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VSEBINA / CONTENTS

STRUCTURE OF HOP FARMS IN THE EU AFTER THE YEAR 2000

Martin PAVLOVIČ[1]

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Abstract

A study of the structure of hop farms in the EU based on an expert questioner's survey and a review of the literature available was carried out in 2009. The hop-producing countries included in the survey were members of the International Hop Growers Convention (IHGC). The results demonstrate that the production structure in the hop industry sector varies greatly across EU countries. In addition, the structure is changing due to a market-driven structural adjustment aimed at being more competitive. The number of farms growing hops in the main hop-producing countries in the EU declined significantly during the 2000-2008 period. More than an estimated 1,350 growers in the EU-27 stopped growing hops during the period 2001-2007. As a result, the average farm size increased in almost all EU member states. The rate of specialization of hops farms is generally increasing. Briefly, hop farmers are slowly becoming entrepreneurs, and most try to attain a farm size that makes production more profitable.

Key word: hop industry, farming structure, number of holdings, IHGC

STRUKTURA HMELJARSKIH KMETIJ V EU PO LETU 2000

Izvleček

Raziskava o strukturi hmeljarskih kmetij v EU temelji na rezultatih ekspertnega vprašalnika in pregleda obstoječe literature ter vključuje države članice Mednarodne hmeljarske organizacije (IHGC). Rezultati kažejo, da se struktura posestev, na katerih se ukvarjajo s pridelavo hmelja, med državami EU precej razlikuje. Spreminja se pretežno zaradi prilagoditev na tržne razmere z namenom povečevanja njihove konkurenčnosti. Med letoma 2000 in 2008 se je število kmetij v pomembnejših državah pridelovalkah bistveno zmanjšalo. Velja ocena, da je v EU-27 med 2001 in 2007 preko 1.350 hmeljarjev prenehalo s pridelavo. Posledično se je v skoraj vseh državah povečala povprečna površina hmeljišč na kmetijah. Tudi stopnja specializacije hmeljarskih kmetij se je povečala. Hmeljarji postopno postajajo podjetniki in težijo k povečevanju obsega pridelave, kar bi jim zagotavljalo dobičkonosno kmetijsko proizvodnjo.

Ključne besede: hmeljarstvo, struktura kmetij, število posestev, IHGC

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1 INTRODUCTION

Hops (*Humulus lupulus* L.) essentially contribute to the quality of the taste of beer and its flavor. Hops are a specialty crop produced for the female flowers (cones), which either raw or processed, are an essential ingredient in the production of beer. Lupulin glands on the hop cones contain soft resins (alpha acids and beta acids), essential oils that impart bitterness, flavor, aroma, foam (head) characteristics, and preservative qualities to beer. The total amount and percentage composition of these compounds vary with variety, region, growing conditions, and production technique. Because the brewing industry depends on hops to provide distinctive and proprietary characteristics to beer, a stable supply of high-quality hops is a high priority [5,14].

Hop plants in the European Union (EU) are grown on a wire and cable trellis usually suspended about 6 to 7 meters above the ground on a regular arrangement of wooden or concrete poles. Anchors, attached to trellis cables, surround the yard and hold the trellis upright under the weight of the developing crop. Plant spacing depends mostly on hop variety and growing area, with 2.4 to 3.2 m between rows and about 1.1 to 1.7 m between plants within rows. Once established, the hop rootstock will produce indefinitely although industry practice is to rotate plantings every 15-20 years. The timing of the rootstock replacement is influenced by declining yield caused by insects, disease and pests and by merchants', i.e., brewers', demand for specific varieties. The major production practices used annually to produce hops include pruning, stringing, training, irrigating, protecting plant against pests and diseases, harvesting, drying as well as processing and packing according to market demands [1,3,5].

The hop industry is one of the highest capital- and work-intensive types of agricultural production. It is estimated that on EU competitive hop farms (more than 10 ha of hops) the initial capital investment required for hop fields with wirework is more than 15,000 EUR/ha. Additional investments for specialized mechanization such as spraying and picking machines as well as a hop kiln with all necessary equipment would require at least an additional 25,000 EUR/ha. The amount of machine and labor hours varies related to the level of mechanization. The amount ranges between 60 and 80 machine hours and 200 and 350 labor hours per ha [6,10]. Based on the model SIMAHOP, 39% of the variable costs in hop production involve hop picking and drying, 26% stringing and training of hop bines, 13% plant protection, 12% winter and spring activities in hop fields, etc. with 10 ha of hops and an average yield of 1,800 kg/ha [10,11]. The total model production costs in Slovenia in 2009 were 5.25 EUR/kg of dry hops, while the model average variable costs were 3.93 EUR/kg [13].

The European Union is the main player in the world hops market. Hops are produced by fourteen EU member states although together Germany and the Czech Republic account for more than 80% of the total EU production by volume. Poland is the only other member state to account for more than 5% of total EU production. Traditional hops production areas can be found within each hop-producing member state, including Bavaria, Saxony, and Bitburg in Germany; Bohemia in the Czech Republic; the Lublin region in eastern Poland; Savinja Valley, Ptuj, and the Koroška region in Slovenia; the Kent and Hereford area in England; the León area in Spain; Alsace in France; the Horna Streda region in Slovakia; the Poperinge area in Belgium, etc [6,8].

In the period 2001-2008, the hops-growing surface area in EU countries varied from 32,569 ha (21,554 ha of aroma hops and 11,015 ha of bitter hops) in 2001 to 29,705 ha (19,756 ha of aroma hops and 9,949 ha of bitter hops) in 2008 [6,7].

In 2008, the total EU hop production was about 57,000 t, more than 50% of the world hops production. The largest producer within the EU is Germany (39,676 t), followed by the Czech Republic (6,753 t), Poland (3,446 t), Slovenia (2,359 t), France (1,469 t), the UK (1,410 t), etc. Hops acreage is decreasing steadily in the EU, with a 16% reduction since 2001. Bitter varieties are grown in about one-third of the area. This percentage has been constant throughout the last eight years [6,7].

Hop growers must respond to the ever-changing needs of the brewing community by providing appropriate varieties at a certain quality demanded by the market as well remain competitive in the global hop industry [14]. An important issue related to competitiveness is the production structure in the hop industry sector (number of holdings, average farm size, and rate of specialization), which will be discussed in this paper.

2 METHODOLOGY

The research was carried out as a part of the Evaluation of the CAP Measures Related to Hops project under the Framework contract No 30-CE-0219319/00-20 for the EU DG-AGRI in 2009 [4,7]. To collate data about the change in the farm structure and organization in the hop industry in EU countries, various methods were used. First, a questionnaire related to farming structure and national organizations in the hop industry was sent to the 11 national representatives of the IHGC member countries. Second, two 2-day field trips to the most important EU hop-producing countries such as Germany and the Czech Republic were organized and carried out by the author to collect additional detailed information. Third, supplementary telephone interviews with 7 EU national hop experts were conducted. In addition, a business report and text from hop merchant companies were analyzed [2,6].

3 RESULTS AND DISCUSSION

3.1 Number of hop farms (holdings) in EU

During the 2000-2008 period, the number of holdings growing hops declined significantly in the main hop-producing countries (Table 1). The reduction ranges from 10.9% in Poland to 37.7% in Spain. In Germany, the decrease was 22.9%, with a loss of 446 farms.

While the number of holdings has decreased, the average acreage per holding has increased in all the listed countries from +2.5% in the Czech Republic to +31.6% in Germany. These data series show a large variability in average acreage across member states. The largest holdings are in the Czech Republic (40.7 ha per holding in 2008), and the smallest are in Spain and Poland (around 2 ha per holding).

Table 1: Number of hop farms and average acreage per farm in major hop-producing countries (2002-2008)

Preglednica 1: Število hmeljarskih kmetij in povprečna površina hmeljišč na posestvo v pomembnejših državah pridelovalkah (2002-2008)

Country	Indicators	2002	2003	2004	2005	2006	2007	2008	2000/08 Change (%)
Germany	Nr. of farms	1943	1710	1698	1611	1554	1510	1497	-22.9
	ha/farm	9.5	9.7	10.3	10.7	11.1	11.7	12.5	+31.6
Czech Rep.	Nr. of farms	185	165	162	145	145	139	131	-29.2
	ha/farm	40.0	36.0	36.0	39.0	37.0	39.0	41.0	+2.5
Poland	Nr. of farms	1191	1129	1121	1144	1113	1066	1061	-10.9
	ha/farm	1.9	1.9	2.0	2.0	2.0	2.0	2.1	+10.5
Slovenia	Nr. of farms	189	186	176	176	150	140	140	-25.9
	ha/farm	9.6	8.9	8.8	8.8	10.1	11.0	11.0	+14.6
UK-England	Nr. of farms	85	76	60	60	60	60	58	-31.8
	ha/farm	21.4	19.0	22.6	17.9	17.4	17.7	18.5	-13.6
France	Nr. of farms	111	100	96	96	96	90	89	-19.8
	ha/farm	7.4	8.2	8.2	8.4	8.3	8.8	9.3	+25.7
Spain	Nr. of farms	398	400	395	353	325	248	248	-37.7
	ha/farm	1.7	1.7	1.7	1.9	1.9	2.0	2.0	+17.7
Belgium	Nr. of farms	52	49	47	45	44	42	29	-44.2
	ha/farm	4.8	4.7	4.4	4.6	4.5	4.4	5.8	+20.7
Portugal	Nr. of farms	14	12	12	12	7	4	4	-71.4
	ha/farm	2.6	3.1	3.1	3.3	2.6	5.3	5.0	+89.2
Austria	Nr. of farms	72	73	70	70	67	65	63	-12.5
	ha/farm	3.1	3.0	3.0	3.0	3.0	3.1	3.3	+9.6
USA	Nr. of farms	60	60	52	52	56	62	74	
	ha/farm	196.3	188.6	216.0	227.3	212.7	201.7	267.0	

Source: [4,6,7]

In the period 2004-2007, according to the data available for all member states, more than 480 farms abandoned hop production. Comparable data for the period 2001-2007 were not at hand. However, if we keep the number of farms abandoning hops growing in the new member states (which makes a conservative estimate) constant, we estimate that more than 1,350 farms in Europe stopped producing hops in the period 2001-2007.

Growers mostly exit the hop sector as their farms and hop gardens are not able to guarantee a sufficient income. This phenomenon is affecting old farmers, whose farms are not continued by younger generations, and farmers who have small farms. Land abandonment is thought to occur rarely, but no figure exists for hops. Farmers who stop growing hops normally sell their hop gardens to other hop growers, who continue to grow hops.

3.2 Average size of hop farms

The average hop acreage per farm increased in almost all the member states because several farmers stopped growing hops. The farmers mostly stopped because of ageing rather than for economic reasons, according to the interviewees. However, the economic component might be stronger than what the interviewees suggested. Related to measures of the EU Common Agricultural Policy (CAP) in the hop sector 2004-2008, some growers of the countries

adopting full decoupling may wait to leave the hops sector until they face the next heavy investment (for instance, when renewing hop gardens) and exit at this point, keeping the decoupled support. No figures are available at the national level on the causes of the cessation of hops production, so the estimates are based on personal assumptions made by the interviewees. The hops gardens were mainly sold to other farmers who stayed in business.

Interviewees have confirmed that the increase in average farm size and increase in specialization trend existed before the CAP reform (before 2004); the stakeholders visited link this restructuring to market pressure (mainly to more competition and to the cost structure of hop farming, which can be extremely onerous) rather than to the CAP reform. Hop farmers are slowly becoming entrepreneurs; thus, most try to attain a farm size that makes production more profitable. The main concern of farmers, according to data gathered in the interviews, is to be able to spread the high fixed costs generated by hop growing over a sufficient number of hectares, so that the farmers can make profits per hectare. When this is not possible, hop growers are slowly stopping hop production, according to the interviewees. Spain, for instance, is an emblematic case in this sense.

Most Spanish and Polish hop holdings are extremely small (< 2 ha) so farmers do not find it convenient to invest in machinery and in new technology. In the long term, farmers will either abandon hop growing or will expand their business to become specialized. The size threshold that makes a farm profitable varies across countries. In Germany, a holding having 10 ha of hops starts being economically viable (once one takes subsidies into account). A similar size is estimated for Slovenia.

European hop farms (holdings) are becoming larger stepwise. The farm structure varies greatly across the EU countries. The main reason lies in economic competitiveness at the international level. No effect of the CAP reform after 2004 on a farming structure was discovered. The difference in the average size of European farms depends on historical and agronomic reasons. In the Czech Republic, the current farms are the heritage of the enormous socialist collective farms; thus, Czech farms are much bigger than the European average. On the other hand, hop farms in Poland and Slovenia used to be much smaller and predominantly in the hands of independent farmers during the socialist period. In Slovenia, the hop farms on average were significantly enlarged after structural changes when the company Hmezad kmetijstvo collapsed in 1999, from 3.5 ha to 10 ha per farm on average. Consequently about 1,000 ha of hop fields were part of local cooperatives and purchased by approximately 70 local hop farmers in the Savinja Valley [9]. On the other hand, in Western European countries hops were traditionally only one of the products grown by mixed farms [2].

The Tables 2 and 3 present in more detail the information synthesized. They provide a distribution in terms of class size for hop farms in 2003 and 2007. In this section, quantitative data for the countries - for which these data exist and the time series are complete - are provided.

Therefore, the average hop acreage per farm in Europe is increasing but is still much lower than in the USA. This may affect the competitiveness of European hops in the medium term.

Table 2: Hop farm structure by class size in 2003
Preglednica 2: Struktura hmeljarskih kmetij po velikosti v 2003

2003	< 2 ha	2<ha<5	5<ha<10	10<ha<20	20<ha<30	30<ha<50	50<ha<100	>100 ha	TOTAL
Belgium	0.0%	0.0%	0.0%	25.0%	25.0%	50.0%	0.0%	0.0%	100%
Czech R.	0.0%	0.0%	0.0%	6.3%	12.5%	6.3%	12.5%	62.5%	100%
Germany	0.0%	3.3%	11.5%	31.7%	20.8%	23.0%	7.1%	2.7%	100%
Spain	31.6%	57.9%	10.5%	0.0%	0.0%	0.0%	0.0%	0.0%	100%
France	0.0%	0.0%	16.7%	16.7%	8.3%	25.0%	25.0%	8.3%	100%
Austria	0.0%	0.0%	11.1%	33.3%	33.3%	22.2%	0.0%	0.0%	100%
Poland	8.7%	26.1%	41.7%	17.4%	2.6%	1.7%	0.9%	0.9%	100%
Slovenia	6.3%	6.3%	25.0%	25.0%	25.0%	12.5%	0.0%	0.0%	100%
UK	0.0%	0.0%	0.0%	6.3%	6.3%	12.5%	25.0%	50.0%	100%

Source: [4,7]

Table 3: Hop farm structure by class size in 2007
Preglednica 3: Struktura hmeljarskih kmetij po velikosti v 2007

2007	< 2 ha	2<ha<5	5<ha<10	10<ha<20	20<ha<30	30<ha<50	50<ha<100	>100 ha	TOTAL
Belgium	0.0%	0.0%	0.0%	25.0%	25.0%	50.0%	0.0%	0.0%	100%
Czech R.	0.0%	0.0%	0.0%	7.7%	0.0%	7.7%	15.4%	69.2%	100%
Germany	0.0%	3.2%	10.9%	28.2%	19.9%	23.1%	12.2%	2.6%	100%
Spain	30.8%	53.8%	15.4%	0.0%	0.0%	0.0%	0.0%	0.0%	100%
France	0.0%	0.0%	0.0%	16.7%	8.3%	50.0%	25.0%	0.0%	100%
Austria	0.0%	0.0%	50.0%	25.0%	0.0%	25.0%	0.0%	0.0%	100%
Slovenia	0.0%	10.0%	20.0%	20.0%	30.0%	20.0%	0.0%	0.0%	100%

Source: [4,7]

The productive structure of U.S. farms is more competitive than European farms. For an idea of the competitive advantage enjoyed by the United States in terms of production structure, the 12,510 ha devoted to hops in 2007 in the US were spread over 62 farms. This works out to 202 ha per farm, 18 times the average German farm and more than five times the average Czech farm.

3.3 Rate of specialization of hops farms

The rate of specialization of EU hop farms is generally increasing. The interview results showed that hop farms tend to become more specialized in Germany and Czech Republic. In Germany, the specialization rate (defined as the amount of revenues determined from hops of the overall farm revenues) for hop-producing farms increased from 42% in 2003 to 59% in 2006. A similar trend can be observed in the Czech Republic, with the specialization rate increasing from 16% in 2004 (the first year for which data were available) to 25% in 2006. As these data come from the FADN database, they are limited to these two countries.
Other EU countries had no hop sector FADN data available. However, a number of interviewees in other member states have confirmed this trend. Interviewees also linked the increased level of specialization to the high revenues that hops provide if cultivated on an adequate scale.

4 CONCLUSIONS

The EU hop industry sector, similar to the global hop trade and the world brewing industry, is facing a trend toward a concentration in capital investment and decision making. The following main findings related to the hop industry farming structure can be stated:

The production structure in the EU-27 is changing, which is mostly due to market-driven structural adjustment aimed at being more competitive. Growers are exiting the hop sector as their farms and hop gardens are not able to guarantee a sufficient income. No evidence regarding the influence of the CAP reform after 2004 on the production structure was discovered.

The average hop farm size is increasing in all EU member states. The growth in the average size is mainly due to the reduction in the number of growers, while the reduction in hop area is less pronounced. Small hop-producing countries with weak or no sector-linked national research and development support have seen a sharper decrease in growing area and in the number of farmers. In some countries, such as Spain, Belgium, Bulgaria, Portugal, and the UK, the reduction in the number of growers has endangered the very existence of the hops sector. The few farms left are becoming more specialized in hops in terms of equipment and other investments. However, the farms are still much smaller than in the U.S., and this could affect the competitiveness of European hops in the medium term.

With the exception of Germany, hop acreage in Europe is dwindling, following the global trend. This is mainly due to the launch of new bitter hop varieties by the USA and Germany that provide a higher yield per hectare so that less acreage is needed for the same amount of alpha acids, required by the global brewing industry. However, the acreage reduction was insufficient to prevent an oversupply of hops in 2009 and 2010. Again, farmers' on-time business decisions linked to making forward contracts for their crop production play a crucial role in the farmers' hop supply competitiveness at the end of the decade investigated [12].

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INFLUENCE OF ROW DISTANCE, PLANT DENSITY AND NUMBER OF TRAINING BINES ON YIELD AND ALPHA ACID CONTENT IN HOPS (*Humulus lupulus* L.) cv. DANA

Barbara ČEH[2]

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Abstract

The influence of row distance (2.4 m and 2.8 m), plant density (2,800 and 3,200 plants per hectare) and number of traininig bines per string (3, 4, 5) on the yield of hop cones and alpha acids content of cv. Dana was studied during the three years of hop vegetation, since 2007 till 2009. The field experiment was carried out on the experimental field of Slovenian Institute of Hop Research and Brewing in Žalec, designed as a random block field trial in three replications. Hop cultivar Dana is a middle late hop cultivar, bred at the Slovenian Institute of Hop Research and Brewing. It is a representative of so called high alpha hop cultivars. Weather conditions had the highest influence on the yield of hop cones, content of α-acids and α-acids yield. Lower plant density (2,800 plants/ha) would be recommendable in the hop field with 2.4 m row distance and higher plant density (3,200 plants/ha) would be recommendable at 2.8 m row distance in the conditions of the conducted experiment.

Key words: hops, *Humulus lupulus* L., hop cv. Dana, plant density, yield, alpha acids, training

VPLIV MEDVRSTNE RAZDALJE, GOSTOTE RASTLIN IN ŠTEVILA NAVITIH POGANJKOV NA PRIDELEK IN VSEBNOST ALFA KISLIN PRI HMELJU (*Humulus lupulus* L.) SORTE DANA

Izvleček

V letih 2007 do 2009 smo v poljskem bločnem poskusu v treh ponovitvah preučevali vpliv medvrstne razdalje (2,4 m in 2,8 m) in gostote rastlin (2.800 in 3.200 rastlin/ha) ter števila navitih poganjkov (3, 4, 5) na pridelek, pridelek alfa kislin in vsebnost alfa kislin v storžkih sorte Dana. Sorta Dana je žlahtnjena na IHPS, predstavnica sort z višjo vsebnostjo alfa kislin. V polnorodnem nasadu hmelja so imele največji vpliv na preučevane parametre vremenske razmere. Pri medvrstni razdalji 2,4 m je bila bolj priporočljiva manjša gostota rastlin (2.800 rastlin/ha), pri medvrstni razdalji 2,8 m pa zaradi značilno večjega pridelka večja gostota rastlin (3.200 rastlin/ha).

Ključne besede: hmelj, *Humulus lupulus* L., sorta Dana, gostota rastlin, pridelek, alfa kisline, napeljava

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1 INTRODUCTION

Hop breeding program of Slovenian Institute of Hop Research and Brewing was started some 50 years ago. Nowadays, 13 of reached hop cultivars are planted on more than 95 % of Slovenian hop fields. In the comparison with well-known aromatic Slovenian hop cultivars such as Aurora and Celeia, the newest hop cultivar Dana, released in 2007, has primarily bitter characteristics. Two important traits of cv. Dana, such as high content of α-acids and at the same time pleasant aroma, fit the variety into the recent qualitative demands of beer industry.

As for each new released cultivar, agrotechnique needs to be assessed to gain as much as possible from it. Among agro-technological factors plant density and plant spacing impact the yield and its quality at cultivated plants, as well.

Investigations about the impact of plant density and plant spacing on the yield of hops and its quality are not published for hops, while results of the investigations for other cultivated plants showed, for example, that more squared arrangement of plants has more positive impact on the yield compared to more rectangle plant distribution at the same plant density. It was confirmed in the experiment by Čeh at al. [4] for oilseed pumpkins, too. Other investigators report the impact of plant spacing on different parameters of cultivated plants as well [5,6,7].

In the presented paper the impact of plant density, plant spacing and the number of training bines at hops cv. Dana on the yield and its quality is presented.

2 MATERIAL AND METHODS

2.1 Material

The experiment was carried out in the hop field of cv. Dana, a middle late hop cultivar, bred at the Slovenian Institute of Hop Research and Brewing, representative of high alpha varieties. Its cones contain 12.5 % to 19.3 % alpha acids in dry matter. Technological maturity is reached in the last days of August or in the beginning of September. Essential oil content ranges from 3.1 ml/100g to 4.6 % in dry matter, the expected yield is between 1800 kg/ha and 2800 kg/ha. It is middle resistant to downy mildew (*Pseudoperonospora humuli* (Miyabe et Takahashi) G.W. Wilson) and mild form of hop *Verticillium* wilt, low resistant to powdery mildew (*Podosphaera macularis*), botrytis (*Botrytis cinerea*), and lethal form of *Verticillium* wilt (*Verticillium albo-atrum* Reinke et Berthold in *Verticillium dahliae* Klebahn) [8].

2.2 Experiment layout

Field experiment was conducted at the experimental field of Slovenian Institute of Hop Research and Brewing (IHPS) in two hop fields with different row distances (2.4 and 2.8 m) in 2006 and continued at the same plots in 2007 to 2009 as a random block trial in three replications. The size of plots was approximately 200 m^2. There were six treatments, as presented in Table 1, investigated in both hop fields. In 2006 hop plants were planted, measurements were performed in the following years (2007 to 2009).

Table 1: Plant density and number of trained bines in hop fields of cv. Dana with row distances 2.4 m and 2.8 m
Preglednica 1: Gostota rastlin in število navitih poganjkov v poskusu v hmeljiščih z medvrstno razdaljo 2,4 m in 2,8 m

Treatment	Distance between rows (m)	Distance between plants in a row (m)	Plant density (plants/ha)	No. of trained sprouts per string
1a	2.4 m	1.3	3200	3
2a	2.4 m	1.3	3200	4
3a	2.4 m	1.3	3200	5
4a	2.4 m	1.5	2800	3
5a	2.4 m	1.5	2800	4
6a	2.4 m	1.5	2800	5
1b	2.8 m	1.1	3200	3
2b	2.8 m	1.1	3200	4
3b	2.8 m	1.1	3200	5
4b	2.8 m	1.3	2800	3
5b	2.8 m	1.3	2800	4
6b	2.8 m	1.3	2800	5

The rest of the agrotechnique was the same for all plots and performed in the terms of good agricultural practise.

Both experiments were harvested plot by plot at the time of technological maturity (the first decade of September). For the evaluation the inner three rows were considered. Number of plants and strings per plot were counted, surface under harvested plots was measured. Yield per plots was weighted, samples for moisture and alpha acid content were taken and delivered to the laboratory of Slovenian Institute of Hop Research and Brewing.

2.3 Chemical analyses and data processing

Moisture content in hop cones was detected according to EBC Analytica (1998) [1], alpha acid content in cones according to EBC Analytica (2000) [2]. Results were statistically processed by the computer programs Excel and Statgraphics, differences among treatments were detected by Duncan multiple range test ($p<0.05$).

2.4 Soil and weather conditions

Soil of the experimental field is sandy clay loam; pH value at the conduction of the experiment was 5.8, phosphorus supply excessive, potassium supply adequate (Table 2).

Table 2: Soil analyze results (AL method) at the conduction of the experiment
Preglednica 2: Analiza tal (metoda AL) pred postavitvijo poskusa

pH in KCl	P_2O_5 (mg/100 g soil)	K_2O (mg/100 g soil)
5.8	36.0	24.9

There were above average temperatures in the season of 2007. Extremely high temperatures were in the second half of July when maximum daily temperatures exceeded 35ºC. High temperatures oscillations were also observed; temperatures raised and decreased in a short time period even for 10°C. Average temperature from the beginning of April to the end of August was 18.2ºC; what is by almost 2 degrees higher compared to the long term average. April was warmer by 3.2ºC, May by 1.9ºC, the second decade of June by 4.4ºC and August by 0.3ºC compared to the long term average. 473 mm of precipitation were detected in the season of 2007; what is 116 mm less compared to the long term average. On the other hand precipitations were not equally disposed with regard to location, time and quantity.

In the growth season of 2008 (April to August) there was 713 mm precipitation, what is by 124 mm more compared to the long term average. Precipitation was locally and timely not uniform disposed; there were lots of showers and storms with hail which appear already in May. In the period from June to August there was 83% of all seasonal precipitation. There was only 47 mm precipitation in May and as much as 228 mm in June. Average temperature from the beginning of April to the end of August was 16.4°C, what is by more than 1°C higher compared to the long term average. April was warmer by 0.7°C and May by 1.6°C. Temperatures in June were very variable. The second decade was by 1.2°C colder compared to the long term average (minimum daily temperature was 9.9°C) but then, in the last decade of the month there were extremely high temperatures; in the majority of the days maximum daily temperature exceeded 30°C. Average temperature was higher by 4.6°C compared to the long term average. Warm weather, but not with temperatures over 30°C, continued in July and August.

In 2009 there were relatively high temperatures in May and then they decreased suddenly at the end of the month. That was expressed in non-uniform and long flowering and consecutive in non-uniform ripening. Different stages of ripeness were observed even on the same plant. Compared to the long term average, there was more precipitation in June 2009 (174 mm) and at the beginning of July. At the beginning of August there were relatively high temperatures.

3 RESULTS AND DISCUSSION

3.1 Second year of the experiment

There were no significant differences in the yield of cones per hectare, yield of cones per plant and alpha acids yield per hectare among treatments (1a to 6a, and 1b to 6b) in the second year of the experiment. At the same time there were significant differences in alpha acids content in hop cones of different treatments in the hop field with 2.4 m row distance, while not in the hop field with 2.8 m row distance (data not shown). In the hop field with 2.4 m row distance significantly the highest alpha acid content was reached with 2800 plants/ha and 5 trained sprouts per string (12.7% in DM). With regard to yield and alpha acids yield, it was indicated in both hop fields (row distances 2.4 m and 2.8 m) that in the second year of hop plantation higher plant density (3200 plants/ha) is favourable compared to lower plant density (2800 plants/ha).

3.2 A mature, productive hop garden

In the third year of hop plantation and later, it is considered as a mature, productive hop garden. In our experiment in the third and fourth season weather conditions had the highest impact on all examined parameters (yield of cones, alpha acids content, and alpha acids yield) (Table 3). In 2008 more favourable conditions for hops cv. Dana prevailed compared to 2009. With exception of the yield in hop field with 2.8 m row distance, all the other examined parameters were significantly higher in 2008 compared to 2009, namley.

Because plant density had no significant impact on all the examined parameters except yield per plant in the hop field with 2.4 m row distance (Table 3), lower plant density (2800 plants/ha) would be recommendable; it means lower expenses for plant material, strings, and less working hours for training, namely.

Table 3: Yield, alpha acids content and alpha acids yield in the hop fields with 2.4 m and 2.8 m row distances with regard to plant density (2800 plants/ha and 3200 plants/ha), number of training bines per string (3, 4, 5) and year (2008, 2009) in a mature plantation of hops cv. Dana

Preglednica 3: Pridelek, vsebnost alfa kislin in pridelek alfa kislin v hmeljiščih z medvrstnima razdaljama 2,4 m in 2,8 m glede na gostoto rastlin (2800 rastlin/ha and 3200 rastlin/ha), število navitih poganjkov (3, 4, 5) in leto raziskave (2008, 2009) v polnorodnem nasadu hmelja cv. Dana

	Yield (kg/ha DM)		Yield (kg/plant DM)		Alpha acid content (% in DM)		Alpha acid yield (kg/ha)	
Row distance:	2,4 m	2,8 m	2,4 m	2,8 m	2,4 m	2,8 m	2,4 m	2,8 m
Plant density 3.200	1487 *a**	1733 *b*	0,47 *a*	0,44 *a*	15,3 *a*	14,6 *a*	230 *a*	253 *a*
Plant density 2.800	1480 *a*	1599 *a*	0,53 *b*	0,49 *b*	14,9 *a*	14,8 *a*	225 *a*	236 *a*
Year 2008	1734 *b*	1660 *a*	0,58 *b*	0,56 *b*	16,2 *b*	15,8 *b*	281 *b*	263 *b*
Year 2009	1234 *a*	1672 *a*	0,42 *a*	0,37 *a*	14,0 *a*	13,6 *a*	173 *a*	227 *a*
3 trained bines	1457 *a*	1665 *a*	0,49 *a*	0,46 *a*	15,3 *a*	14,8 *a*	226 *a*	247 *a*
4 trained bines	1489 *a*	1648 *a*	0,50 *a*	0,46 *a*	15,0 *a*	14,8 *a*	227 *a*	244 *a*
5 trained bines	1505 *a*	1685 *a*	0,51 *a*	0,47 *a*	15,0 *a*	14,5 *a*	228 *a*	244 *a*

* The same letter in the column within one parameter (density, year, and number of traininig bines) indicates that there is no significant difference between treatments (Duncan multiple range test, $p<0.05$).

On the other hand, in the hop field with wider row distance (2.8 m), plant density had a significant impact on the yield per hectare and yield per plant. Significantly higher yield per plant was reached at lower plant density (2800 plants/ha) compared to 3200 plants/ha. But the difference in yield per plant was not that high to cover the lower number of plants at lower plant density, so at the end there was significantly higher yield per hectare at higher plant density (3200 plants/ha) compared to 2800 plants/ha (Table 3). So, with the goal of higher yield, higher plant density (3200 plants/ha compared to 2800 plants/ha) would be recommendable. But, actual hop prices are very low, thus the differences in yield between the

two investigated plant densities have to be compared with expenses in the hop fields, caused by higher number of plants (higher expenses for plant material and strings and more working hours for training).

With regard to results the number of trained bines per string did not have a significant impact on the examined parameters (Table 3), what should be the matter of further investigations.

4 ACKNOWLEDGEMENT

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VPLIV DOGNOJEVANJA Z GNOJEVKO NA PRIDELEK IN KAKOVOST HMELJA (*Humulus lupulus* L.)

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Izvleček

V letu 2010 smo na Inštitutu za hmeljarstvo in pivovarstvo Slovenije postavili bločni poljski poskus s štirimi obravnavanji v treh ponovitvah, s katerim želimo ugotoviti možnost uporabe gnojevke za drugo ter tretje dognojevanje hmelja s hkratno defoliacijo spodnjega dela rastlin. Sicer razlik med obravnavanji nismo mogli statistično dokazati, se pa je v prvem letu poskusa nakazal ugoden vpliv drugega dognojevanja z gnojevko na pridelek hmelja in njegovo kakovost, kljub temu da je bila skupna količina apliciranega dušika v rastlinam dostopnih oblikah manjša kot pri kontroli, kjer smo dognojevali s KAN-om. Eden od ključnih dejavnikov za ugodno delovanje gnojevke je bilo tudi dejstvo, da gnojevka vsebuje vodo, kar je v suhem juniju v letu 2010 pripomoglo ne samo k večji razpoložljivosti vlage ampak tudi večji možnosti absorbcije hranil iz tal. Poleg tega gnojevka v primerjavi s KAN-om vsebuje tudi druga hranila. Na podlagi enoletnih rezultatov se je tudi uporaba gnojevke za tretje dognojevanje nakazala kot smiselna. Predstavljeni rezultati so enoletni, poskus bomo nadaljevali v prihodnjih sezonah.

Ključne besede: hmelj, *Humulus lupulus* L., dognojevanje, gnojevka, pridelek, alfa-kisline

IMPACT OF HOPS (*Humulus lupulus* L.) FERTILIZATION BY LIQUID MANURE ON YIELD AND ITS QUALITY

Abstract

In 2010 field experiment was conducted at the Slovenian institute of hop research and brewing to investigate possibility of second and the third nitrogen fertilization of hops in the form of liquid manure. Together with the third nitrogen fertilization by liquid manure defoliation of the lower part of hop plants was performed. Although there was no significant difference among treatments in the first year of the research, positive impact of the second nitrogen fertilization in the form of liquid manure compared to amonnitrate on the yield and its quality (alpha-acid and nitrates content) was detected, even though the nitrogen amount applied was lower. One of the factors for good impact of the liquid manure in 2010 was that June was drier compared to the long term average, so water from this type of fertilizer impacted positive on the investigated parameters, allowing better water and so also better nutrients supply of hop

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plants. And, it contains also other nutrients. The third nitrogen fertilization of hops by liquid manure was indicated as replaceable with amonnitrate in the first year of the experiment which should be continued in the following seasons.

Key words: hops, *Humulus lupulus* L., fertilization, liquid manure, yield, alpha-acids

1 UVOD

Po navedbah Leskoška in Miheliča [6] 1 m^3 goveje gnojevke vsebuje približno 5 kg N, 2 kg P_2O_5 in 7 kg K_2O. S stališča potreb hmelja po fosforju in kaliju torej lahko potrosimo letno 26 m^3/ha goveje gnojevke, pa bodo zadovoljene potrebe hmelja po teh dveh hranilih. To je dobra rešitev v primeru, če so tla v hmeljišču dobro preskrbljena (razred preskrbljenosti C) tako s fosforjem kot kalijem. Obenem s to količino gnojevke v hmeljišče vnesemo okrog 130 kg dušika. Od tega predvidevamo, da ga je okrog 50% v amonijski obliki, ostalo pa predstavlja organsko vezan dušik. Če gnojevko mešamo z vodo, se razredči, in moramo mešanice aplicirati ustrezno več.

Ker se fosfor in kalij iz gnojevke izkoriščata enako dobro kot iz mineralnih gnojil, moramo hranila, ki smo jih vnesli v hmeljišče z gnojevko, odšteti od skupne letne potrebe po hranilih in le razliko pognojiti z mineralnimi gnojili. Z gnojevko obenem vnašamo v hmeljišča tudi dragoceno organsko snov. Zaradi velikega deleža amonijskega dušika v gnojevki moramo z njo gnojiti enako kot z dušikovimi mineralnimi gnojili – torej takrat, ko rastlina dušik dejansko potrebuje. Računamo, da je kratkoročno hmelju na voljo 50% dušika (amonijska oblika), ostali del, ki je organsko vezan, pa se sprošča kasneje. Gnojevke ne vozimo na suha tla ali tla, zasičena z vodo, in ne v sončnem, vročem vremenu, saj pride sicer do izgube hranil, po drugi strani pa po nepotrebnem obremenjujemo okolje. Pri prvem in drugem dognojevanju je priporočljivo gnojevko neposredno deponirati v tla oziroma mora biti ustrezno razredčena (vsaj 1:1) in jo takoj po aplikaciji vdelamo v zemljo. Če pa gnojevko uporabljamo za tretje dognojevanje, je obenem herbicid in defoliant, s katerim poskrbimo za defoliacijo spodnjega dela rastlin hmelja (do višine 1 m). V tem primeru jo v tla zadelamo z osipanjem [5,6,7].

Z raziskavo smo želeli ugotoviti, kakšen vpliv ima dognojevanje hmelja sorte Aurora z gnojevko na pridelek in njegovo kakovost.

2 MATERIAL IN METODE

V letu 2010 smo postavili bločni poljski poskus s štirimi obravnavanji v treh ponovitvah, s katerim želimo ugotoviti možnost uporabe gnojevke za drugo in tretje dognojevanje hmelja s hkratno defoliacijo spodnjega dela rastlin.

V obravnavanja smo vključili:

1 = kontrola (brez gnojevke, vsa tri dognojevanja s KAN-om 50+70+50 kg/ha N), defoliacija klasična;
2 = prvo in drugo dognojevanje kot pri kontroli, tretje dognojevanje z gnojevko v količini 26 m^3/ha, obenem defoliacija spodnjega dela rastlin, osipamo po aplikaciji gnojevke;

3 = prvo in tretje dognojevanje kot pri kontroli, drugo dognojevanje z gnojevko v odmerku 26 m^3/ha, gnojevko takoj vdelamo v tla, defoliacija klasična;

4 = prvo dognojevanje kot pri kontroli, drugo in tretje dognojevanje z gnojevko (26 m^3/ha); po drugem dognojevanju gnojevko vdelamo v tla, pri tretjem dognojevanju obenem z aplikacijo defoliacija, potem hmelj osipamo.

Ostala agrotehnika je bila enaka za vse parcele in je potekala po načelih dobre kmetijske prakse. V poskusu smo tedensko merili višino rastlin in nastop razvojnih faz. Škropljenje s fitofarmacevtskimi sredstvi je potekalo po napovedi prognostične službe in v skladu s škropilnim programom enako za cel poskus (23.6.2010 = folpan, kohinor, 3.7.2010 = delan, vertimec, pepelin, 20.7.2010 = fulpan, pepelin, nisorrun, karate, 12.8.2010 = cuprablau, pepelin).

Pred postavitvijo poskusa smo vzeli vzorec tal za osnovno analizo (preglednica 1). Ker so bila tla ekstremno preskrbljena s fosforjem in dobro s kalijem, smo pognojili celoten poskus s kalijevim kloridom v količini 180 kg/ha K_2O. Apnjenje glede na analizo tal ni bilo potrebno. Hmeljišče se nahaja na oglejeniih obrečnih rjavih tleh. Tla so nastala na peščeno prodnatem nanosu reke Savinje in potoka Lava. Po teksturi so razvrščena v teksturni razred PGI (peščeno glinasta ilovica), kar jih uvršča med srednje težka tla (raziskave IHPS).

Preglednica 1: Osnovna analiza tal (0-25 cm) pred postavitvijo poskusa z gnojevko spomladi 2010

Table 1: Plant available phosphorus and potassium in the upper layer of the soil (0-25 cm) and pH of soil before the conduction of experiment in spring 2010

Globina (cm)	pH v KCl	P_2O_5 (mg/100 g tal)	K_2O (mg/100 g tal)
0-25	7,0	46,0	22,6

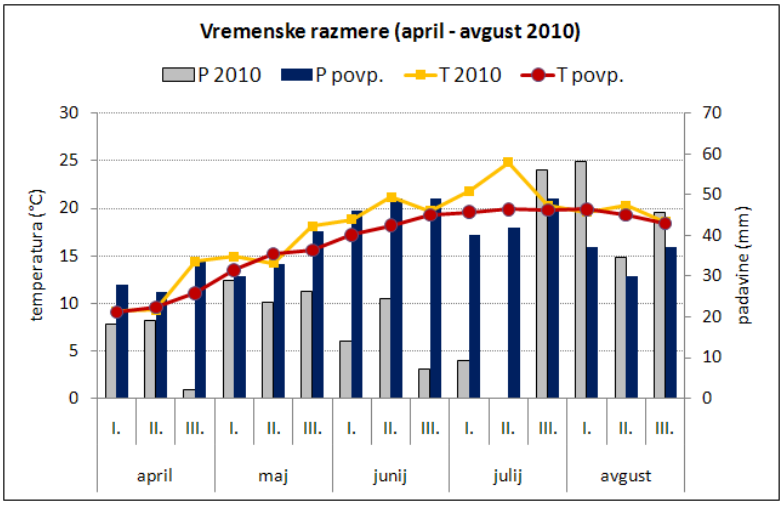
Prvo dognojevanje smo izvedli 19. maja enako za cel poskus v količini 50 kg/ha N v obliki KAN-a, drugo dognojevanje 15. junija glede na plan poskusa: parcele obravnavanj 3 in 4 smo dognojevali z gnojevko v količini 26 m^3/ha, parcele obravnavanj 1 in 2 pa ročno s KAN-om (70 kg/ha N). Po dognojevanju smo izvedli kultiviranje in drugo osipanje. Tretje dognojevanje smo opravili 12. julija; obravnavanji 2 in 4 z gnojevko (26 m^3/ha), obravnavanji 1 in 3 s KAN-om v količini 50 kg/ha N. Takoj je sledilo osipanje hmelja. Sestava gnojevke za drugo in tretje dognojevanje je predstavljena v preglednici 2.

Vzorčenje tal za analizo na vsebnost dostopnega dušika v zgornjem sloju tal (0 do 25 cm) smo izvedli pred drugim dognojevanjem (9. junija), pred tretjim dognojevanjem (8. julija) in po obiranju (13. september). V svežih vzorcih smo s hitrim talnim testom [8] določili vsebnost nitratne in amonijske oblike dušika. 23. julija smo na vseh parcelah posejali podsevek oljna redkev.

V času tehnološke zrelosti smo poskus obirali ločeno po parcelah. Izločili smo robne vrste, parcele premerili, prešteli število rastlin in število vodil na parcelo. Pridelek smo stehtali za vsako parcelo posebej in odvzeli vzorce storžkov za analizo na vsebnost vlage [1], alfa-kislin [2] in nitratov [4].

Preglednica 2: Sestava goveje gnojevke za drugo in tretje dognojevanje hmelja v poskusu v letu 2010
Table 2: Analyse of liquid manure, used for the second and the third hops fertilization in the field experiment in 2010

		Drugo dognojevanje		Tretje dognojevanje	
Parameter	Enota	V suhem vzorcu	V svežem vzorcu	V suhem vzorcu	V svežem vzorcu
Vlaga	%	-	95,9	-	92,2
pH v sveži gnojevki	-	-	6,9	-	6,4
NH_4-N v sveži gnojevki	mg/kg	-	712	-	1283
P - celokupni fosfor	%	0,71	0,03	0,52	0,04
K - celokupni kalij	%	4,34	0,18	3,71	0,29
Ca - celokupni kalcij	%	1,69	0,07	1,44	0,11
Mg - celokupni magnezij	%	0,79	0,03	0,57	0,04
N - celokupni dušik	%	2,66	0,11	2,30	0,18
Sušina	g/kg	-	40,7	-	78,4



Slika 1: Količina padavin in povprečne dekadne temperature v rastni sezoni hmelja v letu 2010 v primerjavi z dolgoletnim povprečjem (Žalec)
Figure 1: Precipitation amount and average decade temperatures in the hop growth season in 2010 compared to the long term average (Žalec)

Po podatkih z referenčne postaje v Žalcu (IHPS) je v rastni sezoni hmelja (april do avgust) v letu 2010 padlo le 434 mm padavin, kar za 189 mm manj v primerjavi s 40-letnim povprečjem (slika 1). V zadnjih desetih letih je to druga najnižja vsota padavin v rastnem obdobju hmelja. Padavine so bile tudi časovno, količinsko in krajevno zelo neenakomerno razporejene. Največji primanjkljaj padavin glede na dolgoletno povprečje je bil v mesecih junij in julij, ko je bilo 130 mm manj padavin, kot jih je bilo povprečno v tem obdobju. Tretjina padavin je padla v mesecu avgustu (slika 1). Leto 2010 je bilo nadpovprečno toplo leto z ekstremno visokimi temperaturami v sredini julija. Povprečna temperatura od začetka marca do konca

avgusta je bila 15,7ºC, kar je za dobro stopinjo več od dolgoletnega povprečja. Glede temperatur je najbolj izstopalo prvih dvajset dni v juliju. Prva dekada je bila glede na 40-letno povprečje za 2,2ºC toplejša, druga dekada pa za 4,9ºC. Po 25 juliju je sledila ohladitev, povprečne dnevne temperature so bile od 15,4ºC do 20,9ºC. Hladno vreme se je nadaljevalo še v prvi dekadi avgusta [3].

3 REZULTATI Z DISKUSIJO

3.1 Rast in razvoj

Med obravnavanji v rasti in razvoju nismo opazili razlik.

3.2 Vsebnost hranil v gnojevki

Goveja gnojevka za drugo dognojevanje je bila zelo razredčena; vsebovala je le okrog 4% suhe snovi (preglednica 2). V 1 m^3 je vsebovala le 1,1 kg N, 0,3 kg P in 1,8 kg K. S 26 m^3/ha smo torej pri drugem dognojevanju pognojili okrog 29 kg/ha N, 18 kg/ha P_2O_5 in 57 kg/ha K_2O, kar je manj, kot smo predvidevali glede na vsebnost hranil, ki naj bi jih vseboval 1 m^3 goveje gnojevke po virih [5,6]. Od celokupnega dušika ga je bilo okrog 64% v amonijski obliki, ostala količina je bil organsko vezan dušik, torej ga je bilo hmelju trenutno na voljo le 19 kg/ha.

V primerjavi z gnojevko istega hmeljarja za drugo dognojevanje je bila gnojevka za tretje dognojevanje manj razredčena in je vsebovala več hranil na prostorninsko enoto (preglednica 2). V 1 m^3 je vsebovala 1,8 kg N, 0,4 kg P, 2,9 kg K in 0,4 kg Mg. S 26 m^3/ha smo torej pri tretjem dognojevanju aplicirali okrog 47 kg/ha N, 23 kg/ha P_2O_5 in 90 kg/ha K_2O. Od celokupnega dušika ga je bilo 71% v amonijski obliki, torej smo s tretjim dognojevanjem vnesli v hmeljišče okrog 33 kg/ha rastlinam dostopnega dušika, kar je zopet manj kot pri kontroli, kjer smo gnojili s KAN-om (50 kg/ha N).

Pri izbiri gnojevke za dognojevanje je torej priporočljivo narediti analizo le-te, preden jo apliciramo, da lahko količino reguliramo glede na dejanske potrebe hmelja. Potrebno je tudi upoštevati, kako jo bomo pred aplikacijo razredčili, in na podlagi tega narediti izračun po potrebni količini gnojevke za določeno površino.

Če računamo z letnim odvzemom 50 do 60 kg/ha P_2O_5 in 180 kg/ha K_2O, potem pri obravnavanju 4 (drugo in tretje dognojevanje z gnojevko) hmeljišče nismo pognojili z več fosforja in kalija, kot ga odvzame hmelj v enem letu, saj je bila gnojevka zelo razredčena. V primeru, če bi z gnojevko vnesli v hmeljišče več fosforja in kalija, kot ga odvzame hmelj v enem letu, pa bi morali to upoštevati pri gnojenju z mineralnimi gnojili v prihodnjih letih. Problem aplikacije organskih gnojil je v hmeljiščih, ki so pretirano ali celo ekstremno preskrbljena z določenimi hranili, kot je tudi v našem primeru (ekstremno preskrbljena tla s fosforjem).

3.3 Vsebnost rastlinam dostopnega dušika v tleh

Pred drugim dognojevanjem je bilo v zgornjem sloju tal (0 do 25 cm) 179 kg/ha rastlinam dostopnega dušika, od tega 154 kg/ha v nitratni obliki (preglednica 3).

Preglednica 3: Rezultati analize tal na rastlinam dostopni dušik v zgornjih 25 cm tal v letu 2010 v poskusu z gnojevko glede na obravnavanje in datum vzorčenja
Table 3: Results of Nmin analyse in the upper layer of the soil (0-25 cm) in 2010 with regard to treatment and date of sampling

Datum vzorčenja	Obravnavanje	NO_3 – N (kg/ha)	NH_4 – N (kg/ha)	Skupni rastlinam dostopni N (kg/ha)
9.6.2010	1, 2, 3, 4	154	25	179
8.7.2010	1	150	15	165
	2	198	10	208
	3	126	12	138
	4	138	11	149
13.9.2010	1	24	20	44
	2	20	18	38
	3	24	14	38
	4	20	16	36

V začetku julija je bila večja količina rastlinam dostopnega dušika v zgornjem sloju tal (0 do 25 cm) pri obravnavanjih 1 in 2, kjer smo drugo dognojevanji opravili s KAN-om, manjša pa pri obravnavanjih, kjer smo drugo dognojevanje opravili z gnojevko (preglednica 3). Delno je to pripisati dejstvu, da je bila za drugo dognojevanje uporabljena gnojevka bolj razredčena, kot smo pričakovali, in je zato vsebovala manj rastlinam dostopnega dušika, kot smo ga potrosili pri obravnavanjih s KAN-om. Nepričakovano pa je bila med obravnavanji v tleh podobna količina amonijske oblike dušika; pričakovati bi je bilo več pri variantah, kjer smo za drugo dognojevanje uporabili gnojevko. Količina rastlinam dostopnega dušika v tleh pa je bila pri vseh obravnavanjih relativno visoka za obdobje pred tretjim dognojevanjem.

Po obiranju hmelja pri nobeni od preučevanih variant ni v tleh ostalo več kot 50 kg/ha rastlinam dostopnega dušika (preglednica 3).

3.4 Pridelek in kakovost pridelka

Razlik med obravnavanji sicer nismo mogli statistično dokazati, med drugim najbrž tudi zaradi nehomogenosti hmeljišča (sredinski blok je statistično značilno pozitivno odstopal od ostalih dveh blokov), se pa je nakazoval pozitiven vpliv drugega dognojevanja z gnojevko (obravnavanji 3 in 4) na vse preučevane parametre (preglednica 4), kljub temu da je bila skupna količina apliciranega dušika (če računamo pri gnojevki dostopno - amonijsko obliko) v teh dveh primerih manjša kot pri kontroli, kjer smo dognojevali trikrat s KAN-om (obravnavanje 1).

Drugo dognojevanje z gnojevko se je nakazalo kot ustrezno tudi s stališča kakovosti pridelka; pri obravnavanjih, kjer je bila gnojevka uporabljena za drugo dognojevanje, se je namreč nakazala manjša vsebnost nitratov v storžkih v primerjavi z obravnavanjema, kjer je bil za drugo dognojevanje uporabljen KAN (razlik med obravnavanji sicer nismo mogli statistično dokazati). Pozitivna stran drugega dognojevanja z gnojevko je bila med drugim tudi v tem, da gnojevka vsebuje vodo, kar je v suhem juniju v letu 2010 (slika 1) pripomoglo ne samo k večji ponudbi vlage ampak tudi k večji možnosti absorbcije hranil iz tal. Poleg tega gnojevka v primerjavi s KAN-om vsebuje tudi druga hranila.

Preglednica 4: Pridelek storžkov (suha snov) na hektar in na rastlino ter vsebnost alfa-kislin in nitratov v poskusu z gnojevko v letu 2010 glede na obravnavanje (1, 2, 3, 4)

Table 4: Yield (kg/ha and kg/plant dry matter), alpha-acids and nitrates content in hop cones at harvest in 2010 with regard to treatment (1, 2, 3, 4)

Obr.	Pridelek storžkov (kg/ha SS)	Pridelek na rastlino (kg SS)	Vsebnost alfa-kislin (% v SS)	Pridelek alfa-kislin (kg/ha)	Pridelek Alfa-kislin (kg/rastlino)	Nitrati v SS (mg/100 g)
1	1312 a*	0,46 a	0,092 a	120 a	0,043 a	1522 a
2	1438 a	0,54 a	0,095 a	137 a	0,052 a	1582 a
3	1639 a	0,60 a	0,093 a	152 a	0,056 a	1406 a
4	1626 a	0,60 a	0,096 a	157 a	0,057 a	1446 a

* Enaka črka v stolpcu pomeni, da med obravnavanjema ni statistično značilne razlike (Duncanov test mnogoterih primerjav, $p<0,07$).

4 SKLEPI

Razlik med obravnavanji sicer nismo mogli statistično dokazati, se pa je v prvem letu poskusa nakazal ugoden vpliv drugega dognojevanja z gnojevko na pridelek hmelja in njegovo kakovost, kljub temu da je bila skupna količina apliciranega dušika v rastlinam dostopnih oblikah manjša kot pri kontroli, kjer smo dognojevali s KAN-om. Eden od ključnih dejavnikov za ugodno delovanje gnojevke je bilo tudi dejstvo, da gnojevka vsebuje vodo, kar je v suhem juniju v letu 2010 pripomoglo ne samo k večji ponudbi vlage ampak tudi večji možnosti absorbcije hranil iz tal. Poleg tega gnojevka v primerjavi s KAN-om vsebuje tudi druga hranila. Na podlagi enoletnih rezultatov se je tudi uporaba gnojevke za tretje dognojevanje hmelja nakazala kot vsaj zamenljiva z dognojevanjem v obliki KAN-a.

Predstavljeni rezultati so enoletni, poskus bomo nadaljevali v prihodnjih sezonah.

5 ZAHVALA

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IMPACT OF MICRONIZED CALCITE ON THE YIELD AND QUALITY OF HOPS (*Humulus lupulus* L.)

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Abstract

A result of a special technological process of mechanical milling of calcite and collision at high frequency are particles of micro and submicro sizes (0.2-50 μm) with highly increased specific surface. These energized particles can be sprayed finely to the leaves and can be taken in plants directly through stomata and as a result a reduced water requirement of plants is expected because CO_2 is converted from calcite inside the leaves. Other elements that micronized calcite contains should have positive impact on plant physiology. In a two year investigation with the use of micronized calcite spraying of hops cv. Aurora it was indicated that it has positive impact on the yield in a season with more frequent stress conditions (high temperatures oscillations, lack of precipitation), but in a season with average weather conditions and enough precipitation its positive impact was not detected. In both investigated seasons it was shown that N, P, K fertilization can't be avoided, even if spraying by micronized calcite is performed, if the same yield as at conventional production wants to be reached.

Key words: hops, *Humulus lupulus* L., fertilization, micronized calcite, yield, alpha acids

VPLIV MIKRONIZIRANEGA KALCITA NA PRIDELEK IN KAKOVOST HMELJA (*Humulus lupulus* L.)

Izvleček

Rezultat posebne tehnologije mehanskega mletja kalcita in kolizije delcev pri visokih frekvencah so delci mikro in submikro velikosti (0.2-50 μm) z zelo povečano specifično površino. Delci, raztopljeni v vodi, se pri pršenju fino nanesejo na liste rastline in prehajajo v liste neposredno skozi reže, zato naj bi se zaradi CO_2, v katerega se pretvori proizvod znotraj rastline, zmanjšala potreba po vodi. Druga hranila, ki jih proizvod vsebuje, naj bi pozitivno vplivala na fiziologijo rastline. V dvoletni raziskavi s pršenjem hmelja z mikroniziranim kalcitom med rastno sezono se je nakazal pozitiven učinek (razlike sicer niso bile statistično značilne) tega ukrepa na pridelek hmelja v sezoni z več stresnimi situacijami (velika temperaturna nihanja, malo padavin), ne pa v sezoni s povprečnimi vremenskimi razmerami in večjo količino padavin. V obeh sezonah se je nakazalo, da če želimo doseči enak pridelek kot

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v konvencionalni pridelavi, se kljub pršenju hmelja z mikroniziranim kalcitom gnojenju z N, P, K gnojili ne moremo izogniti.

Ključne besede: hmelj, *Humulus lupulus* L., gnojenje, prehrana rastlin, mikroniziran kalcit, pridelek, alfa kisline

1 INTRODUCTION

Calcite milled in dry or wet system in micronized size can be used in paper, plastic, paint, cable, construction, adhesive, ceramic, carpet,… sectors and also as food additive in biscuits, breads and chewing gums. Due to its chemical purity, colour and low price, it is used as a source of calcium [8]. The result of a special technological process of mechanical milling of calcite and collision at high frequency are particles of micro and submicro sizes (0.2-50 μm**)** with highly increased specific surface. Micronized calcite (MC) is 100 % natural product from limestone deposits. It contains $CaCO_3$ (around 94 %) and other nutrients (MgO, SiO_2, Fe_2O_3,...) with regard to its natural origin. For plant nutrition it is used in a 0.3-0.5 % water suspension for spraying [4,5,6]. These energized particles can be sprayed finely to the leaves and can be taken into plants directly through stomata and as a result a reduced water requirement of plants is expected because CO_2 is converted from calcite inside the leaves. Other elements that the product contains should impact positive on plant physiology.

2 MATERIAL AND METHODS

2.1 Experimental layout and realization

Field experiment was conducted at the experimental field of Slovenian Institute of Hop Research and Brewing in the season 2007 and continued at the same plots in 2008 as a random block trial in three replications. The size of plots was 380 m^2, so the whole size under the experiment was approximately 4500 m^2. There were three treatments investigated:

K: Control treatment; conventional fertilization by phosphorus (P) and potassium (K) according to the soil analyse, conventional fertilization by nitrogen (N) (50+70+50 kg/ha N; 20th May, 10th June and 10th July respectively), no foliar fertilizers, spraying by insecticides and fungicides (PPP) according to the spraying program*.

K+MC: Conventional fertilization by P and K according to the soil analyse, conventional fertilization by N, spraying by PPP according to the spraying program, after each spraying by PPP spraying by MC (3 kg/ha) (3-times in season 2007, 5-times in season 2008).

MC+PPP: No fertilization by P, K and N, spraying by PPP according to the spraying program, after each spraying by PPP spraying by MC (3 kg/ha) (3-times in season 2007, 5-times in season 2008).

*In 2007: 12th June insecticides Kohinor and Vertimec and fungicide Cuprablau, 2nd July Cuprablau, 18th July insecticides. In 2008: 11th June Cuprablau and Confidor, 3rd July Delan,

Confidor and Nisurun, 17th July Cuprablau, 26th July Vertimec, Folpan and Silvet, 12th August Cuprablau and Pepelin.

The rest of the agrotechnique was the same for all plots and performed in the terms of good agricultural practise. Fertilization by nitrogen in the form of KAN was performed manually, spraying by PPP and by MC was performed manually when hop plants were still small, but when they reached the top of the trellis, spraying was performed by machinery.

Measurements of plant growth and detection of growth stages were detected once to twice a week. Soil sampling for quick soil test Nmin (nitrate and ammonium) [7] was performed on 20th May (before 1st N fertilization at control), on 5th July (before 3rd N fertilization at control) and after harvest. Before harvest border rows of hop plants were removed, the inner two rows were harvested plot by plot and evaluated. Plot size was measured, number of plants and number of strings per plots were counted. Yield per plots was weighted, samples of cones were taken and delivered to the laboratory of Slovenian Institute of Hop Research and Brewing.

2.2 Chemical analyses

Moisture content in hop cones was detected according to EBC Analytica (1998) [1], alpha acid content according to EBC Analytica (2000) [2] in 2007 and 2008, in 2008 also nitrate content in hop cones was analysed (according to DIN/EN (1998) [3]).

2.3 Processing of statistical data

Results were statistically processed by the computer programs Excel and Statgraphics, differences among treatments were detected by Duncan multiple range test ($p<0.05$).

2.4 Weather and soil conditions

There were above average temperatures in the season of 2007. Extremely high temperatures were in the second half of July when maximum daily temperatures exceeded 35ºC. There were also high temperatures oscillations; temperatures raised and decreased in a short time period even for 10°C. Average temperature from the beginning of April to the end of August was 18.2ºC; for almost 2 degrees higher compared to the long term average. April was warmer for 3.2ºC, May for 1.9ºC, the second decade of June for 4.4ºC and August for 0.3ºC compared to the long term average. There was 473 mm of precipitation in the season of 2007; 116 mm less compared to the long term average. On the other hand precipitations were not equally disposed with regard to location, time and quantity.

In the season 2008 there was 713 mm precipitation, what means 124 mm more than in a long term average. Precipitation was locally and timely not uniform disposed; there were lots of storms with hail which appeared already in May. In the period from June to August there was 83 % of all seasonal precipitation. There was only 47 mm precipitation in May and as much as 228 mm in June. Average temperature from the beginning of April to the end of August was 16.4°C, what is by 1°C more compared to the long term average. April was warmer by 0.7°C, May by 1.6°C. In June there were very variable temperatures; the second decade was by 1.2°C colder compared to the long term average, minimum daily temperature was 9.9°C.

But, in the last decade of June, high temperatures were detected. In the most of the days daily temperature exceeded 30^0C, average decade temperature was by 4.6^0C higher compared to the long term average. July and August were comparable to the long term average.

Soil of the experimental field is sandy clay loam, pH value at the conduction of the experiment was 5.8, phosphorus supply was excessive, potassium supply was adequate (Table 1).

Table 1: Soil analyze results (AL method) at the conduction of the experiment
Preglednica 1: Rezultati analize tal (AL metoda) pred postavitvijo poskusa

pH in KCl	pH in Ca-acetate	P_2O_5* mg/100 g soil	K_2O* mg/100 g soil
5.8	6.5	36.0	24.9

3 RESULTS AND DISCUSSION

3.1 Plant available nitrogen in soil

In the first investigated year (2007) there was around 80 kg/ha plant available N in the upper layer of soil (0-25 cm) in May (Table 2).

At the end of July 2007 there were differences among treatments, as expected. At treatments K and K+MC there was higher quantity of plant available N in soil (142-150 kg/ha N) compared to the treatment MC+PPP (49 kg/ha N), where no N fertilization was included. At treatments where N fertilization was included (K and K+MC) plant available N quantity in soil was increased by around 55 kg/ha from May to the end of July, although plants absorbed N in that time, too. At the treatment MC+PPP plant available N quantity was lower by around 30 kg/ha N.

After harvest in 2007 differences among treatments in quantity of plant available N in the upper layer of the soil were lower compared to the end of July; it was decreased by around 50 kg/ha at treatments K and K+MC and stayed almost at the same level at treatment MC+PPP (Table 2). At treatments K and K+MC quantity of plant available N in soil was relatively high for that time.

In May 2008 there was comparable quantity of plant available N in the upper layer of the soil among treatments (17-24 kg/ha N; Table 2).

In the first days of July 2008, before the third N fertilization at treatments K and K+MC, there was a bit higher quantity of N in soil compared to quantity in May; 42-66 kg/ha N. Compared to the first sampling in May there was higher quantity of plant available N in soil at all treatments, also treatments with no N fertilization, what points to the good mineralisation of N from soil organic matter in that time. As expected there was lower quantity of plant available N at treatment MC+PPP where no N was fertilized, compared to treatments K and K+MC where 120 kg/ha N was fertilized in May and June.

Table 2: Quantity of plant available N in the upper layer of soil according to Nmin analyse in 2007 and 2008 with regard to treatment and sampling date (kg/ha)
Preglednica 2: Vsebnost rastlinam dostopnega dušika (Nmin) v zgornjem sloju tal glede na obravnavanje in datum vzorčenja v letih 2007 in 2008

		2007			2008		
Date of sampling	Treatment	NO_3–N (kg/ha)	NH_4–N (kg/ha)	NO_3–N + NH_4–N (kg/ha)	NO_3 – N (kg/ha)	NH_4–N (kg/ha)	NO_3–N + NH_4–N (kg/ha)
20th May	K	71	25	96	16	5	21
	K+MC	71	12	83	20	4	24
	MC + PPP	67	12	79	12	5	17
31st July in 2007, 5th July in 2008	K	119	31	150	51	15	66
	K+ MC	123	19	142	40	18	58
	MC + PPP	40	10	49	28	14	42
After harvest	K	83	11	94	36	11	47
	K+ MC	83	12	95	28	8	36
	MC + PPP	32	10	41	16	10	26

In the soil of all treatments there was not more than 50 kg/ha of plant available N after harvest (Table 2). With regard that the same quantity of mineral N was fertilized at treatments K and K+MC and that there was lower quantity of plant available N in the soil of the treatment K+MC, a bit higher absorption of N at plants of the treatment K+MC is assumed.

3.2 Plant growth and development

In 2007 plants at the control treatment (K) grew the slowest; the fastest grew plants at treatments K+MC. But at the end of June plants of all treatments reached the top of the construction. Flowering started a week sooner at treatments K and K+MC compared to MC+PPP, while flowering ended approximately at the same time at all treatments in 2007.

In 2008 there were no important differences among treatments.

3.3 Yield

Although differences between treatments K and K+MC could not be statistically confirmed, in drier season with high temperatures oscillations (2007) higher yield was indicated at treatment K+MC (Table 3) what suggests the conclusion that spraying with MC is more appropriate in that kind of season (stress conditions). Significantly lower yield was detected at the treatment with no N, P, K fertilization (MC+PPP).

In the season with above average precipitation quantity, especially in June and July (2008), spraying by MC did not impact the yield of hops significantly, too; there were no significant difference among all investigated treatments. In both seasons it was shown that N, P, K fertilization can't be avoided, even if spraying by MC is performed, if the same yield as at K wants to be reached.

Table 3: Yield of cones (kg/ha DM, kg/plant DM), alpha acids content (% DM) and alpha acids yield (kg/ha) in the experiment in 2007 and 2008
Preglednica 3: Pridelek (kg/ha suhe snovi, kg/rastlino), vsebnost alfa kislin (% v suhi snovi) in pridelek alfa kislin (kg/ha) v poskusu v letu 2007 in 2008

	Yield of cones (kg/ha DM)		Yield of cones (kg/plant DM)		Alpha acid content (% DM)		Alpha acid yield (kg/ha)	
	2007	2008	2007	2008	2007	2008	2007	2008
K	1301 *b**	1780 *a*	0,43 *bc*	0,61 *a*	6,9 *a*	10,3 *a*	90 *b*	183 *a*
K+MC	1394 *b*	1637 *a*	0,49 *c*	0,58 *a*	6,9 *a*	10,6 *a*	96 *b*	174 *a*
MC+PPP	1067 *a*	1452 *a*	0,36 *ab*	0,52 *a*	6,4 *b*	10,9 *a*	68 *a*	159 *a*

* The same letter in the column indicates that there is no significant difference between treatments (Duncan multiple range test, $p<0.07$).

3.4 Alpha acid content and alpha acid yield

There was significantly lower alpha acids content at treatment MC+PPP compared to the other two investigated treatments in 2007. In 2008 there was no significant difference in alpha acid content among treatments (Table 3). The characteristic of the season 2007 compared to 2008 was that hop cones contained less alpha acids.

In the yield of alpha acids the same results were obtained as in the case of the yield of cones. N fertilization was indicated to impact positively on the yield of alpha acids, although differences among treatments could not be statistically confirmed (differences among blocks were too high).

3.5 Nitrate content

In 2008 nitrate content in hop cones at the technological maturity was analyzed with regard to treatment. The highest nitrate content was indicated at K and the lowest at the treatment where no N fertilization was carried out (MC+PPP) (Table 4).

Table 4: Nitrate content in hop cones with regard to treatment in 2008
Preglednica 4: Vsebnost nitratov v storžkih glede na obravnavanje v letu 2008

	Nitrate content (mg/100 g DM)
K	1253
K+MC	871
MC+PPP	494

Spraying by MC was indicated to lower nitrate content in hop cones. But, because investigation of nitrate content in hop cones was not performed by plots but only by treatments, statistic could not be done to confirm these findings, so these starting points are left for the future investigations.

4 CONCLUSIONS

In a two year investigation it was indicated that spraying by MC has positive impact on the yield of hops cv. Aurora and its quality in a season with more frequent stress conditions (high temperatures oscillations, lack of precipitation), but in a season with more average weather conditions and enough precipitation positive impact was not detected. In both investigated seasons it was shown that N, P, K fertilization can't be avoided, even if spraying by MC is performed, if the same yield as at K wants to be reached.

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PHYSIOLOGICAL RESPONSE OF HOP (*Humulus lupulus* L.) PLANTS TO DROUGHT STRESS

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Abstract

The response of plants to water stress is well known in some agricultural crop species while in hop very little is known about the topics. In the research presented the response of hop plants vars. Savinjski golding and Aurora to drought stress was investigated. In a pot experiment the physiological response of hop plants was measured using total radical trapping potential (TRAP), chlorophyll *a* fluorescence and reflectometry. Based on the results obtained the hypothesis was confirmed – the variety Aurora is more drought tolerant compared to the variety Savinjski golding and has a higher regenerative capability. The results form a good basis for further investigations.

Keywords: hop, *Humulus lupulus* L., drought, physiological response, variety

FIZIOLOŠKI ODZIV RASTLIN HMELJA (*Humulus lupulus* L.) NA SUŠNI STRES

Izvleček

Odziv rastlin na vodni stres je dobro raziskan pri nekaterih kmetijskih rastlinah, medtem ko je pri hmelju o tem le malo znanega. V raziskavi smo proučevali odziv rastlin hmelja sort Savinjski golding in Aurora na sušni stres. V lončnem poskusu je bil merjen fiziološki odziv rastlin z uporabo TRAP testa (total radical trapping potential), meritve fluorescence klorofila *a* in reflektometrije. Rezultati potrjujejo hipotezo – sorta Aurora je bolj tolerantna na sušo v primerjavi s Savinjskim goldingom in ima višjo regenerativno sposobnost. Rezultati raziskave so dobra osnova za nadaljnja proučevanja.

Ključne besede: hmelj, *Humulus lupulus* L., suša, fiziološki odziv, sorta

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1 INTRODUCTION

Hop (*Humulus lupulus* L.) is a dioecious cone-bearing plant cultivated for commercial use, predominantly beer brewing [5]. In the cones of female plant the lupulin glands contain bitter acids and essential oils which impart bitterness, flavour and preservation to beer. Commercial hop cultivation occurs in many parts of the world, including Europe, North America, South Africa, Australia and New Zealand. Breeding programs are focused to develop new and improved cultivars, with quantity and quality of yield and disease resistance as main goals. Resistance to abiotic stress is not well known in hops, compared to pest and disease resistance. Much less is also known about genetics of resistance to abiotic constraints or physiological stress. Abiotic stress resistance is typically governed by polygenic inheritance and may be conditioned by multiple, interacting mechanisms. These and other factors make abiotic stress resistance especially difficult to study, both physiologically and genetically. In other agricultural species varieties with enhanced resistance to drought already exist on the market.

The effect of stress can be measured by determining specific or unspecific stress symptoms. Stress can be measured as the change of enzymatic activity, accumulation of osmotics, presence of stress hormones, inhibition of photosynthesis, etc. Drought stress in plants leads to gradually failing of cell antioxidative defense mechanism and subsequently accumulation of reactive oxygen species (ROS). The main consequences of ROS are oxidation of membrane lipids, proteins, nucleic acids and changed redox condition in the cell [6]. ROS are present in all aerobic cells, in equilibrium with antioxidants. When this balance is disturbed oxidative stress occurs. Aerobic organisms respond to oxidative stress by either non-enzymatic or enzymatic defense responses. Non-enzymatic defense involves glutathione, ascorbic acid, α-tocopherol, β-carotene and other compounds capable of quenching ROS. Enzymes involved in defense include superoxide dismutases, catalases, peroxidases, glutathione reductase and $NADP^+$ reducing enzymes [4, 8].

One of the methods for detecting ROS, particularly the formation of hydrogen peroxide, is luminol assisted chemiluminescence [1]. With this method it is also possible to determine ROS quenching activity indirectly in a biological sample, if an exogenous source of ROS is added. The resulting parameter is the total radical trapping potential (TRAP), which represents the cumulative action of intracellular and intercellular enzymatic and non-enzymatic antioxidants, active under experimental conditions.

The TRAP assay is based on a chemiluminescence signal, which occurs when enzyme horseradish peroxidase is oxidized by hydrogen peroxide and loses two electrons. When the horseradish peroxidase oxidizes luminol to recover the missing electrons, the latter becomes unstable and emits chemiluminescence [1].

Chlorophyll fluorescence is a widely used method for accessing the physiological status of the photosynthetic apparatus. PAM (pulse amplitude modulated) fluorometry has become a common tool in plant physiology and ecophysiology to access photosynthetic performance of plants non-invasively. Monitoring of primary photochemistry is extremely useful in the cases of plant stress caused by high temperature, chilling, high light and drought [3]. The photosynthetic parameters potential photochemical efficiency (Fv/Fm), photochemical

quenching (qp) and non-photochemical quenching (qn) are well described in several articles including [9].

Vegetation indexes are robust, empirical measures of vegetation activity at the land surface. They are designed to enhance the vegetation signal from measured spectral responses by combining two (or more) different wavebands, often in the red (600-700 nm) and Near-IR wavelengths (700-1100 nm). Stress is indicated by progressive decrease in Near-IR reflectance due to water loss and increase in red reflectance due to lower rates of photosynthesis.

In the research, the results of hop plant response to drought stress on physiological level using TRAP, PAM fluorometry and reflectometry (normalized difference vegetation index - NDVI) were obtained for the very first time. The good indication for higher tolerance to drought in the variety Aurora was obtained as it was expected.

2 MATERIAL AND METHODS

2.1 Material

In the experiment two well known Slovenian hop varieties were included which differ in drought tolerance in field conditions, Aurora as a more tolerant variety and Savinjski golding as a less tolerant or susceptible variety to drought.

2.2 Methods

2.2.1 The experiment in growth chamber

The experiment was conducted in the year 2009. Plants were grown in pots (substrate Gramoflor, Germany; volume 4 L); every plant was grown in a separate pot at the beginning of the experiment we provided the same volume density of substrate. Four different treatments were undertaken, with 20 plants in each of them:

1. Aurora – drought
2. Aurora – control
3. Savinjski golding – drought
4. Savinjski golding – control

The plants were grown in controled conditions (growth chamber RK - 13300CH, Kambič Laboratory equipment, Slovenia), different regimes were applied for each treatment:

- Control treatment: Normal lightness (15000 lux); relative air humidity 70 %; the day length 15 h; day temperature: 26 °C, night temperature: 20 °C.
- Drought treatment: Normal lightness (15000 lux); relative air humidity 70 % in the beginning of the trial and 55 % under drought conditions.

The plants were optimally irrigated based on tensiometer measurements and with the pot weighting every 3 days as well. At each sampling the soil humidity was determined gravimetrically in each treatment.

All plants were irrigated for the last time on 33rd day of the trial. The control plants were optimally watered every 3 days, while the plants in drought treatment were not watered till regeneration phase started (58th day). The sampling was performed on:

1. 39th day of the trial
2. 51st day of the trial
3. 58th day of the trial
4. 78th day of the trial (regeneration)

After the 3rd sampling (58th day, 25 days after the last watering of plants in drought treatment) all plants (control and drought treatment) were optimally watered every 3 days (310 ml/pot) till the end of the trial.

2.2.3 Physiological measurements

2.2.3.1 Total radical trapping potential (TRAP)

The TRAP assay is based on a chemiluminescence signal, which is produced when horseradish peroxidase is oxidized by H_2O_2 and loses two electrons. When the horseradish peroxidase oxidizes luminol to recover the missing electrons, the latter becomes unstable and emits chemiluminescence [1]. The samples for TRAP measurement were prepared in the following manner: Approximately 100 mg of fresh leaf tissue per sample was frozen in liquid nitrogen, homogenized in 1 ml of potassium phosphate buffer (50 mM, pH 7.0) and centrifuged (10 min, 10000 RPM, 4 °C). The supernatant was frozen and kept at -80°C until the measurement of luminescence. The luminescence reaction mixture contained horseradish peroxidase (0.13 units ml^{-1}) and 350 µM luminol in 0.1 M potassium phosphate buffer, pH 8.5. Into the reaction mixture first the sample and then hydrogen peroxide (final concentration of 870 mM) was added to start the reaction. The mixture was shaken for 15 s followed by 5 s of incubation at 20°C. Chemiluminescence was then recorded as relative light units every 20 s for 25 min with a Victor X5 Multilabel reader 2030 (Perkin Elmer). The TRAP value of plant tissue homogenates was determined as the quotient of blanks to treatments according to

$$TRAP = \frac{\sum I_0}{\sum I},$$

where I_0 represents the measured chemiluminescence of the blank sample (luminol, buffer, horseradish peroxidase and H_2O_2) and I represents the chemiluminescence of the tested sample (supernatant of homogenized plant tissue, luminol, buffer, horseradish peroxidase and H_2O_2).

2.2.3.2 Chlorophyll fluorescence

Chlorophyll fluorescence was measured using a pulse amplitude modulated fluorometer from Opti Sciences (USA) OS-5 using a kinetic test protocol. Before measurement a dark clip was utilized for 10 minutes, then the F_0 of dark adapted leaves was obtained. The saturation pulse of the intensity 8000 µmol $(m^2s)^{-1}$ was switched on for 0,8 s and Fm was obtained. The steady state parameters Fs and Fm were measured after 3 minutes of actinic illumination of 160 µmol $(m^2s)^{-1}$.

From the measured fluorescence several physiological parameters were obtained: maximum quantum efficiency of PSII photochemistry $F_v/F_m=(F_m-F_0)/F_m$, photochemical quenching $qP=(F_m'-F')/(F_m'-F_0)$ and nonphotochemical fluorescence quenching $qN=(F_m-F_m')/(F_m-F_0)$ [9].

2.2.3.3 Reflectometry

Reflectance spectra was measured using an Ocean Optics HL-2000 Tungsten Halogen Light Source, the reflectance probe Ocean optics QR400-7-UV/BX and Ocean optics USB2000 spectrometer with the wavelength range from 400 – 1200 nm. Reflection probe was mounted 20 mm from the leaf at the 45 degrees incidence angle.

Reflection spectra was obtained using a SpectraSuit application software. First the dark spectra was subtracted. Light spectra was measured when 1 mm thick teflon sheet was inserted instead of the leaf. Reflection spectra was calculated as follows:

$R=(I_{leaf}-I_{noise})/(I_{teflon}-I_{noise})$

Every leaf was illuminated for 30 s before measurement to allow for the stabilization of reflectance in the red part of the spectrum.

NDVI was calculated as

$$NDVI = \frac{NIR - R}{NIR + R},$$

where NIR was taken at 800 ± 5 and R at 580 ± 5 nm.

The NDVI values were averaged between 5 plants with the same treatment.

2.2.3.4 Statistical analysis

The TRAP, Fv/Fm, qP, qN and NDVI values were averaged between 5 plants within the same treatment. The statistical significance was evaluated using a student t-test with a=0.05. All data groups were tested against their initial value at the start of the experiment. Additionally, averaged control and drought groups were tested for the significance of difference of their mean values.

3 RESULTS

3.1 Total radical trapping potential (TRAP)

As can be observed from the Figure 1, in the variety Savinjski golding, the TRAP values in control plants decrease in the third week as a consequence of non-optimal conditions in the growth chamber (too strong air ventilation), and again increase during the regeneration phase. The TRAP values in plants under drought conditions increase in the second week (as an active defense against ROS), while during the regeneration they are returned to the control level.

In the Aurora variety, the TRAP values decrease during the trial (as a consequence of the non-optimal conditions in the growth chamber) while in drought plants during the regeneration phase they reach the control level.

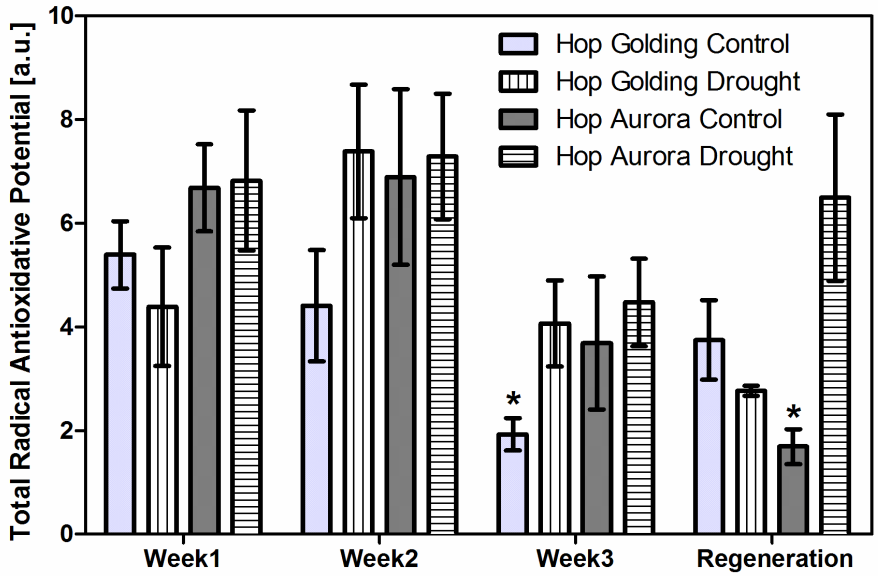

Figure 1: Total radical antioxidative potential (TRAP) of varieties Savinjski golding and Aurora in relation to drought stress duration. The watering was discontinued six days prior to 'Week1' time point. After 25 days of drought, the plants were watered to test their regeneration capability (after 20 days of re-watering). Data shown are mean values ± standard error of a single experiment performed in 5 replicates (n=5). Asterisk (*) represents statistically significant difference between control and drought exposed plants of the same variety at the same time point.

Slika 1: Rezultati TRAP testa sort hmelja Savinjski golding in Aurora, v odvisnosti od trajanja sušnega stresa. Zalivati smo prenehali šest dni pred "Week1" časovno točko. Po 25. dnevih suše smo rastline zalili, da smo preizkusili njihovo sposobnost regeneracije (po 20. dnevih ponovnega zalivanja). Prikazani podatki so povprečne vrednosti ± standardna napaka enega eksperimenta izvedenega v petih ponovitvah (n = 5). Zvezdica (*) predstavlja statistično značilne razlike v TRAP vrednostih, pri rastlinah, izpostavljenih suši in kontrolnimi rastlinami, iste sorte ob istem času.

3.2 Chlorophyll fluorescence

At the start of the experiment we obtained the theoretical optimum value of 0.83 for the parameter Fv/Fm (Figure 2a). In the second week the value dropped for drought exposed Aurora plants, but their state improved during the regeneration. The Fv/Fm value of control plants of Aurora variety dropped during regeneration showing non-optimal environmental conditions in the growth chamber.

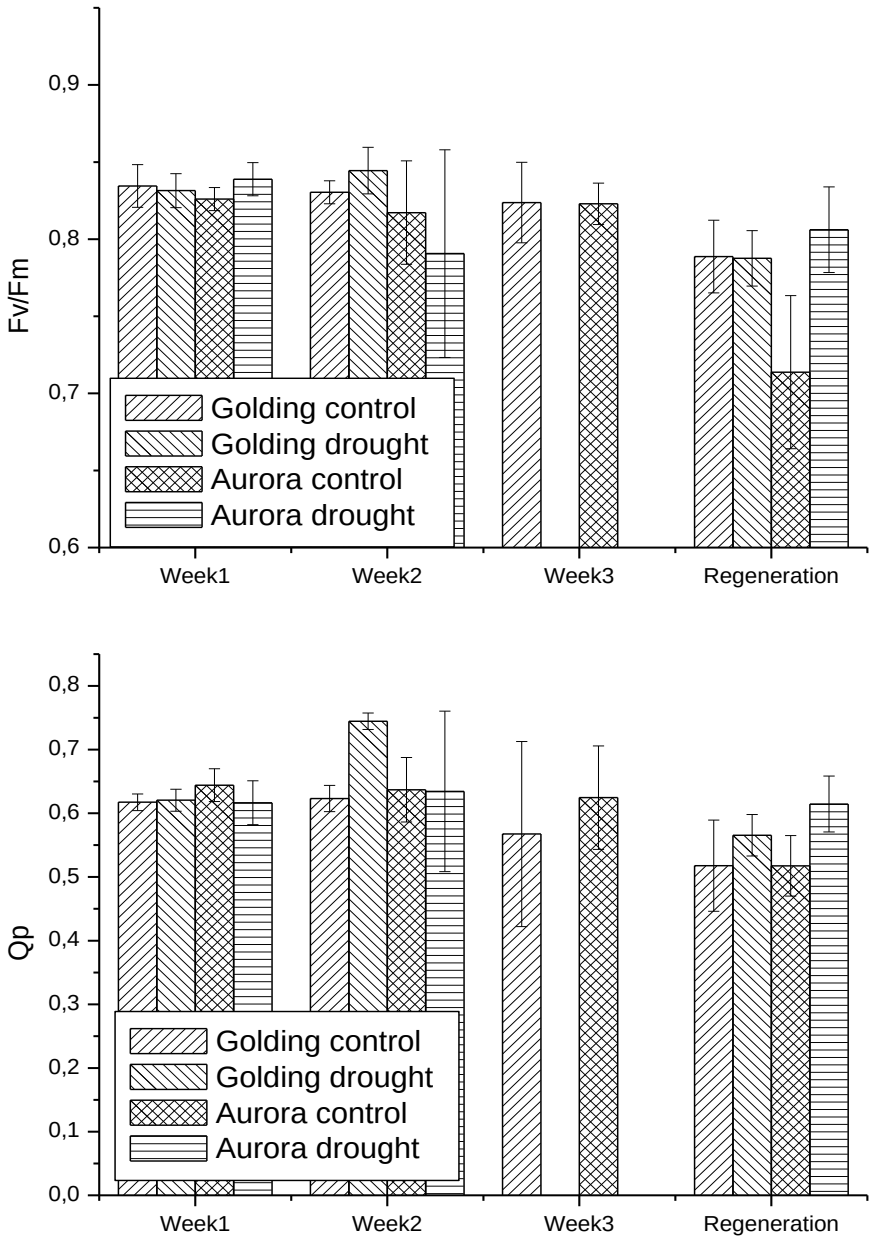

Figure 2a: Photochemical parameters Fv/Fm and qP of hop varieties Savinjski golding and Aurora after different time of drought exposure. Data shown are mean values ± standard deviation of a single experiment performed in 5 replicates (n=5).

Slika 2a: Fotokemična parametra Fv/Fm in qP pri sortah hmelja Savinjski golding in Aurora, po različnem času trajanja suše. Prikazani podatki so povprečne vrednosti ± standardni odklon enega poskusa, izvedenega v petih ponovitvah (n = 5).

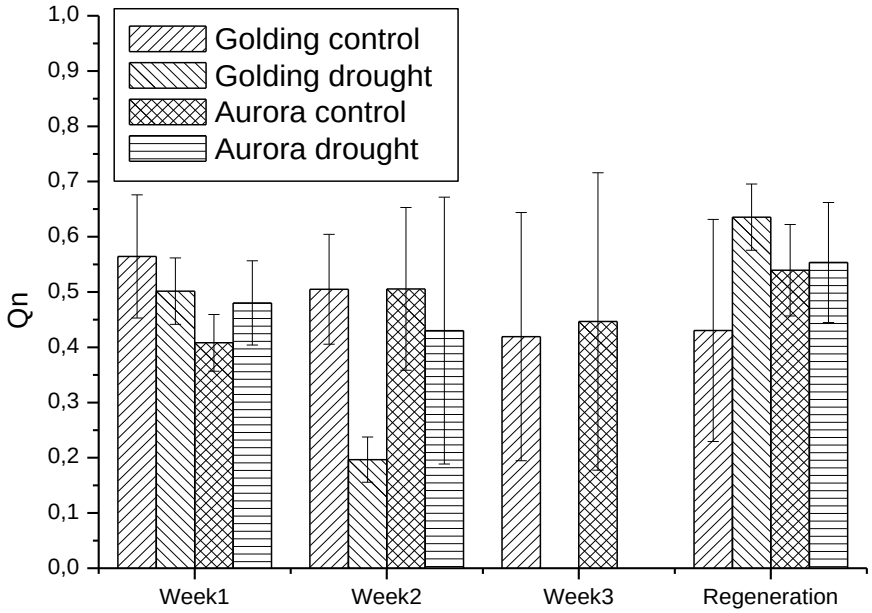

Figure 2b: Photochemical parameter qN of hop varieties Savinjski golding and Aurora after different time of drought exposure. Data shown are mean values ± standard deviation of a single experiment performed in 5 replicates (n=5).

Slika 2b: Fotokemični parameter qN pri sortah hmelja Savinjski golding in Aurora, po različnem času trajanja suše. Prikazani podatki so povprečne vrednosti ± standardni odklon enega poskusa, izvedenega v petih ponovitvah (n = 5).

We concentrated on the data from the regeneration stage which can give us some indication of the damage caused by drought. The increase of the photochemical quenching qP of the drought exposed plants during the regeneration show that the fitness of these plants increased after the stress has been removed. Although the high qN value of the Savinjski golding variety show some damage of the photosynthetic apparatus.

3.3 Reflectometry (NDVI)

The NDVI values of Savinjski golding variety are lower already at the beginning of the experiment. There was a drop in NDVI for the Aurora variety, which was not statistically significant. During the regeneration phase we obtained similar values for the control and drought exposed plants, but the difference between the varieties remained (Figure 3).

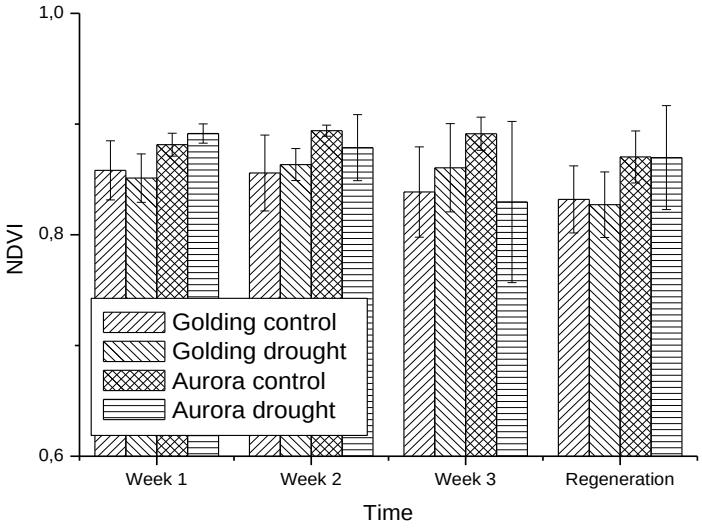

Figure 3: Measured values of the reflectometric index NDVI of hop varieties Savinjski golding and Aurora after different timef drought exposure. Data shown are mean values ± standard deviation of a single experiment performed in 5 replicates (n=5).

Slika 3: Izmerjene vrednosti reflektometričnega indeksa NDVI sort hmelja Savinjski golding in Aurora po različnem času trajanja suše. Prikazani podatki so povprečne vrednosti ± standardni odklon enega poskusa, izvedenega v petih ponovitvah (n = 5).

4 DISCUSSION

The results obtained from TRAP test revealed that the variety Savinjski golding exhibited a typical stress response, as the antioxidative network was transiently induced in the stressed plants. This suggests higher activity of antioxidative enzymes and/or increased concentrations of low molecular antioxidants, such as glutathione [7, 8]. The TRAP value later gradually decreased and reached the lowest point in the drought-stressed regenerated plants (Figure 1). This indicates that antioxidative network in the Savinjski golding plants was overburdened and that the plants could not undo the damage caused by the drought induced oxidative stress.

In contrast, the TRAP value of drought-stressed plants of the variety Aurora decreased progressively in three weeks of drought conditions, however the same was observed for the control plants (Figure 1). During regeneration the TRAP value in drought-stressed plants increased to the initial values and surpassed the control plants' TRAP value. This demonstrates that the antioxidative network of the Aurora plants was not irreversibly damaged during drought stress. Therefore the Aurora variety is more drought tolerant compared to Savinjski golding variety and has a higher regenerative capability.

The results of the regeneration phase can point out some insight into the physiology of the photosynthetic apparatus. The most robust parameter Fv/Fm representing maximum quantum efficiency of photosystem II shows that drought exposed plants managed to avoid permanent damage as expected in the case of mild water stress [2, 6]. The good regeneration capacity is

especially evident for the Aurora variety. During the regeneration phase we obtained the same values for non-photochemical quenching in control and drought exposed plants.

NDVI is a measure of the water content in leaf [10]. The difference between the two varieties at the beginning of the experiment shows higher water content in the Aurora variety. There was some drop of the NDVI during the drought for Aurora but the difference was not significant and quickly returned to the start values during the regeneration phase showing that there was no irreversible damage to the plants.

Based on the results obtained in this preliminary investigation we can conclude that variety Savinjski golding appeared to be more drought sensitive than the variety Aurora. The variety Aurora shows better regeneration capacity as well. The same indication was obtained from the results of TRAP and PAM fluorometry. In the further research the experiment will be conducted in optimal conditions. Finally, the presented results confirm the data obtained from the observations in field conditions as was expected. The use of optimized TRAP method for drought selection in hop breeding program will be studied in the future.

5 ACKNOWLDEGEMENT

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PHYSIOLOGICAL RESPONSE OF COMMON BEAN (*Phaseolus vulgaris* L.) TO DROUGHT STRESS

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Abstract

Exposure to drought stress induces a cluster of physiological changes and has detrimental effects on several cell functions. Common bean is sensitive to drought stress, which can cause yield losses of more than 50 %. Different approaches to study physiological changes in leaves of two common bean varieties, Tiber and Starozagorski, subjected to drought stress were implemented. These included Total Radical-Trapping Potential (TRAP) test, Pulse-Amplitude-Modulation (PAM) fluorometry and reflectometry. The results of TRAP measurements and reflectometry indicate higher drought tolerance of the variety Tiber. Drought stress was not so intense as to cause irreversible damage of the photosynthetic apparatus in any of the varieties.

Keywords: common bean, *Phaseolus vulgaris* L., drought, physiological response

FIZIOLOŠKI ODZIV NAVADNEGA FIŽOLA (*Phaseolus vulgaris* L.) NA SUŠNI STRES

Izvleček

Izpostavljenost sušnemu stresu povzroči niz fizioloških sprememb v rastlinah in ima lahko uničujoč vpliv na številne celične funkcije. Navadni fižol je občutljiv na sušni stres, pridelek se lahko zmanjša do 50%. Za študij fizioloških sprememb v listih dveh sort navadnega fižola, Tiber in Starozagorski, so bili uporabljeni različni pristopi, ki so vključevali TRAP (Total Radical-Trapping Potential) test, PAM (Pulse-Amplitude-Modulation) fluorometrija in reflektometrija. Rezultati meritev TRAP in reflektometrije so pokazali, da je sorta Tiber bolj odporna na sušni stres. Vendar sušni stres pri nobeni sorti ni bil tako močan, da bi povzročil nepovratne spremembe fotosintetskega aparata.

Ključne besede: fižol, *Phaseolus vulgaris* L., suša, fiziološki odziv

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1 INTRODUCTION

Drought is a major factor affecting the growth and development of plants and may cause severe reductions in crop yields in many countries in the world. Its importance is likely to increase in response to the effect of global change and increased competition for water. The first signs of drought are visible in leaves, which appear prematurely senescent, although earlier changes, both morphological and metabolic, occur in roots, the first tissues to experience the reduction in water supply. These changes reflect, not merely a progressive reduction of water content in the plant, but qualitative and quantitative changes in its metabolism, suggesting a number of mechanisms by which plants can, within different limits, tolerate drought and recover from its effects. During evolution, plants have developed both physiological and biochemical responses to promote their survival under stress.

Drought stress can lead to increased reactive oxygen species (ROS) production. Drought induced stomatal closure inhibits CO_2 uptake, which under high light conditions results in over-reduced electron transport chains leading to photo-oxidative stress [27]. The plant cells can contain drought induced imbalance between pro- and antioxidants only to a certain degree, after which irreversible oxidative damage occurs [8]. Some plants, however, are more adapted to drought. Pastore et al. [18] have demonstrated that durum wheat mitochondria have mechanisms for dissipation of membrane potential, which inhibits ROS production. Other mechanisms of drought avoidance and oxidative damage control include solute accumulation, cell wall modification and the synthesis of protective proteins [31]. ROS are present in all aerobic cells, in equilibrium with antioxidants. When this balance is disturbed oxidative stress occurs. Aerobic organisms respond to oxidative stress by either non-enzymatic or enzymatic defence responses. Non-enzymatic defence reactions involves glutathione, ascorbic acid, α-tocopherol, β-carotene and other compounds capable of quenching ROS. Enzymes involved in defence include superoxide dismutases, catalases, peroxidases, glutathione reductase and $NADP^+$ reducing enzymes [14, 21, 24]. One of the methods for detecting ROS, particularly the formation of H_2O_2, is luminol assisted chemiluminescence [3]. With a slight modification of this method it is possible to determine ROS quenching activity in a biological sample, if an exogenous source of ROS is added. The resulting parameter, Total Radical Antioxidant Potential (TRAP), represents the cumulative action of intracellular and intercellular enzymatic and non-enzymatic antioxidants active under assay conditions [30]. The assay of luminol assisted chemiluminescence has been used for determining the antioxidant activity of pure chemicals [9] as well as biological samples [17, 20].

Chlorophyll fluorescence has become a common tool in plant physiology and ecophysiology to access photosynthetic performance of plants non-invasively. Monitoring of primary photochemistry is extremely useful in the cases of plant stress caused by high temperature, chilling, high light and drought [12]. The photosynthetic parameters Fv/Fm, qP and qN are well described [22].
The decrease in relative water content of leaves cause stomatal closing inhibiting the supply of CO_2 to the mesophyll cells. Such conditions are not expected to influence fluorescence induction parameters such as Fv/Fm [2]. After severe water stress the inhibition of the photosynthesis occurs resulting in the reduction of photochemical quenching qP [16] and increase in nonphotochemical quenching [11]. Fv/Fm is the indicator of the extreme stress causing the photoinhibition of PSII [12].

Reflectometry in the visible and near-infrared spectrum is a popular tool for studying plant stress, yield and biomass in the field conditions. Reflectometry is especially suited for the remote sensing applications from airplanes and satellites. One of the major goals for the remote sensing is detection of the water stress caused by drought. There are several vegetation indexes used to indentify leaf water content including normalized difference vegetation index (NDVI), developed by Rouse et al. [23]:

$$NDVI = \frac{NIR - R}{NIR + R},$$

where R and NIR are reflectances in the red (0.6-0.7 μm) and Near-IR wavelengths (0.7-1.1 μm) respectively. NDVI was used for several field studies including screening of drought tolerance in *Solanum* [25], peanut genotypes [26] and mixed vegetation [7].

Common bean is widely exposed to drought and many modifications associated with stress responses generally affect plant growth and yield. Abiotic stress resistance is by its nature more complex, is typically subject to large environmental effects and has been less well studied than biotic stress resistance in common bean [19]. Therefore, compared to pest and disease resistance, much less is known about the genetics of resistance to abiotic constraints or physiological stress [13] Abiotic stress resistance is typically governed by polygenic inheritance and may be conditioned by multiple, interacting mechanisms. These and other factors make abiotic stress resistance especially difficult to study, both physiologically and genetically. Several genes whose expression responds to drought have already been identified in common bean [28, 29]. In a study that was performed at the Agricultural Institute of Slovenia changes in gene expression in the leaves at different levels of dehydration were identified by differential display reverse transcriptase PCR and confirmed by quantitative real-time PCR [10].

A better understanding of the physiological basis of differences in drought stress resistance is very important in selection of new varieties of crops to obtain a better productivity under stress conditions. Studies of the effect of drought on the yield, photosynthesis, growth, osmotic adjustment and also about adaptation of common bean were described by Montalvo-Hernández et al. [15] and Aydi et al. [1]. In many studies the identification of tolerant and susceptible cultivars is based on a few physiological measures related to drought response. The physiologically relevant integrator of drought effects are the water content and the water potential of plant tissues which are widely published [6]. Experience in other crops indicates that abiotic stress tolerance may be the key to improving yields of common bean in both stressed and unstressed environments. Although disease resistance remains an important objective for most bean breeding programs, selection for greater tolerance to abiotic stress such as drought is expected to gain importance in response to climate change [4]. Breeding for drought resistance has a long history in many national bean programmes but only limited progress has been made in the improvement of cultivars [4]. The reasons for slow improvement are genetic complexity and the complex mechanism of water stress tolerance. However, there have been a few recent reports on development of cultivars and breeding lines with enhanced tolerance to drought [5]. Local adaptation is an important component of drought resistance in common bean. Development of cultivars with improved resistance to abiotic stress is one of the goals of the common bean breeding program at the Agricultural Institute of Slovenia.

The aim of the research was to implement different approaches to study physiological changes in common bean plants subjected to drought stress, which included TRAP test, PAM fluorometry and reflectometry.

2 METHODS

2.1 Plant material

Two common bean varieties, Starozagorski (susceptible to drought) and Tiber (tolerant to drought) were included in the analysis. The samples for TRAP measurement were excised from the 5th pair of leaves (bottom up), whereas PAM and NDVI measurements were performed on the 3rd or 4th pair of leaves (bottom up), each time on the same leaf.

2.2 The experiment in growth chamber

The experiment was conducted under controlled conditions in the growth chamber (RK - 13300CH, Kambič Laboratory equipment, Slovenia) in the year 2009. Plants were grown in pots (substrate Gramoflor, Germany; volume 4 l); in each pot one plant was grown. At the beginning of the experiment the same volume density of substrate was provided. Plants were subjected to three different levels of dehydration followed by a regeneration phase. Different regimes were applied for each treatment, control (well-watered plants) and drought (plants under different levels of dehydration) (Table 1). Twenty plants were used per each treatment. For the control treatment the regime was as follows: normal lightness (15000 lux); relative air humidity 70 %; the day length 15 h; day temperature: 26 °C, night temperature: 20 °C. The regime for drought treatment was: normal lightness (15000 lux); relative air humidity 70 % in the beginning of the experiment and 55 % under drought conditions.

The plants were optimally irrigated based on tensiometer measurements and with the pot weighting every three days. Control plants followed these regimes until the end of the experiment, while watering of drought-stressed plants was stopped when plants were 24, 31 and 38 days old. After 21 of water deprivation all plants (control and drought treatment) were optimally watered again every third day (310 ml/pot) till the end of the experiment. At each sampling the soil humidity was determined gravimetrically for each treatment.

Table 1: Plant age at collection for control (well-watered), drought-stressed plants and at the regeneration phase with duration of water deprivation.

Preglednica 1: Starost rastlin ob vzorčenju kontrolnih rastlin (dobro zalite), rastlin pod sušnim stresom in rastlin v fazi regeneracije s trajanjem odvzema vode

Treatment	Plant age at collection	Water deprivation days
Control	24, 31, 38, 57	
Drought - week 1	24	7
Drought - week 2	31	14
Drought - week 3	38	21
Regeneration phase	57	21

2.3 TRAP

The TRAP assay is based on a chemiluminescence signal, which is produced when horseradish peroxidase is oxidized by H_2O_2 and loses two electrons. When the horseradish peroxidase oxidizes luminol to recover the missing electrons, the latter becomes unstable and emits chemiluminescence [3]. The samples for TRAP measurement were prepared in the following manner: Approximately 100 mg of fresh leaf tissue per sample was frozen in liquid nitrogen, homogenized in 1 ml of potassium phosphate buffer (50 mM, pH 7.0) and centrifuged (10 min, 10000 RPM, 4 °C). The supernatant was frozen and kept at -80°C until the measurement of luminescence. The luminescence reaction mixture contained horseradish peroxidase (0.13 units ml^{-1}) and 350 µM luminol in 0.1 M potassium phosphate buffer, pH 8.5. To the reaction mixture, the sample and then hydrogen peroxide (final concentration of 870 mM) were added to start the reaction. The mixture was shaken for 15 s followed by 5 s of incubation at 20°C. Chemiluminescence was then recorded as relative light units every 20 s for 25 min with a Victor X5 Multilabel reader 2030 (Perkin Elmer). The TRAP value of plant tissue homogenates was determined as the quotient of blanks to treatments according to

$$TRAP = \frac{\sum I_0}{\sum I},$$

where I_0 represents the measured chemiluminescence of the blank sample (luminol, buffer, horseradish peroxidase and H_2O_2) and I represents the chemiluminescence of the tested sample (supernatant of homogenized plant tissue, luminol, buffer, horseradish peroxidase and H_2O_2).

The samples of variety Starozagorski from the first week of the experiment unfortunately thawed during the transportation to the laboratory, therefore we omitted those results.
Note: The results of TRAP test of Starozagorski variety in the first week of drought were excluded from the analysis due to power failure of the ultra-freezer in which they were stored.

2.4 Chlorophyll fluorescence

Chlorophyll fluorescence was measured using a pulse amplitude modulated fluorometer from Opti Sciences (USA) OS-5 using a kinetic test protocol. Before measurement a dark clip was utilized for 10 minutes, and then the F_0 of dark adapted leaves was obtained. The saturation pulse of the intensity 8000 µmol $(m^2s)^{-1}$ was switched on for 0.8 s and Fm was obtained. The steady state parameters Fs and Fm were measured after 3 minutes of actinic illumination of 160 µmol $(m^2s)^{-1}$.

From the measured fluorescence several physiological parameters were obtained: maximum quantum efficiency of PSII photochemistry $F_v/F_m=(F_m-F_0)/F_m$, photochemical quenching $qP=(F_m'-F')/(F_m'-F_0)$ and nonphotochemical fluorescence quenching $qN=(F_m-F_m')/(F_m-F_0)$ [22].

2.5 Reflectometry

Reflectance spectra was measured using a Ocean Optics HL-2000 Tungsten Halogen Light Source, the reflectance probe Ocean optics QR400-7-UV/BX and Ocean optics USB2000

spectrometer with the wavelength range from 400 – 1200 nm. Reflection probe was mounted 20 mm from the leaf at the 45 degrees incidence angle.

Reflection spectra were obtained using SpectraSuit application software. First the dark spectrum was subtracted. Light spectra were measured when 1 mm thick teflon sheet was inserted instead of the leaf. Reflection spectra were calculated as follows:

$R=(I_{leaf}-I_{noise})/(I_{teflon}-I_{noise})$

Every leaf was illuminated for 30 s before measurement to allow for the stabilization of reflectance in the red part of the spectrum.

NDVI was calculated as

$$NDVI = \frac{NIR - R}{NIR + R},$$

where NIR was taken at 800 ± 5 and R at 580 ± 5 nm.The NDVI values were averaged between 5 plants with the same treatment.

2.6 Statistical analysis

The TRAP, Fv/Fm, qP, qN and NDVI values were averaged from five plants within the same treatment. The statistical significance of plants exposed to drought compared to the control plants was evaluated using a student t-test. The results were considered statistically significant when $p<0.05$.

3 RESULTS

3.1 TRAP

A transient significant difference between control and drought stressed plants of variety Tiber was measured in week 3 of drought stress (Figure 1). The TRAP value of the drought stressed plants was 154% of the control value. The TRAP value in the leaves of the variety Tiber increased in the regeneration phase, but the difference between the control and drought-stressed plants diminished.

The TRAP value of the drought-stressed plants of variety Starozagorski insignificantly decreased from week 2 to week 3 (Figure 1). The TRAP value significantly differed between the control and the drought-stressed plants at the regeneration phase; it was 2.57-fold higher in drought stressed plants compared to control plants.

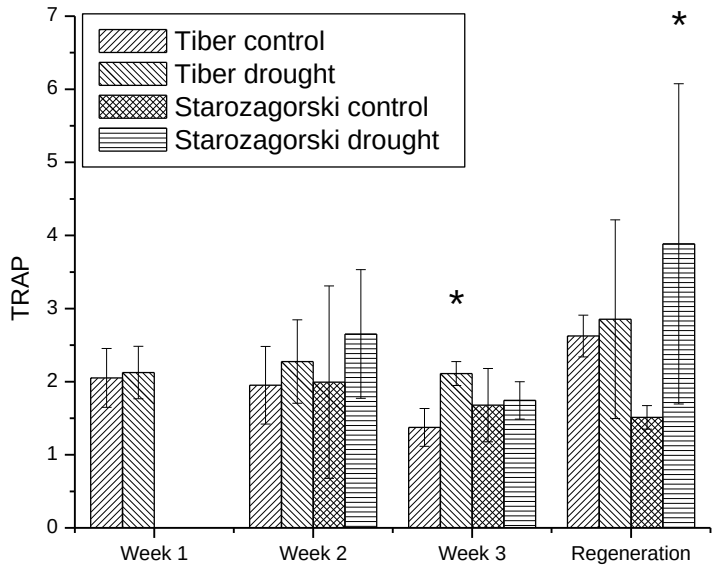

Figure 1: Total radical antioxidative potential of common bean plants, varieties Tiber and Starozagorski in relation to the duration of drought stress. The watering was discontinued seven days prior to 'Week1' time point. After three weeks of drought, the plants were watered to test their regeneration capability. Data shown are the mean values ± standard error of a single experiment performed in 5 replicates (n=5). Asterisk (*) represents statistically significant difference between control and drought exposed plants of the same variety at the same time point.

Slika 1: Rezultati TRAP testa fižolovih rastlin, sort Tiber in Starozagorski, v odvisnosti od trajanja sušnega stresa. Zalivati smo prenehali sedem dni pred "Week1" časovno točko. Po treh tednih suše smo rastline zalili, da smo preizkusili njihovo sposobnost regeneracije. Prikazani podatki so povprečne vrednosti ± standardna napaka enega poskusa, izvedenega v petih ponovitvah (n = 5). Zvezdica (*) predstavlja statistično značilne razlike med rastlinami, izpostavljenim suši in kontrolnimi rastlinami, iste sorte ob istem času.

3.2 Chlorophyll fluorescence

We observed a decrease of qP in week 3 for the stressed samples in both varieties. The difference was statistically significant in the variety Starozagorski. The effect was reversed but non significant in the regeneration phase (Figure 2a).

There was no statistically significant difference of nonphotochemical quenching qN between the control and drought exposed plants during the drought induction (Figure 2b). During the regeneration phase qN of the drought exposed variety Starozagorski dropped significantly compared to the control.

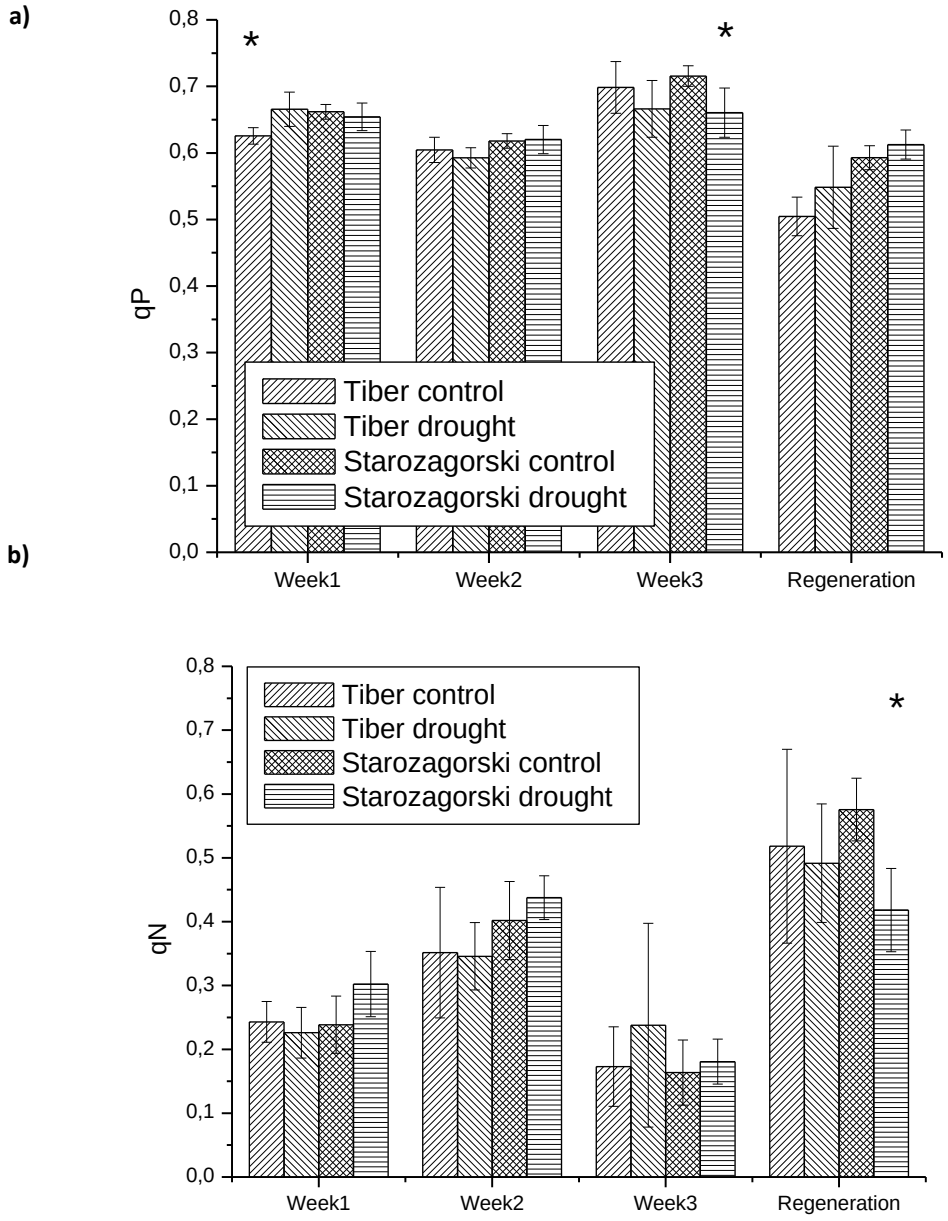

Figure 2: Photochemical parameters qP (a) and qN (b) of two common bean varieties, Tiber and Starozagorski after different duration of drought. Data shown are mean values ± standard deviation of a single experiment performed in 5 replicates (n=5). Asterisk (*) represents statistically significant difference between control and drought exposed plants of the same variety at the same time point.

Slika 2: Fotokemična parametra qP (a) in qN (b) dveh sort navadnega fižola, Tiber in Starozagorski, po različnem času trajanja suše. Prikazani podatki so povprečne vrednosti ± standardni odklon enega poskusa, izvedenega v petih ponovitvah (n = 5). Zvezdica (*) predstavlja statistično značilne razlike med rastlinami, izpostavljenim suši in kontrolnimi rastlinami, iste sorte ob istem času.

Parameter Fv/Fm was stable at the theoretical optimum value 0.83 except for the drought exposed plants of the variety Starozagorski in week 3, where the measured Fv/Fm value was 0.8 (Figure 3). The effect diminished during the regeneration.

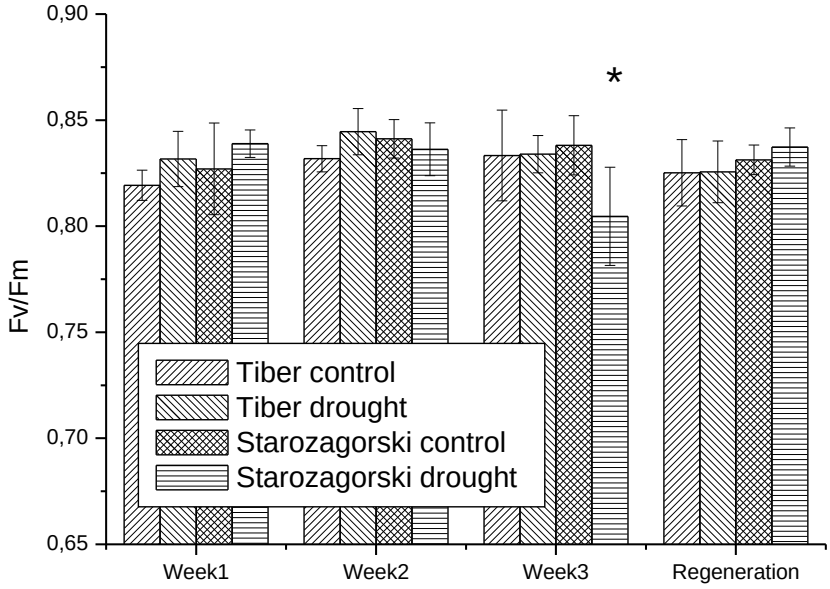

Figure 3: Photochemical parameter Fv/Fm of two common bean varieties, Tiber and Starozagorski after different duration of drought. Data shown are mean values ± standard deviation of a single experiment performed in 5 replicates (n=5). Asterisk (*) represents statistically significant difference between control and drought exposed plants of the same variety at the same time point.

Slika 3: Fotokemični parameter Fv/Fm dveh sort navadnega fižola, Tiber in Starozagorski, po različnem času trajanja suše. Prikazani podatki so povprečne vrednosti ± standardni odklon enega poskusa, izvedenega v petih ponovitvah (n = 5). Zvezdica (*) predstavlja statistično značilne razlike med rastlinami, izpostavljenim suši in kontrolnimi rastlinami, iste sorte ob istem času.

3.3 Reflectometry

In the first two weeks (week 1, week 2) there was no difference between the groups with the NDVI being approximately 0.91. In week 3 there was a slight drop of NDVI in the drought exposed variety Starozagorski. The difference was reduced in the regeneration phase.

4 DISCUSSION

The results of TRAP measurements of common bean plants under drought stress indicate higher drought tolerance of the variety Tiber. The TRAP value in the leaves of drought stressed Tiber plants was significantly higher than in the control plants in the third week of drought stress. This indicates active struggle against drought-induced oxidative stress [20]. During the regeneration phase the difference of TRAP value between drought-stressed and control plants diminished. The opposite is true for the variety Starozagorski, where the TRAP

value was significantly induced in the drought stressed plants during the regeneration phase. This indicates that the antioxidative network was still induced after three weeks of regeneration and that the plants were still struggling against drought-induced oxidative stress [20].

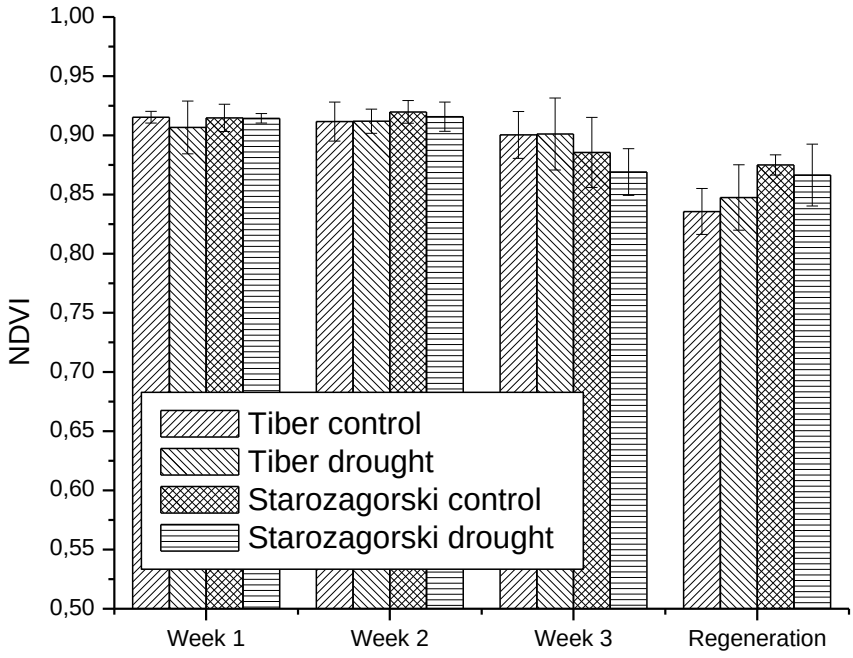

Figure 4**:** Measured values of the reflectometric index NDVI of leaves of two common bean varieties Tiber and Starozagorski s after different times of drought induction. Data shown are the mean values ± the standard deviation of a single experiment performed in five replicates (n=5). Asterisk (*) represents statistically significant difference between control and drought exposed plants of the same variety at the same time point.

Slika 4: Izmerjene vrednosti reflektometričnega indeksa NDVI v listih dveh sort fižola, Tiber in Starozagorski, po različnem času trajanja suše. Prikazani podatki so povprečne vrednosti ± standardni odklon enega poskusa, izvedenega v petih ponovitvah (n = 5). Zvezdica (*) predstavlja statistično značilne razlike med rastlinami, izpostavljenim suši in kontrolnimi rastlinami, iste sorte ob istem času.

First effects of drought on photosynthesis were observed after three weeks of drought. The stress manifested itself via the decrease of the photochemical quenching, which can be a direct consequence of stomatal closing and lower mesophyll CO_2 concentration [2]. There was no observable increase in nonphotochemical quenching showing the ability of the stressed plants to utilize the actinic illumination for the photosynthesis. The damage caused by drought was indicated in Starozagorski variety where parameter Fv/Fm was decreased. Such effect is not expected in the case of mild water stress according to Lima et al. [11]. The drought stress was not so intense as to cause irreversible damage to the plants. During the regeneration phase the qP value in the drought-stressed plants was higher than in the control plants. The opposite was

true at week 3. Additionally, the Fv/Fm value of the drought-stressed plants of the variety Starozagorski surpassed the control values during the regeneration indicating that the plants actively repaired the photosynthetic apparatus.

NDVI was very stable in the first two weeks of the experiment, showing the decrease in the variety Starozagorski in third week. The lower value in the drought exposed plants of Starozagorski indicates a drop in the leaf water content. The drop of NDVI values in the regeneration phase indicates damage to the structure of the leaves in the variety Starozagorski since the effect was not fully reduced during the regeneration phase. For the variety Tiber there was no observable difference between the plants before and during the regeneration phase. NDVI shows higher drought resistance in the variety Tiber and indicates that reflectometry can be used to distinguish drought tolerant and intolerant plants as in the study of Sullivan and Holbrook [26] conducted on peanuts plants.

The decrease in NDVI value in the variety Starozagorski in the third week showed the onset of drought stress. This is in accordance with a significant decrease of the Fv/Fm parameter. During the regeneration phase the photosynthetic performance of the drought-stressed plants of variety Starozagorski improved as represented by the increase of qP and Fv/Fm and reduction of qN. However, the significantly increased TRAP value shows that the antioxidant apparatus of plants of the variety Starozagorski was still active in protecting the plants from drought-induced oxidative damage. In contrast, in the variety Tiber we did not measure any significant decrease of the Fv/Fm value and the average NDVI index was very similar in the control and drought stressed plants in the third week of drought. Additionally, the TRAP value was not induced during the regeneration phase. This indicates that the strategy of the variety Tiber for coping with drought stress is stress avoidance.

The results provide new insights into physiological pathways of drought tolerance in common bean and revealed that the methods applied to measure physiological changes in leaves of plants exposed to drought could be applicable in the selection for drought tolerance in the breeding program of common bean.

4 ACKNOWLDEGEMENT

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UČINKOVITOST FUMIGANTOV KLORPIKRINA IN DAZOMETA ZA ZATIRANJE GLIVE *VERTICILLIUM ALBO-ATRUM* V TLEH

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Izvleček

Talni škodljivi organizmi spadajo med pomembne omejujoče dejavnike rastlinske proizvodnje, saj intenzivna in monokulturna pridelava povzroča naraščanje njihovega infekcijskega potenciala. Nadaljevanje proizvodnje na okuženih območjih poleg odstranjevanja obolelih rastlin temelji predvsem na uničevanju in nižanju talnega infekcijskega potenciala. V prispevku predstavljamo poskus, v katerem smo preizkušali učinkovitost kemičnega razkuževanja tal s fitofarmacevtskima pripravkoma na osnovi aktivne snovi klorpikrin in dazomet. Učinkovitost razkuževanja smo določali s spremljanjem nivoja umetno pripravljenega infekcijskega potenciala glive *Verticillium albo-atrum* in določanjem vpliva na pojav zapleveljenosti poskusnih površin.

Ključne besede: hmelj, kemično razkuževanje tal, talne glive

EFFICACY OF FUMIGANTS CHLOROPICRIN AND DAZOMET TO CONTROL *VERTICILLIUM ALBO-ATRUM* IN SOIL

Abstract

Soil borne pathogens present limitation factors of plant production, because intense and monoculture production causes increasing of their infection potential. Besides destroying of infected plants, continuation of production on contaminated areas is based on elimination or lowering of soil infection potential. The manuscript presents efficacy trial of chemical soil disinfection by using two phyto-pharmaceutical products based on active ingredients, chloropicrin and dazomet. The disinfection efficacy was evaluated by determination of level of artificially prepared infection potential of *Verticillium albo-atrum* and assessments of weed populations in trial plots.

Key words: hop, chemical soil disinfestations, soil fungi

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1 UVOD

Talni škodljivi organizmi spadajo med pomembne omejujoče dejavnike rastlinske proizvodnje, saj intenzivna in monokulturna pridelava povzroča prerazmnožitve in naraščanje njihovega infekcijskega potenciala. Največji delež škod na rastlinah, ki se izražajo v obliki gnitja koreninskega sistema, uvelosti, padavic in trohnob, povzročajo talne glive iz rodov kot so: *Fusarium, Verticillium, Sclerotinia, Rhizoctonia, Thielaviopsis* in oomicete iz rodov *Phytium* ter *Phythopthora*. Pogoste so tudi okužbe s talnimi patogenimi bakterijami kot so *Erwinia carotovora* in vrstami iz rodov *Pseudomonas, Xanthomonas* in *Clavibacter*. Med zelo pomembne talne škodljive organizme spadajo tudi fitofagne ogorčice, ki poleg neposrednega parazitizma rastlin ustvarjajo sinergistične interakcije s patogenimi talnimi glivami in tako pospešujejo razvoj bolezenskih stanj. V Sloveniji po obsegu škode izstopa pojav zelo virulentnega patotipa PV1 talne glive *V. albo-atrum*, ki povzroča hitro odmiranje rastlin oz. letalno obliko verticilijske uvelosti hmelja. Od odkritja prvega žarišča leta 1997 je bolezen v 12 letih na območju Savinjske doline prizadela 195 ha hmeljišč, od katerih jih je bilo zaradi prevelike stopnje okuženosti uničenih več kot 100 ha [12].

Nadaljevanje proizvodnje na okuženih območjih poleg odstranjevanja obolelih rastlin temelji predvsem na uničevanju in nižanju talnega infekcijskega potenciala. Zaradi dolgotrajnega ohranjanja glive *V. albo-atrum* v tleh se tako na uničenih hmeljiščih izvaja obvezna štiriletna karantenska premena (Uradni list RS, št. 65/01, 117/02 in 31/04), ki vključuje pridelovanje ne-gostiteljskih rastlin kot so žita in trave. Čas trajanja karantenske premene temelji na osnovi preživitvene sposobnosti trajnih organov glive *V. albo-atrum* v tleh brez navzočnosti gostiteljskih rastlin [14]. Štiriletni izpad proizvodnje hmelja na uničenih hmeljiščih in vsakoletni pojav novih bolezenskih žarišč, ki zahtevajo izvajanje dodatnih ukrepov (kompostiranje hmeljevine, prilagojena agrotehnika, …) potiska pridelovalce na okuženih območjih v nezavidljiv in nekonkurenčen položaj.

Zaradi omenjenega se je v svetu za zatiranje talnih škodljivih organizmov zelo razširilo kemično razkuževanje tal s fumiganti, med katerimi je v obdobju 1960 - 2005 zaradi visoke stopnje učinkovitosti prevladoval metilbromid. Ugotovitve o škodljivem vplivu substance na zemeljsko ozonsko plast in o visoki stopnji strupenosti je prispevala k zavezi o opuščanju uporabe metilbromida [16] in posledično širši uporabi fumigantov kot so klorpikrin, dazomet, 1,3-dikloropropen, metam-kalij in metam-natrij ter razvoju alternativnih bioloških metod dezinfestacije. Učinkovitost posameznih fumigantov se razlikuje glede na tarčni organizem in je močno odvisna od načina aplikacije ter pogojev v času razkuževanja. V letu 2010 smo opravili poskus v katerem smo želeli preizkusiti možnost zatiranja glive *V. albo-atrum* na izkrčenih hmeljiščih z uporabo kemičnih fumigantov na osnovi aktivne snovi (a.s.) klorpikrin in dazomet.

Klorpikrin (triklornitrometan, CCl_3NO_2) je bil prvotno razvit za vojaške namene kot solzivec, po prvi svetovni vojni pa so ugotovili njegovo uporabno vrednost tudi za namene talne fumigacije [13], predvsem v kombinaciji z metilbromidom. Danes je široko uporabljen fumigant, ki se uporablja v obliki injekcijske »shank« aplikacije in aplikacije preko namakalnega sistema predvsem pri pridelavi vrtnin in jagodičevja. Poleg dobrega delovanja na talne glive ga odlikuje hitra degradacija v tleh (razpolovni čas 1-2 uri) in relativna neškodljivost razpadnih produktov [18].

Dazomet (tetrahidro-3,5-dimetil-1,3,5-tiadiazin-2-tion) spada v skupino fumigantov, ki ob razgradnji tvorijo plin metil-izotiocianat, ki je toksičen za večino organizmov kot so glive, oomicete, ogorčice in semena rastlin [9]. V kmetijstvu se uporablja za razkuževanje tal že od leta 1970 dalje, najdemo pa ga tudi v industriji pri proizvodnji kartona, izdelkov iz gume in različnih strojnih transmisijskih elementov.

Učinkovitost razkuževanja smo določali s spremljanjem umetno pripravljenega infekcijskega potenciala glive *V. albo-atrum* in določanjem vpliva FFS na pojav zapleveljenosti poskusnih površin.

2 MATERIAL IN METODE

2.1 Poskusno polje in aplikacija fumigantov

Poskus je potekal v kraju Gomilsko v Savinjski dolini na izkrčenem hmeljišču z oznako Klinca 1 na površini velikosti 0,5 ha. Poskusno polje je bilo v mesecu aprilu preorano do globine 35 cm in obdelano s predsetvenikom. Z namenom varstva pred pleveli smo poskusno polje po obdelavi poškropili s herbicidom Primextra TZ Gold 500 SC (odmerek 40 ml/100 m^2). Rezultati osnovne kemične in pedološke analize so pokazali naslednje ugotovitve: pH = 6,0; P_2O_5 = 40,2 mg/100 g tal; K_2O = 21,7 mg/100 g tal; 2 % organske snovi; 80 % rastlinam dostopne vode na dan aplikacije (22. 6. 2010); srednje težka tla (PG – peščena glina).

V poskusu smo preizkušali pripravek Tripicrin (94 % a.s klorpikrin; TRIS International) v odmerku 20 g/m^2 in 40 g/m^2 ter pripravek Basamid Granulat (98 % a.s. dazomet; Kanesho soil treatment SPRL/BVBA) v odmerku 50 g/m^2, ob uporabi prozorne neprepustne plastične folije (VIP; angl. virtually impermeable folia). Vsako od 5-ih obravnavanj (preglednica 1) smo izvedli v 4 ponovitvah z naključno razporeditvijo parcel velikost 25 m^2 (5 x 5m). Pripravek Tripicrin smo v tla vnesli v obliki »shank« injekcijske aplikacije na globino 25 cm in razmaku 30 cm, medtem ko smo Basamid Granulat ročno raztresli po površini poskusne parcele in nato s kultivatorjem zadelali na globino 25-30 cm. V primeru obravnavanj, ki so vključevale VIP folijo, smo le-to nanesli takoj po aplikaciji in jo nato odstranili po 7 dneh.

Preglednica 1: Opis obravnavanj preizkušanja talnih fumigantov v letu 2010
Table 1: Treatment descriptions of fumigations trial in year 2010

Št.	Obravnavanje	Delež aktivne snovi	Formu-lacija*	Način aplikacije
0	Netretirano	/	/	/
1	TRIPICRIN 20 g/m^2+ VIP folija	94 % klorpikrin	GA	»shank« injiciranje
2	TRIPICRIN 40 g/m^2+ VIP folija	94 % klorpikrin	GA	»shank« injiciranje
3	Basamid Granulat 50 g/m^2 + VIP folija	98 % dazomet	MG	Raztros s kultiviranjem
4	Basamid Granulat 50 g/m^2	98 % dazomet	MG	Raztros s kultiviranjem

*GA-plin; MG-mikrozrna

2.2 Priprava infekcijskega potenciala

Za zagotovitev uniformne talne infestacije z visokim infekcijskim potencialom smo pripravili umetno pripravljen inokulum glive *V. albo-atrum* (patotip M, genotip PG1) v obliki mikrobioloških sond. Osnovo sond so predstavljale 150 ml vreče izdelane iz štirih slojev visoko kakovostne poliestrske tkanine (velikost por 16 µm; Sefar), v katerih se je nahajal inokulum. Inokulum smo pred pripravo sond pripravili iz posušenih delov hmeljnih trt, ki smo jih čez noč namakali v raztopini 1 % glukoze in 0,2 % kalijevega nitrata. Namakano zmes smo nato sterilizirali in okužili s kulturami izolata glive *V. albo-atrum* z oznako »Rec«, ki smo jih predhodno namnožili v tekočem umetnem gojišču [17] z enotedensko inkubacijo na rotacijskem stresalniku. Sledila je štiri tedenska inkubacija v temi pri sobni temperaturi s tedenskim mešanjem, da smo zagotovili enakomerno razraščanje glive po trtah. V posamezno sondo smo vnesli 10 trt, dolžine 5 cm z dobro preraščenim micelijem. Sonde smo z namenom stabilizacije kultur v tleh tri dni pred aplikacijo fumigantov vkopali v tla na globino 35 cm v obsegu 1 sonda/parcelo in njihovo lokacijo primerno označili.

2.3 Mikrobiološka analiza sond in določanje fungicidne učinkovitosti

Mikrobiološke sonde smo iz tal izkopali 22 dni po aplikaciji fumigantov in jih nato takoj shranili pri temperaturi 4 °C. Določanje infekcijskega potenciala sond smo pred in po izkopu opravili s tehniko serijskih redčitev na modificiranem Komada gojišču [1]. Vsebino sond smo s tresenjem najprej sprali v 200 ml sterilne destilirane vode in ustrezne redčitve (1:10, 1:100, 1:1000, 1:10000) v volumnu 200 µl z razmazom enakomerno nanesli na gojišče v 2 podvzorcih. Gojišča smo 7 dni inkubirali v temi pri sobni temperaturi, čemur je sledila mikroskopska identifikacija in štetje kolonij glive *V. albo-atrum*. Infekcijski potencial sond smo izrazili v enotah CFU (colony forming units)/g. Podatke smo statistično ovrednotili z analizo variance, kjer smo uporabili Duncanov test mnogoterih primerjav ter izračunali odstotek učinkovitosti po Abbott-u [11].

2.4 Določanje herbicidne aktivnosti in fitotoksičnosti

V poskusu smo proučevali tudi herbicidno učinkovitost apliciranih fumigantov in potencialni fitotoksični vpliv na posevek lucerne (*Medicago sativa*; cv. Krima). Lucerno smo 36 dni po aplikaciji fumigantov posejali s poskusno sejalnico Wintersteiger v odmerku semen 6 g/m^2. Ocenjevanja smo opravili v fazi višine rastlin 25-30 cm. Na vsaki parceli smo na površini 1 m^2 določili število rastlin lucerne in število posameznih plevelnih vrst. Fitotoksičnost na rastlinah smo določali kot delež deformiranih, slabo rastočih rastlin ali rastlin z znamenji kloroze.

3 REZULTATI IN DISKUSIJA

3.1 Fungicidna aktivnost

Pri določanju fungicidne aktivnosti talnih fumigantov klorpikrina in dazometa smo uporabili metodo mikrobioloških sond, ki zagotavlja visok in uniformen infekcijski potencial v tleh z omogočanjem točkovnega merjenja uspešnosti postopkov aplikacije. Pred vnosom sond v tla je začetno stanje infekcijskega potenciala glive *V. albo-atrum* znašalo 110 x 10^4 CFU/g, ki pa

je zaradi stabilizacije kultur in vpliva ostalih organizmov po 22 dneh v tleh (netretirane parcele) padel na 67,8 x 10^4 CFU/g oz. se je znižal za 38 %. Injekcijska »shank« aplikacija pripravka Tripicrin v odmerkih 20 g/m^2 in 40 g/m^2 z uporabo VIP folije, ki jo odlikuje visoka stopnja zadrževanja plinov, se je izkazala s 100 % stopnjo učinkovitosti, saj v nobeni od sond nismo potrdili preživetja kultur glive *V. albo-atrum*. V primeru razkuževanja s pripravkom Basamid granulat v uradno registriranem odmerku 50 g/m^2 v Republiki Sloveniji [3] smo ob uporabi VIP folije dosegli 87,8 % učinkovitost, medtem ko je učinkovitost brez VIP folije znašala 53,1 % (preglednica 2). Slabše delovanje pripravka Basamid granulat brez uporabe VIP folije je bilo pričakovano, saj na ta način organizmom toksični plin metil-izotiocianat hitreje izpareva iz tal, kar je še posebno izrazito pri višjih temperaturah, tako kot v primeru našega poskusa, ki je potekal v poletnih mesecih (od 22. 6. do 14. 7. 2010).

Preglednica 2: Učinkovitost fumigantov za zatiranje glive *V. albo-atrum* v tleh v letu 2010
Table 2: Efficacy of fumigants to control *V. albo-atrum* in soil in 2010.

Obravnavanje	Infekcijski potencial glive *V. albo-atrum* (CFU/g x 10^4)				Povprečje[x]	Učinkovitost po Abbott-u (%)
	Sonde					
	1	2	3	4		
Netretirano	82,8	47,8	42,6	97,8	67,8[a]	/
TRIPICRIN 20 g/m^2+ VIP folija	0,0	0,0	0,0	0,0	0,0[b]	100,0
TRIPICRIN 40 g/m^2+ VIP folija	0,0	0,0	0,0	0,0	0,0[b]	100,0
Basamid Granulat 50 g/m^2 + VIP folija	16,0	8,6	3,8	4,6	8,3[bc]	87,8
Basamid Granulat 50 g/m^2	6,1	24,6	32,1	64,4	31,8[c]	53,1

[x]Skupine z enako črko pri posameznem obravnavanju se med seboj statistično značilno ne razlikujejo (Duncan-ov test mnogoterih primerjav, α = 5 %).

Rezultati poskusa so potrdili predhodne raziskave o dobrem fungicidnem delovanju a.s klorpikrin in dazomet na patogene glive iz rodu *Verticillium*. Talboys in sod. [15] so med prvimi poročali o uporabi klorpikrina za zatiranje glive *V. dahliae*. Pri tem so zelo dobro učinkovitost dosegli v odmerku 42 ml/m^2 brez prekrivanja tal s folijo po aplikaciji. Harris [6] je dosegel enako stopnjo učinkovitosti kot Talboys s sodelavci [15] le s polovičnimi odmerki klorpikrina in z odmerkom dazometa 45 g/m^2, vendar z uporabo plinsko neprepustne folije po aplikaciji. Pri nadaljnjih poskusih zatiranja talnih patogenov je Harris [7] uspešno zatiral glivo *V. dahliae* v odmerkih klorpikrina 7.5, 15 in 30 ml/m^2 ter odmerkih dazometa 38 in 57g/m^2. Zatiranje oomicet *Phytophthora fragariae* in *P. cactorum* je bilo je v istih poskusih uspešno le z dazometom v odmerku nad 38 in 57 g/m^2. Gullino in sod. [4] so prav tako potrdili dobro učinkovitost klorpikrina za zatiranje gliv *V. dahliae*, *F. oxysporum* f.sp. *lycopersisci* in *F. oxysporum* f.sp. *radicis-lycopersici* v odmerku nad 30 g/m^2 z uporabo »shank« aplikacije.

3.2 Herbicidna aktivnost in fitotoksičnost

Herbicidno aktivnost uporabljenih fumigantov smo ocenjevali z določitvijo deleža zapleveljenosti posevka lucerne, ki smo ga posejali na poskusne parcele 36. dan po aplikaciji. Pri pregledu parcel, kjer nismo uporabili fumigantov, smo ugotovili povprečno stopnjo

zapleveljenosti (57,5 %). Največji delež populacije plevelne vegetacije je predstavljal vejičasti rogovilček (*Galinsoga ciliata*) (49 %), sledili so navadna loboda (*Atriplex patula*) (37 %), navadni plešec (*Capsella bursa-pastoris*) (6 %), hrapava škrbinka (*Sonchus asper*) (5 %), enoletna latovka (*Poa annua*) (2 %) in srhkodlakavi ščir (*Amaranthus retroflexus*) (1 %). V primeru parcel, kjer smo uporabili fumigante, smo ugotovili dobro herbicidno delovanje, saj se je učinkovitost gibala med 87,1 – 97,3 %. Med pripravki je boljše herbicidno delovanje izrazil pripravek Basamid granulat, pri katerem smo ob uporabi VIP folije dosegli 97,3 % učinkovitost, brez folije pa 91,9 %. V primeru pripravka Tripicrin smo opazili slabše delovanje predvsem na enoletni plevel hrapavo škrbinko (*Sonchus asper*), kar je razlog za nižjo stopnjo učinkovitosti (preglednica 3).

Preglednica 3: Učinkovitost fumigantov za zatiranje plevelov v letu 2010
Table 3: Efficacy of fumigants to control weeds population in year 2010.

Obravnavanje	Zapleveljenost (%)				Povp. (%)	Učinkovitost po Abbott-u (%)	Fitotoksičnost
	1	2	3	4			
Netretirano	53,0	85,0	41,4	50,4	57,5[a]	/	Nismo zaznali
TRIPICRIN 20 g/m^2+ VIP folija	12,5	6.0	4,0	7,0	7,4[b]	87,1	Nismo zaznali
TRIPICRIN 40 g/m^2+ VIP folija	5,0	6,0	3,0	2,0	4,0[b]	93,0	Nismo zaznali
Basamid Granulat 50 g/m^2 + VIP folija	1,0	1,0	2,0	2,0	1,5[b]	97,3	Nismo zaznali
Basamid Granulat 50 g/m^2	5,0	2,0	2,0	10,0	4,8[b]	91,6	Nismo zaznali

[x]Skupine z enako črko pri posameznem obravnavanju se med seboj statistično značilno ne razlikujejo (Duncan-ov test mnogoterih primerjav, $\alpha = 5$ %)

Rezultati poskusa tako potrjujejo predhodne raziskave o herbicidni aktivnosti klorpikrina in dazometa, ki po učinkovitosti in zatiranju spektra plevelnih vrst dajejo prednost slednjemu [7, 8]. O selektivnosti klorpikrina na viabilnost semena plevelov poročajo tudi Haar in sod. [5]. Pri tem so ugotovili dobro delovanje na seme navadne zvezdice (*Stellaria media*), navadnega tolščaka (*Portulaca oleracea*) in ptičjo dresen (*Polygonum aviculare*), medtem ko viabilnost semena slezenovca (*Malva parviflora*) in navadnega čapljevca (*Erodium cicutarium*) ni bila prizadeta. Podobne raziskave so bile opravljene tudi pri testiranju dazometa, za katerega so dokazali herbicidni vpliv na širši spekter plevelnih vrst [2, 9].

4 ZAKLJUČEK

Za uspešno sanacijo tal pred talnimi glivami je pomembno stremeti k popolni eliminaciji, ki zagotavlja minimalno tveganje za nastanek novih bolezenskih izbruhov. Pri določanju učinkovitosti zatiranja glive *V. albo-atrum* v tleh smo z injekcijsko »shank« aplikacijo pripravka na osnovi klorpikrina dosegli 100 % učinkovitost že z odmerkom 20 g/m^2, medtem ko smo pri uporabi pripravka na osnovi dazometa v registriranem odmerku 50 g/m^2 s prekrivanjem parcel s folijo določili 87,7 % učinkovitost. Rezultati poskusa tako dajejo prednost klorpikrinu za potencialno kemično sanacijo okuženih in izkrčenih hmeljišč v prihodnosti. Poleg doseganja visoke stopnje učinkovitosti klorpikrina, aplikacijo izvajajo izurjene in izkušene ekipe s posebej prilagojeno aplikacijsko tehniko, kar zmanjša tveganje

nepravilne uporabe. Pri določanju herbicidne aktivnosti je dazomet po pričakovanjih izrazil višjo stopnjo učinkovitosti, predvsem preko zatiranja kalivosti širšega spektra plevelnih vrst. Klorpikrin je herbicidno deloval na najpogosteje zastopane plevele, neučinkovito delovanje pa se je izrazilo pri semenu hrapave škrbinke (*Sonchus asper*). Pri uporabi fumigantov je potrebno upoštevati tudi tip in vlago tal ter delež organske snovi. Na splošno fumiganti dosegajo višjo stopnjo učinkovitosti v poroznih in peščenih tleh z nižjim deležem organske snovi. Prav tako se občutljivost gliv in semena rastlin viša z deležem vlage v tkivu [10], kar pomeni, da moramo biti zelo pozorni na nivo vlage v zemljišču, ki ga razkužujemo.

5 ZAHVALA

Avtorja članka se zahvaljujeva podjetju TRIS International, Via Palestro, 97019 Italija, in podjetju Cinkarna Metalurško Kemična Industrija Celje d.d, Kidričeva 26, 3001 Celje, Slovenija, za pomoč pri izvedbi poskusa.

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DOLOČITEV RASE KORUZNE VEŠČE (*Ostrinia nubilalis* Hübner) V SAVINJSKI DOLINI; PRELIMINAREN POSKUS SPREMLJANJA MOŠKIH METULJEV S FEROMONSKO VABO

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Izvleček

Koruzna vešča (*Ostrinia nubilalis*) je v Savinjski dolini že dolgo znana škodljivka koruze in hmelja, ki v zadnjem času povzroča pomembno gospodarsko škodo. S pomočjo plinske kromatografije (GC), opremljene s FID detektorjem, smo določili, da imamo na območju Savinjske doline E raso koruzne vešče, in sicer tako na koruzi (*Zea mays* L.) kot tudi na hmelju (*Humulus lupulus*). Za spremljanje koruzne vešče smo uporabili feromon E (E11-14Ac) in feromonski vabi različnih oblik; vaba deltoidne oblike z ravnim lepljivim dnom ter vaba deltoidne oblike s pregibnim lepljivim dnom. Raziskave na prostem so pokazale, da se moški osebki na feromonske vabe pri obeh pasteh slabo lovijo. V raziskavi smo ugotovili, da se je na vabe deltoidne oblike z lepljivim in pregibnim dnom ulovilo približno 2-krat več moških osebkov kot na vabe deltoidne oblike z lepljivim dnom.

Ključne besede: monitoring, svetlobna vaba, hmelj, *Humulus lupulus* L., koruza, *Zea mays* L., koruzna vešča, *Ostrinia nubilalis*, feromonska vaba

DETERMINATION OF THE STRAIN OF EUROPEAN CORN BORER (*Ostrinia nubilalis* Hübner) IN SAVINJA VALLEY; PRELIMINARY MONITORING OF MALE EUROPEAN CORN BORER WITH PHEROMONE BAIT

Abstract

The European corn borer (*Ostrinia nubilalis*), EBC, has been known as a corn and hop pest in Savinja valley for a long time which has lately caused significant economic damage. Using gas chromatography (GC) equipped with FID detector, E strain of EBC was identified in Savinja valley on corn (*Zea mays*) and on hop (*Humulus lupulus*) as well. Pheromone-baited traps with E strain (E11-14Ac) were used for monitoring the EBC flight; delta sticky traps and

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sticky wing traps. Field studies showed that EBC males were not optimally attracted and were poorly captured by pheromone bait on both traps. The results showed that sticky wing traps captured approximately two times more EBC males than sticky delta traps.

Key words: monitoring, light trap, hop, *Humulus lupulus* L., European corn borer, corn, *Zea mays* L., *Ostrinia nubilalis*, pheromone-bait

1 UVOD

Koruzna vešča je polifagna vrsta, saj se prehranjuje z mnogimi rastlinami. Pri nas povzroča največjo škodo predvsem na koruzi in hmelju [10, 11], čeprav jo vse pogosteje srečamo tudi pri pridelavi zelenjave (npr. paprike, paradižnika, fižola) kot tudi na okrasnih rastlinah (npr. dalijah, krizantemah, gladiolah).

V preteklosti je omenjena škodljivka na hmelju le občasno povzročala značilno gospodarsko škodo [11]. V zadnjih 10 letih pa v Sloveniji opažamo povečan vsakoletni pojav koruzne vešče, kar lahko pripisujemo relativno visokim temperaturam, z znatnim odstopanjem od dolgoletnih povprečij kot tudi neizvajanju fitosanitarnih-higienskih ukrepov [1, 9, 10]. Že v preteklosti so se naši predhodniki zavedali pomena izvajanja fitosanitarnih-higienskih ukrepov za prepečevanje širjenja koruzne vešče. Zato je bil od leta 1978 v veljavi Odlok o zatiranju prosene (koruzne) vešče na območju SR Slovenije (Ur. l. SRS, št. 20/1978), ki je s spremembo Zakona o zdravstvenem varstvu rastlin v letu 1995 prenehal veljati. Po tem letu se je populacija koruzne vešče znantno povečala.

Na hmelju gosenice prve generacije povzročajo škodo predvsem z vrtanjem v hmeljne trte, gosenice druge generacije pa se zavrtajo tudi v listne peclje in storžke. S prisotnostjo gosenic v steblih rastlin je oviran pretok vode in hranil, kar je še posebno opazno v sušnih in vročih letih. Napadene rastline hmelja zaostanejo v rasti, so šibkejšega habitusa (imajo obliko stožca ali ozkega valja), storžki so drobnejši. Velikokrat listi na napadenih rastlinah rumenijo, venijo posamezni deli rastline, lahko pa se posuši tudi cela rastlina. V eni trti je lahko več gosenic, ob močnem napadu tudi do 20. Gosenice druge generacije vrtajo rastline hmelja na višini 4 metrov ali višje. Zelo pogosto se gosenice zavrtajo v vretenca že oblikovanih storžkov hmelja, pri čemer izjedajo od spodaj navzgor. Storžki se najprej posušijo na spodnjem delu, z napredovanjem gosenice pa se posuši in porjavi celoten storžek. Ob močnejših napadih zato prihaja do znatnega zmanjšanja količine in kakovosti pridelka hmelja [10].

Populacijo koruzne vešče, kot nočnega metulja, že preko 30 let spremljamo s svetlobno vabo v Žalcu, na Inštitutu za hmeljarstvo in pivovarstvo Slovenije. Spremenjene klimatske razmere močno vplivajo na razvoj koruzne vešče. Svetlobna vaba se je izkazala za najbolj zanesljivo metodo spremljanja metuljev koruzne vešče [2, 8], po dosedanjih podatkih so feromoni namreč manj zanesljivi. Pri uporabi feromonov je potrebno vedeti, katero raso koruzne vešče imamo na določenem območju in kakšno obliko vabe uporabimo za njeno spremljanje [7, 8]. Vrabl [11] je predpostavil, da imamo na hmelju v Sloveniji morda drugo raso koruzne vešče kot na koruzi. V Franciji so ugotovili, da imajo na hmelju E raso koruzne vešče [8].

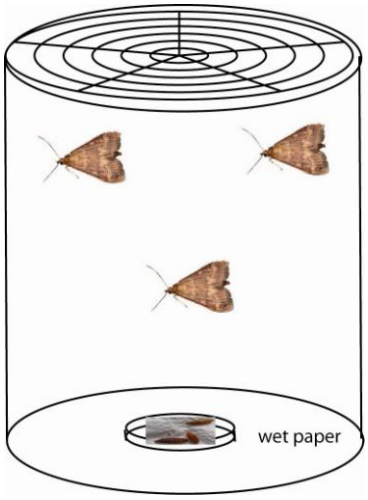
Med najbolj zanesljive vabe za spremljanje koruzne vešče na koruzi sodijo vabe iz mreže v obliki stožca [8]. Poleg oblike vabe je pomembna tudi njena postavitev, kar je odvisno od višine rastline. Najbolje je, da je feromonska vaba postavljena 10 cm nad višino rastline [8]. Pri hmelju je težko postaviti vabo nad 6 m visoko (ko doseže hmelj vrh opore). Kljub temu pa je potrebno pristopiti k raziskavam uporabe in optimizacije različnih feromonskih vab v hmelju za spremljanje populacije koruzne vešče. S pomočjo feromonskih vab bi lahko spremljali njeno populacijo na več lokacijah z različno mikro klimo. Tako bi rezultati monitoringa doprinesli k zanesljivi in natančni uporabi sredstev za zatiranje gosenic koruzne vešče na hmelju.

2 MATERIAL IN METODE

2.1 Določitev rase koruzne vešče

2.1.1 Žuželke

V mesecu marcu smo v okolici Žalca, na Rojah, na njivi koruze (iz koruznih stebel) nabrali ličinke koruzne vešče (*Ostrinia nubilalis*) v zadnjem larvalnem stadiju (L5). Enako smo v juniju na hmelju (na hmeljnih steblih) v hmeljišču v neposredni bližini Žalca nabrali ličinke koruzne vešče, ki so bile v stadiju L2-L3. V laboratoriju na Švedskem (Swedish University of Agricultural Sciences) smo ličinke dali v polipropilenske posode velikosti 30 x 30 x 30 cm z odprtino na vrhu, katere smo nato gojili v rastni komori v nadzorovanih razmerah s temperaturo 25 °C, 70 % relativno zračno vlago ter s fotoperiodo L:D = 17:7. Po razvoju bub smo te odstranili iz stebel (ločeno iz hmelja in koruze) ter jih ločili po spolu. Ženske bube smo dali v cilinder s premerom 10 cm (ločeno od pridobljenih gostiteljskih rastlin), katerega smo nato prenesli v komoro v enake pogoje kot smo gojili ličinke. V cilindru smo spremljali razvoj odraslih metuljev ženskega spola (slika 1). Na dno cilindra smo dali v manjšo petrijevko vlažen filter papir, katerega smo redno vlažili in s tem ustvarili dodatno vlažnost, kar ustreza samičkam.



Slika 1: Valj za gojenje metuljev koruzne vešče (*Ostrinia nubilalis*)
Figure 1: Cylinder for growing European corn borer (*Ostrinia nubilalis*)

2.1.2 Ekstrakcija feromonske žleze

Feromonske žleze so bile pridobljene iz 1-3 dni starih samic koruzne vešče. Ekstrakcijo feromonskih žlez smo opravili v zadnji uri skotofaze (zadnja temnega dela fotoperiode), kajti takrat samice proizvedejo največ feromona [5]. Za ekstrakcijo so bile primerne samice, ki so kazale znake ovipozicije - odlaganja jajčec, te smo odstranili iz cilindra. Samice smo obrnili na trebuh, tako da jim je konica zadka štrlela v zrak, nato smo z ostrim rezilom zarezali v feromonske žleze. Feromonske žleze smo ločeno dali v stekleno kapilaro v kateri je bilo 7 µl n-heksana (dvakrat destiliran, 99,9 % čistoče, proizvajalca Labscan, Malmö iz Švedske) kot univerzalno organsko topilo, in sicer za 5 min. Ekstrakt smo prenesli v drugo kapilaro, katero smo zamrznili na -60 °C do nadaljnje analize s plinsko kromatografijo (GC).

2.1.3 Identifikacija feromona na plinskem kromatografu

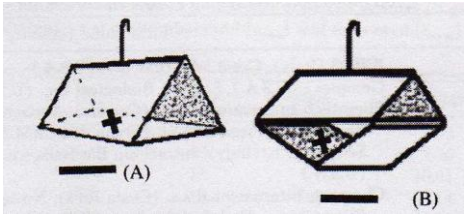
Ekstrakt smo injicirali v GC opremljen s FID detektorjem. Določili smo retenzijski čas sintetično izdelane komponente E11-tetradecinil acetat (E11-14Ac) in Z11-tetradecinil acetat (Z11-14Ac), ki sta standarda za E in Z raso koruzne vešče [8].

2.2 Spremljanje koruzne vešče s svetlobno vabo

Na Inštitutu za hmeljarstvo in pivovarstvo Slovenije v Žalcu smo v hmeljišču SN 5 postavili svetlobno vabo opremljeno s 160 W žarnico za mešano svetlobo proizvajalca Osram. Metulje smo v letu 2010 dnevno spremljali od maja do sredine septembra. Vsak večer smo nalili v zbiralno posodico, ki je nameščena pod svetlobno vabo, od 15 do 35 ml kloroforma. Različna količina kloroforma je odvisna od temperature zraka in posledično izhlapevanja; višja kot je temperatura, večje je izhlapevanje, več kloroforma smo odmerili. Kloroform umori ulovljene žuželke. V laboratoriju smo determinirali koruzne vešče in jim določili spol [3].

2.3 Spremljanje moških metuljev koruzne vešče s feromonsko vabo različnih oblik

V letu 2010 smo na prostem spremljali metulje koruzne vešče s feromonom tipa E (Z11-14Ac:E11-14Ac = 1:99), proizvajalca Isagro Italija, pri čemer smo uporabili dve različni vabi, in sicer deltoidno vabo z lepljivo podlago, proizvajalca Pherobank iz Nizozemske, in deltoidno vabo z lepljivo podlago ter s pregibnim dnom. Slednjo proizvaja Isagro, Italija (slika 2).



Slika 2: Feromonske vabe za lovljenje samcev koruzne vešče (*Ostrinia nubilalis*): A – vaba v obliki deltoida z lepljivo podlago, B – deltoidna vaba z lepljivo podlago ter pregibnim dnom

Figure 2: Pheromone – baited traps for capture of European corn borer (*Ostrinia nubilalis*) males: A – sticky delta trap, B – sticky wing trap

Feromonske vabe smo izobesili na različna mesta ob robu hmeljišč, blizu njive, kjer je bila koruznica zelo napadena s koruzno veščo ter blizu svetlobne vabe na Inštitutu. Na vsaki

opazovani lokaciji smo izobesili obe obliki vabe (A in B), med katerimi je bila razdalja več kot 50 m. Feromonski vabi, ki smo ju postavili na Inštitutu sta bili od svetlobne vabe oddaljeni 120 m. Vse vabe smo postavili 27. maja in sicer na višino 150 cm od tal. Hmelj je bil v času postavitve vab v povprečju visok 4 do 5 m. Feromone smo menjali na 14 dni.

V preglednici 1 so navedene lokacije spremljanja moških osebkov koruzne vešče s feromonom rase E in dveh različnih oblik vab.

Preglednica 1: Lokacije in vrsta vabe za spremljanje koruzne vešče (*Ostrinia nubilalis*) s feromonom rase E v letu 2010
Table 1: Locations and traps for monitoring European corn borer (*Ostrinia nubilalis*) with pheromone strain E in 2010

Št. obravnavanja	Lokacija	Vrsta vabe (angl. izraz)
1 A	Roje pri Žalcu- poljedelska njiva	A - sticky delta trap
1 B		B - sticky wing trap
2 A	Roje pri Žalcu – rob hmeljišča	A - sticky delta trap
2 B		B - sticky wing trap
3 A	Žalec na Inštitutu v hmeljišču SN 10	A - sticky delta trap
3 B		B - sticky wing trap

3 REZULTATI IN DISKUSIJA

3.1 Rasa koruzne vešče na območju Savinjske doline

Raso koruzne vešče smo določali s pomočjo plinske kromatografije. Ker so bile nabrane ličinke na koruzi v zadnjem larvalnem stadiju (L5) je bila njihova umrljivost manjša in tako smo zlahka izolirali feromonske žleze iz 10 samičk koruzne vešče (preglednica 2). Gosenice koruzne vešče, ki smo jih nabrali na hmelju so bile v stadiju L2-L3, kar je pri gojenju imelo za posledico večjo umrljivost. Tako smo v nadzorovanih razmerah vzgojili 6 metuljev koruzne vešče nabrane na hmelju, od katerih je bila le ena samička (preglednica 2).

Za vse samice nabrane na koruzi, z zagotovostjo trdimo, da sodijo v E raso, saj njihove feromonske žleže proizvajajo v večini primerov nad 97 % E11-tetradecinil acetata (E11-14Ac) (preglednica 2). Prav tako je tudi samica koruzne vešče pridobljena iz hmelja, v okolici Žalca, izločala E11-tetradecinil acetat, medtem ko feromona Z11-tetradecinil acetat (Z11-14Ac) nismo zasledili. Seveda je na osnovi ene samice težko zanesljivo trditi, so pa do enakih rezultatov na hmelju prišli tudi v Franciji [8]. Tako lahko ovržemo tezo, da na hmelju ni druge rase koruzne vešče kot na koruzi, kar je predpostavil Vrabl [11].

3.2 Ulovi moških osebkov koruzne vešče na svetlobno vabo

Metulje koruzne vešče smo s svetlobno vabo v Žalcu v letu 2010 pričeli spremljati 4. maja. Prve metulje smo na svetlobno vabo ulovili 15. maja. Let metuljev je bil sprva zaradi neugodnih vremenskih razmer (nizkih temperatur, dežja) slabši. Proti koncu meseca maja in v začetku junija pa so bili metulji koruzne vešče množično prisotni (tudi do 10 metuljev na noč), kar je za prvo generacijo koruzne vešče izredno veliko. V primerjavi z letom 2009 je bil

potencial koruzne vešče zelo velik (slika 2). V okolici Žalca, v bližini hmeljišč, je bila nepospravljena koruza na nekaterih njivah vse do 9. junija 2010. Posledica tega je bil tudi velik ulov metuljev koruzne vešče na svetlobno vabo, saj smo 22. in 23. julija ulovili tudi do 178 metuljev na noč (slika 2). To je rekord v več kot 30 letnih spremljanjih koruzne vešče v Žalcu. Let metuljev koruzne vešče druge generacije je bil v avgustu sicer manjši kot v letu 2009, vendar smo vseeno pozvali hmeljarje k pravočasnemu ukrepanju. Metulje koruzne vešče smo na svetlobni vabi našli vse do sredine septembra. Na svetlobno vabo se je skozi celotno leto 2010 skupno ulovilo 840 metuljev, v primerjavi z letom 2009, ko smo na svetlobno vabo ulovili le 420 metuljev. V letu 2010 se je na svetlobno vabo ulovilo skupno 572 moških osebkov. Razmerje med spoloma ulovljenih metuljev je tako bilo ženski:moški = 1:2,1.

Preglednica 2: Določitev rase koruzne vešče (*Ostrinia nubilalis*) iz ženskih žlez, nabrane na koruzi in hmelju v okolici Žalca, z GC-FID

Table 2: Determination of strain of European corn borer (*Ostrinia nubilalis*) on corn and hop from individual female gland extract with GC-FID, around Žalec

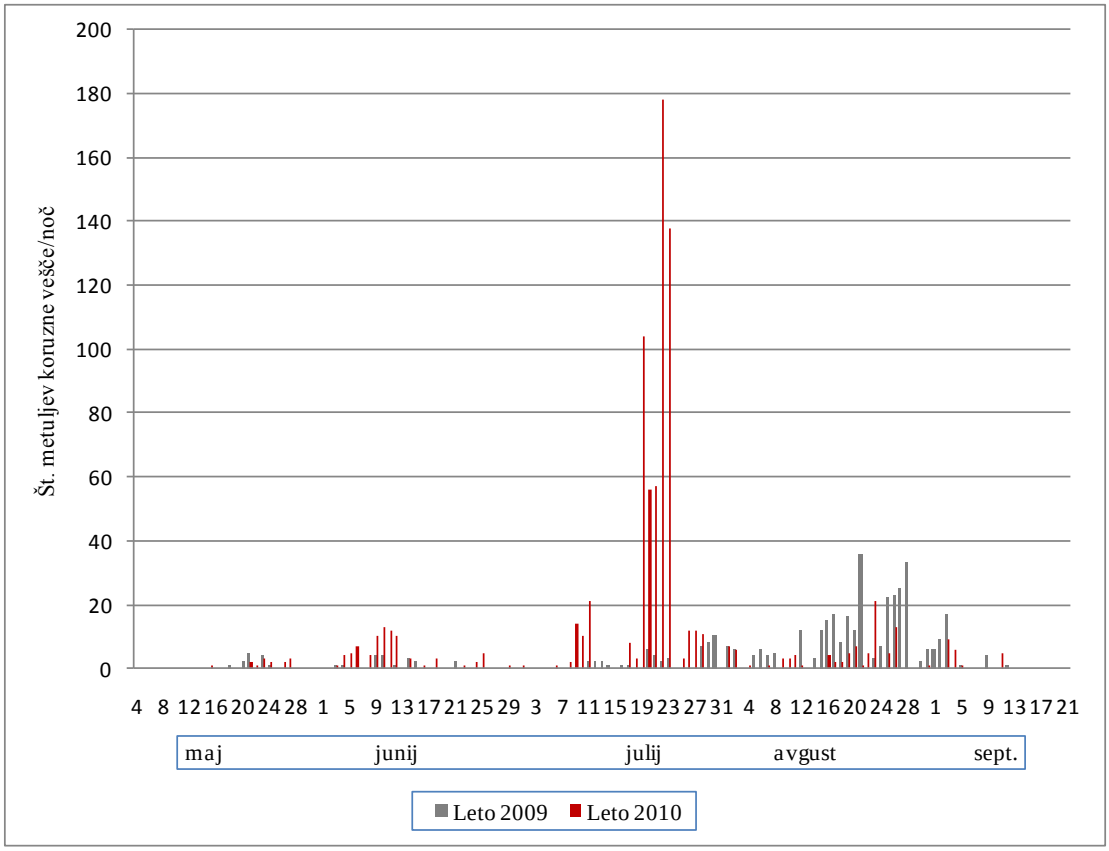
Št. samičke	RT E11-14Ac (min)	RT Z11-14Ac (min)	Masa E11-14Ac (ng)	Masa Z11-14Ac (ng)	Razmerje E11-14Ac:Z11-14Ac
Gosenice koruzne vešče iz koruze					
1	15,203	15,436	20,15	0,57	97,23:2,77
2	15,189	15,419	8,11	0,19	97,69:2,30
3	15,186	-	1,34	-	/
4	15,195	15,427	3,71	0,14	96,28:3,72
5	15,188	-	0,59	-	/
6	15,196	15,427	15,99	0,25	98,47:1,53
7	15,192	-	0,40	-	/
8	15,191	15,419	2,04	0,11	94,67:5,33
9	15,192	-	1,26	-	/
10	15,2	15,428	5,69	0,12	97,86:2,14
Samice koruzne vešče iz hmelja					
1	15,197	-	1,35	-	/

RT – retenzijski čas

Površina- površina pod vrhom na GC-kromatogramu

3.3 Ulovi moških osebkov koruzne vešče na feromonsko vabo

Ulovi na obe vrsti feromonskih vab so bili izredno slabi, na obeh lokacijah na Rojah (okolica Žalca) ter v Žalcu na Inštitutu, 120 m stran od svetlobne vabe. Neposredno lahko primerjamo ulove moških osebkov koruzne vešče na svetlobno vabo v Žalcu, kjer se je od 27. maja do 8. avgusta 2010 ulovilo 475 moških osebkov, za razliko od obeh vrst feromonskih vab (A in B), kjer nismo ulovili nobenega osebka (preglednica 3). Na lokaciji Roje pa smo z vizualnimi pregledi koruze ugotovili, da je bil potencial koruzne vešče izredno velik, saj je do 9. junija bila na njivi, kjer smo spremljali veščo s feromonskimi vabami, še koruza od predhodnega leta, katera je bila napadena od 20 - 85 % z gosenicami koruzne vešče [6].



Slika 2: Primerjava ulova metuljev koruzne vešče (*Ostrinia nubilalis*) na svetlobni vabi v Žalcu v letih 2009 in 2010

Figure 2: Comparison of captures of moths of European corn borer (*Ostrinia nubilalis*) on light trap in Žalec in 2009 and 2010

Prav tako smo v hmeljišču, kjer smo spremljali veščo s feromoni na hmelju ugotovili veliko prisotnost gosenic 1. generacije. Če primerjamo ulove na feromonski vabi deltoidne oblike (A), se je ulovilo približno 2-krat manj moških osebkov koruzne vešče kot na vabi deltoidne oblike s pregibnim dnom (B). Tudi nekateri drugi raziskovalci so ugotovili, da so feromonske vabe deltoidne oblike z lepljivo podlago manj zanesljive od vab deltoidne oblike z lepljivim in pregibnim dnom [7, 8]. Postavlja se tudi vprašanje, katera višina postavitve vabe je primerna za spremljanje koruzne vešče na hmelju. Morda je višina 150 cm neprimerna za spremljanje koruzne vešče 1. generacije kot tudi druge. To je v bodoče potrebno preučiti.

Ulov na feromonske vabe je bil letos slab oziroma ga sploh ni bilo, kljub temu, da smo imeli nekatere vabe izobešene ob robu hmeljišč, kjer je bil potencial vešče izredno velik, kar je bilo razvidno iz poškodb na hmelju in koruzi kot tudi ulov na svetlobni vabi (slika 2). V bodoče želimo optimizirati spremljanje populacije koruzne vešče s feromoni in ustreznimi vabami. Tako bi lahko vsak lastnik hmeljišč sam spremljal populacijo koruzne vešče na svojih lokacijah. To bi hmeljarjem omogočilo bolj zanesljivo in pravočasno zatiranje gosenic koruzne vešče. Z optimizacijo uporabe feromonskih vab v hmelju, bi lahko razširili mrežo

opazovanj in spremljanj koruzne vešče, kar bi imelo pozitiven vpliv na pravočasno ukrepanje zoper gosenice koruzne vešče.

Preglednica 3: Ulovi metuljev koruzne vešče (*Ostrinia nubilalis*) na prostem s feromonom E rase in z deltoidno vabo z lepljivim dnom (A) ter deltoidno vabo z lepljivo podlago ter pregibnim dnom (B) v letu 2010

Table 3: Field captures of the European corn borer (*Ostrinia nubilalis*) male moths with pheromone E-strain, sticky delta trap (A) and sticky wing trap (B) in 2010

Št. obrav.	Št. ulovljenih moških metuljev koruzne vešče									
	2. junij	4. junij	9. junij	11. junij	15. junij	24. junij	1. julij	15. julij	8. avg.	**Skupno**
1 A	1	1	0	0	0	0	0	0	0	**2**
1 B	3	7	1	1	1	1	0	0	0	**14**
2 A	0	0	0	0	1	0	0	0	0	**1**
2 B	3	0	2	1	1	0	0	0	0	**7**
3 A	0	0	0	0	0	0	0	0	0	**0**
3 B	0	0	0	0	0	0	0	0	0	**0**
Skupno	**7**	**8**	**3**	**2**	**3**	**1**	**0**	**0**	**0**	**24**

A – deltoidna vaba z lepljivim dnom (sticky delta trap)
B- deltoidna vaba z lepljivo podlago ter pregibnim dnom (sticky wing trap)

4 ZAKLJUČKI

Na podlagi pridobljenih podatkov pri spremljanju koruzne vešče (*Ostrinia nubilalis*) lahko zaključimo:

- Na območju Savinjske doline imamo E raso koruzne vešče, ki je ista na koruzi kot tudi na hmelju.
- V letu 2010 so se na svetlobno vabo množično lovili metulji koruzne vešče.
- Razmerje med spolom ulovljenih osebkov koruzne vešče na svetlobni vabi je v prid moških, in sicer jih je bilo 2,1-krat več kot ženskih metuljev.
- Ulov moških osebkov koruzne vešče z E feromonom (Z11-14Ac:E11-14Ac = 1:99), z dvema različnima vabama, je bil zelo slab. Če primerjamo ulov na obe različni vabi, je bil na vabi deltoidne oblike z lepljivim in pregibnim dnom (slika 2 B) večji za približno 2-krat, za razliko od klasične feromonske vabe deltoidne oblike z lepljivim dnom (slika 2 A), ki ni primerna za spremljanje koruzne vešče.
- V hmelju bomo v bodoče preverili učinkovitost uporabe feromonskih vab ostalih oblik (npr. vaba iz mreže v obliki stožca) kot tudi različne postavitve višine vab.

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PRIDELEK RAZLIČNIH SORT ŽAMETNICE (*Tagetes erecta* L.)

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Izvleček

Pridelek žametnice (*Tagetes erecta* L.) predstavljajo posušeni jezičasti cvetovi. Zdravilni učinek ima zaradi vsebnosti karotenoidov (provitamin A) v cvetovih, zlasti heleniena, ki ugodno vpliva na vid, uporablja pa se tudi v druge namene. Namen predstavljene raziskave je bil ugotoviti pridelek (masa svežih socvtij, masa suhih jezičastih cvetov) pri različnih sortah žametnice (Sierra orange, Tall American F1 double eagle orange, Mum F1 Orange in Marigold Xantophyll Scarletade) v naših pridelovalnih razmerah. Največji pridelek svežih socvetij je dosegla sorta Tall American F1 double eagle orange. Sorti Marigold Xantophyll Scarletade in Sierra orange nista dosegli niti polovice pridelka svežega socvetja v primerjavi s to sorto, sorta Mum F1 Orange pa je dosegla 59% pridelka v primerjavi s sorto Tall American F1 double eagle orange. Tudi v pridelku mase jezičastih cvetov je statistično značilno izstopala sorta Tall American F1 double eagle orange, ki je imela najvišjo maso jezičastih cvetov pri vsakem obiranju v avgustu. Delež suhih jezičastih cvetov glede na maso socvetij (svež pridelek) je bil največji pri sorti Tall American F1 double eagle orange, sledile so sorte Marigold Xantophyll Scarletade, Mum F1 Orange in Sierra orange. Po pridelku heleniena pa je dosegla najboljši rezultat sorta Marigold Xantophyll Scarletade, sledile so ji sorte Tall American F1 double eagle orange, Sierra orange in Mum F1 Orange.

Ključne besede: žametnica, *Tagetes erecta* L., pridelek, zdravilne rastline

YIELD AT DIFFERENT VARIETES OF MARIGOLD (*Tagetes erecta* L.)

Abstract

Marigold (*Tagetes erecta* L.) is grown for its ray florets which have medicinal effect; they contain caroteniodes (provitamin A), mainly helenien which has positive impact on eyesight and is used also for many other purposes. The aim of presented research was to investigate yield (fresh flowers yield, dry ray floret yield) of different marigold varieties (Sierra orange, Tall American F1 double eagle orange, Mum F1 Orange in Marigold Xantophyll Scarletade) in our pedoclimatic conditions. The highest yield of fresh flowers was provided by variety Tall American F1 double eagle orange; varieties Marigold Xantophyll Scarletade and Sierra

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orange reached less than a half of the yield of this variety, variety Mum F1 orange 59%. Also, the yield of dry ray floret was significantly higher at the variety Tall American F1 double eagle orange compared to the other investigated varieties; it had significantly higher yield at each sampling in August. The share of dry ray florets compared to the fresh weight of flowers was the highest at Tall American F1 double eagle orange, followed by varieties Marigold Xantophyll Scarletade and Mum F1 Orange; the lowest was at the variety Sierra orange. The yield of helenien was the highest at Marigold Xantophyll Scarletade, followed by Tall American F1 double eagle orange, Sierra orange and Mum F1 Orange.

Key words: marigold, *Tagetes erecta* L., yield, medicinal plants

1 UVOD

Žametnica (*Tagetes erecta* L.) je enoletna zdravilna in okrasna rastlina iz družine nebinovk (Asteraceae). V rod Tagetes spada vsaj 50 različnih vrst rastlin [7]. Običajno se pridelujeta dve različni vrsti: *Tagetes erecta* L. (afriška žametnica, včasih tudi ameriška žametnica) in *Tagetes patula* (francoska žametnica) in njuni križanci, ki so triploidi. Obe vrsti izvirata iz Nove Mehike in Arizone (ZDA) ter južno od tega območja proti Argentini [4].

Višina rastline je odvisna od sorte; visoke dosežejo tudi do 80 cm. Imajo pokončno, delno votlo steblo z vzdolžnimi brazdami. Steblo se vejasto razrašča. Listi so pernati, z globoko narezanimi listnimi ploskvami. Na koncu pokončnih poganjkov so socvetja, ki so sestavljena iz jezičastih cvetov na obodu in cevastih cvetov v sredini. Barva cvetov je rumena do oranžna, obstajajo pa tudi temno rdeči, rjavkasti in cvetovi s kombinacijami teh barv ter beli. Cveti od junija do septembra. Seme je črno, podolgovato, sploščeno, dolgo okoli 10 mm in široko 2-3 mm. Korenine so vlaknate in sestavljajo koreninsko grudo. Rastline imajo značilen oster vonj [7]. Rastline vrste *Tagetes erecta* L. so večje in višje rasti in imajo večje cvetove kot rastline vrste *Tagetes patula*, ki so nižje rasti (do 20 cm) in imajo manjše in manj številne dvojne cvetove [4]. Kot okrasna rastlina krasi gredice, v vrtu pa jo pridelujemo zaradi insekticidnega delovanja, saj pozitivno vpliva na zmanjšanje potenciala škodljivcev v tleh. Priljubljena je pri vrtnarjih, saj cveti celo poletje do jeseni v živahnih barvah. Cvetovi so lahko v zelo različnih velikostih in različnih tipih [6].

Pridelek – drogo predstavljajo posušeni jezičasti cvetovi. Zdravilni učinek ima zaradi vsebnosti karotenov (provitamin A) v cvetovih, zlasti heleniena, ki ugodno vpliva na vid. V živilski industriji uporabljajo žametnico kot vir ksantofilov – karotenoidnih barvil, ki jih uporabljajo za obarvanje živil; od namazov do pijač. V Mehiki jo pridelujejo kot krmno rastlino (zempa) [6]. V perutninski industriji uporabljajo zmlete cvetne lističe kot krmni dodatek, ki pozitivno vpliva na oranžno barvo jajčnih rumenjakov in rumeno kožo perutnine. Lutein ima tudi dodatne farmakološke učinke na oftamotološkem področju [2]. Kot posebno pomemben dodatek so zmleti cvetovi tudi pri gojenju lososov in morskih rakcev [7].

Žametnica je za pridelovanje skromna rastlina. Uspeva na različnih tleh, najbolj pa ji prijajo s hranili srednje preskrbljena tla z večjim deležem glinastih delcev. Je rastlina sončnih leg. Razmnožuje se s semenom, ki ga v marcu posejemo v rastlinjaku [7]. Semena *Tagetes erecta* L. kalijo 3 do 5 dni pri povprečni temperaturi 23 do 26°C. Triploidi potrebujejo nekaj dni več. Primeren pH substrata je med 5,8 in 6,5. Kakovostne sadike vzgojimo pri 15 do 18°C nočne

temperature in pri 18 do 22°C dnevne temperature. Nočne temperature lahko po 7 do 10 dneh po presajanju znižamo pod 15°C [4]. Konec aprila, začetek maja, ko rastline ustvarijo primerno koreninsko grudo, jih presadimo na stalno mesto. Razdalja v vrsti in medvrstna razdalja je odvisna sorte (višine rastlin) in je od 30-50 cm. Kot so v triletnem poskusu ugotovili Bosma in sod. [2] presajene rastline dajejo večji pridelek v primerjavi z direktno setvijo. Dognojevanje z dušikom je različno vplivalo na parametre pridelka, vsekakor pa ni vplivalo na povečanje pridelka pigmenta in ga zato ne priporočajo. Potrebno pa je dobro pripraviti tla, kamor se presaja, da je v rastni sezoni na voljo dovolj dostopnih hranil. Pleti je potrebno po potrebi, zlasti spomladi, ko so rastline še majhne. Polno razvite rastline preprečujejo razvoj plevela. Cvetove obiramo redno, da spodbujamo razvoj novih, s čimer pozitivno vplivamo na pridelek [7]. Pri ročnem obiranju je pridelek večji v primerjavi s strojno žetvijo, vendar bo potrebno tehnologijo strojnega obiranja dodelati do te mere, da bo ekonomsko upravičena, če bomo žametnico začeli obravnavati kot alternativno kulturno rastlino [2].

Za proizvodnjo pigmentov žametnico pridelujejo v Mehiki, Peruju in Indiji [2]. Prideluje pa se tudi v različnih Evropskih državah. Relevantnih podatkov o površinah in o količini pridelane droge nismo našli. Pridelujejo se zlasti sorte, ki imajo intenzivno oranžne cvetove, ker je uporabna sestavina karoteniod (lutein) oranžne barve. Tudi selekcija in pridobivanje novih sort gre v smeri čim bolj intenzivnih oranžnih cvetov. Širša komercialna uporaba karotenoidov bi lahko povečala zanimanje za pridelavo *Tagetes erecta* L. kot alternativne poljščine. Zaželene so sorte, ki dobro prenašajo lokalne vremenske razmere in konstantno proizvajajo veliko število oranžnih cvetov, katerih barva se ne spreminja in so relativno odporne na bolezni in škodljivce [2].

Namen predstavljene raziskave je, ugotoviti pridelek žametnice (*Tagetes erecta* L.) (masa svežega socvetja, masa suhih jezičastih cvetov) pri različnih sortah (Sierra orange, Tall American F1 double eagle orange, Mum F1 Orange in Marigold Xantophyll Scarletade) v naših pridelovalnih razmerah.

2 MATERIAL IN METODE

Poskus je bil zastavljen kot bločni poskus s štirimi obravnavanji v štirih ponovitvah. Izveden je bil na poskusnem posestvu Inštituta za hmeljarstvo in pivovarstvo Slovenije (IHPS). Velikost osnovne parcele je bila 2,4 m^2 (10 rastlin pri medvrstni razdalji 60 cm in razdalji v vrsti 40 cm). V raziskavo smo vključili štiri sorte žametnice (*Tagetes erecta* L.) z oranžnimi cvetovi (slika 1), in sicer: Sierra orange, Tall American F1 double eagle orange (v nadaljevanju Tall American), Mum F1 Orange (v nadaljevanju Mum Orange) in kontrolno sorto Marigold Xantophyll Scarletade (v nadaljevanju Marigold).

Sorta Marigold je visoka okrog 60 cm. Ima rdečkasto steblo in razrasle poganjke, na katerih so socvetja. Socvetja so velika od 30 do 50 mm, včasih tudi večja. So intenzivno oranžne barve. Sorta Sierra Orange je visoka okoli 70 cm. Ima zeleno steblo in razrasle poganjke, na katerih so socvetja. Socvetja so velika od 30 do 55 mm. Imajo velike jezičaste cvetove. Barva jezičastih svetov je svetlo oranžna do oranžna. Sorta Tall American je visoka okoli 70 cm. Ima zeleno steblo in razrasle poganjke, na katerih so socvetja, ki so velika od 35 do 60 mm. Socvetje vsebuje mnogo jezičastih cvetov. Socvetja so svetlo oranžna do oranžna [3]. Sorta

Mum Orange je visoka okoli 40 cm. Ima zeleno do rahlo rdečkasto steblo. Socvetja so velika 40 do 60 mm, imajo jezičaste cvetove na obrobju. Proti sredini socvetja so jezičasti cvetovi zviti v cevke, tako da dajo zanimiv videz. Socvetja so intenzivno oranžne barve [3].

Slika 1: Socvetja sort žametnice (*Tagetes erecta* L.), vključenih v poskus (1 = Marigold, 2 = Sierra Orange, 3 = Tall American, 4 = Mum Orange) (foto: N. Ferant)

Figure 1: Flowers of four marigold (*Tagetes erecta* L.) varieties (1 = Marigold, 2 = Sierra Orange, 3 = Tall American, 4 = Mum Orange), included in the research (foto: N. Ferant)

Sadike smo vzgojili v setvenih ploščah v rastlinjaku v skladu z ekološko pridelavo. Presajanje na njivo je bilo ročno. Tla na poskusni površini so bila pretirano preskrbljena s fosforjem in dobro preskrbljena s kalijem, vrednost pH je bila 6,7 (preglednica 1). Po predsetveni obdelavi smo gnojili glede na rezultate analize tal (preglednica 1) in glede na odvzem hranil (50-70 kg/ha P_2O_5 in 180-250 kg/ha K_2O) v skladu s Smernicami za strokovno utemeljeno gnojenje [5]. Apnjenje glede na analizo tal ni bilo potrebno.

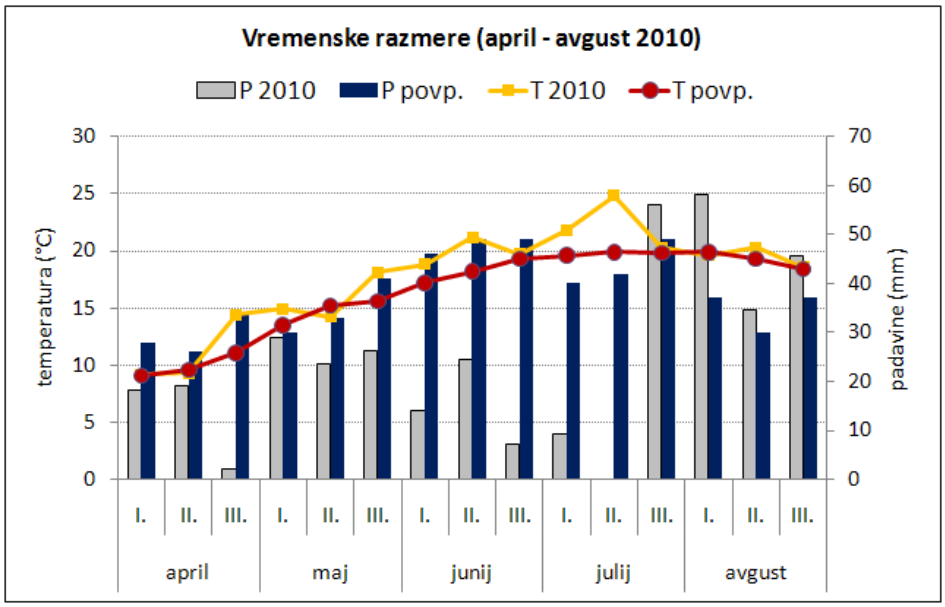
Poskus smo zasadili 21. maja 2010 in obenem vzpostavili kapljično namakanje. Dognojevanje rastlin smo izvajali s fertigacijo v dveh terminih, in sicer 16. junija ter 13. avgusta s Kristalon^{TM} (17:6:18:2) v količini 2,5 kg v raztopini 1000 l vode. Nasad smo dvakrat okopali (24.6. in 6.8. 2010), namakanje pa smo izvajali glede na potrebe oziroma vremenske razmere (slika 2). Poskus smo redno opazovali. Pojava bolezni in škodljivcev nismo zasledili.

Socvetja smo začeli pobirali ločeno po parcelah od 12. julija 2010 dalje, v času polnega cvetenja treh sort, vključenih v poskus, pri sorti Mum Orange, ki v tem času še ni imela razvitih socvetij, pa smo začeli socvetja pobirati v zadnji dekadi julija. Socvetja smo obirali enkrat tedensko do 6. septembra 2010. Stehtali smo pridelek sveže nabranih socvetij po parcelah in pred sušenjem ločili cvetno čašo od jezičastih cvetov, ki smo jih sušili v sušilni omari pri 55°C 8 do 10 ur. Vsebnost vlage smo določili po metodi Analytica EBC, 7.2. [1].

Preglednica 1: Rezultat kemične analize tal pred postavitvijo poskusa spomladi 2010
Table 1: Results of chemical soil analyse before experiment conduction in spring 2010

pH v KCl	P_2O_5 mg/100 g tal	K_2O mg/100 g tal	Organska snov %
6,7	31,0 D	21,4 C	4,3

*črke ob številčnih vrednostih označujejo stopnjo založenosti tal s hranili:
C: dobro preskrbljena tla, D : pretirano preskrbljena tla



Slika 2: Količina padavin in povprečne dekadne temperature v rastni sezoni žametnice (*Tagetes erecta* L.) v letu 2010 v primerjavi z dolgoletnim povprečjem (podatki z avtomatske meteorološke postaje javne službe zdravstvenega varstva rastlin za lokacijo Žalec IHPS)
Figure 2: Precipitation and average decade temperatures in the growth season of marigolds (*Tagetes erecta* L.) in 2010 compared to long term average (Žalec, IHPS)

Rezultate (masa svežih socvetij in masa suhe snovi jezičastih cvetov) smo obdelali z računalniškima programoma Excel in Statgraphics plus. Za zaznavanje razlik med obravnavanji smo uporabili Duncanov test mnogoterih primerjav (p=0,05).

Vreme v preučevanem letu 2010 je odstopalo od dolgoletnega povprečja predvsem po višjih temperaturah in manjši količini padavin. Od druge dekade maja do konca julija je bilo manj

padavin v primerjavi z dolgoletnim povprečjem, temperature pa so bile višje od dolgoletnega povprečja (slika 2). Zlasti visoke povprečne temperature so bile v prvih dveh dekadah julija, ko je bila obenem količina padavin zelo majhna, velik odklon od dolgoletnega povprečja pa je bil tudi v zadnji dekadi maja in v sredini junija. V zadnji dekadi julija in v avgustu so bile vremenske razmere podobne dolgoletnim.

3 REZULTATI

3.1 Masa svežih socvetij

V preglednici 2 in na sliki 3 je prikazana masa svežih socvetij pri preučevanih sortah (Marigold, Sierra orange, Tall American in Mum Orange) v letu 2010 glede na datum pobiranja.

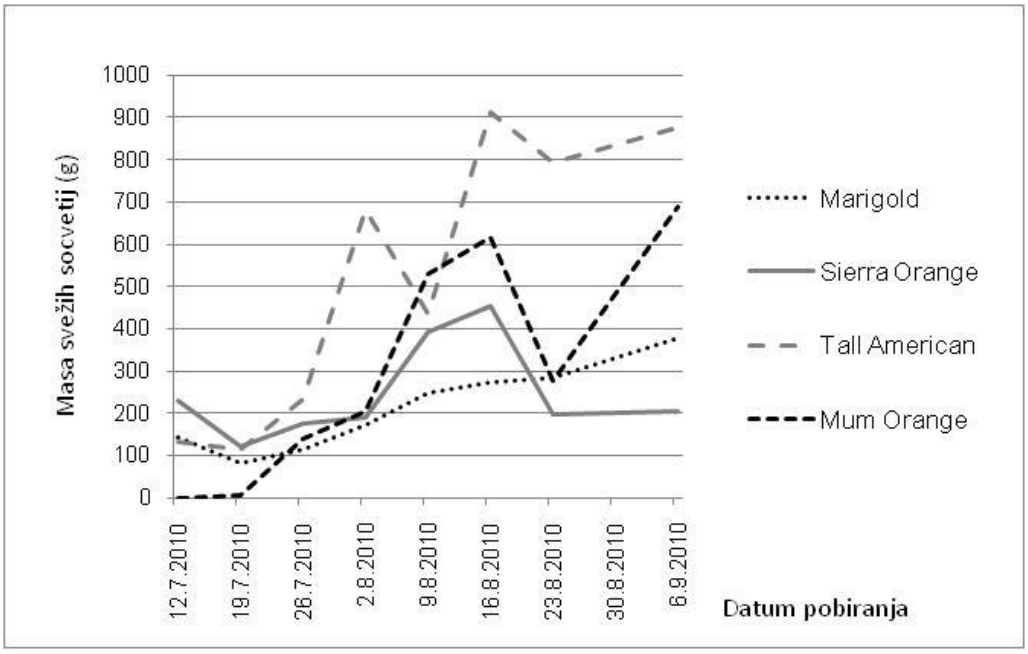
Preglednica 2: Masa svežih socvetij (g/parcelo) glede na sorto (Marigold, Sierra orange, Tall American in Mum Orange) in datum pobiranja (IHPS, 2010)
Table 2: Yield of fresh flowers of marigolds (g/plot) with regard to the examined variety (Marigold, Sierra orange, Tall American in Mum Orange) and sampling date (IHPS, 2010)

	Datum pobiranja								
Sorta	12.7.	19.7.	26.7.	2.8.	9.8.	16.8.	23.8.	6.9.	Skupaj
Marigold	143 b	82 b	115 a	174 a	249 a	273 a	283 a	377 a	1696
Sierra orange	229 b	121 b	175 a	191 a	394 ab	453 ab	196 a	206 a	1965
Tall American	131 b	115 b	234 a	683 b	436 ab	913 c	794 b	875 c	4181
Mum Orange	0 a	5 a	139 a	205 a	531 b	617 b	278 a	687 b	2462

*a - Enaka črka v stolpcu pomeni, da se obravnavanji med seboj statistično značilno ne razlikujeta (Duncanov test mnogoterih primerjav, $p<0,05$).

Pri prvem pobiranju socvetja (12. julij) se pridelek med sortami Marigold, Sierra orange in Tall American ni statistično značilno razlikoval, med tem ko rastline sorte Mum Orange v tem času še niso bile v razvojni fazi polno cvetenje. Tudi 19. julija se pridelek med prvimi tremi sortami med seboj ni statistično značilno razlikoval, medtem ko je bil pridelek značilno manjši pri sorti Mum Orange. Konec julija (26. julij) je tudi sorta Mum Orange prešla v fazo polnega cvetenja; pridelek po sortah je bil v tem času med vsemi sortami primerljiv. V začetku avgusta je začela pozitivno izstopati sorta Tall American, ki je v vseh pobiranjih v mesecu avgustu in tudi v začetku septembra imela večji pridelek v primerjavi z ostalimi sortami, v nekaterih pobiranjih statistično značilno. Po pridelku pa je v avgustu začela zaostajati sorta Marigold, v zadnji dekadi avgusta tudi sorta Sierra orange.

V skupnem seštevku je imela največji pridelek socvetja sorta Tall American, sledila ji je sorta Mum Orange, ki pa je v preučevanem letu dosegla le 59% pridelka sorte Tall American. Ostali dve preučevani sorti, Marigold in Sierra orange, nista dosegli niti polovice pridelka v primerjavi s sorto Tall American.



Slika 3: Masa svežih socvetij po sortah (Marigold, Sierra orange, Tall American in Mum Orange) glede na datum pobiranja (od 12. julija do 6. septembra 2010; IHPS)

Figure 3: Yield of fresh flowers of marigolds with regard to variety (Marigold, Sierra orange, Tall American in Mum Orange) and sampling date (from 12th July to 6th September 2010; IHPS)

3.2 Masa suhih jezičastih cvetov

V preglednici 3 in na sliki 4 je prikazan pridelek suhe snovi (g/parcelo) jezičastih cvetov po sortah (Marigold, Sierra orange, Tall American in Mum Orange) glede na datum pobiranja socvetij. Kot v primeru mase socvetij je tudi v pridelku mase jezičastih cvetov statistično značilno pozitivno izstopala sorta Tall American, ki je imela značilno večjo maso jezičastih cvetov v primerjavi z ostalimi tremi sortami pri vsakem pobiranju v avgustu. V juliju je pri prvih dveh pobiranjih negativno izstopala sorta Mum Orange, ki do pobiranja konec julija še ni bila v razvojni fazi polnega cvetenja. Kasneje je bil pridelek jezičastih cvetov pri tej sorti primerljiv s sortama Marigold in Sierra orange. V skupnem seštevku med sortami Marigold, Mum Orange in Sierra orange ni bilo bistvenih razlik.

Delež suhih jezičastih cvetov glede na maso socvetij (svež pridelek) je bil največji pri sorti Tal American, sledili sta sorti Marigold in Mum Orange, najnižji pa je bil pri sorti Sierra orange (preglednica 4).

3.3 Vsebnost in pridelek heleniena

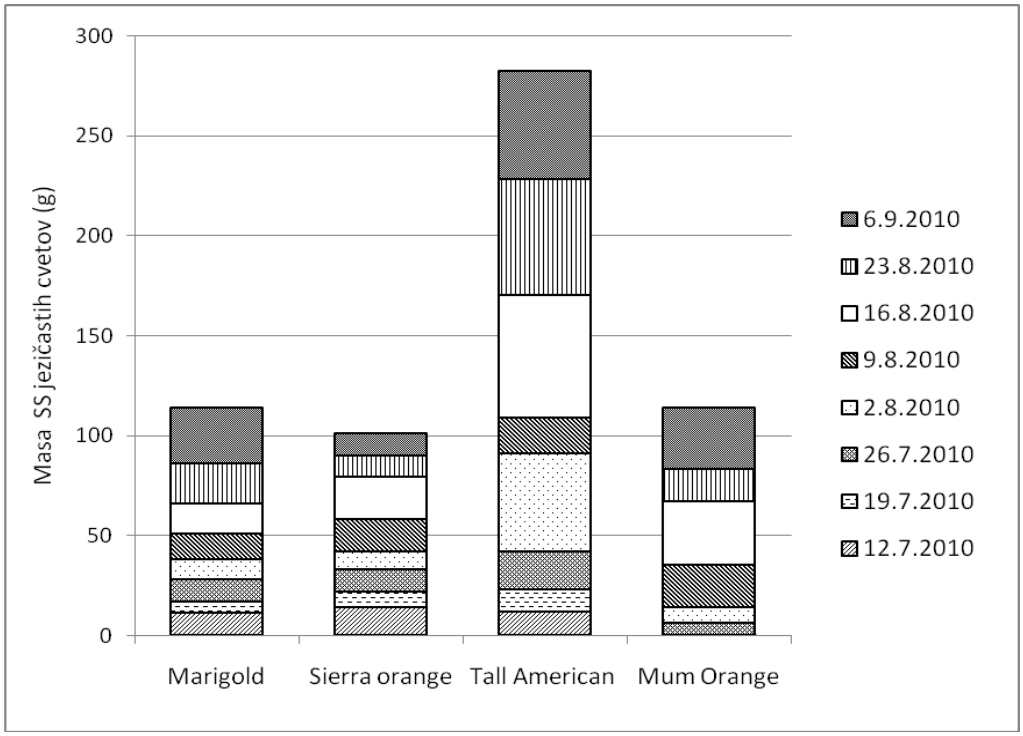
Sorta Tall American je v preučevanih razmerah po pridelku značilno pozitivno odstopala od ostalih sort, bilo pa je opaziti, da ima ta sorta v primerjavi z ostalimi bolj rumene, manj oranžne jezičaste cvetove. Intenzivnost oranžne barve socvetij nakazuje vsebnost barvila v cvetovih, kar je potrdila tudi analiza vsebnosti heleniena, ki je bila pri tej sorti najnižja, in

sicer 1,94 % (preglednica 4). Najvišja vsebnost heleniena je bila v preučevanih razmerah določena pri standardni sorti Marigold (5,61 % v SS), ki je dosegla tudi najvišji pridelek heleniena (23,8 kg/ha). Po pridelku heleniena so ji sledile sorte Tall American, Sierra orange in z najmanjšim pridelkom heleniena sorta Mum Orange.

Preglednica 3: Pridelek suhe snovi (g/parcelo SS) jezičastih cvetov glede na sorto (Marigold, Sierra orange, Tall American in Mum Orange) in datum pobiranja
Table 3: Yield of dry matter (g/plot) of marginal flowers with regard to variety (Marigold, Sierra orange, Tall American in Mum Orange) and sampling date

	Datum pobiranja								
Sorta	12.7.	19.7.	26.7.	2.8.	9.8.	16.8.	23.8.	6.9.	Skupaj
Marigold	11 b*	6 b	11 ab	10 a	13 a	15 a	20 a	28 a	114
Sierra orange	14 b	8 b	11 ab	9 a	16 a	21 a	11 a	11 a	101
Tall American	12 b	11 b	19 b	49 b	18 b	61 b	58 b	54 b	282
Mum Orange	0 a	1 a	5 a	8 a	21 ab	32 a	16 a	31 a	114

*a - Enaka črka v stolpcu pomeni, da se obravnavanju med seboj statistično značilno ne razlikujeta (Duncanov test mnogoterih primerjav, p<0,05).



Slika 4: Pridelek suhe snovi (g/parcelo) jezičastih cvetov glede na sorto (Marigold, Sierra orange, Tall American in Mum Orange) in datum vzorčenja
Figure 4: Yield of of marginal flowers (g DM/plot) with regard to variety (Marigold, Sierra orange, Tall American in Mum Orange) and sampling date

Preglednica 4: Delež suhih jezičastih cvetov glede na maso socvetij, pridelek droge (kg/ha), vsebnost heleniena in pridelek heleniena (kg/ha) po preučevanih sortah (IHPS, 2010)

Table 4: Share of dry marginal flowers with regard to the weight of fresh flowers, yield of marginal flowers (kg/ha), helenien content (%) and yield of helenien (kg/ha) (IHPS, 2010)

	Marigold	Sierra orange	Tall American	Mum Orange
Delež suhih jezičastih cvetov (%)	56,5	46,3	68,1	51,7
Pridelek droge (kg/ha)	475	435	1184	509
Vsebnost heleniena (% v SS)	5,61	4,42	1,94	2,68
Pridelek heleniena (kg/ha)	23,8	17,2	20,6	12,2

4 SKLEPI

V preučevanih razmerah je največji pridelek svežega socvetja dosegla sorta Tall American. Sorti Marigold in Sierra orange nista dosegli niti polovice pridelka svežega socvetja v primerjavi s sorto Tall American. Sorta Mum Orange pa je dosegla 59% pridelka v primerjavi s sorto Tall American. Tudi v pridelku droge (suhe mase jezičastih cvetov) je statistično značilno izstopala sorta Tall American, ki je imela najvišjo maso jezičastih cvetov pri vsakem obiranju v avgustu. Pridelek droge pri gostoti sajenja 41.500 rastlin/ha, preračunan na hektar glede na rezultate predstavljenega enoletnega poskusa, je bil pri sorti Marigold 475 kg/ha, pri sorti Sierra orange 435 kg/ha, pri sorti Tall American 1184 kg/ha in pri sorti Mum Orange 509 kg/ha. Tudi delež suhih jezičastih cvetov glede na maso socvetij (svež pridelek) je bil največji pri sorti Tall American, sledili sta sorti Marigold in Mum Orange, najnižji pa je bil pri sorti Sierra orange.

Analiza vsebnosti heleniena pa je pokazala, da je bila le-ta najnižja pri sorti Tall American, in sicer 1,94 %, kar jo je po pridelku heleniena uvrstilo na drugo mesto (za standardno sorto Marigold).

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USE OF AROMATIC PROFILES AS A TOOL FOR DETERMINING THE AUTHENTICITY OF FRUIT JUICES

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Abstract

The purpose of presented work was to develop the analytical and chemometric methodology that will enable authenticity characterization of fruit juices on the base of their aroma profiles. For the experimental part, juices and nectars of Slovenian and foreign producers were selected. Solid phase microextraction (SPME) technique was chosen as the technique for sampling volatile components. Sampling, optimal elution conditions and determination with gas chromatography-mass spectrometry was optimized according to the standard procedures. Peak areas of particular compounds in chromatograms were input parameters for the chemometric analysis (principal component analysis, group analysis and discriminant analysis). The purpose of chemometric part of work was to calculate the model which would be capable to discriminate between the groups of fruit juices. Results of all three chemometric methods were the best in the case of discriminating samples of fruit nectars and in the case when we want to discriminate samples of 100 % orange juices and samples of orange nectars. Worse, but still satisfying results were obtained when different samples of 100 % fruit juices and when samples of 100 % apple fruit juices with samples of apple nectars were compared.

Keywords: aromas, volatile components, fruit juice, SPME, GC-MS, chemometrics

UPORABA AROMATSKIH PROFILOV KOT ORODJA ZA DOLOČANJE PRISTNOSTI SADNIH SOKOV

Izvleček

Namen predstavljenega dela je bil razviti analizne in kemometrijske metode, ki bi karakterizirale pristnost sadnih sokov na osnovi njihovih aromatskih profilov. Za raziskovalno delo smo uporabili sokove in nektarje slovenskih in tujih proizvajalcev. Izbrana tehnika vzorčenja oziroma zajemanja hlapnih komponent arome je bila mikroekstrakcija na trdnem nosilcu - SPME tehnika. Postopke vzorčenja, optimalne pogoje za ločbo in določevanje s plinsko kromatografijo z masno detekcijo smo optimirali po standardnih postopkih za tovrstne analize. Površine vrhov posameznih spojin v kromatogramih so tvorile vstopne parametre za kemometrijsko analizo (metoda glavnih osi, analiza grup in diskriminantna analiza), katere

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namen je bil izdelava modela, ki bo sposoben z dovolj visoko zanesljivostjo razločevati med posameznimi sadnimi sokovi. Rezultati vseh treh kemometrijskih analiz so bili najbolj primerljivi v primeru ločevanja vzorcev sadnih nektarjev in v primeru, ko smo želeli med seboj razlikovati vzorce 100 % sokov pomaranč z vzorci pomarančnih nektarjev. Nekoliko slabše, vendar še vedno dovolj dobre, rezultate pa smo dobili pri medsebojnem ločevanju vzorcev 100 % sadnih sokov in medsebojnem razločevanju med vzorci 100 % sokov jabolk in vzorci jabolčnih nektarjev.

Ključne besede: arome, hlapne komponente, sadni sok, SPME, GC-MS, kemometrijske metode

1 INTRODUCTION

Compounds that contribute to the flavour characteristics of food products are mostly organic compounds belonging in several chemical classes such as esters, terpenoids, poliols, organic acids… Their common characteristic is the volatility [14]. The concentration ranges of a particular compound depend of a plant species, plant variety and of geoclimatic conditions although for a particular fruit juice the characteristic profiles are specific. Determination of the profiles of standard fruit juice samples enables determination of concentration ranges of some, most important compounds, which are usually present at higher concentrations. It is possible to assume that the profiles of volatile fruit juice compounds are similar.

Gas capillary chromatography (GC) with different detectors and gas chromatography coupled to the mass spectrometry (GC-MS) are the methods of choice for the analysis of volatile compounds in food products. One of the analytical approaches to determine volatiles is the analysis of gas phase which is in equilibrium with the sample. For the characterisation of a particular samples the use of multivariate chemometric methods is needed, since with such complex samples only the combination of different variables enable the better view in the nature of a sample. Basic approach in resolving of such questions is the formation of data bank. Such bank consists of a sample data sets belonging to the standard samples which are defined as those with the wanted characteristics. The bank should be extensive enough that it is able to show all ranges of particular compounds that are still in the acceptable limits. On the other hand bank should contain also the samples with evident faults. In most cases that means the higher content of undesirable compounds or the lack of favourable ones. Sufficient number of data in bank enables the construction of models which could be used further on as a tool during the regular quality control or as a tool for finding and evaluation of flavour composition of unknown samples.

Such approaches are already successfully used in many fields of food production. The use of EU isotopic data bank of wine or honey may serve as an example [5,9]. At our Institute we have long term experiences with a similar bank for the composition of hop essential oils which is successfully used in the case of hop variety studies [3,7]. Similarly the use of red wine anthocyanins profiles determined by liquid chromatograpy has been presented in the case of red vine varieties studies. The use of white wines profiles determined with NMR and ICP spectroscopy in the case of determination of the geographical origin and the variety is the another example [1,4,6].

In the literature a lot of examples of use of different chemometric methods PCA, HCA, LDA in combination with different analytical techniques which enable determination of different parameters in connections with the determination of the authenticity of a wide range of food products as wine, olive oil, beer, fruit juices could be find [2,8,10-13].

2 MATERIAL AND METHODS

The work was divided into several parts as optimization of the process of binding the volatile flavors of fruit juices on the selected fiber by varying the temperature, the ratio of volume between liquid and gas phase and the time of balancing/bonding. The objective of optimization was to determine the bonding time, temperature, volume and an acceptable amount of certain compounds related to the fiber. These parameters were further used for a complete analysis of all juice samples. We analyzed 53 different fruit juices of Slovenian and foreign producers, available on the domestic Slovenian market. Of these, 26 fruit juices with 100% fruit content and 27 nectars. Fruit juices are juices made from different fruits and have 100 % fruit base, while nectars are essentially the same except they are diluted with water that is usually followed by the addition of sugar and aromas of natural or artificial sources. The second part of presented work was getting aromatic profiles using SPME and subsequent determination by GC-MS.

25 mL of samples were placed in a 40 mL vials and thermostated at 50 °C for 60 min. A 100 μm polydimethylsiloxane (PDMS) SPME fiber was inserted through the septum and exposed for 50 min at 50 °C. The compounds adsorbed on the fiber were analyzed by GC-MS with a Varian STAR 3400 CX GC system coupled to an ion trap mass selective detector (Varian SATURN 2000); transfer line 260 °C, source 180 °C, ionization potential 70 eV. The fiber was inserted manually into the injector port (180 °C), desorbed and chromatographed on a nonpolar column (5% diphenyl and 95% dimethylsiloxane) (HP–5MS, 30 m, 0.25 mm, 0.25 μm; Agilent Technologies). Helium at a constant pressure of 240 kPa was used as carrier gas. After fiber insertion, the column temperature was increased from 60 to 190 °C at a rate of 2.5 °C/min followed by increasing to 240°C at the rate of 50 °C/min and ended with the final stage of 10 min at 240 °C. Detector temperature was set to 280 °C.

Chemometric evaluation of obtained results was the last part of our work. All the calculations where done by SCAN statistical program (Minitab Inc., USA).

3 RESULTS AND DISCUSSION

3.1 Qualitative evaluation of chromatographic peaks

First, we recorded the chromatograms of samples of different kinds of fruit juices (orange, apple, pineapple, pear, grapefruit and lemon) under the semi-quantitative conditions and identified the individual chromatographic peaks (Fig. 1). For the identification of compounds, we used the mass spectra of standard solutions, a library of mass spectra and literature data. These chromatograms are of great value to continue the work as they served as standards. For reliable identification, we identified and confirmed compounds, for which we had available

standard solutions as hexyl alcohol, α - pinene, hexyl acetate, limonene, linalool, methyl caprilate, β - caryophyllene, α - humulene.

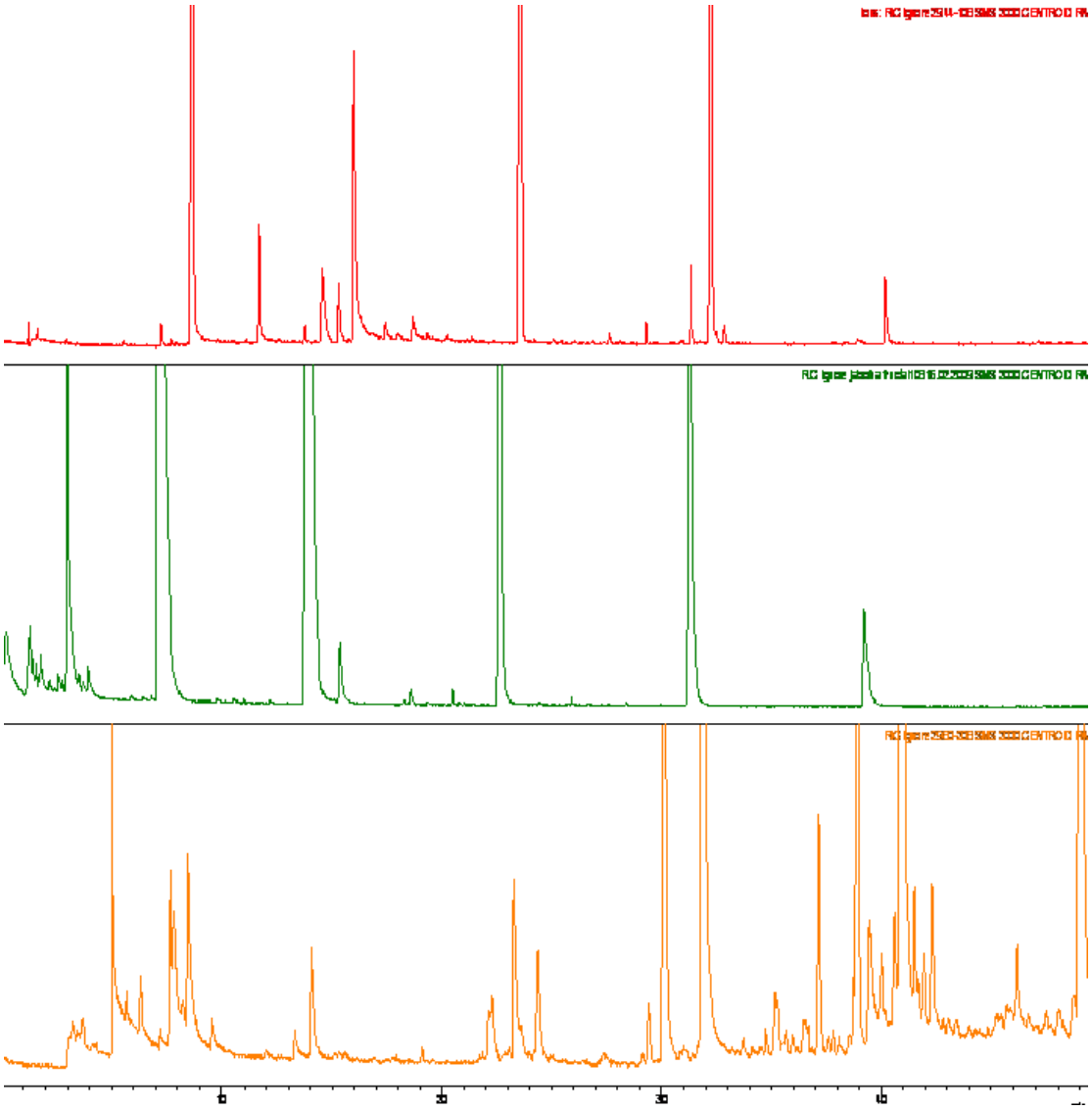

Figure 1: Comparison of GC chromatograms of 100% fruit juices of different fruits. From the top to the bottom chromatograms of juices or oranges, apples and pinepple are presented as an example.

Slika 1: Primerjava GC kromatogramov 100 % sadnih sokov različnega sadja. Od vrha proti dnu si kot primeri sledijo kromatogrami sokov pomaranč, jabolk in ananasa.

3.2 Chemometric analysis

In the second part microextraction of volatile compounds on solid medium was performed and 53 samples of fruit juices were analyzed under the semiquantitative GC/MS method. 26 samples, representing 100% juices and 27 samples of nectars of different kinds of fruits

(orange, apple, pineapple, pear, grapefruit and lemon). This was followed by chemometric analysis (principal component analysis, hierarchical clustering and discriminant analysis of groups) through which we created a model that was able with sufficient confidence to distinguish between different fruit juices.

With the use of principal component analysis basic information about similarities and differences of 25 samples of 100% juices and 26 samples of nectars was searching. First, we were interested in the similarity or difference between the 25 samples of 100% juices. 100% pear juices formed their own group. Other 100% juices (orange, apple, pineapple and grapefruit) are close to each other, but groups of certain types of juices are indicated (Fig. 2). It is also evident that one orange juice and pineapple juice are outliers of their groups.

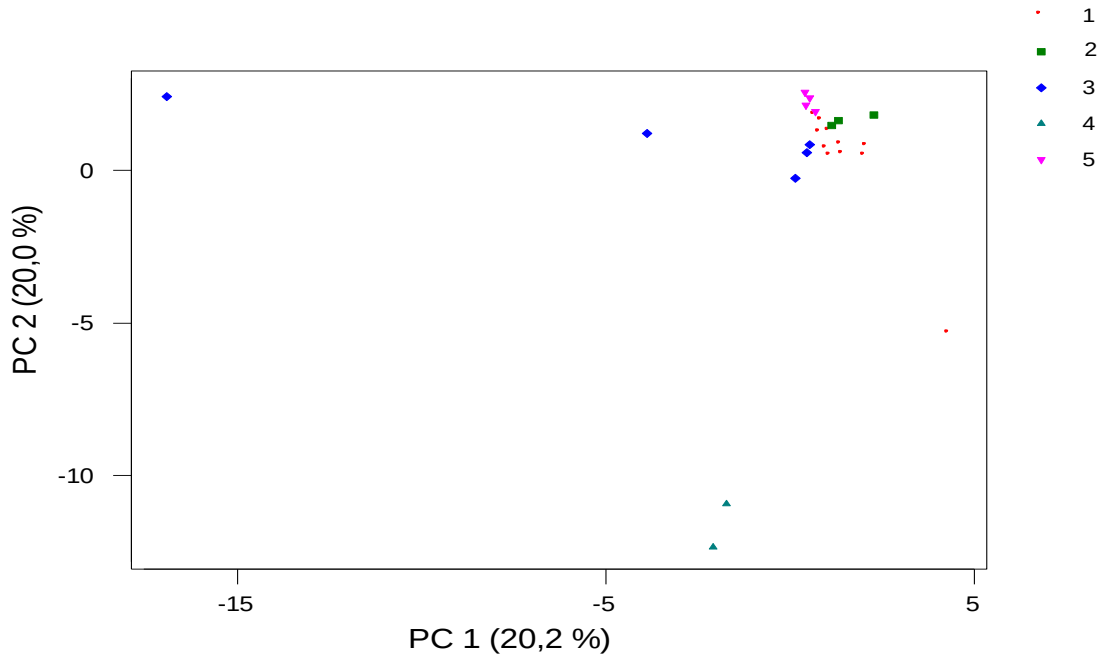

Figure 2: Projection of the 25 samples of 100% fruit juice in the plane defined by the first two principal components PC1 and PC2, which together represent 40.20% of the variance. The numeric codes are: 1 oranges, 2 apples, 3 pineapple, 4 pears, 5 grapefruit.

Slika 2: Projekcija 25 vzorcev 100 % sadnih sokov v ravnini definirana s prvima dvema glavnima osema PC1 in PC2, ki skupaj predstavljata 40,20 % variance. Številčne oznake so: 1 pomaranče, 2 jabolka, 3 ananas, 4 hruške, 5 grenivke.

Further on we were interested in the similarity or difference among the 26 nectar samples of different kinds of fruit (orange, apple, pineapple and pear). In this case, we have used principal component analysis on the basis of 25 variables. Fruit nectars are classified into three groups. The first group consisted of apple nectars (with the exception of one nectar - outlier), which was close to other groups. The second group is consisted of orange and three pear nectars. The third group is formed by pineapple nectars and two pear nectars.

In the third case, we were interested in the similarity or difference between 100% orange juices and samples of orange nectars. Juices and nectars were divided into three distinct

groups. The first group qualified for all orange nectars including three 100% orange juices. The second group consisted of three 100% orange juices and a third group are the remaining five samples of 100% orange juice. In this case we get three 100% fruit juices, which classified in the group of nectars and it was anticipated that these juices were actually not 100% juices but nectars.

In the fourth case, we were interested in the similarity or difference among samples of 100% apple juice and apple nectar samples. Juices and nectars were divided into two separate groups with the exception of one nectar sample that is placed close to the 100% apple juices.

The results of the analysis by hierarchical clustering were virtually the same as in the case of the use of the principal component analysis. Therefore in this paper we will not discuss these results in details. Results showed the same classification and found the same patterns, which represented outliers as described above.

Figure 3: Classification of 25 samples of 100% fruit juices on the basis of the calculated model with RDA (A) and the validation of the model done by cross validation (B). The numeric codes are: 1 oranges, 2 apples, 3 pineapple, 4 pears, 5 grapefruit.

Slika 3: Klasifikacija 25 vzorcev 100 % sadnih sokov na osnovi izračunanega modela z RDA (A) in prikaz rezultatov validacije modela, ki je bil narejen z navzkrižno validacijo (B). Številčne oznake so: 1 = pomaranče, 2 = jabolka, 3 = ananas, 4 = hruške, 5 = grenivke.

The last step of chemometric analysis was the use of regularised discriminant analysis (RDA). Within the 25 samples of 100% juice, we got 92% correct classification results, since only two of 25 samples ranked outside of their group. One outlier (pineapple) was the same as in the two previous methods, while another one (grapefruit) by this method was otherwise classified.

Among 26 nectar samples 92.3% correct classification results were obtained, because in this case, only two of 26 samples of nectars were classified wrong.

In the last case within 100% of apple juice and apple nectar samples we obtained 100% correct classification results. In this case, we got slightly different results, as with the earlier methods, where in both cases, one of the nectars ranked among the 100% juices.

The results of all three chemometric analysis were the most comparable in the case of separation of 27 samples of nectars and where we wanted to distinguish among 100% orange juices and nectars. Slightly less, but still enough good results were achieved in the separation of 26 samples of 100% fruit juices (Fig. 3).

4 CONCLUSION

With the chemometric analysis very good results were achieved. Even if some differences among the results is noticed, the values where regularised discriminant analysis was used were high (about 90%), which assures that we have created a model that is able to distinguish among different fruit juices with sufficient confidence.

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