani, kemikalije so za parazitoida sinomoni in iz nekaterih interakcij med gostiteljem in rastlino, ko izločene kemikalije za parazitoida delujejo kot sinomoni (Hatano in sod., 2008; van Alphen in Jervis, 1996, cit. po Jervis in Kidd, 1996).

Večina parazitoidov se odziva na vonjalne kairomone ali sinomone za lociranje gostitelja na velike razdalje. Alomone pa lahko izloča samica parazitoida, ki odloži jajčeca v gostitelja, le-tega zaznamuje in tako odvrne drugo samico, da bi odložila njena jajčeca v istega gostitelja (Godfray, 1994). To so lahko tudi obrambne kemikalije (odvračala, toksini). Kemikalije, ki se izločajo iz rastlinskih tkiv ob napadu herbivorov, lahko pozitivno vplivajo na parazitoide (terpeni, terpenoidi, indoli; acetaldehid, etanol (izločki gliv, ki rastejo na sadju – gnitje sadja): kvas: glive na lesu) (Godfray, 1994).

Škodljivci imajo pomembno vlogo pri privabljanju naravnih sovražnikov, saj sami oddajajo kemične signale, ki na naravne sovražnike delujejo kot

kairomoni. Te kemične informacije pa lahko delujejo kot sinomoni za sekundarne parazitoide, ki delujejo v korist škodljivcev.

Rastline aktivno izločajo kemične signale, ki privabljajo naravne sovražnike herbivorov (Dicke in Vet, 1999) in imajo podoben vpliv na parazitoide kot dražljaji samih gostiteljev. Vendar pa so signali rastlin lažje izsledljivi na velike razdalje zaradi relativno velike biomase rastlin. Le določeni herbivori lahko izločajo kemične signale, ki lahko izhajajo le na poškodovanem mestu rastline ali pa sistemsko tudi iz drugih nepoškodovanih delov rastlin. Umetno povzročene poškodbe rastlin ne izzovejo enakega odziva kot pa poškodbe zaradi hranjenja rastlinojedov na rastlinah, ki so prostorsko in časovno urejene (Thompson, 1996).



Slika 1: Razvojni krog parazitoida listnih uši in nekatere pomembne povezave med gostiteljsko rastlino, ušjo in parazitoidom s semiokemikalijami (K. Kos).

Količina izločenih hlapljivih snovi iz rastlin je lahko preprosto rezultat obsega poškodb. Veliki škodljivci navadno povzročijo večjo škodo od manjših. Hlapljive snovi različnih vrst gojenih rastlin lahko vplivajo tudi na število parazitoidov, ki se odzovejo na kemične dražljaje (Kalule in sod., 2004).

Hlapljive snovi, izločene iz rastlin ob napadu herbivorov, imajo lahko vlogo sinomonov (slika 1) ali kairomonov. V prvem primeru je izločanje koristno tako za rastlino kot za parazitoida, v drugem primeru pa gre tudi za privabljanje herbivorov. Sinomoni so pomembni za iskanje gostiteljev parazitoidov zaradi njihove zaznavnosti, saj vsebujejo dovolj podatkov za iskanje specifičnega gostitelja. Kairomoni in vizualni dražljaji povečajo specifičnost iskanja, ko parazitoid že prispe v mikrohabitat gostitelja. Snovi rastlin, izločene zaradi napada herbivorov, lahko parazitoidu posredujejo tudi oceno približnega števila zastopanih gostiteljev v določenem okolju (Thompson, 1996).

Parazitoidi, ki iščejo habitat gostitelja, se lahko opirajo na hlapljive signale napadenih in nenapadenih gostiteljskih rastlin. Lo Pinto in sod. (2004) so proučevali vpliv hlapljivih stimulantov na vedenje parazitoidov *Lysiphlebus testaceipes* (Cresson) in *Aphidius colemani* Viereck ob iskanju gostitelja. Za gostitelja so uporabili bombaževčevo uš (*Aphis gossypii* Glover) na kumarah. Poskusi so bili izvedeni v laboratoriju v vetrovnem tunelu, kjer so parazitoide »izpostavili« trem virom: a) kompleksu kumar in bombaževčeve uši, b) nenepadenim kumaram, in c) kartonskim "lažnim" kumaram. Ugotovili so, da sta obe vrsti parazitoidov raje leteli proti pravim kakor k

"lažnim" rastlinam, medtem ko med letom na napadene in nenapadene rastline niso ugotovili razlik. To si lahko razlagamo s tem, da parazitoidi iščejo potencialne habitate, kjer bi lahko bili gostitelji in jih privlačijo snovi, ki ne izhajajo neposredno iz gostiteljev. Sposobnost lociranja gostiteljev s strani parazitoidov variira z različnimi gostitelji in gostiteljskimi rastlinami, saj tako izločajo različne mešanice hlapljivih snovi (Lo Pinto in sod., 2004).

Ko parazitoid prispe v potencialni habitat gostitelja, prestopi v naslednji stadij iskanja gostitelja. Žuželke se pogosto odzivajo na kairomone z majhno intenziteto vonja, ki ostane za gostitelji na substratu. Snovi, ki vsebujejo te kairomone, vključujejo izločke žlez slinavk ali mandibularnih žlez, medeno roso enakokrilcev in izločke kutikule (van Alphen in Jervis, 1996, cit. po Jervis in Kidd, 1996). Kairomoni gostitelja privlačijo parazitoida in tako povečajo možnost odkritja gostitelja. Lociranje gostitelja pa je odziv na nekemične, torej vizualne in čutilne dražljaje.

Vrsta *Aphidius ervi* pri lociranju gostiteljev izrablja alarmne feromone listnih uši. Nekateri jajčni parazitoidi pa izrabljajo spolne feromone odraslih osebkov gostiteljev, saj je jajčece najtesneje povezano z odraslim osebkom, ki ga izleže. Spolni feromoni se adsorbirajo in ostanejo na listnem površju gostiteljskih rastlin in tako posredujejo informacije o pretekli prisotnosti spolno aktivnih odraslih osebkov gostitelja (Godfray, 1994).

Poškodbe na rastlinah, nastale zaradi napada herbivora in izločena medena rosa, so pomembni viri odvračalnih in privabilnih dražljajev za parazitoide. Ravno tako pa so pomembni tudi izločki mandibularnih in labralnih žlez herbivorov (Godfray, 1994). Odziv parazitoidnih vrst na rastlinske snovi variira glede na stopnjo in tip poškodbe rastlin. Celo bližnje sorodne vrste imajo lahko na poškodovane oz. nepoškodovane rastline in glede na vir poškodbe različne odzive (Hare, 2002).

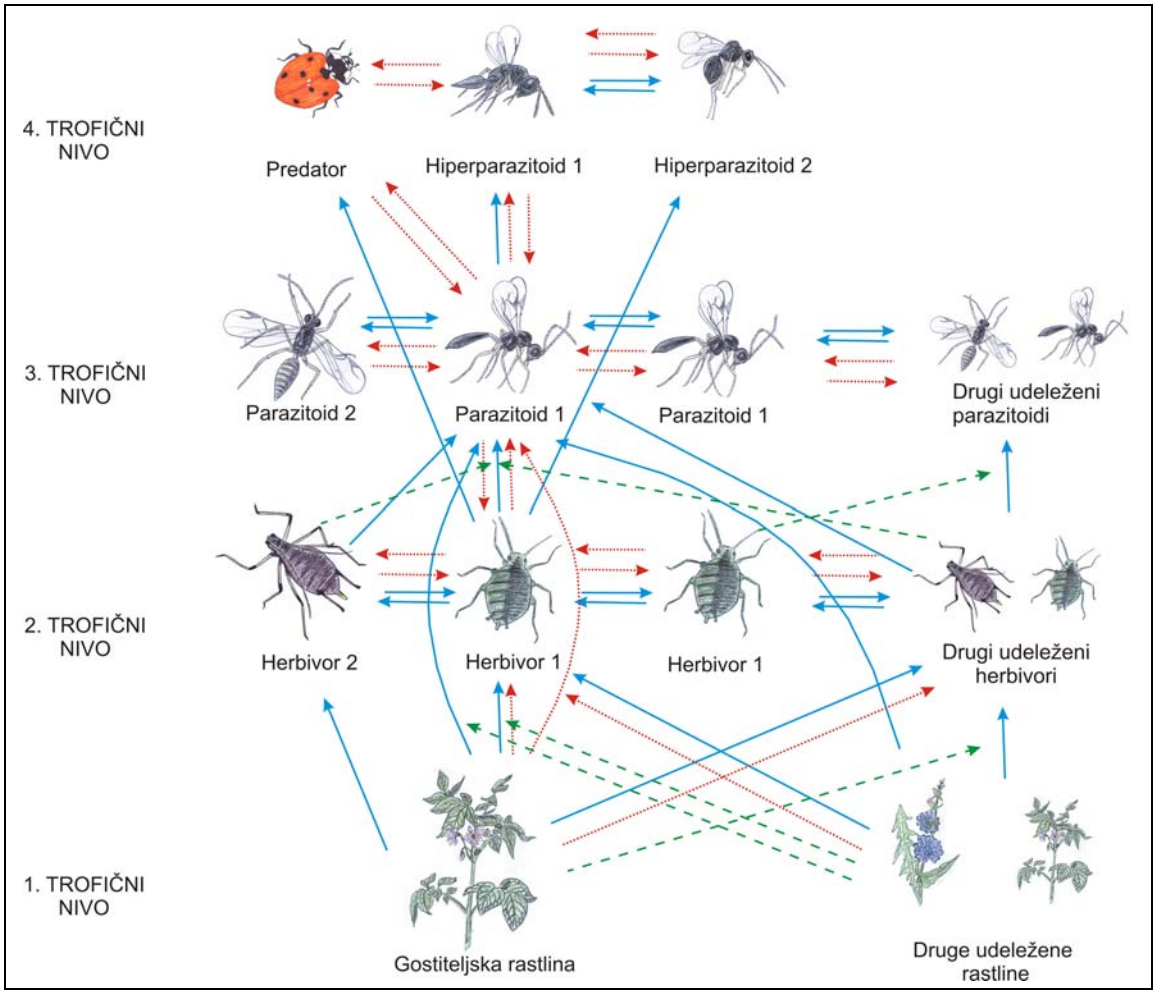
Kemični dražljaji, ki so samici parazitoida služili za iskanje gostitelja, imajo pomembno vlogo tudi pri določanju ustreznosti gostitelja za ovipozicijo. Poleg tega so lahko nehlapljive snovi, ki so prisotne na površju gostitelja, ključni dražljaj, ki pogojuje ovipozicijo. Parazitoidi lahko potencialne gostitelje pregledajo od zunaj in od znotraj; od zunaj, ko sledijo kemičnim dražljajem in ko s tipalkami ter s stopalci pretipajo gostitelja, od znotraj pa, ko leglico zabodejo v gostitelja, vendar še ne izležejo jajčec. Leglica je navadno pokrita s čutnicami, ki služijo za ugotavljanje ustreznosti gostitelja (Godfray, 1994).

Prav tako kot lahko gostiteljska rastlina in parazitoid vzpostavita nekakšen mutualističen odnos prek sinomonov, ki jih izloča napadena rastlina in s tem privabi parazitoide, se lahko vzpostavi tudi mutualističen odnos med herbivorom in hiperparazitoidom. To se zgodi ob povečani parazitiranosti herbivorov - ti izločajo kemične snovi (v medeni rosi) - ki privlačijo hiperparazitoide. Tako zmanjšajo stopnjo parazitiranosti in število primarnih parazitoidov, hkrati pa povečajo razmnoževalno sposobnost uši (Budenberg, 1990).

Samce parazitoidnih osic vrste *Diaeretiella rapae* (McIntosh) privlačijo hlapljive snovi, ki jih izločajo druge samice, hkrati pa te snovi privlačijo tudi hiperparazitoida *Alloxysta brassicae* (Ashmead). Pogosto hiperparazitoida same rastline in listne uši ne privlačijo, svojo pozornost usmerijo le na parazitirane kolonije uši (Godfray, 1994).

Slika 2 prikazuje kemične interakcije med štirimi nivoji prehranjevalne verige. Herbivore privlačijo hlapljive snovi rastlin (modre črte), parazitoidi pa se lahko pozitivno odzovejo na isto spojino. Vendar pa so toksične snovi v herbivoru, pridobljene iz gostiteljskih rastlin, lahko učinkovita obramba pred naravnim sovražnikom (rdeče črte). Toksične snovi so rastlinski sekundarni metaboliti, ki herbivora sicer odvračajo, vendar pa če ta odpor premaga in se vseeno hrani s to rastlino, parazitoida ta snov še toliko bolj odbija in takšnega herbivora ne bo napadel (Price, 1984).

Hlapljive snovi rastline lahko na herbivora delujejo repelentno, vendar če le-ta premaga odpor in rastlino vseeno poje, lahko ista snov deluje posredno kot repelent za naravnega sovražnika, ki ga zato ne pleni, deluje pa lahko tudi neposredno na tretji trofični nivo (rdeče črte). Tudi znotraj istega trofičnega nivoja delujejo interakcije, ki žuželke med seboj bodisi privlačijo (spolni, agregacijski feromoni) (modre črte) ali pa odvračajo (alarmni feromoni, alomoni) (rdeče črte). Tudi na hiperparazitoide lahko delujejo sinomoni, ki jih oddajajo herbivori (modre črte). Podobno kot gostiteljske rastline, pa lahko na vse trofične nivoje delujejo tudi druge (asociativne) rastline (zelene črte), ki pa lahko povzročijo tudi vmešavanje drugih dražljajev in tako motijo žuželke pri iskanju njihovih gostiteljev, tako rastlin kot tudi žuželk.



Slika 2: Skupnost štirih trofičnih nivojev vsebuje kemične interakcije s semiokemikalijami. Puščice nakazujejo smer dražljaja proti organizmu, ki se nanj odzove. Modre neprekinjene črte kažejo privlačnost na dražljaje (herbivora privlači rastlina), rdeče pikaste črte pa odpor na dražljaje. Zelene črtkane črte kažejo posredni vpliv (motnje – vmešavanje drugih odzivov) (Slika: K. Kos, po Price, 1984).

3 ZAKLJUČKI

Rastline proizvajajo semiokemikalije kot notranjo obrambo pred herbivori, hkrati pa vplivajo tudi na tretji trofični nivo, kar se kaže kot tritrofična interakcija. Rastlina proizvaja tudi hrano za naravne sovražnike, kot nektar in cvetni prah, ter jim s tem pomaga najti tudi herbivora kot plen/gostitelja, ki se hrani na isti rastlini. Posredno rastline skrbijo tudi za kakovost medene rose, kot hrane za naravne sovražnike, ki jo izločajo enakokrilci (Ahmad in sod., 2004). Za gostiteljske rastline je pomembno, da parazitoidi s svojim vedenjskim vzorcem iskanja gostitelja s pomočjo različnih kemičnih dražljajev, izvajajo selekcijski pritisk tudi na rastline, saj sinomoni, ki jih rastline izločijo kot posledice napada herbivorov, privabijo več parazitoidov in drugih naravnih sovražnikov in tako vplivajo na večjo smrtnost herbivorov (Tentelier in Fauvergue, 2007).

Za odrasle samice parazitoidov je ključnega pomena, da so sposobne prepoznati in razbrati fizične in kemične dražljaje, ki jih nudijo njihovi gostitelji in gostiteljske rastline. Specifične kemične snovi iz določenih rastlin lahko nakažejo na prisotnost določenegaa rastlinojedega gostitelja, če je le-ta specialist na tej vrsti rastlin. Tako parazitoida listnih uši *Diaeretiella rapae*, ki prednostno parazitira listne uši, ki se hranijo na kapusnicah, privabijo izotiocianati kapusnic, ki so tipične kemikalije teh rastlin (Baer in sod., 2004). Za svoje potomstvo morajo najti ustrezne prehranske vire, saj morajo locirati in izbrati ustrezne gostitelje; ličinke pri parazitoidih so namreč večinoma omejeno mobilne in so v tesnem odnosu s svojim gostiteljem. Tako je uspešnost preživetja potomstva vezana na sposobnosti samice, da razloči kemične in fizične dražljaje okolice. Kemični

dražljaji gostiteljev in gostiteljskih rastlin tako vodijo samice do ustreznega okolja z njihovimi gostitelji ter zagotovijo ustreznost gostiteljev, ki pri solitarnih vrstah ne smejo biti predhodno parazitirani (Hilker in McNeil, 2008). Kemični dražljaji tako močno pogojujejo uspešnost parazitiranja parazitoidnih osic, ki jih uspešno uporabljajo v biotičnem varstvu rastlin pred škodljivimi organizmi že več kot stoletje.

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Agrovoc descriptors: foods,decision making,consumer behaviour,consumer surveys,environmental protection,data collection,market research,marketing,value systems,cultural values,motivation

Agris category code: E50, E73

Odločitveni dejavniki pri nakupu živil v Sloveniji

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IZVLEČEK

V spletni anketni raziskavi nas je zanimalo, kateri dejavniki so odločilni za nakup živil v osrednjem in SZ delu Slovenije (Gorenjska). Anketni vzorec je sestavljalo 249 anketirancev. Ugotovili smo, da 79 % anketirancev prideluje pretežno sadje in zelenjavo na svojem vrtu. Pri tem jih večina uporablja le organska gnojila (76 %), kar kaže na visoko ozaveščenost ljudi v zvezi z ohranjanjem okolja. Kar 94 % vprašanih nakupuje v večjih trgovinah (supermarketih), 88 % pa jih ni nikoli kupovalo živil prek interneta. Pri nakupu potrošniki na pomembno mesto dajejo poznan oziroma slovenski izdelek in ekološki izdelek, medtem ko cena izdelka ne sodi med pomembnejše motive za nakup. Rezultati so pokazali, da se za nakup ekološkega proizvoda anketiranci raje odločajo v ekoloških trgovinah kot v supermarketih.

Ključne besede: živila, navade potrošnikov, trženje, anketa

ABSTRACT

DECISIVE FACTORS WHEN BUYING FOODSTUFF IN SLOVENIA

In the survey that was performed over internet we were looking for factors that are the most important for buying foodstuff products in central and NW part of Slovenia (Gorenjska region). Sample: 249 individuals. It was discovered that 79% of respondents on their gardens grow mostly fruits and vegetables. Mostly (76%) just organic fertilisers are used that shows high level of conscience towards environment friendly behaviour. As much as 94% of respondents are buying in supermarkets (bigger points of sale), 88% of them have never bought foodstuf over net. Knowing the product, ecological product and Slovenian origin are three important characteristics, where price is supposed not to be a motivator of a buying decision. Respondents prefer to buy ecological products in an ecological store and not to buying them in supermarkets.

Key words: foodstuff, consumers' habits, marketing, questionnaire

1 UVOD

Hrana je bila vedno ena izmed osnovnih potreb človeštva in je bila v vsej njegovi zgodovini med njegovimi prioritetami. Zato si vsaka država prizadeva, kljub globalizaciji trga s hrano, da bi pridelala dovolj varne, kakovostne in čim cenejše hrane. Kljub količinsko zadostni svetovni pridelavi hrane je še vedno blizu milijarda ljudi v svetu podhranjenih, kar se ocenjuje z indeksom WHI od 0 (brez lakote) do 100 (najhujša oblika lakote). V evropskem prostoru sta omenjeni le Albanija in Hrvaška z najnižjim indeksom lakote, pod 4,9 (Welthunger-Index, 2009). Projekcije za leto 2020 kažejo, da se bo svetovno prebivalstvo v primerjavi z letom 1990 povečalo za 50 % in se približalo 8 milijardam ljudi. Leta 2050 bo na Zemlji živelo že skoraj 9 milijard ljudi, to pa utegne biti ob zdajšnji distribuciji hrane velik problem, saj se bo po projekciji rast proizvodnje kmetijskih pridelkov do leta 2018 povečala le za 10 % (Sušnik, 2008).

Evropa pridela trenutno dovolj hrane in je drugi največji svetovni izvoznik hrane. Z dvostranskimi trgovinskimi sporazumi (barcelonski proces, Afrika, Karibi in države Pacifika) se bo količina hrane v Evropi samo še povečevala, s tem pa v prihodnosti lahko pričakujemo večja nihanja na kmetijskih trgih, in to tako glede kakovosti ponujene hrane kot pri cenah proizvodov (Landwirtschaft und Ernaehrung, 2010). Pri tem pa niso upoštevane podnebne spremembe, zaradi katerih se

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razmere na trgu še dodatno lahko zaostrijo, tako glede količine ponujenih proizvodov kot glede cen (Sušnik, 2008).

Slovenija v svoji strategiji med prednostne naloge postavlja stabilno pridelavo varne, kakovostne in čim cenejše hrane ter zagotavljanje prehranske varnosti in čim višje stopnje samooskrbe. Poleg proizvodne funkcije ima kmetijstvo tudi okoljsko in socialno vlogo, to pa odločilno prispeva k vitalnosti podeželja (Nacionalni strateški načrt ..., 2009; Strategija razvoja kmetijstva, 2009). Hrano pridelujemo na 456.214 ha kmetijskih površin, od tega je 62 % travnikov in pašnikov, le 33 % je njiv, preostalo so sadovnjaki (2 %) in vinogradi (3 %) (Statistični urad RS, 2009a). Pri rastlinskih pridelkih pridelamo največ žita, 579.634 ton, 132.342 ton sadja, 78.194 ton zelenjave in 78.955 ton grozdja. Pri živalskih proizvodih pridelamo približno 132.000 ton različnih vrst mesa, 500 milijonov litrov mleka ter približno 127.400 jajc. Kritična stopnja samooskrbe je samo pri zelenjavi, saj je v letu 2008 znašala le 36 % (Poročilo o stanju kmetijstva ...., 2009).

V zadnjih letih svetovni trgi doživljajo velike spremembe, predvsem gre za močno povečanje ponudbe ekološke hrane, v letu 2008 v primerjavi z letom 2007 za 4 bilijone USD. Skupni svetovni trg ekološke hrane pa je bil v letu 2008 vreden 50 bilijonov USD (IFOAM EU Group and FiBL, 2009).

Tudi v Sloveniji se je v zadnjih letih opazno povečalo zanimanje za ekološko hrano, posebej za doma pridelane kmetijske proizvode. V trgovski skupini Mercator so leta 2001 prodali 200 kg ekološko pridelane hrane, v letu 2004 pa že 90 ton (Padar-Lazarevič, 2006). Po raziskavi, opravljeni v letih 2002 in 2003, smo v letu 2003 v Sloveniji pridelali približno 700 ton žita in oljnic, malo manj krompirja (662 t) in zelenjave (661 t), približno 500 kg mesa, 2.300.000 ton mleka, 3.400 ton sadja in 980.000 milijonov jajc (Slabe, 2005).

Na področju trženja trgovske družbe sistematično spremljajo potrošnika (zaznava za izdelek) in ga skušajo usmerjati (učenje in prepričevanje) k nakupu novih izdelkov (motivacija). (Možina in sod., 2010). Prehranske navade slovenskega potrošnika se ne razlikujejo bistveno od navad povprečnega evropskega potrošnika, nekaj razlik je le pri sadju in zelenjavi (Regoršek, 2005).

V raziskavi nas je zanimalo, kako se na dogajanje na trgu odzivajo slovenski kupci, in kateri dejavniki so odločilni pri nakupu živil.

2 MATERIAL IN METODE

Sestavili smo internetni vprašalnik s programom Google form v spletnem brskalniku Google ter ga poslali posameznikom in inštitucijam po vsej Sloveniji. Anketa je bila oblikovana v Biotehniškem centru Naklo v sodelovanju z Biotehniško fakulteto Univerze v Ljubljani. Anketiranje je potekalo v novembru in decembru 2009. Skupaj smo postavili 30 vprašanj, na katera je bilo treba odgovoriti na trditve z eno ali več možnostmi ali se opredeliti glede trditev. Stopnjo strinjanja s trditvami v vprašalniku smo merili s petstopenjsko ocenjevalno lestvico, na kateri je ocena 1 vedno pomenila najmanjše strinjanje, ocena 5 pa največje strinjanje (Likertova lestvica). Za obdelavo podatkov smo uporabili statistični program SPSS 12.

Anketirance smo razvrstili v 3 glavne skupine, in sicer glede na status in spol, kraj bivanja in mesečni dohodek na družinskega člana

Spol in status
Pri tem vprašanju smo anketirance razdelili glede na:
- spol (moški, ženske);
- starost v letih (od 18 do 29, od 30 do 49, od 50 do 64 let in nad 65 let);
- status (samski, poročen brez otrok, poročen z otroki);
- izobrazbo (osnovna šola, srednja šola, višja in visoka šola, magisterij in doktorat);
- vrsto dela (javna uprava, kmetijstvo, predelovalna in storitvena dejavnost, finančni sektor, dijak in študent, nezaposlen, upokojenec).

Kraj bivanja
Anketirance smo razdelili glede na:
- kraj bivanja (do 500 prebivalcev, od 500 do 1.000, od 1.000 do 3.000, od 3.000 do 5.000, od 5.000 do 10.000 in nad 10.000 prebivalcev);
- statistično regijo bivanja (upoštevali smo regionalno razdelitev Slovenije na 11 regij).

Mesečni dohodek na družinskega člana
Anketirance smo razvrstili v šest razredov:
1. nižji razred – do 400 EUR,
2. od 400 do 600 EUR,
3. od 600 do 1.000 EUR,
4. od 1.000 do 1.500 EUR,
5. od 1.500 do 2.000 EUR in
6. nad 2.000 EUR.

3 REZULTATI IN DISKUSIJA

Analitična predstavitev lastnosti vzorca

Na vprašalnik se je odzvalo 249 oseb, od tega 76 % žensk in 24 % moških. Največ jih je bilo poročenih (82 %) in z zaključeno srednjo šolo (80 %). Največ anketirancev prihaja iz javne uprave (58 %), 16 % pa iz predelovalnih in storitvenih dejavnosti. Njihova starost se giblje od 30 do 49 let (61 %), 29 % jih je starih od 50 do 64 let, po 5 % pa do 29 oziroma nad 65 let.

32 % anketirancev živi v manjših naseljih z do 1.000 prebivalci, 26 % anketirancev v večjih naseljih s 1.000 do 5.000 prebivalci, medtem ko v velikih naseljih z več kot 5.000 prebivalci živi 42 % anketirancev. Večina anketirancev prihaja iz Gorenjske statistične regije (51 %) in Osrednje Slovenije (24 %), preostale regije pa jima sledijo s 6 % ali manj odstotki.

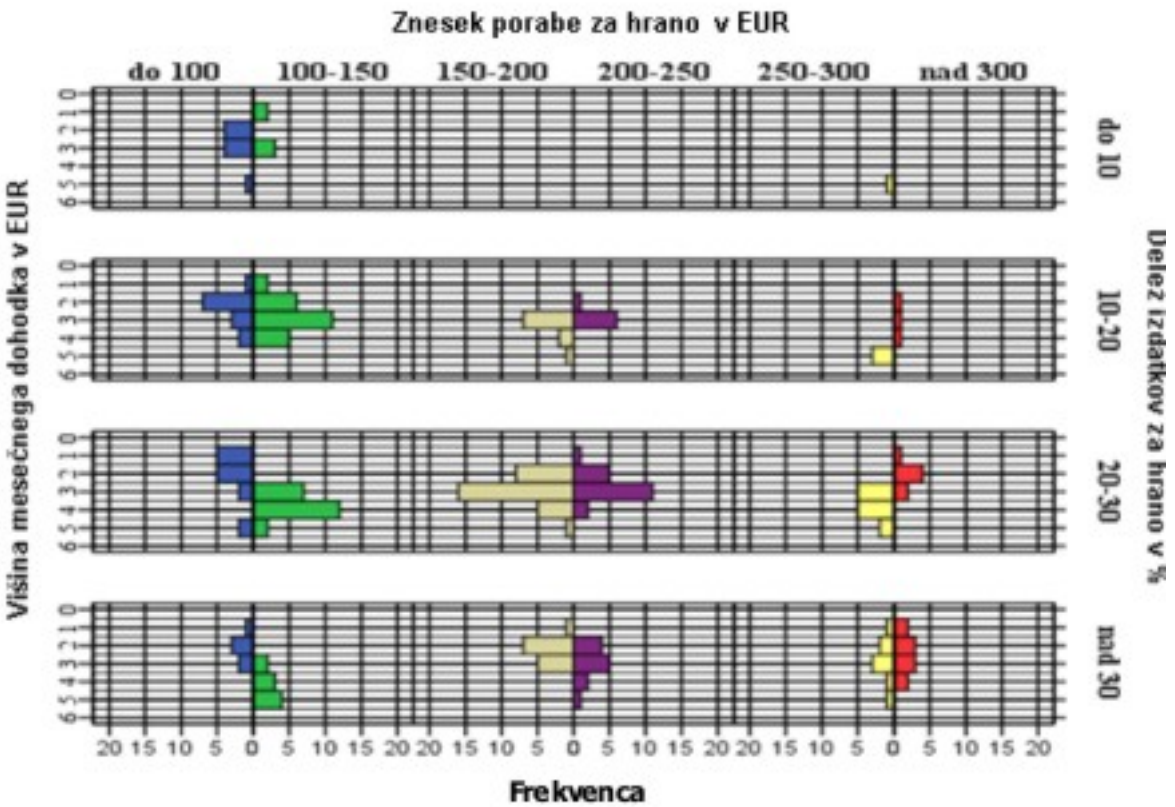
35 % anketirancev je ocenilo, da imajo mesečni dohodek na družinskega člana do 600 EUR, pri 41 % vprašanih se dohodek giblje med 600 in 1.000 EUR, 24 % pa jih ima nad 1.000 EUR mesečnega dohodka.

Višina porabe in delež izdatkov za hrano na družinskega člana

Vprašali smo o mesečni višini porabe hrane na družinskega člana (do 100 EUR, od 100 do 150 EUR, od 150 do 200 EUR, od 200 do 250 EUR, od 250 do 300 in nad 300 EUR).

Iz Slike 1 lahko razberemo, da za hrano do 200 EUR na družinskega člana porabi kar 66 % vprašanih, nad 300 EUR pa le dobrih 8 % vprašanih. Večina anketirancev (43 %) porabi za hrano od 20 do 30 % mesečnega dohodka, 26 % jih nameni za hrano med 10 in 20 % dohodka, medtem ko je 24 % vprašanih pripravljenih odšteti za hrano več kot tretjino dohodka. Le 7 % vprašanih porabi za hrano manj kot 10 % sredstev.

Rezultati naše ankete se značilno razlikujejo od podatkov Statističnega Urada Republike Slovenije (Statistični urad RS, 2009b), ki navaja, da je delež izdatkov za hrano na gospodinjstvo v Sloveniji 13,9 %. Razlog za razhajanje med uradnimi statističnimi in našimi podatki bi lahko našli v tem, da »uradna« statistika v svojih poročilih ne upošteva lastne pridelave hrane.



Legenda: 1- do 400 EUR; 2- od 400 do 600 EUR; 3- od 600 do 1.000 EUR; 4- od 1.000-1.500 EUR; 5- od 1.500 do 2.000 EUR; 6- nad 2.000 EUR

Slika 1: Višina mesečnega dohodka, znesek porabe in delež izdatkov za hrano

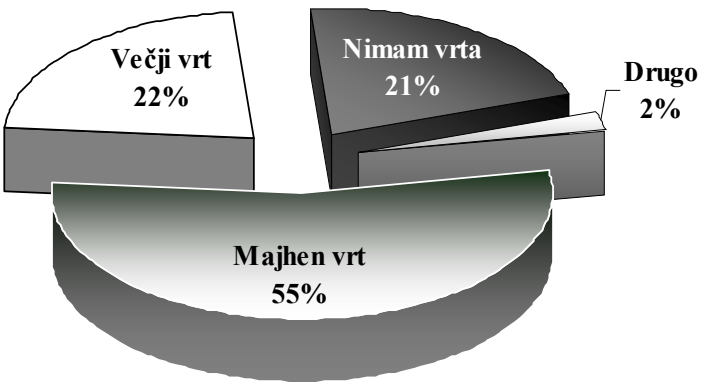
Figure 1: The amount of monthly income, the amount of costs and the portion of food expenditure

Lastna pridelava hrane

Anketirance smo vprašali o pridelovanju hrane na lastnem vrtu (brez vrta, majhen vrt, velik vrt, drugo). Po podatkih Statističnega urada Republike Slovenije je v Sloveniji 22.409 manjših kmetijskih enot, ki so v povprečju velike 22 arov. Med uporabljeno površino prevladujejo sadovnjaki (45 %) in vinogradi (18 %) (Statistični urad RS, 2009a).

Samo 21 % anketirancev hrane ne prideluje doma oziroma nima vrta, kar 55 % jih ima manjši vrt, 22 % pa jih trdi, da večino zelenjave in sadja pridelajo doma (Slika 2). To dejstvo moramo upoštevati pri ocenah izdatkov in cenovne elastičnosti povpraševanja po teh proizvodih (Regoršek, 2005).

Večina anketirancev (Preglednica 1) zelo redko uporablja mineralna gnojila (μ = 1,48) in sintetična fitofarmacevtska sredstva (μ = 1,44), veliko jih uporablja naravna organska gnojila (μ = 4,01) in bistveno manj naravna sredstva za varstvo rastlin (μ = 2,68).



Slika 2: Pridelava hrane na lastnem vrtu

Figure 2: Food production in backyard gardens

Preglednica 1: Uporaba gnojil in fitofarmacevtskih sredstev pri lastni pridelavi hrane

Table 1: The use of fertilizers and plant protection products in individual food production

Dejavniki	Število	Ar. sredina	St. odklon	St. napaka
Uporaba mineralnih gnojil	182	1,48	0,711	0,053
Uporaba organskih gnojil	200	4,01	1,098	0,078
Uporaba sintetičnih fitofarmacevtskih sredstev	171	1,44	0,661	0,051
Uporaba naravnih fitofarmacevtskih sredstev	189	2,68	1,363	0,099

Izračun korelacijske povezave (Preglednica 2) med posameznimi dejavniki (spremenljivkami) nam kaže zmerno statistično značilno odvisnost (0,598) med uporabo mineralnih gnojil in uporabo sintetičnih fitofarmacevtskih sredstev in med uporabo organskih gnojil in uporabo naravnih fitofarmacevtskih sredstev (0,342) ter manjšo, vendar značilno odvisnost med uporabo sintetičnih in naravnih fitofarmacevtskih sredstev (0,210). Pri vprašanju lastne pridelave, ki jo izvaja skoraj 80 % anketirancev, se pojavlja značilna odvisnost (0,206) pri uporabi organskih gnojil; ta gnojila po podatkih ankete uporablja pogosto oziroma stalno 76 % anketirancev. Tudi nekatere druge raziskave kažejo, da vrtičkarji v večji meri uporabljajo naravna sredstva za pridelavo rastlin (Vrtičkarstvo v Ljubljani, 2008).

Preglednica 2: Korelacijska matrika med obravnavanimi spremenljivkami

Table 2: Correlation matrix between considered variables

Spremenljivka	Lastna pridelava	Uporaba min. gnojil	Uporaba org. gnojil	Uporaba sintet. FFS	Uporaba naravnih FFS
Lastna pridelava	1				
Uporaba min. gnojil	–0,026	1			
Uporaba org. gnojil	0,206**	–0,080	1		
Uporaba sintet. FFS	0,071	0,598**	0,138	1	
Uporaba naravnih FFS	0,114	0,065	0,342**	0,210**	1

** Vrednosti so statistično značilne pri p > 0,05
* Vrednosti so statistično značilne pri p > 0,01

Preglednica 3: Srednje vrednosti dejavnikov, ki vplivajo na nakup živil

Table 3: Mean values for factors influence the purchase of foodstuff

Dejavniki	Število	Aritm. sredina	Stand. odklon	Stand. napaka
Supermarket	246	4,56	0,696	0,044
Specializirana trgovina	201	3,00	1,107	0,078
Ekološka trgovina	217	2,82	1,310	0,089
Tržnica	223	3,13	1,281	0,086
Kmetija	213	2,59	1,254	0,086
Dostava na dom	201	1,58	0,892	0,063
Internet	206	1,22	0,660	0,046
Slovenski proizvod	246	4,01	0,848	0,054
Ekološka hrana	249	3,90	1,015	0,064
Blagovna znamka	246	3,61	1,050	0,067
Videz izdelka	233	2,48	1,005	0,066
Poznan izdelek	246	4,39	0,659	0,042
Deklaracija	243	3,56	1,000	0,064
Promocija izdelka	234	2,15	0,967	0,063
Cena izdelka	234	2,29	1,045	0,068

Dejavniki pri nakupu živil

Anketiranci, od tega je bilo 76 % žensk, so odgovarjali po Likerjevi lestvici (od 1 do 5) kje kupujejo prehranske izdelke in kateri dejavniki vplivajo na njihov nakup (Preglednica 3).

Večina anketirancev (94 %) kupuje hrano od enkrat do štirikrat na mesec v supermarketih (μ = 4,56), v ekološki trgovini pa jih kupuje le 36 % (μ = 2,82). Po mnenju Brelih in sod. (2006) trend nakupovanja v večjih trgovskih centrih raste že nekaj let. Še leta 1991 je npr. po raziskavi, ki jo je opravil Pavlovič (2001), v

celjski regiji 250 anketirancev dajalo prednost manjši trgovini (več kot 48 %).

Zelo nizko je ocenjen nakup prek interneta, kar 88 % anketirancev hrane namreč ni še nikoli kupovalo prek spleta. Prav tako v glavnem ne uporabljajo dostave na dom, saj se le 3 % anketirancev odloča za pogosto in stalno dostavo hrane na dom. Pri nakupu živil anketiranci dajejo prednost poznanemu (μ = 4,39) in slovenskemu proizvodu (μ = 4,01) ter ekološki hrani (μ = 3,90), nizko pa ocenjujejo vpliv promocije (μ = 2,15), cene (μ = 2,29) in videza izdelka (μ = 2,48). Da cene izdelka anketiranci ne ocenjujejo kot najpomembnejšega dejavnika nakupa, potrjujejo tudi raziskave Pavlovič (2001), Verhovec-Kajtner (2003) ter Ogorevc-Račič in sod. (2010), medtem ko sta Hribar in Bojnec (2010) v svoji raziskavi prišla do nasprotnih rezultatov.

Z multivariantno faktorsko analizo smo skušali ugotoviti, ali obstajajo skupni dejavniki (faktorji), s katerimi lahko pojasnimo odvisnost med dejavniki, ki vplivajo na nakup živil (preglednica ni prikazana). Iz korelacijske matrike smo razbrali, da je največja povezanost med ekološko trgovino in nakupom ekološke hrane (0,529), med ekološko hrano in blagovno znamko (0,508), med promocijo in videzom izdelka (0,494) ter med specializirano in ekološko trgovino (0,438). Ekološka trgovina je povezana s tržnico (0,467), blagovno znamko (0,351) in kmetijo (0,272), negativno pa s ceno (–0,241) in promocijo izdelka (–0,238). Kupovanje na tržnici je povezano z ekološko hrano (0,294), kmetijo (0,235) in blagovno znamko (0,204). Kupovanje na kmetiji je povezano z ekološko hrano (0,307) in dostavo na dom (0,223). S slovenskim proizvodom so povezani blagovna znamka (0,375), ekološka hrana (0,359) in videz izdelka (0,228). Vpliv cene na nakup izdelkov je v večini primerov negativen, pozitivno povezanost zasledimo le pri promociji izdelka (0,224) in pri nakupu v supermarketu (0,197).

Z namenom, da bi ugotovili, v kakšni soodvisnosti so izbrani dejavniki oziroma spremenljivke, smo uporabili statistično metodo PCA. Pri metodi PCA smo za izračun glavnih komponent uporabili korelacijsko matriko, ki je nastala iz 11 osnovnih merjenih spremenljivk. Iz korelacijske matrike (Preglednica 5) je v paru razvidno, kateri dve spremenjivki sta korelirali pozitivno, nista korelirali ali sta korelirali negativno. Bartlettov test je pokazal, da ničelno domnevo, ki pravi, da je korelacijska matrika enaka identiteti, zavrnemo brez tveganja (p = 0,00) in je torej uporaba metode PCA utemeljena. Tudi vrednost mere KMO (0,674) je potrdila to trditev. Preverjali smo več metod, metodo glavnih osi (Principal Axis Faktoring), metodo največjega verjetja (Maksimum Likelihood), poševno rotacijo (Direct Oblim), pri kateri so faktorji odvisni med seboj, in pravokotno rotacijo (Varimax), pri kateri so rotirani faktorji med seboj neodvisni.

Iz Preglednice 4 lahko razberemo, da so glavne komponente urejene po padajoči velikosti variance. Prva glavna komponenta je določena tako, da pojasni kar se da velik del celotne variance (26,6 %) osnovnih spremenljivk. Druga glavna komponenta je določena tako, da je neodvisna od prve in pojasni kar se da velik del še nepojasnjene variance (16,6 %) itd. Velja pravilo, da če so osnovne spremenljivke dovolj povezane, pojasnijo »pozne« glavne komponente majhen delež celotne variance in jih lahko zanemarimo.

Preglednica 4: Lastna vrednost, odstotek pojasnjene variabilnosti in odstotek celotne pojasnjene variabilnosti za 10 glavnih komponent

Table 4: Eigenvalue, % of variance explained and % of total variance explained for 10 principal components

	F1	F2	F3	F4	F5	F6	F7	F8	F9	F10	F11
Lastna vrednost	2,924	1,831	1,083	1,027	0,970	0,754	0,718	0,558	0,435	0,377	0,323
Variabilnost (%)	26,586	16,646	9,843	9,339	8,819	6,857	6,526	5,069	3,951	3,428	2,936
Skupno (%)	26,586	43,252	53,075	62,414	71,234	78,091	84,617	89,685	93,636	97,064	100,000

Pri analizi z metodo glavnih komponent (Preglednica 5) smo prvi faktor imenovali **ozaveščenost**, v največjem deležu pa se kaže kot ekološka trgovina (76,7 %) in ekološka hrana (74,9 %). V negativnem delu pa je kupovanje hrane v supermarketu (–43,9 %). Drugi faktor smo poimenovali **marketing**, kaže pa se v glavnem v videzu (79,9 %) in promociji izdelka (75,0 %). Tretji faktor v tem delu ni izrazit, v 48,1 % je pri nakupovanju na kmetiji ter v 43,7 % pri ceni izdelka, zato ga nismo poimenovali.

Pri metodi največjega zaupanja je pri prvem faktorju ozaveščenosti izrazita ekološka trgovina z 99,9 %, medtem ko se preostali dejavniki gibljejo pri manjšem obsegu. Pri drugem faktorju marketingu se dejavnika samo zamenjata, na prvo mesto pride promocija izdelka s 73,1 %, temu pa sledi videz izdelka s 70,2 %. Tretji faktor smo poimenovali **zaupanje**, kaže pa se v dejavniku ekološka hrana (54,5 %) in blagovni znamki (52,4 %), v nekoliki manjši meri pa tudi v slovenskem proizvodu (42,0 %).

Pri poševni in pravokotni rotaciji ostaja pri prvem faktorju, ozaveščenosti, v ospredju ekološka trgovina (več kot 98 %). Pri drugem faktorju, marketingu, sta pri poševni rotaciji v ospredju promocija izdelka (80,7 %) in videz izdelka (71,2 %), pri tretjem faktorju, zaupanju sta na prvem mestu blagovna znamka (69,5 %) in ekološki proizvod (66,6 %). Pri pravokotni rotaciji pride na drugo mesto zaupanje, tretji faktor pa je marketing. Pri rotacijah se pri marketingu pojavi s približno 20 % še cena izdelka.

Preglednica 5: Analiza glavnih komponent*

Table 5: Principal component analysis

	Metoda glavnih komponent			Metoda največjega zaupanja			Metoda največjega zaupanja z rotacijsko metodo Oblimin s Kaiserjevo normalizacijo (poševna rotacija)								
	Component Matrix[a] (1)			Factor Matrix[a] (2)			Strukturna matrika (Pattern Matrix[a] (3)			Strukturna matrika (Structure Matrix) (4)			Rotacijska faktorska analiza (Rotated Factor Matrix)[a] (5)		
	Faktorji			Faktorji			Faktorji			Faktorji			Faktorji		
	1	2	3	1	2	3	1	2	3	1	2	3	1	2	3
Supermarket	**–0,439**	**0,117**	–0,104	**–0,366**	0,017	–0,102	**–0,333**	0,063	–0,055	**–0,373**	0,133	–0,214	**–0,341**	–0,137	0,097
Special. trgovina	**0,428**	0,085	0,185	**0,413**	0,111	0,025	**0,439**	0,088	–0,003	**0,419**	–0,005	0,209	**0,411**	0,111	0,041
Ekološka trgovina	**0,767**	–0,124	0,289	**0,999**	0,000	–0,001	**–0,572**	–0,023	–0,148	0,**990**	–0,247	0,359	**0,982**	0,125	–0,138
Tržnica	0,**569**	–0,172	0,401	**0,434**	-0,095	0,151	**0,370**	–0,159	0,078	**0,441**	–0,236	0,254	**0,393**	0,167	–0,195
Kmetija	0,402	0,130	**0,481**	**0,242**	0,101	0,151	**0,195**	0,034	0,159	**0,264**	–0,005	0,253	**0,223**	0,205	0,017
Slovenski proizvod	0,355	**0,555**	–0,389	0,106	0,347	**0,420**	–0,048	0,172	**0,543**	0,177	0,189	**0,523**	0,068	**0,518**	0,189
Ekološka hrana	**0,749**	0,093	–0,155	0,482	0,058	**0,545**	0,246	–0,162	**0,549**	0,544	–0,206	**0,665**	0,390	**0,592**	–0,173
Blagovna znamka	**0,580**	0,481	–0,257	0,300	0,383	**0,524**	0,110	0,162	0,**640**	0,384	0,148	**0,695**	0,245	**0,651**	0,165
Videz izdelka	–0,166	**0,799**	0,286	–0,149	**0,702**	–0,092	–0,012	**0,712**	0,108	–0,109	**0,716**	0,112	–0,066	0,115	**0,711**
Promocija izdelka	–0,342	**0,750**	0,219	–0,242	**0,731**	–0,249	–0,028	**0,802**	–0,044	–0,218	**0,807**	–0,047	–0,129	–0,036	**0,798**
Cena izdelka	**–0,555**	0,001	**0,437**	–0,213	0,042	**–0,450**	0,005	**0,216**	**–0,457**	–0,260	**0,208**	**–0,452**	–0,132	**–0,438**	**0,203**

* Vrednosti v mastnem tisku so statistično značilne pri p > 0,05; (1) Extraction Method: Principal Component Analysis: a - 3 components extracted; (2) Extraction Method: Maximum Likelihood: a - 3 factors extracted, 6 iterations required; (3) Extraction Method: Maximum Likelihood, Rotation Method: Oblimin with Kaiser Normalization (Rotation converged in 6 iterations); (4) Extraction Method: Maximum Likelihood, Rotation Method: Oblimin with Kaiser Normalization; (5) Extraction Method: Maximum Likelihood. Rotation Method: Varimax with Kaiser Normalization: a - Rotation converged in 4 iterations.

4 SKLEPI

Raziskava je pokazala, da potrošniki v Sloveniji podobno kot v Evropi vse več posegajo po ekološko pridelani hrani, pri tem pa slovenski potrošniki bolj zaupajo v slovenske izdelke. Velikim trgovskim družbam je uspelo pridobiti kupce za nakupovanje v večjih trgovskih centrih, s tem pa so spravile v podrejen položaj proizvajalce, ki jim dobavljajo izdelke po najnižjih možnih cenah. Pridelovalci in predelovalci se bodo morali bolje organizirati in ponudbo bolj prilagoditi željam potrošnikov. Pri tem morajo paziti na kakovost in ceno izdelka.

Za druge načine prodaje je sedaj najboljša priložnost pri prodaji ekološke hrane, za katero trg v Sloveniji še ni razvit. Proizvajalci uvajajo neposredne načine trženja (dostavo na dom ali prodajo na domu), pri katerih bodo lahko dosegli višje cene. V zvezi s tem se kaže priložnost tudi za podjetnike, ki bi prodajali v manjših ekoloških trgovinah.

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CONTENT ANALYSIS OF THE PAPERS IN THE ACTA AGRICULTURAE SLOVENICA

VSEBINSKA OBDELAVA PRISPEVKOV V ACTA AGRICULTURAE SLOVENICA let. 95 št. 2

Tomaž BARTOL[a], Karmen STOPAR[b],

SUBJECT INDEX BY AGRIS CATEGORY CODES

VSEBINSKO KAZALO PO SKUPINAH ZNANJA (PREDMETNIH KATEGORIJAH)

SUBJECT INDEX BY AGROVOC DESCRIPTORS

PREDMETNO KAZALO PO DESKRIPTORJIH AGROVOC

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b B.Sc., M.Sc., ibid

Recenziji

Jože MAČEK

Dušan Čamprag: **Razmnožavanje štetočina ratarskih kultura u Srbiji i susednim zemljama tokom 20. veka.** Srpska akademija nauka i umetnosti, Ogranak u Novom Sadu. Novi sad 2007, 348 strani.

V kontinentalnih, poleti toplih območjih, kakor je npr. pri nas Panonska nižina so, kot je znano, škodljivci v fitomedicini poglaviten problem. Dodatno omenjeni problem otežuje še večinoma monokulturni način rastlinske pridelave v teh pokrajinah, ki ga neizbežno spremljajo talni škodljivci. Seveda so v takih razmerah načini zatiranja poglavitnih vrst škodljivcev na prvem mestu. Toda pri tem imajo odločilno vlogo načini razmnoževanja teh škodljivcev. Tej sila zapleteni problematiki je namenil pričujočo knjigo dolgoletni profesor aplikativne entomologije na agronomski fakulteti v Novem sadu, akademik prof. dr. Dušan Čamprag.

V uvodu je omenjeno, da je v knjigi obdelano 160 vrst škodljivcev na poljščinah. V okviru razreda žuželk so zastopani Orthoptera s 5,3 %, Homoptera z 12,6 %, Heteroptera s 7,9 %, Thysanoptera z 2,0 %, Coleoptera z 32,4 %, Lepidoptera z 28,5 %, Hymenoptera z 1,7 % in Diptera z 8,2 %. Posebej so obdelane razmere v Srbiji, Bolgariji, Romuniji in na Hrvaškem, ki mejijo z Vojvodino.Iz teh držav so navedeni tudi avtorji, ki so pisali o tej problematiki. V 1. poglavju so opisani dejavniki, ki opredeljujejo nihanje populacij škodljivcev poljščin. Na prvem mestu so opisani ekološki dejavniki, nato sledijo abiotični dejavniki. Znotraj njih je podrobno opisan vpliv klimatskih elementov, predvsem toplote s številnimi navedbami zahtev, ki jih imajo škodljivci. Nato sledi vpliv vlage, nato pa kompleksno delovanje toplote in vlage. Opisan je vpliv vetra in astronomskih dejavnikov, predvsem vpliv Sonca. Opis slednjega vpliva je posebno zanimiv, ker se v literaturi najde le redko. Naposled sledi še opis kako na škodljivce vpliva zemljišče. Med biotičnimi dejavniki je obdelan vpliv prehrane na škodljivce, delovanje naravnih sovražnikov, delovanje človeka (struktura setve, kolobar, prostorska izolacija, velikost polja, sorte, vpliv gnojil, vpliv fitofarmacevtskih sredstev, plevelov, namakanje in osuševanje, obdelava zemljišča, spravljanje pridelkov).

Sledi kratek oris populacijskih teorij. Tu je nakazano, da večina ekologov soglaša, da številčnost škodljivcev opredeljujejo vplivi iz sedmih skupin: 1. klime, 2. količine razpoložljive hrane, 3. konkurence v okviru vrste, 4. konkurence za hrano in prostor, 5. delovanja parazitoidov, 6. delovanja predatorjev, 7. spremembe genetskih lastnosti populacije škodljivcev. Po kompleksih dejavnikov, ki jim posamezni avtorji populacijskih teorij pripisujejo največji vpliv, se razlikujejo fizikalne teorije (odločilen vpliv toplote in vlage), biotične teorije (odločilni vpliv imajo biotični dejavniki, zlasti tekmovalnost), sintetične teorije (ki upoštevajo vse dejavnike, ki vplivajo na nihanje populacije), parazitska teorija (največji vpliv imajo naravni sovražniki), nadalje obstajajo še biocenotska teorija, klimatska teorija in teorija prenaseljenosti. Najboljša je Schwerdtfegerjeva sintetična teorija gradocenov iz šestdesetih let prejšnjega stoletja, ki skuša združevati vplive vseh naštetih dejavnikov. Zelo izčrpno so opisane možnosti za razmnoževanje škodljivcev poljščin v raznih območjih Srbije, kar tukaj ne kaže podrobneje predstavljati. Sila zanimiv je pregled hkratnih množičnih pojavov nekaterih škodljivcev v Srbiji in sosednih državah. Širše zanimiv je nadalje pojav introduciranih migratornih škodljivcev na omenjenem velikem območju. Naj med škodljivci poljščin navedemo samo nekatere: *Lixus scabricollis, Tanymecus dilaticollis, Leptinotarsa decemlineata, Diabrotica virgifera virgifera, Scrobipalpa ocellatella, Loxostege sticticalis, Grapholitha delineana, Autographa gamma, Agrotis ipsilon, Spodoptera exigua, Helicoverpa armigera, Vanessa cardui. Eurygaster integriceps* in *Pemphigus integriceps.* Med škodljivci sadnega drevja je omenjen *Quadriaspidiotus perniciosus,* polifagne vrste slinarjev *Arion rufus in A. lusitanicus* ter zaradi izkopavanja rovov pomembna škodljivka obrežij potokov, rek in kanalov, pižmovka *Ondatra zibethica.*

Glede na globalno otoplitev sveta zaradi toplogrednih plinov se obetajo tudi nevarne gradacije škodljivcev. Tem, razen nekaj izjem, toplota nasploh zelo prija, posebej pri procesih razmnoževanja. Tej tematiki je namenjeno obsežno, aktualno in glede literature povsem ažurirano poglavje.

Sledi najobsežnejše poglavje o škodljivcih poljščin. Med poljščinami so zajeta strna žita, koruza, sladkorna pesa, sončnice, soja, oljna ogrščica, tobak, konoplja, hmelj, črna detelja in lucerna. Pri vsaki poljščini so navedeni vsi pomembni in manj pomembni škodljivci,

Nato sledi podroben opis vseh pomembnejših škodljivcev iz razreda žuželk, ki ga tu ni mogoče predstavljati. V tem poglavju pa so vključeni tudi škodljivi organizmi iz drugih skupin živalskega sveta. Tako so obdelane ogorčice (*Heterodera avenae*, *H. schachtii, Anguina tritici* in *Ditylenchus dipsaci*), polž (*Deroceras agreste*) in pršici (*Tetranychus atlanticus* in *T. urticae*), sesalci (*Cricetus cricetus, Microtus arvalis, Mus musculus hortulanus. Apodemus silvaticus, Citellus citellus* in še nekaj vrst, ki so se na obravnavanem območju pojavile v obdobju od 1965 do 1989. Nato sledi poglavje z navedbami literature, ki obsega kar 31 strani. Med njimi pa skoraj ni slovenskih avtorjev, ker se pri nas s tematiko škodljivcev poljščin ni nihče znanstveno ukvarjal. Sledi kratek povzetek v angleškem jeziku in register latinskih imen škodljivih organizmov.

V tej recenziji smo lahko le shematično prikazali bogato vsebino te knjige, njene prave vrline pa se lahko odkrijejo šele pri podrobnejšem študiju. Posebej dragocen je seznam literature, ki nudi dobro izhodišče za poglobljeno raziskovanje škodljivcev kmetijskih rastlin na obravnavanem območju.

Jože Maček

Pero Štrbac, Ragheb Thalji, Bruno Toscano: **Homoptera Sternorrhyncha Aphidodidea. Ekonomski važnije vrste vaši u biljnoj proizvodnji.** Univerzitet u Novom Sadu, Poljoprivredni fakultet Novi sad. Novi Sad: 2009, 212 strani.

Kot izhaja iz naslova založnice je knjiga sicer zamišljena kot monografija, bo pa z lahkoto služila tudi kot dopolnilni univerzitetni učbenik entomologije za poglavje o listnih ušeh študentom agronomije, biologije in sorodnih disciplin pri diplomskem in podiplomskem študiju. Zaradi zanimivega prikaza in številnih ilustracij pa bo dostopna tudi bolj razgledanim neposrednim gojiteljem kmetijskih rastlin in številnim entomološkim amaterjem.

Knjiga je po zapisu recenzentke prof dr. Tatjane Kereši razdeljena v deset poglavij in sicer: Uvod, Sistematika Homoptera, Morfologija listnih uši, Načini lova in preučevanja listnih uši, Ocena napadenosti in škodljivosti listnih uši pri poljščinah, Ocena napadenosti in škodljivosti listnih uši pri vrtninah, Ocena napadenosti in škodljivosti listnih uši pri sadnem drevju, jagodičevju in vinski trti, Pregled listnih uši s kratkim opisom, Metode zatiranja listnih uši, Literatura in Dodatek.

V poglavju Sistematika Homoptera je na podlagi podatkov več domačih piscev prikazana razdelitev reda enakokrilcev, ki je preprosta in praktična za uporabo, s kratkim opisom podredov in družin. V poglavju o Morfologiji listnih uši je opisan zunanji videz krilatih in nekrilatih osebkov, poudarjene so poglavitne značilnosti za identifikacijo vrst, prikazan pa je tudi popoln ali nepopoln ciklus razvoja. Nato sledi opis metod lova in preučevanja uši na rastlinah, njihovo spravljanje z rastlin, štetje, spremljanje leta in preučevanje listnih uši kot vektorjev virusov. V naslednjih treh poglavjih je pri treh skupinah kmetijskih rastlin prikazano ocenjevanje napadenosti in škodljivosti (strna žita, koruza, sladkorna pesa, sončnice, tobak, mak in hmelj, krompir, zelje, grah, nizki in visoki fižol, kumare, dinje in lubenice, jablana, hruška, sliva, breskev in marelica, vinska trta). Najobsežnejše je seveda poglavje Pregled listnih uši s kratkim opisom, kar 96 strani, ki zajema gospodarsko najpomembnejše vrste v rastlinski pridelavi, kakor tudi nekatere druge manj znane vrste, katerih pojav je mogoč v Panonski nižini. Pri 52 vrstah listnih uši so prikazani razširjenost, značaj in pomen, morfologija in taksonomija, na kratko so razloženi tudi razvojni krog, škodljivost in načini zatiranja. To poglavje je tudi bogato ilustrirano z risbami, kjer so ponazorjene morfološke značilnosti listnih uši. S tem je omogočeno sorazmerno lahko in hitro ter dovolj zanesljivo razpoznavanje posameznih vrst listnih uši.

V poglavju o literaturi je navedeno 206 enot, od tega 98 domačih in 108 tujih in sicer le tistih, ki v glavnem besedilu niso bile posebej omenjene. Od slovenskih avtorjev sta navedeni le dve objavi rajnega prof. dr. Franca Janežiča in proti pričakovanju angleška poljudna strokovna knjiga A. Brooksa in A. Halsteada, ki sem jo podpisani prevedel leta 1985 in je v založbi Kmečkega glasu izšla pod naslovom Bolezni in škodljivci vrtnih rastlin. Bolezni, škodljivci in motnje pri sadnem drevju, vrtninah, okrasnih rastlinah in tratah.

Kot sklep lahko povzamemo, da je avtorjem uspelo napisati znanstveno zanimivo in široko uporabno knjigo. Agronomski fakulteti v Novem Sadu pa velja izreči pohvalo za njen založniški podvig.

Jože Maček

NAVODILA AVTORJEM

Prispevki

Sprejemamo izvirne znanstvene članke, predhodne objave in raziskovalne notice s področja agronomije, hortikulture, rastlinske biotehnologije, raziskave živil rastlinskega izvora, agrarne ekonomike in informatike ter s sorodnih področij v slovenskem, angleškem in nemškem jeziku, znanstveno pregledne članke samo po poprejšnjem dogovoru. Objavljamo prispevke, podane na simpozijih, ki niso bili v celoti objavljeni v zborniku simpozija. Če je prispevek del diplomske naloge, magistrskega ali doktorskega dela, navedemo to in tudi mentorja na dnu prve strani. Navedbe morajo biti v slovenskem in angleškem jeziku.

Pri prispevkih v slovenskem jeziku morajo biti preglednice, grafikoni, slike in priloge dvojezični, povsod je slovenščina na prvem mestu. Naslovi grafikonov in slik so pod njimi. Slike in grafikoni so v besedilu. Priloženi morajo biti tudi jasno označeni izvirniki slik. Na avtorjevo željo jih vračamo, s tem da je želja pisno sporočena ob oddaji gradiva in ponovno v teku 30 dni po izidu. Latinske izraze pišemo ležeče. V slovenščini uporabljamo decimalno vejico, v angleščini decimalno piko. Prispevki v angleščini morajo imeti povzetek v slovenščini in obratno. Prispevki v nemščini morajo imeti tudi povzetka v slovenščini in angleščini.

Prispevki naj bodo strnjeni, kratki, praviloma največ 12 strani. Uporabljamo Microsoft Word 97 (Windows); pisava Times New Roman, velikost strani 16,2 x 23,5 cm, velikost črk besedila 10, v obsežnih preglednicah je lahko 8; izvlečki in metode dela Arial velikost 8, levi in desni rob 2,1 cm, zgornji rob 1,3 cm, spodnji rob 1,6 cm,

Prva stran

Na prvi strani prispevka na desni strani označimo vrsto prispevka v slovenščini in angleščini, sledi naslov prispevka, pod njim avtorji. Ime avtorjev navedemo v polni obliki (ime in priimek). Vsak avtor naj bo označen z indeksom, ki ga navedemo takoj pod avtorji, in vsebuje polni naslov ustanove ter znanstveni in akademski naslov; vse v jeziku prispevka. Navedemo sedež ustanove, kjer avtor dela. Če je raziskava opravljena drugje, avtor navede tudi sedež te inštitucije. Na željo avtorjev bomo navedli naslov elektronske pošte.

Pod naslovi avtorjev je datum prispetja in datum sprejetja prispevka, ki ostaneta odprta. Sledi razumljiv in poveden izvleček z do 250 besedami. Vsebuje namen in metode dela, rezultate, razpravo in sklepe. Sledijo ključne besede.

Izvlečku v jeziku objave sledi naslov in izvleček s ključnimi besedami v drugem jeziku.

Viri

V besedilu navajamo v oklepaju avtorja in leto objave: (priimek, leto). Če sta avtorja dva, pišemo: (priimek in priimek, leto), če je avtorjev več, pišemo: (priimek in sod., leto). Sekundarni vir označimo z "navedeno v" ali "cv.". Seznam virov je na koncu prispevka, neoštevilčen in v abecednem redu. Vire istega avtorja, objavljene v istem letu, razvrstimo kronološko z a, b, c. Primer: 1997a. Navajanje literature naj bo popolno: pri revijah letnik, leto, številka, strani; pri knjigah kraj, založba, leto, strani. Za naslove revij je dovoljena uradna okrajšava, za okrajšanimi besedami naj bodo vedno pike. Navedbo zaključimo s piko. Za primere upoštevajte objave v Zborniku BFUL.

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Avtorji prispevke oddajo v dveh izvodih, enega z dvojnim razmakom med vrsticami in največ 35 vrst na strani, in na disketi. Priložijo tudi izjavo s podpisi vseh avtorjev, da avtorske pravice v celoti odstopajo reviji.

Prispevke recenziramo in lektoriramo. Praviloma pošljemo mnenje prvemu avtorju, po želji lahko tudi drugače. Če uredniki ali recenzenti predlagajo spremembe oz. izboljšave, vrne avtor popravljeno besedilo v 10 dneh v dveh izvodih, enega z dvojnim razmakom. Ko prvi avtor vnese še uredniške pripombe, odda popravljeno besedilo v enem izvodu in na disketi ter vrne izvod z uredniškimi popravki.

Prispevke sprejemamo vse leto.

NOTES FOR AUTHORS

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Papers in Slovenian language should have tables, graphs, figures and appendices in both languages, Slovenian language being the first. Titles of graphs and figures are below them. Figures and graphs are part of the text. Clearly marked origins of figures should be added; they can be returned if author desires. Latin expressions are written in italics. Decimal coma is used in Slovenian and decimal point in English. Papers in English should contain abstract in Slovenian and *vice versa*. Papers in German should contain abstracts in German, Slovenian and English.

The papers should be condensed, short and usually should not exceed 12 pages. Microsoft Word 97 (Windows) should be used, fonts Times New Roman, paper size 16.2 x 23.5 cm, font size in main text 10; in large tables size 8 could be used, abstracts and material and methods Arial size 8, right and left margin 2.1 cm, upper margin 1.3 cm and lower margin 1.6 cm.

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The type of the paper should be indicated on the first page on the right side in Slovenian and English language following by title of the paper and authors. Full names of authors are used (first name and surname). Each name of the author should have been added an index, which is put immediately after the author(s), and contains address of the institution and academic degree of the author, in the language of the paper. The address of the institution in which the author works is indicated. If the research was realised elsewhere, the author should name the headquarters of the institution. E-mail is optional.

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