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**Agris category code:** f01, f30

## Genetic diversity and correlation estimates for grain yield and quality traits in Kosovo local maize (*Zea mays* L.) populations

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### ABSTRACT

The aim of the presented investigation was to estimate the genetic diversity, and correlation analysis among yield and quality traits in twenty local maize populations. The experiment was based on randomized complete block design (RCBD) with three replications. In the study we compared grain yield, and quality traits such as protein, oil and starch content in grain. The results showed that there were significant differences among populations. The overall mean grain yield was  $79.33 \text{ g plant}^{-1}$  with the highest grain yield in population GBK-7 ( $105.13 \text{ g plant}^{-1}$ ). The protein and oil contents ranged between 11.02 to 13.02% and 2.56 to 5.57%, respectively. The starch content varied from 68.58 to 70.92%. The first two canonical discriminant functions were significant at  $p < 0.01$ . It is important to point out the great relevance of the first two discriminant functions justifying 95.80% of the variability among populations. There were also big differences regarding phenotypic correlations. The study suggests that the quality traits are phenotypically and genotypically highly variable and therefore very useful for breeding process

**Key words:** local maize populations, protein, oil, starch

### IZVLEČEK

### GENETSKA RAZNOLIKOST IN KORELACIJE MED PRIDELOKOM ZRNJA IN KAKOVOSTNIMI LASTNOSTMI ZRNJA LOKALNIH POPULACIJ KORUZE (*Zea mays* L.) NA KOSOVEM

Namen raziskave je bil proučiti genetsko raznolikost in korelacijske med pridelkom in nekaterimi kakovostnimi lastnostmi zrnja (vsebnost beljakovin, olja, škroba in pepela v zrnju) 20 lokalnih populacij koruze na Kosovem. Poskus je bil postavljen po metodi naključnih blokov v 3 ponovitvah. Za vse lastnosti so ugotovljene statistično značilne razlike med proučevanimi populacijami. Povprečni pridelek zrnja vseh populacij je znašal  $79,33 \text{ g rastlino}^{-1}$ , najvišji pridelek je imela populacija GBK-7 ( $105,13 \text{ g rastlino}^{-1}$ ). Vsebnost beljakovin se je gibala med 11,02 in 13,02 %, olja med 2,56 in 5,57 % in škroba med 68,58 in 70,92 %. Z diskriminantno analizo je ugotovljeno, da sta prvi dve komponenti pojasnili 95,80 % varabilnosti med populacijami. Med nekaterimi lastnostmi so za proučevane populacije ugotovili signifikantne korelacije. Glede na veliko genetsko variabilnost smatramo, da bi proučevane lokalne populacije lahko bile koristne v nadalnjem žlahtnjenju genotipov koruze z dobrimi kakovostnimi lastnostmi zrnja.

**Ključne besede:** lokalne populacije koruze, Kosovo, pridelek zrnja, beljakovine, olje, škrob

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## 1 INTRODUCTION

Maize (*Zea mays* L.) is considered to be a major source of food for livestock and humans due to its relatively high content of proteins, oil, starch and some other important vitamins such as vitamin B and B12. Yellow maize can provide a substantial amount of vitamin A, and the maize germ is rich in vitamin E (Okoruwa *et al.*, 1996).

The maize crop is considered to be an integral part of Kosovo's agricultural production system and has a potential to compete with other crops. In order to have access to the global market, there is a need to improve the yield quality with judicious use of inputs (Saleem *et al.*, 2008).

Maize is commonly used in animal feed as an energy source for its high starch content (Oliveira *et al.*, 2006). Several million people, particularly in developing countries, derive their protein and calorie requirements from maize. Some of the most important traits of interest in the maize market are protein and oil content. The protein content (PC) is a quantitative trait and several studies have pointed out that there is a great number of genes involved in its control (Mittelman *et al.*, 2003). Protein is an expensive but necessary constituent of both food and feed. Grain protein quantity in ordinary maize is relatively low ( $80\text{--}110 \text{ g kg}^{-1}$ ) and of poor quality because of low levels of amino acids, lysine and tryptophan (Bjarnason and Vasal, 1992). Maize protein which ranges from 6 to 12% is regarded to be inferior because it is low in lysine and tryptophan (FAO, 1988). This may cause nutritional deficiencies when maize is used as an exclusive protein source, without the addition of supplements (Glover and Mertz, 1987). Some proteins in maize have anti-fungal qualities. The basis of resistance to fungal infection may lie with protein content, type, or distribution in the tissue (Guo *et al.*, 1997).

The existence of genetic variability and the prospect of selection for protein content in maize have been demonstrated in several studies (Miću *et al.*, 1995). Most of the oil is in the germ of the kernel. Oil and protein contents have been increased to levels almost twice as high as those of the original grain (Jugenheimer, 1961). High-oil corn (HOC) inbreds were first developed in 1896 and some hybrids containing 6–8% oil (Haumann, 1996), and affected by the size of embryo, maturity, and position of the kernel on the ear (Lambert 2001). Breeding studies in maize to enhance fatty acid composition started in 1970's; however, they have not continued. Research in this area dealt with different aspects of grain quality, focusing mainly on determination of the grain quality traits and characterization of maize genotypes in terms of fatty acid composition (Egesel *et al.*, 2011).

The major use of HOC is in livestock feed because of its higher metabolizable energy value (Weber, 1987). The developing countries have more areas dedicated to maize cultivation than developed countries, but yield in the latter is about four times higher. While most production in developing countries is used for human consumption, in developed world, it is mainly used for animal feed and industry (FAO, 1988). As indicated earlier, maize has three possible uses such as food, feed for livestock and raw material for industry.

Maize is one of the most important grain crops in Kosovo, with over 60,000 hectares in production (MAFRD, 2010) where the area under local maize populations is more than 5%. In most cases, the cultivation of local populations is associated with their adaptability to specific environments and with their nutritional value. It is frequently used for human consumption as corn bread that can be consumed together with milk.

The main objective of the present study was to describe and analyze twenty local maize populations and to identify the quality traits with more differentiating ability.

## 2 MATERIAL AND METHODS

### **Plant material and experimental design:**

Twenty local maize populations (LMP), collected from different regions of Kosovo, were used in this study (Table 1). The experiment was carried out in a randomized complete block design with three replicates in Prishtina  $42^{\circ}38'29.76''$  N and  $21^{\circ}07'16.49''$  E on 571 m of altitude in 2010. The climate of the region is semiarid with hot summers. The

soil in the experimental area is classified as vertisol (black soil). Standard agronomic practices were used to provide adequate nutrition and keep the plots disease free. Each plot consisted of a row 5 m long with an inter and intra row plant spacing of  $0.75 \times 0.25$  m, resulting in a population density of 53,000 plants  $\text{ha}^{-1}$ . Area of individual plots in each replication was  $15 \text{ m}^2$ .

**Table 1.** Geographical data of collected Kosovo local maize populations

Populations	Geographical origin	Longitude	Latitude	Elevation
GBK-1	Drenas	$020^{\circ}44'43''$	$42^{\circ}41'50''$	567
GBK-2	Malishevë	$020^{\circ}44'09''$	$42^{\circ}28'12''$	562
GBK-3	Malishevë	$020^{\circ}43'22''$	$42^{\circ}27'56''$	576
GBK-4	Drenas	$020^{\circ}45'53''$	$42^{\circ}41'35''$	694
GBK-5	Prishtine	$021^{\circ}04'00''$	$42^{\circ}35'05''$	810
GBK-6	Kamenice	$021^{\circ}31'32''$	$42^{\circ}34'16''$	766
GBK-7	Kamenice	$021^{\circ}25'32''$	$42^{\circ}33'56''$	812
GBK-8	Lipjan	$021^{\circ}07'20''$	$42^{\circ}31'45''$	551
GBK-9	Podujeve	$021^{\circ}12'12''$	$42^{\circ}33'39''$	598
GBK-10	Drenas	$020^{\circ}54'06''$	$42^{\circ}34'50''$	585
GBK-11	Vushtrri	$021^{\circ}59'26''$	$42^{\circ}50'46''$	557
GBK-12	Ferizaj	$021^{\circ}09'39''$	$42^{\circ}22'15''$	580
GBK-13	Suhareke	$020^{\circ}49'02''$	$42^{\circ}21'45''$	388
GBK-14	Vushtrri	$020^{\circ}58'30''$	$42^{\circ}33'38''$	518
GBK-15	Drenas	$020^{\circ}42'32''$	$42^{\circ}39'21''$	586
GBK-16	Drenas	$020^{\circ}42'46''$	$42^{\circ}39'30''$	565
GBK-17	Skenderaj	$020^{\circ}48'23''$	$42^{\circ}45'00''$	623
GBK-18	Skenderaj	$020^{\circ}47'39''$	$42^{\circ}44'39''$	597
GBK-19	Skenderaj	$020^{\circ}48'04''$	$42^{\circ}44'39''$	603
GBK-20	Shtime	$020^{\circ}07'06''$	$42^{\circ}44'40''$	610

**Laboratory studies:** At harvest time, five random ears were selected from each plot, resulting in a total of 15 ears per individual population. Grains were carefully removed by hand. From each population, an equal number of grains was taken from each plot, mixed together in order to form a balanced sample

and then subjected to analyses in the laboratory. The grains obtained were grounded to form a fine powder. The chemical analyses included protein content (PC), starch content (SC) and oil content (OC). Analyses were based on standard methods: PC was determined by the Kjeldahl, while OC was

determined by extraction using Soxhlet method (using petroleum ether at boiling point 40-60 °C). Ash content of each sample was determined by drying samples at 550 °C.

**Statistical analyses:** All statistical analyses were performed with the SPSS software (version 15.0, SPSS Inc., 2006). Mean values and variation coefficients were used in the statistical analyses. Effects of the studied traits

were evaluated by ANOVA. In order to assess the differentiation of local maize populations (LMP's) based on all variables that were measured, the Canonical Discriminant Analyses (CDA) was applied. CDA is a technique for classifying a set of observation into predefined classes. Relationship among different variables of the quality analyses were tested by Pearson's correlation test.

### 3 RESULTS AND DISCUSSION

Significant differences among the LMP's for grain yield and grain quality such as content of

oil, protein, starch, ash and moisture were determined (Table 2).

**Table 2:** Mean squares for grain yield and quality parameters of 20 Kosovo local maize populations

Sources of variation	d.f.	Yield per plant (g)	Oil Content (%)	Protein Content (%)	Starch Content (%)	Ash Content (%)	Moisture Content (%)
LMP's	19	2.895 **	2.863 **	18.593 **	4.584 **	2.863 **	247.49 **
CV%		18.26	13.37	4.40	1.49	3.10	3.62
Error	38	217.46	0.0019	0.0437	0.728	0.0019	0.003

\*\* Significant at  $p = 0.01$ .

The analysis of variance (ANOVA) showed that the LMP's differed in most characteristics (Table 3). Mean values of all measured characteristics are presented in Table 3. The mean values of the grain yield per plant at LMP's were 79.33 g plant<sup>-1</sup>. It is evident from our results that local maize population GBK-7 had maximum grain yield per plant (105.13 g plant<sup>-1</sup>) which is 25.8 g plant<sup>-1</sup> or 32.52% higher than the mean values, whereas LMP's for GBK-13 had the lowest grain yield (59.62 g plant<sup>-1</sup>) which is 19.71 g plant<sup>-1</sup> or 24. 84% lower than the mean value (79.33 g plant<sup>-1</sup>). High oil content maize is a special type that has been bred to have higher percent oil content (OC) than regular yellow maize. Typically, oil content of yellow maize varies

from 3.5 to 4%. Ideally, high oil content maize should contain from 7 to 8% of oil (Heiniger, 1997). Kernel oil content is considered to be a quantitative trait controlled by numerous genes with small effects (Dudley, 1977). The data showed a relatively wide range among the LMP's for OC. The overall mean value for the OC was 4.44%. The genotype GBK-5 exhibited maximum OC value with 5.57%, while GBK-13 had the lowest OC value (2.56%). Different range of variation (5.26 and 7.17%) was observed by Berardo *et al.*, (2009). Has *et al.*, (2009) reported different values which varied from 0.04 to 12.3%. Significant values of OC were also reported by Saleem *et al.* (2008).

**Table 3:** Mean values of grain yield and quality characters in Kosovo local maize populations

Local maize populations	Ash content (%)	Oil content (%)	Moisture content (%)	Protein content (%)	Starch content (%)	Yield (plant <sup>-1</sup> g)
GBK-1	1.44±0.020	4.37±0.032	14.48±0.030	13.02±0.215	68.58±0.535	69.69±1.060
GBK-2	1.38±0.028	4.53±0.316	14.14±0.040	11.69±0.049	69.87±0.640	88.97±9.763
GBK-3	1.33±0.080	3.83±0.520	14.56±0.005	12.50±0.205	70.70±0.362	102.55±7.230
GBK-4	1.38±0.025	4.01±0.198	14.84±0.085	11.75±0.280	70.96±0.753	93.30±5.863
GBK-5	1.39±0.055	5.57±0.456	14.16±0.045	11.59±0.176	69.97±0.761	86.29±14.657
GBK-6	1.34±0.051	4.30±0.445	14.85±0.020	11.72±0.200	70.19±0.223	82.08±21.328
GBK-7	1.34±0.035	4.78±0.413	14.14±0.040	11.32±0.202	70.43±0.355	105.13±11.017
GBK-8	1.28±0.045	4.31±0.518	14.50±0.078	11.05±0.435	72.15±1.162	82.43±4.787
GBK-9	1.35±0.015	4.49±0.181	14.67±0.075	11.02±0.258	70.02±0.920	99.86±8.741
GBK-10	1.35±0.032	4.27±0.208	14.45±0.005	11.64±0.081	70.23±0.309	78.34±17.001
GBK-11	1.34±0.043	4.37±0.457	14.56±0.046	11.59±0.134	70.92±0.453	69.83±17.969
GBK-12	1.31±0.052	4.40±0.705	14.07±0.015	11.15±0.223	70.68±0.850	75.77±17.339
GBK-13	1.36±0.030	2.56±0.181	16.42±0.111	11.99±0.161	70.36±0.420	59.62±16.243
GBK-14	1.36±0.023	4.66±0.408	14.61±0.077	11.92±0.217	70.34±1.125	78.43±3.458
GBK-15	1.45±0.070	4.44±0.866	14.19±0.043	12.49±0.308	68.63±1.341	85.47±16.479
GBK-16	1.43±0.032	4.89±0.394	14.24±0.062	12.52±0.070	67.39±0.747	67.36±29.061
GBK-17	1.38±0.026	4.67±0.050	14.01±0.075	11.86±0.160	69.41±0.660	72.67±15.445
GBK-18	1.36±0.060	5.25±0.728	14.10±0.010	11.67±0.081	69.33±1.236	67.21±14.839
GBK-19	1.37±0.023	4.85±0.496	14.63±0.026	12.11±0.209	69.24±1.400	74.39±18.798
GBK-20	1.41±0.047	4.34±0.699	14.18±0.095	11.60±0.081	70.40±1.013	47.33±10.565
Mean	1.36	4.44	14.48	11.81	69.90	79.33
CV %	3.10	13.37	3.62	4.40	1.49	18.26

The variation for protein content (PC) was found to be higher than the variation for OC. The ANOVA indicated that the differences among the LPM's for PC were highly significant (Table 3). The mean value of PC observed in the present study was 11.81%. Some of LMP's were identified with high grain PC ranging from 11.02 to 13.02%. This can be regarded as a relatively high level of PC. The LMP's also showed a high genetic variation (16.93%). The variation for the protein content has been well demonstrated by numerous studies. Has *et al.*, (2009) at some LMP's reported variation from 11.2 to 15.6%;

while Prasanna *et al.*, (2001) presented different results which varied from 8.9 to 10.2%. From the data shown in Table 3 it is evident that the starch content (SC) of maize kernels depends to a very large extent on genotype of population. The starch content (SC) ranging between 67.39 and 72.15%, while the grand mean values of SC was 69.90%. There were significant differences at  $p = 0.01$ . Similar results (58.1 to 72%) for SC in different LMP were reported by Has *et al.*, (2009). Also ash content (AC) ranged from 1.28 to 1.45%.

**Table 4:** Summary statistics for canonical discriminant standardized functions

		Discriminant functions				
	1	2	3	4	5	6
Eigenvalue	179.22	12.26	5.24	1.72	1.14	0.29
Percentage of variation	89.70	6.10	2.60	0.90	0.60	0.10
Cumulative percentage	89.70	95.80	98.40	99.30	99.90	100.00
Canonical correlation	0.99	0.96	0.91	0.79	0.73	0.47
Wilks' Lambda	0.00	0.002	0.021	0.132	0.360	0.771
Chi-square	535.17	296.24	177.31	93.04	46.98	11.97
df	114	90	68	48	30	14
Significance	<0.001	<0.001	<0.001	<0.001	0.025	0.608
<i>Elements of Structure Matrix</i>						
Ash Content (%)	0.003	0.291	-0.065	-0.209	-0.029	0.931 *
Oil Content (%)	-0.095	-0.005	0.133	-0.431	-0.145	0.876 *
Moisture (%)	0.789 *	-0.549	0.261	-0.019	-0.070	0.042
Protein Content (%)	0.053	0.752 *	0.503	-0.135	-0.188	-0.354
Starch Content (%)	0.015	-0.296	-0.004	0.685 *	0.471	-0.470
Grain yield (%)	-0.019	-0.082	0.092	0.506	-0.800 *	0.296

\* Largest absolute correlation between each variable and any discriminant function

In table 4 the canonical discriminant functions are described, the eigenvalue, percentages of variation of each function and the cumulative variance of the six discriminant functions. Table presents as well the standardized elements of structure matrix. The first two canonical discriminant functions were significant at  $p < 0.01$ . It is important to point out the great relevance of the first two

discriminant functions justifying 95.80% of the variability. The first discriminant function showed a significant positive correlation with the moisture (0.789) following by protein and starch content (0.053 and 0.015). But, the negative correlation (-0.019) was determined between first discriminant function and grain yield (Table 5).

**Table 5:** Pearson's correlations between yield and grain quality traits

Trait	Yield	Oil Content (%)	Protein Content (%)	Starch content (%)	Ash Content (%)
Oil Content (%)	0.12				
Protein Content (%)	-0.19	-0.12			
Starch Content (%)	0.33 *	-0.15	-0.68 *		
Ash Content (%)	-0.30 *	0.17	0.69 *	-0.58 *	
Moisture Content (%)	-0.15	-0.80 **	0.09	0.14	-0.15

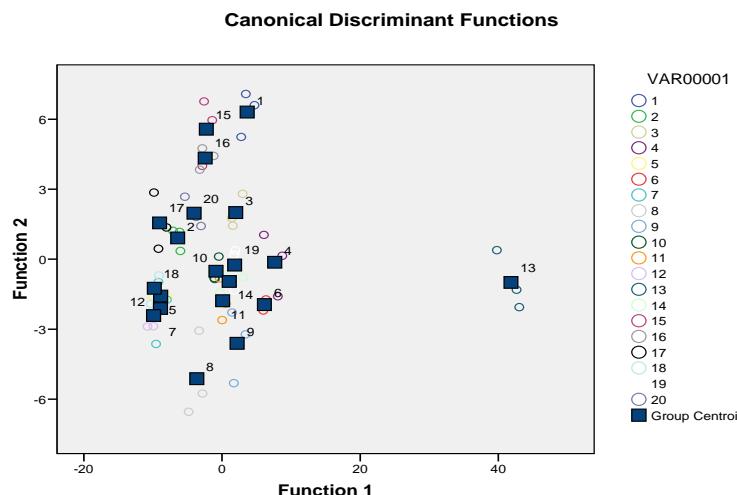
\* = significant at  $p = 0.05$ . and  $p = 0.01$

Maize breeders expect that kernel protein and oil content should be negatively correlated with plant yield. In our study, grain yield per plant was positively and significantly correlated with starch content ( $r = 0.33^*$ ), positively and nonsignificantly correlated with

oil content ( $r = 0.12$ ) and was negatively correlated with protein content ( $r = -0.19$ ).

Also, the significant correlation was obtained between Ash content (AC) and protein content (PC) on value 0.69. Table 5. The possible cause for observed correlation was probably due to reduction of starch content in the grains.

The presented results are in partial agreement *et al.*, (2008). with the results of Has *et al.*, (2009) and Salem



**Figure 1:** Canonical discriminant analyses of local maize populations by grain yield and quality characteristics applied (4 - 7 mismatches allowed at the 3' end of primers).

The canonical discriminant analysis of the traits is presented in Figure 1. The first canonical functions described 89.7% and a second canonical function is 6.1% of the

existing variance. Ash content had the strongest influence in the Function 1, while the Functions 2 was mostly influenced by the oil content followed by protein content and starch content.

#### 4 CONCLUSIONS

The study showed that there was a significant genetic variability for many traits among local maize populations. High variability was determined for PC, OC and SC. Positive and significant correlations were found between yield and starch contents. Negative correlation was found between yield and protein content. The first canonical function described 89.7% and a second canonical function is 6.1% of the

existing variance. The investigated maize populations could be considered as a source of new genetic variability, and could be successfully used for the development of maize inbred lines in the Kosovo breeding program. The evaluation of plant genetic resources has been considered of prime importance, especially in those species having economic importance.

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**Agrovoc descriptors:** prunus avium, cherries, fruit crops, stone fruits, varieties, land varieties, fruits, quality, rootstocks, orchards, fruit growing, flowering, growth, plant developmental stages, biological development, proximate composition, plant breeding, climatic factors, adaptation

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## Testing of 'Gisela 5' and 'Santa Lucia 64' cherry rootstocks in Bosnia and Herzegovina

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### ABSTRACT

Cherry cultivation has a long tradition in Bosnia and Herzegovina mainly due to favorable climatic conditions for cherry growing in this region. However, current cherry production is insufficient because of prevailing old cultivars and rootstocks. Modern intensive production of sweet cherry (*Prunus avium* L.) requires planting of high quality cultivars on dwarfing rootstocks planted in high density orchards. Cherry rootstock breeding programs worldwide require data on tolerance and performance of their rootstocks in different climatic conditions. Therefore, the influence of two cherry rootstocks ('Gisela 5' and 'Santa Lucia 64') on phenological events (blooming), growth and pomological properties of two cherry cultivars ('Stella' and 'Burlat') planted in modern orchard (managed according to standard commercial practice for integrated fruit production), near Sarajevo was evaluated. The trees grafted on 'Gisela 5' rootstocks were planted in 2004 and on 'Santa Lucia 64' in 2005. All trees were trained in a spindle system and the analyzed parameters were monitored in 2010. Rootstocks greatly influenced blooming time, growth and fruit characteristics of both investigated cultivars. The weaker rootstock was 'Gisela 5', which stimulated earlier blooming and caused statistically significant better fruit characteristics (fruit size, % flesh of fruit as well as total soluble solids content in fruit). The results of the analysis showed that both cherry cultivars reached better fruit quality on 'Gisela 5'. 'Stella' had better fruit quality than 'Burlat'. 'Santa Lucia 64' proved a better rootstock for 'Burlat' than for 'Stella'.

**Key words:** cherry, cultivar, dwarfing rootstocks, trunk cross section area (TCSA), 'Stella', 'Burlat', 'Gisela 5', 'Santa Lucia 64'

### IZVLEČEK

#### PREIZKUŠANJE ČEŠNJEVIH PODLAG 'GISELA 5' IN 'SANTA LUCIA 64' V BOSNI IN HERCEGOVINI

Gojenje češenj v Bosni in Hercegovini ima dolgo tradicijo. Glavni razlog so ugodne klimatske razmere za gojenje češenj. Vendar pa je trenutna pridelava češenj premajhna zaradi starih sort in njihovih podlag. Sodobna intenzivna pridelava češenj (*Prunus avium* L.) zahteva sajenje visokokakovostnih sort na šibkih podlagah v nasadih z gostim sajenjem. Programi vzojo novih češnjevih podlag po svetu potrebujejo podatke o odpornosti in obnašanju podlag v različnih klimatskih razmerah. Ugotavljalci smo vpliv dveh češnjevih podlag ('Gisela 5' in 'Santa Lucia 64') na fenološke lastnosti (cvetenje), rast in pomološke lastnosti dveh češnjevih sort ('Stella' in 'Burlat'), ki sta bili posajeni v sodobnem nasadu blizu Sarajeva, oskrbovanem po načelih integrirane pridelave. Drevesa na podlagi 'Gisela 5' so bila posajena leta 2004 in na podlagi 'Santa Lucia 64' leta 2005. Drevesa smo opazovali v letu 2010, vzgojena so bila v gojitveni obliki vrtenast grm. Podlagi sta značilno vplivali na cvetenje, rast in na značilnosti plodov pri obeh obravnavanih sortah. Šibkejša podlaga je bila 'Gisela 5', kjer je bilo cvetenje zgodnejše in plodovi so imeli boljše značilnosti (velikost ploda, delež mesa kakor tudi vsebnost suhe snovi v plodu). Analiza je pokazala, da sta obe češnjevi sorte razvili bolj kakovostne plodove na podlagi 'Gisela 5'. Sorta 'Stella' je imela kakovostnejše plodove kot sorta 'Burlat'. Za sorto 'Burlat' je podlaga 'Santa Lucia 64' bolj primerna kot za sorto 'Stella'.

**Ključne besede:** češnja, sorta, šibke podlage, ploščina preseka debla, 'Stella', 'Burlat', 'Gisela 5', 'Santa Lucia 64'

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## 1 INTRODUCTION

Cherry growing has a long tradition in Bosnia and Herzegovina. However, the current sweet cherry production is quite small. In recent years the government and research institutions have made efforts to improve cherry production in the region. Now, the producers can get more information concerning modern systems of cultivation, recent cherry cultivars, rootstocks, etc.

Modern, high-productive cherry cultivars, which require the application of appropriate agrotechnical practices in order to achieve high yields with a good fruit quality, were introduced in cherry production in Bosnia and Herzegovina in the past years. In addition to planting new cultivars, new rootstocks must also be introduced as they are essential for high density cherry planting.

The proper choice of rootstock is one of the key factors for successful cherry production. The rootstock has a direct impact on nutrition, vegetative growth, longevity, yield, fruit quality and resistance of fruit trees (Usenik et al., 2010a). In Bosnia and Herzegovina, the cherry was traditionally grafted on generative rootstocks (*Prunus avium* and *Prunus mahaleb*), which are characterized by strong vigor resulting in higher harvesting costs.

Cherry rootstock breeding programs worldwide collect data on rootstocks tolerance to different soil properties as well as select rootstocks for "high density" orchard design and early production (Callesen, 1998; Sansavini et al., 1994, Sansavini and Lugly, 1998).

For achieving lower tree vigor it is important to select fertile land and to apply all agro-pomotechnical measures for intensive cherry production (Riesen and Ladner, 1998).

Adaptation to regional climates and soil condition is also an important criterion during the evaluation of cherry rootstocks. Big differences can exist in the vigor habits and yield of trees even between areas in close vicinity (Siegler et al., 2000). The choice of rootstock influences the growth, precocity, blooming date, yield and fruit quality (Beckman et al., 1992; Sugar et al., 1999). Therefore, new dwarfing and semi-dwarfing rootstock for cherry must reduce vigor, be graft compatible and site adaptable, and should have good fruit quality without reduction in fruit size and quality (Reighard et al., 2006).

The aim of this study was to evaluate two cherry cultivars grafted on two rootstocks in the ecological conditions of Bosnia and Herzegovina.

## 2 MATERIALS AND METHODS

The cherry cultivars 'Stella' and 'Burlat' were grafted on two rootstocks: 'Gisela 5' and 'Santa Lucia 64'. Evaluation was carried out in the cherry orchard located in Podlugovi - Ilijas municipality, nearby Sarajevo. The parameters were monitored from February to June 2010.

The trees grafted on 'Gisela 5' rootstocks were planted in 2004 at a spacing of 4.0 m x 3.0 m and the trees grafted on rootstock 'Santa Lucia

64' were planted in 2005 at a spacing of 4.0 m x 3.5 m. All tress were trained to a spindle.

The experiment was arranged as a random design with five trees in 2 replications (10 trees per rootstock). The orchard was managed according to standard commercial practice for integrated fruit production. According to its textural classification, the soil was classified as medium heavy to heavy. The phenological

properties (swelling buds, first bloom, full bloom, bloom end) were monitored according to Winter (2002). Trunk circumference was measured 20 cm above the graft union and the data were used for calculation of the trunk cross section area (TCSA). Flower index (FI) was calculated as a number of flower buds in relation to the number of buds. Titratable acids

were measured with Titrino 719 S (Metrohm) and expressed as % of malic acid. All quantitative parameters were analyzed statistically and the values obtained in terms of further evaluation were tested by mathematical - statistical tests (using one-way analysis of variance - ANOVA test) at  $p<0.05$ .

### 3 RESULTS AND DISCUSSION

Phenological observations are presented in Table 1.

**Table 1:** Flowering phenology of 'Stella' and 'Burlat' cherry cultivars grafted on two rootstocks

Phenophase	'Stella'/'Gisela 5'	'Burlat'/'Gisela 5'	'Stella'/'Santa Lucia 64'	'Burlat'/'Santa Lucia 64'
Swelling buds	27. 3. 2010	27. 3. 2010	29. 3. 2010	30. 3. 2010
Green cluster	30. 3. 2010	1. 4. 2010	31. 3. 2010	2. 4. 2010
White bud	5. 4. 2010	6. 4. 2010	07. 4. 2010	8. 4. 2010
First bloom	7. 4. 2010	9. 4. 2010	09. 4. 2010	10. 4. 2010
Full bloom	12. 4. 2010	15. 4. 2010	13. 4. 2010	16. 4. 2010
Bloom end	19. 4. 2010	22. 4. 2010	21. 4. 2010	23. 4. 2010
Flowering duration (days)	12	13	12	13

The flowering is a very important parameter affecting cherry productivity. This is a variable quantity, strongly dependent on genetic characteristics of the cultivar and growing conditions during the flowering. Stancevic (1984) researched phenology data of 48 cherry cultivars and reported that flowering duration for cherry is 11-17 days. According to Pirnat et al. (1980) the flowering period of cherry is 12 days. The results of our study showed 12 days flowering duration in 'Stella' and 13 days in 'Burlat'. It can be concluded that 'Stella' and 'Burlat' are sufficiently overlapped in the duration of flowering. This information is important for pollination, because 'Burlat' is a

self – sterile cultivar. Usenik and Stampar (2011) reported that 'Burlat' had earlier first bloom (4<sup>th</sup> April) and longer flowering duration (15 days).

The pomological characteristics of analyzed cherry fruits are presented in Table 2. The average fruit weight ranged from 4.30 g to 6.69 g; the highest average fruit weight of both analyzed cultivars (6.69 g and 6.49 g) was measured on 'Santa Lucia 64' rootstock. However, cultivars grafted on 'Gisela 5' developed fruit with significantly higher fruit weight, than those grafted on 'Santa Lucia 64' rootstock.

**Table 2:** Average value of pomological characteristics of 'Stella' and 'Burlat' cherry cultivars grafted on two rootstocks<sup>a</sup>

Cultivar	Rootstock	Fruit weight (g)	Fruit height (cm)	Fruit width (cm)	Fruit diameter (cm)	Stone weight (g)	% flesh of fruit
'Stella'	Gisela 5	4.3±0.80 a	2.1±0.08 a	2.32±0.2 a	2.03±0.1 a	0.47±0.7 a	92.5±1.3 a
	Santa Lucia 64	6.49±0.6 b	1.79±0.1 b	1.94±0.5 b	1.61±0.6 b	0.37±0.7 b	91.3±1.4 b
	Average	5.35±1.3 a	1.94±0.2 a	2.13±0.4 a	1.82±0.3 a	0.42±0.9 a	91.9±1.2 a
'Burlat'	Gisela 5	4.45±0.5 a	2.1±0.09 a	2.33±0.8 a	1.88±0.7 b	0.43±0.7 a	93.5±1.2 a
	Santa Lucia 64	6.69±0.7 b	1.79±0.1 b	1.94±0.8 b	1.63±0.8 b	0.4±0.08 a	90.8±2.3 b
	Average	5.57±1.3 a	1.94±0.2 a	2.13±0.4 a	1.76±0.5 b	0.42±0.7 a	92.2±2.8 a
Average of 'Gisela 5'		6.54±0.7 a	6.54±0.7 a	2.1±0.08 a	2.32±0.1 a	1.96±0.1 a	0.45±0.7 a
Average of 'Santa Lucia 64'		4.38±0.7 b	4.38±0.7 b	1.79±0.2 b	1.94±0.7 b	1.62±0.7 b	0.39±0.8 b
Cultivar x Rootstock		NS	NS	NS	***	*	*

<sup>a</sup> Average values ± standard error are presented. Different letters in columns indicate significantly different values at  $p < 0.05$ ; NS – no significant influence; \*\*\* - indicate significant influence at  $p < 0.001$ ; \* - indicate significant influence at  $p < 0.05$ .

Both evaluated cherry cultivars had an equal average fruit height (Table 2). On the other hand, a significantly higher fruit height (2.1 cm) was measured in cultivars grafted on the 'Gisela 5' rootstock compared to those grafted on 'Santa Lucia 64' (1.79 cm). Similar differences between the cultivars were detected in fruit width with the widest fruit determined in 'Burlat' grafted on the 'Gisela 5' rootstock (2.33 cm). The minimum average fruit width was measured on cultivars grafted on the 'Santa Lucia 64' rootstock (1.94 cm). Analysis of variance revealed that only the rootstock had a significant effect on the expression of fruit size (weight, height and width). For all three analyzed fruit size parameters, the fruits from trees grafted on 'Gisela 5' rootstock had significantly higher mean values compared to fruit from trees grafted on 'Santa Lucia 64'. Similarly, pruning, which indirectly reduces the number of fruiting spurs, also results in increased fruit size and fruit weight (Usenik et al., 2008), but it also reduces total yield (Andersen et al., 1999; Lang, 2001; Kappel, 2002; Whiting and Lang, 2004). As 'Gisela 5' significantly alters the growth potential of cherry cultivars it also

positively affects the external fruit quality characteristics.

Fruit weight of both cultivars was somewhat lower in relation to the results of other studies. Aliman (2008) stated that the average weight of 'Burlat' was 5.98 g and of 'Stella' 7.67 g. Albertini and Della Strada (1996) reported that the fruit weight of 'Burlat' was 6.65 g.

Albertini and Della Strada (1996) reported the average fruit height of 'Stella' fruit below 2.32 cm, and Aliman (2008) measured fruit height of this cultivar in the range of 2.33 cm. Radičević et al. (2011) reported that the average fruit height of the 'Burlat' was 2.23 cm and Aliman (2008) measured 2.10 cm fruit height in this cultivar.

Aliman (2008) reported higher fruit width for 'Stella' (2.47 cm) and 'Burlat' (2.34 cm), while Albertini and Della Strada (1996) reported slightly lower average fruit width for 'Stella' (2.38 cm). Radičević et al. (2011) reported an average fruit width of 2.43 cm for 'Burlat'.

The average fruit diameter of 'Stella' grafted on the 'Santa Lucia 64' rootstock was smaller

(1.61 cm) compared to 'Burlat' grafted on the same rootstock (1.63 cm). Cultivar and rootstock, as well as their interactions, exhibited statistically significant influences on fruit diameter. 'Stella' had a significantly higher fruit diameter in relation to 'Burlat'. The fruits from trees grafted on 'Gisela 5' had a significantly higher fruit diameter than those grafted on 'Santa Lucia 64' rootstock. The fruit diameter of 'Burlat' was much lower compared to 2.01 cm reported by Radičević et al. (2011) and 2.11 cm measured by Zhivondov (2011).

The analyzed cultivars grafted on the 'Gisela 5' rootstock developed averagely significantly heavier stone compared to the 'Santa Lucia 64' rootstock. The average stone weight of the cv. 'Burlat' grafted on the 'Santa Lucia 64' was higher (0.40 g) in relation to the 'Stella' grafted on the same rootstock (0.37 g). The obtained data of 'Burlat' stone weight was slightly higher in relation to the data reported by Zhivondov (2011), Aliman (2008) and Radičević et al. (2011).

**Table 3:** Average content of total soluble solids ( $^{\circ}$  Brix) and titratable acids (%) in fruits of 'Stella' and 'Burlat' cherry cultivars grafted on two rootstocks<sup>a</sup>

Cultivar	Rootstock	Total soluble solids ( $^{\circ}$ Brix)	Titratable acids (% of malic acid)
'Stella'	'Gisela 5'	13.0 a $\pm$ 0.29	0.29 a $\pm$ 0.10
	'Santa Lucia 64'	16.0 b $\pm$ 0.20	0.23 b $\pm$ 0.08
	Average	14.5 a $\pm$ 1.70	0.26 a $\pm$ 0.04
'Burlat'	'Gisela 5'	13.0 a $\pm$ 0.28	0.22 a $\pm$ 0.10
	'Santa Lucia 64'	12.5 a $\pm$ 0.50	0.33 b $\pm$ 0.08
	Average	12.8 b $\pm$ 0.48	0.28 b $\pm$ 0.03
Average of 'Gisela 5'		14.3 a $\pm$ 0.44	0.26 a $\pm$ 0.04
Average of 'Santa Lucia 64'		13.0 b $\pm$ 1.95	0.28 b $\pm$ 0.08
Cultivar x Rootstock		***	NS

<sup>a</sup> Average values  $\pm$  standard error are presented. Different letters in columns indicate significantly different values at  $p < 0.05$ ; NS – no significant influence; \*\*\* - indicate significant influence at  $p < 0.001$ .

The average total soluble solids content ranged from 12.5  $^{\circ}$ Brix in 'Burlat' grafted on the 'Santa Lucia 64' rootstock to 16  $^{\circ}$ Brix in 'Stella' grafted on the 'Gisela 5' rootstock. Cultivar and rootstock exhibited significant influence on the content of total soluble solids

The cherry cultivar did not significantly influence the percentage of flesh in the fruit but average fruit ratio was significantly higher in cultivars grafted on the 'Gisela 5' rootstock (93 %) compared to those grafted on 'Santa Lucia 64' (91.1 %). Usenik et al. (2010a) reported that 'Lapins' grafted on 'Gisela 5' rootstock had an average percentage of flesh fruit weight of 91.8 %.

Stancevic (1976) and Aliman (2008) reported slightly higher average values of fruit ratio (% of fruit flesh) of 'Stella' (94.91 and 94.88 %). The obtained average fruit ratio of 'Burlat' was also lower compared to the data reported by Aliman (2008) and Radičević et al. (2011), where the fruit ratio was between 93.73 and 91.85 %.

In Table 3 the results of total soluble solids (TSS) and titratable acids content in fruits of cherry cultivars are presented.

in cherry fruits. The fruits of cv. 'Stella' had significantly higher content of soluble solids in relation to fruits of 'Burlat'. The fruits from the trees grafted on 'Gisela 5' rootstock had significantly higher content of total soluble solids in relation to the fruits from trees grafted

on 'Santa Lucia 64'. The results of the average content of soluble solids for the cultivar 'Stella' (14.5 °Brix) are similar to results of Aliman (2008). Albertini and Della Strada (1996) and Stancevic (1976) obtained higher values of this parameter (15.2 and 17.2 °Brix). The obtained results of the total soluble solids in 'Burlat' fruit are lower in relation to Radičević et al. (2011) and Aliman (2008), which reported that the average content of total soluble solids of 'Burlat' was 13.77 and 15.90 °Brix. Usenik et al. (2010b) reported that leaf/fruit ratio also had significantly influences total soluble solids content in 'Lapins' fruit grafted on 'Gisela 5' rootstock.

**Table 4:** Average values of yield/tree (kg), TCSA ( $\text{cm}^2$ ) and flower index (FI) of 'Stella' and 'Burlat' cherry cultivars grafted on two rootstocks<sup>a</sup>

Cultivar	Rootstock	Yield/tree (kg)	TCSA ( $\text{cm}^2$ )	Flower index (FI)
'Stella'	'Santa Lucia 64'	21.00 a ± 6.94	47.61 a ± 9.60	0.50 a ± 0.15
	'Gisela 5'	22.50 a ± 7.53	54.42 b ± 9.47	0.54 a ± 0.24
	Average	21.75 a ± 7.24	51.01 a ± 9.92	0.52 a ± 0.20
'Burlat'	'Santa Lucia 64'	20.00 a ± 6.78	50.64 c ± 10.32	0.26 b ± 0.06
	'Gisela 5'	21.50 a ± 7.06	61.64 d ± 10.55	0.60 c ± 0.14
	Average	20.75 a ± 6.92	56.14 b ± 11.62	0.42 b ± 0.21
Average of 'Gisela 5'		22.00 a ± 7.30	58.03 a ± 10.44	0.57 a ± 0.20
Average of 'Santa Lucia 64'		20.75 a ± 7.01	49.13 b ± 9.82	0.37 b ± 0.17
Cultivar x Rootstock		NS	NS	***

<sup>a</sup> Average values ± standard error are presented. Different letters in columns indicate significantly different values at  $p < 0.05$ ; NS – no significant influence; \*\*\* - indicate significant influence at  $p < 0.001$ .

The average yield of 'Stella' (Table 4) ranged from 21.0 ('Santa Lucia 64' rootstock) to 22.5 kg/tree ('Gisela 5' rootstock). The trees of 'Burlat' averagely yielded from 20.5 to 21.5 kg/tree. There were no significant differences between rootstocks as well as between cultivars in average yield per tree. The results of average yield of 'Gisela 5' rootstock are slightly lower than the results reported by Usenik et al. (2010a). Analyses of variance showed that both factors (cultivar and rootstock) had a statistically significant influence on the TCSA. Trees grafted on 'Gisela 5' rootstock had significant higher average of TCSA ( $58.03 \text{ cm}^2$ ) compared to

Cultivar and rootstock had a significant influence on the fruit titratable acids content. Significantly higher average levels of titratable acids were measured in 'Burlat' cultivar than in 'Stella'. The investigated rootstock 'Santa Lucia 64' caused significant increase of titratable acids content of cherry cultivars compared to the 'Gisela 5' rootstock.

In table 4 the results of trunk cross section area (TCSA) and flower index (FI) of 'Stella' and 'Burlat' cherry cultivars grafted on two rootstocks are presented.

trees grafted on the 'Santa Lucia 64' rootstock ( $49.13 \text{ cm}^2$ ). However, trees grafted on 'Gisela 5' were one year older than trees grafted on the 'Santa Lucia 64' rootstock.

Radunic et al. (2011) obtained  $40.1 \text{ cm}^2$  TCSA for 7 year-old cherry trees trained in a spindle bush training system. The same author reported  $40.8 \text{ cm}^2$  TCSA for cherry trees trained in the "V" training system. Fajt and Komel (2008) measured an average of  $63 \text{ cm}^2$  TCSA in 8 year-old cherry cultivars grafted on 'Gisela 5' rootstock.

Cultivar and rootstock had a statistically significant influence on the flower index (FI). The average value of 'Stella' flower index was significantly higher compared to the 'Burlat' cultivar. The trees grafted on 'Santa Lucia 64'

rootstock had significantly higher average flower index than the trees grafted on 'Gisela 5'. Cultivar 'Stella' grafted on 'Gisela 5' rootstock had a higher average FI compared to 'Burlat' grafted on the same rootstock.

#### 4 CONCLUSIONS

Analyses of pomological and phenological characteristics of cherry cultivars 'Burlat' and 'Stella' grafted on the 'Santa Lucia 64' and 'Gisela 5' rootstocks showed that the choice of rootstock had a significant influence on the fruit and tree characteristics. The analyzed

cultivars generally developed better fruit quality when grafted on 'Gisela 5' rootstock compared to 'Santa Lucia 64' rootstock. Also, cultivar 'Stella' had a better fruit quality compared to 'Burlat'.

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**Agris category code:** f01, e16

## Research of Energy use efficiency for maize production systems in Izeh, Iran

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### ABSTRACT

This study was carried out in Khuzestan province in Iran and aim of investigation was energy analysis (input-output) of maize production systems in Izeh County of Iran. Data were collected from 30 maize farms by using a face to face questionnaire method in 2011. The results revealed that in maize production systems total energy input was  $34.640 \text{ MJ.ha}^{-1}$ . The highest share of energy consumed was recorded for N fertilizer (20.80%) which is a nonrenewable resource. Output energy was  $102.973 \text{ MJ.ha}^{-1}$ . Accordingly, energy use efficiency (output-input ratio) was 2.97, energy productivity calculated as  $0.20 \text{ kg.MJ}^{-1}$  and net energy was observed as  $68.333 \text{ MJ.Kg}^{-1}$ . Also, agrochemical energy ratio was 48.97% which is high ratio of input energy in this agro ecosystems.

**Key words:** Energy use efficiency, Energy productivity, Maize, Agrochemical

### IZVLEČEK

#### RAZISKAVA ENERGETSKE UČINKOVITOSTI PRIDELAVE KORUZE V IZEHU, IRAN

Raziskava energetske učinkovitosti pridelave koruze je bila opravljena v provinci Khuzestan, okrožja Izeh v Iranu. Podatki za raziskavo so bili pridobljeni z ustnim anketiranjem 30 pridelovalcev koruze v letu 2011. Rezultati so pokazali, da je bil celokupen vnos energije v ta pridelovalni sistem  $34,640 \text{ MJ.ha}^{-1}$ . Največji delež porabljene energije odpade na dušikova gnojila (20,80 %), kar predstavlja neobnovljivi vir. Izplen energije je znašal  $102,973 \text{ MJ.ha}^{-1}$ . Učinkovitost izrabe energije (vnos/iznos) je bila 2,97, izračunana energetska produktivnost je bila  $0,20 \text{ kg.MJ}^{-1}$ , neto energija pridelka je znašala  $68,333 \text{ MJ.Kg}^{-1}$ . Agrokemijsko energijsko razmerje je bilo 48,97 %, kar predstavlja dokaj velik vnos energije v tem agro-ekosistemu.

**Ključne besede:** izkoristek energije, energetska učinkovitost, koruza, agrokemikalije

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## 1 INTRODUCTION

Maize (*Zea mays L.*) is an important cereal crop of Khuzestan province, Iran. It is grown for fodder as well as for grain purpose in Iran. Approximately 320000 hectares of field corn were grown with a production of 2.560.000 tons an average grain yield of 8000 kg per hectare in Iran in 2010. (*Anonymous, 2010*). Energy has been a key input of agriculture since the age of subsistence agriculture. It is an established fact worldwide that agricultural production is positively correlated with energy input (*TaheriGaravand et al., 2010*). Agriculture is both a producer and consumer of energy. Energy input-output analysis is usually used to evaluate the efficiency and environmental impacts of production systems (*Ozkan et al., 2004*).

Energy use in agriculture has been increasing in response to increasing population, limited supply of arable land, and a desire for higher standards of living (Kizilaslan, 2009). In modern agriculture system input energy is very much higher than in traditional agriculture system, but energy use efficiency has been reduced in response to no affective use of input energy. Efficient use of energies helps to achieve increased productivity and contributes to the economy, profitability and competitiveness of agriculture sustainability in rural areas (Ozkan et al., 2004; Singh et al., 2002).

The main objective of this study is analysis of energy use and energy indicator of maize production systems in Izeh county of Khuzestan province in Iran.

## 2 MATERIALS AND METHODS

Izeh(Izeh County (49 31' N, 52 49' E), south of Iran), is one of the important maize production areas in the south part of Iran in Khuzestan province. In this region maize is grown as second crop.

For this study data were collected from 30 farms applying a face-to-face questionnaire. Other information was collected from the Ministry of Agriculture. Total energy input and output in maize production systems was estimated by using questionnaires and data analysis. It should be clearly stated that parameters listed in Table 1 were obtained from the questionares and data analysis. Basic information on energy inputs and maize yield were entered into Excel spreadsheets and then energy indicators were calculated according Table 1.

Finally energy use efficiency, specific energy, energy productivity and net energy were

determined applying standard equations (*Hairili et al., 2006; Mohammadi et al., 2010*)

$$\text{Energy use efficiency} = (\text{output energy (MJ.ha}^{-1}\text{)}) / (\text{input energy (MJ.ha}^{-1}\text{)}) \quad (1)$$

$$\text{Specific energy} = (\text{input energy (MJ.ha}^{-1}\text{)}) / (\text{maize yield (kg.ha}^{-1}\text{)}) \quad (2)$$

$$\text{Energy productivity} = (\text{maize yield (kg.ha}^{-1}\text{)}) / (\text{input energy (MJha}^{-1}\text{)}) \quad (3)$$

$$\text{Net energy} = \text{output energy (MJ.ha}^{-1}\text{)} - \text{input energy (MJ.ha}^{-1}\text{)} \quad (4)$$

Agrochemical energy ratio was calculated by applying Equations 5 (*Khan et al., 2009*):

$$\text{Agrochemical energy ratio} = \text{input energy of agrochemicals (MJha}^{-1}\text{)} / \text{total input energy (MJ.ha}^{-1}\text{)} \quad (5)$$

**Table 1.** Energy equivalents for input and output of Maize production systems in Izeh county

	Unit	Energy equivalents	Reference
<b>A. Inputs</b>			
1. Human labor	h	1.96	[Mohammadi and Omid, 2010]
2. Machinery	h	62.7	[Samavatean et al., 2010]
3. Diesel fuel	l	51.33	[Samavatean et al., 2010]
4. Chemical fertilizer	Kg		
(a) Nitrogen	Kg	66.14	[Erdal et al., 2007]
(b) Phosphate ( $P_2O_5$ )	Kg	12.44	[Erdal et al., 2007]
(c) Potassium ( $K_2O$ )	Kg	11.15	[Mohammadi and Omid, 2010]
5. Chemicals	Kg	120	[Demircan et al., 2006]
6. Electricity	Kwh	3.6	[Rafiee et al., 2010]
7. Water for irrigation	$m^3$	0.63	[Hatirli et al., 2005]
8. Seed	Kg	50.0	[Erdal et al., 2007]
<b>B. Output</b>			
1. Maize	Kg	14.7	[Ozkan et al, 2004; Mandal et al., 2002]

The input energy was divided into direct, indirect, renewable and non-renewable energies [Kizilaslan, 2009; Samavatean et al., 2010]. Direct energy covered human labour, diesel fuel, water for irrigation, and electricity used in the corn production while indirect energy consists of seeds, pesticide, fertilizers,

and machinery energy. Renewable energy consists of human labor and seeds and nonrenewable energy includes diesel, pesticide, fertilizers, electricity and machinery. Also in the last part of the research, economic analysis of corn production was investigated.

### 3 RESULTS AND DISCUSSION

#### 3.1 Energy use in maize production systems in Izeh County

The inputs used and output in maize production systems, their energy equivalents, and percentages in the total energy input presented in Table 2. The results revealed that total energy input in maize production systems was  $3464002 \text{ MJ.ha}^{-1}$ . N fertilizer used in maize production systems had a high share

with 20.80%. Diesel fuel energy used in maize production systems ranked in the second place with 31.60% in the total energy input. The lowest share of total energy was recorded for human labour (0.56%) which is a renewable resource of energy. In this study maize grain yield was  $7005 \text{ kg.ha}^{-1}$  that total energy equivalents for this amount was  $102.97 \text{ MJ.ha}^{-1}$ .

**Table 2:** Energy inputs, outputs and the ratio of maize production in Izeh county

Inputs and output - (unit)	Quantity per unit area (ha)	Total Energy equivalents(%)	
<b>A. Inputs</b>			
1. Human- labour (h)	99.32	194.68	0.56
2. Machinery (h)	17.50	1097.25	3.17
3. Diesel fuel (L)	213.51	10959.47	31.60
4. Chemical fertilizer (kg)			
(a) Nitrogen (N)	108.68	7188.10	20.80
(b) Phosphate ( $P_2O_5$ )	61.98	771.03	2.23
(c) Potassium ( $K_2O$ )	37.84	421.92	1.22
5. Chemicals(kg)	9.25	1110	3.20
6. Electricity(Kwh)	2000	7200	20.80
7. Water for irrigation( $m^3$ )	7327.13	4616.10	13.30
8. Seed (kg)	21.63	1081.50	3.12
Total input energy		34640.02	100
<b>B. Output</b>			
1. maize grain yield (kg)	7005	102973.5	100
Total output energy		102973.5	100

Results of energy indicators for maize production systems are shown in Table 3. Accordingly, energy efficiency (output-input ratio) was 2.97. Lower energy use efficiency in maize production systems is due to higher energy inputs in these systems for example N fertilizer consumed. Such indicator was reported 2.8 for wheat production systems (*Streimikiene et al., 2007*) and 25.75 for sugar beet (*Erdal et al., 2007*) in Turkey.

Energy productivity (grain yield per energy input) and specific energy in maize production systems were  $0.20 \text{ kg.MJ}^{-1}$  and  $9.95 \text{ MJ.kg}^{-1}$  respectively. System net energy (output minus input) was as  $68333 \text{ MJ.ha}^{-1}$ . Agrochemical energy ratio was 48.97% which is a high portion of input energy of maize production systems. It should be mentioned that maize production in south part of Iran could be limited using amounts of chemical fertilizers and pesticides.

**Table 3:** Indicators of energy use of Maize production systems in Izeh county

Indicators	Unit	Quantity
Inputs energy	$\text{MJ.ha}^{-1}$	34640
Output energy	$\text{MJ.ha}^{-1}$	102973
Energy use efficiency		2.97
Energy productivity	$\text{kg.MJ}^{-1}$	0.20
Net energy balance	$\text{MJ.ha}^{-1}$	68333

### 3.2 Energetic of producing maize systems in Izeh County

The total means energy input as direct and indirect, renewable, and non-renewable forms for greenhouse and open-field maize production was given in Table 4. The total energy input necessary for maize production was 34640.02 MJ/ha. Out of all 33.74% of the

total energy, input use in maize production was in the form of indirect energy. The remaining part of energy input use (66.26%) was in the form of direct energy. On the other hand the research results indicate that the total energy input used in maize production systems was mainly dependent on non-renewable energy forms (Table 4). As can be seen from the table, on an average, the non-renewable form of energy input was 83.02% in maize

production systems of the total energy input while the 16.98% of input energy was renewable energy resource. The high rate of non-renewable and direct energy inputs

indicates an intensive use of chemical fertilizer and diesel fuel consumption in these agroecosystems.

**Table 4:** Total energy input in form of direct, indirect, renewable and non-renewable for maize production in Izeh county

Indicators	Quantity (MJ ha <sup>-1</sup> )	Percentage (%)
Direct energy <sup>a</sup>	22952.48	66.26
Indirect energy <sup>b</sup>	11687.54	33.74
Renewable energy <sup>c</sup>	5881.88	16.98
Non-renewable energy <sup>d</sup>	28758.14	83.02
Total energy input	34640.02	100

<sup>a</sup> Includes human labour, diesel, water for irrigation, electricity, <sup>b</sup> Includes seeds, fertilizers, pesticides, machinery. <sup>c</sup> Includes human labor, seeds, and water for irrigation. <sup>d</sup> Includes diesel, pesticides, fertilizers, electricity and machinery.

## 4 CONCLUSION

The important following conclusions are drawn;

1. Total energy input and output in maize production systems were 34640 and 102973 MJ. ha<sup>-1</sup>.

2. That the highest share of input energy was reported for nitrogen fertilizer, diesel fuel, and water for irrigation (20.80, 31.60 and 13.30%) respectively.

3. The energy use efficiency, energy productivity, specific energy, net energy of maize production systems were 2.97, 0.20 kg MJ<sup>-1</sup>, 10.63 MJ.kg<sup>-1</sup> and 68333 MJ.ha<sup>-1</sup> respectively.

4. The share of total input energy as direct, indirect, renewable and nonrenewable forms were 66.26, 33.74, 16.98 and 83.02% respectively.

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**Agris category code:** f30, f70

## Collection and genetic characterization of *Vitis vinifera* 'Žilavka' by microsatellites and AFLP markers

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### ABSTRACT

'Žilavka' has been grown in Bosnia and Herzegovina since the XIVth century and is exploited for wine production. Although not sufficiently studied, this grapevine cultivar has high economic potential for the country. Five survey missions resulted in the collection of eighty 'Žilavka' accessions that varied in terms of names and characteristics. Because of the unknown origin of the cultivar, these accessions were analyzed with microsatellites markers in order to obtain a standard 'Žilavka' genotype. AFLP markers were used to investigate the genetic basis of variability within the cultivar. 'Žilavka' grapevines were screened on 14 microsatellite loci, thus revealing 4 different genotypes arising from mutations observed at 10 polymorphic loci. AFLP analysis of 52 'Žilavka' accessions revealed 35 different genotypes, with an average polymorphism of 57 %. Cluster analysis showed no grouping of different Žilavka accessions according to their names, characteristics or collection locations. The standard 'Žilavka' genotype was further compared to 211 cultivars from Slovenia (49), Austria/Germany (20), France (13), Portugal (27), Croatia (19), Greece (32), Spain (21) and Italy (30) in order to assess their genetic relationships. In pairwise comparisons, the highest genetic similarity was found with Slovenian cultivars 'Glera' and 'Briška Glera' (64 %) and the highest genetic dissimilarity (100 %) with two Italian cultivars, 'Nebbiolo Lampia' and 'Vespolina'. Inventory, collection and genetic characterization of 'Žilavka' accessions are important steps towards cultivar standardization, identification of parental cultivars and investigation of cultivar origin, required for its sustainable use.

**Key words:** grapevine, microsatellite, standard genotype, variability, AFLP

### IZVLEČEK

### ZBIRANJE VINSKE TRTE SORTE ŽILAVKA (*Vitis vinifera* L.) IN GENETSKA KARAKTERIZACIJA Z MIKROSATELITNIMI IN AFLP MARKERJ

'Žilavka' je pomembna vinska sorta z območja Bosne in Hercegovine kjer jo gojijo že od XIV stoletja. V preteklosti ni bila dovolj proučevana vendar ima velik ekonomski potencial za omenjeno območje. Akcesije žilavke (80), ki imajo različna imena in se razlikujejo po nekaterih osnovnih karakteristikah so bile nabранe na različnih področjih Bosne in Hercegovine. Za pridobitev standardnega genotipa žilavke smo uporabili mikrosatelitne markerje in z analizo 14 lokusov odkrili mutacije na 10 lokusih in določili 4 različne genotipe. Vse akcesije (52) katerim smo na osnovi mikrosatelitov določili standardni genotip smo nadalje analizirali z AFLP markerji in tako določili 35 različnih genotipov. Povprečni polimorfizem pri AFLP analizi je bil 57 %. S klastrsko analizo nismo odkrili skupin povezanih z različnimi karakteristikami, poimenovanjem ali izvorom akcesij žilavke. Standardni genotip žilavke smo nadalje primerjali z genotipi 211 kultivarjev iz Slovenije (49), Avstrije/Nemčije (20), Francije (13), Portugalske (27), Hrvaške (19), Grčije (32), Španije (21) in Italije (30), da bi ugotovili kakšna je sorodnost z njimi. V analizi primerjave parov smo ugotovili, da je žilavka najbolj sorodna (64 %) s slovenskima kultivarjem 'Glera' in 'Briška Glera' ter najbolj genetsko oddaljena od italijanskih kultivarjev 'Nebbiolo Lampia' in 'Vespolina'. Zbiranje, vrednotenje in genetska karakterizacija akcesij žilavke je pomembna za standardizacijo kultivarjev, analizo starševstva in izvora sorte.

**Ključne besede:** vinska trta, mikrosateliti, standardni genotip, variabilnost, AFLP

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## 1 INTRODUCTION

*Vitis vinifera* ‘Žilavka’ is a white grapevine cultivar and is used traditionally for wine production. In the region of Herzegovina (Bosnia and Herzegovina), this cultivar has been grown for more than 600 years, the Bosnian King Tvrtko having been mentioned as drinking Žilavka wine in the 14<sup>th</sup> century (Vuksanović and Kovačina 1984). In the 19<sup>th</sup> century, due to the grape quality and, particularly, to its resistance to bunch rot (*Botrytis cinerea* Pers.), the Austro-Hungarians exploited this cultivar by the production of a special dessert wine of the *Malaga* type (Mijatović 1988). Apart from its historic importance and long tradition, Žilavka wine has high economic potential for Bosnia and Herzegovina, since it is exported and is popular in neighboring countries. It is also grown in adjacent regions of Croatia, Macedonia, Montenegro and Serbia. Vuksanović and Kovačina (1984), Mijatović (1988), Tarailo (1991) and Cindrić *et al.* (2000) have provided an ampelographic description of this cultivar, which is characterized by high morphological heterogeneity of various traits: the shape, size and compactness of the cluster, the color, shape and size of the berry, vegetative potential, grape and wine quality, and resistance to bunch rot (*Botrytis cinerea* Pers.). In 1978 and 1991, clonal selection was performed at various locations and different types of ‘Žilavka’ were mentioned. Tarailo (1991), who described the clonal selection of ‘Žilavka’, also reported four different ‘Žilavka’ types and claimed that they are

different genotypes. ‘Žilavka’ heterogeneity might be a result of ecological/growth factors or genetic mutations. Many authors designate ‘Žilavka’ as an autochthonous cultivar to Herzegovina (Cindrić *et al.* 2000; Milosavljević 1998; Burić 1985; Vuksanović and Kovačina 1984; Licul and Premužić 1979; Tarailo *et al.* 1978; Avramov 1974), where it can be found under various synonymous names: ‘Žilavka Mostarska’ (Cindrić *et al.* 2000; Milosavljević 1998; Mijatović 1988), ‘Žilavka Hercegovačka’ (Cindrić *et al.* 2000; Avramov 1974), ‘Žilavka Bijela’ (Mijatović 1988; Avramov 1974), ‘Žilavka Žutka’ and ‘Žilavka Zelenka’ (Aničić *op cit.* Mijatović 1988). In our study, two different marker systems, microsatellites (SSR – short sequence repeats) and AFLP (amplified fragment length polymorphism), were applied for genetic analysis, thus providing a complementary tool to ampelographic descriptions. Microsatellites are among the most frequently used DNA markers for cultivar identification, revealing synonyms and homonyms and geographical origin, studying genetic relationships within large groups of cultivars and for clonal variability characterization. AFLP has been found to be an efficient and sensitive method for establishing genetic intra-varietal variability. A combination of SSR and AFLP molecular markers is used to obtain a more accurate and complete framework for genomic identity of different accessions within the cultivar (Vignani *et al.* 2002; Labra *et al.* 2001).

## 2 MATERIALS AND METHODS

### 2.1 Plant material and DNA extraction

Survey expeditions were organized to the region of Herzegovina as the traditional centre of ‘Žilavka’ cultivation. ‘Žilavka’ was collected from 5 different locations, which

were inventoried according to the information received from local people. Four locations belong to the Mostar area (43°20'58"N; 17°48'45"E) and one is from the Trebinje area (42°42'40"N; 18°20'44"E). Three of them are

old vineyards established during the seventies, one is a collection vineyard and one is a modern vineyard. Fresh leaves were collected from each of 80 chosen accessions. Plant material of accessions with different characteristics and names ('Starinska Žilavka', 'Žilavka', 'Stara Žilavka', 'Žuta Žilavka', 'Zelena Žilavka') were included in the study (data available upon request).

Total genomic DNA was extracted from fresh leaf tissue by CTAB (cetyltrimethylammonium bromide) extraction buffer (Kump and Javornik 1996), re-suspended in TE buffer (1 M Tris-HCl, 0,5 M EDTA, pH 8.0) and stored at 4 °C.

## 2.2 Microsatellite analysis

Eighty 'Žilavka' accessions were analyzed at 14 microsatellite (SSR) loci: VVS2 (Thomas and Scott 1993), VVMD5 and VVMD6 (Bowers *et al.* 1996), VVMD24 24, VVMD25 25 and VVMD27 27 (Bowers *et al.* 1999), VrZAG21, VrZAG47, VrZAG62, VrZAG64, VrZAG67 and VrZAG79 (Sefc *et al.* 1999), and Uch11 and Uch29 (Lefort *et al.* 2001). The PCR reaction in a total volume of 10 µl contained 20 ng of extracted DNA, 10 × PCR buffer (Fermentas), 0.2 mM dNTP's (Fermentas), 2 mM MgCl<sub>2</sub> (Fermentas), 0.5 µM of each primer and 0.5 U of *Taq* polymerase (Fermentas). One of each of the primer pairs was labeled with fluorescent Cy-5 dye. Amplification of SSR loci was done in a Whatman Biometra T-Gradient thermocycler with the following steps: hot start for 5 minutes at 95 °C; 26-40 cycles of: denaturation at 94 °C for 30-45 seconds, annealing at 50-56 °C for 30-45 seconds and an extension step at 72 °C for 90 seconds. Amplification of loci Uch11 and Uch29 was done by a tailing protocol using three different primers: 0.2 µM of each of unlabelled Uch primers and 0.075 µM of 18 bp M13 tail sequence attached to the forward primer for subsequent fluorescent labeling (5' TGTAAAACGACGGCCAGT '3). An optimized touchdown protocol was

used for PCR of these two loci: initial denaturation of 94 °C for 5 minutes: 5 cycles of 94 °C for 45 seconds, 60 °C for 30 seconds with a decrease of -1 °C per cycle and 72 °C for 1.5 minutes; followed by 25 cycles at the annealing temperature of 55 °C. PCR reactions were completed at 72 °C for 8 minutes (incubation). Amplified SSR fragments were denatured for 4 minutes at 95 °C and separated on 6 % polyacrylamide gel electrophoresis containing 7 M urea and detected by an ALFexpress DNA automated sequencer (GE Healthcare). The allele sizes were analyzed with AlleleLocator version 1.03 software (Amersham Pharmacia Biotech, 1998). Alleles were precisely sized against ALFexpress sizer 50 - 500 bp (GE Healthcare) and by internal DNA standards of various sizes amplified from plasmid.

## 2.3 AFLP Analysis

Fifty-two 'Žilavka' accessions were analyzed by 6 different AFLP primer combinations: *MseI*-CAA/*PstI*-ACA, *MseI*-CAA/*PstI*-AAC, *MseI*-CAT/*PstI*-ACA, *MseI*-AG/*PstI*-AGA, *MseI*-CT/*PstI*-ACA and *MseI*-AG/*PstI*-ACA. Isolated DNA (250 ng) was digested by 2.5 U of *PstI* and 2.5 U of *MseI* restriction endonucleases (New England Biolabs) in a reaction volume of 40 µl at 37 °C for 3 h. After digestion, 5 pmol of *Pst* and 50 pmol of *Mse* double stranded adaptors were ligated (1 Weiss U of T4 DNA ligase) to the sticky ends of the genomic fragments in a final volume of 50 µl at 37 °C for 3 h. Adaptors were obtained by mixing equimolar amounts of primers *Pst*RI linker1 and *Pst*RI linker2 for *Pst* adapters and *Mse*I linker1 and *Mse*I linker2 for *Mse* adapters. The pre-amplification procedure was done in a total reaction volume of 50 µl containing: 5 µl of ligation reaction serving as template, 1× PCR buffer, 1.5 mM MgCl<sub>2</sub>, 200 µM each dNTP's, 1.25 U *Taq* polymerase, 50 ng of both *PstI* and *MseI* primers with one selective nucleotide (*Pst*I+A and *Mse*I+C). Reactions were amplified in a Whatman Biometra T-Gradient thermocycler, repeating 20 cycles of: 94 °C for 30 seconds, 56 °C for 60 seconds and

72 °C for 105 seconds. The PCR products from the pre-amplification step were diluted 10-fold and 5 µl of the dilution was used for selective amplification. Selective amplification was done by 15 ng of both *Pst*I and *Mse*I primers with two or three selective nucleotides at the 3' end and *Pst*I primers were Cy5 labeled at their 5' end to allow automated laser fluorescence analysis. Selective reactions were performed in a 10 µl PCR reaction mix with the same reaction components as for the pre-amplification step, except that 0.3 U of *Taq* polymerase was used. The reaction was amplified with an initial touch down protocol: 94 °C for 30 seconds, 65 °C for 30 seconds and 72 °C for 60 seconds with a touchdown of - 0.7 °C of annealing temperature per cycle, followed by 23 cycles at 94 °C 30 s, 56 °C 30 s and 72 °C 1 minutes, and the samples were immediately transferred onto ice. The amplification reaction was mixed with an equal volume (10 µl) of formamide loading buffer (5 mg dextrane blue in 1 ml of formamide), denatured by heating to 94 °C for 4 minutes. Five µl of sample was loaded onto 5% polyacrilamide denaturating gel (5% acrylamide-bisacrylamide 19:1, 1×TBE, 7 M urea) and electrophoresed on an ALFexpress DNA automated sequencer (GE Healthcare). An external size standard ranging from 50 to 500 bp was run together with samples to allow further sizing of the fragments. AFLP bands ranging from 50 to 500 base pairs were analyzed using AlleleLocator version 1.03 software, (Amersham

Pharmacia Biotech, 1998). In the first instance, all 80 accessions were included in AFLP testing but only 52 produced scorable fingerprints and were included in the AFLP analysis.

## 2.4 Data analysis

Microsatellite and AFLP amplified bands across all analyzed loci were scored by state as present and given numerical value 1 or absent 0, making a presence-absence matrix of bands. This matrix served as a basis for clustering and assessing overall similarity among the analyzed accessions. A similarity index was calculated from band sharing data of each pair of accessions using the Jaccard (1908) coefficient of similarity. A dendrogram was constructed from the matrix of pairwise distances using an unweighted pair group method (UPGMA) for clustering in the NTSYS-PC software package, version 1.80.

The obtained microsatellite profile of 'Žilavka' was further compared to 211 already genotyped cultivars in Europe in order to assess their genetic relationships.

For AFLP analysis, the percentage of polymorphism was calculated for each primer pair combination from the total number of amplified bands and the number of polymorphic bands.

## 3 RESULTS AND DISCUSSION

### 3.1 Microsatellite analysis

Eighty 'Žilavka' accessions were screened on 14 microsatellite loci. These analyses resulted in 4 different genotypes arising from mutations observed at 10 polymorphic loci (Tab. 1). Seventy-seven accessions had the same allelic

profile across all 14 loci and could be considered as a standard genotype for 'Žilavka'. Three accessions (9/2, 10/2 and 11/2), which were thought to be 'Žilavka', resulted in polymorphism at 5 to 6 loci (Tab. 1) and could not be considered to be 'Žilavka'.

**Table 1:** Standard ‘Žilavka’ genotype profiled at 14 microsatellite loci with three distinguished genotypes and ‘Chardonnay’ as reference cultivar.

<b>Genotype</b>	<b>‘Žilavka’</b>	<b>9/2</b>	<b>10/2</b>	<b>11/2</b>	<b>‘Chardonnay’</b>
VVS2	132:152	n.a.	132:152	132:152	137:152
VVMD5	226:238	226:238	226:238	226:238	232:236
VVMD6	208:208	208:208	190 <sup>a</sup> :208	208:208	198:208
VVMD24	208:208	208:208	208:208	208:208	208:216
VVMD25	240:242	250 <sup>a</sup> :250 <sup>a</sup>	n.a.	240:242	240:256
VVMD27	179:194	179:194	181 <sup>a</sup> :194	179:194	n.a.
VrZAG21	201:203	201:206 <sup>b</sup>	n.a.	201:206 <sup>b</sup>	200:206
VrZAG47	158:173	158:173	158:173	158:173	160:168
VrZAG62	189:189	189:189	189:189	189:205 <sup>a</sup>	188:196
VrZAG64	144:144	160 <sup>b</sup> :164 <sup>b</sup>	144:144	160 <sup>b</sup> :164 <sup>b</sup>	160:164
VrZAG67	151:161	141 <sup>b</sup> :151	141 <sup>b</sup> :141 <sup>b</sup>	141 <sup>b</sup> :151	140:153
VrZAG79	249:249	237 <sup>b</sup> :249	242 <sup>a</sup> :258 <sup>a</sup>	237 <sup>b</sup> :249	244:246
Uch11	235:235	235:235	235:235	235:249 <sup>a</sup>	248:263
Uch29	208:295	208:295	295:306 <sup>a</sup>	208:295	289:297

<sup>a</sup>new alleles different from standard ‘Žilavka’ genotype,<sup>b</sup>alleles shared between distinguished ‘Žilavka’ genotypes, n.a. no amplification

However, detailed morphological description of these accessions should be performed for clear definition. Grapevine cultivars are vegetatively propagated and individuals of one cultivar are expected to be genetically identical to each other, but some cultivars may comprise several different genotypes due to a polyclonal origin (Kozjak *et al.* 2003) or somatic mutations can be accumulated over years of clonal propagation.

Comparison of standard ‘Žilavka’ from our analyses with ‘Žilavka’ from Croatia, genotyped by Maletić *et al.* (1999) resulted in the same allelic profiles at all 7 comparable loci, which confirmed the trueness to type of the ‘Žilavka’ genotype.

The standard ‘Žilavka’ genotype was further compared to 211 cultivars, from Slovenia (49), Austria/Germany (20), France (13), Portugal (27), Croatia (19), Greece (32), Spain (21) and Italy (30) in order to assess their genetic relationships. Comparison was enabled by using one reference cultivar, ‘Chardonnay’, for allele size standardization. The proportion of

shared alleles was used as the basis for distance measurement among analyzed varieties. In pairwise comparisons of ‘Žilavka’ with cultivars from the various European regions, the highest genetic similarity was found with a Slovenian cultivar ‘Glera’ (64 %) and the highest genetic dissimilarity (100 %) with two Italian cultivars, ‘Nebbiolo Lampia’ and ‘Vespolina’. A phenogram of all 211 genotypes was constructed (data available on request) in order to illustrate the genetic relationships between ‘Žilavka’ and other European cultivars. ‘Žilavka’ clustered together with Slovenian old cultivars ‘Glera’, ‘Briška Glera’, ‘Vitovska’, ‘Zunek’, ‘Duranja’ and ‘Popetre’.

### 3.2 AFLP analysis

Fifty-two accessions of standard ‘Žilavka’ genotype analyzed at 14 microsatellite loci were included in further AFLP analysis. Six primer pair combinations generated 163 scorable bands, of which 87 (57%) were polymorphic, expressing intra-varietal variability (Tab. 2).

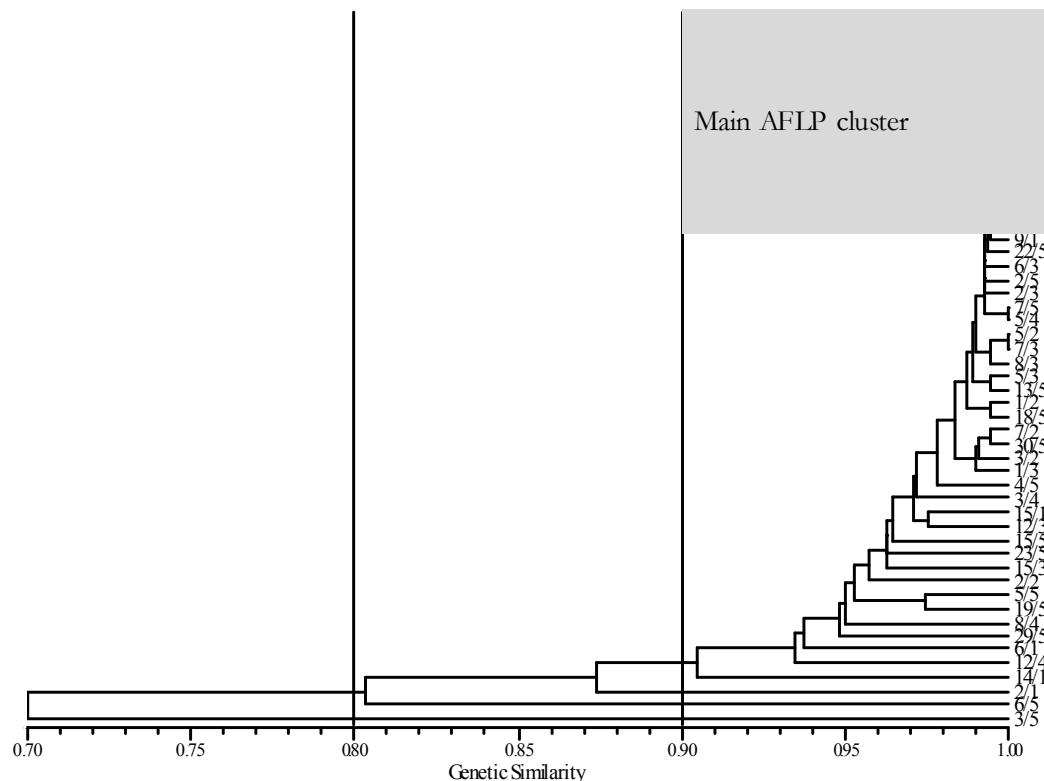
**Table 2:** Characteristics of analyzed AFLP loci expressed by number of amplified bands, polymorphic bands and % of polymorphism.

Primer pair combination	Number of amplified bands	Number of polymorphic bands	Polymorphism (%)
<i>MseI-CAA / PstI-ACA</i>	15	13	87
<i>MseI-CAA / PstI-AAC</i>	20	18	90
<i>MseI-CAT / PstI-ACA</i>	13	5	38
<i>MseI-AG / PstI-AGA</i>	44	27	61
<i>MseI-CT / PstI-ACA</i>	36	11	31
<i>MseI-AG / PstI-ACA</i>	35	13	37
<b>Total</b>	<b>163</b>	<b>87</b>	<b>57 (Average)</b>

The degree of polymorphism was higher than reported previously for some cultivars, e.g., ‘Carmenere’ (2.3%) (Moncada and Hinrichsen 2007), ‘Cabernet Sauvignon’ (5.9%) (Moncada *et al.*, 2005) or ‘Traminer’ (34%) (Imazio *et al.* 2002). The high level of detected polymorphism might be explained by the accumulation of somatic mutations over

years of cultivation. ‘Žilavka’ was spontaneously propagated for many years without formal selection of clones, which might also have resulted in higher heterogeneity within the cultivar.

Based on AFLP data, the 52 ‘Žilavka’ accessions revealed 35 genotypes, which are clustered on the dendrogram (Fig. 1).

**Figure 1:** Dendrogram showing genetic similarity among ‘Žilavka’ accessions generated from AFLP data

The clustering of accessions did not correlate with their names, characteristics or sampling

locations. The main AFLP cluster consists of 16 non-differentiated accessions of the

standard ‘Žilavka’ genotype, which had previously been differentiated by their different names, characteristics or sampling locations (data available upon request), which means that AFLP analysis revealed no type specific marker.

Three out of 52 accessions had an AFLP genetic similarity lower than 0.90 (33/5, 6/5 and 2/1). On the basis of some reports (e.g.,

Cervera *et al.* 2000, 1998), accessions showing similarities > 0.90 can be considered to belong to the same cultivar, while the cultivars that are different show similarities between 0.65 - 0.90). These three accessions all belong to the standard ‘Žilavka’ genotype according to the SSR analysis, but further detailed morphological analyses should be done before reaching any final conclusions.

#### 4 DISCUSSION

Identification and distinction of ‘Žilavka’ accessions was possible on the basis of two marker systems. Microsatellite markers enabled determination of a standard ‘Žilavka’ genotype and, together with AFLP markers, revealed intra-varietal polymorphism. The two molecular markers, SSR and AFLP, explore different parts of the genome and have a different structure in terms of primer sequences and amplified motifs, so they are both valuable for obtaining reliable results on accession variability.

Molecular analysis has shown that ‘Žilavka’ is not a highly homogenous cultivar (high polymorphism is observed), which can be explained by non-formal clonal selection of Žilavka in Bosnia and Herzegovina and also by the long cultivation period, resulting in an accumulation of mutations. Microsatellite genotyping enabled us to exclude the accessions that are different from the true-to-type ‘Žilavka’, since it is known that different cultivars show at least four allelic differences, while clones show fewer differences but can

also be distinguished in some cases by SSR analysis (Laucou *et al.* 2011). In our study, 80 different accessions of ‘Žilavka’ were sampled from 5 locations (data available on request) but molecular analysis revealed no clustering of ‘Žilavka’ accessions according to the observed characteristics, names or locations. Detailed research on ampelographic data needs to be performed to explain the high AFLP polymorphism obtained for three accessions (33/5, 6/5 and 2/1) and to confirm their different origin. The remaining accessions analysed with AFLP markers expressed lower AFLP polymorphism (< 0.90) explaining their intra-varietal variability and true-to-type identity, which was also confirmed by SSR genotyping.

On the basis of the results obtained by molecular analysis, we can clearly distinguish accessions genetically different from the true-to-type ‘Žilavka’, which is a step toward cultivar identification, standardization and collection.

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**Agrovoc descriptors:** solanum tuberosum, potatoes, root vegetables, sprayers, nozzles, application methods, chemical control, control methods, fungicides, plant protection, disease control, equipment, plant protection equipment, equipment, equipment parts

**Agris category code:** h20, n20

## Improved quality of fungicide deposition and coverage of potato leaves using flat fan air-injector nozzle IDK

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### ABSTRACT

The aim of our experiment was to improve fungicide spray deposition and coverage of potato leaves by using air-injector nozzle types. We used two standard nozzle types - a flat fan nozzle ST and a hollow cone nozzle TR, as well as a couple of air-injector nozzle types - an air-injector compact nozzle IDK and a symmetric double flat fan air-injector nozzle TWIN (with a 30° forward and a 30° backward spray jet angle). Water-sensitive paper was placed on the upper, middle and lower part of the plant in order to determine the quality of fungicide deposition and the ability of droplets to penetrate lower parts of the plant. When using the air-injector compact nozzle IDK, potato leaves were covered well at all three levels of the plant. The use of the above-mentioned nozzle resulted in the lowest reduction in coverage value and droplet impression area from the top towards the lowest part of the plant. Furthermore, the nozzle created large enough droplets with sufficient mass and speed to penetrate the dense canopy all the way to the lowest part of the plant. When using the symmetric double flat fan air-injector nozzle TWIN, the angle of both spray jets was excessively wide in order for the droplets to reach the lower part of the plant despite a very large droplet impression area. The use of both standard nozzle types resulted in a poor spray mixture coverage of the middle and lower part of the plant. This predominantly occurred due to insufficient droplet size and the subsequent lack of kinetic energy. Results show that the use of a newer air-injector compact nozzle IDK improves the deposition and coverage of potato leaves with spray mixture.

**Key words:** deposition, coverage, nozzles, potato, fungicide

### IZVLEČEK

#### IZBOLJŠANJE DEPOZICIJE IN POKRITOSTI LISTOV KROMPIRJA S ŠKROPILNO BROZGO PRI UPORABI INJEKTORSKE ŠPRANJASTE ŠOBE IDK

Namen poskusa je bil izboljšanje nanosa in pokritosti listov krompirja s škropilno brozgo pri uporabi novejših izvedb injektorskih šob. Uporabili smo dve standardni izvedbi šob, špranjasto šobo ST in vrtinčno šobo TR ter dve injektorski izvedbi šob, šobo IDK in šobo z dvojnim simetričnim curkom TWIN (škropilni curek pod kotom 30° naprej in 30° nazaj). Na zgornji, srednji in spodnji del rastline smo namestili na vodo občutljive lističe, da bi pri uporabljenih šobah ugotovili kakovost fungicidnega nanosa in sposobnost prodiranja kapljic v spodnje dele rastlin. Ugotovili smo, da je bila najboljša pokritost listov krompirja na vseh etažah pri uporabi injektorske šobe IDK. Pri tej šobi je bilo najmanjše zmanjšanje odstotka pokritosti in površine odtisa posamezne kapljice od vrha proti spodnjemu delu rastline. Ta šoba tvori dovolj velike kapljice, ki imajo veliko hitrost in zadostno maso, da lahko prodrejo skozi gost listni sestoj do spodnjega dela rastline. Pri injektorski šobi z dvojnim simetričnim curkom TWIN je bil kot obeh škropilnih curkov prevelik, da bi kapljice zadele spodnji del rastline, kljub temu da je bila površina odtisa posamezne kapljice zelo velika. Pri obeh standardnih izvedbah šob je bila preslabna pokritost s škropilno brozgo v srednjem in spodnjem delu rastline predvsem zaradi premajhnih kapljic in posledično premajhne kinetične energije. Rezultati kažejo izboljšanje depozicije in pokritosti listov krompirja s škropilno brozgo pri uporabi novejše injektorske šobe IDK.

**Ključne besede:** nanos, pokritost, šobe, krompir, fungicid

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## 1 INTRODUCTION

Spray deposition on the target surface proved to be the decisive factor in spraying with plant protection products (PPP). It is possible to affect the quality of spray deposition on the target surface by changing droplet size, volume application rate, pressure and driving speed. Fine droplets can provide better fungicide coverage, however, with a less-effective droplet penetration through the canopy (Stangl, 2009). Required volume application rate and adequate fungicide deposition rate result in a good spray coating on the target surface and represent the basic requirement for an effective disease control. Lower volume application rate results in a smaller number of droplets and a smaller spray coating on the target surface (Brune, 2011).

Potato has a different morphology than other cultivated plants. Its leaf-area index (LAI) is 4 and it has a complex system of intertwined stems and leaves at different levels which proves to be problematic for the penetration of fungicides through the canopy. Spray deposit should equally cover all parts of the plant in the highest possible degree. Potato has villous leaves and stems with cuticles enabling a quality fungicide deposition. Potato epidermis, on the other hand, does not have an extra epicuticular wax layer, characteristic of some other plants, such as oilseed rape, cabbage etc. (Strasburger, 1991). When spray droplets touch the leaf surface, they stick to it and finally rearrange throughout the surface. There is no risk of droplets bouncing off the leaves or trickling away (Luckhard and Brune, 2011).

In the potato late blight control, timely spraying and high-quality fungicide deposition are of extreme importance. The chemical control should involve nozzles with medium droplet size (VMD 250-350 µm). Nozzles with a spray jet at a certain angle from the vertical allow a better leaf coverage, particularly during the main stem elongation and all the way to the point where plants meet between

the rows (BBCH 301-309) (Spray Application Technique, 2003).

Nowadays, new spraying techniques are being introduced and tested in the potato late blight control. These involve reduced volume application rate (less than 50 l/ha) in Danfoil sprayers, use of standard nozzles on air-assisted sprayers, under leaf fungicide deposition and use of nozzles with different spray jets on classic sprayers (Kryger Jensen, 2007).

Several authors determined the effect of various nozzle types on the coverage of potato leaves with fungicides. According to Kierzek and Wachowiak (2009), the best coverage of potato leaves can be achieved by using drift-reducing nozzles and double flat fan nozzles. On the lower part of the plant, coverage of the upper part of the leaves was 3 to 5 times better than on the lower part of the leaves. Furthermore, Kierzek and Wachowiak (2007) determined the highest spray deposit and the best coverage of potato plant with the use of a special flat fan nozzle with a spray jet revolved backwards at a 30 ° angle from the vertical. An air-assisted sprayer was used in the trial. Air-injector flat fan nozzle with a single spray jet and standard flat fan nozzle were both less effective in the coverage of potato leaves.

Kryger Jensen (2007) stated a similar biological efficacy of air-injector nozzles in comparison with standard and drift-reducing nozzles. Backward angled nozzles proved to reach a better coverage of potato leaves than nozzles with a vertical spray jet. At times, air-assisted sprayers prove to be more biologically effective. With Danfoil sprayers, smaller volume application and fungicide deposition rates may be used. This could, however, result in reduced biological efficacy. Under-leaf deposition of spray mixture may improve the efficacy but it would simultaneously reduce

the area efficiency and raise the expenses (Kryger Jensen, 2007).

According to Klausen (2007), air-injector nozzles proved to be more effective in the coverage of potato leaves in comparison with standard nozzles. The coverage on the upper part of the plant was better when air-assisted sprayers and Danfoil sprayers had been used. The use of Danfoil sprayers resulted in the best coverage of the middle part of the plant. With a reduced volume application rate (120 l/ha), the largest coverage was reached by air-assisted sprayers and Danfoil sprayers.

Kierzek (2007) compared the fungicide deposition quality on potato plants between a standard nozzle with a vertical spray jet and a nozzle with a 45° spray jet. In comparison with the standard nozzle, the latter reached an approximately 50% better coverage of the upper part of the leaves. Coverage of the lower part of the leaves with the nozzle having a 45° spray jet was 3-5 times better.

Gajkowski et al. (2005) determined that, in comparison with air-injector nozzles, the use of standard nozzles results in a better coverage of potato leaves. With the pressure raised from 2 to 4 bars, the number of droplets per cm<sup>2</sup> when air-injector nozzles had been used was reduced under the allowed limit (20 droplet impressions per cm<sup>2</sup>).

Stallinga et al. (2010) wanted to determine the effect of driving speed and various nozzle types on spray deposition quality and biological efficacy of Shirlan fungicide (AI fluazinam) in potato late blight control. At the referential 100% application rate, there were no statistically significant differences. They did, however, occur with smaller fungicide and volume application rates (65 and 135 l/ha respectively). Standard nozzles reached better results than air-injector nozzles. An increase of the driving speed from 2 to 4 m/s reduced biological efficacy of the fungicide used at the referential 100% application rate. Furthermore,

droplet impression number per cm<sup>2</sup> dropped by 40-75%. Authors discovered that the droplet impression number per cm<sup>2</sup> lower than 120 reduces biological efficacy. Coverage value and droplet impression number per cm<sup>2</sup> were reduced, from the top towards the lower part of the plant.

Knewitz and Koch (2010) established that, in comparison with single spray jet air-injector nozzles, the use of symmetric double flat fan air-injector nozzles resulted in a better coverage of potato plant. The coverage reached by using the asymmetric double flat fan air-injector nozzle was, however, slightly smaller than with other air-injector nozzle types.

Luckhard and Brune (2011) confirmed a much better spray deposition on the upper two thirds of the plant in comparison with the lower third of the plant, regardless of the nozzle type. In comparison with other air-injector nozzle types, the use of symmetric double compact air-injector nozzles resulted in a better coverage of potato plant. Asymmetric and symmetric double flat fan air-injector nozzles reached better coverage values than single spray jet air-injector nozzles. Due to a smaller number of droplets, the coverage of potato plant at a 150 l/ha volume application rate was much smaller than at 350 l/ha. Due to a smaller number of coarse droplets, droplet impression mass on the upper third of the plant was relatively high, whereas the coverage value was small. Despite the extremely coarse (450-575 µm) and coarse (350-450 µm) volume median diameter (VMD) of droplets formed by nozzles, spray deposition on the leaves was good due to a special leaf surface structure of potato plant. Fungicide deposition quality proved to be better at a 350 l/ha than at a 150 l/h volume application rate.

The aim of the trial was to determine the spray deposition quality on potato leaves according to various nozzle types. Furthermore, we wanted to research the coverage of potato

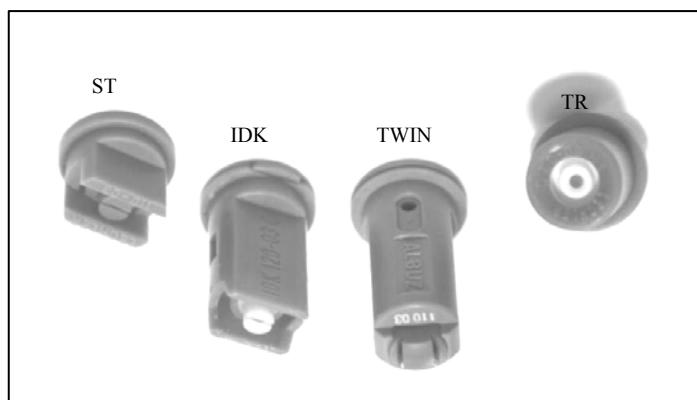
leaves with spray mixture on the lower part of the plant and to determine which nozzle type is the most suitable one for penetrating the thick canopy, from the top towards the lower part of the plant. Two standard nozzle types were used in the trial, namely a flat fan nozzle ST and a hollow cone nozzle TR. We also included two newer nozzle types – a single spray jet air-injector compact nozzle IDK and a symmetric double flat fan air-injector nozzle AVI-TWIN. In Slovenia, it is customary to use standard nozzles. Air-injector nozzles, on the

other hand, are used less frequently. We were interested in finding out whether the new nozzle types ensure a better spray deposition on potato leaves in comparison with standard nozzles. We put forth a hypothesis that, particularly in the middle and lower part of the plant, the use of the two air-injector nozzle types ensures a better spray deposition in comparison with the two standard nozzle types. Moreover, we presumed that both air-injector nozzle types are able to penetrate the canopy better than the standard nozzle types.

## 2 MATERIALS AND METHODS

4 different nozzle types - 2 standard ones and 2 air-injector ones – were used in the potato spraying trial (Figure 1). Among the standard nozzle types, there were a standard flat fan nozzle ST 110-03 and a standard hollow cone nozzle TR 80-03 C, while air-injector nozzle

types included a flat fan air-injector nozzle IDK 120-03 C and a symmetric double flat fan air-injector nozzle AVI TWIN 110 03. The latter had a 30 ° forward and a 30 ° backward spray jet in accordance with the spraying direction.



**Figure 1:** Nozzles used in the trial

The trial was performed on lighter soil, in Dol pri Ljubljani, in the year 2009. The trial was based on 3 random blocks with 3 repetitions within trial units. Each of three block was 12 m wide and 5 m long. 10 m divider strips were created among the blocks. Within each block, various nozzle types were randomly distributed along the spray boom. Nozzles of the same type were placed together in groups of 4 or 5, it means 2 to 2.5 m wide. In the trial, a medium-to-late Aladin potato cultivar was

used at a 75 cm inter row width. Potatoes were planted at a distance of 29.6 cm, creating an exact tuber density of 45,000/ha.

During the inflorescence emergence, when approximately 40% of flowers were already open (BBCH 604), potato was sprayed with systemic and contact fungicide Melody Duo (AI iprovalicarb and propineb) at a 2.5 kg/ha application rate. This fungicide is used in potato late blight (*Phytophthora infestans*) and

early blight (*Alternaria solani*) control during the period of intensive growth. During the spraying, plants were 70 cm high. Each treatment involved three randomly selected plants in the same row. The chosen plants were always located in the middle, on the spot corresponding to the third nozzle (out of five) of each nozzle type. We thus managed to avoid the edge effect of spray jets from the adjacent nozzle types on our measuring place. We attached water-sensitive paper to the upper leaf surface in the upper (70 cm from the ground), middle (40 cm from the ground) and lower part (10 cm from the ground) of the plant (Figure 2). This was done with the aid of paperclips. There was approx. 30 cm of vertical distance among various measuring papers. Measuring papers were 76 mm long

and 26 mm wide. Each treatment involved 9 measuring papers. Due to a possible effect of tractor/sprayer passage on the spray deposition on measuring papers, measurements were not performed along the tramlines. Tractor mounted sprayer with a 600 l tank capacity and a 12 m wide spray boom was used in the trial (Figure 3). Spray boom was located 50 cm from the target surface. Driving speed during the spraying was 4.1 km/h and working pressure amounted to 4.0 bars. There was a 400 l/ha volume application rate. Volume median diameter (VMD) of droplets for each nozzle type is shown in Table 1. Air temperature during the spraying was 15 °C, with a 67% relative air humidity and a 0.8 m/s wind speed. After the spraying, measuring papers were collected and analysed.



**Figure 2:** Water-sensitive paper on the upper parts of plants



**Figure 3:** Spraying the field trial with tractor mounted sprayer

**Table 1:** VMD of droplets and droplet distribution according to BCPC and ASAE for each nozzle used in the trial, the pressure of 4.0 bars was used in all cases

Nozzle type	VMD ( $\mu\text{m}$ )	Droplet distribution according to BCPC and ASAE
ST 110-03	200	fine
IDK 120-03 C	380	coarse
AVI-TWIN 110-03	410	coarse
TR 80-03 C	100	very fine

Droplet impressions on measuring papers were analysed with Optomax Image Analyser. Three measurements were performed on each measuring paper. In the APA 2001 V5.1 program, we calculated the coverage value and the droplet impression number per  $\text{cm}^2$ . Based on this data, impression area of a single droplet was calculated as a quotient of coverage value and droplet impression number per  $\text{cm}^2$ . In addition, relative reductions in coverage value and impression area of a single droplet, from the top towards the lowest part of the plant, were analysed. Coverage value and impression area of a single droplet on the upper part of the plant meant 100%.

Statistical analysis was performed according to the procedure valid for random blocks with repetitions within the trial units (Košmelj,

2001; Hadživuković, 1991). We initially examined homogeneity of variance, using Hartley's test. Coverage value data, relative reduction in coverage value and relative reduction in impression area of a single droplet were then transformed with the asin (sqrt) function. Analysis of variance and Duncan's Multiple Range Test were performed at  $\alpha = 0.05$ . A separate analysis of the upper, middle and lower part of the plant was performed. It was followed by a joint analysis of separate parts of the plant. This gave us a more accurate picture on the spray deposition on the target surface according to individual nozzle types. If present, statistically significant differences among various treatments were marked with different letters. All statistical analyses were performed by the Statgraph 4.0 program (Statistical Graphics Corp., Manugistics, Inc.).

### 3 RESULTS AND DISCUSSION

In comparison with the symmetric double flat fan air-injector nozzle TWIN (37%), the standard hollow cone nozzle TR reached better coverage value (47%) on the upper part of the plant (Figure 4). It is presumed that the slightly higher coverage value of the standard hollow cone nozzle TR was the result of a higher droplet impression number per  $\text{cm}^2$  (65) when compared with both air-injector nozzle types, namely IDK and TWIN (with 40 and 42 droplet impressions respectively). Compared to the TR nozzle, the IDK nozzle forms a smaller number of droplets, which are, however, coarser, causing the lack of statistically significant differences in the coverage value. TR nozzle forms a larger number of very fine droplets with the volume median diameter of 100  $\mu\text{m}$ . Results of the impression area of a single droplet amounting to 0.72  $\text{mm}^2$  partially prove that fact (Figure 6). Volume median diameter of the symmetric double flat fan air-injector nozzle TWIN was probably excessive (approx. 410  $\mu\text{m}$ ), causing the coverage value of this nozzle to be slightly lower. Higher pressure values and a slightly higher number of droplets per  $\text{cm}^2$  would raise the coverage value resulting from the use of this nozzle. According to nozzle producer Agrotop (2010), TWIN nozzles allow a better spray deposition on the vertically positioned plant parts in comparison with the horizontal parts, i.e. leaves on the upper part of the plant, which might be the cause of a slightly worse coverage value. Gajtkowski *et al.* (2005) stated that good fungicide deposition onto potato plants should involve at least 50 droplet impressions per  $\text{cm}^2$  and a coverage value higher than 15%. In our trial, both air-injector nozzle types proved to deposit less than 50 droplets per  $\text{cm}^2$ . On the other hand, the coverage value they reached largely exceeded the above-mentioned 15%. Due to this fact, the quality of spray deposition was very high with air-injector nozzles as well. Results of coverage value indicate a very good quality of

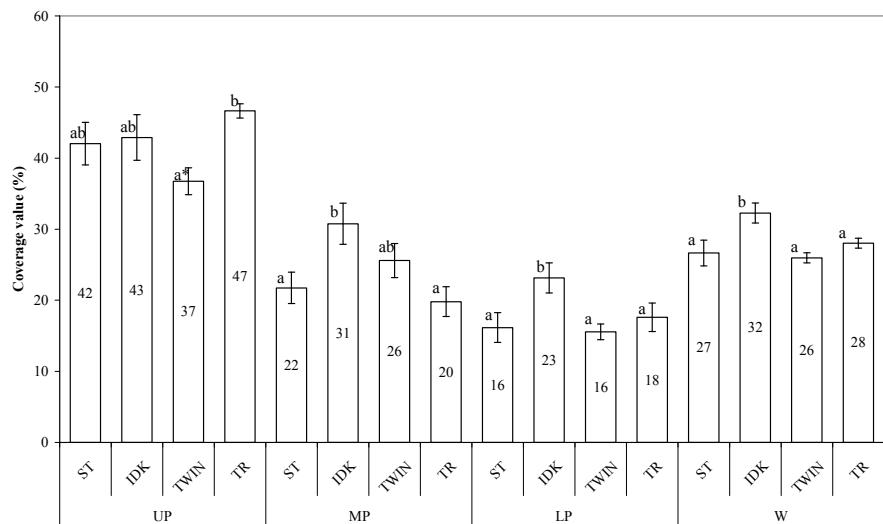
fungicide deposition on the upper part of the plant with all nozzle types used in the trial. This was concluded due to the fact that the upper part of the plant does not impede the droplets to reach the target surface. Moreover, its leaves are well exposed to the spray deposition. Our results partially correspond to the results of Gajtkowski *et al.* (2005), who stated that, in comparison with air-injector nozzles, the use of standard nozzles results in a better coverage of potato leaves. In our trial, this held true merely for the standard hollow cone nozzle TR in the upper part of the plant.

In the middle part of the plant, the single spray jet air-injector compact nozzle IDK reached a significantly higher coverage value (31%) than standard ST and TR nozzles (with 22 and 20% respectively). (Figure 4). Due to a larger volume median diameter, IDK nozzles seem to be more efficient in the penetration through the canopy, whereas the droplets formed by standard ST and TR nozzles are too small and do not have enough speed and kinetic energy to penetrate deeper into the lower parts of the plant. These results partially correspond to Klausen's findings (2007). The latter proved that the use of air-injector nozzles results in a better coverage of potato plant in comparison with the use of standard nozzles. In our trial, this held true for the middle part of the plant, and not the upper one. As anticipated, in the middle part of the plant, both ST and TR standard nozzle types proved to have a higher droplet impression number per  $\text{cm}^2$  than the IDK and TWIN air-injector nozzles (Figure 5). Nevertheless, the droplets formed by both ST and TR standard nozzles were much smaller than those of the IDK and TWIN air-injector nozzles. This was, furthermore, proved by the results of the impression area of a single droplet. With both IDK and TWIN air-injector nozzles, the impression area of a single droplet proved to be bigger (0.45 and 0.60  $\text{mm}^2$  respectively) than with the ST and TR

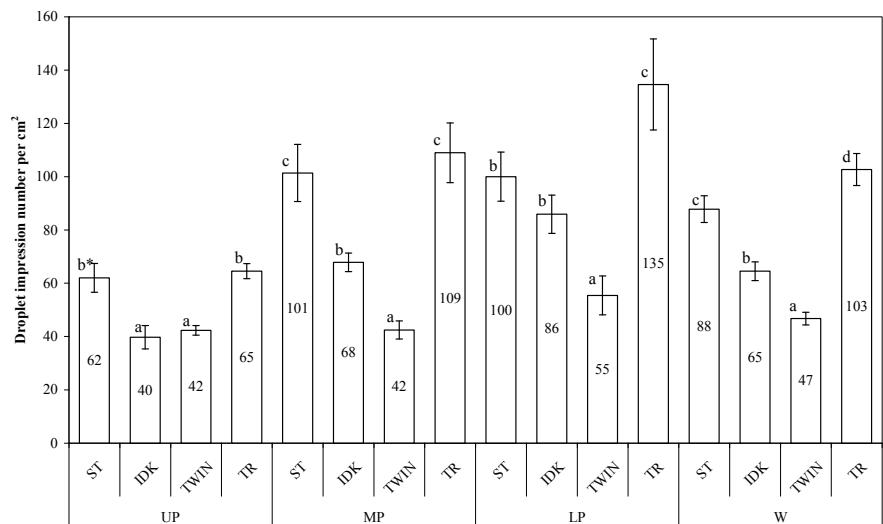
standard nozzles ( $0.21$  and  $0.18 \text{ mm}^2$  respectively) (Figure 6). When comparing the two air-injector nozzles, the single spray jet IDK nozzle proved to reach a higher droplet impression number per  $\text{cm}^2$  (68) than the symmetric double flat fan nozzle TWIN (42). It is thus possible to conclude that both spray jets of the TWIN nozzle have an excessive forward and backward angle which disabled them from sending the droplets deeper into the canopy. In comparison with the upper part of the plant, the middle part had a lower coverage value regardless of the nozzle type. This was anticipated as leaves present a physical obstacle in droplet penetration to the lower-lying parts of the plant. The velocity of fine standard nozzle droplets is lower, causing them to stop earlier on their way in comparison with coarser air-injector nozzle droplets. The latter have a bigger mass, a greater velocity and, therefore, a larger kinetic energy. This is why they are able to penetrate the lower-lying parts of the plant. The increase of the vertical distance between the nozzle and the target surface resulted in a higher droplet impression number per  $\text{cm}^2$ . It can be assumed this occurred due to a bigger overlap of spray patterns from the adjacent nozzles and finer droplets.

In comparison with the upper and middle part, the lower part of the plant reached the lowest coverage value with all nozzle types (Figure 4). In this aspect, the results correspond to the Luckhard and Brune's results (2011). The authors determined that, on the upper two thirds of the plant, the quality of spray

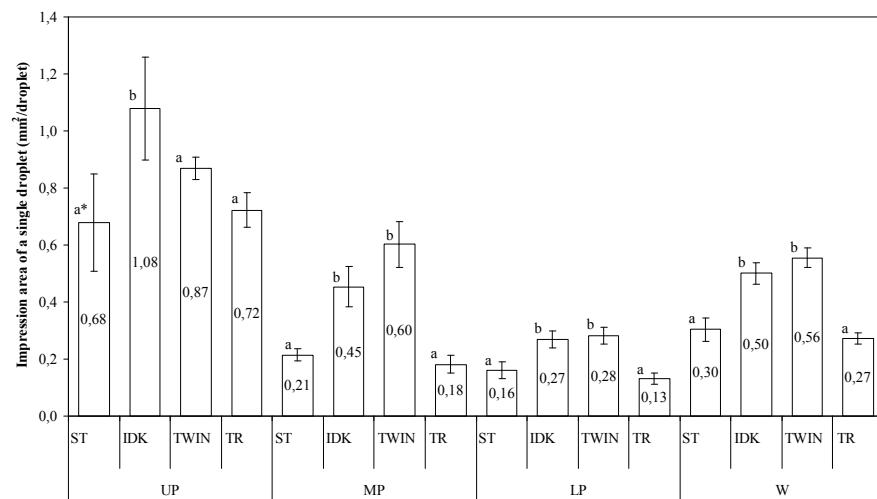
deposition is much higher than on the lower third of the plant regardless of the nozzle type used. Similar to the middle part of the plant, the use of the single spray jet air-injector compact nozzle IDK resulted in a better coverage value (23%) of the lower part of the plant in comparison with other nozzle types (16-18%). Reasons for a quality spray deposition on the lower part of the plant by the IDK nozzle are identical to those for the middle part of the plant. The nozzle has a single vertical spray jet of coarse droplets at a high velocity, which are able to penetrate all the way to the lowest parts of the plant. The ST, TR and TWIN nozzles all reached coverage values which were only slightly higher than 15%. According to Gajtkowski *et al.* (2005), that is the lower limit for a quality fungicide application onto potato plants. Moreover, with the majority of nozzle types, the droplet impression number per  $\text{cm}^2$  was higher on the lower part of the plant than on the middle part of the plant (Figure 5). As already mentioned, this is connected with the increase of distance between the target surface and the nozzle which causes an even bigger overlap of spray patterns from the adjacent nozzles and the reduction in droplet size. With all nozzle types, impression area of a single droplet was smaller on the leaves of the lower part of the plant than in its middle (Figure 6). However, the impression area of a single droplet reached with both IDK and TWIN air-injector nozzle types ( $0.27$  and  $0.28 \text{ mm}^2$  respectively) was statistically higher than with both ST and TR standard nozzles ( $0.16$  and  $0.13 \text{ mm}^2$  respectively).



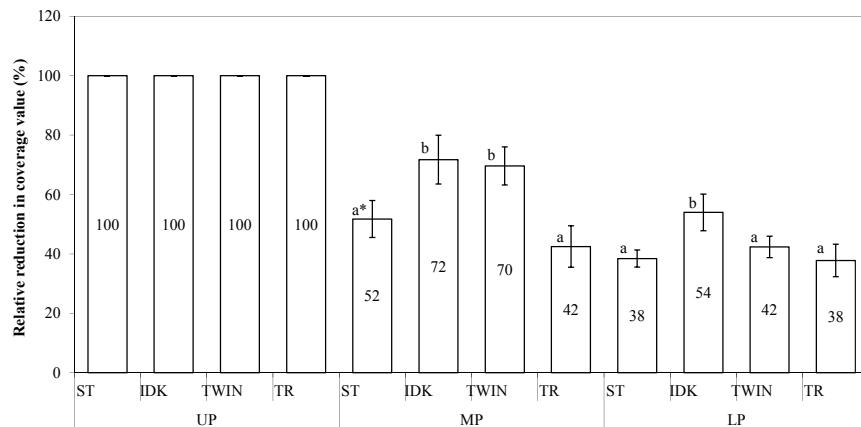
**Figure 4:** Coverage value on the upper, middle and lower part of the plant and on the whole plant according to the nozzle type; UP – upper part of the plant; MP – middle part of the plant; LP – lower part of the plant; W – the whole plant; \* means, at the same part of the plant, followed by different letters are significantly different ( $p < 0.05$ ); bars represent standard errors.



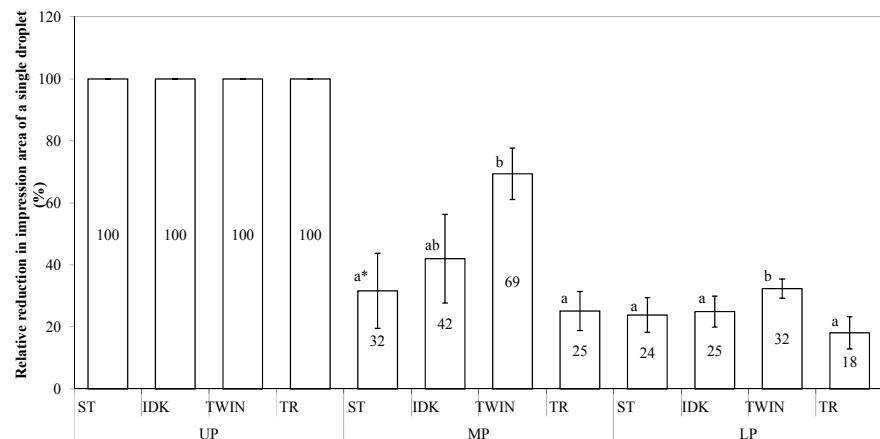
**Figure 5:** Droplet impression number on the upper, middle and lower part of the plant and on the whole plant according to the nozzle type; UP – upper part of the plant; MP – middle part of the plant; LP – lower part of the plant; W – the whole plant; \* means, at the same part of the plant, followed by different letters are significantly different ( $p < 0.05$ ); bars represent standard errors.



**Figure 6:** Impression area of a single droplet on the upper, middle and lower part of the plant and on the whole plant according to the nozzle type; UP – upper part of the plant; MP – middle part of the plant; LP – lower part of the plant; W – the whole plant; \* means, at the same part of the plant, followed by different letters are significantly different ( $p < 0.05$ ); bars represent standard errors.



**Figure 7:** Relative reduction in coverage value on the middle and lower part of the plant in comparison with the upper part of the plant according to the nozzle type; UP – upper part of the plant; MP – middle part of the plant; LP – lower part of the plant; W – the whole plant; \* means, at the same part of the plant, followed by different letters are significantly different ( $p < 0.05$ ); bars represent standard errors.



**Figure 8:** Relative reduction in impression area of a single droplet on the middle and lower part of the plant in comparison with the upper part of the plant according to the nozzle type; UP – upper part of the plant; MP – middle part of the plant; LP – lower part of the plant; W – the whole plant; \* means, at the same part of the plant, followed by different letters are significantly different ( $p < 0.05$ ); bars represent standard errors.

In comparison with the upper part of the plant (100%), the use of IDK and TWIN air-injector nozzle types reduced the coverage value on the middle part to 72 and 70% respectively (Figure 7). A much bigger reduction occurred with the two standard nozzle types. The ST nozzle reduced the coverage value to 52%, while the reduction of the TR nozzle amounted to 42%. The reduction in coverage value on the lower part of the plant was even more distinctive than on the upper part. Once again, the use of standard ST and TR nozzles resulted in a higher reduction (38% of value in comparison with the upper part) than the two air-injector nozzle types – IDK and TWIN (with a 54 and 42% reduction in comparison with the upper part). The above-cited results show that, predominantly with the air-injector nozzle IDK, the coverage value drops throughout the whole plant height downwards by slightly less than 50%. Based on this it is possible to conclude that, when this nozzle is used, a sufficient number of droplets penetrate all the way to the lower part of the plant in order to cover a sufficiently large area. With other nozzle types, particularly the standard ST and TR nozzles, the coverage value is reduced by slightly less than 66%. When comparing the relative reduction in impression

area of a single droplet, from the upper towards the lower part of the plant, the relative reduction was the lowest both in the middle part (reduced to 69% of the upper part value) and the lower part of the plant by the TWIN nozzle (reduced to 32% of the upper part value) (Figure 8). Among other nozzle types (ST, IDK and TR), relative reduction in the impression area of a single droplet on the middle and lower part of the plant proved to be bigger than with the TWIN nozzle. There were, however, no statistically significant differences among the three above-stated nozzle types. Based on we concluded that thick canopy represents a great obstacle for the droplets on their way downwards. Furthermore, the impression area of a single droplet is, moreover, dependent upon the vertical distance between the nozzle and the target area. In comparison with the upper part, middle and lower parts of the plant were reached predominantly by a range of finer droplets. It is important to ensure that the difference in the size of droplets falling on the top of the plant and those reaching its lower part is not excessive. With both standard

nozzle types (ST and TR), impression area of a single droplet was very small already in the middle, but even more in the lower part of the plant. These two nozzles form a range of fine and very fine droplets extremely sensitive to drift. Droplets are considered driftable if their volume median diameter (VMD) is smaller than 150 µm. According to Lešnik (2007), the nozzles which form very fine droplets (e.g. the TR nozzle) create 50-60% of driftable droplets. Nozzles forming fine droplets (e.g. the ST nozzle) have 20 to 50% driftable droplets.

On the whole, flat fan air-injector nozzle IDK proved to have the best fungicide deposition quality on potato leaves at all the levels (upper, middle and lower part of the plant). This nozzle reached the highest coverage value in the middle and lower part of the plant. A sufficient number of droplets managed to penetrate the lowest parts of the plant and there was a less than 50% reduction in coverage of the lower part in comparison with the upper part. Moreover, impression area of a single droplet and subsequent droplet size were also sufficiently large. This confirmed the hypothesis that air-injector nozzle types ensure better spray deposition on potato plants. These results partially correspond to Klausen's findings (2007) of a better spray deposition with air-injector nozzles in comparison with standard nozzles. Nonetheless, our results are not directly comparable with his findings since his trial involved an air-assisted sprayer and a Danfoil sprayer, while our own included a standard sprayer. The hypothesis was only confirmed for the IDK nozzle, and not the symmetric double flat fan air-injector nozzle TWIN. The coverage value of this nozzle type was insufficient particularly in the lower part of the plant. Despite a sufficient impression area of a single droplet, droplet impression number per cm<sup>2</sup> proved to be too small. It is assumed that spray jet angles of the TWIN nozzle (30 ° forward angle and 30 ° backward angle) are too wide, thus reducing the

possibility for the droplets to penetrate the lower parts of the plant. Our findings are different to those of Knewitz and Koch (2010), Kierzek and Wachowiak (2009), and Luckhard and Brune (2011), all determining that the use of air-symmetric double flat fan injector nozzles results in a better coverage in comparison with the single spray jet air-injector nozzles.

Standard ST and TR nozzles reached worse coverage values of the middle and lower part of the plant. Our results on this point are in accordance with Stangl's statement (2009) that nozzles with fine droplets are not able to sufficiently penetrate the thick canopy. Both nozzle types form relatively fine droplets which do not have sufficient velocity and enough kinetic energy to penetrate the thick canopy. Due to this fact, droplets are much more subject to drift. The ST and TR nozzles bear the most pronounced reduction in coverage value, particularly in the middle part of the plant where it dropped to 52% (ST) and 42% (TR) in comparison with the upper part value. Similar reduction occurred with the impression area of a single droplet. On the middle part of the plant, the latter decreased by 68% (ST) and 75% (TR) in comparison with the upper part value. Our results on this point do not correspond to the results of Gajtkowski *et al.* (2005), and Stallinga *et al.* (2010) who determined that, in comparison with air-injector nozzles, the use of standard nozzles results in a better spray deposition on potato plants.

As anticipated, coverage value decreased, from the top towards the lower part of the plant, regardless of the nozzle type. This corresponds to the findings of Stallinga *et al.* (2010). Contrary to their statements, however, the droplet impression number per cm<sup>2</sup> increased. It is assumed that this was caused due to a larger overlap of spray patterns from the adjacent nozzles and finer droplets at a

larger vertical distance between the nozzle and the middle/lower part of the plant.

Our findings are limited to the fungicide deposition, excluding the research on the

biological efficacy of the fungicide in potato late blight (*Phytophthora infestans* L.) and early blight (*Alternaria solani* L.) control. Nonetheless, potato late blight and early blight infections did not occur in our trial.

#### 4 CONCLUSION

Results of the trial show the flat fan air-injector nozzle IDK achieved the best fungicide coverage of potato leaves in the upper, middle and lower part of the plant. This nozzle reached the highest spray mixture coverage value while retaining a sufficient impression area of a single droplet in the middle and lower part of the plant. Furthermore, the coverage with spray mixture applied by the nozzle dropped by 28% in the middle part of the plant and by 46% in the lower part, which is less than with other nozzle types. The IDK nozzle forms a single vertical spray jet of coarse droplets with enough velocity and kinetic energy for the droplets to penetrate the thick canopy. Results show that the angles of both spray jets of symmetric double flat fan air-injector nozzle TWIN (the 30 ° forward and the 30 ° backward angle) are excessively wide in order for the droplets to penetrate all the way to the lower parts of the plant. This nozzle did thus not confirm the proposed hypothesis. The use of both standard

nozzle types resulted in a worse coverage and impression area of a single droplet despite them having the largest droplet impression number per cm<sup>2</sup>. The two standard nozzles form a larger number of fine and very fine droplets which are excessively light and do not have enough speed to penetrate all the way to the lower parts of the plant. Among these droplets, there is a large percentage of driftable droplets (< 150 µm) which are extremely sensitive to drift.

Our conclusions refer to annual results. In order to confirm results as a whole, additional field trials should be performed in the years to come. Further research should involve biological efficacy of fungicides according to various nozzle types. This would give us an even clearer picture on the effect of various nozzle types and subsequent spray deposition quality on the efficacy of potato late blight (*Phytophthora infestans*) and early blight (*Alternaria solani*) control.

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**Agrovoc descriptors:** water balance, groundwater, lysimeters, evapotranspiration, measuring instruments, equipment, meteorological instruments, plant cover, water use, precipitation, resource management, water management, water resources, water pollution, groundwater pollution

Agris category code: p10

## Determination of water balance components with high precision weighing lysimeter in Kleče

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### ABSTRACT

Components of the basic water balance equation and water balance calculation for July 2011 for weighing lysimeter on water supply pumping station in Kleče, Ljubljana are presented. Lysimeter and outflow mass measured with high precision weighing cells and precipitation as determined from changes in the mass of the lysimeter were used in calculation. Precipitation measurements in the same time resolution as the lysimeter mass measurements would be needed for correct calculation of actual evapotranspiration. In time of high plant water requirements only substantial precipitation events directly contribute to immediate groundwater recharge. The low water retention of the aquifer sediments shows susceptibility of the aquifer to ground water pollution.

**Key words:** weighing lysimeter, water balance, lysimeter station

### IZVLEČEK

### DOLOČITEV ČLENOV VODNE BALANCE Z NATANČNIM TEHTALNIM LIZIMETROM V KLEČAH

Obračnavani so členi in izračun osnovne enačbe za vodno平衡 za julij 2011 za tehtalni lizimeter na črpališču pitne vode Kleče v Ljubljani. Pri izračunu so bile uporabljene meritve mase lizimetra in iztoka, merjene z natančnimi tehtalnimi celicami ter padavin, izračunane iz spremembe mase lizimetra. Za pravilen izračun dejanske evapotranspiracije bi bile potrebne meritve padavin v enakem časovnem razkoraku kot potekajo meritve na tehtalnem lizimetru. V času visoke porabe vode s strani rastlinskega pokrova k bogatitvi podtalnice prispevajo le večji, zaporedni padavinski dogodki. Nizke zadrževalne sposobnosti sedimentov vodonosnika kažejo občutljivost vodonosnika in ogroženost podzemne vode zaradi onesnaženja.

**Ključne besede:** tehtalni lizimeter, vodna bilanca, lizimetska postaja

## 1 INTRODUCTION

Lysimeters are used for the measurement of amount and quality of water percolation beneath plant's root zone, water loss via evapotranspiration, as well as numerous ranges of applications in agriculture and environment (Meissner et al., 2010). Quantification of soil

water flow is a prerequisite for accurate prediction of solute transfer within the unsaturated zone (Meissner et al., 2010) providing answers to both scientific and practical questions regarding protection of groundwater and groundwater recharge.

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Practical application of groundwater models for urban water management can be hampered by the lack of knowledge of the complex urban groundwater recharge patterns (Vižintin et al., 2010). Groundwater from Ljubljansko polje aquifer, which is in the center of Slovenia, is fresh water source for the 270 000 inhabitants of Slovenia's capital (Vižintin et al., 2010). Above the aquifer intensive vegetable production is ongoing in close proximity to or even within the protected groundwater zone, posing a threat to groundwater quality (Zupanc et al., 2011).

In the recent years lysimetry has evolved significantly and improved techniques, which enable accurate measurements of water flow and water balance parameters (Meissner et al., 2010, von Unold and Fank, 2008), and can be used for investigating hydrological processes such as precipitation, infiltration, or deep percolation with respect to groundwater recharge.

In this article we address water balance for July 2011 by using data from the lysimeter station in Ljubljana Kleče, and some of the challenges arising from calculation and data interpretation.

## 2 MATERIALS AND METHODS

Lysimeter station in Ljubljana Kleče Water Pumping station was constructed in the eighties for the water balance measurements (Brilly and Gorišek, 1985), however outflow measurements indicated structural damage (Zupanc et al., 2005). In 2010 a new and technically advanced weighing lysimeter was installed at the drinking water pumping station in Ljubljana Kleče, Slovenia (308 m altitude, 46°5'11'' N, 14°29'56'' E), that enables state-of-the-art measurements of water balance parameters. The type of lysimeter is a scientific lysimeter (von Unold and Fank, 2008), designed to solve the water balance equation by measuring the mass of the lysimeter monolith as well as that of outflow tank with high accuracy and high temporal resolution. The soil monolith (2 m height, surface area 1 m<sup>2</sup>) was taken from sandy gravel sediments on the area of the water pumping station (Fluvisol), plant cover is extensive grass. The lysimeter weighing facility detects mass changes as small as 30 g, which corresponds to a water head of 0.03 mm. Inside the monolith T8 tensiometers and TDR Trime probes were installed to measure

soil water status, namely soil water tension (hPa) and soil water content (%), respectively (Table 1, Figure 1). In addition, water sampling from monolith horizons under field conditions is possible to describe the solute fluxes inside the soil profile (von Unold and Fank, 2008). Figure 1 shows the structure of the lysimeter facilities (lysimeter vessel, outflow tank, pump, and data logger), and data management processes, as well as the lysimeter bottom boundary conditions subroutine. The latter is necessary in order to adapt the water dynamics inside the lysimeter as a closed system to the conditions in an undisturbed field soil profile. Outflow water is collected with suction cups and pumped into outflow storage tank. Boundary conditions are controlled through soil water status (soil water tension, Figure 1) on the bottom of the lysimeter and in the field on the same depth (190 cm). If necessary, water is pumped back into the lysimeter to maintain the same soil water status. Total water mass of the full outflow tank is 30 kg, drainage capacity is 20 kg, boundary conditions water supply 10 kg.

**Table 1:** Hydrological quantities measured on the lysimeter station in Kleče, Ljubljana

	Parameter	Units	Time step
Weighing Lysimeter 2010	Water tension – lysimeter (50, 100, 150, 190 cm)	hPa	10 min
	Water tension – field (190 cm)	hPa	10 min
	Water content (50, 100, 150 cm)	Vol %	10 min
	Soil temperature – lysimeter (50, 100, 150, 190 cm)	°C	10 min
	Soil temperature – field (190 cm)	°C	10 min
	Lysimeter mass	kg	1 min
	Outflow tank mass	kg	1 min
Environmental Agency of Slovenia	Precipitation	mm	24h sum

Components of the basic water balance equation for the lysimeter are precipitation ( $P$ ), outflow ( $O$ ), evapotranspiration ( $ET$ ) and change of water in the monolith ( $\Delta S$ ), written as

$$P - ET - O - \Delta S = 0 \quad (1)$$

If the lysimeters' mass is recorded in certain time steps, with precipitation and outflow amount measured separately, actual evapotranspiration can be deduced from their mass change (Young et al. 1996).  $ET_a$  should then be calculated after

$$ET_a = (P_{i+1} - P_i) - (W_{i+1} - W_i) - (O_{i+1} - O_i), \quad (2)$$

where  $ET_a$  is actual evapotranspiration (mm),  $P_i$  precipitation (mm),  $W_i$  lysimeter mass (kg) and  $O_i$  mass of the outflow tank (kg),  $i$  is the time step. All quantities should have the same temporal resolution.  $W$  and  $O$  were measured at the lysimeter facility in 1-minute-intervals (Table 1).  $P$  was measured on-site ( $P_{site}$ ), and at the meteorological station of the Environmental Agency of Slovenia (ARSO) in Ljubljana ( $P_{city}$ ) (299 m altitude, 46°3'57" N, 14°31'2" E) with standard pluviograph, which gives the sum of precipitation for 24 hours (from 7 am to 7 am). Therefore  $ET_a$  was determined on a daily base using Eq. 2, with  $i$  being 24hrs.

Furthermore,  $P$  was determined directly from the lysimeter weighing data ( $P_{lys}$ ).  $P_{lys}$  was calculated by first determining positive  $W$  change with the help of graph, then subtracting  $W$  immediately before mass increase from the maximum  $W$  before the latter began decreasing (which is due to either  $ET$  or  $O$ ). The basic approach is that in short time intervals either  $P$  (positive mass change) or  $ET$  (negative mass change) occur, with  $O$  being taken into account (von Unold and Fank, 2008). Obviously, this method can provide only an estimation of  $P$ , because during rainfall also evaporation and transpiration take place, which can have significant influence if precipitation event is small and the surface hot. Due to high spatial variability of the storms (Barros and Lettenmeier, 1994), measurements on lysimeter's micro location is necessary. Weighing lysimeters with the same precision have given good results for dew measurements (Meissner et al., 2007, Xiao et al., 2009), and they deliver proper results if  $P$  from standardized pluviographs is not representative (remote location), malfunctioning or inadequate in terms of temporal resolution of measurements.

Reference evapotranspiration ( $ET_0$ ) was calculated by ARSO according to FAO-Penman-Monteith (Allen et al., 1998) based on weather data from the meteorological station in Ljubljana (299 m altitude, 46°3'57" N, 14°31'2" E).

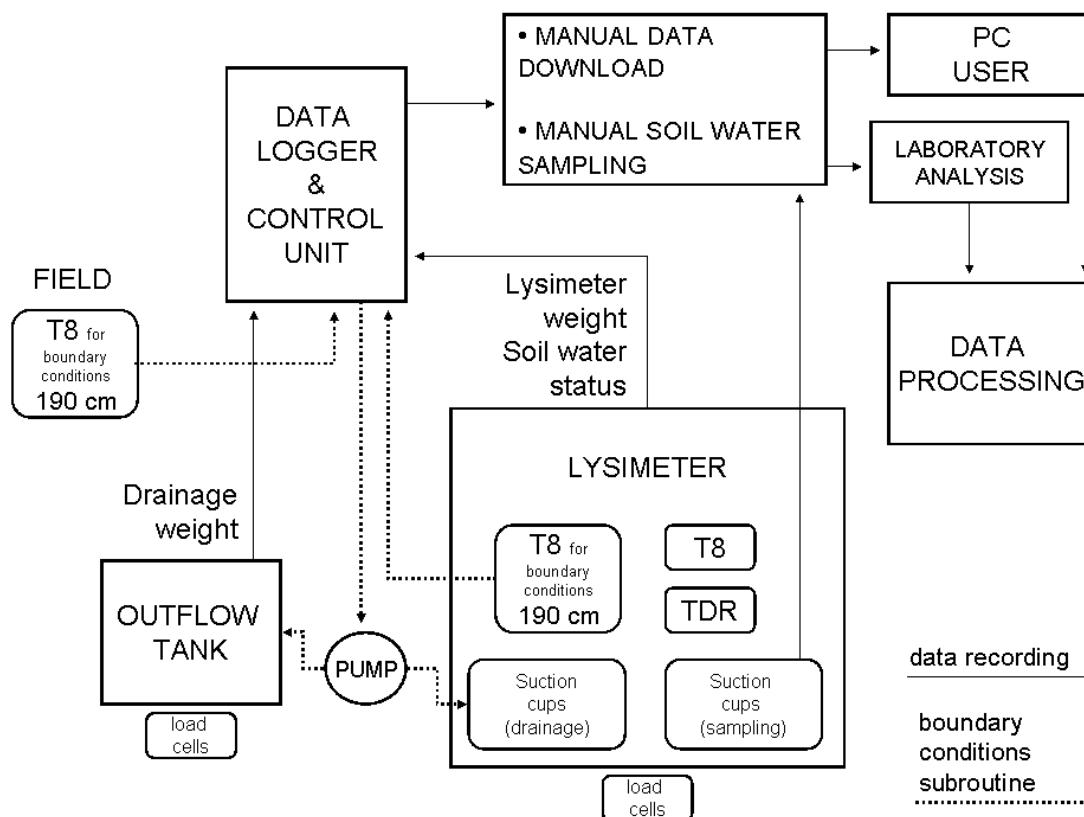


Figure 1: Flow chart of Kleče weighing lysimeter station elements and sensors, as well as data transfer and storage

### 3 RESULTS AND DISCUSSION

Figure 2 shows the mass changes of the lysimeter monolith and the outflow tank. The mass of the lysimeter was between 4030.6 kg (minimum on the 17<sup>th</sup> of July) and 4219.9 kg (maximum on the 24<sup>th</sup> of July, Figure 2) representing a change in profile water content of about 190 mm. Outflow was about 130 mm in the same period. Based on the mass changes 15 precipitation events were determined (Figure 2, Table 2), two substantial: one on the 17<sup>th</sup> of July and a second on the 23<sup>th</sup> of July. Total monthly precipitation amount was 246.5 mm. The precipitation from 22<sup>nd</sup> to 24<sup>th</sup> of July caused high outflow – the outflow tank was emptied six times – that lasted for several days and disguised the subsequent precipitation event on the 27<sup>th</sup> of July (3.9 mm, as determined from the data). The amount of

monthly outflow was about 60 % of the rainfall in July.

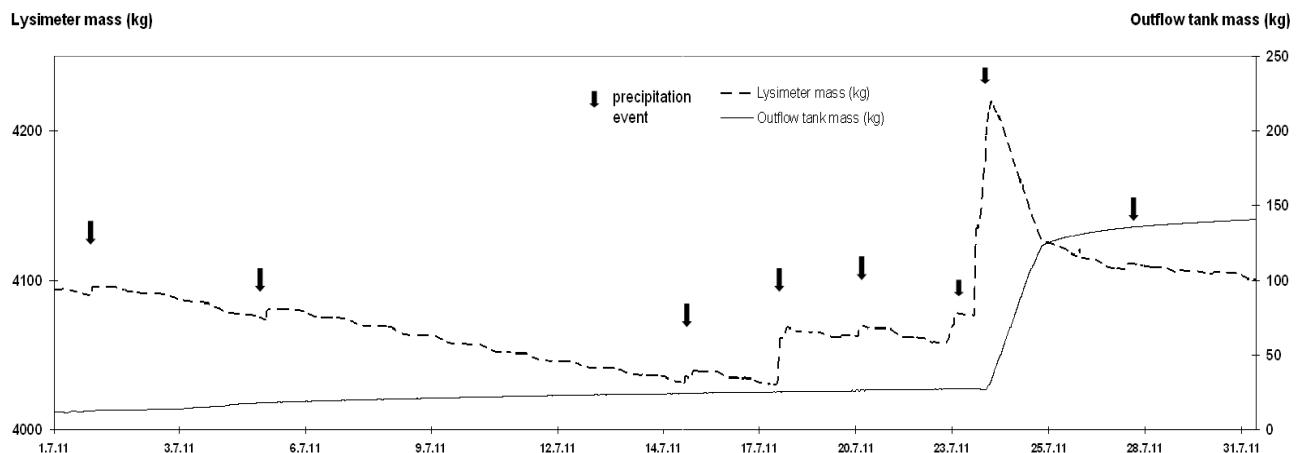
The pluviograph on the lysimeter station delivered no data ( $P_{site}$ ) after 23<sup>rd</sup> of July (Table 2), presumably malfunction occurred due to high intensity of the event. Namely, precipitation event on the 23<sup>rd</sup> of July begun at 20:17 ( $W = 4076.6$  kg, Figure 2) and lasted until 22:09 ( $W = 4134.7$  kg, Figure 2) with high intensity ( $31.1 \text{ mm} \cdot \text{h}^{-1}$ ) that was observed for example in precipitation events in Julian Alps (Žagar et al., 2004), then after a short break continued until the next day at 8:08 ( $W_{max} = 4219.9$  kg) with lower intensity ( $8.5 \text{ mm} \cdot \text{h}^{-1}$ ) (Figure 2). Measurements from the ARSO meteorological station ( $P_{city}$ ) were significantly different, thus they were not

representative. Consequently, actual  $ET_a$  was calculated using  $P_{lys}$ .

The first half of July was hot and dry with an average  $ET_0$  of 5.4 mm per day. In the same period,  $ET_a$  was always lower with an average of 4.5 mm per day (Table 2). An explanation for the differences is that  $ET_0$  was calculated using data from the ARSO meteorological station in the city (ca. 4 km away), and micro climatic conditions of the Kleče lysimeter station differ due to the surrounding forest. It is also possible that the differences between calculated  $ET_0$  and measured  $ET_a$  are that the

grass cover does not fulfill the requirements for comparing Penman-Monteith calculation (Allen et al., 2011).

The second half was affected from several rainfall events as mentioned before.  $ET_a$  exceeded  $ET_0$  after rainfall, likely due to interception and evaporation losses. On 23<sup>rd</sup> and 24<sup>th</sup> the water balance resulted in improper negative  $ET_a$ . This problem should be solved by optimizing interpretation of outflow data, especially at high outflow rates with several emptying processes of the outflow tank.



**Figure 2:** Mass of lysimeter (kg) and outflow tank (kg) for lysimeter station in Kleče in July 2011, black arrows mark precipitation events

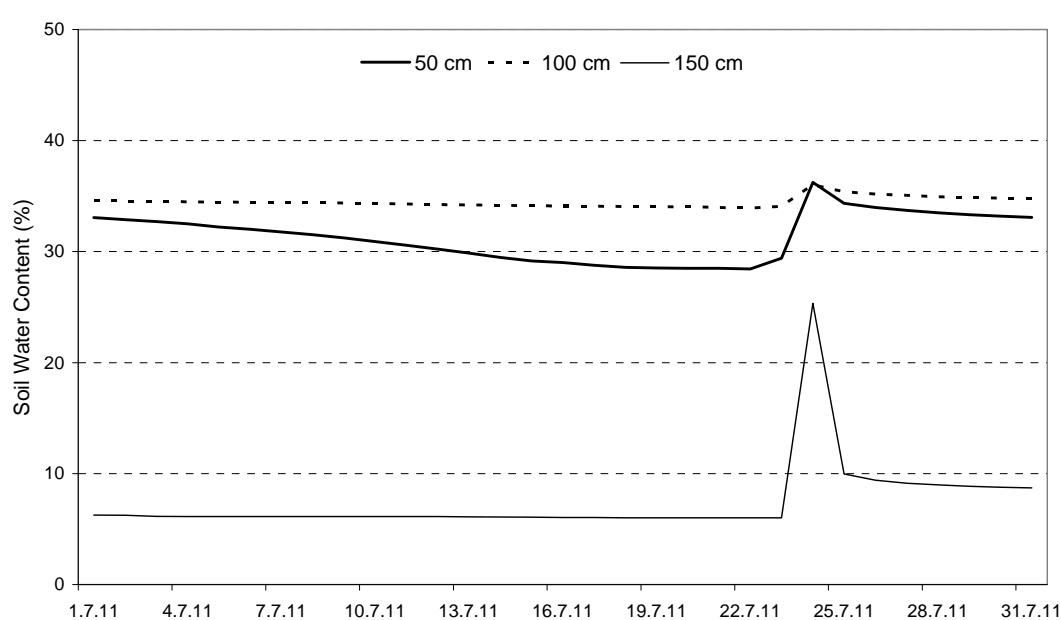
**Table 2:** Water balance parameters: mass change of lysimeter  $dW_{7h}$  and outflow tank  $dO_{7h}$ , precipitation measured by ARSO in the city  $P_{city}$  and on the site  $P_{site}$ , and  $P_{lys}$  as determined from  $W$  data,  $ET_0$  and  $ET_a$  calculated from Eq. 2 using  $P_{lys}$ 

Date	$dW_{7h}$ (mm)	$dO_{7h}$ (mm)	$P_{city}$ (mm)	$P_{site}$ (mm)	$P_{lys}$ (mm)	$ET_0$ (mm)	$ET_a$ (mm)
1.7.2011	1.3	1.7			5.8	3.8	2.9
2.7.2011	-4.5	0.6	4.2	5.6		4.1	3.9
3.7.2011	-5.9	1.5				5.1	4.5
4.7.2011	-7.9	2.5				5.9	5.4
5.7.2011	3.2	1.3			8.0	4.2	3.4
6.7.2011	-5.5	0.9	10.3	7.4	0.2	5.0	4.8
7.7.2011	-5.9	0.8				5.8	5.0
8.7.2011	-6.0	0.7			0.1	6.2	5.4
9.7.2011	-6.0	0.8				6.2	5.2
10.7.2011	-5.9	0.7				6.5	5.2
11.7.2011	-5.2	0.5				6.1	4.8
12.7.2011	-4.8	0.7				5.4	4.1
13.7.2011	-4.9	0.3				5.7	4.6
14.7.2011	-4.7	0.4				5.9	4.3
15.7.2011	7.3	0.4			10.3	3.0	2.6
16.7.2011	-4.3	0.4	7.4	7.8		4.3	4.0
17.7.2011	27.1	0.2			31.0	5.2	3.7
18.7.2011	3.9	0.4	21.8	28.0	6.8	3.9	2.5
19.7.2011	-2.0	0.2	4.5	6.6		4.0	1.7
20.7.2011	4.3	0.2	1.3	1.8	8.9	3.5	4.4
21.7.2011	-6.2	0.4	6.5	6.6		5.5	5.8
22.7.2011	13.1	0.3			17.6	4.7	4.1
23.7.2011	140.9	2.9	20.2	-	143.8	2.4	0.0
24.7.2011	-57.4	64.4	65.5	-	4.7	1.7	-2.3
25.7.2011	-35.5	32.2	7.8	-		2.1	3.3
26.7.2011	-8.9	5.0		-		3.4	3.9
27.7.2011	-2.7	3.1		-	3.9	4.6	3.5
28.7.2011	-3.0	2.0	5.2	-	0.5	2.3	1.4
29.7.2011	-2.4	1.4	0.5	-	2.3	3.6	3.3
30.7.2011	-1.0	1.4	0.8	-	2.6	3.3	2.1
31.7.2011	-4.7	0.9	1.2	-		3.4	3.9
<b>Sum</b>	<b>5.8</b>	<b>-129.1</b>	<b>157.2</b>	*	<b>246.5</b>	<b>136.8</b>	<b>111.5</b>

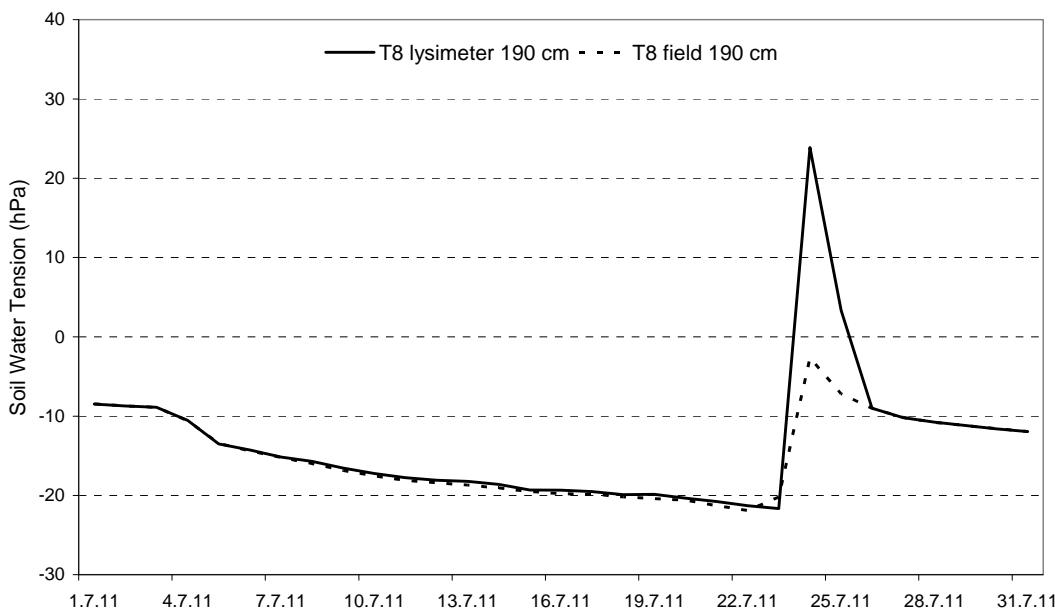
Sensors for monitoring water status inside the lysimeter showed high soil water content (SWC) in 50 and 100 cm depth (Figure 3). SWC in 50 cm decreased slightly until rainfall from 22<sup>nd</sup> onwards. Neither previous rainfall events nor characteristics of plant water uptake – usually expressed as stepwise decrease of SWC representing typical day-night-effects – were displayed. It can be concluded that higher soil water dynamics occurred in the upper soil layer and the rooting zone, respectively. Decrease of SWC in 50 cm can be explained by water movement to upper soil layers compensating ET losses, or water flow to deeper layers, mainly due to gravitation forces. This question cannot be explained satisfactorily without information on water potential in the respective soil layers. SWC in 100 and 150 cm did not change visibly until rainfall from 22<sup>nd</sup> onwards (Figure 3), however, downward water fluxes must be assumed, because of the constant outflow at the bottom boundary (Figure 2). Low SWC (between 6 and 9 %) of the 150 cm layer indicates poor water retention capacity (Figure

3), typical for gravelly soils found on Ljubljana aquifer (Vižintin et al., 2009). The spike in SWC after 24<sup>th</sup> of July for 36 hrs represented water from rainfall moving towards outflow.

Measurements of the soil water tension in 190 cm depth inside the lysimeter and in the field (Figure 4) showed unsaturated conditions in the lysimeter and in the field (between -9 and -20 hPa). During high precipitation between 22<sup>nd</sup> and 24<sup>th</sup> of July the outflow through the suction cups at the bottom of the lysimeter was not sufficiently fast to match the flow of the water through the soil layers outside in the field conditions, causing temporary water logging of the lower layers, expressed as positive water pressure 25 hPa in the lysimeter (Figure 4). Water pressure in 190 cm in the field remained negative (-3 hPa, Figure 4), indicating that the water flow following the storm did not saturate the lower layers but has moved through the gravelly sediments in a few hours.



**Figure 3:** Soil water content (%) in three soil depths (50, 100 and 150 cm) inside the Kleče lysimeter measured by TDR-probes in July 2011



**Figure 4:** Soil water tension (hPa) in 190 cm depth inside the Kleče lysimeter and in the field for boundary conditions management in July 2011

Only short period was evaluated, however the chosen month demonstrates weather extremes of the local climate – relatively dry periods, followed by high precipitation amount. In time of high plant water requirements only subsequent substantial precipitation events directly results in water flow towards lower layers. At the same time, the stony, gravelly layers of the deeper parts of the sediments have little or no capacity for water retention,

and in the event that water line leaves top soil, water flow moves downwards fairly quickly. On one hand this confirms high recharge capacity of Ljubljansko polje aquifer from precipitation (Vižintin et al., 2009) on green areas; on the other hand it shows tremendous susceptibility of the aquifer to pollution and reinforces the position of groundwater protection zones above aquifer.

#### 4 CONCLUSIONS

Practical execution of the commonly applied water balance equation is not as straightforward. Precipitation measurement in the same time resolution as the lysimeter mass measurements is needed for correct calculation of reference evapotranspiration. It is also possible that the outflow rate, which is determined by water flow through suction cups

by pump activity, should be re-set. The results show that in time of high plant water requirements only substantial precipitation events directly contribute to immediate groundwater recharge as well as susceptibility of the aquifer of Ljubljansko polje to ground water pollution.

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**Agrovoc descriptors:** agriculture, developing countries, appropriate technology, information processing, data collection, data processing, information services, information technology, information systems, communication technology, social change, socioeconomic development, education, extension activities, adult education, advisory officers

**Agris category code:** a01, c20, c30

## E-readiness of Rural ICT Offices for Rice e-marketing in Rasht Township, Iran

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### ABSTRACT

Applying Information and Communication Technology (ICT) in different economic sectors, especially the agricultural sector is increasing in developing countries. Rural ICT offices in Iran have an important role in decreasing the digital divide by representing internet, post and bank services; however they are not yet active in agricultural services. The present study seeks to survey the electronic readiness of 68 rural ICT offices in the rural area of Rasht in Guilan province, in the northern part of Iran. A five-point Likert type scale questionnaire was prepared, to survey socio-cultural, legal, connection and back-up services indices. Results indicated that intention to adopt, and legal aspects, required development, but the socio-cultural index had a better situation in comparison with others, and receptivity to agricultural electronic commerce (e-commerce) showed a high percentage among respondents. So this points to the necessity of creating an integrated website for rural offices to represent agricultural services. Also, the level of education and age of managers did not show any significant influence on receptivity to e-commerce, but internet skills and farmers visiting the offices have been important factors in receptivity to e-commerce.

**Key words:** E-readiness, rural ICT offices, e-marketing, Rasht

### IZVLEČEK

### PRIPRAVLJENOST PODEŽELSKIH IKT SVETOVALCEV ZA E-MARKETING RIŽA NA OBMOČJU MESTA RASHT, IRAN

Uporaba informacijske in komunikacijske tehnologije (IKT) v različnih gospodarskih sektorjih, še posebej v kmetijstvu, se v državah v razvoju stalno povečuje. Podeželski IKT svetovalci imajo v Iranu pomembno vlogo v zmanjševanju digitalne pregrade tako, da posredujejo internetne, poštne in bančne usluge, niso pa še aktivni pri posredovanju elektronskih uslug za kmetijstvo. Predstavljena študija želi preučiti pripravljenost 68 podeželskih IKT svetovalcev v območju provinc Rasht in Guilan v severnem Iranu. Pripravljen je bil vprašalnik s petstopenjsko Likertovo lestvico s katerim smo ocenjevali kulturno-sociološke, pravne, povezovalne in podporne storitvene kazalnike. Rezultati kažejo, da namen za sprejetje storitev in pravna vprašanja zahtevajo dodatni razvoj, medtem ko imajo kulturno-socialni kazalniki, v primerjavi z ostalimi, višje vrednosti, kar velja tudi za sprejemljivost elektronsko trgovjanje v kmetijstvu. To nakazuje potrebo po oblikovanju celovite spletne storitve za kmetijstvo, ki jo bodo podeželski svetovalci lahko uporabljali. Stopnja izobrazbe in starost podeželskih svetovalcev ni pokazala nobenega statistično značilnega vpliva na njihovo pripravljenost za elektronsko trgovjanje, obratno pa velja za poznavanje interneta in pogostnost obiskov kmetov.

**Ključne besede:** E-pripravljenost, podeželski IKT svetovalci, e-marketing, Rasht

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## 1 INTRODUCTION

The world has witnessed the birth of a new era - the information age, a global wave sweeping through all corners of the world (Ifinedo, 2005). Information and Communication Technology (ICT) consists of some latest technologies such as the computer, internet, cell phone and satellite. Older technologies like radio, television, telephone and technology centers, employers, and investment are also in this zone (Dada, 2006, ITU, 2010). E-readiness (electronic readiness) assesses the quality of a country's ICT infrastructure and the ability of its consumers, businesses and governments to use ICT to their benefit. According to APEC (1999) e-readiness is the degree to which an economy or community is prepared to participate in the digital economy. McConnell International (2000) defines e-readiness as the capacity to participate in the global digital economy. In 2010, e-readiness was renamed as the digital economy (EIU, 2010).

In 2006 a study attempted to critically assess the concept of e-readiness (electronic readiness), and suggest ways to bridge the limitation of this tool, with a special focus on developing countries. Results showed that there were both positive and negative aspects of such measures. It was discovered that although e-readiness measures provide a useful overview of the environmental situation, they do not completely reflect the possibility of achieving development from ICT in developing countries. However, there are implications for further research in this area (Dada, 2006).

Ramayah et al. (2005) studied SME e-readiness in Malaysia. They reported that SMEs in Northern Malaysia are ready to go for e-business, e-commerce and Internet in general. They also showed that in general top management commitment and infrastructure and technology have significant impact on

SMEs' e-readiness. However, human capital, resistance to change, and information security do not have significant impact or contribution on e-readiness in SMEs.

The government of Zimbabwe commissioned an e-readiness survey in 2005 in conjunction with the National Economic Consultative Forum (NECF) to assess the country's readiness to embrace ICT. Such findings will later inform a broader national policy. However it does not explain whether in the absence of a national ICT policy, there were sector specific policies, as having such a policy is imperative. In order to bridge the digital gap, Zimbabwe needs to build the infrastructure to allow ICT to be accessible. There is a need to consider non-quantifiable variables, such as poverty and social justice. The other factor is the need for Zimbabwe's government to address current political and economic problems, as this isolation would impact heavily on the need to be interconnected (Mhlanga, 2006).

In a survey about digital development of rural areas in the USA, Maleki (2003) noted that Information Technology (IT) could be very efficient. However this efficiency is based on two factors which do not usually exist in rural sections-economies of scale and end-to-end service capability. He mentioned that investment in infrastructure would be the first step, and rural areas have been less considered in this issue. Not only investing in communication systems is important for rural development, but also educating villagers and local leaders. Information technology could develop rural business, and could be effective in job-creation (Maleki, 2003).

Case study evidence from various developing countries suggests that use of ICTs – especially mobile phones – is increasing among rural enterprises, and makes a positive

contribution to enterprise growth in rural areas. In the case of small farmers, diverse information needs can be identified along the value chain in connection with their primary and support activities;

- Inbound logistics - information concerning the sourcing and purchasing of seeds for a specific crop.
- Operations - information concerning seeding, preparing, planting, growing and harvesting.
- Marketing and sales - information that helps identify customers for the output. It is also important for the farmer to know when, and in which, markets to buy and sell.
- Outbound logistics - information concerning packaging, storing and transport.
- After-sales service - feedback from customers.
- Support activities - information on access to extension services, credit and insurance.

Information needs in rural enterprises directly linked to the poor can be considerable. A study of the value chain of poor vegetable farmers in Sri Lanka measured the information search costs for all core enterprise operations, such as land preparation, growing and harvesting, as well as for seed purchase and selling. It found that the relative proportion of information search costs in the total costs of production were highest in the early decision stages and the latter selling stages. Overall, information search costs amounted to 70 per cent of all transaction costs, and the transaction costs themselves were recorded as fifteen per cent of the total costs incurred. There is growing evidence that enhanced access to ICT has helped farmers address some or all of these needs. In many instances, this has been achieved through the spontaneous uptake of mobiles by farmers; in other cases information supply has improved as a result of deliberate assistance by government or other actors. There are also many examples of ICT initiatives aimed at improving relevant information that have failed to produce the desired results (UNCTAD, 2010).

In 2004 a study was conducted to evaluate the e-readiness of non-profit organizations (NPOs) in the Western Cape. The assessment of 100 NPOs, spread throughout the province, helped to identify the constraints to greater ICT adoption among NPOs, and also to determine if geographic location - inside or outside the City of Cape Town Municipality - has an influence on NPO e-readiness. Based on the results of the survey, appropriate recommendations were made to the government for its ICT enablement programs (Vosloo and Paul, 2004).

The Hawaii Department of Agriculture in a study called, "the feasibility of a farmer based e-commerce market", studied factors such as farmers' computer skills, internet structure and the way of delivering the product to the market, and then a website was designed. Farmers could deliver products to farm businesses, and then a central company would deliver the products to the customer (Hawaii Department of Agriculture., 2006).

FAO reported some examples of using rural ICT at different countries, in a report named 'Electronic Agriculture'.

Egypt VERCON (Virtual Extension and Research Communication Network) - Government researchers and extension workers in institutes and rural villages are now actively using a web-based portal system to exchange critical information with district offices and Ministries in Cairo. Researchers and extension workers presently access information resources such as extension brochures, statistical databases, decision support systems, and can participate in special interest forums, online discussions, news and events. An online query system called 'Farmer's Problems' answers technical questions raised by smallholder farmers.

FoodNet - This network in Uganda comprises a national system that was established to gather and disseminate agricultural market

price information via newspapers, the Internet, radio and mobile phones (SMS). FoodNet is a typical example of an agriculture application that can be found in many countries, showing how small-scale farmers in rural communities can overcome their marginalization through a mix of media including ICT based information access.

Rural Radio, ICTs and Food Security - for billions of people in rural areas, where illiteracy rates are high and access to electricity, phones and Internet is marginal to say the least, radio is still the most accessible, economical, and popular means of communication. FAO is working with the World Association of Community Broadcasters, AMARC, an international community radio network, and the Developing Countries' Farm Radio Network (DCFRN) to enhance networking and sharing by assisting radio stations to use the Internet to access alternative information on a range of subjects of interest to rural communities, including health, education, credit and local projects (FAO, 2005).

Establishment of rural ICT offices in Iran began in 2004 in villages with more than 150

families, and it developed from 963 rural offices in 2005, to 10,000 offices in 2010. Agriculture has the highest percentage of employment in Guilan province. It seems necessary to use the capacities of rural ICT offices to help with agricultural activities. The number of villages in Guilan province is more than 2600, of which 533 villages are equipped with ICT offices. Also the Rasht Township area has 292 villages, of which 92 have rural ICT offices. These offices have concentrated on areas of internet services, bank services and postal services in villages (Iran Statistics Centre, 2010).

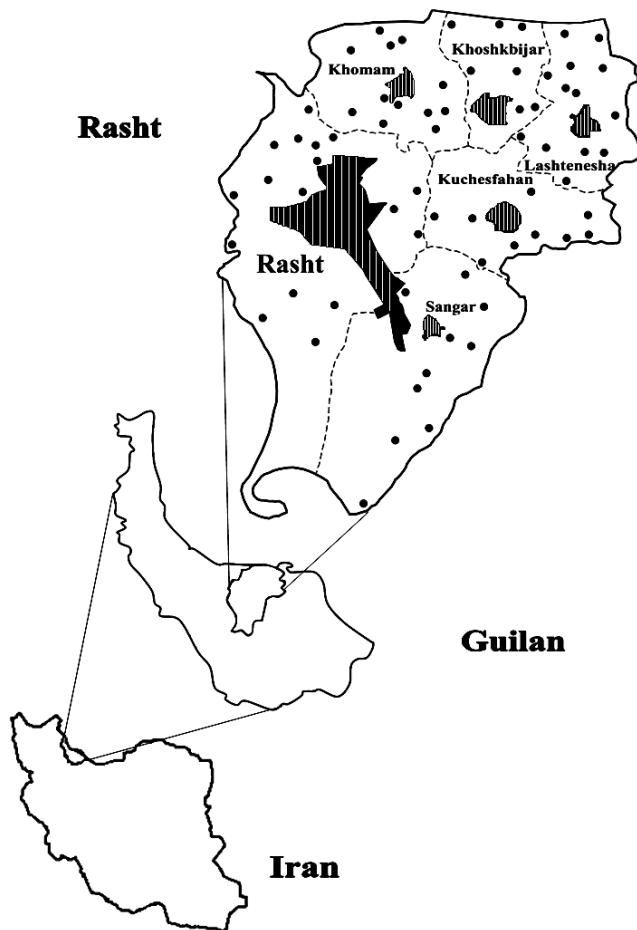
The main purpose of this study was to determine the e-readiness of rural ICT offices for rice e-marketing in Rasht Township, Iran. For this the following objectives were considered:

- 1 - Identifying the e-readiness of rural ICT offices.
- 2 - Identifying the effective factors in adoption of electronic marketing.
- 3 - Identifying attitudes of ICT office managers toward agricultural e-marketing.
- 4 - Identifying attitudes of ICT office managers about the e-marketing network.

## 2 MATERIALS AND METHODS

The Rasht Township is located in the north of Iran in Guilan Province and has six urban districts (the hachured areas in Figure 1). At the time of this study, 24 rural ICT offices among 92 offices were not active, and 68 offices rendered services which are shown in black points on the map. All 68 offices were

interviewed. As can be observed, these offices have a wide dispersion in the area of villages. Establishment of these offices has simplified accessibility to communication services for villagers, so decreasing their traffic to urban areas.



**Figure 1:** Site of study and dispersion of rural ICT offices

The researchers used survey research methods in which data was gathered by the questionnaire. Respondents were selected from rural ITCs offices in Rasht Township. Distributing 68 questionnaires related to rural ICT management was achieved in three forms; thirty-five offices by fax, 22 offices by e-mail, and eleven offices by post. Before the questionnaires were sent, managers were called by phone and some details about the purpose of the survey were offered. To determine the validity of the questionnaire, ICT experts' comments were used. To

measure questionnaire reliability, ten of them were distributed to rural ICT offices, which were randomly selected, and finally, its reliability was established at 0.83, through the use of Cronbach's alpha coefficient. Data were collected from February to May, 2011, and coded and entered onto an Excel spreadsheet, imported into a PASW Statistics 18 data file, and analyzed using PASW Statistics 18. Calculations were of frequencies, percentages, means, standard deviations, cross-tabulations, Kurskal-Wallis test, and correlations.

### 3 RESULTS

Table 1 shows that among managers of ICT offices, most (60.3 per cent) were aged between 20 to 30. More than 70 per cent of

families were engaged in agricultural activities.

**Table 1:** Personal characteristics

Age:	frequencies	percent
20 – 30	41	60.3
31 – 40	19	27.9
41 – 50	6	8.9
≤51	2	2.9
<hr/>		
Farmer family:		
Yes	49	72.1
No	19	27.9
<hr/>		
Level of education:		
High school diploma	26	38.2
2 years college	17	25.0
Bachelor's degree	25	36.8

#### 3.1 Identifying the level of e-readiness of rural ICT offices

To identify the level of e-readiness of rural ICT offices in Rasht Township, three indices with six questions in 5-point Likert type were asked from ICT officers. In the socio-cultural index and in the sub-index of adoption of electronic marketing, 77.9 per cent of managers had a good and very good tendency to cooperate with electronic marketing. Even in the sub-index of Internet skills, the condition was acceptable, and more than 50 per cent of managers reported 'good' and 'very good' status for their internet skills (table 2). Also, about level of education as a sub index of socio-cultural index, 38 per cent held a high school diploma, and 62 per cent held an academic degree (table 1).

Regarding the legal index related to electronic commerce and banking which was asked respondents to show their answers regarding the level of current laws to support e-marketing, 45.6 per cent of respondents reported at 'moderate' level and 23.5 per cent reported at 'good' situation.

In the back-up services index there was three sub indices. In the sub-index of e-banking education, 44 per cent of ICT offices managers reported an 'average' education. In post services, 69 per cent reported a condition between 'moderate' to 'good' situation. In addition, half of respondents reported a 'moderate' to 'good' condition for technical services (table 2).

**Table 2:** Socio-cultural, Legal, Backup services

index	Sub index		Very good	Good	Moderate	Weak	Very weak	M	SD
Socio-cultural	Adoption	frequencies	20	33	11	1	3	3.97	0.96
		percent	29.4	48.5	16.2	1.5	4.4		
Legal	Internet skill	frequencies	10	27	26	4	1	3.60	0.86
		percent	14.7	39.7	38.2	5.9	1.5		
Backup services	e-commerce legal	frequencies	1	16	31	19	1	2.95	0.79
		percent	1.5	23.5	45.6	27.9	1.5		
	E-banking education	frequencies	2	7	30	20	9	3.58	0.94
		percent	2.9	10.3	44.1	29.4	13.2		
	Post services	frequencies	9	20	27	9	3	3.33	1.01
		percent	13.2	29.4	39.7	13.2	4.4		
Technical services	Technical services	frequencies	3	8	29	20	8	2.64	0.94
		percent	4.4	11.8	42.6	29.4	11.8		

Most offices have ADSL broadband internet, and bandwidth in 50% of offices is 64 kb/s.

Also, approximately 90% of offices are connected to a banking network (table 3).

**Table 3:** connectivity Index

Variable	Internet:	frequencies	percent
Connectivity	DIAL UP	10	14.9
	ADSL	56	83.6
	WIMAX	1	1.5
	Bandwidth:		
	64 Kb/s	33	50.8
	128 Kb/s	24	36.9
	256 Kb/s	8	12.3
Banking Network:	Banking Network:		
	Yes	61	89.7
	No	7	10.3

### 3.2 Identifying the effective factors in receptivity to electronic marketing

Age, level of education and e-banking education of ICT officers had no effect on

receptivity to e-marketing, but three factors had effects on receptivity. These were, ‘experience in offering internet services to farmers’ ( $p = 0.04$ ), ‘internet skill’ ( $p = 0.01$ ) and ‘connectivity’ ( $p = 0.04$ ) (table 4).

**Table 4:** The Kurskal Wallis test - effective factors in receptivity to e-marketing

Factors	Chi-Square	df	p-value
Level of education	4.31	3	0.22
Age	4.04	3	0.25
Internet skill	12.28	4	0.01 *
Experience in offering internet services to farmers	15.64	4	0.04 *
E-banking education	2.45	4	0.65
Connectivity	15.24	4	0.04 *

### 3.3 Identifying Attitudes of ICT Office Managers towards Agricultural e-marketing

Managers of ICT offices believe that agricultural e-marketing may have the most efficiency to decrease the role of the middleman. However, the option ‘price

reduction’ had the least mean among respondents ( $M = 3.58$ ,  $SD = 0.88$ ). The score for option ‘very weak’ was one, and the score for option ‘very good’ was five. The mean of 68 managers’ response for each question is represented in the table 5.

**Table 5:** Identifying agricultural e-marketing

Role of E-marketing in		Very good	Good	Moderate	Weak	Very weak	M	SD
Middleman decrease	frequencies	23	36	7	2	0	4.17	0.73
	percent	33.8	52.9	10.3	2.9	0		
Ease in acquisition	frequencies	29	25	11	3	0	4.17	0.86
	percent	42.6	36.8	16.2	4.4	0		
Sale increase	frequencies	22	35	10	1	0	4.14	0.71
	percent	32.4	51.5	14.6	1.5	0		
Demand increase	frequencies	17	35	13	3	0	3.97	0.79
	percent	25	51.5	19.1	4.4	0		
Price reduction	frequencies	9	29	25	3	2	3.58	0.88
	percent	13.2	42.6	36.8	4.4	2.9		

### 3.4 Identifying Attitudes of ICT Offices Managers to the e-marketing network

Table 6 shows that 70.6% of respondents choose rural ICT offices for agricultural e-

marketing as the best among three possible networks, including public offices network, rural offices network and rural cooperatives network.

**Table 6:** Agricultural e-marketing network

Network	frequencies	percent
Public offices network	2	2.9
Rural ICT offices network	48	70.6
Rural cooperatives network	18	26.5

## 4 DISCUSSION AND CONCLUSION

Conclusions of some studies have shown that awareness and receptivity to electronic agriculture enjoys a better condition in Asia in comparison to Europe/North America. EAWG,

or the e-agriculture Working Group (2007) studied 4000 people in 135 countries worldwide, in cooperation with FAO and several other world organizations in a poll

titled, 'Familiarity with the term e-agriculture'. In a question related to respondents' familiarity with electronic agriculture in Asia, the percentage of those who were familiar with e-agriculture was more than in other continents, as 37 per cent of responses were positive. However in North America 29 per cent and in Europe 32 per cent of answers were positive. Shehata *et al.* (2006) interviewed 147 managers of cooperatives in a study of feasibility assessment for cooperative e-commerce. Conclusions of the polls showed that approximately 46 per cent are interested in e-commerce (Shehata, 2006), while receptivity to e-commerce was 80 per cent in this study. Postal service for the fast sending of goods from rural offices to other areas has a moderate status, while e-marketing required postal services with minimum time. Twelve per cent of rural offices have a fast internet

speed (256Kb/s), 37 per cent of them have an acceptable internet speed, (128Kb/s), but 51 per cent need to develop their internet speed (64Kb/s) to begin e-commerce. At the present time, rural ICT are managed with a proper dispersion in areas of villages by personnel who are from farming families and have a suitable capacity to help agricultural activities through internet, post and bank services. It is considered necessary to create an integrated system of rural ICT offices.

Rural ICT offices network could be a proper pattern in this regard. Farmers should refer to ICT offices where they live and represent specification of their products so that office operators register the information after they enter the rural office site. They would send products through that office and the post after customer purchase and receiving orders.

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**Agrovoc descriptors:** citrus sinensis, sweet oranges, fruit crops, culture media, embryonic development, seedlings, regeneration, callus, nucleus, fruit, plant growth substances, maturation, plant embryos, plant developmental stages, growing media

**Agris category code:** f62

## Somatic embryogenesis and plantlet regeneration from nucleus tissues of Local orange (*Citrus sinensis* (L.) Osbeck)

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### ABSTRACT

Somatic embryogenesis and plantlet regeneration were achieved in callus cultures of nucellus tissues derived from undeveloped ovules of immature fruits of local orange (*Citrus sinensis* (L.) Osbeck). Four types of culture media were used and all produced embryogenic callus. Somatic embryos were developed on MS medium supplemented with BA only. Embryo maturation took place on MS medium supplemented with BA and 2,4-D. Plantlets were regenerated from those somatic embryos on half strength MS medium free of plant growth regulators. The well-developed plantlets were transferred to a potting mix containing sand and peat moss (2:1) and grown for 8 months, with a survival rate of 100 %.

Abbreviation: BA: 6-benzyl amino purine; 2,4-D: 2,4-dichlorophenoxy acetic acid; NAA:  $\alpha$ -naphthalene acetic acid

**Key words:** *citrus sinensis*, embryogenic callus, somatic embryogenesis, nucleus tissues, plantlet regeneration

### IZVLEČEK

### SOMATSKA EMBRIOGENEZA IN REGENERACIJA IZ NUCELARNEGA TKIVA LOKALNE POMARANČE (*Citrus sinensis* (L.) Osbeck)

Somatska embriogeneza in regeneracija je bila dobljena iz kalusne kulture nucelarnega tkiva pridobljenega iz nerazvitih semenskih zasnov nezrelih plodov lokalne pomaranče (*Citrus sinensis* (L.) Osbeck). Uporabljena so bila štiri gojišča in na vseh je nastal embriogeni kalus. Somatski embriji so se razvili na MS gojišču obogatenim samo z BA. Razviti embriji so bili prestavljeni na MS gojišče z dodatkom BA in 2,4-D. Rastline so nastale iz vseh somatskih embrijev na polovičnem MS gojišču brez rastlinskih hormonov. Dobro razvite rastline so bile prestavljene v mešanico substrata, ki je vsebovala pesek in šoto (2 : 1) in 8 mesečno rast so 100 % preživele.

Kratice: BA: 6-benzil amino purin; 2,4-D: 2,4-diklorofenoksi ocetna kislina; NAA:  $\alpha$ -naftalen ocetna kislina

**Ključne besede:** *Citrus sinensis*, embriogeni kalus, somatska embriogeneza, nucelarno tkivo, regeneracija

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## 1 INTRODUCTION

Citrus is an important fruit crop worldwide. Although selected genotypes can be multiplied through grafting and budding methods, however, the rate of multiplication is very low. Thus, to meet the demands of planting materials, it is necessary to develop a suitable protocol for mass propagation from existing elite cultivars. Somatic embryogenesis is an efficient method of plant regeneration allowing rapid production of large number of plants within short period. So far, no information is available on somatic embryogenesis in local orange (*Citrus sinensis*

(L.) Osbeck), which is the most widely grown citrus crop in Iraq.

Successful somatic embryogenesis from nucellus tissues of different citrus species was achieved by several authors (Rangan *et al.*, 1968; Chen *et al.*, 1990; Miah *et al.*, 2002).

The present investigation was undertaken to develop the *in vitro* suitable protocol for micropropagation of local orange (*Citrus sinensis* (L.) Osbeck) from nucellus tissues through somatic embryogenesis.

## 2 MATERIALS AND METHODS

The experiment to be described was carried out at Plant Tissue Culture Laboratories, Date Palm Research Centre, Basrah University, Basrah, Iraq.

### Source of plant material:

Mature trees of local orange (*Citrus sinensis* (L.) Osbeck), grown in a private orchard were used as the source of the plant material.

### Preparation of explants:

Immature fruits (8 weeks old after anthesis) were washed thoroughly under running tap water to reduce dust and surface contaminants. Then, they were surface disinfected by immersion in 70 % (v/v) ethanol for five minutes, then soaked for 10 minutes in 20 % commercial chlorax solution containing 1.05 % sodium hypochlorite, and finally rinsed 5 times with sterilized distilled water.

The fruits were then cut open under aseptic conditions, and immature seeds were separated. For somatic embryogenic callus induction, the immature seeds were cut by a scalpel and nucellus halves were separated as described by Juarez and Navarro (1977). The nucellus tissues were then kept in an anti-

oxidant solution containing 100 mg l<sup>-1</sup> ascorbic acid and 150 mg l<sup>-1</sup> citric acid for 24 hrs. The nucellus tissues were afterwards rinsed in sterilized water for 5 times, and then surface sterilized with 20 % commercial chlorax solution containing 1.05 % sodium hypochlorite and a drop of tween 20 for 15 minutes. The nucellus tissues, were then rinsed in sterile distilled water 3 times.

### Callus induction:

Nucellus tissues (Fig. 1a) were cultured on four culture media, basically all contained MS salts and vitamins (Murashige and Skoog, 1962). The first medium, called M1 consisted of MS medium supplemented with 500 mg l<sup>-1</sup> malt extract. At the same time, nucellus tissues were cultured on MS + 5.0 mg l<sup>-1</sup> NAA + 1.0 mg l<sup>-1</sup> BA (M2), MS + 5.0 mg l<sup>-1</sup> 2,4-D + 1.0 mg l<sup>-1</sup> BA (M3), MS + 1.0 mg l<sup>-1</sup> 2,4-D + 5.0 mg l<sup>-1</sup> BA (M4). The nucellus halves were incubated in conical flasks containing five samples, and five replicates for each medium. The flasks were incubated in darkness, in a growth chamber (25±2 °C) for four weeks.

**Formation of the primary callus:**

The primary callus was formed on all nucellus tissues in all media. This callus was then divided and incubated on two growth media (M2 and M3), containing half strength of MS medium for callus proliferation. The process of primary callus proliferation continued for 12 weeks, with sub-culturing every 4 weeks (Fig. 1b).

**Embryogenic callus induction:**

The white globular callus was divided and incubated on half strength MS medium supplemented with BA at  $1.0 \text{ mg l}^{-1}$  and 2,4-D at  $5.0 \text{ mg l}^{-1}$  for 4 weeks. At the end of this period, the embryogenic callus was obtained (Fig. 1c).

**Induction of somatic embryos:**

The embryogenic callus obtained from the previous step was incubated on half strength MS medium, supplemented with BA at  $1.0 \text{ mg l}^{-1}$  for the induction of somatic embryos. Somatic embryos were obtained after 4 weeks of culture (Fig. 1d, e).

**Embryo maturation:**

Somatic embryos were cultured on half strength MS medium, supplemented with BA

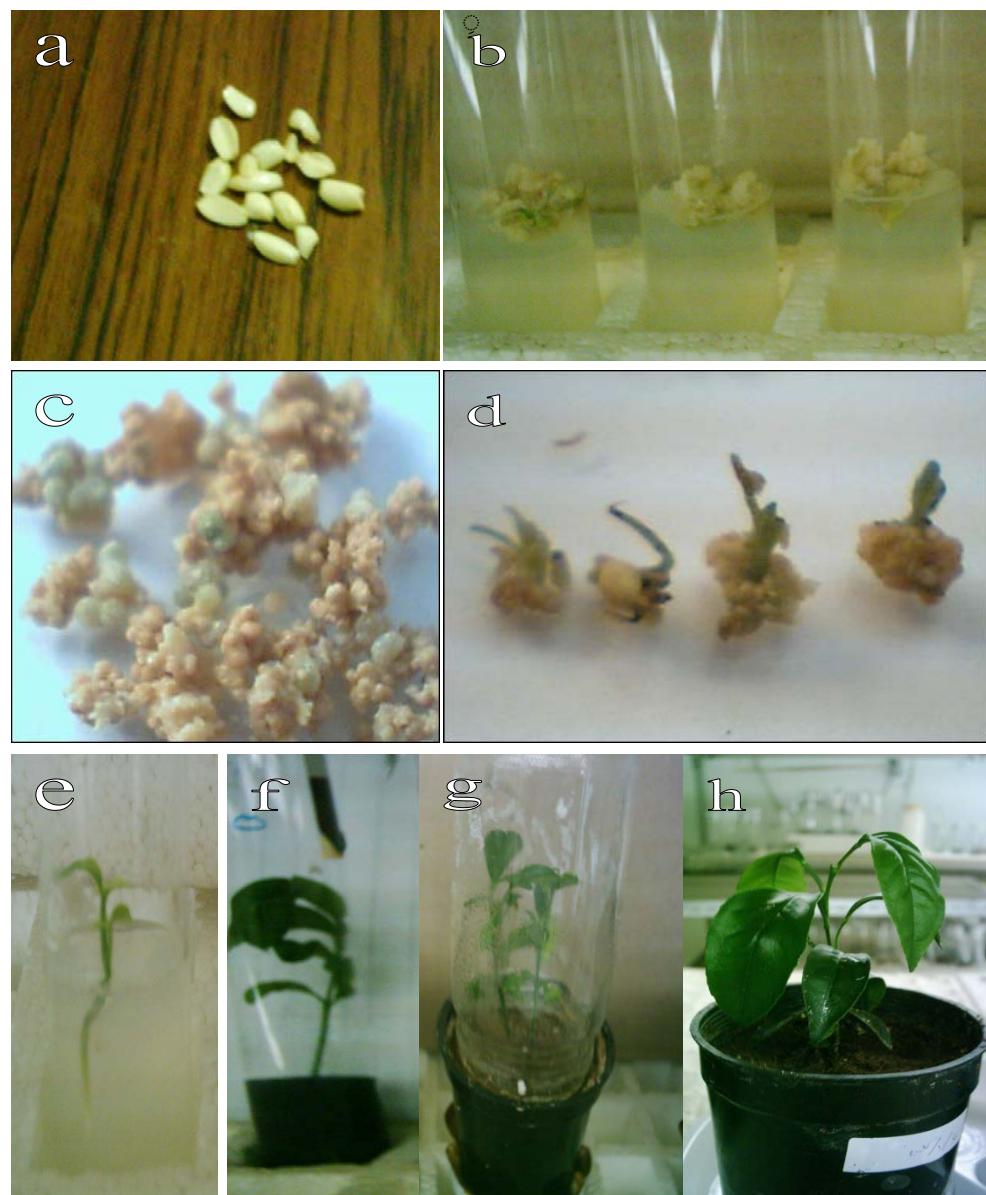
at  $1.0 \text{ mg l}^{-1}$  and 2,4-D at  $5.0 \text{ mg l}^{-1}$  for embryo maturation. This step took about four weeks.

**Plantlet regeneration:**

The fully developed somatic embryos (Fig. 1e) were cultured, each in a test tube containing half strength MS medium, free of plant growth regulators. Fully developed plantlets were obtained (Fig. 1f).

**Plantlet acclimatization:**

The process of acclimatization was carried out on plantlets having good shoot and root system. The plantlets were removed from the culture vessels and washed with sterilized water to clean the root system from the remains of the growth medium. The plantlets were then placed in glass tubes containing half strength MS medium, ensuring the submergence of the root system. The glass tubes were then closed with aluminum foil and placed in a growth chamber for 24 hrs. Then, the plantlets were planted in an autoclaved soil mix containing sand and peat moss (2 : 1). The plantlets were then covered with glass cover for 3 – 4 weeks (Fig. 1g) and then glass covers were removed gradually. The process of acclimatization continued for 8 months, and the rate of survival was 100 % (Fig. 1h).



**Figure 1.:** The time line of somatic embryogenesis and plantlet regeneration in *citrus sinensis* .cv. Local orange; a, isolated nucelus tissue; b, primary callus; c, embryogenic callus; d, e, germinating embryos; f, plantlet; g, plantlet during acclimatization; h, plant in pot after acclimatization.

### 3 RESULTS AND DISCUSSION

It is evident from Fig. 1, that local orange (*Citrus sinensis* (L.) Osbeck) can be clonally mass propagated *in vitro* using somatic embryogenesis from nucellus derived callus. In the present work, successful embryogenic calli were developed from all culture media. Similar results were obtained by Pasqual and

Ando (1988) with sweet orange cv. Vallencia. However, Miah *et al.* (2002), working with nucellus tissues of *Citrus macroptera*, found that embryogenic callus was obtained only from MS medium supplemented with malt extract. However, such differences in the

response of *Citrus* species to culture media are probably genetically determined.

It is obvious from the present work, that half strength MS media supplemented with BA at 1.0 mg l<sup>-1</sup> induced the formation of somatic embryos (Fig. 1d, e). The importance of the benzyl adenine for the induction of somatic embryo has also been reported by Praveen *et al.* (2003) for Kinnow mandarin.

Embryo germination and maturation occurred on half strength MS medium supplemented with BA at 1.0 mg l<sup>-1</sup> and 2,4-D at 5.0 mg l<sup>-1</sup>. Similar results were obtained by Miah *et al.* (2002) for *Citrus macroptera* and Bhargara *et al.* (2003) for date palm, who showed the importance of benzyl adenine and auxins, for embryo germination and maturation. After embryo germination and maturation, they were

transferred to half strength MS medium free of plant growth regulators and complete plantlets were obtained within 12 weeks (Fig. 1f) from the start of culturing the nucellus tissues, and to the time point when they were ready for acclimatization. The plantlets thus obtained through somatic embryogenesis were transferred to a soil mixture (sand : peat moss, 2 : 1) and the rate of survival was 100%.

In conclusion, the results obtained in the present work demonstrate efficient cloning of local orange (*Citrus sinensis* (L.) Osbeck), through somatic embryogenesis. Furthermore, this *in vitro* culture method is useful not only for plant breeding and genetic studies, but also for studying the tolerance of orange trees to abiotic stresses, in particular, soil salinity, which is generally detrimental to plant growth and productivity in Iraq and worldwide.

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**Agrovoc descriptors:** lycopersicon esculentum, tomatoes, varieties, fruit vegetables, plant production, cultivation, data collection, surveys, farm surveys, farmers, rural population, land ownership, ownership, development indicators, postharvest losses, crop management

**Agris category code:** f01, e20, e51

## Assessment of production practices of small scale farm holders of tomato in Bagrote Valley, CKNP region of Gilgit-Baltistan, Pakistan

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### ABSTRACT

The primary data used for the investigation were obtained through a questionnaire. One hundred and twenty (120) farmers were randomly selected; their education level, area of cultivation, time of planting, intercropping, varieties grown, fertilizer used, time of picking, packing, transportation and processing were looked at. It was found that out of 120 respondents, 52% were literate, and 48% were illiterate. 90% land holders were owners while remaining were tenants. Roma variety (46%) was preferred variety followed by Rio-Grind (38%) and Heirloom variety (16%). In terms of picking time, majority of farmers picked tomatoes in the afternoon (38%) or evenings (34%) and rest picked them in the mornings (28%). All the farmers transported tomatoes to local market either in wooden boxes (76%) or traditional baskets (24%). Majority of the farmers responded positively to drying tomato, but (27%) were unaware of processing procedures. Over all loss of tomato crop to fungal and viral diseases was estimated to be 24%.

**Key words:** Tomato, production practices, post-harvest losses, assessment

### IZVLEČEK

### OCENJEVANJE PRIDELOVANJA PARADIŽNIKA NA MAJHNIH KMETIJAH V DOLINI BAGROTE, CKNP OBMOČJE GILGIT-BALTISTANA, PAKISTAN

Podatki za raziskavo so bili pridobljeni z anketnim vprašalnikom. Naključno je bilo izbrano 120 kmetov glede na njihovo izobrazbo, velikost obdelovalne površine, čas sajenja, uporabo medposevkov, sorte paradižnika, uporabo gnojil, čas pobiranja, način pakiranja, transporta na trg in predelave. Raziskava je pokazala, da je bilo 52 % pridelovalcev pismenih in 48 % nepismenih. 90 % pridelovalcev so bili lastniki zemljišč, ostali so bili najemniki. Najbolj priljubljena sorta je bila 'Roma' (46%), sledili sta ji sorte 'Rio-Grind' (38 %) in 'Heirloom' (16%). Kmetje so najbolj pogosto pobirali paradižnike ob popoldnevih (38%) ali zvečer (34%), ostali zjutraj (28%). Večina kmetov je transportirala paradižnik na lokalne trge v lesenih zaboljih (76%) ali v tradicionalnih košarah (24%). Večina pridelovalcev se je odzvala pozitivno na možnost sušenja paradižnikov, le manjši del (27%) ni poznal tega postopka predelave. Celokupna izguba pridelka zaradi glivnih in virusnih okužb je bila ocenjena na 24%.

**Ključne besede:** paradižnik, tehnologija pridelave, izguba pridelka po obiranju, ovrednotenje

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## 1 INTRODUCTION

Tomato (*Lycopersicum esculentum*) is a staple fruit vegetable. Fresh fruits and vegetables are very important source of vitamins and minerals which are essential for human health.

Tomato is one of the most important vegetables in the world. It is considered as an important cash and industrial crop in many parts of the world (Babalola *et al.*, 2010).

Tomatoes are grown as fresh market and as processing tomatoes. They are important for food industry as they serve as raw material for production of value added products (Soe, 2003). The domestic consumption and demand for tomato is growing due to increase in population. It is very important vegetable with substantial nutritional value. Moreover it is available at low price as compared to other vegetables. It is consumed in every home in different modes, such as vegetable, salad, ketchup, chatni and it is part of every delicious cuisine. Due to wide seasonal variations in Pakistan tomato is available throughout the year.

Pakistan produces two crops annually first in spring and second in autumn. However, in southern Pakistan tomato can be grown throughout the year. Pakistan produced 560,700 tones tomatoes in 2008-9. Production/hectare was 10.50 tones. During 2008-09 total area under cultivation were 53,400 hectares (Agric Stat, 2008-09). While Gilgit-Baltistan produced 6,455 metric tons tomatoes. Of which 3194 MT were consumed, 2293 MT were marketed, and 968 M.T were wasted. While, in Gilgit-Baltistan tomatoes were cultivated on 805 hectare (Agric Stat, 2009).

Despite the remarkable progress made in increasing world food production at the global level, approximately half of the population in the third world does not have access to adequate food supplies. There are many reasons for this. For instance food losses to pests, diseases, lack of awareness of modern production practices, seed selection and post harvest problems. Evidence suggests that these losses tend to be highest in countries where the need for food is greatest (FAO, 1989; Oyewole and Oloko, 2006; Babalola *et al.*, 2008).

Optimum production of tomato requires intensive management practices that conserve and manage soil nutrients needed for maintaining soil fertility and water quality and for sustaining tomato production (Yafa *et al.*, 2000).

It is also common knowledge that production of tomatoes is declining due to some bacterial, fungal diseases and pest attacks. Furthermore, lack of resistant varieties and poor marketing add to farmer's woes. Therefore, this crop needs special attention of extension workers, researchers, policy makers and growers to identify the constraints, improve production methods and suggest remedial measures to enhance tomato production in Pakistan. This study was conducted to achieve following objectives.

To review the production practices followed by the tomato producers in Bagrote valley.

To propose new and innovative methods of replacing existing traditional production practices.

## 2 MATERIALS AND METHODS

### **2.1 Study area**

Bagrote valley is situated in the North-West of Gilgit at two hours drive from the city. The valley has fertile lands and is famous for the production of seasonal crops, like wheat, potato, maize and vegetables. Bagrote valley consists of eight villages. Irrigation system of valley consists of water canals. This valley is famous for tomato production but in last few years production has steadily declined, so current study was conducted to assess production practices of small scale Tomato growers.

### **2.2 Data collection**

This study was based on primary data collected from tomato producers during Kharif 2010-11. Sample survey was carried out and personal interviews were held to collect the information. Before launching, the survey

questionnaire was pre tested and was improved accordingly. Key informant technique was also followed to get authenticated information.

### **2.3 Sample size**

The survey included 120 randomly selected tomato growers. There are many estimation techniques. Frequency tables are very useful in knowing the trend related to a particular variable. The same technique was followed in analyzing results of this study. Percentage method was used to analyze farmers' responses related to production practices and post-harvest losses. The analysis was carried out in relation to percentage analysis on tomato; including education level, cultivation time, fertilizer use, intercropping, picking time, varieties grown, mode of transportation, processing, sale point and disease prevalence.

## 3 RESULTS AND DISCUSSION

### **3.1 Education level of farmers and their tenant status**

Literacy is one of the important characteristics of farmers that greatly influences the adoption of new technology. Data with respect to the literacy rate of sampled farmers were collected on the basis of their schooling years. Those who had completed school education were categorized as literate and those who had no formal school education were deemed as

illiterate. It was observed that out of 120 farmers, 52% were literate while remaining 48% growers were illiterate. Tenancy is an agreement between land owners and farmers under which land owners lease out their land to tenants who work the land and pay agreed rent or hand over agreed share of crop to land owners (Amanullah, 1999). From our studies it was observed that 90% tomato growers were owners. Whereas, remaining 10% were tenants (Table 1).

**Table1:** Number of farmers classified by their education level and tenure status

Education Level	Frequency	Percentage
Literate	62	52
Illiterate	58	48
Total	120	100
Tenure Status	Owner	Tenants
	90%	10%

### 3.2 Time of cultivation & operational area held by the farmers

Majority of the farmers planted tomatoes in August. However, some planted them in march as well (Table 2).Operational holding of farmers plays a significant role in the adoption of new technology. A number of researchers reported that there is an inverse relationship between farm size and productivity (Maureithi

*et al.*, 1991 and Kapronczai and Tomka, 1991). While, other studies do not support this contention that farm size affects the productivity and inputs (Thakur *et al.*, 1990). Data regarding operational land holdings showed that 71% of farmers had tomato patches measuring from 1-10 Marlas (one marla = 50 sq. yards),14% farmers had fields up to 10-20 Marlas and 15% had fields upto 20-30 Marlas.

**Table 2:** Numbers of farmers classified by time of cultivation of tomato

Time of Cultivation	Frequency	Percentage
March	20	17
August	100	83
Total	120	100

### 3.3 Intercropping in tomato crop

Our survey showed that majority of the farmers (54%) practiced intercropping in order to minimize the risks and maximize returns.

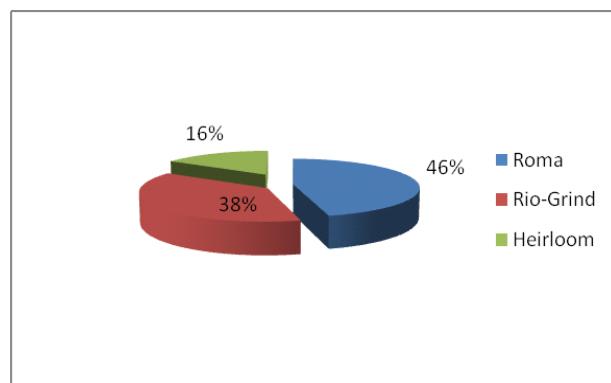
28% farmers cultivated leafy vegetables, 12% Beans, 13% Cabbage, 3% Radish and 4% Corundum crops, while the remaining 46% did not practice intercropping in their fields.

### 3.4 Tomato varieties grown by the farmers

Our study showed that majority of the farmers used Roma variety (46%) followed by Rio-Grind (38%) and

Heirloom (16%). Farmers prefer Roma and Rio-Grind tomato varieties because of their good shape, flesh and weight. These tomatoes are easy to market and farmers can get better returns. Heirloom variety was grown for home consumption and processing (Fig. 1).

**Figure 1:** Percentage of different varieties grown in Bagrote valley

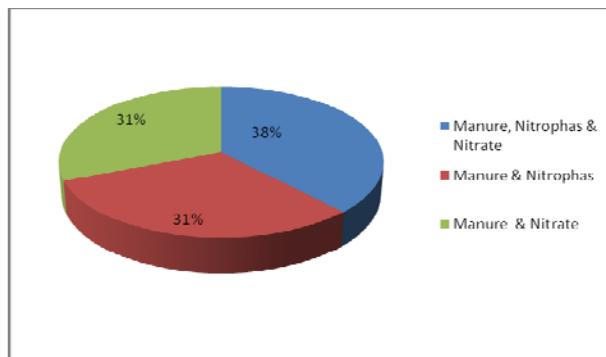


### 3.5 Tomato varieties

Balanced use of fertilizers in proper quantities and their timely application is inevitable in getting a good yield. Research has shown that growing same crops on the same piece of land for years depletes soil nutrients and therefore, for raising good crop it becomes imperative to replenish the soil fertility with the application of organic and inorganic fertilizers. Commercial fertilizers are the major source of readily available nutrients to enhance crop production and achieve food security. The application of fertilizers in balanced ratio not only guarantees a good crop but also maintains

the natural fertility of the soil. Gurmani *et al.*, 2003 reported that proper supply of nitrogen to crop is associated with the enhancement of photosynthetic activity, vigorous growth and dark green colour of plant leaves. Being a vital part of the building blocks of genes and chromosomes, phosphorus controls all the living processes in plants. It is not only essential for cell division but also for energy transport system in all cells (Saleem, 2003). It was observed in the present study that out of 120 farmers 38% farmers used manure (cow dung) Nitrophas, and Nitrate, 31% used manure & Nitrophas. While 31% used manure & Nitrate (Fig. 2) only.

**Figure 2:** Number of farmers classified by use of fertilizers in tomato field



### 3.6 Picking time of tomatoes

The time of picking is considered most important factor in post - harvest losses. Our study indicated that picking time is determined by the commitments of farmers with the transporters for short and long distance transportation. It was observed that the farmers, who transported their produce to the

local and nearby markets, generally pick tomato early in the morning (28%). But most farmers, pick tomato in morning for drying as well. While the farmers who transport their produce to distant markets, pick their tomato in afternoon (38%) and evening time (34%) in order to make the produce ready for transportation and available for sale in the wholesale markets.

**Table 3:** Numbers of farmers classified by time of tomato picking

Time of Picking	Frequency	Percentage
Morning	33	28
Afternoon	46	38
Evening	41	34
Total	120	100

### 3.7 Packaging material used for tomato crop

Packing of mechanically injured or fungus infested fruit increases probability of considerable deterioration (De Lucia and Assennato, 1994). Efforts were also made to develop a relationship between fungal infection and distance from the market.

Remove bruised and damaged fruits and pack fruits with similar maturity stage in one container. Separate ripe fruits since these produced ethylene that could hasten the

ripening process of unripe fruits. There are different modes of packaging. Generally, container-packing is considered ideal for packing fruits or vegetables because these are easy to handle, provide good protection from mechanical damage, have adequate ventilation and convenient for merchandising. For local markets tomatoes can be packed in wooden boxes or other traditional crates assuring careful handling. From the present study it was observed that for local markets cardboard boxes or wooden crates (76%) 70 to 80 kg and Traditional baskets (24%) with 30-35 kg capacity were used (Table 4).

**Table 4:** Numbers of farmers classified by use of packaging material of tomato

Material Used	Frequency	Percentage
Wooden boxes	91	76
Traditional Baskets	29	24
Total	120	100

### 3.8 Sale points

It was found that all the tomato growers brought produce to local market for sale and

none of the growers transported their produce to distant markets.

**Table 5:** Numbers of farmers classified by sale points for tomato produce

Sale Points	Frequency	Percentage
Local Market	120	100
Outside	0	0
Total	120	100

### 3.9 Mode of transportation

Most tomato growers (86%) transported their product to markets by jeeps while the remaining (14%) farmers used other sources to transport their produce. During transportation fruit passes through ripening, which is a complex phenomenon. It involves maturation, color change, production of ethylene, tissue

softening, and change in respiration rate, fruit membrane thickness and permeability. During transportation the produce should be immobilized by proper packaging and stacking, to avoid excessive movement or vibration. Vibration and impact during transportation (Table 6) may cause severe bruising or other types of mechanical injury to the food (Gregor, 1987).

**Table 6:** Numbers of farmers classified by mode of tomato transportation

Mode of Transportation	Frequency	Percentage
Jeeps	103	86
Others	17	14
Total	120	100

### 3.10 Knowledge of tomato processing

Large quantities of tomato fruits are harvested each season, but post - harvest processing and preservation techniques are ineffective. Therefore, fruits spoil very early because of lack of appropriate system of preservation and processing (Francois, 1995). To reduce the post-harvest losses and over supply to the markets, it is essential that the surplus and

over ripe produce be separated and processed. In order to know the farmers' knowledge of processing, tomato growers were asked whether they knew what can be produced from tomato crop. Of the total respondents, 27% responded negatively and the remaining 73%, responded positively (Table 7). They were using drying techniques and hence were aware of processing.

**Table 7:** Numbers of farmers classified by tomato processing techniques

What can be produced	Frequency	Percentage
Drying	88	73
Others	0	0
Nothing	32	27
Total	120	100

### 3.11 Post - harvest losses in tomato crop

The post harvest loss of tomato crop in Bagrote valley is 24% and the remaining is available with the growers for consumption and marketing.

(Raja and Khokhar, 1993) stated that postharvest losses in fruits and vegetables range from 25-40% or even greater (Iqbal, 1996). The post harvest losses of tomato crop in Peshawar valley was 20% (Manzoor *et al.*, 2006). Seasonal post harvest losses of fruits and vegetables are high in the tropics due to hot environmental conditions and moisture levels (MAEP, 1999).

The deterioration of fruits and vegetables starts right after their harvest, if not properly harvested. Generally accepted methods for

evaluating post - harvest losses of fresh produce do not exist. In the appraisal of an existing marketing operation, the accurate evaluation of losses is a problem. It may be conjectured that losses are huge, but there may be no figures to support this view (FAO, 2002).

### 3.12 Storage

During the survey it was observed that most of the farmers picked their crop in afternoon and transported their produce to local markets in wooden Crates using jeeps as a mode of transportation. The estimated post harvest losses of tomato crop in Bagrote valley were 24% of the total production. These losses may occur during picking, handling transportation, etc.

Due to poor storage conditions resistance of fruit and vegetables to natural disease usually declines, leading to infection by pathogens (Tefera *et al.*, 2007). Many horticultural crops have a relatively short harvesting season. Storage is needed to extend the marketing period. Air-cooled common storage houses are often used in this regard. To delay ripening in tomato temperature should be greater than 26-27 °C as they are harvested at different stages and stored. Although fully ripened tomatoes may be held at 2-5 °C before consumption for short time (Maul *et al.*, 2000) but the mature green tomatoes should not be placed at temperature below 12 °C as it causes chilling injury which adversely affects quality and ripening (Saltveit, 2001). There is significant loss of weight and firmness in tomatoes during storage due to the effect of temperatures (Van Dijk *et al.*, 2006) and there is relationship between the harvest and firmness changes in tomato. Different techniques are employed to improve the shelf life of fresh commodities. One of the techniques to improve shelf life of tomatoes is edible coatings (Baldwin *et al.*,

1995). These edible coating can be used to preserve vegetables by providing barrier to moisture, oxygen and carbon dioxide and also improve its handling properties and contribute to the production of volatile aroma (Oliva *et al.*, 2005). Absence of proper storage and marketing facilities, farmer is forced to sell their products at throw away prices. Sometimes farmers do not even get the two ways transportation costs back, so they would rather dump their produce near the market area than taking them back to home.

### **3.13 Tomato field diseases and insect damage**

During field visits three fungal, two bacterial and two insect pests causing damage were observed (Table 8). A study conducted in Sindh during 2004 revealed that various fungal, bacterial and viral diseases attack tomato crop. It was also reported that *Rhizoctonia solani* was isolated with highest frequency i.e. 60.0% (Rajput, 2004).

**Table 8:** Field diseases of tomato crop

Early blight	<i>Phytophthora infestans</i>	Fungal
Late Blight	<i>Alternaria solani</i>	Fungal
Gray leaf spot	<i>Stemphylium solani</i>	Fungal
Fusarium wilt	<i>Fusarium oxysporum</i>	Fungal
Bacterial spot	<i>Xanthomonas campestris</i>	Bacteria

## **4 CONCLUSION**

Based on the results and observations made during the survey, it seems that the current farming system of tomatoes, in Bagrote valley of CKNP region is inadequate. The farmers lack fundamental knowledge about production practices, disease and post harvest handling practices.

**Therefore the following recommendations are made:**

- Farmers must be provided extension lectures on new and innovative production methods.

- Young and educated persons must be encouraged to take to farming and they must be provided financial incentives.
- Farmers should be educated to adopt modern technologies for tomato production and they must be assured that it will work to their advantage.
- Farmers must be helped with farm infra structures and provided with post-harvest farm facilities in order to avoid post-harvest losses.

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**Agrovoc descriptors:** dietary fibres ,human nutrition, chemicophysical properties, nutrition physiology, physiological functions, mankind, health, therapeutic diets, disease control

**AgriS category code:** s20, s30

## Pomen prehranske vlaknine v prehrani človeka

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### IZVLEČEK

Prispevek podaja definicijo in opredelitev prehranske vlaknine ter njeno vlogo v prehrani človeka. Predstavljena je delitev prehranske vlaknine glede na fizikalno-kemijske lastnosti in fiziološke učinke. Prikazani so fiziološki učinki topnih in netopnih komponent vlaknine, priporočila glede dnevno zaužite količine vlaknine in njena energijska vrednost. Prikazani so pozitivni učinki uživanja prehranske vlaknine na zdravje ljudi in na zaščito pred različnimi obolenji. Nakazani so dvomi o pretiranem pomenu prehranske vlaknine v prehrani.

**Ključne besede:** prehranska vlaknina, fiziološki učinki prehranske vlaknine, zdravje, živila rastlinskega izvora

### ABSTRACT

#### THE IMPORTANCE OF DIETARY FIBRE IN HUMAN NUTRITION

The paper provides a definition of dietary fiber and describes its role in a human nutrition. Dietary fiber is presented in the respect of the physico-chemical properties and physiological effects. The recommendations for daily intake of fiber and the energy value of dietary fiber are stated. The positive effects of dietary fiber on human health and protection against various diseases are shown. The doubts about the exaggerated importance of dietary fiber in the diet are mentioned.

**Key words:** dietary fibre, physiological effects of dietary fiber, health, plant-derived food

### 1 UVOD

O prehrani človeka je veliko polemičnih razglabljanj, objavljenih različnih znanstvenih raziskav, pa tudi strokovnih prispevkov. Po navedbah Kendalla in sod. (2010) je meso v prehrano vključeno sicer že 2,6 milijona let, vendar naj bi naši davni predniki uživali v glavnem rastlinsko hrano, sestavljeno iz listne zelenjave, poganjkov, sadja, semen in oreškov. Ta hrana je bila bogata s prehransko vlaknino in imela nizek glikemični nivo.

Industrijska revolucija, ki ji je sledil hiter tehnološki napredek, je dramatično spremenila življenjski slog ljudi. V zadnjih 200 letih je hrana postala vse bolj predelana, kar ima za posledico občutno manjši vnos prehranske vlaknine. Različni načini vzreje živali, katerih glavni cilj je bil znižanje stroškov, so vplivali na občutno povečanje potrošnje hrane živalskega izvora, za katero pa je značilno, da ne vsebuje vlaknine in da je bogata z

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nasičenimi maščobami in s holesterolom. Prav ta drastična sprememba v naši prehrani naj bi bila vzrok, da je današnja populacija podvržena različnim kroničnim boleznim, kot so srčna obolenja in diabetes tipa 2 (Kendall in sod., 2010).

Mnenja strokovnjakov o vlogi vlaknine v prehrani človeka so bila do nedavnega precej nasprotujoča. V preglednem članku Rodrígueza in sod., objavljenega v Trends in Food Science and Technology (2006), je podan kronološki pogled o vlogi prehranske vlaknine v prehrani. Navaja, da je Kellogg že 1923. govoril o pozitivnem delovanju vlaknine, medtem ko sta McCance in Lawrence leta 1929 opozarjala, da nerazgradljivi deli rastlin, ki sestavljajo prehransko vlaknino, dražijo (iritirajo) črevo. Nekoliko kasneje, leta 1947, naj bi Walker navajal, da večja vsebnost vlaknine pospešuje delovanje prebavil, Cleave pa je leta 1956 povezal nekatere bolezni s pomanjkanjem

vlaknine, govoril je o sindromu pomanjkanja vlaknine ('deficiency of fibre syndrome'). Šele v 70-ih letih prejšnjega stoletja so številni znanstveniki poudarili pomen vlaknine in njeno vlogo pri zmanjšanju kardiovaskularnih bolezni, diabetesa in nekaterih oblik raka. Od tedaj so bile izpeljane številne raziskave, ki so poudarjale vlogo vlaknine pri preprečevanju in lajšanju kroničnih bolezni. Podpora vlogi vlaknine v preventivi in preprečevanju kroničnih bolezni še raste (Rodriguez in sod. 2006). O pomenu uživanja zadostne količine prehranske vlaknine v otroštvu in negativnih posledicah pri otrocih pri premajhnem vnosu vlaknine govorji Williams (2006) v prispevku, objavljenem v The Journal of Pediatrics. Vzporedno pa zasledimo različne raziskave, v katerih poudarjajo, da je zaščitna vloga vlaknine pred pojavom različnih oblik raka predvsem posledica širokega spektra drugih, t.i. funkcionalnih komponent, ki jih vsebujejo živila, bogata z vlaknino in da slednji ne smemo pripisovati prevelikega pomena.

## 2 OPREDELITEV PREHRANSKE VLAKNINE

Prehranske vlaknine ne predstavlja določena kemijska spojina, ampak jo sestavlja kombinacija kemijsko zelo različnih snovi, kot so: celuloza, hemiceluloza, pektin, lignin, gume in polisaharidi iz morskih alg ali bakterij. Celuloza, hemiceluloza in pektin so sestavni deli celičnih sten rastlin, zato jih imenujemo tudi strurni polisahari. Med druge polisaharide, ki jih prav tako uvrščamo v

prehransko vlaknino, čeprav niso sestavine celičnih sten, spadajo: gume (npr. gumi arabikum), rezervne gume (guar gume) in polisaharidi iz morskih alg (karagenan, agar, alginati); nekateri raziskovalci štejejo sem tudi rezistentni škrob (del škroba, ki ga encimi tankega črevesa ne razgradijo). Viri različnih komponent vlaknine so navedeni v preglednici 1.

**Preglednica 1:** Naravni viri različnih komponent prehranske vlaknine (Gray, 2006)

**Table 1:** Natural sources of dietary fiber components (Gray, 2006)

Komponenta vlaknine	Glavni vir
celuloza	zelenjava, lesnate rastline, žitni otrobi
hemiceluloza	žitna zrna
lignin	žitni otrobi, luščine riža in stročnic, lesnate rastline
β-glukan	zrna (ovsa, ječmena, rži, pšenice)
pektin	sadje, zelenjava, stročnice, sladkorna pesa, krompir
gume	stročnice, morske alge, mikroorganizmi (guar, karagenan, ksantan, guma arabika)
inulin in fruktooligosaharidi	cikorija, artičoka, čebula
oligosaharidi	humano mleko, zrnate stročnice
rezistentni škrob	semena stročnic, žitna zrna, semena, surov krompir, zelene banane, staran kruh, koruzni kosmiči, ohlajen kuhan krompir, riž

Glede na kompleksnost komponent, ki jih uvrščamo v vlaknino, se je tekom različnih obdobjij spremjalna tudi definicija za vlaknino. Večina definicij označuje analitične kriterije (predvsem topnost v vodi), nič pa ne pove o viskoznosti in fermentabilnosti, torej o fizikalno-kemijskih lastnostih, ki lahko pomembno vplivajo na delovanje prebavnega trakta. Še danes številni strokovnjaki navajajo, da je natančna opredelitev prehranske vlaknine sporna. Medtem ko ena izmed fizioloških definicij pravi: »prehranska vlaknina so ostanki rastlinskih celičnih sten, ki so odporne na encimsko hidrolizo v tankem črevesu«, pa ena izmed kemijskih opredelitev definira: »prehranska vlaknina so neškrobeni polisaharidi« (Theubadin in sod., 1997; Slavin, 2003). Najpogosteje uporabljeni definiciji je naslednja: »prehranska vlaknina je kompleksna mešanica neprebavljivih polisaharidov (celuloze, hemiceluloz, oligosaharidov, pektina, gum), voskov in lignina, ki jih najdemo predvsem v rastlinskih celičnih stenah. To so snovi, ki jih človekovi prebavni encimi ne razgradijo do komponent, ki bi se absorbirale v zgornjem prebavnem

traktu«. Tosh in Yada (2010) navajata, da je pomen uživanja prehranske vlaknine za zdravje v veliki meri posledica različnih fizioloških učinkov in je zato ta vidik vključen tudi v definicijo ameriškega združenja AACC (American Association for Clinical Chemistry), ki se glasi: »prehranska vlaknina so užitni deli rastlin ali ogljikovim hidratom podobne spojine, ki so odporne na prebavo in absorpcijo v človeškem tankem črevesu in se popolno ali delno fermentirajo v debelem črevesu. Prehranska vlaknina so polisaharidi, oligosaharidi, lignin, in druge rastlinske snovi. Prehranska vlaknina ima ugodne fiziološke učinke, pospešuje prebavo, in/ali vpliva na zniževanje nivoja holesterola in/ali glukoze v krvi«.

V izogib različnim definicijam je v Pravilniku o spremembah Pravilnika o označevanju hranične vrednosti živil (U.I. 87, 2009 z dne 02. 11. 2009) skladno z Direktivo Komisije 2008/100/ES (z dne 28. oktobra 2008) o spremembah Direktive Sveta 90/496/EGS podana naslednja opredelitev snovi, ki predstavljajo prehransko vlaknino:

*Prehranska vlaknina so polimeri ogljikovih hidratov s tremi ali več monomerskimi enotami, ki se ne prebavijo niti absorbirajo v tankem črevesu človeka in spadajo v naslednje kategorije:*

- *užitni polimeri ogljikovih hidratov, naravno prisotni v živilih v obliki, v kateri se zaužijejo,*
- *užitni polimeri ogljikovih hidratov, ki so bili pridobljeni iz surovine za živilo s fizikalnimi, encimskimi ali kemijskimi sredstvi in, ki imajo ugoden fiziološki učinek, dokazan s splošno sprejetim znanstvenim dokazom,*
- *užitni sintetični polimeri ogljikovih hidratov, ki imajo ugoden fiziološki učinek, dokazan s splošno sprejetim znanstvenim dokazom.*

V istem pravilniku je navedena tudi energijska vrednost prehranske vlaknine, ki znaša 8 kJ/g oziroma 2 kcal/g. Torej moramo pri vrednotenju hranične vrednosti živila, bogatega z vlaknino, poznati njeno vsebnost in izračunati njeno energijsko vrednost. Do nedavnega smo prehransko vlaknino pri

nepoznavanju njene vsebnosti upoštevali kot del ogljikovih hidratov, pri poznavanju njene vsebnosti, pa smo le-to odšteli od skupne vsebnosti ogljikovih hidratov in nato le za izkoristljive ogljikove hidrate izračunali energijsko vrednost.

## 2.1 Kje najdemo prehransko vlaknino

Vlaknino najdemo le v živilih rastlinskega izvora. Glede na obliko, v kateri ljudje uživamo prehransko vlaknino, govorimo o:

- živilih rastlinskega izvora: sadje, zelenjava, oreščki, žita in njihovi izdelki; komponente vlaknine se nahajajo predvsem v celičnih stenah zunanjih delov plodu ali zrna (v perikarpu, alevronski plasti), precej manj v celičnih stenah endosperma,
- izdelkih z dodano vlaknino (npr. kruh, v katerem je del pšenične moke nadomeščen z otrobi, z ječmenovo ali ovseno moko);
- nadomestkih hrane (prehranskih dopolnilih); tu mislimo na vlaknino v obliki kapsul ali tablet.

## 3 DELITEV PREHRANSKE VLAKNINE

Vlaknino delimo glede na njene fizikalno-kemijske lastnosti in fiziološko vlogo na (Gray, 2006):

- topno in netopno vlaknino,
- naravno prehransko vlaknino in funkcionalno vlaknino,
- viskozno in neviskozno vlaknino,
- fermentirajočo in nefermentirajočo vlaknino.

### 3.1 Topna in netopna prehranska vlaknina

Večina znanstvenikov se strinja glede delitve vlaknine na topno in netopno v vodi. Ta podatek je pomemben tako za analizo vsebnosti vlaknine kot za razumevanje različnih funkcij, ki jih imajo posamezne komponente vlaknine v telesu. Med topno

vlaknino uvrščamo gume, psilium, pektin,  $\beta$ -glukane in nekatere hemiceluloze, med netopno pa lignin, celulozo, hemiceluloso in nekatere pektine.

Topno vlaknino najdemo v vseh živilih rastlinskega izvora, vendar v zelo različnih količinah (preglednica 2). Živila, bogata s topno vlaknino so: ovjeni kosmiči, ječmen, stročnice (grah, soja, bob), nekatere vrste sadja in sadni sokovi (slive, jagode, banane, jabolka, agrumi, marelice, datliji, rozine, slivov sok), nekatere vrste zelenjave (brokoli, korenje, pesa, sladek krompir, čebula) in laneno seme. Glavna značilnost topne vlaknine je njena topnost v vodi in s tem povezane različne pozitivne fiziološke funkcije.

Tudi netopno vlaknino najdemo v različnih deležih zastopano v širokem spektru živil rastlinskega izvora. Bogat vir netopne vlaknine so zlasti: pšenični in koruzni otrobi, polnozrnatna živila, oreščki in semena, zelenjava (stročji fižol, cvetača, bučke,

zelena), nekatere vrste sadja (avokado in banane) in olupki nekaterih vrst sadja, krompirja, paradižnika, čebule. Komponente netopne vlaknine so v vodi netopne in imajo pomembno vlogo pri prehodu hrane skozi prebavni trakt.

**Preglednica 2:** Vsebnost prehranske vlaknine v nekaterih živilih rastlinskega izvora (Souci in sod., 2008)

**Table 2:** Dietary fiber content in some plant-derived foods (Souci et al., 2008)

Živilo	Vsebnost prehranske vlaknine (g/100 g)		
	topna prehranska vlaknina	netopna prehranska vlaknina	skupna prehranska vlaknina
jabolko	0,48	1,54	2,02
hruška	0,61	2,66	3,27
banana	0,62	1,20	1,82
krompir	0,92	1,15	2,07
krompir, kuhan z lupino	0,74	0,96	1,70
korenje	1,74	1,89	3,63
korenje, kuhan	1,17	1,30	2,47
brokoli	1,30	1,70	3,00
brokoli, kuhan	0,50	2,23	2,73
endivija	0,18	1,04	1,22
rdeča pesa	0,48	2,05	2,53
belo zelje	1,33	1,62	2,95
kislo zelje, odcejeno	0,84	1,30	2,14
stročji fižol	0,88	1,01	1,89
čebula	0,29	1,52	1,81
ovseni kosmiči	4,95	5,08	10,00
koruzni kosmiči	1,20	2,80	4,00
oreh	0,84	5,30	6,14
lešnik	0,42	7,80	8,22

### 3.2 Naravna prehranska vlaknina in funkcionalna vlaknina

S pojmom naravna prehranska vlaknina označujemo komponente, ki se nahajajo v naravnih prehranskih virih. Sem štejemo: celulozo, lignin, hemicelulozo, pektin, gume,  $\beta$ -glukane, inulin in oligofruktozo ter neprejavljivi škrob.

Kot funkcionalno vlaknino pa opredeljujemo izolirano naravno ali sintetično vlaknino, ki jo lahko dodajamo živilom in ima v telesu dokazan pozitiven fiziološki učinek. Med funkcionalno vlaknino spadajo poleg vseh zgoraj naštetih izoliranih komponent vlaknine tudi hitin (nahaja se v skeletu hrustančnic, npr. rakov), psilium (nahaja se v lupinicah trpotčevih semen) ter fruktooligosaharidi, polidekstroza, poliolji in neprejavljivi

dekstrini, ki jih uporabljamo kot aditive v proizvodnji živil.

Na International Life Science Institute-ILSI Europe (1999) so definirali, da lahko »živilo štejemo za funkcionalno, če je bilo dokazano, da ima poleg hraničnega učinka blagodejen vpliv na eno ali več fizioloških funkcij, pri čemer je ta pomembna za izboljšanje zdravja ljudi in/ali zmanjšanje tveganja za pojav nekaterih bolezni«. Z rezultati številnih raziskav so potrdili različne učinke prehranske vlaknine, na osnovi katerih jo lahko obravnavamo kot funkcionalno sestavino hrane (Elleuch in sod. 2011; Tosh in Yada, 2010; Cui in sod., 2011; Slavin, 2005). Poleg tega so v različnih raziskavah proučevali dodajanje vlaknine kot funkcionalnega dodatka: npr. Elleuch je s sod. (2008) proučeval dodajanje ekstraktov datljev; Tosh

in Yada (2010) sta proučevala vpliv dodatka moke iz stročnic (fižola, čičerike, leče in graha); Galanakis (2011) in Anil (2007) navajata možnost uporabe vlaknine iz stranskih produktov pri predelavi oljk (ostanek po stiskanju olja) oziroma pri praženju lešnikov (ovojnica); Peerajit in sod. (2012) so proučevali funkcionalne lastnosti ekstrakta vlaknine iz lupine limet. Pri obogatitvi izdelkov z vlaknino velja opozoriti na zahtevno nalogu živilskih tehnologov zaradi velike različnosti v kemijski strukturi in različnih funkcionalnih lastnosti posameznih komponent vlaknine (Cui in sod., 2011).

### 3.3 Viskozna in neviskozna prehranska vlaknina

Nekatere komponente vlaknine tvorijo v kombinaciji z vodo viskozne raztopine, ki vplivajo na počasnejšo absorpcijo nekaterih hranil, na počasnejše praznjenje želodca, na nižji nivo krvnega holesterola in na uravnavanje nivoja glukoze v krvi (Jenkins in sod., 2004). Med viskozno vlaknino spadajo nekatere gume, psilium, pektin in  $\beta$ -glukani.

Kristensen in Jensen (2011) sta v reviji Appetite objavila članek o pomenu viskoznosti vlaknine pri uravnavanju apetita in na občutek sitosti. Poudarjata, da lakoto in vnos hrane regulirajo sicer različni fiziološki vplivi, med katerimi pa je viskoznost prehranske vlaknine eden pomembnejših.

### 3.4 Fermentirajoča in nefermentirajoča prehranska vlaknina

Nekatere komponente vlaknine v črevesju s pomočjo bakterij fermentirajo, pri čemer nastajajo plini in kratkoverižne maščobne kisline, ki se lahko absorbirajo in so tako vir energije. Med visoko fermentirajočo vlaknino spadajo: inulin, oligofruktosa, pektin,  $\beta$ -glukani in nekatere gume. Medtem ko spadata hemiceluloza in rezistentni škrob med delno ali slabo fermentirajočo, pa se lignin in celuloza smatrata za nefermentirajoči komponenti. Živila z veliko vsebnostjo fermentirajoče vlaknine so ječmen, oves, sadje in zelenjava, nefermentirajočo vlaknino pa vsebujejo žita in otrobi oz. živila z veliko vsebnostjo celuloze (Rodríguez in sod., 2006).

## 4 PRIPOROČILA GLEDE VNOSA PREHRANSKE VLAKNINE

Referenčne vrednosti za vnos hranil (2004) navajajo orientacijsko vrednost za vnos prehranske vlaknine pri odraslih najmanj 30 g na dan, to je približno 3 g/MJ oziroma 12,5 g/1000 kcal pri ženskah in 2,4 g/MJ oziroma 10 g/1000 kcal pri moških. Po priporočilih prehranskih strokovnjakov je dnevno zaužita količina vlaknine med 20 in 40 g. Glede na sodobni način prehranjevanja večina ljudi uživa hrano, pripravljeno iz predelanih in prečiščenih sestavin, z veliko mesa in sladkarij, zato tega odmerka ni tako

lahko doseči. Strokovnjaki opozarjajo, da je vnos vlaknine s prehrano v zahodnih deželah pod priporočili, manjši kot ga potrebujemo za zdravo življenje (Baixauli in sod., 2008). Če želimo povečati vnos vlaknine v telo, je to potrebno storiti postopoma, in sicer za največ 5 g v 14-ih dneh. Priporočajo, da bi uživali pestro hrano (sadje, zelenjavno, žita ter stročnice), ker bi s tem zagotovili organizmu tako po sestavi kot po učinkovanju različne komponente prehranske vlaknine.

## 5 VPLIV PREHRANSKE VLAKNINE NA ZDRAVJE LJUDI IN BOLEZNI

Na osnovi rezultatov številnih znanstvenih raziskav je sprejeto prepričanje, da ima

prehranska vlaknina pomembno vlogo pri preprečevanju številnih bolezni, in da prehrana

z veliko vsebnostjo vlaknine, oziroma, da hrana, ki je bogata z žiti, s sadjem in z zelenjavno, pozitivno vpliva na zdravje. Večje uživanje vlaknine povezujejo tudi z zmanjšano incidento za več vrst raka (Peerajit in sod., 2012).

Kljub temu, da je večina sestavin prehranske vlaknine neprebavljivih, ker jih encimi, prisotni v debelem črevesu, ne morejo razgraditi, so izpostavljene aktivnostim bakterijskih encimov, ki jih lahko delno razgradijo. Kot poudarjajo Rodríguez in sod. (2006), je obseg te razgradnje odvisen od vrste bakterijske flore. Dejavnika, ki so ju znanstveniki v povezavi z vlaknino proučevali, sta bila čas prehoda skozi debelo črevo in viskoznost.

Čas prehoda skozi črevo vpliva na trajanje stika med bakterijskimi encimi in komponentami prehranske vlaknine, ki omejujejo obseg njihove razgradnje (30-90 % polisaharidov, večina hemiceluloze ter pektin). Ta razgradnja se začne z zunanjim celično hidrolizo, ki pretvarja polisaharide v mono- in disaharide, temu pa sledi anaerobna glikoliza, katere končni produkti so acetat, butirat in propionat.

Glavni učinek v tankem črevesu je povezan z viskoznostjo polisaharidov, komponent topne vlaknine, kot so pektin in gume, ki zmanjšujejo asimilacijo hranljivih snovi, medtem ko netopne komponente tu ne igrajo pomembne vloge. Iz visoko fermentirajočih snovi (pektina), ostankov slabše fermentirajočih polimerov (celuloze in hemiceluloze) in vode, ki jo te komponente vežejo in zadržujejo, nastaja bakterijska masa, ki je odgovorna za povečanje mase blata (Rodríguez in sod., 2006).

## 5.1 Prehranska vlaknina in telesna teža

V prispevku, objavljenem v reviji Nutrition, je Slavin (2005) prikazal pregled raziskav o vplivu prehranske vlaknine na telesno težo in

mehanizme njenega delovanja. Podaja teoretične razloge, s katerimi prehranska vlaknina pomaga pri vzdrževanju telesne teže. Pravi, da deluje prehranska vlaknina kot fiziološka ovira vnosa energije z najmanj tremi mehanizmi: nadomesti hranila in izkoristljive kalorije v hrani; vpliva na povečano žvečenje, kar omeji vnos hrane in pospeši izločanje sline in prebavnih sokov; zmanjša absorpcijo hranil v tankem črevesu.

Z vlaknino bogata hrana ima manjšo energijsko vrednost. Torej, če uživamo konstantno količino hrane, ki ima na račun večje vsebnosti vlaknine nižjo energijsko vrednost, vplivamo na zmanjšanje teže. Ker pa ima vlaknina zaradi sposobnosti povečanja volumna in viskoznosti vpliv na občutek sitosti in nasitno vrednost, smo z uživanjem enake količine hrane z nizko energijsko vrednostjo in veliko vsebnostjo vlaknine prej siti.

Poleg tega je dokazano, da vlaknina lahko blokira ali omeji absorpcijo makrohranil in s tem pomaga pri kontroli telesne teže. Celo zelo majhne spremembe v absorpciji hranil imajo dolgoročno značilen vpliv na telesno težo.

Tudi počasnejše praznjenje želodca je učinek, ki ga pripisujemo uživanju hrane bogate z vlaknino, predvsem z viskozno vlaknino. Upočasnjeno praznjenje želodca poveča občutek polnosti, upočasni absorpcijo glukoze in drugih hranil in tako vpliva na telesno težo.

Lluch in sod. (2010) so ugotovili, da je poleg tega, da je še dve uri po obroku, ki je vseboval manj maščob in bil obogaten z beljakovinami in vlaknino zmanjšan tek, manjša tudi zaužita količina naslednjega obroka.

## 5.2 Prehranska vlaknina in čas prehajanja skozi črevesje

Prehranska vlaknina velja za najpomembnejši dejavnik, ki vpliva na maso blata in hitrost peristaltike. Problem zaprtja obravnavamo običajno kot posledico uživanja nezadostne

količine prehranske vlaknine. Thebaudin in sod. (1997) omenjajo, da je fiziološki učinek vlaknine v črevesju odvisen od deleža netopne oziroma topne vlaknine. Netopna vlaknina poveča maso blata in pogostost praznjenja, mehča blato in skrajšuje čas prehajanja skozi črevesje. Sposobnost vlaknine za zadrževanje vode je pomembna pri formiraju blata. Povečanje mase blata pripisujejo povečanju bakterijske mase, neprebavljenim vlakninskim ostankom in količini vode v blatu. Učinki vlaknine na maso blata so različni glede na vrsto in vir vlaknine, deloma pa vpliva tudi metoda priprave hrane. Vlaknini iz žit običajno pripisujejo večjo učinkovitost pri večanju teže blata kot vlaknini iz sadja. Topna vlaknina, kot je pektin, ne vpliva bistveno na funkcijo debelega črevesa ali skrajšanje časa prehoda; črevesna mikroflora jo popolnoma razgradi.

### 5.3. Prehranska vlaknina in rak

Obstajajo rezultati številnih raziskav, v katerih so proučevali vpliv prehrane z veliko vsebnostjo vlaknine na zdravje in na pogostnost obolenja za različnimi oblikami raka. Domnevno zaščitno vlogo pred rakom pri uživanju hrane bogate z vlaknino je močno podprt Burkitt (1973). Tudi Willett in sod. (1992) so v obsežni raziskavi v Veliki Britaniji preučevali učinke vnosa vlaknine skupaj z drugimi sestavinami hrane na pojavnost raka debelega črevesa pri ženskah. Ugotovili so, da je zmanjšano tveganje za pojav raka debelega črevesa povezano z večjim vnosom vlaknine s sadjem.

Vzporedno pa lahko spremljamo rezultate raziskav, v katerih opozarjajo na težave pri oceni epidemioloških študij in o direktni povezavi med uživanjem hrane bogate z vlaknino in manjšo pojavnostjo raka debelega črevesa. Saj z uživanjem sadja, zelenjave, žit vnašamo v organizem tudi druge zaščitne snovi, kot so vitamin C, vitamin E in  $\beta$ -karoten. Rezultati 13-ih primerjalnih študij so

pokazali, da je vsaka komponenta posamično ( $\beta$ -karoten, askorbinska kislina in vlaknina) pomembno povezana z zmanjšanim tveganjem za pojav raka in težko zaključimo, da je to posledica samo vnosa vlaknine (Thebaudin in sod., 1997).

Nekateri raziskovalci so predlagali naslednji mehanizem, s katerim lahko prehranska vlaknina varuje pred rakom debelega črevesa: netopna vlaknina (npr. pšenični otrobi) poveča volumen blata in razredči njegovo vsebino, kar zmanjšuje interakcije med črevesno sluznico in vsemi rakotvornimi snovmi v blatu; krajši čas prehoda skozi črevesje zmanjša možnost interakcij mutagenov iz blata s črevesnim epitelom; vlaknina, kot fermentacijski substrat, spremeni tudi delovanje prebavne mikroflore, kar privede do spremembe ali zmanjšanja nastanka mutagenov; nekatere vrste vlaknine lahko celo adsorbirajo mutagene agense in s tem pospešijo njihovo izločanje (Thebaudin in sod., 1997).

Možen mehanizem zaščitnega učinka vlaknine proti raku dojk je, da večji vnos vlaknine z večjim izločanjem blata vpliva na večje izločanje estrogenov, ki jih povezujejo s povečanim tveganjem za pojav raka dojk. Vendar opozarjajo, da so podatki o tem vplivu še nejasni. Tudi z raziskavo, v kateri so medicinske sestre uživale 22 g vlaknine na dan, niso dokazali varovalnega učinka vlaknine (Willet, 1992). V obsežnem pregledu izsledkov raziskav je bilo ugotovljeno, da iz dosedanjih literaturnih podatkov ne moremo zaključiti, da ima vlaknina zaščitni učinek niti pred rakom debelega črevesa niti rakom dojke, kljub temu, da so številne študije pokazale trende v tej smeri. Potrebne so še dodatne raziskave.

### 5.4 Vlaknina in presnova ogljikovih hidratov, beljakovin in maščob

Ugotovljeno je, da lahko vlaknina vpliva na razpoložljivost ogljikovih hidratov v

prebavnem traktu. Ta učinek je bil potrjen pri bolnikih s slatkorno boleznijo, pri katerih se je raven glukoze v krvi zmanjšala ob prehrani bogati z vlaknino. Theubaudin in sod. (1997) menijo, da netopna prehranska vlaknina nima vpliva na presnovo ogljikovih hidratov, medtem ko topna in viskozna vlaknina vplivata na zmanjšanje nivoja postprandialne glukoze v krvi.

Glede vpliva vlaknine na presnovo beljakovin pa so številne študije pokazale, da je povečan vnos vlaknine v prehrani povezan s povečanim izločanjem dušika z blatom. Dušikove snovi so v veliki meri odvisne od bakterijskega izvora; povečanje mase blata pa kaže pri hrani bogati z vlaknino na povečano rast bakterij. Avtorji menijo, da imamo zaenkrat še premalo dokazov o tem, da bi prehranska vlaknina vplivala na presnovo in absorpcijo beljakovin. Poudarjajo nujnost nadalnjih raziskav (Thebaudin in sod., 1997). Eggum (1992) ugotavlja, da je vpliv prehranske vlaknine na presnovo beljakovin v veliki meri odvisen od strukture in kemijske sestave vlaknine.

Bistveno bolj pa so proučevani učinki vlaknine na presnovo lipidov. Ugotovljeno je bilo, da vlaknina vpliva na absorpcijo lipidov, saj močno zavira delovanje lipaz trebušne slinavke, ki sodelujejo pri presnovi lipidov. Po drugi strani pa prehranska vlaknina prispeva k zmanjšanju ravni holesterola in lipoproteinov nizke gostote v plazmi, kar je povezano z redčenjem in z večjim izločanjem žolčnih kislin (Rodríguez, 2006). V raziskavi o učinkih topne vlaknine na serumske lipide so ugotovili, da topna vlaknina vpliva na znižanje ravni lipoproteinskega holesterola nizke gostote LDH v serumu. Vezanje soli žolčnih kislin na komponente prehranske vlaknine spremeni presnovo holesterola, oziroma izločanje holesterola iz telesa. Z vezavo žolčnih kislin nastajajo žolčne soli, ki se s pomočjo blata odvajajo iz telesa. Tako je onemogočena resorpacija žolčnih kislin iz tankega črevesa nazaj v jetra, kjer se iz njih tvori holesterol LDL, povzročitelj

arterioskleroze. Gallaher in sod. (1992) pripisujejo ta pozitiven učinek predvsem prehranski vlaknini iz ječmena in sladkorne pese.

Pri proučevanju vpliva vlaknine na presnovo maščob je potrebno omeniti kratko verižne maščobne kisline (SCFA), ki nastajajo v debelem črevesu pri bakterijski razgradnji prehranske vlaknine. Številni raziskovalci menijo, da SCFA (zlasti propionat) zavirajo sintezo jetrnega holesterola (Roberts in sod., 1994).

## 5.5 Prehranska vlaknina in biološka izkoristljivost vitaminov in mineralov

Vnos hrane, bogate z vlaknino, vpliva na delovanje encimov in podaljša čas prehoda hrane, kar naj bi vplivalo na povečano absorpcijo vitaminov. Vendar pa nekatera živila vsebujejo tako imenovane zaviralce izkoriščanja vitaminov (npr. inhibitorji proteaz), ki zmanjšujejo njihovo topnost/sproščanje. O učinkih prehranske vlaknine na biološko izkoristljivost vitaminov je bilo opravljenih le malo raziskav; največ pozornosti je bilo namenjene vitaminoma E in D. Ugotovili so, da so imele podgane, krmljene z grobimi pšeničnimi otrobi, nižji nivo tokoferola v jetrih kot podgane, krmljene s finimi gladkimi pšeničnimi otrobi ali celulozo. Nekatere študije so pokazale, da lahko velika vsebnost vlaknine v hrani vodi do večjega izločanja vitamina D. To razlagajo z nizkim statusom vitamina D pri vegetarijancih in pri makrobiotični dieti (Dagnelie, 1991).

Biološka izkoristljivost mineralov je odvisna od količine zaužite hrane, od količine hrane, ki se v telesu absorbira in izkoristi. Domnevajo, da velik vnos vlaknine lahko zmanjša absorpcijo mineralov zaradi interakcij med minerali in vlaknino in je zato težko napovedati biološko izkoristljivost mineralov. Vpliv vlaknine na biološko izkoristljivost mineralov je odvisen predvsem od vrste vlaknine. Večina raziskav je vključevala

pšenične otrobe. Najbolj raziskane so interakcije prehranske vlaknine z železom, s cinkom in s kalcijem. Številne študije so pokazale škodljiv vpliv vlaknine iz pšenice in koruze, pa tudi posameznih komponent vlaknine (guar gume, lignina in psilium sluzi) na absorpcijo železa in cinka. Medtem ko so rezultati drugih študij pokazali, da vlaknina ne zavira absorpcije železa in cinka. Te nasprotuječe ugotovitve so morda posledica tega, ker se vlaknina pogosto pojavi skupaj s

fitatom, dokazanim zavircem absorpcije železa in cinka pri ljudeh in podganah. Dokazali so, da odstranitev fitata (kar je med predelavo izvedljivo) izboljšuje biološko izkoristljivost železa, cinka in kalcija. Tudi Thebaudin in sod., (1997) poudarjajo, da nikakor ne smemo slabše absorpcije mineralov pripisati le tvorbi kelatov med vlaknino in minerali, da na biološko izkoristljivost mineralov oziroma zmanjšano absorpcijo mineralov vpliva poleg vlaknine tudi fitat.

## 6 ZAKLJUČEK

Čeprav je o lastnostih prehranske vlaknine veliko znanega, ostajajo še vedno številni nepojasnjeni vidiki, predvsem glede vpliva posameznih komponent vlaknine na nekatere patološke pojave. Ugotavljamo, da je danes jasno opredeljena le definicija vlaknine, dobro pa so dokumentirani tudi različni koristni učinki na zdravje ljudi, ki omogočajo, da vlaknino opredeljujemo kot funkcionalno sestavino, s širokim potencialom uporabe.

Po pregledu različnih študij o prehranski vlaknini lahko povzamemo, da je na tem raziskovalnem področju še veliko vidikov, ki so potrebni nadalnjih preiskav. Smatramo, da

so za boljše poznavanje natančne strukture posameznih komponent prehranske vlaknine in razumevanje njihovih fizioloških učinkov na človeško telo potrebne kompleksne študije, v katerih bi sodelovali raziskovalci iz različnih področij znanosti: kemije, biokemije, biotehnologije, biologije, fiziologije, prehrane in medicine. Z rezultati take raziskave bi izgradili večjo in multidisciplinarno bazo, ki bo omogočala univerzalni dogovor o opredelitvi prehranske vlaknine, definirala metode za analizo njene vsebnosti ter natančno opredelila fiziološko vlogo posameznih komponent.

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**Agrovoc descriptors:** scaphoideus titanus, pest insects, vineyards, grapevines, vitis vinifera, pest insects, pest surveys, surveys, monitoring, data collection, traps, pest control equipment, plant nurseries

**Agris category code:** h10

## Migration of *Scaphoideus titanus* Ball from the surrounding vineyards into the nursery

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### ABSTRACT

A one-year study of the mobility of leafhopper *Scaphoideus titanus* Ball (ST), vector of Flavescence dorée phytoplasma, was conducted in 2009 and included three Slovenian nurseries in the Primorska winegrowing region, as well as two Italian and one Swiss nursery, and was aiming at evaluating the risk of spreading ST with vine propagating material. The presence of ST was monitored by the means of yellow sticky traps that were placed at three distances in the nurseries and the nearby vineyards on three parallel lines (replicates). The traps were equidistantly placed from the nurseries' and vineyards' borders in order to determine the possible distance gradient. The monitoring started in the latest nymphal stages (middle of June) and terminated in the first decade of October. Insecticide treatments with deltamethrin (Decis 2.5 EC) were applied in nurseries, according to the instructions for use and recommendations for suppression of ST. On all Slovenian plots in the vineyards, the population level of ST was high (1796 adults in average / vineyard or 18 adults per trap / week), which indicated a great migration potential of the vector towards the nurseries. Catches were very low (54 adults in average / nursery or 0.4 adults / trap / week) on two nurseries' plots (Lože and Slap), although high numbers of ST adults were caught in the adjacent untreated vineyards. On the Poreč nursery plot, insecticide treatments were not sufficient to avoid high numbers of ST catches (in total 481 adults or 3 adults / trap / week). This could be related to the sub-optimal timing of the first insecticide treatment and/or migration of adults from two nearby untreated vineyards located at a distance of 70 and 150 m. The distance between the nurseries and vineyards did not affect ST catches in the nurseries.

**Key words:** *Scaphoideus titanus*, Flavescence Dorée, migration, nursery

### IZVLEČEK

#### MIGRACIJA AMERIŠKEGA ŠKRŽATKA (*Scaphoideus titanus* Ball) IZ BLIŽNJIH VINOGRADOV V TRSNICO

Z namenom preučitve nevarnosti širjenja prenašalca zlate trsne rumenice z razmnoževalnim materialom vinske trte smo v letu 2009 izvedli raziskavo mobilnosti ameriškega škržatka (*Scaphoideus titanus* Ball) (ST) v treh trsnicah v vinorodni deželi Primorska. V manjšem obsegu je bil poskus izведен tudi pri partnerskih institucijah v Italiji in Švici. Zastopanost in nalet ST smo ugotavljali z rumenimi lepljivimi ploščami, ki so bile postavljene na treh različnih razdaljah od roba trsnice in vinogradov v 3 vzporednih ponovitvah. S postavitvijo lepljivih plošč v enakomernih razmakih od roba trsnice in vinogradov v globino smo želeli ugotoviti morebitni gradient naleta. Z monitoringom naleta smo začeli, ko je bil škodljivec v 5. stadiju ličinke (sredi junija) in ga zaključili v prvi dekadi oktobra. Tretiranje z deltametrinom (Decis 2.5 EC) je bilo izvedeno v trsnici, skladno z navodili za uporabo insekticida in s priporočili za zatiranje ST. Populacija ST v vinogradih je bila na vseh treh lokacijah v Sloveniji visoka (v povprečju 1796 odraslih osebkov / vinograd oziroma 18 osebkov / ploščo / teden), kar je predstavljalo velik migracijski potencial prenašalca proti trsnicam. Kljub visoki populaciji ST v bližnjih vinogradih je bilo število ujetih ST v trsnicah na lokaciji Slap in Lože nizko (v povprečju 54 osebkov / trsnico oziroma 0,4 osebka / ploščo / teden). Število ujetih odraslih osebkov na lokaciji Poreče je bilo kljub tretiranju z insekticidom visoko (skupno 481 osebkov ali 3 osebki / ploščo / teden). Vzrok za to gre iskati v neustremnem času prvega tretiranja z insekticidom ali v dodatnem naletu ST iz bližnjih vinogradov, ki so bili oddaljeni 70 in 150 m. Razdalja med trsnico in vinogradom ni vplivala na število ujetih ST v trsnici.

**Ključne besede:** ameriški škržatek, zlata trsna rumenica, migracija, trsnica

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## 1 INTRODUCTION

The efficacy of insecticide treatments against *Scaphoideus titanus* Ball (ST) (Hemiptera, Cicadellidae), the vector of Flavescence Dorée phytoplasma (FD), in nurseries depends on the term of treatment regarding the vector's development stage, on the proportion of its mortality, insecticide residues on plants and on the migration of adults from the surrounding vineyards. The previous pesticide registration against ST considered only the situation in spacious vineyards, but did not make any allowances for small-sized nurseries (Boudon-Padieu, 2003). This aspect has to be considered also in adopting a suitable insecticide treatment program. Also the distances between vineyards and nurseries and the mobility range of ST are crucial for determining the risk of spread of ST with the vine grafted plants.

ST, a Nearctic leafhopper, was introduced into Europe from the North America before 1958. This univoltine species overwinters in the egg stage. Eggs are deposited into the bark of two or more years old vine branches (Vidano, 1964), rarely also in one-year branches. Nymphs start emerging in the second half of May and adults occur in mid-summer (in the Slovenian climate, at the end of June). They feed exclusively on *Vitis* spp. and cause indirect damages by transmitting FD from infected vines. Although insecticide treatment against FD vectors is mandatory to prevent the progression of the disease, new contaminations

may occur with the movement of infested planting material or ST migrations (Boudon-Padeau et al, 2005). The movement of ST is rather limited to the vine's canopy and is not very likely to fly for more than 2.50 m in high (Lessio F., Alma A., 2004). Seasonal and daily fly of ST is partly influenced by increased temperatures and humidity (Lessio F., Alma A., 2004).

In order to determine the risk of FD vector-spreading with the planting material, a one-year study of the mobility of ST was conducted in 2009 in three nurseries in the Slovenian Primorska winegrowing region. The occurrence of ST was monitored using yellow sticky traps. They were placed at three distances in the nurseries and the nearby vineyards in three parallel lines (replicates). The traps were equidistantly placed from the nurseries' and vineyards' borders in order to determine the possible distance gradient. The main part of this study was carried out in Slovenia, where ST is widely spread in all winegrowing regions and the population rates in some vineyards were still fairly large. A small-scale experiment was also conducted in Italy and Switzerland.

The objective of the work was to evaluate the effectiveness of insecticide treatments against ST adults in nurseries in order to reduce the risk of immigration of adults from the nearby vineyards.

## 2 MATERIALS AND METHODS

### 2.1 Slovenian trials

In Slovenia, the monitoring was carried out in 2009 at three locations in the Primorska winegrowing region, winegrowing district of Vipava valley (Slap - 45° 50' 73,55" N, 13° 56' 31,57" E, 99 m altitude; Lože - 45° 49' 52,80" N, 13° 56' 43,68" E, 109 m altitude; Poreče –

45° 50' 57,72" N, 13° 56' 20,30" E, 95 m altitude) with four evaluating plots. Distances between the vineyards and nurseries were different, because the major part of vineyards were grown on hilly sides, while nurseries were situated along the river Vipava. Distances between nurseries and vineyards

included in experimental plots are shown in the Table 1.

**Table 1:** Sizes of experimental plots and distances between them

Location (size)	Vineyard nr. 1	Vineyard nr. 2
1. Slap		
Nursery (0.8 ha)	126 m	188 m
Vineyard nr. 1 (0.65 ha)	/	
Vineyard nr. 2 (2.0 ha)		/
2. Poreč		
Nursery (4.5 ha)	150 m	70 m
Vineyard nr. 1 (0.5 ha)	/	
Vineyard nr. 2 (1.1 ha)		/
3. Lože		
Nursery nr. 1 (2.5 ha)	18 m	
Nursery nr. 2 (0.6 ha)		18 m
Vineyard nr. 1 (2.2 ha)	/	
Vineyard nr. 2 (0.43 ha)		/

Except for the second location where a tree-barrier was grown between the nursery and vineyards, no other spatial obstacles were noticed. Yellow sticky traps (Unichem, 17 x 24 cm) were placed on the plots on 17 June. The traps were placed on the nurseries' and vineyards' edges and in at least three parallel lines (replications). They were placed on the inner side of borders, considering the distance of 10 m between traps. Replications were designated in order to cover the whole area of the vector's mobility; distances between them were 20 m.

In vineyards, planted with varieties Merlot, Sauvignon and Cabernet sauvignon, traps were attached with a wire inside the leaf canopy between 1.3 and 1.8 m altitude, depended on the growing type. In nurseries, traps were fixed on 0.5 m long iron stakes. The traps were checked weekly and replaced every three weeks. The monitoring was finished on 8 October. In nurseries, two insecticide treatments against ST were applied. Application data are given in Table 2.

**Table 2:** Data of insecticide treatments in nurseries in Slovenian experiment in 2009

Plot	First treatment	Second treatment
Slap	13. 7.	6. 8.
Poreč	9. 7.	31. 7.
Lože	20. 7.	7. 8.

For all treatments, the insecticide deltametrin (Decis 2.5 EC) in dosage 0.5 L of the product per hectare was used.

### Data analysis

Data were analyzed using nested analysis of variance, where time of sampling were nested within trap distance from border, distance were nested within a plot use (nursery and

vineyard), and plot use were nested in location. With this analysis the amount of variability contributed by each factors were estimated. Analysis was performed using STATGRAPHIC 5.0 statistical software.

A small-scale experiment was conducted also in Switzerland (one plot) and Italy (two plots). In order to facilitate trial activities and compare results between the three countries, a

common protocol had been established before starting the activities.

## 2.2 Italian trials

The experiment performed by the Italian partner (IVC) was done on two plots (Pordenone - 46° 2' 48" N, 12° 52' 23" E, 70 m altitude and Verona - 45° 33' 19" N; 10° 46' 42" E; 200 m altitude). The trials started on 16 June 2009 in Verona and on 30 June 2009 in Pordenone, and were finished on 1 October. Insecticide treatments were applied in nurseries and in vineyards. The period of application as well as the number of treatments was different for the locations, as presented here below:

### Pordenone

Vineyard - four treatments (18 April – deltametrin, 23 May indoxacarb -, 26 June and

21 July chlorpyrifos ethyl). Nursery - three treatments (deltametrin, chlorpyrifos ethyl).

### Verona

Vineyard – one treatment (chlorpyrifos methyl – 14 July). Nursery - seven treatments (20 May - fenazaquin, 29 May - spinosad, 10 June - chlorpyrifos ethyl, 25 June - spinosad, 10 July, 30 July and 20 August - chlorpyrifos ethyl)

## 2.3 The Swiss trial

A small-scale experiment was carried out on one plot (Anieres – 6° 13' 23,5" E, 46° 16' 24,9" N, 412 m altitude). Traps were set on 3 July 2009 and the trial finished on 5 October. Two insecticide treatments against the leafhopper were applied only in the nursery: on 13 July and 27 July. The insecticide used was lambda-cyhalotrine (Kararate with Zeon Technology (9.43% 100 g/l) in dosage 1.2 l/ha.

## 3 RESULTS

### 3.1 Slovenian trials

On all three Slovenian plots, the population level of ST in vineyards was very high, which indicates a high immigration potential of the

vector towards the nurseries. It should also be stressed that all Slovenian experimental locations are exposed to frequent and strong NO-SW winds that may influence the movement direction and distance of ST.

**Table 3:** Results from nested ANOVA analyses of ST catches

Source	Sum of squares	Df	Mean square	F	Variance component	% variance component
Location	6640.6	2	3320.30	0.12	0.00	0.00
Manner of land use	82043.1	3	27347.70	23.34 **	116.86	28.75
Trap distance from border	16403.7	14	1171.69	1.90 *	8.64	2.13
Time of sampling	172895.0	280	617.48	3.34 **	96.14	23.65
Error	194083.0	1050	184.84		184.84	45.47

Significance of F values indicated as follows: \* $p < 0.05$ ; \*\* $p < 0.01$

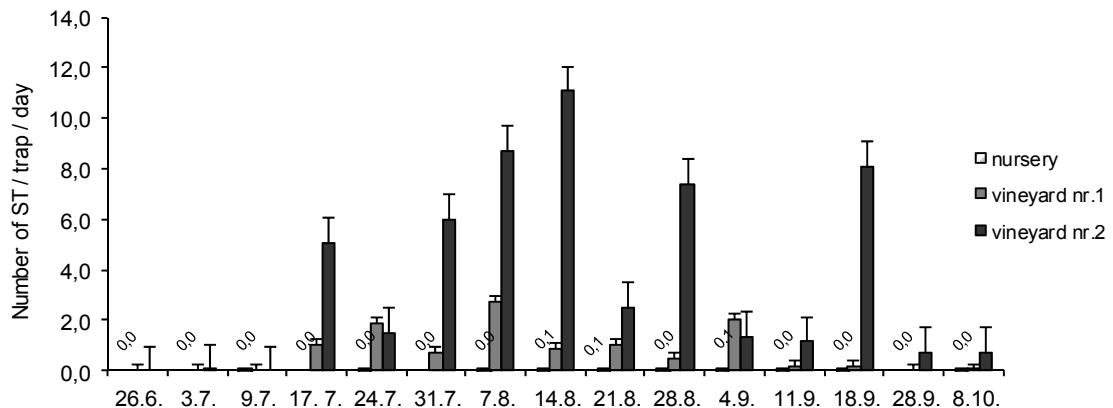
The results of nested ANOVA indicate that manner of land use and time of sampling contributes significantly

( $p < 0.01$ , F statistic) to the total variance of number of ST. Manner of land use contribute 28.75% of the total variation, and time of

sampling contributed 23.65%. The large variance component for the error (45.47%) indicates large variation among replicates within time of sampling. Trap distance from

border is significant at  $p < 0.05$ , but not at  $p < 0.01$  and contribute only 2.13% of the total variation.

## Slap location

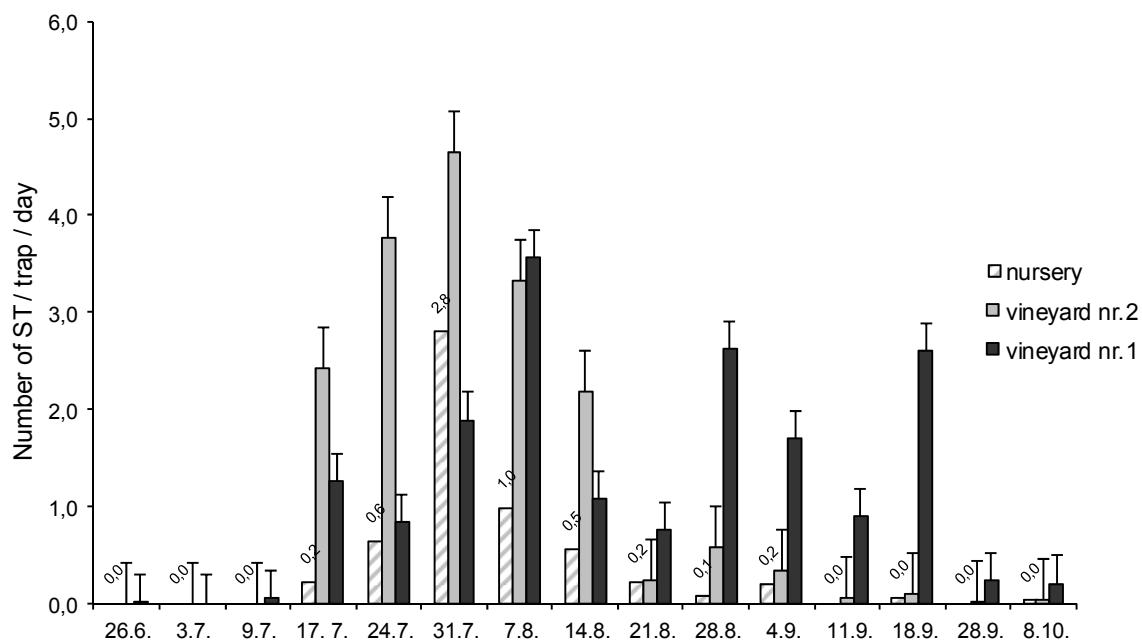


**Figure 1:** Number of captured adults of ST per trap and day in location Slap

At the first location, the number of captured ST in the nurseries was very low (34 adults in total, 0.0 adults / trap / day) in contrast to the captures in the two vineyards in the neighbourhood, where the average number of adults per trap per day was 0.7 for the vineyard No. 1 and 3.6 for the vineyard No. 2. In this case, the high population rate of ST in vineyards didn't imply the population of ST in the nursery; the reason might be fairly long distances between the nursery and vineyards (126 m and 188 m). Also the position of traps regarding the distance from the nursery edge didn't show differences. Insecticide treatments didn't show reduction of population level, especially the second application of 5. August.

## Poreč location

The trial conducted on the second plot revealed a high number of ST captures (481 adults in total or 0.4 adults / trap / day in average) in the nursery, with a pick at the end of July (49% of all captures in the nursery). Since the nursery was treated two times with an insecticide, the high number of ST captures could be explained either by the movement of ST from the neighbourhood untreated vineyards (Vineyard nr. 2) or by insufficient efficacy of the first treatments. After the second treatment (31. July), the population rate in the nursery dropped considerably, but still relatively numerous captures afterwards suggest that one or two additional treatments against ST would be necessary.

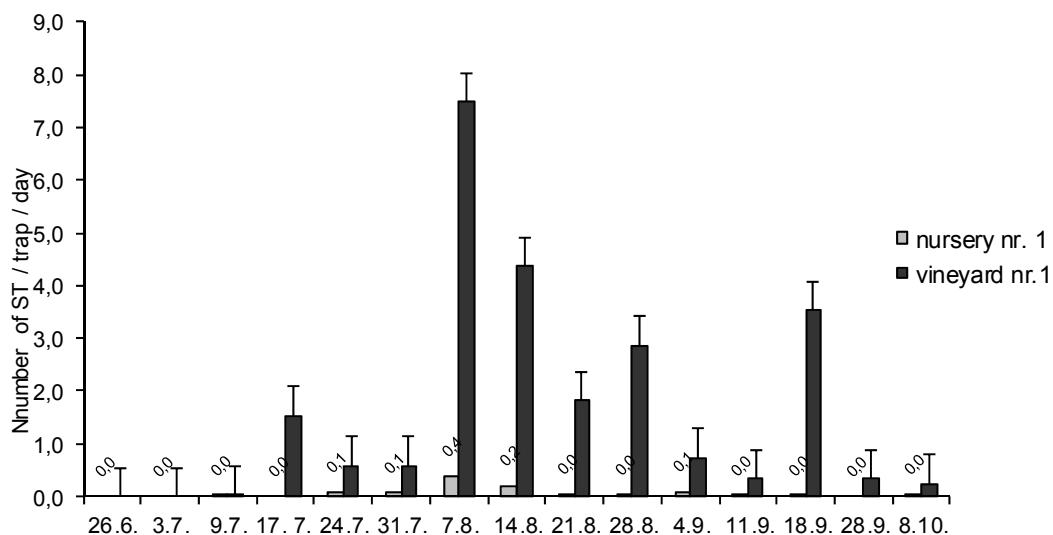


**Figure 2:** Number of captured adults of ST per trap and day in location Poreč

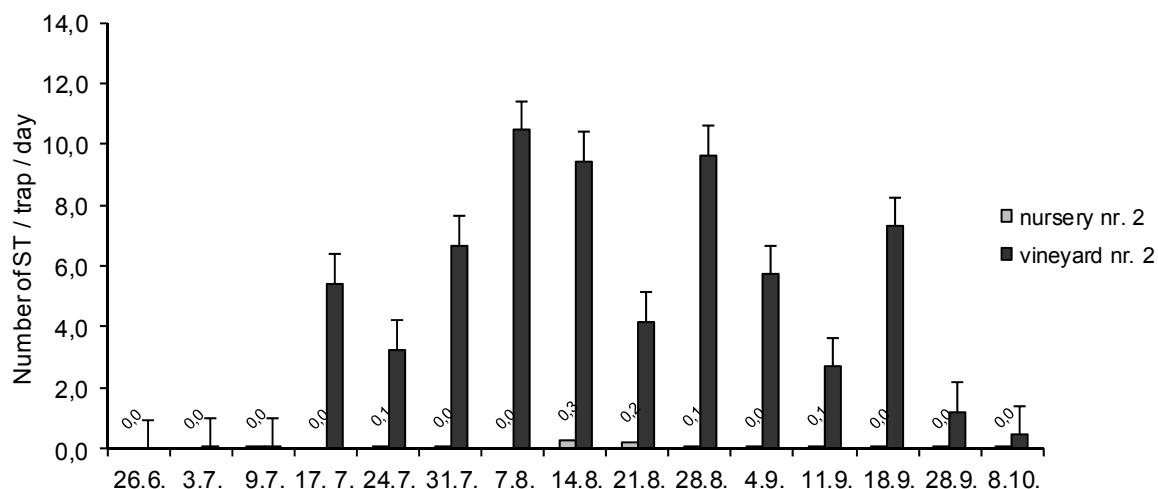
The highest number of adults was captured on the traps placed 20 meters from the nursery border (182 adults in total or 0.6 adults / trap / day in average). On traps placed on the border line, the mean capture was 0.4 adults / trap / day. 45% of all adults captured at the end of July were found 20 meters from the border line.

### Lože location

At this location, two different plots were evaluated because of the different position of the vineyards. In contrast to the previous locations, in this case the distance between the nursery and vineyard was quite short (18 m) so that the possibilities for the movement of ST from the adjacent vineyard to the nursery were much more reliable.



**Figure 3:** Number of captured adults of ST per trap and day in Lože nursery nr.1

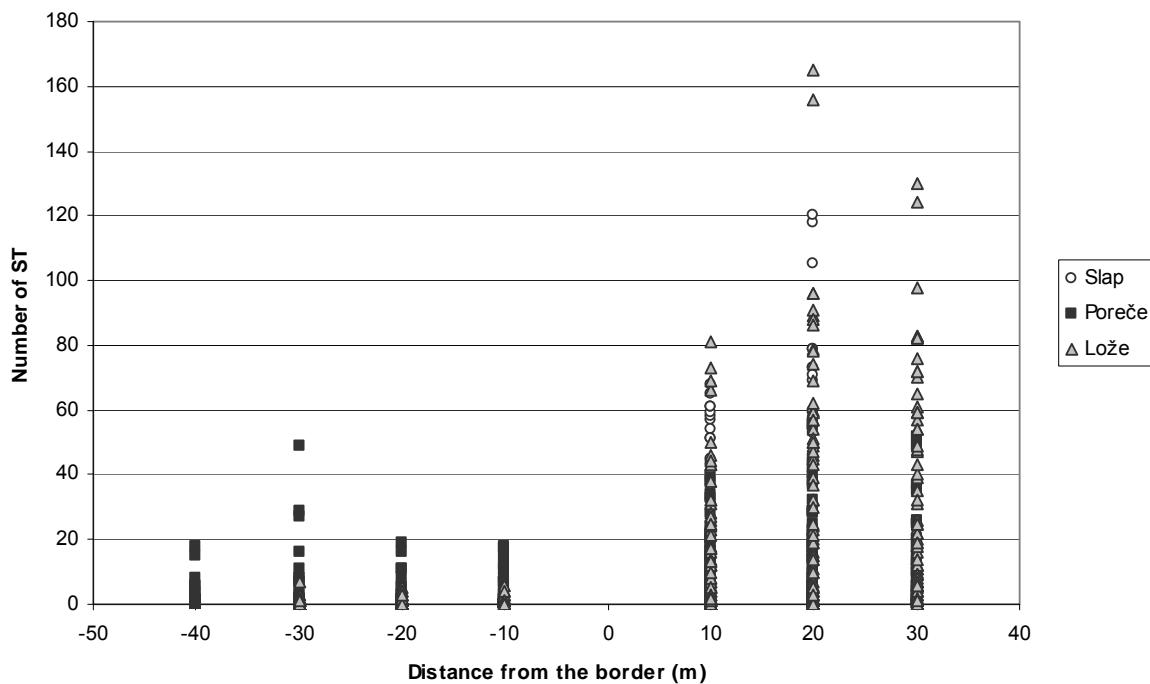


**Figure 4:** Number of captured adults of ST per trap and day in Lože nursery nr.2

Data gathered show a low number of ST captures in the nursery plot (64 adults in the nursery nr. 1 and 63 adults in the nursery nr. 2 in total; 0.1 adults / trap / day in average), in spite of a very high immigration potential from the vineyards in the nearest neighbourhood. It seems likely that in this case, insecticide treatments kept the ST population in the nursery at a relatively low level. Nevertheless, an additional treatment in mid-August would be necessary. Considerable differences in the population levels between vineyard No. 1 (1.6 adults / trap / day) and vineyard No. 2 (4.4

adults / trap / day) could be explained by irrigating technology that was applied in the vineyard No. 2.

Gradient distribution data revealed the same situation on the plot No. 1 as in other nurseries (the highest number of adults was captured 10 m and 20 m from the border). The gradient distribution for the nursery No. 2 shows just the opposite situation, where the highest number of adults was captured at the border line.



**Figure 5:** Scatterplot for the number of ST versus the distance of traps from the border

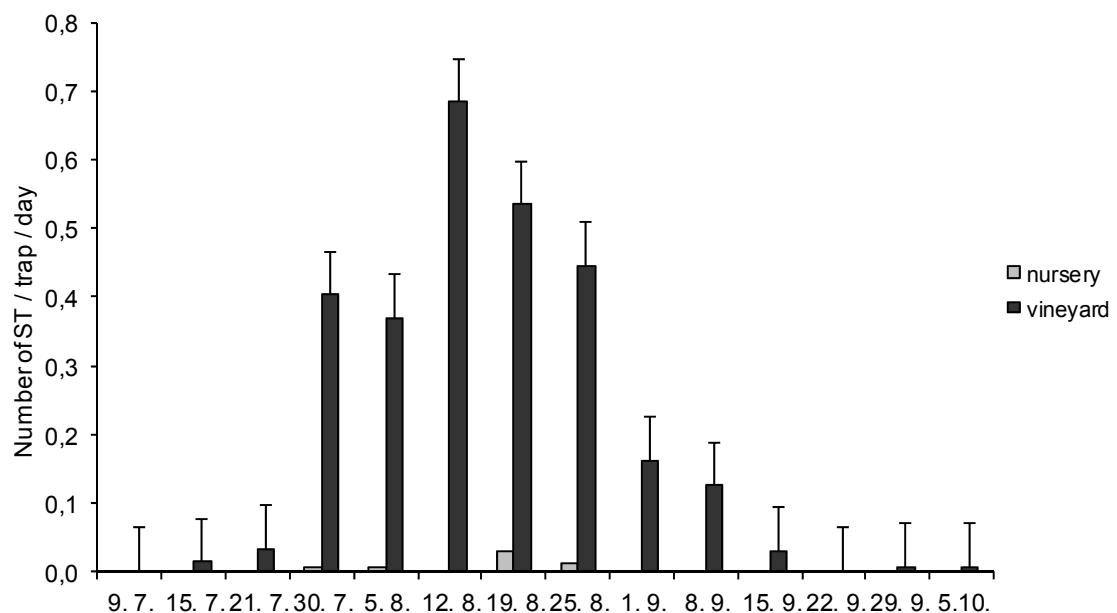
The number of ST catches dropped rigorously at traps posted 30 m from the border in vineyards towards the nursery. As it is shown in Figure 5, the highest number of ST was captured 30 m away from the nursery border.

### 3.2 Italian trials

In trials done by the Italian partner, no relevant data on mobility was obtained because only 1

ST male adult was captured 30 meters from the border of the vineyard. The situation was more rigorous at the location of Verona, where no catches were detected. The absence of ST catches in nurseries is the consequence of numerous insecticide treatments applied both in vineyards (up to four) and nurseries (up to seven).

### 3.3 Swiss trials



**Figure 6:** Number of captured adults of ST per trap and day in location Anieres

In Switzerland, two insecticide applications had an important impact on adults' catches in the nursery (98% less catches compared to the vineyard). However, a slight peak of catches could be observed in August, three weeks after the last insecticide application, suggesting immigration from the vineyard. In the nursery, catches on border traps facing the vineyard represented 25% of all captured adults and the highest number of adults was observed on the trap located 70 m from the border (37.5% of catches). In the vineyard, the numbers of captured adults on border traps facing the nursery were the lowest, while they were the highest 10 meters from the border (16% of all

catches). However, from the traps-distance-distribution point of view, adult catches did not show a significant gradient.

Sex-ratio of the captured adults was determined in the nursery in order to check the hypothesis if females were more mobile than males. Among the total of 8 catches in the nursery, only 1 adult was identified as a female.

A side differentiation of trap-catches was also done in the nursery to determine if the mobility of ST could be greater than in the vineyard. The results showed that only 2 adults were captured at the vineyard side.

## 4 DISCUSSION

Immigration risk of ST from nearby vineyards and the efficacy of insecticide treatments in nurseries were monitored. On two Slovenian locations, catches were fairly low in the nursery plots although high numbers of ST adults were caught in the adjacent untreated vineyards. On the third plot (Poreč), insecticide treatments were not sufficient to

avoid important catches in the nursery. This could be related to the sub-optimal timing of the first insecticide treatment and/or immigration of adults from two nearby untreated vineyards located at a distance of 70 and 150 m. Population rate in vineyards showed a small influence on the population of ST in nurseries. That could be explained by

the preference of ST to remain in vineyards, where leaf canopy provides for easier feeding and reproduction of the vector. Nevertheless, vineyards present the source of ST infection for nurseries.

Insecticide treatments reduced sufficiently the ST population in nurseries in almost all trials except on one Slovenian plot. This case suggests that additional insecticide treatments are necessary if the immigration pressure of ST from adjacent vineyards is high (Boudon-Padieu E., 2005). With four to seven insecticide applications in Italian trials, Italians managed to completely suppress the population of ST in nurseries and vineyards and eliminated the risk of vectors' immigration. Swiss trials showed that males seem to be more mobile than females and that they are trapped in higher numbers in nurseries. If this behaviour is confirmed, the risk of egg-laying on propagation material could be considered as very low.

The variance components analysis shows that the location of the nursery doesn't contribute to the variability of the ST number. About 28% of the variability came from different uses of the plot (nursery vs. vineyard) and 23% from the time of capture of ST. Only 2% of the variability came from the position of catches. Regarding the distance between the

vineyards and the nursery, we can conclude that the population of ST in nurseries doesn't depend on this factor but rather on the season and the use of the plot where the technology (insecticide treatment) is also included. Irrespective of the vineyard being 18 m or 126 m far from a nursery, it could be the source of infestation.

As all Slovenian experimental plots were subject to continuous strong wind we conclude that abiotic factors can also enhance the movement range of ST adults towards nurseries.

### Main conclusions

- Distance between the nursery and nearby vineyard lower than about 200 m didn't influence the mobility of ST from the vineyard to the nursery.
- Applied suppression technology against ST, particularly proper timing of insecticide treatments, affected the population level of ST in the nursery.
- The population level of ST adults in vineyards did not seem to have a direct effect on the mobility range.
- A decreasing flight gradient of ST from vineyards to nurseries could not be established at distances monitored in these trials.

### 5 ACKNOWLEDGEMENTS

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**Agrovoc descriptors:** harmonia axyridis, identification, biological control, biological control organisms, beneficial organisms, natural enemies, pest control, ecology, damage, urban areas, urban environment, hypersensitivity, allergens

**Agris category code:** h10

## Pisana polonica (*Harmonia axyridis* [Pallas], Coleoptera, Coccinellidae) - invazivna koristna vrsta

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### IZVLEČEK

V začetku novembra 2011 smo v okolici Ljubljane na zidovih posameznih stanovanjskih hiš opazili množičen pojav polonice. Nekaj primerkov smo izlovili, da bi določili vrsto. Na podlagi stereomikroskopskega pregleda in ob uporabi dostopne literature smo potrdili vrsto *Harmonia axyridis* (Pallas). Vrsta pri nas še nima slovenskega poimenovanja, zato v tem prispevku predlagamo uporabo imena pisana polonica, ki izhaja iz angleškega poimenovanja Harlequin ladybird. V prispevku je opisana bionomija vrste in njen pomen za Slovenijo. Pisana polonica je bila v Evropo vnesena z namenom uporabe v biotičnem varstvu rastlin. V nekaterih državah poročajo o škodljivem hranjenju odraslih osebkov pisane polonice z mehkimi plodovi nekaterih sadnih vrst, kot tudi grozdnimi jagodami. Vrsta *H. axyridis* postaja nadležna v urbanem prostoru. Nekateri tuji viri navajajo, da s svojimi izločki onesnažuje zidove in opremo, povzroča lahko tudi alergijske reakcije pri ljudeh.

**Ključne besede:** *Harmonia axyridis*, pisana polonica, bionomija, Slovenija, biotično varstvo

### ABSTRACT

#### MULTICOLOURED ASIAN LADY BEETLE (*Harmonia axyridis* [Pallas], Coleoptera, Coccinellidae) - invasive beneficial species

In the beginning of November 2011, massive occurrence of lady beetles was established on the walls of individual dwelling houses in the vicinity of Ljubljana. Some individuals were sampled for determination purposes. Based on stereomicroscopic survey and the use of accessible scientific literature we confirmed the coccinellid *Harmonia axyridis* (Pallas). Up to now this species did not have Slovenian name, therefore we suggest the name 'pisana polonica', which originated from English name Harlequin ladybird. In this paper the bionomics of the species and its importance in Slovenia are presented. Multicoloured Asian lady beetle was introduced to Europe for biological control purposes. In some countries the adults of this insect species cause damage on soft fruits and grape berries. In addition, *H. axyridis* also became inconvenient in urban areas. Some foreign sources mention that this coccinellid soil the walls of dwelling houses and equipment with their secretions, furthermore it can cause the allergic reactions of humans.

**Key words:** *Harmonia axyridis*, multicoloured Asian lady beetle, bionomics, Slovenia, biological control

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## 1 UVOD

Pisana polonica (*Harmonia axyridis* [Pallas], Coleoptera, Coccinellidae) izvira iz območja srednje in vzhodne Azije (Koch, 2003) in velja za izjemno učinkovitega plenilca listnih uši (Aphidina), bolšic (Psyllina) in nekaterih vrst kaparjev (Coccina), zato se v številnih državah uporablja kot naravni sovražnik za zatiranje vrst iz omenjenih podredov škodljivih žuželk (Ferran *et al.*, 1996). Vrsta je bila vnesena v Severno Ameriko (1916), Evropo (1982) in Južno Ameriko (2002) z namenom uporabe v biotičnem varstvu rastlin (Gordon, 1985; Koch, 2003). Kljub temu, da so se v preteklosti vnosi nekaterih tujerodnih vrst polonic (vrsta *Rodolia cardinalis* za zatiranje pomarančevega kaparja [*Icerya purchasi* Maskell]) izkazali kot učinkovita alternativna

metoda pri zatiranju škodljivih žuželčjih vrst, pa vrsta *H. axyridis* velja za invazivno vrsto, ki z domorodnimi plenilskimi vrstami tekmuje za hrano (Majerus *et al.*, 2006) in jih tako izpodriva iz domačega okolja (preglednica 1). V nekaterih državah tudi poročajo o škodljivem hranjenju odraslih osebkov pisane polonice z mehkimi plodovi nekaterih sadnih vrst, kot tudi z grozdnimi jagodami (Kovach, 2004). Vrsta *H. axyridis* je postala nadležna tudi v urbanem prostoru. Po več sto ali celo tisoč se jih prikrade na stavbe, kjer iščejo zimsko zatočišče. Nekateri tuji viri navajajo, da s svojimi izločki onesnažijo zidove in opremo, povzročijo pa tudi alergijske reakcije pri ljudeh (Kovach, 2004).

**Preglednica 1:** Vrste neciljnih in ciljnih organizmov, ki jih pleni pisana polonica (*Harmonia axyridis*) (Koch, 2003)

Neciljni organizmi	Ciljni organizmi
<i>Adalia bipunctata</i> L.	<i>Acyrthosiphon pisum</i> (Harris)
<i>Aphidoletes aphidimyza</i> Rondani	<i>Aphis gossypii</i> Glover
<i>Aphidius ervi</i> Haldyday	<i>Aphis glycines</i> Matsumura
<i>Brachiacantha ursina</i> (Fabricius)	<i>Aphis nasturtii</i> Kaltenbach
<i>Coccinella septempunctata</i> (L.)	<i>Aphis spiraecola</i> Patch
<i>Coccinella transversoguttata</i> Brown	<i>Diaphorina citri</i> Kuwayama
<i>Coccinella undecimpunctata</i> L.	<i>Diaprepes abbreviatus</i> (L.)
<i>Coleomegilla maculata</i> DeGeer	<i>Macrosiphum euphorbiae</i> Thomas
<i>Chrysoperla carnea</i> Stephens	<i>Macrosiphum rosae</i> (L.)
<i>Cycloneda munda</i> (Say)	<i>Matsucoccus resinosae</i> Bean and Godwin
<i>Cycloneda sanguinea</i> L.	<i>Monellia caryella</i> (Fitch)
<i>Hippodamia convergens</i> Guérin-Méneville	<i>Monelliopsis pecanis</i> Bissel
<i>Tamarixia radiata</i> (Waterston)	<i>Myzus persicae</i> Sulzer
	<i>Panonychus citri</i> (McGregor)
	<i>Paraproctiphilus tessellatus</i> (Fitch)
	<i>Phorodon humili</i> (Shrank)
	<i>Rhopalosiphum maidis</i> (Fitch)

## 2 TAKSONOMIJA

Vrsta *H. axyridis* je trenutno uvrščena v družino Coccinellidae (Kovar, 1996), njena taksonomska umestitev pa se je v preteklosti spremenjala. Leta 1773 jo je Pallas poimenoval *Coccinella axyridis*. Njeno latinsko ime se je v naslednjih desetletjih večkrat spremenilo: *Coccinella bisex-notata* Herbst 1793, *Coccinella 19-sinata* Faldermann 1835, *Coccinella conspicua* Faldermann 1835, *Coccinella aulica* Faldermann 1835, *Harmonia spectabilis* Faldermann 1835, *Coccinella succinea* Hop 1845, *Anatis circe*

Mulsant 1850 in *Ptychantis yedoensis* Takizawa 1917 (Koch, 2003). Leta 1885 je Weise predlagal umestitev vrste v rod *Harmonia*. Ameriško entomološko društvo (Entomological Society of America) je vrsto *H. axyridis* poimenovalo z izrazom večbarvna azijska polonica (angl. multicoloured Asian lady beetle). Ker slovenskega poimenovanja omenjena vrsta še nima, predlagamo domače ime 'pisana polonica', ki izhaja iz poslovenjenega izraza »harlekinska« (angl. harlequin = pisan) (Milevoj, 2011a).

## 3 OPIS IN BIONOMIJA VRSTE

Hrošček pisane polonice meri od 5 do 8 mm (Koch, 2003). Barvno so imagi zelo raznoliki. Pokrovke imajo svetlo rumenooranžne do črne (Koch, 2003), na katerih je od 0 do 19 (po drugih podatkih prek 20) pik, ki so navadno črne, lahko pa tudi rdeče (Korschefsky, 1932) (slika 1). Predel za glavo (pronotum) je rumen s črnimi madeži v sredini. Ti madeži se lahko pojavljajo kot štiri črne pike, dve vijugasti črti, črni madež v obliki črke M ali madež v obliki trapezoida. Lateralni del zaglavja je rumen z ovalno piko (Chapin in Brou, 1991).

Samica dnevno odloži približno 25 jajčec, ki so ovalna in rumena ter dolga okoli 1,2 mm (El-Sebaey in El-Gantiry, 1999). V celotnem življenjskem obdobju lahko samica odloži tudi do 4000 jajčec. Dan pred izleganjem ličink jajčeca postanejo sivočrna. Ličinke se trikrat levijo in merijo od 1,9 ( $L_1$ ) do 10,7 mm ( $L_4$ ) (Sasaji, 1977). Različne razvojne stopnje ličink se med seboj ločijo tako po velikosti kot tudi obarvanosti. Ličinke prve razvojne stopnje ( $L_1$ ) so temno obarvane in imajo rdečo piko na šestem abdominalnem segmentu (El-Sebaey in El-Gantiry, 1999). Druga larvalna stopnja ( $L_2$ ) se po barvi le malo razlikuje od prve, razlike so vidne le v obarvanosti prvega in drugega abdominalnega segmenta, ki sta na dorzalno-lateralni strani oranžno obarvana (Sasaji,

1977). Dorzalno-lateralna stran prvega, drugega in petega abdominalnega segmenta je pri tretjestopenjskih ličinkah ( $L_3$ ) obarvana oranžno (Sasaji, 1977). Ličinka četrte razvojne stopnje ( $L_4$ ) ima četrti in peti abdominalni segment v celoti oranžen. Ličinke vseh razvojnih stopenj imajo po telesu trnaste izrastke. Buba je oranžna. Iz bube se izleže imago, ki je še brez pik. Celotni razvoj lahko traja od enega do treh mesecev, nekateri avtorji pa navajajo, da je lahko v manj ugodnih razmerah življenjski krog zaključen šele po enem do treh let (Koch, 2003).

Barvni polimofizem vrste *H. axyridis* je najverjetneje rezultat serije multiplih alelov (Honek, 1996). Nekateri drugi avtorji navajajo, da je možen vzrok različne obarvanosti v kakovostni in količinski sestavi hrane ličink (Grill in Moore, 1998) in v temperaturah, katerim so izpostavljene bube. Več kot 40-letno preučevanje pisane polonice je pokazalo, da na barvni polimorfizem vrste vpliva čas (sezona) (Osawa in Nishida, 1992) kot tudi kraj pojavljanja. Temne forme odraslih osebkov, ki so zelo pogoste na območju Azije (Dobzhansky, 1933), so na območju Severne Amerike zelo redke (LaMana in Miller, 1996).

Pisana polonica ima popoln holometabolni razvojni krog. LaMana in Miller (1998) navajata, da pri  $26^{\circ}\text{C}$  in pri hrانjenju samic z grahovo ušjo (*Acyrthosiphon pisum* [Harris]) traja razvoj jajčeca 2,8 dni, razvoj prvostopenjskih ličink 2,5 dni, razvoj drugostopenjskih ličink 1,5 dni, razvoj tretjestopenjskih ličink 1,8 dni, medtem ko traja pri omenjenih razmerah razvoj četrstopenjskih ličink 4,4 dni. Razvoj bube traja 4,5 dni. Odrasli osebki navadno živijo od 30 do 90 dni, odvisno od temperature (El-

Sebaey in El-Gantiry, 1999). Rezultati raziskav v Evropi (Francija) so pokazali, da pri temperaturnem pragu  $10,5^{\circ}\text{C}$  pisana polonica potrebuje vsoto efektivnih temperatur  $231,3^{\circ}\text{C}$  za razvoj od jajčeca do imaga (Schanderl in sod., 1985). Pisana polonica je bivoltina vrsta (Koch, 2003). Detzel (2008) navaja, da pri vrsti ni znana obligatna diapavza in da se umakne prezimovat šele ob nastopu nizkih jesenskih temperatur. Slednje smo tudi mi ugotovili.





Slika 1: Odrasli osebki pisane polonice (*H. axyridis*) (foto: J. Rupnik)

Kanibalizem ima velik pomen pri populacijski dinamiki vrste *H. axyridis* (Osawa, 1993). Dokazano je bilo, da sta množični pojav listnih uši in kanibalizem obratno sorazmerna (Hironori in Katsuhiro, 1997). Najbolj množičen je pojav jajčnega kanibalizma (50 %), medtem ko do pojava kanibalizma med ličinkami pride predvsem zaradi morebitnega pomanjkanja plena (Wagner in sod., 1999). Dokazano je bilo, da je pojav jajčnega kanibalizma bolj pogost, če jajčeca pisane polonice niso odložena v bližino kolonije listnih uši. Hironiri in Katsuhiro (1997) v svoji raziskavi zaključujeta, da se stopnja prezivetja

pisane polonice od jajčeca do imaga giblje v intervalu med 0 in 16 %. Poleg kanibalizma ima pomembno vlogo pri preživetju pisane polonice tudi bakterija iz rodu *Spiroplasma* (Majerus in sod., 1999), ki okužuje predvsem samce. Okuženi samci hitreje poginejo in tako ne pride do uspešne ploditve s samicami. Med naravnimi sovražniki pisane polonice velja omeniti dve parazitoidni vrsti: *Phalacrotophora* sp. in *Strongygaster triangulifera* (Loew) (Koch, 2003). Pisana polonica predstavlja plen tudi nekaterim vrstam ptic (Koch, 2003).

#### 4 ISKANJE PLENA IN NJEGOVO PLENJENJE

Pisana polonica ima izjemno visoko sposobnost sledenja listnih uši, tako v času kot v prostoru (Osawa, 2000). Evans (2003) je v svoji raziskavi preučeval načine iskanja plena pri različnih vrstah polonic iz družine Coccinellidae. Vrsta *H. axyridis* doseže vrh ovipozicije navadno pred vrhom vivipozicije listnih uši ali hkrati z njihovo vivipozicijo (Osawa, 2000; Evans, 2003). Če imajo polonice vrh ovipozicije v času, ko se kolonije listnih uši zmanjšujejo, lahko pride do

stradanja ličink polonic. Yasuda s sod. (2000) so ugotovili, da samice vrste *H. axyridis* s posebnimi receptorji zaznajo semiokemikalije, ki jih izločajo listne uši, na ta način pa ocenijo starost kolonije in se na podlagi dražljajev odločijo ali je kolonija primerna, da vanjo ali njeno bližino odložijo jajčeca. Iskanje plena sprva poteka ekstenzivno (zaznavanje kemičnih dražljajev), ko je plen zaznan, pa pisana polonica preide v stadij intenzivnega premikanja (Kawai, 1976) s plezanjem po

rastlini predvsem na račun vizualnih dražljajev iz okolice. Harmon s sod. (1998) so ugotovili, da je plenjenje učinkovitejše podnevi, ko je vizualno zaznavanje dražljajev iz okolice boljše.

Pisana polonica pleni vrste iz družin Aphididae (Hodek, 1996), Tetranychidae (Lucas s sod., 1997), Psyllidae (Michaud, 2001), Coccidae (McClure, 1986), mlajše razvojne stadije hroščev iz družin Chrysomelidae (Koch, 2003) ter Curculionidae (Stuart *et al.*, 2002) in predstavnike iz reda

Lepidoptera (Koch *et al.*, 2003). Vrsta *H. axyridis* se hrani tudi s cvetnim prahom in nektarjem (Lamana in Miller, 1996), lotila se je tudi glukoze, ki smo ji ga v prezimovališču ponudili. Za nekatere organizme (*Arion* sp., *Epilachna varivestis* Mulsant) je bilo ugotovljeno, da izločajo alkaloide, ki delujejo antifidno na pisano polonico. V povprečju ličinka pisane polonice med razvojem zaužije od 90 do 370 listnih uši, odrasel osebek pa dnevno zaužije od 15 do 65 listnih uši (Lucas s sod., 1997).

## 5 PRISTOPI BIOTIČNEGA VARSTVA RASTLIN IN PISANA POLONICA

Klasično biotično varstvo se izvaja na prostem. Gre za načrten vnos tujerodne koristne vrste za zatiranje tujerodnega škodljivega organizma, ki se je razširil od drugod in v novem okolju nima učinkovitih naravnih sovražnikov (Milevoj, 2011b). Pisana polonica je bila z namenom klasičnega biotičnega varstva vnesena v Severno Ameriko, predvsem za zatiranje nekaterih škodljivcev na citrusih, tobaku in soji (Koch, 2003). V Evropi poročajo o načrtnem vnosu pisane polonice v Ukrajino (1964), Francijo (1982), na Azore (1993), v Grčijo (1993), Veliko Britanijo (2003) in Nemčijo (2003) (Koch, 2003) predvsem z namenom zatiranja škodljivcev na citrusih. V Evropi od leta 1995 številna podjetja v zahodni Evropi tržijo vrsto *H. axyridis* z namenom biotičnega varstva rastlin, ki je z omenjeno vrsto trenutno dovoljeno v 13 evropskih državah (Brown s sod., 2008). Brown s sod. (2008) poročajo o zastopanosti pisane polonice v 26 evropskih državah. Avtorji omenjenega članka v zaključku navajajo, da je vrsta najverjetnejše zastopana tudi na Poljskem, Madžarskem, Slovaškem in Sloveniji, vendar dotlej v omenjenih državah še ni bila potrjena.

Preplavno biotično varstvo se izvaja v prostorih ločenih od narave. Vključuje vnos

domorodne ali tujerodne koristne vrste, laboratorijsko namnožene v večjem številu, da preplavijo rastline in ciljni prostor, zaradi hitrega zmanjševanja populacije škodljivega organizma (Milevoj, 2011). Za omenjeni pristop biotičnega varstva je bila pisana polonica vnesena v Aziji (Seo in Youn, 2000), Evropi (Trouve s sod., 1997) in Severni Ameriki (LaRock in Ellington, 1996). Liu in Qin (1989) poročata, da je pisana polonica ustrezен kandidat za preplavno biotično varstvo pri zatiranju blede jagodove listne uši (*Chaetosiphon fragaefolii* [Cockerell]) in rožne uši (*Macrosiphum rosae* [L.]), vendar pa vnos vrste *H. axyridis* ni primeren za zatiranje bombaževčeve uši (*Aphis gossypii* Glover) ter hmeljeve uši (*Phorodon humuli* [Schrank]).

Varovalno biotično varstvo je usmerjeno v varovanje domorodnih koristnih organizmov in v vzpodbujanje njihovega razmnoževanja in naselitve, z uporabo okolju prijazne agrotehnike in fitofarmacevtskih sredstev (FFS), s setvijo vmesnih posevkov ali privabilnih rastlin, ki vzpodbujajo razmnoževanje koristnih vrst (Milevoj, 2011b). Nekatere predhodne raziskave so pokazale, da so sintetični piretroidi manj škodljivi za pisano polonico kot za listne uši (Cho s sod., 1997; Wells s sod., 2001).

Michaud (2002) poroča, da aktivne snovi spinosad, indoksakarb ter piriproksifen ne vplivajo na pisano polonico, podobno kot tudi ne azadirahitin ter entomopatogena gliva *Beauveria bassiana* (Bals.- Criv.) Vuill. (Smith in Krischik, 2000). Aktivna snov

karbaril se je v sorodni raziskavi izkazala kot zelo toksična za vrsto *H. axyridis* (Smith in Krischik, 2000). Rezultati raziskav so pokazali, da občutljivost pisane polonice na insekticide varira predvsem na račun razvojnega stadija vrste (Koch, 2003).

## 6 POMEN ZA SLOVENIJO

Med zakonskimi podlagami za uporabo koristnih organizmov v biotičnem varstvu morajo izvajalci biotičnih varstvenih ukrepov upoštevati 51., 52., in 53. člen Zakona o zdravstvenem varstvu rastlin (Uradni list RD, št. 62/07 – Uradno prečiščeno besedilo in 36/10) in Pravilnik o biotičnem varstvu rastlin (Uradni list RS, št. 45/06), ki določa postopke ravnanja/ uporabe organizmov za biotično zatiranje (Milevoj, 2011b). Pravilnik obravnava pogoje za vnos, uporabo in gojenje koristnih organizmov, pogoje glede prostorov, opreme in kadrov, ki jih mora izpolnjevati tisti, ki želi koristne organizme gojiti in pogoje glede trženja koristnih vrst. Pravilniku sta dodana tudi seznama domorodnih in tujerodnih koristnih organizmov, katerih vnos in uporaba so dovoljeni na območju Slovenije. Svetovati, oglaševati in priporočati se sme le organizme, ki so navedeni v obeh seznamih (Milevoj, 2011b).

V začetku novembra 2011 smo v okolici Ljubljane (Občina Škofljica; 45°59'15,35"N, 14°34'20,95"E, okrog 300 m nad morjem) na zidovih posameznih stanovanjskih hiš opazili množičen pojav polonic, pred katerimi so nekateri trdno zapirali okna in vrata. Nekaj primerkov smo izlovili, da bi določili vrsto. Na podlagi stereomikroskopskega pregleda in ob uporabi dostopne literature smo potrdili vrsto *H. axyridis* tudi pri nas. Vrsta je pri nas še slabo znana, čeprav so jo v minulih letih

ponekod v Sloveniji že zasledili (Milevoj, 2011 b).

Seznam domorodnih koristnih organizmov trenutno vključuje 24 vrst, medtem ko je na Seznamu tujerodnih koristnih organizmov 71 vrst. Vrste *H. axyridis* ne najdemo na nobenem od obeh seznamov, zato njena uporaba za namene biotičnega varstva v Sloveniji ni dovoljena. Med domorodnimi koristnimi žuželkami je več takšnih vrst, s katerimi se lahko hrani tudi pisana polonica: dvopika polonica (*Adalia bipunctata* L.), sedempikčasta polonica (*Coccinella septempunctata* [L.], navadna tenčičarica (*Chrysoperla carnea* Stephens), parazitoidna osica *Aphidius ervi* Haliday in plenilska hržica *Aphidolethes aphidimyza* Rondani) (preglednica 1), zato bi bil njen namenski vnos v naše okolje vprašljiv. Omenjene koristne vrste žuželk se tudi v tujini uporabljajo v programih biotičnega varstva rastlin. V dosedanjih domačih raziskavah so spremljali prehranjevanje treh pri nas najbolj razširjenih vrst polonic: *C. septempunctata*, *A. bipunctata* in *Propylea quatuordecimpunctata* (Milevoj, 2001). Rezultati so pokazali (preglednica 2), da se hranijo s podobnimi vrstami listnih uši kot pisana polonica. Z vidika varovanja domorodnih vrst namenski vnos pisane polonice v naše okolje tudi v prihodnosti ni potreben. Zavedati se moramo, da je tu in, da jo je treba načrtno spremljati, zaradi njene morebitne ustalitve in vpliva na okolje.

**Preglednica 2:** Prehranjevalne zveze med polonicami in listnimi ušmi v Sloveniji (Milevoj, 2001)

Plenilska vrsta	Živali gostitelji
<i>Coccinella septempunctata</i>	<i>Aphis fabae</i> Scopoli
	<i>Aphis pomi</i> Degeer
	<i>Acyrthosiphon pisum</i> (Harris)
	<i>Hyalopterus pruni</i> (Geoffroy)
	<i>Macrosiphum rosae</i> (L.)
	<i>Myzus persicae</i> (Sulzer)
	<i>Rhopalosiphum padi</i> (L.)
	<i>Sitobion avenae</i> (Fabricius)
<i>Adalia bipunctata</i>	<i>Aphis gossypii</i> Glover
	<i>Aphis pomi</i> Degeer
	<i>Acyrthosiphon pisum</i> (Harris)
	<i>Dysaphis devecta</i> (Walker)
	<i>Hyalopterus pruni</i> (Geoffroy)
	<i>Myzus cerasi</i> Fabricius
<i>Propylaea quatuordecimpunctata</i>	<i>Myzus persicae</i> (Sulzer)
	<i>Acyrthosiphon pisum</i> (Harris)
	<i>Rhopalosiphum padi</i> (L.)
	<i>Rhopalosiphum</i> spp.

**7 ZAKLJUČEK**

V številnih državah, v katere je bila vrsta *H. axyridis* v preteklosti vnesena z namenom biotičnega varstva rastlin in je danes zastopana v njihovem naravnem okolju, se sprašujejo, ali je bil njen vnos v naravno okolje gospodarsko upravičen? V Slovenijo je omenjena vrsta polonice prišla nenadzorovano in bi lahko v prihodnosti povzročila populacijski upad nekaterih domačih vrst koristnih organizmov, ki jih v Sloveniji lahko uporabljamo za namene biotičnega varstva rastlin. Nekatere države so izdelale ocene tveganja (Lonsdale s sod., 2001), s katerimi želijo lažje določiti pomen nekaterih, sicer v osnovi koristnih, tujerodnih organizmov za okolje, ki pa imajo

lahko tudi neželeno neciljno delovanje na koristne domorodne organizme. V Sloveniji je s Pravilnikom o biotičnem varstvu rastlin zakonsko urejeno vnašanje tujerodnih organizmov v naravno okolje, vendar pa pri napakah, ki so bile v drugih državah storjene v preteklosti (vnos polonice v Evropo datira v 80' leta prejšnjega stoletja), njene aktivnosti na tem področju žal nimajo vpliva. Narava ne pozna meja, zato ima vnos tujerodnega organizma v državo, kjer so zakonski akti na področju vnosa živalskih ali rastlinskih organizmov manj natančno določeni kot pri nas, lahko tudi poznejši dolgoročen vpliv za širše območje.

**8 ZAHVALA**

Prispevek je nastal s finančno pomočjo Ministrstva za kmetijstvo in okolje –

Fitosanitarne uprave RS v okviru strokovnih nalog s področja zdravstvenega varstva rastlin.

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**Agrovoc descriptors:** drought, monitoring, data collection, models, water balance, plant water relations, precipitation deficit, zea mays, cereal crops, maize, maize, soil water retention, water requirements, temperature, thermal stress, climatic change, irrigation, vegetative period, weather data, weather hazards

**Agris category code:** p10, f06

## Tools for agricultural drought detection in the frame of Drought Management Centre for Southeastern Europe – DMCSEE

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### ABSTRACT

Numerous specialized indices have been proposed to assess drought severity, but the precise quantification of drought is a difficult challenge. The purpose of this paper is to evaluate two indices: the well known Standardized Precipitation Index (SPI) and the Net Irrigation Requirements (NIR) derived by WinISAREG model. WinISAREG water balance model is tested in the frame of working activities of Drought Management Centre for Southeastern Europe (DMCSEE). The drought assessment for areas with different climatic characteristics in Slovenia is achieved using both indices for maize crop in the period from 1961 to 2010. A simple water balance was used for the comparison with the both indices. Results of the indices were compared with information about drought available in the agrometeorological reports of the Environmental Agency of the Republic of Slovenia for analysed time period. For the comparison among indices qualitative analysis for the vegetation season (April–September) was performed. For this reason 1-month and 6-month Standardized Precipitation Index were used. Soil type with low soil water holding capacity was chosen for the study. Seasonal NIR generally responds in a similar fashion to the 6-month Standardized Precipitation Index. With both indices extremely dry periods were validated by agrometeorological reports. There are some years with drought which are only confirmed by NIR and not confirmed by SPI6. SPI6 performs relatively well as indicator of long-term meteorological droughts caused by the lack of precipitation while NIR identifies also long-term agricultural droughts provoked by high evapotranspiration triggered by temperature stress. Study showed that at all locations NIR in the last ten years (2001–2010) increased on soils with low water holding capacity. NIR could be proposed as indicator for agricultural drought detection in the frame of DMCSEE.

**Key words:** drought, crop water balance, WinISAREG model, Standardized Precipitation Index, Net Irrigation Requirements, climate variability

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### IZVLEČEK

#### ORODJA ZA UGOTAVLJANJE KMETIJSKE SUŠE V OKVIRU CENTRA ZA UPRAVLJANJE S SUŠO V JV EVROPI – DMCSEE

Intenzivnost suše je, kljub številnim specifičnim kazalcem, zelo težko natančno vrednotiti. Namen članka je ovrednotenje dveh indeksov: že dobro poznanega Standardiziranega padavinskega indeksa (SPI) in Neto namakalne potrebe (NIR), izračunane s programom WinISAREG. Vodno bilančni model WinISAREG je preizkušen v okviru delovnih aktivnosti Centra za upravljanje s sušo v jugovzhodni Evropi - DMCSEE. V študijo so bile vključene lokacije po Sloveniji, z različnimi podnebnimi značilnostmi, za primer koruze, v obdobju 1961–2010. Za primerjavo obeh indeksov je bila uporabljena enostavna vodna bilanca.

Vrednosti indeksov za analizirano obdobje so bile primerjane z arhivskimi podatki o sušah, dostopnih v agrometeoroloških zapisih na Agenciji Republike Slovenije za okolje. Analizirana so bila vegetacijska obdobja, ki trajajo od aprila do septembra, zato sta bila uporabljeni 1-mesečni in 6-mesečni SPI. V študiji so bila uporabljena tla s slabo zadrževalno kapaciteto za vodo. Sezonski NIR (obdobje rasti rastline) v splošnem odgovarja 6-mesečnem Standardiziranem padavinskem indeksu (SPI6).

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Agrometeorološki zapisi so potrdili ekstremno sušna leta glede na oba indeksa. Povajili pa so tudi primeri, ko so bila nekatera sušna leta potrjena z NIR-om, z SPI6 pa ne in obratno. Oba indeksa dobro zaznata dolgotrajne suše, SPI6 meteorološke suše na račun pomanjkanja padavin, NIR pa upošteva tudi visoko izhlapevanje povzročeno s temperaturnim stresom. Študija je pokazala, da se trend vrednosti NIR-a na vseh izbranih lokacijah v zadnjem desetletju (2001-2010) povečuje na tleh s slabo zadrževalno kapaciteto za vodo.

V okviru DMCSEE NIR lahko predlagamo kot kazalec določanja kmetijske suše.

**Ključne besede:** suša, vodna bilanca rastlin, WinISAREG model, Standardizirani padavinski indeks, Neto namakalne potrebe, podnebna spremenljivost

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## 1 INTRODUCTION

Drought is at least occasionally a normal part of climate in virtually all regions of the world. Southeastern Europe is no exception; in past decades the drought-related damages have had large impact on the economy and welfare.

The climate of a region or a country is determined by long-term average, frequency and extremes of several weather parameters, notably precipitation and temperature. Drought, in particular, is considered by many to be the most complex but least understood of all natural hazards. The American Meteorological Society (1997) groups drought definitions and types into four categories: meteorological or climatological, agricultural, hydrological, and socioeconomic. A prolonged (e.g., of several months or years duration) *meteorological drought* – the atmospheric conditions resulting in the absence or reduction of precipitation – can develop quickly and end abruptly (in some cases, the transition can occur almost literally overnight). Short-term (i.e., a few weeks duration) dryness in the surface layers (root zone), which occurs at a critical time during the growing season, can result in an *agricultural drought* that severely reduces crop yields, even though deeper soil levels may be saturated. Hot temperatures, low relative humidity, and desiccating winds often add to the impact of the lack of rainfall. The onset of an agricultural drought may lag that of a meteorological drought, depending on the prior moisture status of the surface soil layers.

Precipitation deficits over a prolonged period that affect surface or subsurface water supply, thus reducing streamflow, groundwater, reservoir, and lake levels, will result in a *hydrological drought*, which will persist long after a meteorological drought has ended. *Socioeconomic drought* associates the supply and demand of some economic good with elements of meteorological, agricultural, and hydrological drought. (Heim, Jr., 2002)

In the last century, many drought indices were formulated by integrating weather variables such as rainfall, evapotranspiration and temperature into a single number. Drought indices, in general, enable the detection of the onset of drought events and the measurement of their severity, thereby allowing an examination of the spatial and temporal characteristics of drought, and comparison between different regions. An important feature of drought is timescale, which can vary substantially. A single month of deficient rainfall can adversely affect rainfed crops while having small or no impact on large reservoirs.

Drought indices are important elements of drought monitoring while they interrelate among many climate and climate – related parameters. Attempts to coordinate and facilitate the development, assessment, and application of drought monitoring tools is also important task of Drought Management Centre

for Southeastern Europe (DMCSEE) which was established in Slovenia in 2006.

At present, the most commonly used drought index in the frame of DMCSEE is the Standardized Precipitation Index (SPI) (McKee et al., 1993; 1995) which takes into account the role of antecedent conditions in quantifying drought severity. It is based on the consideration that each component of a water resources system reacts to a deficit in

precipitation over different time scales. Beside SPI, simple water balance (WB) and Net Irrigation Requirement (NIR) derived by WinISAREG model were analysed for Slovenia. Given the high potential of the SPI to detect drought periods in southeastern Europe, the present study was focused on investigating the applicability of three indicators of agricultural drought in four agricultural regions in Slovenia in the period 1961-2010.

## 2 MATERIALS AND METHODS

### 2.1 Site descriptions

In Slovenia three climatic belts are met: continental climate in the northeast, southeast

and partly in central Slovenia, alpine climate in the mountainous region in the north and sub-Mediterranean climate in the southwest.



**Figure 1:** Site locations  
**Slika 1:** Lege postaj

This study was conducted in main agricultural areas in Slovenia: Ljubljana, Murska Sobota and Novo mesto are situated in central, northeast and southeast Slovenia (respectively) and Bilje is situated in southwest (Figure 1).

The average annual (vegetation season) air temperature in the period 1971–2000 ranges from 12 °C (17.6 °C) at Bilje to around 10 °C (16 °C) at the other three stations. The lowest

monthly average air temperature is in January and the highest in July. Annual (vegetation season) precipitation amounts are the lowest at the east and increasing towards the west. The average precipitation ranges from 805 mm (Murska Sobota) to 1446 mm (Bilje) for annual and from 502 mm (Murska Sobota) to 754 mm (Bilje) for vegetative season. The calculated evapotranspiration (ETP) sums in the seasons are less variable: from 600 mm (Murska Sobota) to 640 mm (Bilje), with increasing trend in recent years.

**Table 1:** Climatic characteristics of selected sites (SEA/ARSO, 2010)**Tabela 1:** Podnebne karakteristike izbranih postaj (SEA/ARSO, 2010)

1971–2000		Average air temperature (°C)	Precipitation (mm)	ETP (mm)	Sunshine duration (h)	Number of days with ETP > 5mm
<b>Ljubljana</b> 46°4' N 14°31' E 299 m. a. s. l.	annual	10.2	1368	762	1798	22.3
	vegetation season	16.5	752	620	1291	
	maximum monthly	23.9	147	154	267	
	minimum monthly	-2.7	71	6	45	
<b>Murska Sobota</b> 46°39' N 16°12' E 188 m. a. s. l.	annual	9.6	805	741	1913	14.6
	vegetation season	16.1	502	599	1343	
	maximum monthly	19.7	104	148	265	
	minimum monthly	-1.2	31	4	57	
<b>Novo mesto</b> 45°48' N 15°11' E 220 m. a. s. l.	annual	9.9	1147	764	1890	20.0
	vegetation season	16.1	663	612	1316	
	maximum monthly	19.9	123	152	272	
	minimum monthly	-0.2	49	5	66	
<b>Bilje</b> 45°54' N 13°38' E 55 m. a. s. l.	annual	12.0	1446	842	2102	24.3
	vegetation season	17.6	754	642	1348	
	maximum monthly	21.7	164	174	279	
	minimum monthly	3.0	74	2	96	

## 2.2 Input data

### 2.2.1. Meteorological data

Daily meteorological data (precipitation, wind velocity, minimum relative humidity and potential ETP calculated by Penman-Monteith equation) for the period 1961–2010 for four climatological stations were retrieved from the archives of the Slovenian National Meteorological Service at Slovenian Environment Agency (SEA/ARSO).

### 2.2.2. Soil data

Soil type with low water holding capacity (LWHC) was chosen for the study. The water holding capacity of each layer is represented by field capacity (FC) and wilting point (WP). Detailed description of soil characteristics is available in Table 2. This soil type is typical for agricultural areas on alluvial plains of north-east Slovenia where frequent damage due to agricultural drought is reported.

**Table 2:** Soil characteristics (SEA/ARSO/AWS, 2010)**Tabela 2:** Lastnosti tal (SEA/ARSO/AWS, 2010)

Soil layers	Top depth (m)	Bottom depth (m)	Layer thickness (m)	FC (%)	WP (%)	Soil texture of superficial layer
1	0.00	0.10	0.10	0.30	0.16	Depth (m)
2	0.10	0.37	0.27	0.22	0.12	Sand (%)
3	0.37	0.60	0.23	0.31	0.16	Clay (%)

### 2.2.3 Crop data

In general vegetation season in Slovenia begins on the first of April and lasts till the end of September. In the study maize crop was analysed. Phenological data for maize (*Zea mays*) hybrid ‘Cisco’, data of rooting zone layers were derived by experimental data (SEA/ARSO/AWS, 2010). Crop coefficient ( $K_c$ ) is dimensionless coefficient used to calculate ETP requirement for a particular crop from the potential ETP for a reference crop (ET0). Crop

coefficients are determined experimentally and take into account leaf area development of the crop and the crop canopy physiology (Australian... 2001). Due to lack of experimental data, data from literature were used (Doorenbos and Kassam, 1986). The phenological development for maize is described by 6 phenological stages. All used data are described in Table 3. Soil depletion fraction for no stress (p) was set to 0.4.

**Table 3:** Maize phenological development rooting depths and crop coefficients (SEA/ARSO/AWS, 2010; Doorenbos and Kassam, 1986)

**Tabela 3:** Fenološki razvoj globin korenin in koeficienti rastlin za koruzo (SEA/ARSO/AWS, 2010; Doorenbos and Kassam, 1986)

Stages	Date	Root depth (m)	p		Date	$K_c$
<b>A – sowing</b>	20.4.	0.01	0.4		30.4.	0.20
<b>B – third leaf</b>	7.5.	0.20	0.4		7.5.	0.50
<b>C – tasseling</b>	9.7.	0.50	0.4		11.5.	0.90
<b>D – milky ripe</b>	16.8.	0.50	0.4		28.6.	1.10
<b>E – fully ripe</b>	8.10.	0.50	0.4		9.7.	1.20
<b>F – harvest</b>	14.10.	0.50	0.4		16.8.	0.60
					20.9.	0.50

## 2.3 Methods

### 2.3.1 Water balance

Simple surface water balance accumulation (denoted as WB and calculated as difference between precipitation and ETP accumulation) is used as one of drought indicators in this paper. 50 vegetation seasons are available in the database records. In the first part of investigation the 30-day WB was used in comparison to SPI1, following by analysis of 10-day WB and NIR in the second part.

### 2.3.2 Standardized precipitation index

The Standardized Precipitation Index makes it possible to quantify the precipitation anomaly

with respect to long time average condition (1971-2000) for a specific month and time scale. The long term precipitation record is fit to a probability distribution, which is then normalised so that the mean (average) SPI for any place and time period is zero. Positive SPI values indicate greater than median precipitation and negative values mean less than median precipitation. Drought is defined when SPI reaches value of -1 or less, while value of 1 or more defines wet spell. Value also classifies severity of drought or wetness (Table 4).

SPI is typical meteorological index, the short time scale (1-month; SPI1) and medium-term time scale (6-month; SPI6) have been chosen for the analysis. SPI1, which includes 30 days

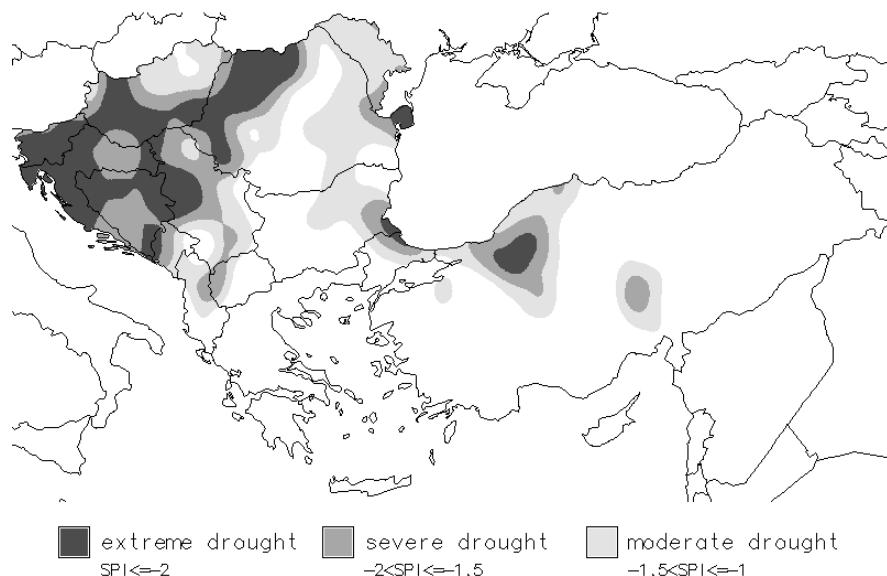
antecedent precipitation, was calculated for each decade in the vegetation seasons. SPI1 is suitable for detecting the presence, in drought event dynamics, of changes that could be particularly important from agricultural

viewpoint. SPI6 was chosen as indicator of cumulative property of vegetation season since SPI6 – if evaluated in September – covers whole period from early spring to harvest of typical summer crops.

**Table 4:** Classification of SPI values (McKee et al., 1993)

**Tabela 3:** Razvrstitev vrednosti SPI (McKee in sod., 1993)

SPI value:	Drought category
2.00 and above	extremely wet
1.50 to 1.99	very wet
1.00 to 1.49	moderately wet
-0.99 to 0.99	near normal
-1.00 to -1.49	moderately dry
-1.50 to -1.99	severely dry
-2.00 and less	extremely dry



**Figure 2:** Spatial distribution of SPI6 for September 2003 (available at: [http://www.dmcsee.org/en/drought\\_monitor/](http://www.dmcsee.org/en/drought_monitor/))

**Slika 2:** Prostorska porazdelitev SPI6 za september 2003 (dostopno na: [http://www.dmcsee.org/en/drought\\_monitor/](http://www.dmcsee.org/en/drought_monitor/))

### 2.3.3. WinISAREG model

With the aim of obtaining an actual description of the changes expected in the agricultural WB of the locations, the time series of simulated maize consumption were computed by model WinISAREG. Model WinISAREG (Pereira et al, 2003; Paredes and Pereira, 2010) is a conceptual WB model for simulating crop irrigation schedules at field level and provides

calculation of irrigation requirements under optimal and/or water stressed conditions. Besides input meteorological data (precipitation, ETP, wind velocity and minimum relative humidity) for WB calculation model demands crop data, soil data and irrigation option data. Also it is optional to define ground water contribution, water supply restrictions and salinity. In this study, these three parameters were not included. Model

includes modul EVAP56 (Pereira et al., 2003) for computation of reference ETP using FAO Penman Monteith method but since ETP calculation is calculated routinely in SEA/ARSO this module was not used. Water balance is performed for a multilayered soil, crop coefficients and root depths at time scale of defined development periods of certain crop. For estimation of crop WB or irrigation, model setting named field, horticultural and tree crops was used. Fixed dates of maize phenological development for year 2010 were set for all the years. According to the experience of Popova and Pereira (2008) it is optional to use the yield response factor  $K_y$  that is derived from field data on yields relative to various irrigation and rainfed regimes. The parameter  $K_y$  introduces in the WinISAREG model, the relation between ETP deficit caused by water stress and the corresponding yield decrease (Paredes and Pereira, 2010). Unfortunately, data for Slovenia are not available therefore crop coefficients from FAO (Doorenbos and Kassam, 1986) were used in the study and adjusted to the local root depth measurements (SEA/ARSO/AWS, 2010). In our analysis we used value 1.25 for  $K_y$  (Doorenbos and Kassam, 1986). The model initializes soil water simulations with initial soil water

content provided by user. Initial total available water (TAW) was set on 100 %. Various time steps of model calculation are adopted depending on weather data availability and required output variable. Model simulation was performed in a way to recognize drought periods according to irrigation requirements. Therefore in WinISAREG model Irrigation Simulation Options mode NIR was used. After simulation model provides information on soil WB or irrigation requirements. In our study daily data were used for ten days and seasonal calculations of NIR, which were input for further investigations.

### 2.3.4. Statistical methods

Results of the crop water simulations were compared with the information available in the agrometeorological reports of SEA/ARSO (1960-2010). Dry seasons detected by study were compared to descriptions in the reports. Beside that, yield decrease and damage due to drought described by the reports of statistical office (Statistical... 2010) were included into discussion and results.

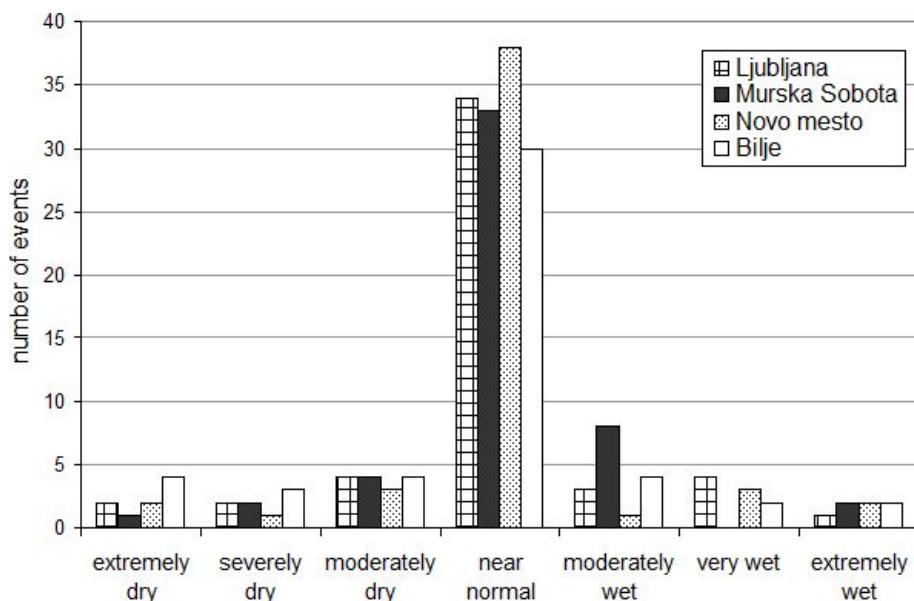
In order to evaluate trends of WB and evapotranspiration at local scale, linear regression was performed.

## 3 RESULTS

### 3.1 Analysis of Standardized Precipitation Index for vegetation seasons 1961–2010

The analysis of the vegetation season time series of SPI6 in the period 1961-2010 shows that from 6 (Novo mesto) to 11 (Bilje) seasons were dry and from 6 (Novo mesto) to 10 (Murska Sobota) seasons were wet. At all locations, except for Bilje, drought in years 2003 and 1992 were detected by SPI6 (Figure 4) as extremely severe. Drought in year 2000 (Ljubljana, Novo mesto, Murska Sobota) and in year 2006 (Bilje) were extreme but more regionalised. The main reason for extreme

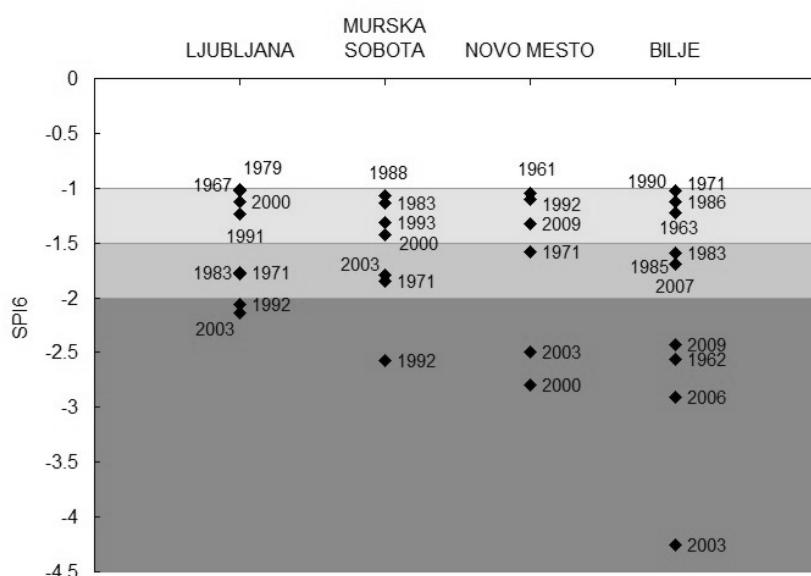
drought detected by SPI6 in the vegetation period in Bilje region was lack of precipitation. In the period from April to July only 190 mm of rain fall, in comparison to other analysed regions were amount of precipitation was more than 400 mm. Above mentioned years were analysed more in details with all the indicators. According to the fact that SPI6 was not appropriate indicator to detect drought dynamics due to its large internal time scale, SPI1 was calculated in ten day updating intervals during the vegetation seasons.



**Figure 3:** Number of vegetation seasons distributed in SPI6 classes

**Slika 3:** Število vegetacijskih sezon porazdeljenih v SPI6 razrede

In the Figure 3 for all the locations number of years in specific SPI class is presented.



**Figure 4:** Vegetation seasons classified according to SPI6 in the range of dry years

**Slika 4:** Sušne vegetacijske sezone razvrščene po SPI6

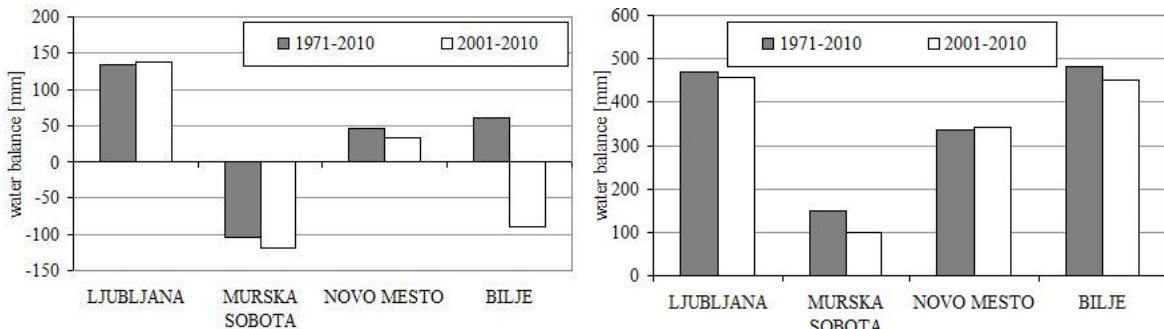
### 3.2 Analysis of water balance

Average 40-year vegetation water balance shows that ETP during vegetation season is

mainly covered by precipitation amount, unfavourable only at Murska Sobota (long term water deficit is 104 mm). In the last ten years the circumstances are getting drier which reflects in slightly lower WB in the eastern Slovenia and significant decrease in western Slovenia (Figure 5). Only in Ljubljana region

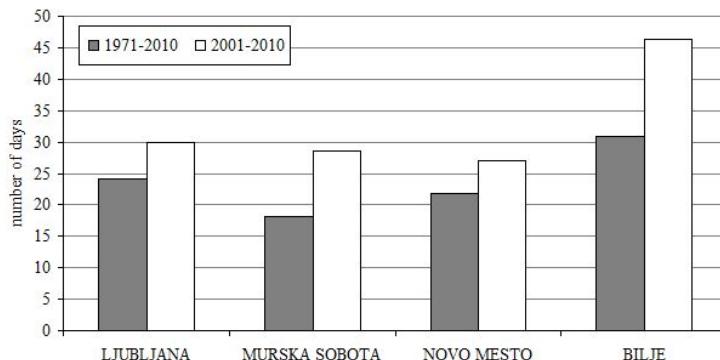
there is a slight increase of WB during vegetation season. In the dormancy small change of WB is in Ljubljana, Novo mesto and Bilje are observed. The largest decrease of WB is in Murska Sobota, 47 mm.

The reason for changing water balance is higher ETP rate (more than 5 mm) in the period 2001-2010 increased from 5 to 16 days in comparison to the period 1971-2000 (Figure 6).



**Figure 5:** Average water balance in vegetation season (left) and in dormancy (right) in periods 1971-2010 and 2001-2010

**Slika 5:** Dolgoletna povprečja vodne bilance vegetacijski sezoni (levo) in v dormanci (desno) v obdobjih 1971-2010 in 2001-2010



**Figure 6:** Number of days with ETP > 5 mm in the period 1971-2010 and 2001-2010 in vegetation seasons

**Slika 6:** Število dni z visokim izhlapevanjem, ETP > 5 mm v vegetacijskih sezona v obdobju 1971-2010 in 2001-2010

### 3.3 Drought analysis using Net Irrigation Requirements

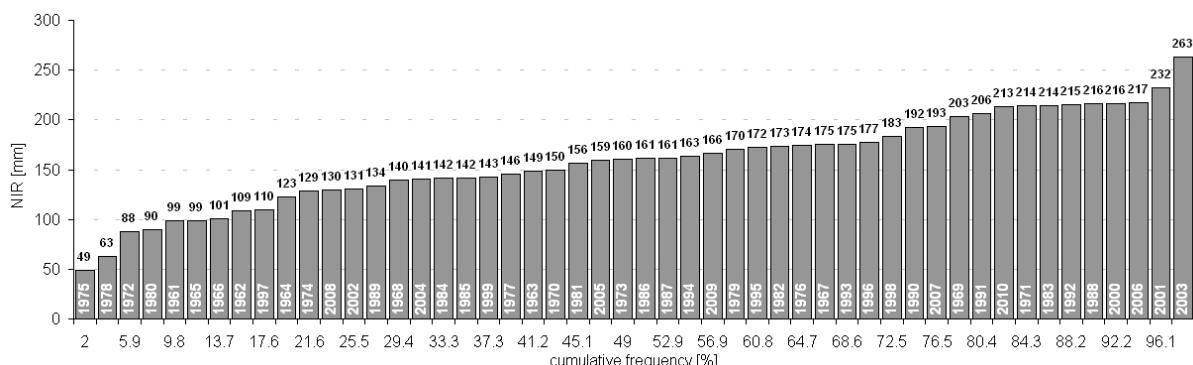
NIRs show similar pattern at specific locations as SPI6. According to the cumulative frequency of NIR, years in the range between 80 % in 100 % were determined as dry. In Ljubljana years 2003, 2001, 2006, 2000 were

detected as dry by NIR but only 2000 and 2003 were detected by SPI6 (Figure 7).

Distribution of dry years by NIR does not fit always with the order using SPI6. The best fit is only with extremely dry years. This result was somehow expected; while during extreme droughts in the vegetation season both severe lack of precipitation and increased ETP appear simultaneously, this is not the case in years that are near to normal conditions. Very important impact on the severity of drought is

distribution of precipitation (drought in year 2006). Shorter heat waves can be interchanged by wet periods so SPI6 (presenting total

precipitation anomaly) and NIR (presenting total irrigation requirements) can show different trends.



**Figure 7:** Classification of NIR according to cumulative frequency in Ljubljana in the period 1961-2010

**Slika 7:** Razvrstitev NIR-a glede na kumulativno frekvenco za Ljubljano v obdobju 1961-2010

### 3.4 Comparison of SPI1 to 30-day water balance and NIR to 10-day water balance

WB, NIR and SPI1 calculations were performed for 10-day periods. Years with extreme droughts are described more in details in sections 3.4.1. Detailed simulations of all indicators are presented in two graphs in Figure 8, 9, 10 and 11. For specific dry vegetation season upper graph represents SPI1 and WB30 describing 30-day weather conditions. In the graph below NIR and WB10 are simulating 10-day circumstances

#### 3.4.1 Case studies – years with extreme droughts

SPI1, NIR and WB for 1992, 2000, 2003 and 2006 vegetation seasons are presented in the Figure 8, 9, 10 and 11.

##### 3.4.1.1. Year 1992

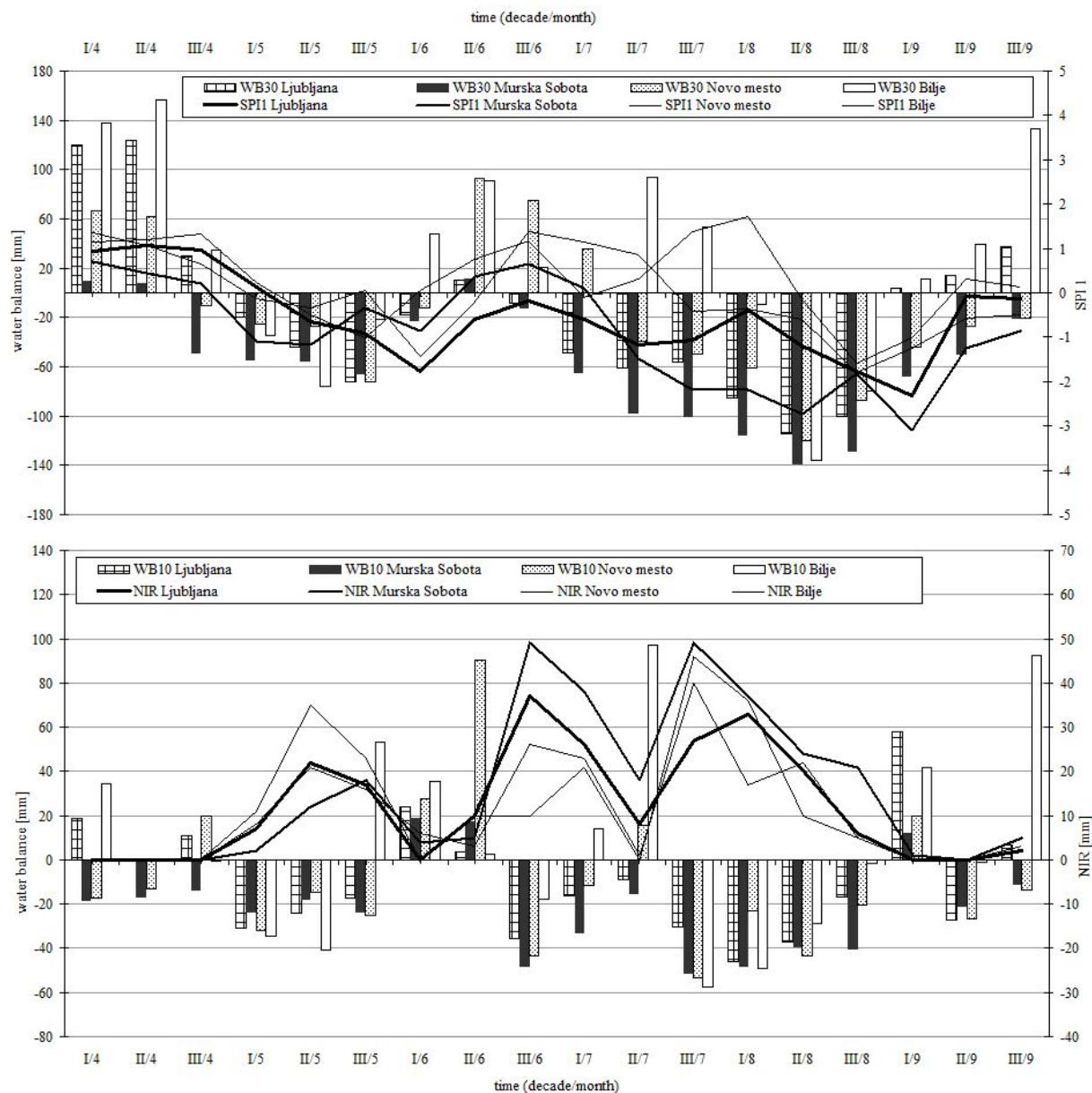
The drought in Murska Sobota has started already in spring. It continued in May and affected besides Murska Sobota region also other areas. The agriculture drought damaged maize crops. At the end of May, instable weather with precipitation interrupted the dry

spell. Precipitation in the first decade of June moistened the soil.

At the end of June second drought appeared. At the end of second decade of June curled leaves of crops have already been noticed in some parts of Slovenia. Drought lasted in Murska Sobota region until the end of August. According to the reports agricultural drought was present also at the other parts in Slovenia, but the most extreme was in region around Murska Sobota. Maize crops on sandy soils were affected the most, from 70 to 90 % of maize crops were damaged due to drought. Most of rainfed agricultural crops ripened forcedly.

In Figure 8 SP1, NIR and WB for vegetation season 1992 is presented. According to SPI1 there were two major dry spells, first at the end of May and second lasting through the whole August, while according to WB and NIR there were three periods of drought appearing in May, June and August. Magnitude of drought in August was classified as the most severe. SPI results imply the lack of precipitation was the highest in Murska Sobota. Comparison of SPI for all sites shows the lowest drought severity in Bilje. The highest magnitude and

the longest duration of drought are identified indices.  
in Murska Sobota also by WB and NIR



**Figure 8:** Ten day SPI1, WB30, NIR and WB10 values for vegetation season 1992

**Slika 8:** Vrednosti SPI1, WB30, NIR in WB10, računane na vsako dekado za vegetacijsko sezono 1992

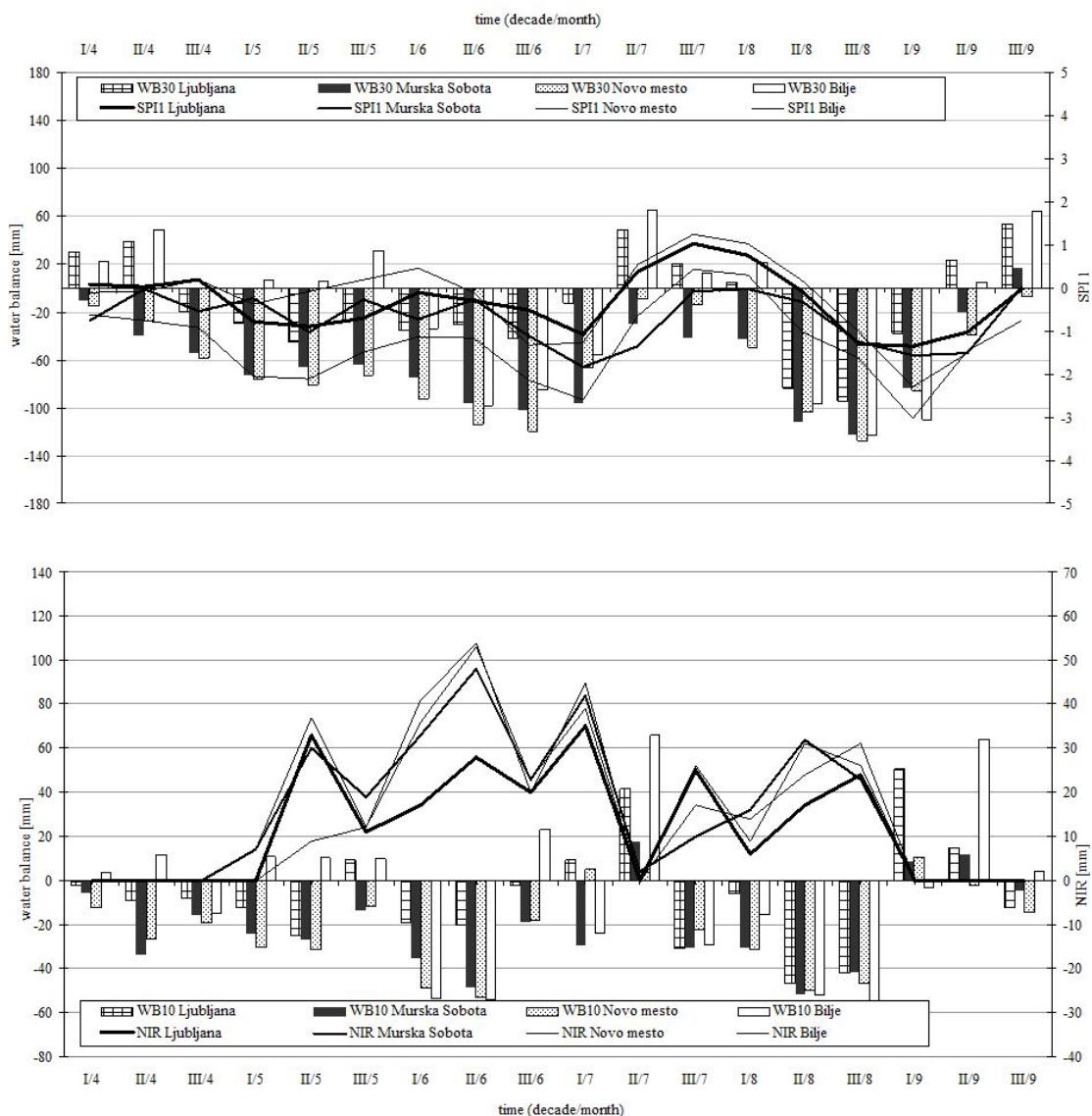
### 3.4.1.2. Year 2000

Due to the lack of precipitation during the winter and early spring, the first interval of agricultural drought was detected in the middle of April. Consequently the winter crops were delayed in the tillering stage.

Results of drought indices calculation for the year 2000 are shown in Figure 9 and confirmed by agrometeorological report survey. The second dry interval appeared in the summer in the major agricultural areas in Slovenia. SPI1 shows period of drought from the beginning of May till the middle of July for Novo mesto. Regarding to SPI1 two short periods with lower drought severity in summer appeared also at other sites. WB for vegetation season is mostly negative with few exceptions at particular individual site. NIR is resulting in a way complementary to WB, as negative soil WB demands irrigation. The short wet interval with positive WB was in the second decade of

July. This was the most severe agricultural drought in the 10-year period from 1990 to 2000.

Drought affected most agricultural plants in the most sensitive phenological stages: maize crops in the tasseling and pollinating stages, setting of cobs and ripening. By the end of June the agricultural drought had halved the normally expected yield, while at the end of the first third of July the consequences attained the extent of a natural disaster. The most distressed regions were the agricultural areas around Novo mesto and Murska Sobota. The maximum damage was recorded in maize crops, which represents the major crop in the agricultural areas of Murska Sobota. Crop yield in the areas of Murska Sobota, Novo mesto and Bilje was reduced by 20 – 30 %. The drought was terminated by abundant precipitation at all sites in the beginning of September.

**Figure 9:** Ten day SPI1, WB30, NIR and WB10 values for vegetation season 2000**Slika 9:** Vrednosti SPI1, WB30, NIR in WB10, računane na vsako dekado za vegetacijsko sezono 2000

### 3.4.1.3 Year 2003

Drought in 2003 lasted from end of April up to second decade of July. It affected the whole country. The damage due to drought attained the extent of a natural disaster which ranked amongst the worst in the previous 50 years. Vegetation season of the year 2003 using SPI1, WB and NIR indices are presented in Figure 10. SPI1 corresponds to precipitation conditions for all sites, continuously below

zero SPI value in the major part of the vegetation season.

SPI1 indicates severe drought at all sites at the beginning of April. Another drought period for all sites except for Murska Sobota started in the second decade of May. According to SPI1 in Bilje drought lasted till the second decade in August, in Novo mesto till the first decade of July, in Ljubljana dry spell also lasted till the first decade of July but was interrupted at the beginning of June. In Murska Sobota drought

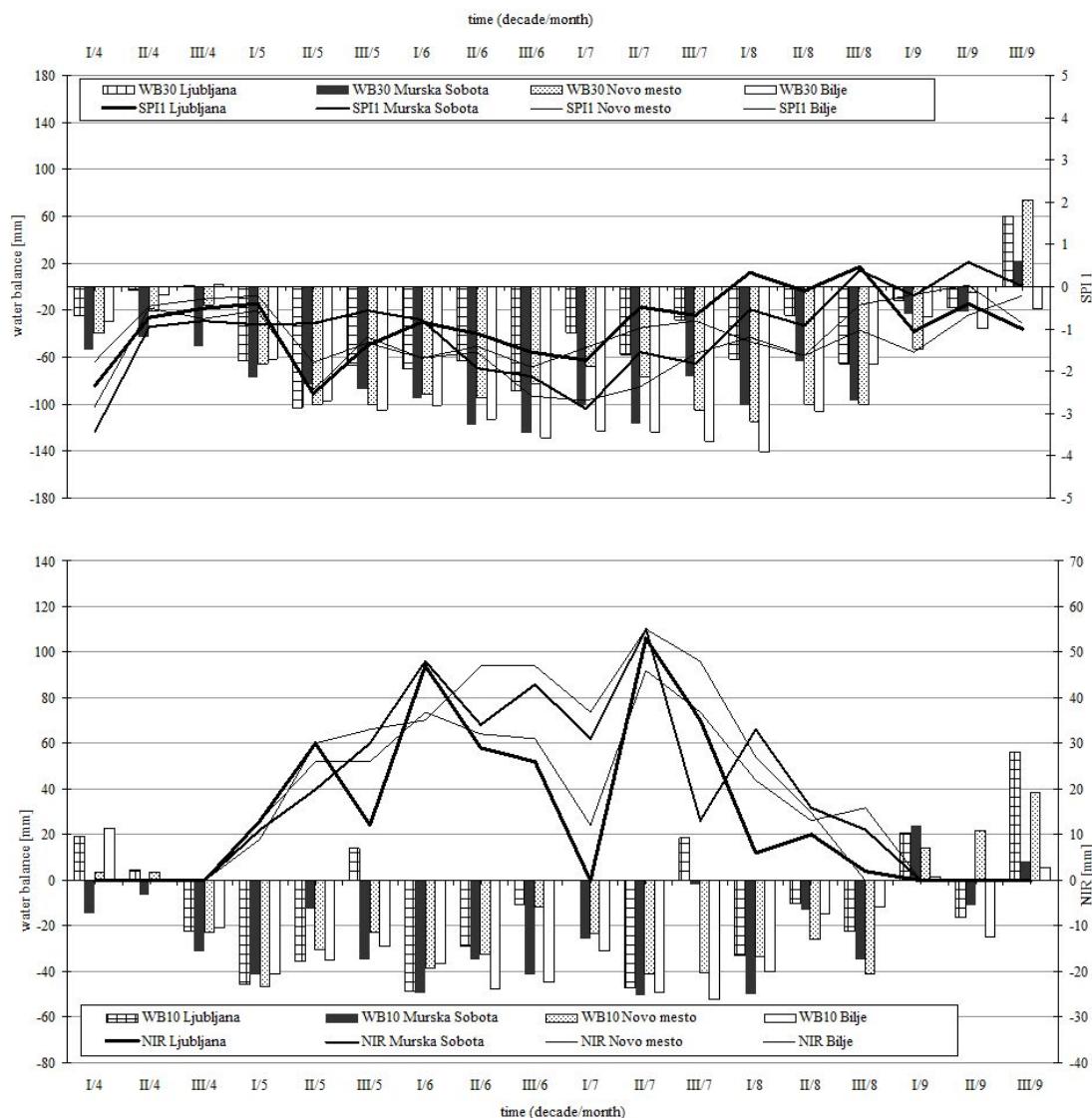
lasted from the second decade of June till the end of July.

The soil water reservoir was not refilled until abundant precipitation in September. However, the WB equilibrium was not restored due to enormous summer water deficit until the end of the year.

The consequences of summer drought were detected also in earlier timing of autumn phenological phases. Long standing water stress heavily influenced the development of

maize. Maize grown on shallow and sandy soils visibly lagged behind the normal growth. The damage was assessed to more than 128 million €. It attained the extent of a heavy natural disaster. About 83 % of Slovenian agricultural area was distressed (Statistical..., 2010).

The drought characteristics were detected also by WB. NIR corresponds to negative WB. With respect to WB values and NIR drought severity was the highest in Bilje following by Murska Sobota and Novo mesto.



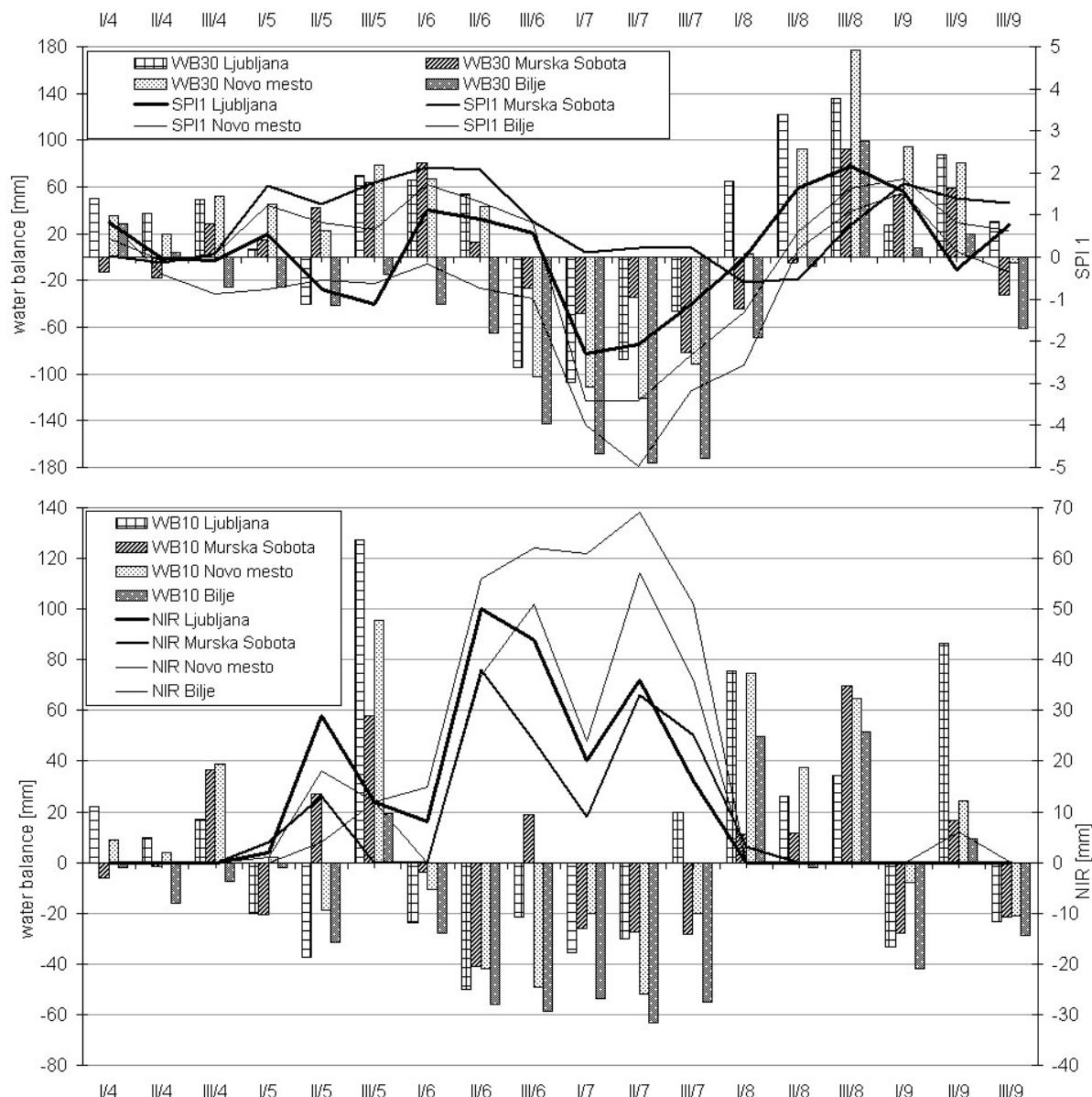
**Figure 10:** Ten day SPI1, WB30, NIR and WB10 values for vegetation season 2003

**Slika 10:** Vrednosti SPI1, WB30, NIR in WB10, računane na vsako dekado za vegetacijsko sezono 2003

### 3.4.1.4 Year 2006

In the last decade of June 2006 the distinctive water shortage by agriculture crops was detected due to the lack of precipitation. Drought was intensified till the end of July at whole Slovenia but the most seriously in the area of Bilje and Novo mesto. At the beginning of August the abundant precipitation ceased two months lasting summer drought. At Bilje region maximum

damage due to drought was recorded. Maize yield was reduced by 40 to 90 %. According to SPI1 drought has not appeared in Murska Sobota in July which neglected the real situation. On the contrary, WB and NIR confirmed dry signal in June and July. SPI1 and NIR indicate the maximum SPI1 and the highest values of irrigation requirements among all analysed years in Bilje.



**Figure 11:** Ten day SPI1, WB30, NIR and WB10 values for vegetation season 2006

**Slika 11:** Vrednosti SPI1, WB30, NIR in WB10, računane na vsako dekado za vegetacijsko sezono 2006

### 3.5. Data verification

Data verification was strictly limited to maize in the vegetation season. Dryness (defined by NIR and SPI6) in the vegetation season was checked by reports about drought agricultural

impacts reported in the bulletins of the SEA/ARSO.

Only years detected as dry by SPI6 and NIR were included in the evaluation process. In Table comparison with reports for dry years is presented. Only year 2003 was confirmed with both indicators and reports.

**Table 5:** Classification of dry years with drought within vegetation season detected by SPI6, NIR and comparison with reports of SEA/ARSO (+ drought, - no drought) for Ljubljana (LJ), Murska Sobota (MS), Novo mesto (NM) and Bilje (BI)

**Tabela 4:** Razvrstitev suhih let, s sušo v vegetacijski sezoni, ugotovljeno z indeksi SPI6 in NIR ter primerjano z arhivom ARSO (+ suša, - ni suše) za Ljubljano (LJ), Mursko Soboto (MS), Novo mesto (NM) in Bilje (BI)

	SPI6				NIR				ARSO				REMARKS
	LJ	MS	NM	BI	LJ	MS	NM	BI	LJ	MS	NM	BI	
1961	-	-	+	-	-	-	-	-	-	-	-	-	NM - precipitation below the average (SPI6), but not low enough for drought
1962	-	-	-	+	-	-	-	-	-	-	-	+	BI - NIR value slightly below chosen threshold, short - term drought
1963	-	-	-	+	-	-	-	-	-	-	-	-	BI - precipitation below the average (SPI6), but not low enough for drought
1967	+	-	-	-	-	-	-	-	-	-	-	-	LJ - precipitation below the average, but not low enough for drought
1971	+	+	+	+	+	+	-	-	+	+	+	+	NM - NIR value slightly below chosen threshold, short - term drought; BI - wet first three months of vegetative season
1973	-	-	-	-	-	-	+	-	-	-	+	-	NM - precipitation (SPI6) near threshold for drought
1976	-	-	-	-	-	-	+	-	-	-	+	-	NM - precipitation on the dry side of normal range (SPI6)
1979	+	-	-	-	-	-	+	-	+	-	+	-	LJ - short - term drought (according to SEA/ARSO); NM - SPI6 near threshold for drought
1981	-	-	-	-	-	+	-	-	-	+	-	-	BI - irregularly distribution of precipitation; large amount of rain in June (130 mm in 3 days) and september
1983	+	+	-	+	+	+	-	-	+	+	-	+	BI - hot and dry condition at the time of pollination influenced on harvest
1985	-	-	-	+	-	-	-	+	-	-	-	+	
1986	-	-	-	+	-	-	-	+	-	-	-	+	
1988	-	+	-	-	+	-	-	-	+	+	-	+	LJ - precipitation near normal value, but not enough for maize; MS - NIR value slightly below chosen threshold; BI - hot and dry condition at the time of pollination influenced on harvest
1990	-	-	-	+	-	-	-	-	-	-	-	+	BI - NIR value slightly below chosen threshold, short - term drought
1991	+	-	-	-	+	-	-	-	+	-	-	-	
1992	+	+	+	-	+	+	-	-	+	+	+	-	NM - NIR value slightly below chosen threshold, short - term drought
1993	-	+	-	-	-	+	+	-	-	+	+	-	NM - precipitation below the average (SPI6), but not low enough for drought
1994	-	-	-	-	-	-	-	+	-	-	-	+	BI - precipitation below the average (SPI6), but not low enough for drought
1996	-	-	-	-	-	-	+	-	-	-	+	-	NM - precipitation below the average (SPI6), but not low enough for drought
2000	+	+	+	-	+	+	+	+	+	+	+	+	BI - precipitation below the average (SPI6), but not low enough for drought
2001	-	-	-	-	+	+	+	+	+	+	+	+	a large amount of precipitation in September affect SPI6
2003	+	+	+	+	+	+	+	+	+	+	+	+	
2004	-	-	-	-	-	-	-	+	-	-	-	-	BI - NIR value equal to chosen threshold
2006	-	-	-	+	+	-	+	+	+	-	+	+	LJ and NM - a large amount of precipitation in August affect on SPI6
2007	-	-	-	+	-	+	-	+	-	+	-	+	MS - a large amount of precipitation in September affect on SPI6
2009	-	-	+	+	-	-	-	+	-	-	+	+	NM - NIR value slightly below chosen threshold
2010	-	-	-	-	+	+	+	-	-	-	-	-	LJ, MS, NM - NIR value near chosen threshold

### 3.6. Trend analysis

Additionally time trends of water components were performed for the period 1961–2010. The hypothesis that cumulative ETP and number of days with high ETP rate (more than 5 mm/day) in the vegetation season are increasing was confirmed. The trend is statistically significant at all locations. The largest increase was recorded in Bilje, absolute change around 7 %/10 years and the minor increase in Novo mesto with almost 2 %/10 years. At all

locations the noticeable increase of crop water consumption in last ten years is observed, from 20 to 40 %.

The slight trend of precipitation decrease during vegetation season was detected. The trend analysis of vegetation season precipitation amount showed that the average trend is around 1 %/10 years at all locations. The WB during the vegetation season showed trend to more intense water deficit in last ten years, only in Ljubljana WB slightly increases.

**Table 6:** Absolute and relative change of ETP and NIR in the period 1961-2010

**Tabela 5:** Absolutna in relativna sprememba ETP in NIR v obdobju 1961-2010

		Ljubljana	Murska Sobota	Novo mesto	Bilje*
<b>ETP in vegetation season</b>	absolute change (mm/10 years) relative change (%/10 years)	14.6 +2.4 **	20.4 +3.4 **	10.1 +1.7	42.5 +6.6 **
<b>ETP &gt; 5 mm in vegetation season</b>	absolute change (NOD/10 years) relative change (%/10 years)	4.1 +18.6 **	4.6 +31.7 **	3.6 +18.1 **	10.0 +41.1 **
<b>NIR in vegetation season</b>	absolute change (mm/10 years) relative change (%/10 years)	13.9 +8.8	14.4 +8.7	11.8 +7.2 %	26.7 +14.0

NOD ... Number of days

\* ... Period 1962-2010

\*\* ... Statistically significant

SPI6 is showing increase of dry vegetation season in the period 2001-2010 in comparison to the period 1971-1990 in Novo mesto and Bilje. In Ljubljana and Murska Sobota SPI6 shows even decrease.

NIR trends for maize during the vegetation season vary a lot, but it is common that all the locations are characterised by the increase of the deficit for 8 – 14 %/10 years (Table 6). In last ten years (2001-2010) in comparison to the reference period (1971-2010) on average increased for 23% in the range from 17 to 38 % (Table 7).

**Table 7:** Average NIR in the period 1971-2000 and relative change of NIR in the period 2001-2010 in comparison to the period 1971-2000**Tabela 6:** Povprečni NIR v obdobju 1971-2000 in relativna sprememba NIR-a v obdobju 2001-2010 v primerjavi z obdobjem 1971-2000

	LJUBLJANA		MURSKA SOBOTA		NOVO MESTO		BILJE	
	1971-2000	2001-2010	1971-2000	2001-2010	1971-2000	2001-2010	1971-2000	2001-2010
NIR (mm)	158	+17 %	166	+20 %	163	+17 %	191	+38 %

#### 4 DISCUSSION

In general, concern about rising frequency and impacts of drought is justified. Economical costs are increasing. In year 2000 almost 79 million € damage due to drought was recorded, in 2001 almost 42 million €, in 2003 more than 128 million € and in 2006 almost 50 million €. A drought monitoring and early warning systems should be established to provide information on the formation, development and end of drought. In our study SPI as indicator was less suitable for detecting

agricultural drought than crop WB and NIR. In order to monitor the occurrence and development of agricultural drought efficiently and provide information on the strength and range of drought more indicators should be included in the monitoring. For agricultural drought monitoring only integrated tools as plant – soil – climate models will dynamically monitor occurrence and evolution of drought over specific region.

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**Agrovoc descriptors:** coccidae, ecology, vitis vinifera, grapevines, vineyards, taxonomy, biosystematics, damage, crop yield, pest insects, insect control, pest control, vectors, hosts, disease transmission, insecticides, biological control, control methods

**Agris category code:** h10, h20

## Kaparji (Hemiptera: Coccoidea) vinske trte (*Vitis vinifera L.*) v Sloveniji in možnosti njihovega biotičnega zatiranja

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### IZVLEČEK

V Sloveniji se na vinski trti pojavljajo kaparji *Neopulvinaria innumerabilis*, *Pulvinaria vitis*, *Parthenolecanium corni* in *Planococcus ficus*. Te žuželke sesajo rastlinski sok in povzročajo prezgodnje odpadanje listov in bolezenske okužbe grozdov, kar vpliva na kakovost pridelka. Kaparji so tudi pomembni prenašalci rastlinskih virusov. Vrsti *P. vitis* in *P. corni* sta razširjena po vsej Sloveniji, vrsta *N. innumerabilis* v vinogradih na Primorskem, vrsto *P. ficus* pa največkrat najdemo v zavarovanih prostorih. Običajno kaparje zatiramo z uporabo insekticidov, vendar so sodobne strategije varstva rastlin usmerjene v naravi prijaznejše načine, kot sta mehansko in biotično zatiranje škodljivcev. Pri nas biotično zatiranje kaparjev še ni dobro raziskano, čeprav se je v srednji Evropi že izkazalo za uspešno.

**Ključne besede:** kaparji, Coccidae, Pseudococcidae, sistematika, morfologija, bionomija, škodljivost, zatiranje, biotično varstvo

### ABSTRACT

#### SOFT SCALES AND MEALYBUGS (Hemiptera: Coccoidea) ON GRAPEVINE (*Vitis vinifera L.*) IN SLOVENIA AND THE POSSIBILITIES OF THEIR BIOLOGICAL CONTROL

In Slovenia *Neopulvinaria innumerabilis*, *Pulvinaria vitis*, *Parthenolecanium corni* and *Planococcus ficus* have been identified on grapevine. These insects cause earlier discolorations on the leaves and defoliation and consequently cause poor growth of plants and reduction of yield and crop quality. They can also transmit important plant viruses. *P. vitis* and *P. corni* are widespread in Slovenia. *N. innumerabilis* was found in vineyards of Primorska, while *P. ficus* was found mainly in greenhouses. Because of their great economic influence insecticides are used for their control. Natural enemies make a significant contribution to biological control of these insects. The biological control of scale insects is not well studied in Slovenia, although it has proved to be successful in Central Europe.

**Key words:** scale insects, mealybugs, Coccidae, Pseudococcidae, systematics, morphology, bionomics, damage, control, biological control

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## 1 UVOD

Kaparji so tujerodne fitofagne žuželke, ki jih uvrščamo v red polkrilcev (Hemiptera), čeprav so bili do nedavnega uvrščeni v red enakokrilcev (Homoptera). Kaparje uvrščamo v naddružino Coccoidea (podred Sternorrhyncha), ki vsebuje 48 družin (Ben-Dov in sod., 2012). Predstavnike devetih družin so potrdili tudi v Sloveniji (Seljak, 2010). Tako v svetu kot pri nas so vrstno najštevilčnejše tri družine, Diaspididae (2479 vrst), Pseudococcidae (2231 vrst) in Coccidae (1133 vrst) (Ben-Dov in sod., 2012). V Sloveniji je bilo na različnih gostiteljskih rastlinah najdenih 43 vrst iz družine Diaspididae, 26 vrst iz družine Coccidae in 18 vrst iz družine Pseudococcidae. Po doslej zbranih podatkih najdemo na vinski trti pri nas le predstavnike zadnjih dveh družin (Seljak, 2010).

V zadnjih 20 letih so v Sloveniji zabeležili porast tujerodnih organizmov. Kaparji predstavljajo kar 41,1 % (37 vrst) vseh polkrilcev, ki so bili v Slovenijo vneseni ali pa so se postopno razširili na to ozemlje (Seljak, 2011). Na splošno so škodljivi predvsem na sadnem drevju, grmičevju in rastlinah v zavarovanih prostorih (Kosztarb in Kozar, 1988). Hranijo se z rastlinskim sokom in s tem oslabijo rastlino, povzročijo razbarvanje in prezgodnje odpadanje listov, deformacije vej, mnogokrat pa so tudi prenašaleci rastlinskih virusov (Kosztarb in Kozar, 1988; Golino in sod., 1999, 2002; Martelli in sod., 2002; Sim in sod., 2003). Posredna škoda, ki jo povzročajo, je izločanje medene rose, kamor se naselijo glive sajavosti (Hamon, 1984; Kosztarb in Kozar, 1988; Gullan in Martin, 2009).

Primarno so kaparji škodljive žuželke, vendar so nekatere vrste med njimi tudi vir mnogih uporabnih snovi, ki jih s pridom izrabljajo druge žuželčje vrste in ljudje. Kaparji poleg

medene rose proizvajajo tudi vosek in naravna barvila (Łagowska in Golan, 2009). Pogosto so predmet preučevanja ekologov in evolucijskih biologov zaradi njihovega simbiotskega odnosa z mravlji. S tem ko mravlje nabirajo medeno roso, odstranijo snovi, potrebne za razvoj gliv sajavosti. Te glive so škodljive ne samo rastlinam, ampak tudi kaparjem, saj jih lahko okužijo in povzročijo njihov pogin (Kosztarb in Kozar, 1988). Za nekatere vrste kaparjev je znano, da imajo mutualistični odnos z neželastimi čebelami (Hymenoptera: Apidae: Meliponinae). Čebelam je medena rosa vir hrane, poleg tega s tem dobijo tudi dodaten vosek za gradnjo satja (Camargo in Perdo, 2002).

Po podatkih Seljaka in Žežline (2007) se v Sloveniji na vinski trti pojavljajo štiri vrste kaparjev. Veliki trtni kapar (*Neopulvinaria innumerabilis* Rathvon), navadni trtni kapar (*Pulvinaria vitis* L.) in češpljev kapar (*Parthenolecanium corni* [Bouché]) sodijo v družino Coccidae, medtem ko smokvinega volnatega kaparja (*Planococcus ficus* Signoret) uvrščamo v družino Pseudococcidae. Poleg naštetih, je na vinski trti gospodarsko škodljiv tudi kapar *Parthenolecanium persicae* Fabricius (Pellizzari, 1997). Vendar ga pri nas na tej rastlinski vrsti še niso potrdili (Seljak, 2010), našli pa so ga na vinski trti v Istri na Hrvaškem (Masten-Milek in sod., 2007).

Navadni trtni kapar in češpljev kapar sta razširjena po vsej Sloveniji, veliki trtni kapar pa le v vinogradih na Primorskem, kjer v bolj vročih letih najdemo tudi smokvinega volnatega kaparja. Ta vrsta je tropskega oz. subtropskega izvora, zato o njeni škodi v Sloveniji le redko poročajo, saj preživi samo v zavarovanih prostorih. Pogosteje se pojavlja v rastlinjakih na baznem materialu vinske trte kot pa na prostem (Seljak in Žežlina, 2007).

## 2 TELESNA ZGRADBA IN RAZVOJNI KROG KAPARJEV (COCCIDAE IN PSEUDOCOCCIDAE)

Pri kaparjih iz družin Coccidae in Pseudococcidae je močno izražen spolni dimorfizem. Klasifikacija temelji večinoma na morfoloških lastnostih odraslih samic (Kondo in sod., 2008).

Segmentacija pri samicah ni vedno razločna, vedno so brez kril, telo je navadno ovalne oblike, noge so petčlenaste in dobro razvite. Za njih je značilna neotenija, oziroma sposobnost razmnoževanja samice že na stopnji nimfe (Ben-Dov, 1997; Miller, 2005). Oči so enostavne in navadno zmanjšane v dve majhni obarvani pegini. Tipalke so dobro razvite z od 1 do 16 segmenti ali pa tipalk sploh nimajo. Zadnjična (analna) odporna je obkrožena s sklerotiziranim analnim obročem, nameščena na zadnjem telesnem obroču (členu). Obdana je s setami ter porami za izločanje voska (Kosztarb in Kozar, 1988). Odrasle samice naddružine Coccoidea lahko živijo od nekaj mesecev do nekaj let (Gullan in Martin, 2009).

Samci kaparjev imajo razvito glavo (*caput*), oprsje (*thorax*) in zadek (*abdomen*). Glava je sklerotizirana ter pri družinah Coccidae in Pseudococcidae z vratom jasno ločena od telesa. Tipalke (*antennae*) so sestavljene iz osmih do desetih členov, nitaste oblike s številnimi setami. Tip oči se med družinami razlikuje, ustnega aparata pa samci nimajo. Telo je sestavljeno iz devetih delov, s štirimi dihalnimi odprtinami vzdolž trupa, razvit je en par kril in močno oščetinjene dolge, vitke petčlene noge s kaveljcem. Na zadku imajo dolge filamentozne sete. Življenska doba odraslih samcev je od nekaj ur do nekaj dni po preobrazbi. V tem času je njihova glavna naloga najti samico in jo oploditi (Kosztarb in Kozar, 1988).

Število rodov na leto je vrstno značilno. Medtem ko imajo v srednji Evropi najpogosteje en rod letno, je za tropske vrste

značilnih več rodov letno (Kosztarb in Kozar, 1988). Samice so hemimetabolne žuželke in imajo najmanj dva razvojna stadija, medtem ko gredo samci skozi pet stopenj holometabolnega razvoja: dva nimfalna stadija, predbubo, bubo in končno fazo odraslega osebka (Kosztarb in Kozar, 1988; Williams, 1997; Miller in sod., 2007).

Novo izlegle nimfe kaparjev niso zavarovane z voščenim poprhom kot so to odrasle samice, zato so zelo občutljive na insekticide. Kmalu po pojavu se premaknejo na spodnjo stran listov, kjer se ustalijo in hranijo. Pred prehodom v tretji razvojni stadij se samci obdajo z belim voščenim ovojem, kjer se preobrazijo v krilate osebke (Kosztarb in Kozar, 1988).

Kaparji se aktivno premikajo v času prve nimfalne stopnje in kot krilati samci po preobrazbi. Premikajo se sicer tudi ostale razvojne oblike nimf in odrasle samice, vendar le v omejenem obsegu. Pasiven prenos navadno poteka z vetrom, zemljo, vodo, pomemben dejavnik prenosa so živali in človek, predvsem s premikanjem rastlinskega materiala (Kosztarb in Kozar, 1988).

### 2.1 Družina Coccidae

Klasifikacija družine Coccidae temelji večinoma na morfoloških lastnostih odraslih samic. Samice imajo enostavnejšo preobrazbo kot samci in preidejo tri ali štiri stadije, samci pa imajo pet razvojnih stadijev (Kosztarb in Kozar, 1988; Williams, 1997).

Nimfe prve stopnje so eliptične oblike, navadno velike le od 0,3 do 1 mm, z dvema sestavljenima (facetnima) očesoma in štirimi dihalnimi odprtinami vzdolž telesa ter z dobro razvitim nogami s krempeljcem na koncu petega člena. Tipalke so obdane s setami in imajo pet ali šest segmentov. Vsaka analna plošča je obdana z

dolgimi apikalnimi setami. Analni obroč je obdan s šestimi setami. Spirakularne sete so najpogosteje nastale iz marginalnih. Večprekatnih por in tubularnih odprtin na trebušni strani nimajo (Kosztarb in Kozar, 1988; Williams, 1997).

Samci in samice so si zelo podobni tudi na drugi razvojni stopnji, vendar tu že prihaja do razlikovanja oziroma do pojava dimorfizma, saj imajo samci na drugi tretjini telesa vzdolž hrbtnih strani tubularne cevke, samice pa ne. Telo obeh spolov je pri tej razvojni stopnji navidezno razčlenjeno (segmentirano) s kožnimi gubami; imajo več set, cevk in por kot nimfe prvega stopnje (Williams, 1997). Telo je ovalno, pri samcih nekoliko bolj podolgovato. Analne plošče so brez dolgih apikalnih set. Tipalke in noge so zakrnele ali pa so dobro razvite.

Samice so v tretjem razvojnem stadiju zelo podobne odraslim osebkom. Če jih ne opazujemo pod mikroskopom, jih lahko velikokrat spregledamo. Telo je ovalno, skoraj okroglo, z zelo dobro razvito analno režo. Tipalke so iz šestih ali sedmih delov ter iz od enega do treh parov set ali pa tipalk sploh nimajo. Lahko so brez nog ali pa so te dobro razvite. Hrbtnne sete so ali niso prisotne, analne plošče z enim ali tremi pari šopov set pa so vedno navzoče. Na analnem obroču je navadno osem set, včasih samo šest. Nimfe tretjega razvojnega stadija imajo na splošno več por, set in ostalih kožnih struktur kot nimfe prejšnjega stadija, vendar manj kot odrasli osebki, tudi telo je manj segmentirano (Williams, 1997). Odrasle samice imajo še več set in por. Tubularne pore so največkrat prisotne, večprekatne pore so na trebušni strani telesa.

Samci tretjega stadija, imenovanega tudi predbuba, se nahajajo v moknatem voščenem ovoju, ki ga oblikujejo na predhodni stopnji. V tej faziji se njihovo telo preobrazi v precej drugačno obliko od samičinega; nimajo analne plošče, oči, hrbtnne pore, tubularnih cevk, niti set okoli dihalnih odprtin (Williams, 1997).

Samci četrtega stadija, imenovanega tudi buba, so podobni prejšnjemu stadiju, z razliko, da so tipalke, spolni organi in noge že bolje oblikovani, še vedno pa nimajo oči. Noge in tipalke so dolge približno kot ena tretjina telesa, vidna je členjenost telesa.

Telo odraslega samca je členjeno v tri regije. Na sklerotizirani glavi je od dva do pet parov enostavnih oči in par lateralnih ocelijev; ustnega aparata nimajo. Samci ostanejo v voskastem zapredku vse dokler niso popolnoma razviti. Zapredek nato poči, samci izletijo ter začnejo iskati samico.

Oplojene samice so ovalne, navadno temno rjave barve, s konveksno, nagubano in močno sklerotizirano hrbtno stranjo. Otrdel eksoskelet mrtve samice služi kot zaščita jajčecem in novo izleglim nimfam (Kosztarb in Kozar, 1988). Predstavniki te družine se v Evropi razmnožujejo večinoma spolno, le pri nekaterih rodovih (npr. *Parthenolecanium*, *Pulvinaria*) poznamo tudi partenogenetsko razmnoževanje (Kosztarb in Kozar, 1988).

Predstavniki iz družine Coccidae so večinoma univoltini, se pravi, da imajo le en rod letno, z izjemo nekaterih vrst v območjih s toplejšim podnebjem, ki razvijejo od dva do tri rodove letno (npr. *Parthenolecanium persicae* in *Parthenolecanium corni*) (Kosztarb in Kozar, 1988). Življenjski krog je vrstno specifičen. Kaparji iz rodu *Pulvinaria* prezimijo kot odrasle samice, pri rodu *Parthenolecanium* predstavljajo prezimni stadij nimfe druge razvojne stopnje, medtem ko pri rodu *Palaelecanium* prezimijo jajčeca (Kosztarb in Kozar, 1988). Pri nas najdemo vrsto *Palaelecanium bituberculatum* Signoret na jablani (*Malus domestica*) in enovratem glogu (*Crataegus monogyna*) (Seljak, 2007).

#### 2.1.1. Veliki trtni kapar (*Neopulvinaria innumerabilis* [Rathvon])

Veliki trtni kapar je eden izmed največjih in najbolj znanih kaparjev. V literaturi ga najdemo tudi pod imenom *Pulvinaria innumerabilis* (Koval in sod., 1996). Gre za polifagno žuželko nearktičnega izvora, ki je razširjena po vsej Ameriki in Kanadi (Pellizzari, 1997). V Evropi je razširjen na Hrvaškem (Masten in sod., 2007), v Rusiji, Franciji in Italiji (CABI, 2012), od koder je bil po vsej verjetnosti zanesen tudi v Slovenijo (Seljak, 1995; Seljak, 2007; Seljak, 2010). Pri nas so ga prvič našli leta 1985 na kakiju (*Diospyros kaki*), navadnem orehu (*Juglans regia*) in vinski trti (*Vitis vinifera*) (Seljak, 1995). Gospodarsko škodo so zabeležili le na vinski trti (Pellizzari, 1997), ki je zanj najpomembnejša gostiteljska rastlina (Seljak, 2007). Po trditvah Seljaka (2007) in Žežline (Seljak in Žežlina, 2007) je veliki trtni kapar pomemben škodljivec vinske trte, ki lahko pri nas povzroči občutno gospodarsko škodo.

*N. innumerabilis* je univoltinla vrsta. Oplojena samica prezimi pretežno na enoletnem lesu (Seljak, 1995). V tem času je telo samice precej ploščato, dolgo okoli 5 mm (Koval in sod., 1996), rjave barve z ozkim grebenom vzdolž ščitka. Samice ostanejo spomladji prisesane na mestu prezimovanja, kjer začnejo pospešeno rasti (Seljak, 1995). Ščitek postane marmorirano rjavosiv in voščena obloga na hrbtnu razpoka v pravokotne ploščice. Marginalne ščetine so povsem podobne stigmatalnim; so tope in krepke.

Proti koncu maja začne samica izlegati jajčeca v jajčno vrečko (slika 1), ki je rahlo prečno nažlebičena, z osrednjo brazdo in voskastimi lepljivimi vlakni. V jajčni vrečki je okoli 3000 (Hadzibejli, 1955; Pellizzari, 1997), po nekaterih podatkih celo okoli 8700 jajčec (Conard, 1966), ki so bledo rožnate barve. Nimfe gredo skozi dve nimfalni stopnji.



Slika 1: Samice *N. innumerabilis* z jajčno vrečko (foto: I. Mavrič Pleško)

Picture 1: The adult females of *N. innumerabilis* with egg sac (photo: I. Mavrič Pleško)

### 2.1.2 Navadni trtni kapar (*Pulvinaria vitis* [L.])

Navadni trtni kapar je polifagna vrsta, najpogosteje pa se pojavlja na vinski trti. Napada liste, korenine in tudi stebla (Kosztarb in Kozar, 1988). Vrsta *P. vitis* je v Evropi splošno razširjena (Ben-Dov in sod., 2012), z gospodarskega stališča pa je precej nepomembna (Masten in sod., 2007).

Samice navadnega trtnega kaparja so manjše od samic velikega trtnega kaparja. Dolge so od 2,5 do 6,5 mm, široke pa od 1,5 do 6,5 mm (Kosztarb in Kozar, 1988). Ščitek imajo enakomerno rjav, marginalne ščetine so tanjše in drugačne kot stigmatalne. Hrbtna stran ne razpoka kot pri velikem trtnem kaparju, temveč ostane cela. Jajčna vrečka nima prečnih žlebičev in vzdolžnega grebena, voskasta vlakna niso lepljiva. Jajčeca v njej so intenzivnejših barv, od oranžnorumene do bledo vinsko rdeče barve.

Mlade samice prezimijo pretežno na dvoletnem, pa tudi starejšem lesu (Seljak, 1995), spomladi pa zelo hitro zrastejo do svoje končne velikosti. Jajčeca odlagajo od konca aprila do začetka junija. Samica v povprečju odloži okoli 3500 jajčec v jajčno vrečko, ki ima bombažni izgled in je nameščena pod ali za telesom samice. Proti koncu maja se začno izlegati mlade nimfe. Te se naselijo ob listne žile na spodnji strani lista. Razvoj navadnega trtnega kaparja poteka prek treh nimfalnih stopenj. V septembru (Pellizzari, 1997) in oktobru (Kosztarb in Kozar, 1988) se odrasle samice parijo in se nato ob koncu oktobra preselijo na veje, kjer prezimijo (Pellizzari, 1997). Vrsta *P. vitis* je univoltinna. Schumutterer (1952) navaja, da se v Evropi navadno razmnožuje spolno, znano pa je, da se lahko razmnožuje tudi partenogenetsko. V Severni Ameriki se razmnožuje le partenogenetsko (Phillips, 1963; Grill, 1988; Kosztarb in Kozar, 1988).

### 2.1.3 Češpljev kapar (*Parthenolecanium corni* [Bouché])

Češpljev kapar ima več poimenovanj, med katerimi sta najpogostejsa *Eulecanium corni* Bouché (Janežič, 1954; Hamon, 1984; Kosztarb in Kozar, 1988;) in *Lecanium corni* Bouché (Hamon, 1984; Kosztarb in Kozar, 1988;).

Kapar *P. corni* je kozmopolitska in polivoltinna vrsta. Letno ima od enega do treh rodov, v razvoju pa se pojavljata dve nimfalni stopnji (Pellizzari, 1997). Število rodov je odvisno od podnebja in gostiteljskih rastlin. Na severu Madžarske so zabeležili en rod, na jugu pa dva rodova na leto. O treh rodovih so poročali iz Kavkaza in iz Srednje Azije (Kosztarb in Kozar, 1988).

Oblika, velikost in obarvanost odraslih samic je odvisna od njihove starosti in gostiteljskih rastlin, na katerih se prehranjujejo. Popolnoma odrasle so dolge okoli šest in široke okoli štiri mm. So rjave barve, hrbtna stran telesa pa je precej konveksna in močno sklerotizirana. Vrsta *P. corni* prezimi v drugi stopnji nimfe. Proti koncu maja se razvijejo odrasli osebki in oplojene samice začnejo odlagati jajčeca. Nov zarod kaparjev se razvije v drugi polovici junija. Mlade nimfe se prehranjujejo na spodnji strani listov, po prehodu v drugo stopnjo pa se preselijo na lesnate dele.

O masovnih izbruhih vrste *P. corni* so poročali iz Evrope po letu 1880, pojavljala pa se je v od deset - do enajstletnih intervalih. Od zadnjega masovnega pojava te vrste leta 1955 je njena številčnost močno upadla, predvidoma zaradi parazitiranja vrste *Blastotrix confusa* Erdös. Večji izbruh je bil nato ugotovljen še leta 1975, vendar v precej manjšem obsegu kot v preteklosti (Kosztarb in Kozar, 1988). V Evropi je češpljev kapar splošno razširjen (Ben-Dov in sod., 2012), v Sloveniji pa je bil prvič najden leta 1954 (Janežič, 1954).

## 2.2 Družina Pseudococcidae

Družina Pseudococcidae je zelo raznolika. Telo samice je navadno ovalne oblike, sestavljen je iz deset segmentov (McKenzie, 1967) in je sive, rožnate ali najpogosteje rumenkaste barve, odvisno od vrste. Tipalke so sestavljene iz dveh do devetih segmentov, oči so kupolaste ali manjkajo. Za razliko od predstavnikov družine Coccidae sta pri tej družini prisotna le dva para dihalnih odprtin. Za predstavnike družine Pseudococcidae so značilni cerariji, strukture nameščene ob robu, vzdolž hrbtne strani telesa. Sestavljene so iz por in set, včasih tudi sklerotizirane (Kosztarb in Kozar, 1988).

Odrasle samice družine Pseudococcidae prepoznamo po vulvi, ki jo najdemo na

trebušni strani med osmim in devetim telesnim segmentom (Kosztarb in Kozar, 1988). Samice izločajo na hrbtne strani voščeni poprh, s katerim se zaščitijo, jajčeca pa izlegajo v jajčno vrečko iz voščenih niti, ki so podobne barve kot samica. Jajčeca so ovalna, rumenkasta, dolga okoli 0,5 mm in široka med 0,2 in 0,3 mm. Novo izlegle nimfe so na prvi pogled zelo podobne jajčecem, le da imajo dobro razvite dolge noge, šestčlenaste tipalke in majhne oči. Nimfe ne izločajo voščenega poprha, spola se na tej razvojni stopnji ne da določiti. Nimfe samic druge razvojne stopnje so podobne prvi, od katere se razlikujejo po nekaj razvitih tubularnih cevkah na obeh straneh telesa in odprtini na zadnjem koncu hrbtne strani, ki je zastopana pri večini primerkov (slika 2).



Slika 2: Ličinke kaparjev iz družine Pseudococcidae različnih razvojnih stopenj (foto: I. Mavrič Pleško)

Picture 2: The immature stages of Pseudococcidae (photo: I. Mavrič Pleško)

Samci druge razvojne stopnje so podobni samicam, a imajo več cevk in razvit dodaten člen na tipalkah. Nimfe tretje razvojne stopnje samic imajo razvitih več por in cevk kot mlajše stopnje, odprtina na zadnjem delu je razvita, prav tako tudi dodaten segment na tipalkah, vendar eden manj kot pri odraslih osebkih. Pri tretji nimfalni stopnji samcev ali t.i. predbubi se noge in tipalke zmanjšajo. Imajo le še nekaj kratkih set in kratke zasnove kril. Naslednji stadij pri samicah je buba, pri

kateri se podaljšajo osnove kril, deseti segment telesa sklerotizira, delno otrdi tudi predel glave in trupa. Telo odraslega samca je podolgovato vretenasto. Navadno ima dobro razvit par kril na mezotoraksu, dva para enostavnih oči in par lateralnih ocelijev. Tipalke in noge so dolge s številnimi setami. Znane so najmanj štiri oblike odraslih samcev: krilati, nekrilati, z reduciranimi krili ali s telesom, ki spominja na nimfo (Kosztarb in Kozar, 1988).

Samica proti koncu maja in v začetku junija izleže v jajčno vrečko več kot 1800 jajčec. Iz teh se v treh do šestnajstih dneh razvijejo nimfe. Sprva se aktivno premikajo in isčejo najustreznejše mesto za prehranjevanje. Nimfe se v drugi razvojni stopnji večinoma vrnejo v razpoke na veje in debla gostiteljskih rastlin na prezimovanje, kjer so zbrane v majhnih voščenih kokonih (Kosztarb in Kozar, 1988). V času prezimovanja lahko preidejo v stadij mirovanja jajčeca, mlade nimfe in odrasle samice (Miller, 2005). Spomladi se nimfe in odrasle samice hranijo na deblih in vejah (Kosztarb in Kozar, 1988). Evropske vrste teh kaparjev imajo na prostem navadno od enega do tri robove na leto, v zavarovanih prostorih pa celo od šest do osem. Tudi nekatere vrste te družine se lahko razmnožujejo partenogenetsko (McKenzie, 1967; Kosztarb in Kozar, 1988). Večina kaparjev družine

Pseudococcidae, ki živijo na vinski trti, tudi vrsta *Planococcus ficus*, lahko sklene razvojni krog v celoti na tej gostiteljski rastlini (Walton in Pringle, 2004b).

### 2.2.1 Smokvin volnati kapar (*Planococcus ficus* [Signoret])

Telo vrste *P. ficus* je svetlo rožnate do svetlo sive barve, pokrito z voščenim poprhom, ki postane med razvojem bolj viden. Odrasle samice so ovalne, dolge približno štiri in široke malo več kot dva mm (Walton in Pringle, 2004b). Tipalke imajo sestavljene iz osmih segmentov. So brez kril. Za razliko od samic so samci krilati, s tremi pari lateralnih ocelijev ter dolgimi filamentoznimi analnimi setami (Kreiger, 1954 povz. po Walton in Pringle, 2004b) (slika 3).



**Slika 3:** Smokvin volnati kapar, odrasla samica (levo) in samec (desno) (foto: M. Štrukelj)  
**Picture 3:** The vine mealybug, adult female (left) and male (right) (photo: M. Štrukelj)

Seljak (2007) je smokvinega volnatega kaparja našel leta 2000 na vinski trti (*Vitis vinifera*) in gumovcu (*Ficus elastica*). Zaradi podobnosti lahko to vrsto zamenjamo s citrusovim kaparjem (*Planococcus citri* [Risso]), in vrstama *Planococcus minor* (Maskell) in *Planococcus halli* Ezzat & McConnell (Miller

in sod., 2007). Te vrste nimajo enakih gostiteljskih rastlin. Vrsto *P. ficus*, kot že prej omenjeno, najpogosteje najdemo na vinski trti, medtem ko je vrsta *P. halli* pogosta na krompirju, vrsta *P. minor* pa je precej polifagna (Miller in sod., 2007).

### 3 ŠKODLJIVOST IN NAČINI ZATIRANJA KAPARJEV

Kaparji so pomembni škodljivci vinske trte, ki so s sesanjem rastlinskega soka neposredno škodljivi in lahko povsem izčrpajo rastline. Oslabljene rastline so dovzetnejše za napade drugih škodljivcev in okužbe s patogenimi organizmi. Posredna škoda nastaja zaradi izločanja medene rose, na katero se naselijo glice sajavosti, ki zmanjšujejo fotosintezo ter vplivajo na razvoj in tržno vrednost rastlin in pridelka.

Kaparji predstavljajo precejšen problem pri pridelavi grozdja, zato jih je potrebno zatirati bodisi z uporabo insekticidov ali z okolju prijaznejšimi načini varstva rastlin. Preden se lotimo njihovega zatiranja moramo dodobra spoznati njihovo bionomijo. Za insekticide so najbolj občutljivi v začetku razvoja, ko so nimfe še popolnoma brez zaščite oziroma voščenega poprha. Navadno so nimfe skrite na spodnji strani listov, pod lubjem in v razpokah, kar otežuje njihovo zatiranje. Kolonije odraslih kaparjev družine Pseudococcidae so zavarovane z voščenim poprhom, predstavniki družine Coccidae pa s ščitkom, kar zmanjšuje učinkovitost insekticidov.

Prvi poskusi kemičnega zatiranja velikega trtnega kaparja so bili v Sloveniji opravljeni v začetku devetdesetih let (Seljak, 1995). Zadovoljivo delovanje proti omenjenemu škodljivcu so dosegli s pripravkoma z aktivno snovjo diazinon (Seljak in Žežlina, 2007), ki pa v Sloveniji ni več na seznamu dovoljenih sredstev (Registrirana..., 2012). Za zatiranje kaparjev so trenutno v Sloveniji registrirani pripravki na podlagi abamektina, acetamiprida,

dimetoata, olja navadne ogrščice, parafinskega olja, piretrina, tiakloprida in tiametoksama (Registrirana..., 2012), vendar njihova učinkovitost pri zatiranju kaparjev še ni bila preučena (Seljak in Žežlina, 2007). Uporaba insekticidov negativno vpliva na naravne sovražnike. Z nepravilno in prepogosto rabo fitofarmacevtskih sredstev lahko povzročimo tudi pojav odpornosti kaparjev, zato moramo večjo pozornost nameniti okoljsko sprejemljivejšim načinom zatiranja teh škodljivcev, na primer mehanskemu in biotičnemu načinu zatiranja.

Mehanskih načinov se poslužujemo takrat, ko škodljivcev na rastlini ni veliko. Odstranimo jih lahko s krtačo ali s spiranjem s toplo vodo (Kosztarb in Kozar, 1988), dovolj učinkovito je tudi odstranjevanje kaparjev z rokavico ali krpo (Seljak in Žežlina, 2007). To pri predstavnikih družine Coccidae navadno napravimo v maju in v začetku junija, ko imajo samice razvito jajčno vrečko in se nimfe še niso izlegle (Seljak in Žežlina, 2007). V kolikor je populacija kaparjev na rastlinah številčnejša, je kemično zatiranje neizogibno.

Med okoljsko sprejemljive načine zatiranja štejemo tudi setev varovalnih posevkov (angl. cover crops) v vrste med glavne posevke. Namen teh posevkov je povečanje populacije koristnih organizmov in bolj raznolik ekosistem, saj organizmom zagotavlja več hrane in prostora (Trdan in sod., 2006; Laznik in sod., 2012). Ti posevki so bili v vinogradih učinkoviti le, če so privabili polonice (Coccinellidae) in mrežekrilce (Neuroptera)

(Bugg in Wassington, 1994). Costello in Daane (1996) pa z raziskavami nakazujeta, da varovalni posevki nimajo bistvenega vpliva na pojavljanje naravnih sovražnikov smokvinega volnatega kaparja v vinogradih.

### 3.1 Naravni sovražniki

Glavni cilj biotičnega varstva rastlin je oblikovanje naravnega ravnoesja med škodljivci in njihovimi naravnimi sovražniki ter s tem preprečitev močnejše namnožitve škodljivcev (Vrabl, 1990). Biotično varstvo rastlin vključuje plenilce in parazitoide, ki so naravni sovražniki kaparjev (preglednice 1-4) in lahko znatno zmanjšajo njihovo populacijo. Naravni sovražniki so navadno škodljivi le nimfam, občasno tudi jajčecem, ptice pa se

hranijo tudi z odraslimi kaparji in njihovimi jajčeci. V Sloveniji biotično zatiranje kaparjev še ni dobro raziskano, čeprav se je biotično varstvo nekaterih kaparjev v srednji Evropi izkazalo za uspešno (Kosztar in Kozar, 1988). Največ tovrstnih raziskav je bilo doslej narejenih v Južni Afriki (Walton in Pringle, 2004ab), Ameriki (Triapitsyn in sod., 2006) ter v Argentini in Iranu (Walton in Pringle, 2004ab).

Walton in Pringle (2004b) trdita, da imajo plenilci pomembnejšo vlogo v biotičnem varstvu vinske trte kot parazitoidi. V splošnem so polonice (Coccinellidae) najpomembnejši plenilci kaparjev. Med parazitoidi prevladujejo parazitske osice iz družin Aphelinidae in Encyrtidae (preglednice 1-4).

**Preglednica 1:** Naravni sovražniki velikega trtnega kaparja (*N. innumerabilis*) (Pellizzari, 1997; Seljak in Žežlina, 2007)

**Table 1:** Natural enemies of cottony maple scale (*N. innumerabilis*) (Pellizzari, 1997; Seljak in Žežlina, 2007)

Red in družina	Vrsta
Coleoptera: Anthribidae	<i>Brachytarsus nebulosus</i> Föster
Coleoptera: Coccinellidae	<i>Exochomus quadripustulatus</i> L.
	<i>Hyperaspis signata</i> Olivier
Hymenoptera: Aphelinidae	<i>Coccophagus lycimnia</i> Walker
	<i>Encarsia lutea</i> Masi
Hymenoptera: Chalcidoidea	<i>Blastothrix britannica</i> Girault
	<i>Blastothrix hungaricus</i> Erdös
Hymenoptera: Encyrtidae	<i>Metaphycus dispar</i> Mereet
	<i>Metaphycus insidiosus</i> Mercet
	<i>Metaphycus punctipes</i> Dalman

**Preglednica 2:** Naravni sovražniki malega trtnega kaparja (*P. vitis*) (Kosztarb in Kozar, 1988; Pellizzari, 1997)

**Table 2:** Natural enemies of cottony grape scale (*P. vitis*) (Kosztarb in Kozar, 1988; Pellizzari, 1997)

Red in družina	Vrsta
Coleoptera: Anthribidae	<i>Brachytarsus nebulosus</i> Föster
Coleoptera: Coccinellidae	Vrste iz rodu <i>Scymnus</i>
Diptera: Chamomyiidae	<i>Leucopis (Leucopomya) silesiaca</i> Egger <i>Leucopis annilipes</i> Zetterstedt <i>Leucopis nigricornis</i> Egger
Hymenoptera: Aphelinidae	<i>Coccophagus gigas</i> Eldos <i>Coccophagus insidiator</i> Dalman <i>Coccophagus lycimnia</i> Walker <i>Coccophagus scutellaris</i> Dalman
Hymenoptera: Encyrtidae	<i>Cheilonerus formosus</i> Boheman <i>Encyrtus albitalis</i> Zetterstedt <i>Metaphycus dispar</i> Mercet <i>Metaphycus insidiosus</i> Mercet <i>Metaphycus punctipes</i> Dalman <i>Microterys duplicatus</i> Nees
Neuroptera: Chrysopidae	<i>Chrysoperla carnea</i> Stephen

**Preglednica 3:** Naravni sovražniki češpljevega kaparja (*P. corni*) (Kosztarb in Kozar, 1988; Pellizzari, 1997)

**Table 3:** Natural enemies of brown scale (*P. corni*) (Kosztarb in Kozar, 1988; Pellizzari, 1997)

Red in družina	Vrsta
Coleoptera: Coccinellidae	<i>Adalia bipunctata</i> L. <i>Chilocorus bipustulatus</i> L. <i>Coccinella septempunctata</i> L. <i>Exochomus quadripustulatus</i> L.
Hymenoptera: Aphelinidae	<i>Coccophagus lycimnia</i> Walker <i>Coccophagus scutellaris</i> Dalman
Hymenoptera: Chalcidoidea	<i>Blastothrix confusa</i> Erdös
Hymenoptera: Encyrtidae	<i>Metaphycus insidiosus</i> Mercet <i>Microterys sylvius</i> Dalman <i>Trichomasthus albimanus</i> Thomson
Neuroptera: Chrysopidae	<i>Chrysopa perla</i> L.

**Preglednica 4:** Naravni sovražniki smokvinega volnatega kaparja (*P. ficus*) (Mgocheki in Addison, 2009; Triapitsyn in sod., 2007; Walton in Pringle, 2004ab)

**Table 4:** Natural enemies of vine mealybug (*P. ficus*) (Mgocheki in Addison, 2009; Triapitsyn in sod., 2007; Walton in Pringle, 2004ab)

Red in družina	Vrsta
Coleoptera: Coccinellidae	<i>Cryptolaemus montrouzieri</i> Mulsant <i>Cydonia lunata</i> F. <i>Hippodamia</i> sp. <i>Hyperaspis felixi</i> Mulsant <i>Nephus angustus</i> Casey <i>Nephus binaevatus</i> Mulsant <i>Nephus quadrivittatus</i> Mulsant <i>Nephus reunioni</i> Fürsch <i>Rhizobiellus</i> sp. <i>Scymnus nubilis</i> Mulsant
Diptera: Chamamelyidae	<i>Leucipis</i> sp.
Hymenoptera: Encyrtidae	<i>Allotropa mecrida</i> Walker <i>Angyrus pseudococci</i> Girault <i>Chartocerus subaeneus</i> Förster <i>Cheilonerus</i> spp. <i>Chrysoplatycerus splenders</i> Howard <i>Clausenia josefi</i> Rosen <i>Coccidoxenoides perminutus</i> Timberlake <i>Lapmastidea abnormis</i> Girault <i>Laptomastix dactylopii</i> Howard <i>Laptomastix flavus</i> Mercet <i>Prochiloneurus pulchellus</i> Silvestri
Hymenoptera: Pteromalidae	<i>Pachyneuron concolor</i> Förster <i>Pachyneuron</i> sp.
Hymenoptera: Signiphoridae	<i>Chartocerus</i> spp.
Neuroptera: Chrysopidae	<i>Chrysoperla carnea</i> Stephen

V Južni Afriki so raziskovalci v dvoletnjem poljskem poskusu uporabili parazita *Coccidoxenoides perminutus* za zatiranje vseh razvojnih stopnej ličink vrste *P. ficus*. Walton in Pringle (2004b) poročata, da je bil tak način zatiranja vsaj tako učinkovit kot zatiranje škodljivca z uporabo insekticidov.

Po podatkih Seljaka in Žežline (2007) sta pri nas naravna sovražnika velikega trtnega kaparja polonica *Exochomus quadripustulatus* in osica *Coccophagus lycimnia*. Ostali plenilci

kaparjev pa v Sloveniji za enkrat še niso preučeni.

Učinkovitost naravnih sovražnikov zmanjšujejo mravlje z branjenjem kaparjev (Buckley in Gullan, 1991; Mgocheki in Addison, 2009). Kaparji z izločanjem medene rose oskrbujejo mravlje s hrano, slednje pa jih zato branijo ter hkrati preprečujejo odlaganje jajčec parazitskih organizmov v telesa kaparjev.

#### 4 ZAKLJUČKI

S pospešeno globalizacijo trgovanja z rastlinami se povečuje vnos žuželk in drugih organizmov v njim tujerodno okolje. Kaparji predstavljajo kar 41,1 % (37 vrst) vseh polkrilcev (Hemiptera), ki so bili v zadnjih 20 letih vneseni ali pa so se postopno razširili v Slovenijo (Seljak, 2011). Škodljivi so predvsem na sadnem drevju, vinski trti, grmičevju in rastlinah v zavarovanih prostorih (Kosztarb in Kozar, 1988). Seljak in Žežlina (2007) izpostavljata štiri vrste kaparjev, ki lahko v Sloveniji povzročajo gospodarsko škodo na vinski trti; velikega trtnega kaparja, navadnega trtnega kaparja, češpljevega kaparja in smokvinega volnatega kaparja. Kaparji se hranijo z rastlinskimi sokovi in povzročajo postopno propadanje rastlin. Na svoje gostitelje ne vplivajo le neposredno, s sesanjem, vendar tudi posredno, s prenosom virusov vinske trte ter izločanjem medene rose, na katero se lahko naselijo glive sajavosti, ki lahko povzročijo precejšnjo gospodarsko škodo. Kaparje lahko zatiramo z insekticidi in mehanskimi ukrepi, vse bolj pa prihaja v ospredje zavedanje o pomenu biotičnega varstva rastlin.

Insekticidi, ki so jih v Sloveniji proti velikemu trtnemu kaparju precej učinkovito uporabljali pred nekaj leti, danes niso več na seznamu dovoljenih sredstev. Na splošno so insekticidi najbolj učinkoviti proti na novo izleglim

nimfam kaparjev, ki niso zaščitene z voščenim poprhom, kot na primer odrasle samice.

Biotično varstvo vinske trte temelji na uporabi plenilcev in parazitoidov, ki so naravni sovražniki kaparjev in lahko precej zmanjšajo njihovo populacijsko gostoto. Po nekaterih navedbah naj bi imeli plenilci, zlasti polonice (Coccinellidae), pri zatiranju kaparjev večji pomen od parazitoidov (predstavnikov družin Aphelinidae in Encyrtidae).

Pri uvajanju biotičnega varstva rastlin, ki temelji na uporabi vrst, ki v našem okolju naravno niso zastopane (klasično biotično varstvo), oziroma na uporabi tako imenovanih tujerodnih generalistov, pa moramo biti izjemno previdni, da ne povzročimo okolju več škode kot koristi. Polonica *Exochomus quadripustulatus* in osica *Coccophagus lycimnia* sta v Sloveniji po navedbah Seljaka in Žežline (2007) naravna sovražnika velikega trtnega kaparja, ostali morebitni plenilci pri nas zaenkrat še niso raziskani. Z ozirom na sodobne usmeritve zdravstvenega varstva rastlin, ki težijo k manjši rabi za okolje manj - ali celo nesprejemljivih fitofarmacevtskih sredstev, je potrebno skupaj z drugimi, okolju prijaznejšimi načini preučiti možnosti uporabe naravnih sovražnikov za zatiranje kaparjev vinske trte.

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# **CONTENT ANALYSIS OF THE PAPERS IN THE ACTA AGRICULTURAE SLOVENICA**

## **VSEBINSKA OBDELAVA PRISPEVKOV V ACTA AGRICULTURAE SLOVENICA let. 99 št. 2**

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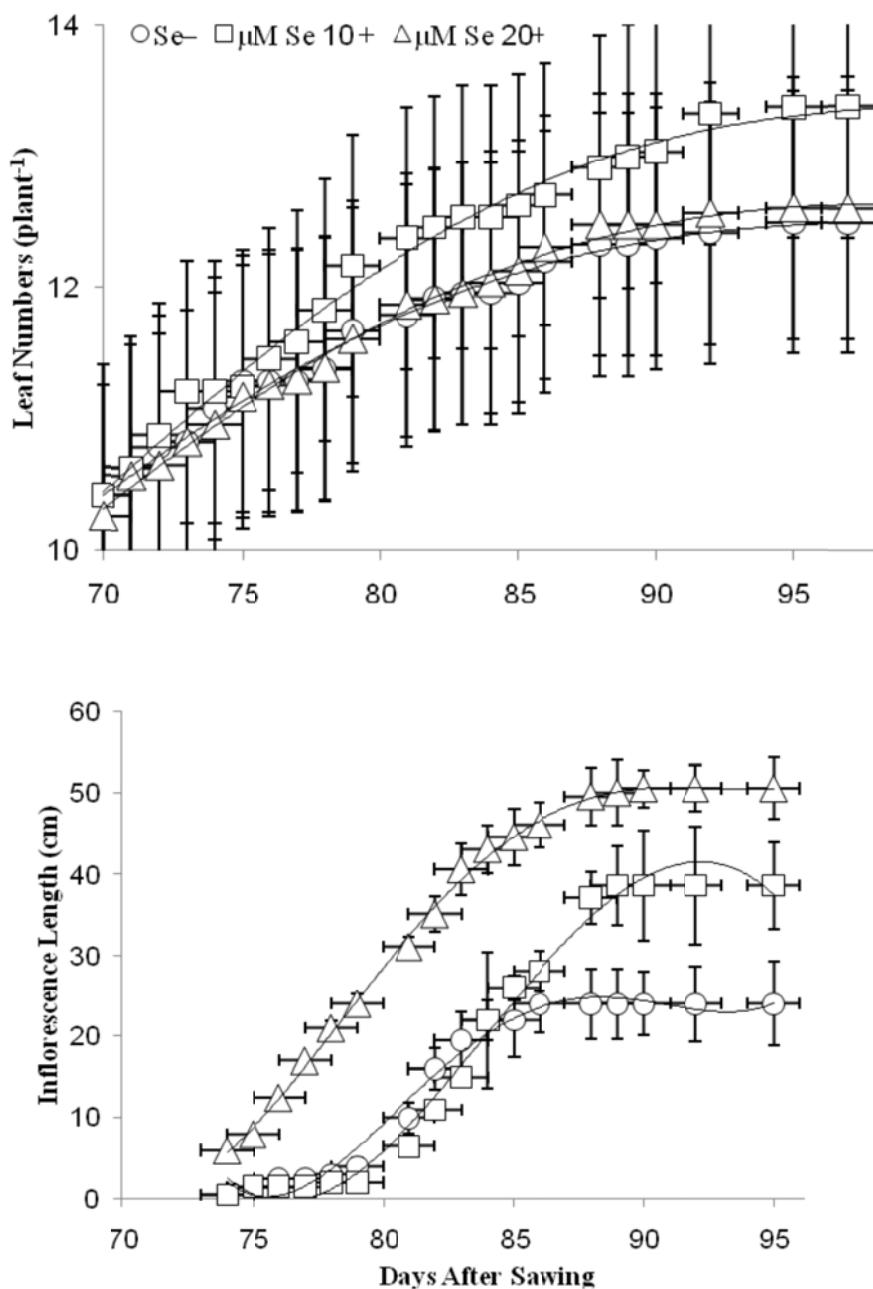
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## POPRAVEK/CORRIGENDUM

Za zvezek 99-1 pravilna slika v članku Selenium supplementation stimulates vegetative and reproductive growth in canola (*Brassica napus* L.) plants (na strani 16).

In issue 99-1 correct picture in article Selenium supplementation stimulates vegetative and reproductive growth in canola (*Brassica napus* L.) plants (on page 16).



**Figure 1:** Effect of Se supplementation on the number of leaves (a) and length of stem (b) in canola (*Brassica napus*) plants grown in greenhouse



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(letniki z liho številko - rastlinska proizvodnja)

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Sprejemamo izvirne znanstvene članke s področja agronomije, hortikulture, rastlinske biotehnologije, raziskave živil rastlinskega izvora, agrarne ekonomike in informatike ter s sorodnih področij - **letniki z liho številko** (npr. 97, 99) - v slovenskem in angleškem jeziku; pregledne znanstvene članke samo po poprejšnjem dogovoru. Objavljamo tudi izbrane razširjene znanstvene prispevke s posvetovanji, vendar morajo taki prispevki zajeti najmanj 30 % dodatnih originalnih vsebin, ki še niso bile objavljene. O tovrstni predhodni objavi mora avtor obvestiti uredniški odbor. 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.

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