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THE BREWING VALUE OF PERSPECTIVE HOP HYBRIDS (A6/58, 285/70, 31/299) AND SLOVENIAN VARIETIES AURORA AND DANA

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ABSTRACT

The brewing value is the most important characteristic of hop varieties since the majority of hops are used in the brewing industry. The brewing value is first evaluated at Slovenian Institute of Hop Research and Brewing as part of Slovenian hop breeding programme. In the past years varieties have also been evaluated in foreign reference laboratory. In this article the results of brewing tests of hop hybrids in the microbrewery at Slovenian Institute of Hop Research and Brewing and varieties in reference laboratory in Germany are presented. It can be concluded from the brewing values that all hop varieties included in the evaluation are comparable to other well known varieties worldwide.

Key words: hop, *Humulus lupulus* L., brewing value, hybrid, variety

PIVOVARSKA VREDNOST PERSPEKTIVNIH KRIŽANCEV HMELJA (A6/58, 285/70, 31/299) TER SLOVENSKIH SORT AURORA IN DANA

IZVLEČEK

Pivovarska vrednost je najpomembnejša lastnost sort hmelja, saj se večina hmelja uporabi v pivovarstvu. Del slovenskega žlahtniteljskega programa hmelja je določanje pivovarske vrednosti na Inštitutu za hmeljarstvo in pivovarstvo Slovenije. V zadnjem letu so bile sorte ocenjene tudi v referenčnem laboratoriju v tujini. V članku so predstavljeni rezultati testiranj križancev hmelja v mikropivovarni na Inštitutu za hmeljarstvo in pivovarstvo Slovenije in sort v referenčnem laboratoriju v Nemčiji. Zaključimo lahko, da je pivovarska vrednost sort in križancev hmelja, vključenih v ocenjevanje, primerljiva z ostalimi dobro poznanimi sortami po svetu.

Ključne besede: hmelj, *Humulus lupulus* L., pivovarska vrednost, križanec, sorta

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1 INTRODUCTION

Despite of the fact that agronomic traits are very important for each hop variety, the brewing value is the most important characteristic that directly affects hop quality and as a result its evaluation. Most of Slovenian hops are exported to global markets therefore the evaluation of Slovenian varieties in foreign reference laboratories or breweries is very important.

The brewing value is determined with a chemical analysis of wort and beer brewed with selected hop variety/hybrid, but the results of the chemical analysis are only a starting point for the value of each variety. When talking about the quality and use of hop, it is necessary to consider organoleptic estimation, and the data obtained through the analysis of final product - beer. The data obtained from all chemical and brewing analysis form a basis for evaluation of used hop, which is particularly important in hop marketing.

Bitterness is an essential beer characteristic. The International Bitterness Units (IBU) scale provides a measure of the biterness of beer and not a measure of isomerised alpha-acids in beer as it is thought by many brewers. It is usually assumed that 1 IBU is normally equivalent to 1 mg of iso-alpha-acids per litre of water or beer.

Beers brewed today show a wide range in their levels of bitterness. Lagers brewed in American large breweries range in biterness from 12 to 15 IBUs while British ales range from16 to 50 IBUs [6].

Commercial brewers measure the alpha-acids content in a laboratory; after a few test batches in the pilot brewery they adjust the hopping rate to the desired IBUs. To ensure consistency, large brewers purchase large quantities of hops and blend it throughout the year to achieve uniform consistency. Furthermore, practically all large breweries blend beer to even out fluctuations in bitterness from batch to batch, and sometimes add hop extracts to further adjust the bitterness [6,7].

Hop utilization is the percentage of alpha-acids that is isomerized and remains in the final beer. The utilization of bitter substances rarely exceeds 40 % in commercial breweries and is often as low as 25 %. Not all of the bitterness potential from the alpha-acids in the hop is utilized, which can be attributed to a number of reasons [6]:

- Form of Hops
  The isomerization rate is initially affected by the form of hops. Isomerisation is slower and at a much lower rate with whole hops or plugs, slightly faster with standard pellets, and greater with extracts.
- Boil Conditions
  Boil conditions can affect isomerization in a number of ways. For example the longer the boil continues, the more isomerization occurs, although eventually the reaction reverses itself, degrading the iso-alpha-acids.
- Hopping Rate
  Isomerization is also affected by a hopping rate; as the hopping rate increases, the rate of isomerization decreases. This effect can be partially offset by adding bittering hops in stages.

- Fermentation Conditions
  Fermentation conditions can affect the amount of iso-alpha-acids that remain in beer in a number of ways. The loss of iso-alpha-acids also occurs during fermentation as they are adsorbed to the yeast cell walls.
- Maturation and Filtration Conditions
  After the fermentation, maturation and filtration conditions affect the extent to which not only bitterness, but also other hop components survive in the final beer.

Dry hopping is the process of adding hops to the primary fermenter, the maturation tank, or the casked beer to increase the aroma and hop character of the final beer. Some brewers believe dry hopping should not be done during primary fermentation because of the risk of contaminating the beer with microorganisms. Dry hopping does not add to beer bitterness and any lingering bitterness will dissipate in a few weeks because alpha-acids are only slightly soluble in cold water. It should also be noted that a beer that has been dry hopped is usually also late hopped in the kettle. British brewers use this method to give a special hop character to cask-conditioned ales [3,6].

To summarise, in Slovenian breeding programme the most perspective hop hybrids are also evaluated at the Institute's microbrewery [4]. In order to receive an estimation from a foreign reference laboratory, some Slovenian varieties were sent to St. Johann microbrewery in Germany. The results of the past years are presented in the article.

2 MATERIAL AND METHODS

2.1 Microbrewery trial at Slovenian institute of hop research and brewing

2.1.1 Material

In the micro-brewing tests at the Slovenian Institute of Hop Research and Brewing the hybrids labelled as 31/299, A6/58 and 285/70 were used. A standard, a combination of Aurora (AU; bitter hop) and Savinjski golding (SG; noble aroma hop) varieties as late addition in kettle was used since both varieties are the most frequently used in Slovenian breweries. Hybrid 31/299 was used in the kettle as aroma hop brewed in combination with Aurora bitter hop, while A6/58 and 285/70 were used as bitter hops and Savinjski golding as a late addition in the kettle. The amount of hop with regard to the alpha-acids content was the same in all combinations (Table 1).

Hop combinations (cones):

1. A6/58 ; SG
2. 285/70 ; SG
3. AU ; 31/299
4. AU ; SG (control)

Production of wort and beer:

- Standard wort from Pivovarna Laško PLC brewery was used
- Amount of hop: corresponding to 120 mg/L alpha-acids
- Hopping: 50 % aroma hop, 50 % bitter hop (corresponding to alpha-acids content)

- Hopping programme: 105 min (3 addings: first 15 min before boiling, second after 45 min, third 15 min before the end)
- Clearing and cooling of wort: 90 min
- Fermentation: 6 days, temp. 12-14 ˚C (yeasts – *Saccharomyces Pastorianus*)
- Maturation: 3 weeks, temp. 2-3 ˚C

Table 1: Hop amount per 30 L of wort
Preglednica 1: Odmerki hmelja na 30 L pivine

	A6/58		**285/70**		**31/299**		**control**	
Hop variety/ hybrid	A6/58	SG	285/70	SG	AU	31/299	AU	SG
Quantity of hops (g)	18.2	52.9	12.9	52.9	17.4	46.2	17.4	52.9

2.1.2 Methods

The following analytical methods to support experimental trials were used in the research. In hop cones moisture, alpha-acids and xanthohumol contents were determined by Analytica-EBC methods [1].

In wort the content of extract, alpha-acids, iso-alpha-acids, polyphenols and antocyanogens was determined. At the same time pH value, bitterness, colour and P.I. value were measured with standard methods from Analytica-EBC and MEBAK Band II [1,2].

After the maturation, the same parameters as in wort with an addition of alcohol content were determined in all beers undergoing trials. For sensorial beer estimation the DLG test from MEBAK, Band II was used [2].

3 RESULTS AND DISCUSSION

3.1 Microbrewery trial at Slovenian institute of hop research and brewing

3.1.1 Analysis of wort

First we analysed the wort before adding hops to get original parameters not influenced by hops. After the hopping, all four types of wort were analysed with analysis protocols listed in the Methods section above (Table 2).

Table 2: Results of wort analysis
Preglednica 2: Rezultati analize sladice in pivine

	wort before hopping	Wort A6/58	Wort 285/70	Wort 31/299	Wort control
Extract (%)	10.99	10.89	11.38	10.96	10.54
pH	5.92	5.82	5.75	5.74	5.83
Colour (EBC)	5.4	7.4	9.1	8.2	7.7
Bitternes (BU)	-	54.3	56.4	56.3	54.2
Alpha-acids (mg/L)	-	39.2	40.6	43.3	41.8
Iso-alpha-acids (mg/L)	-	26.3	26.7	25.1	24.6
Polyphenols (mg/L)	113.3	175.9	171.5	159.7	157.3
Antocianogens (mg/L)	39.6	50.7	52.0	47.8	44.3
P.I.	2.9	3.5	3.3	3.3	3.6

3.1.2 Analysis of beer

After a three-week maturation period all four beers were analysed in accordance with the protocol and with the use of analyses described in the Methods section. The results can be divided into two groups: chemical analyses results and sensorial results.

3.1.2.1 Chemical analysis

In Table 3 the results of beer analysis brewed from different hybrids are shown.

Table 3: Results of beer analysis
Preglednica 3: Rezultati analize piva

	Beer A6/58	Beer 285/70	Beer 31/299	Beer control
Extract (%)	10.66	10.96	10.91	10.50
Alcohol (vol. %)	4.78	4.86	4.69	4.39
pH	4.63	4.56	4.53	4.61
Colour (EBC)	6.3	7.5	7.2	6.5
Bitterness (BU)	19.8	21.9	20.5	20.0
Alpha-acids (mg/L)	2.8	3.9	2.6	2.1
Iso-alpha-acids (mg/L)	20.5	24.1	22.4	20.3
Polyphenols (mg/L)	161.5	159.1	157.3	153.8
Antocianogens (mg/L)	48.4	44.9	39.0	41.3
P.I.	3.3	3.5	4.0	3.7

The content of polyphenols and antocianogens in the wort hopped with hybrid 31/299 was the same as in the control (SG/AU), while in the wort brewed with hybrids A6/58 and 285/70 the content of polyphenols and antocianogens was much higher. The same was also observed when analysing beers, where the content of polyphenols and antocianogens in the beer brewed with 31/299 reached the lowest value, while in the beers brewed with A6/58 and 285/70 the content was slightly higher.

The best efficiency of alpha-acid content, which resulted in the highest value of bitterness, the content of alpha-acids and iso-alpha-acids in the beer, was determined when hybrid 285/70 was used while in other three beers (A6/58, 31/299, control) the values were comparable.

3.1.2.2 Sensorial estimations

Seventy-six randomly selected beer tasters (age groups 20 - 60, males and females) were included in sensorial estimation. Beer kettled with a hop combination of 31/299 hybrid and Aurora variety received the highest grade among all included samples.

In this particular sample, the highest marks that stood out were given to the intensity and quality of bitterness. The intensity and quality of aroma in this sample received a slightly lower grade compared to the control. The beer samples brewed with hybrids A6/58 and 285/70 in combination with Aurora received a slightly lower grade compared to the control and were quite similar within all parameters. The lowest intensity of bitterness was achieved in beer brewed with A6/58 (Table 4).

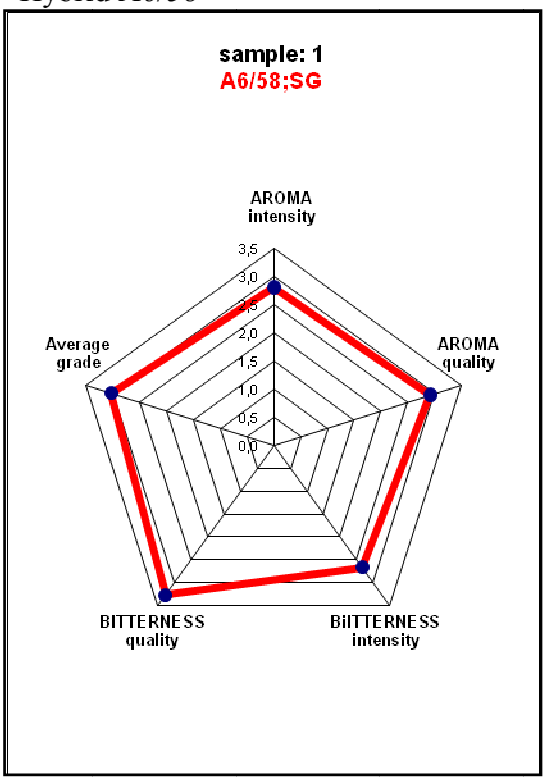
Table 4: The senzorial estimations of beer
Preglednica 4: Senzorična ocena piv

	A6/58	285/70	31/299	control
Hop aroma Intensity	2.8	2.7	2.8	3.0
Hop aroma Quality	2.9	3.0	3.0	3.2
Bitterness Intensity	2.7	3.1	3.1	2.9
Bitterness Quality	3.3	3.3	3.4	3.3
The average grade	3.0	3.1	3.3	3.2
Total	14.7	15.1	15.6	15.5

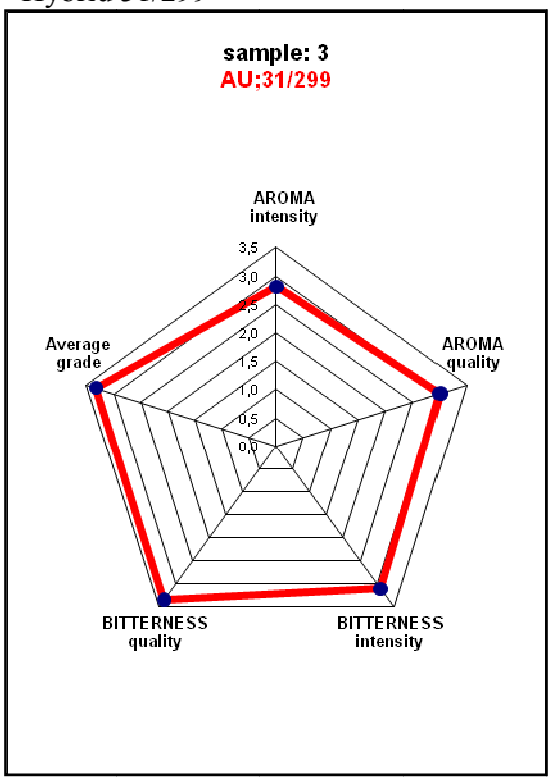
Note: The grades given were from 1 - 5 (where 1 is the minimum and 5 the maximum)
No. of beer tasters: 76

The results of beer tasting are shown in Figures 1 – 4.

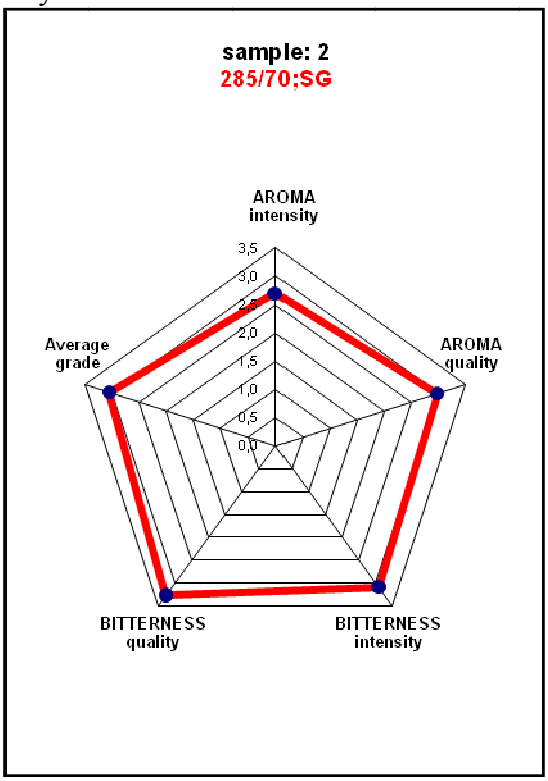
Hybrid A6/58



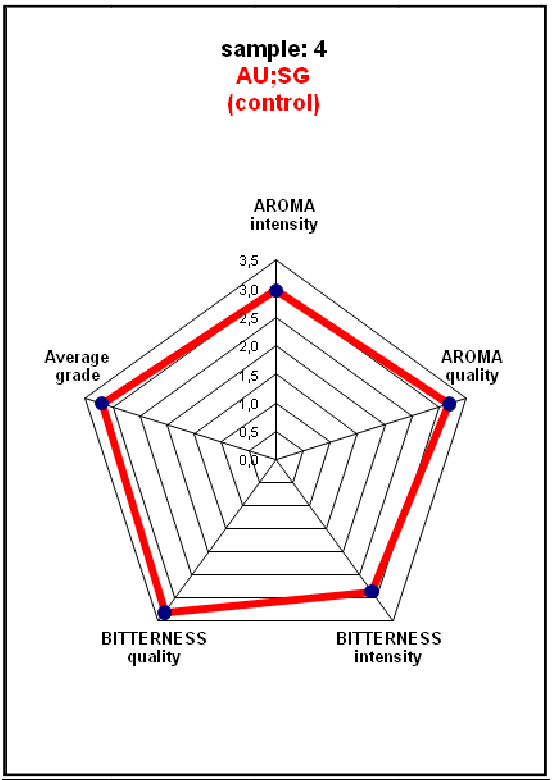
Hybrid 31/299



Hybrid 285/70



Control SG/AU



Figures 1-4: Sensorial evaluation of 4 different beer samples
Slike 1-4: Senzorična ocena 4 različnih vzorcev piva

3.2 Microbrewery trial at St. Johann microbrewery, Germany

In German reference laboratory beer tasters graded Dana and Aurora hop varieties. The aim of this evaluation was to get an independent grade for both varieties from a professional panel of tasters (10 members) and compare it with the results obtained in the microbrewery in Žalec. In St. Johann Research Brewery beer can be filtered, pasteurised and bottled as in macrobrewing (industrial scale) process, which is not possible in microbrewery in Žalec. Both samples were hopped only with one variety.

Both beers, brewed with Dana and Aurora variety received the same grade for the intensity of hop aroma. The quality of hop aroma of the beer brewed with Aurora was graded slightly higher than it was expected, since Aurora is a type of aroma variety.

Beer brewed with Dana variety received a higher grade for intensity of hop bitterness, showing better efficiency of bitter substances. The quality of bitterness was graded slighly worse, which was probably due to a higher percentage of cohumulone in alpha-acids.

To sum up, beers brewed with Aurora or Dana achieved comparable evaluation, slightly higher grades were given to Aurora because of better aroma and the quality of bitterness. Since Aurora is a representative of aroma hops and Dana of bitter hops the comparison between the two cannot be made. In general, the investigated beers cannot be compared directly. In general, beers are brewed with a combination of both aroma and bitter hops, so both investigated varieties received high grades, each in its own category.

4 CONCLUSIONS

It can be concluded from all the samples brewed from hop hybrids at the Slovenian Institute of Hop Research and Brewing that the obtained grades within all investigated parameters did not have a negative effect on the quality of beer; they were also comparable to the control, and brewed with a well known combination of Slovenian Aurora and Savinjski golding varieties. Further analysis of the investigated hybrids together with agronomic traits will be carried out in the future.

In conclusion, the evaluation of German professional tasters in St. Johann microbrewery showed that the new Dana hop variety is a bitter variety with good aroma characteristics, which enhance essential oil and the amount of linalool, contributing to a quite intense hop aroma in final beer. The alpha/beta-acid ratio is around 3, which is characteristic of high alpha variety. Aurora and Dana varieties offer a good brewing value and a pleasant and intense hop aroma, and provide a harmonic and medium to slightly robust bitterness. Their quality is comparable to customary German varieties (ex. Hallertauer Magnum, Hercules) [5].

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STORAGE STABILITY OF HYBRIDS – IMPORTANT HOP QUALITY TRAIT

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ABSTRACT

Storage stability is a very important hop quality trait especially in the time of overproduction. As other important agronomic traits, storage stability is a characteristic observed in all perspective hop hybrids included in the Slovenian breeding programme. Based on the results obtained in the past few years, it can be concluded that hop germplasm at Slovenian Institute of Hop Research and Brewing has a great potential in terms of good storage stability.

Keywords: hop, *Humulus lupulus* L., storage stability, hybrid

SKLADIŠČNA OBSTOJNOST KRIŽANCEV – POMEMBNA LASTNOST KAKOVOSTI HMELJA

IZVLEČEK

Skladiščna obstojnost je zelo pomembna lastnost hmelja zlasti v obdobju viškov hmelja. Kot ostale agronomsko pomembne lastnosti je skladiščna obstojnost lastnost, ki se ocenjuje pri vseh perspektivnih križancih hmelja vključenih v slovenski žlahtniteljski program. Glede na rezultate zadnjih nekaj let se lahko zaključi, da ima dednina hmelja na Inštitutu za hmeljarstvo in pivovarstvo Slovenije dober potencial v smeri skladiščne obstojnosti.

Ključne besede: hmelj, *Humulus lupulus* L., skladiščna obstojnost, križanec

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1 INTRODUCTION

Hops have three main metabolites relevant to brewers: alpha-acids, beta-acids, and essential oils. Brewers normally concern themselves with only two of the three: alpha-acids and essential oils. Hops start to lose their alpha-acids and essential oils as soon as they are harvested. The rate of loss depends on the storage temperature, the amount of air (oxygen) present, and the hop variety, of which the most important factor we have control of is temperature, followed by a hop variety, and finally aerobic or anaerobic storage conditions.

The lower the temperature, the less of the hop metabolites deteriorate. It has been established that the rate of loss halves at every 15 °C drop in the temperature [3].

Oxygen is a non-pleasant parameter in hop storage; oxidized alpha-acids lose their bitterness and cannot be isomerised. They are responsible for the "cheesy" aroma detected in old hops. The compression of hops into bales, pellets, and plugs provides good protection against air exposure to all hop parts except the surface layers, and even then the air penetrates and causes oxidation. Exposure to light hastens hop deterioration as well [6,7].

A **hop variety** also plays a major role in storage. Hops are usually classified as kettle or bitter hops, and aroma hops. Kettle hops have a higher alpha-acid content than aroma hops, and their storage properties are more important. Under identical storage conditions, certain varieties will lose more alpha-acids than others.

As a result of oxidation and auto oxidation processes in hop cones in the time of storage, composition and the amount of hop resins, essential oils and polyphenols changes. The reasons for the difference in storage stability between different varieties are:

- The content of enzyme alpha-acid oxidase,
- The content of endogenous antioxidants,
- Membrane permeability of lupulin glands for oxygen [8].

Determination of storage stability is based on the decrease of alpha-acid content in the given time. For this purpose 2 parameters are used - the alpha-acid content and hop storage index (HSI). HSI parameter is a number obtained by spectrophotometric determinations of alpha-acids; it is a ratio between alpha-acid content and their oxidation products. Nickers and Menary [5] have proposed the following criteria for storage stability of hop varieties stored for 6 months at 20 °C:

- Very good storage stability: the varieties show 80-90 % of initial alpha-acid content
- Good storage stability: the varieties show 60-80 % of initial alpha-acid content
- Poor storage stability: the varieties show less than 50 % of initial alpha-acid content

There is a direct relationship between the losses of alpha-acid content and hop storage index (HSI). If the present alpha-acid content and HSI, or the percentage lost figures for a particular variety is known, the original and future alpha-acid content could be estimated. Hop quality during the storage is identical to the 'degree of freshness' – Table 1 shows the reduction of alpha-acid content and the HSI divided into 5 categories [2].

Table 1: Hop quality expressed as alpha-acid losses and HSI during the storage [2]
Preglednica 1: Kakovost hmelja izražena z izgubami alfa-kislin in HSI med skladiščenjem [2]

Classes of hop freshness	Deterioration of alpha-acids (% rel.)	Hop storage index
Fresh	0 – 10	< 0.32
Slightly aged	11 – 20	0.33 – 0.40
Aged	21 – 30	0.41 – 0.50
Strongly aged	31 – 40	0.51 – 0.60
Overaged	> 40	>0.61

Oils also reduce and oxidize over time. An assumption could be made that the rate of oil loss is directly related to the loss of alpha-acids therefore oil losses can be predicted with the use of alpha-acid loss formulas. Due to the lack of experimental data to support this method, it remains only an assumption [3].

Since storage stability as a parameter of each new variety is an important property, we have included monitoring of both indicators (loss of alpha-acids, HSI) in the breeding process. The aim of prolonged storage period (2 years) was to determine the storage stability of accessions in the time when using hops in breweries is still an option, especially in the time of overproduction of hops.

2 MATERIAL AND METHODS

2.1 Material

In this research perspective hop hybrids from Slovenian hop breeding programme and Dana bitter variety were included (Table 2). Hybrids 279/54, 279/104, 279/122 bred for their bitter acids and Dana were stored for 2 years at 20 °C and 4 °C. The research also included hops stored at 4 °C as nowadays the majority of hops are kept in cold storage. The absence of light was assured in both cases.

The trial started after harvest in 2006. In the last year 5 new perspective hop hybrids labelled as 31/299 (noble hop aroma, high resistance to verticilium wilt), A6/58 (dual purpose, high resistance to verticilium wilt), 285/70 (bitter type), 40/39 (bitter type), A2/132 (bitter type, high resistance to verticilium wilt) were added. The samples were included in the analyses for one year; the trial was carried out after harvest in 2008 (Table 4).

2.2 Methods

All samples were picked in the time of their technological maturity (end of August / beginning of September) on the experimental farm of Slovenian Institute of Hop Research and Brewing in Žalec. In order to obtain the initial values, first determinations of Lead Conductance Value (LCV) and HSI were carried out straight after the harvest. All other determinations were done in the intervals of approximately two months after the harvest time.

To determine LCV value in hop cones Analytica EBC, 7.4 [1] method was used. 50 mL of toluene (S ≤ 0,002 %) (Fluka) was added to the milled hop cones (5 g) and samples were then

shaken for 30 min. After filtration, 40 mL of methanol (ρ = 0,79 g/mL) (Fluka) was added to 10 mL of toluene extract, folowed by titration with the lead acetate solution (min 99,9 %) (Ridel de Haën) on the Conductometer MeThrom 712. The results were evaluated with software TiNet 2.4 (Herisau, Switzerland). Standardization of lead acetate solution before each series of measurements was carried out.

Hop storage index value was determined with ASBC method [4].

3 RESULTS AND DISCUSSION

3.1 Two-year trial

Dana variety

After a six-month storage period at 20 °C the alpha-acid content decreased for 4.3 % (rel.) and for 3.3 % (rel.) when hops were stored at 4 °C. HSI increased by 0.03 up to 0.37 at room temperature and at 4 °C (Table 2). Very good storage stability of the variety was recorded after 6 months.

In one year period alpha-acid content fell by 42.5 % (rel.) and by 27.5 % (rel.) at 20 or 4 °C. The results obtained have shown that Dana variety has good storage properties even after 2 years of storage. During a two-year storage period the alpha-acid content decreased by 60 % (rel.) and by 46.7 % (rel.) at 20 °C or 4 °C respectively (Table 2).

Hybrid 279/122

After a six-month storage period at 20 °C or 4 °C the alpha-acid content decreased for 5.8 % (rel.) and 2.9 % (rel.) respectively. It was observed that in the same time and in the same conditions HSI increased from 0.11 up to 0.50 at room temperature, or from 0.04 up to 0.43 at 4 °C.

It can be concluded that hybrid 279/122 has very good storage stability. After a two-year storage period at 20 °C the alpha-acid content decreased by 68.0 % (rel.) and at 4 °C by 47.6 % (rel.). In the same time the HSI increased to 1.80 and 0.73 at 20 °C or 4 °C respectively (Table 2).

Hybrid 279/104

After a six-month storage period at 20 °C the alpha-acid content decreased for 14.0 % (rel.) when hops were stored at 4 °C alpha-acids content fell by 0.1 % (rel.). HSI increased by 0.03 up to 0.29. All data show that hybrid 279/104 has very good storage stability. After a two-year storage period at 20 °C the alpha-acid content decreased by 46.3 % (rel.) and at 4 °C by 26.4 % (rel.). In the same time HSI increased to 0.53 at 4 °C (Table 2). The results obtained have shown that even after 2 years of storage hybrid 279/104 has very good storage properties.

Hybrid 279/54

After a six-month storage period at 20 °C the alpha-acid content decreased for 13.3 % and by 9.7 % (rel.) at 4 °C. When hops were stored at 20 °C HSI increased by 0.08 up to 0.48. After 6 months hybrid 279/54 has very good storage stability. After a two-year storage period at 20 °C the alpha-acid content decreased by 57.5 % (rel.) and at 4 °C by 44.2 % (rel.). In the same time HSI increased to 1.22 at 20 °C (Table 2).

In 2008 we repeated the trial with the above mentioned hybrids and Dana to confirm the results from the previous year since each production year has different climate characteristics (Table 3). It can be seen from Table 3 that the same results were obtained as in the previous year which means that all three hybrids including Dana variety were characterised as hops with very good storage stability.

3.2 New perspective hybrids

After a six-month storage period at 20 °C the alpha-acid content losses for 5.8 % to 18.0 % were recorded in hybrids **31/299, A6/58, 285/70, 40/39** and **A2/132** (Table 4). HSI increased in the same time in the same conditions by 0.02 up to 0.14, depending on the observed hybrid. After one-year storage period at 20 °C the alpha-acid content decreased from 21.2 % (rel.) to 49.1 % (rel.), and at 4 °C from 5.1 up to 20.9 % (rel.). In the same time the HSI increased from 0.03 up to 0.17 at 4 °C, or from 0.22 up to 0.64 at 20 °C (Table 4).

After a six-month storage period at 20º C all hybrids were classified as hops with very good storage stability.

Table 2: The alpha-acid content and HSI values in samples after a two-year storage period at 4 and 20 °C (harvest 2006)
Preglednica 2: Vsebnosti alfa-kislin in vrednost HSI v staranih vzorcih hmelja tekom dveh let pri temperaturah 4 °C in 20 °C (letnik 2006)

Hybrid			Initial	6 months	9 months	12 months	15 months	19 months	24 months	Storage stability
279D112 (Dana)	α-acids	4	12.0	11.jun	11.jun	8.jul	8.feb	7.maj	6.apr	Very good
		20		11.maj	9.avg	6.sep	6.apr	5.avg	4.avg	
	HSI	4	0.34	0.37	0.39	0.39	0.54	0.74	1.jun	
		20		0.37	0.53	0.64	0.83	0.99	jan.29	
279/122	α-acids	4	10.mar	10.0	10.0	7.sep	7.jul	7.0	5.apr	Very good
		20		9.jul	7.mar	5.avg	5.feb	4.jul	3.mar	
	HSI	4	0.39	0.43	0.43	0.45	0.48	0.56	0.73	
		20		0.50	0.81	0.82	jan.21	0.88	jan.80	
279/104	α-acids	4	12.jan	12.0	11.avg	11.sep	11.jul	10.jun	8.sep	Very good
		20		10.apr	10.apr	8.avg	7.jun	7.feb	6.maj	
	HSI	4	0.26	0.26	0.27	0.28	0.34	0.42	0.53	
		20		0.29	0.34	0.36	0.40	0.57	0.77	
279/54	α-acids	4	11.mar	10.feb	8.feb	7.feb	7.feb	6.avg	6.mar	Very good
		20		9.avg	7.maj	5.maj	5.mar	5.jan	4.avg	
	HSI	4	0.36	0.42	0.51	0.55	0.66	0.72	1.jan	
		20		0.48	0.59	0.65	0.83	0.98	jan.22	

Table 3: The alpha-acid content and HSI values in samples after one-year storage period at 4 and 20°C (harvest 2008)
Preglednica 3: Vsebnosti alfa-kislin in vrednosti HSI v staranih vzorcih hmelja pri temperaturah 4 in 20 °C tekom enega leta (letnik 2008)

Hybrid			initial	3 months	6 months	8 months	10 months	12 months	Storage stability
279D112 (Dana)	α-acids	4	16.sep	16.maj	16.0	15.avg	15.maj	15.apr	Very good
		20		16.feb	15.mar	13.apr	10.mar	9.0	
	HSI	4	0.29	0.29	0.30	0.34	0.38	0.39	
		20		0.35	0.40	0.53	0.71	0.88	
279/122	α-acids	4	15.mar	14.sep	14.maj	14.apr	14.apr	14.mar	Very good
		20		15.jan	14.0	13.sep	13.jun	11.apr	
	HSI	4	0.28	0.28	0.28	0.29	0.32	0.33	
		20		0.29	0.31	0.36	0.46	0.49	
279/104	α-acids	4	15.jan	13.0	13.mar	12.jul	12.jun	12.jun	Very good
		20		13.apr	12.apr	10.maj	9.sep	9.0	
	HSI	4	0.27	0.28	0.29	0.30	0.35	0.37	
		20		0.30	0.32	0.40	0.50	0.56	
279/54	α-acids	4	13.mar	12.avg	12.jul	12.maj	12.maj	12.jan	Very good
		20		12.maj	11.jul	10.maj	7.avg	8.maj	
	HSI	4	0.26	0.27	0.30	0.33	0.39	0.39	
		20		0.32	0.37	0.50	0.67	0.78	

Table 4: The alpha-acid contents and HSI values in hybrids 31/299, A6/58, 285/70, 40/39 and A2/132 after one-year storage period at 4 and 20°C (harvest 2008)
Preglednica 4: Vsebnosti alfa-kislin in vrednost HSI v staranih vzorcih hmelja pri temperaturah 4 in 20 °C križancev 31/299, A6/58, 285/70, 40/39 in A2/132 (letnik 2008)

hybrid			initial	3 months	6 months	8 months	10 months	12 months
31/299	α-acids	4	5.apr	4.jun	4.jun	4.maj	4.apr	4.apr
		20		4.jun	4.maj	4.jan	3.mar	3.feb
	HSI	4	0.28	0.28	0.32	0.33	0.41	0.43
		20		0.37	0.39	0.48	0.66	0.67
A6/58	α-acids	4	10.maj	11.feb	10.feb	9.0	9.mar	8.mar
		20		10.jun	9.mar	9.feb	6.0	5.apr
	HSI	4	0.28	0.29	0.32	0.35	0.42	0.44
		20		0.35	0.42	0.53	0.71	0.88
285/70	α-acids	4	16.jul	16.jun	16.mar	15.sep	15.feb	14.jul
		20		12.sep	12.jul	11.mar	9.jul	8.maj
	HSI	4	0.27	0.27	0.28	0.30	0.34	0.34
		20		0.35	0.39	0.52	0.62	0.68
40/39	α-acids	4	13.jul	13.jul	13.mar	13.0	12.avg	12.feb
		20		12.apr	12.0	11.sep	10.sep	10.avg
	HSI	4	0.25	0.26	0.28	0.28	0.29	0.29
		20		0.29	0.29	0.30	0.38	0.89
A2/132	α-acids	4	15.jan	14.sep	14.avg	14.maj	14.mar	14.0
		20		15.jan	13.sep	13.sep	13.jun	11.apr
	HSI	4	0.27	0.28	0.28	0.29	0.32	0.33
		20		0.29	0.31	0.36	0.46	0.49

4 CONCLUSIONS

It can be concluded that hybrid 279/104 has excellent characteristics after two years of storage; its HSI was 0.77 or 0.53 stored at room or cold temperature respectively. Very good storage stability was also observed in Dana variety. Hybrids 279/54 and 279/122 showed slightly worse results but they were still classified as the ones with good storage properties.

New perspective hybrids 31/299, 285/70, 40/39, A6/58 and A2/132 still show very good storage stability after 1 year of storage in cold conditions.

In the time of overproduction of hops on the global hop market the development of own varieties with very good storage stability is becoming even more important. Storage stability is an important hop trait and an indicator of stability of the main hop metabolites during storage therefore it is essential for maintaining hop quality in the whole hop industry (growers, merchants, brewers).

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EFFECT OF THE STABILIZED NITROGEN FERTILIZER ON THE HOP YIELD AND ITS QUALITY COMPARED TO CALCIUM AMONNITRATE

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ABSTRACT

Of all mineral nutrients N is quantitatively the most important for plant growth, but N recovery from mineral fertilizers is relatively low. Previous investigations show that 3,4-dimethylpyrazol phosphate application with ammonium sulphate nitrate as Entec 26 has reduced N losses due to nitrate leaching and N_2O emissions and had a positive effect on the yield. The results of Aurora hop cultivar presented in the experiment indicated that fertilization with Entec 26 in one split was comparable to conventional fertilization with calcium amonnitrate (KAN) in three splits as far as the yield, alpha acid content, alpha acid yield and nitrate content in hop cones in seasons 2008 and 2009 are concerned.

Key words: hops, *Humulus lupulus* L., nitrogen, fertilization, Entec 26

VPLIV DOGNOJEVANJA HMELJA Z GONJILOM S STABILIZIRANIM DUŠIKOM V PRIMERJAVI S KALCIJEVIM AMONNITRATOM NA PRIDELEK HMELJA IN NJEGOVO KAKOVOST

IZVLEČEK

Med vsemi hranili je dušik najpomembnejši za rast rastlin, vendar je izkoristek tega hranila iz mineralnih gnojil relativno majhen. Dosedanje raziskave so pokazale, da uporaba 3,4-dimetilpirazol fosfata kot zaviralca bakterijske pretvorbe amonijske oblike dušika v nitratno zmanjša izgube dušika zaradi izpiranja nitratov in emisij N_2O ter pozitivno vpliva na pridelek. Rezultati predstavljene raziskave kažejo, da je bilo v preučevanih letih 2008 in 2009 dognojevanje hmelja cv. Aurora z Entec 26 v enem obroku v primerjavi z dognojevanjem s kalcijevem amonnitratom (KANom) v treh obrokih (pri istem odmerku N) primerljivo po pridelku, vsebnosti alfa kislin, pridelku alfa kislin in vsebnosti nitratov v storžkih.

Ključne besede: hmelj, *Humulus lupulus* L., dušik, gnojenje, Entec 26

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1 INTRODUCTION

Of all mineral nutrients nitrogen (N) is quantitatively the most important for plant growth. N uptake from the soil is mainly in the form of ammonium and nitrate, and is regulated not only by the chemical and spatial availability of N in the soil, but also by the activity and number of transport systems at the cellular level, transport from the roots to the shoot, and utilization of growth and storage [3].

Although N recovery from mineral fertilizers is relatively low, especially in the production of rice, cotton and sugarcane where high mineral N rates are experienced and at the same time N losses are high, many farmers tolerate it, and in a good economic situation higher N rates are practised and that increases N losses due to denitrification and volatization [3].

Nitrification inhibitors like 3,4-dimethylpyrazol phosphate (DMPP) are compounds that delay the bacterial oxidation of ammonia to nitrite in the soil (first step of nitrification) for a certain period of time by depressing the activity of *Nitrosomonas* bacteria in the soil [13]. Application of nitrification inhibitors with several ammonium-based fertilizers resulted in a decrease of N_2O emissions [5,14,6]. Investigations showed that DMPP application with ammonium sulphate nitrate (ASN) as Entec 26 has reduced N losses due to nitrate leaching [8,4] and N_2O emissions [16,12].

In the experiment by Paschold et al. [15] on the plots fertilized with Entec 26 the total number of shoots and the number of asparagus spears with diameter >10 mm on the soil surface increased. In 2001 no significant yield differences with a positive trend for Entec 26 were found in the years following. In comparison with ASN the application of Entec 26 led to significant yield increases in 2004. The results of the study by Kołota et al. [11] showed that Entec 26 was an equally valuable source of N for red beet as ammonium nitrate and calcium nitrate and more efficient than ammonium sulphate. In experiment with celeriac, plants supplied with this fertilizer in split doses overyielded those receiving the same amounts of ammonium nitrate. An important advantageous effect of Entec 26 use was a considerable reduction of nitrates accumulation in red beet and in celeriac roots. Results of the study by Kołota and Adamczewska–Sowińska [10] showed that Entec 26 was a better source of nitrogen than ammonium nitrate, providing higher yield of marketable heads and lower nitrate contents in plants of cabbage at harvest. In the experiment by Menéndez et al. [13] it was concluded that DMPP is an efficient nitrification inhibitor that reduces N_2O and NO emissions from grasslands.

The aim of the experiment was to investigate the effect of N fertilization in the form of Entec 26 in one split compared to fertilization with KAN in three splits on the hop yield and its quality. Plant growth, growth stages, Nmin content in soil, yield, alpha content in hop cones, alpha acid yield and nitrate content in hop cones were observed.

2 MATERIAL AND METHODS

2.1 Material

In the experiment Entec 26 was used as a source of N (26% total N; 7,5% nitrate N, 18,5% ammonium N) compared to calcium amonnitrate (KAN) at control treatment. Entec 26 is a granulated mineral fertilizer with stabilized ammonium form of N and water soluble sulphur

(ammonium sulphate: 26+0+0 (+13S)) which differs from other conventional products in DMPP additive. N is made available to plants gradually; the whole amount is loosened in the period of 4 to 10 weeks in accordance with the temperature and moisture of the soil. This way we avoid frequent crossings of the field in order to perform N fertilization because higher doses can be used at a time. The number of doses can be reduced by one or two. Because N losses are reduced, 20-30% lower N rates can be implemented compared to fertilizers with no DMPP stabilizer [9].

2.2 Field experiment and evaluation

The experiment was conducted as a block trial in three replications in the experimental field of Slovenian Institute of Hop Research and Brewing in 2008 and 2009 with hop cultivar Aurora which is planted on approximately 60% of Slovenian hop fields. The size of one plot was 200 m^2. There were two treatments:

Control: Control treatment; conventional fertilization with N in the form of KAN in three splits (50 kg/ha N 20 May + 70 kg/ha N 15 June + 50 kg/ha N 10 July).
ENTEC: Fertilization with N in the form of Entec 26 in one split (170 kg/ha N on 20 May).

The rest of the agrotechnique was the same for all plots and performed in accordance with good agricultural practice. Phosphorus and potassium fertilization was performed in accordance with the soil analysis. No foliar fertilizers were used. Plant protection products were used in accordance with the spraying programme.

In the time of technological maturity the inner two rows on each parcel were evaluated; the number of plants and strings per plot was counted, the plot was measured, hop cone yield was weighed, samples were taken for analysis of moisture, alpha acids and nitrate content. Moisture content in hop cones was determined with Analytica-EBC (1998) method [1], alpha acid content with Analytica-EBC (2000) [2] method, and nitrate content with DIN/EN (1998) [7] method.

The results obtained for yield, alpha acid content, alpha acid yield and nitrate content were statistically processed in Excel and Statgraphics computer programs, and the differences among treatments were determined with Duncan multiple range test ($p<0.05$) for each year separately (as a block trial), because the experiment was not carried out in the same hop field in both years.

2.3 Soil analysis

The experiment was conducted in a hop field with eutric brown soil on sandy – gravel, middle deep, loam–clay texture. In the upper 25 cm the soil was plentifully supplied with plant available phosphorus (43.0 mg P_2O_5/100 g soil; Al method) and well supplied with plant available potassium (27.4 mg K_2O/100 g soil; Al method)), pH value was 6,4 (pH in KCl).

2.4 Weather conditions

In 2008 season there were 713 mm of precipitation, which is 124 mm more than the long term average (Figure 1). With regard to location and time, precipitation was not evenly distributed. There were lots of showers and storms with hail which occurred already in May. From June to August there was 83% of all precipitation in the growth season. There was only 47 mm of precipitation in May and 228 mm in June (Figure 1). All months were warmer compared to the long term average, except the second decade of June which was colder. In the last decade of June extremely high temperatures for the time of the year were recorded; maximum daily temperatures exceeded 30°C. Average temperatures were higher by 4.6°C compared to the long term average. Warm weather continued in July, but maximum daily temperatures exceeded 30°C four times only.

In 2009 the temperatures were relatively high in May and decreased suddenly at the end of the month (Figure 1). This resulted in non-uniform and long flowering and consequently in non-uniform ripening of Aurora cultivar. There were big differences among hop fields as well as among plants in the same hop field. Even on the same plant at the same time we found cones that were over-mature, immature and mature. Compared to the long term average, more precipitation occurred in June 2009 (174 mm) and at the beginning of July. At the beginning of August the temperatures were relatively high.

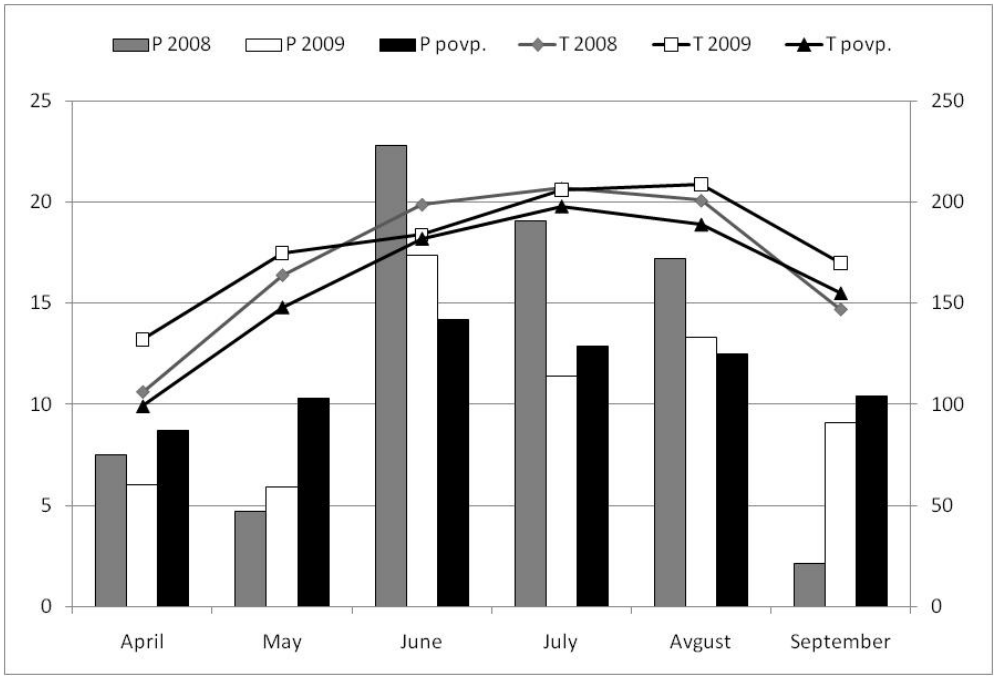

Figure 1: Weather conditions in the growth season of hops in 2008 and 2009 compared to the long term average; P = precipitation amount (mm), P povp. = long term precipitation amount (mm), T = decade average temperature (°C), T povp. = long term average decade temperature (°C)
Slika 1: Vremenske razmere v rastni sezoni hmelja v letih 2008 in 2009 v primerjavi z dolgoletnim povprečjem; P = količina padavin (mm), P povp. = dolgoletna povprečna količina padavin, T = povprečna temperatura dekade (°C), T povp. = dolgoletna povprečna temperatura dekade

2.5 Plant growth and growth stages

Plant height was measured once to twice a week in the time of fast growth. At the same time growth stages were determined with regard to treatment.

2.6 Plant available nitrogen (Nmin) content in soil

Three times in the season (in May – before first N application, at the beginning of July, at harvest) soil was analysed for plant available nitrogen (Nmin; NO_3-N and NH_4-N) (in-house method) in the upper layer of soil (0-25 cm) with regard to treatment (all three replications together).

3 RESULTS AND DISCUSSION

3.1 Plant growth and growth stages

There were no significant differences in the plant growth (Figure 2 and Figure 3) and in the appearance of growth stages in both years. On the other hand, different results were reported for other crops (e.g. in the experiment with asparagus done by Paschold [15] differences in the plant growth were recorded).

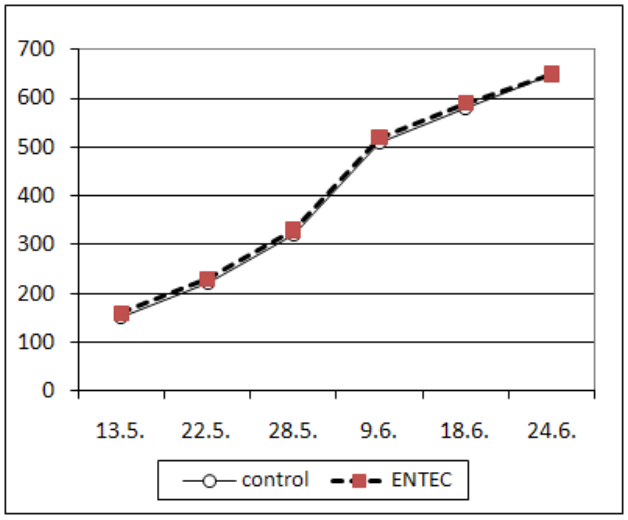

Figure 2: Plant height (cm) with regard to treatment (control, ENTEC) and date in 2008
Slika 2: Višna rastlin (cm) glede na obravnavanje (control, ENTEC) in datum meritve v letu 2008

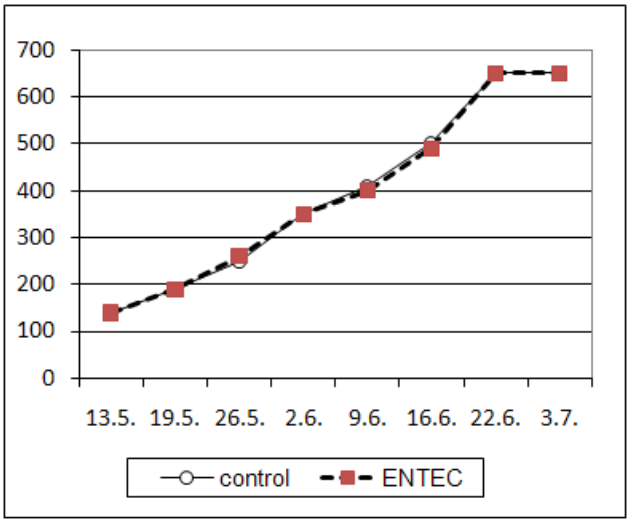

Figure 3: Plant height (cm) with regard to treatment (control, ENTEC) and date in 2009
Slika 3: Višna rastlin (cm) glede na obravnavanje (control, ENTEC) in datum meritve v letu 2009

3.2 Plant available N (Nmin) content in the soil

Nmin (NO_3^- - N and NH_4^+ - N) in the upper layer of the soil (0-25 cm) was between 20 and 40 kg/ha N in May 2008 (Figure 4). At the beginning of July there was an indication that Nmin in the soil was comparable between treatments, but after the harvest a little higher Nmin was detected at treatment with ENTEC compared to control treatment. The results obtained are in

agreement with the experiment done with asparagus where the differences in the supply of nitrogen were detected [15].

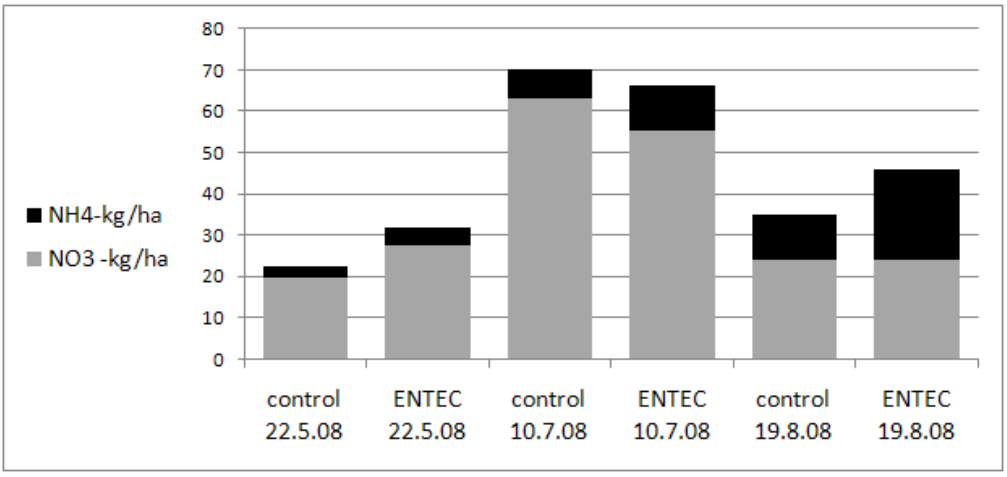

Figure 4: Nmin (NO_3-N and NH_4-N) quantity (kg/ha) in the upper layer of the soil (0-25 cm) with regard to sampling date (22.5.2008, 10.7.2008, 19.8.2008) and treatment (control, ENTEC) in 2008
Slika 4: Količina rastlinam dostopnega dušika (NO_3-N and NH_4-N; kg/ha) v zgornjih 25 cm tal glede na datum vzorčenja (22.5.2008, 10.7.2008, 19.8.2008) in obravnavanje (control, ENTEC) v letu 2008

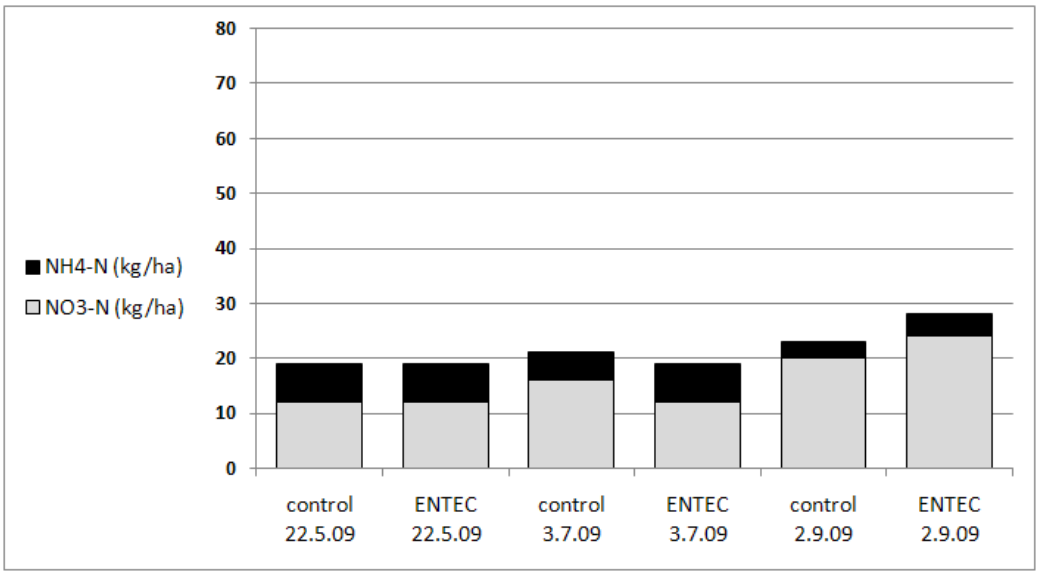

Figure 5: Nmin (NO_3-N and NH_4-N) quantity (kg/ha) in the upper layer of the soil (0-25 cm) with regard to sampling date (22.5.2009, 3.7.2009, 2.9.2009) and treatment (control, ENTEC) in 2009
Slika 5: Količina (kg/ha) rastlinam dostopnega dušika (NO_3-N and NH_4-N, v kg/ha) v zgornjih 25 cm tal glede na datum vzorčenja (22.5.2009, 3.7.2009, 2.9.2009) in obravnavanje (control, ENTEC) v letu 2009

In 2009 Nmin quantity was relatively low at all three samplings (Figure 5). This was probably due to the high amount of precipitation in June (Figure 1), which is the time of fast hop growth and nutrients absorption, and due to the fact that the experiment was carried out in the shallow soil with higher content of skeleton. The differences between the treatments were not recorded.

None of the treatments in both seasons showed higher Nmin quantity in the upper layer of the soil than 50 kg/ha N recorded at harvest.

3.3 Yield, alpha acid content and alpha acid yield

In 2008 there were no significant differences between treatments in the cone yield, alpha acid content and alpha acid yield (Table 1).

Table 1: Cone yield (dry matter - DM) per ha, per plant and per string, alpha acid content in cones (% in DM), alpha acid yield per ha, per plant and per string in 2008
Preglednica 1: Pridelek (kg suhe snovi) storžkov na hektar, na rastlino in na vodilo, vsebnost alfa kislin (% v suhi snovi) in pridelek alfa kislin (kg) v letu 2008

Treatment	Yield (kg/ha)	Yield (kg/plant)	Yield (kg/string)	Alpha acid (% in DM)	Alpha acid yield (kg/ha)	Alpha acid yield (kg/string)	Alpha acid yield (kg/plant)
Control	1771 a*	0.64 a	0.33 a	10.1 a	180 a	0.034 a	0.065 a
ENTEC	1839 a	0.65 a	0.32 a	9.4 a	173 a	0.030 a	0.061 a

** The same letter in a column indicates that there is no significant difference between treatments according to Duncan multiple test (p=0.05)*

Table 2: Cone yield (dry matter - DM) per ha, per plant and per string, alpha acid content in cones (%), alpha acid yield per ha, per plant and per string in 2009
Preglednica 2: Pridelek (kg suhe snovi) storžkov na hektar, na rastlino in na vodilo, vsebnost alfa kislin (% v suhi snovi) in pridelek alfa kislin (kg) v letu 2009

Treatment	Yield (kg/ha)	Yield (kg/plant)	Yield (kg/string)	Alpha acid (% in DM)	Alpha acid yield (kg/ha)	Alpha acid yield (kg/string)	Alpha acid yield (kg/plant)
Control	1527 a*	0.47 a	0.20 a	8.4 a	128 a	0.016 a	0.040 a
ENTEC	1376 a	0.45 a	0.19 a	7.8 a	107 a	0.015 a	0.035 a

** The same letter in a column indicates that there is no significant difference between treatments according to Duncan multiple test (p=0.05)*

Although lower yield, alpha acid content and alpha acid yield was recorded in 2009 at ENTEC treatment compared to the control (Table 2), the differences could not be statistically confirmed.

Lower yield was recorded in 2009 compared to the previous season probably due to unfavourable weather conditions. High amount of precipitation in June was the cause for less nutrients available in lighter soil in the time of the fastest growth and development; warm May with cooling caused uneven flowering, hot beginning of August had a negative effect on the alpha acid formation.

3.4 Nitrate content in hop cones

In both investigated years there were no significant differences in nitrate content in hop cones between treatments (Table 3). In comparison with 2008, lower nitrate content in 2009 was probably due to weather conditions.

Table 3: Nitrate content in hop cones (mg/100 g DM) with regard to treatment (control, ENTEC) in field experiment in 2008 and 2009
Preglednica 3: Vsebnost nitratov v storžkih (mg/100 g suhe snovi) glede na obravnavanje (control, ENTEC) v poskusu v letih 2008 in 2009

	Nitrate content (mg NO_3^-/100 g DM)	
year	2008	2009
Control	1160 a*	599 a
ENTEC	1098 a	775 a

** The same letter in a column indicates that there is no significant difference between treatments according to Duncan multiple test (p=0.05)*

4 CONCLUSION

It was found that the results of Aurora hop cultivar fertilization with Entec 26 in one N split were comparable to conventional fertilization with KAN in three splits as far as the yield, alpha acid content, alpha acid yield and nitrate content in hop cones in seasons 2008 and 2009 are concerned. The result is positive because there was one crossing of the field to perform N fertilization in the case of Entec 26 compared to three crossings in the case of control.

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TREATED MINERAL WATER IN PRODUCTION OF HOPS

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ABSTRACT

The aim of research was to investigate plant growth and development, yield, alpha acid and nitrate content in hop cones, alpha acid yield, and to make observations on pests and hop diseases if treated mineral water (‘*Mineral’*) is included in hop production. Treatments differed in ‘*Mineral green’* quantity used for watering, in inclusion of plant protection products (PPP), in ‘*Mineral yellow’* spraying and in mineral nitrogen (N) fertilization. At treatments where no mineral N was used, the yield was significantly lower compared to the treatments with conventional N fertilization. If ‘*Mineral’* products were included in conventional production, this resulted in a little higher yield compared to the conventional production, but the differences in yield could not be statistically confirmed. Production of hops only with ‘*Mineral’* watering and spraying, without PPP and mineral fertilizers failed in 2009 because of the outbreak of hop downy mildew – primary infection was strong at the start of the growing season. After Al-fosetyl spraying there was no such need any more and finally hop was produced with reduced PPP application on a large scale, but as a result the yield was also lower.

Key words: hops, *Humulus lupulus* L., treated mineral water, Mineral, yield

OBDELANA MINERALNA VODA V PRIDELAVI HMELJA

IZVLEČEK

Namen raziskave je bil preučiti vpliv vključitve obdelane mineralne vode (pripravka Mineral) v pridelavo hmelja na rast in razvoj rastlin, vsebnost alfa kislin in nitratov v storžkih, pridelek storžkov, pridelek alfa kislin in zdravstveno stanje. Obravnavanja se razlikujejo po vključevanju zalivanja s pripravkom '*Mineralom zeleni'*, vključevanju škropljenja s sredstvi za varstvo rastlin (FFS), škropljenju z '*Mineralom rumeni'* in dognojevanju z N. V primerjavi s konvencionalnim dognojevanjem z dušikom je bil pridelek značilno manjši pri obravnavanjih, kjer nismo dognojevali z N. Če smo v konvencionalno pridelavo vključili zalivanje in škropljenje s pripravkoma '*Mineral'*, se je nakazal večji pridelek, vendar razlike niso bile statistično značilne. Poskus ekološke pridelave hmelja cv. Celeia (brez uporabe mineralnih gnojil in FFS) z zalivanjem in škropljenjem s pripravkoma '*Mineral*' v vremenskih razmerah leta 2009 ni popolnoma uspel, saj smo morali dvakrat škropiti s pripravkom na osnovi Al-fosetil. Ostalemu škropljenju s FFS smo se sicer lahko izognili, vendar se je to odrazilo v zmanjšanju pridelka.

Ključne besede: hmelj, *Humulus lupulus* L., obdelana mineralna voda, Mineral, pridelek

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1 INTRODUCTION

Each product has to be practically tested to be approved for wider use. In the investigation Slovenian products '*Mineral green*' and '*Mineral yellow'* – treated mineral water produced by Planet of Health [10] – were tested in different combinations in hop production.

The investigation continued from the previous years [3,4]. In 2008 ecological production of Celeia hop cultivar with '*Mineral'* was estimated as promising. Even though no mineral fertilizers and plant protection products (PPP) were used, the yield of hop cones was around 1200 kg/ha dry matter (DM). Health condition of hops was satisfactory with only '*Mineral'* products used (watering with '*Mineral green'* 320 l/ha in autumn + 50 l/ha in May + 25 l/ha in June) and spraying with '*Mineral yellow*' eight times in the season). But, the results of one year are not conclusive enough to guarantee that this would happen every year because the weather conditions in 2008 were not favourable for the population of spider mite. It could be concluded at that time that the use of '*Mineral'* products has either a positive effect on the natural resistance of hop plants, or causes the natural enemies trying to hold pests back to express themselves even more. An effective combination of '*Mineral'* use in conventional production of Celeia hop cultivar was not determined in 2008. It was decided that the experiment should continue in the years to follow [3,4].

Plant growth and development, yield, alpha acid content, nitrate content in hop cones and alpha acid yield were determined and observations on pests and hop diseases were studied in 2009 to determine an appropriate combination of '*Mineral'* products to be included in conventional hop production and to try to produce hops without PPP and mineral fertilizers and only with the use of '*Mineral'* products.

2 MATERIAL AND METHODS

2.1 Material

The investigated hop cultivar was Celeia – an aromatic, late cultivar with technologic maturity from 6 September – 12 September. Expected yield is 1100 – 3200 kg/ha, alpha acid content in hop cones is between 3.0 % and 8.7 %. It is medium resistant to downy mildew, powdery mildew and gray mould, and susceptible to verticillium hop wilt [8].

'*Mineral*' products are prepared from salted mineral water which is processed with a special method. When mixed in a ratio 1:100 with fresh water it can be used as a natural fertilizer for plants with required 75 minerals for growth. A greater level of soil humidity and lower need for watering are expected. Plants should become more vital and therefore less susceptible to pests and diseases, grow and develop faster and their quality should be enhanced. '*Mineral green'* is used for watering, '*Mineral yellow'* for spraying. The difference between the products is in the way of preparation and treatment of mineral water respectively, so a different main function of product's activity is emphasized [3,4,9].

2.2 Field experiment and evaluation

The experiment was conducted in 2007 as a block trial with five fertilization variants in three replications in drip irrigated experimental field of Slovenian Institute of Hop Research and

Brewing. The size of one plot was 200 m^2, so the area under experiment was approximately 3000 m^2 in size, and in 2008 and 2009 the field experiment continued on the same location. In this paper results of 2009 are presented. Treatments were adjusted according to the previous findings [4] and were in 2009:

1 = Control –fertilization with P and K according to soil analysis, conventional N fertilization (50 + 70 + 50 kg/ha N), no foliar fertilization, spraying with plant protection products (PPP) according to the spraying program (Table 1).

2 = No fertilization with fertilizers that include P, K and N, watering with 50 l/ha '*Mineral green'* at the end of May, spraying with PPP according to the spraying program, spraying with '*Mineral yellow'* four times in the season (BBCH 25, BBCH 37-38, BBCH 61, BBCH 71-75 (Table 1)).

3 = No fertilization with fertilizers that include P, K and N, watering with 50 l/ha '*Mineral green'* at the end of May and 25 l/ha in the last decade of June, reduced spraying with PPP (only with products based on Al-fosetyl on 15 and 25 May to suppress downy mildew primary infection), spraying with '*Mineral yellow'* every 14 days, starting straight after winding up sprouts on strings (BBCH 25).

4 = No fertilization with fertilizers that include P and K, conventional fertilization with N (50 + 70 + 50 kg/ha N), watering with 50 l/ha '*Mineral green'* at the end of May, spraying with PPP according to the spraying program, spraying with Mineral yellow four times in the season (BBCH 25, BBCH 37-38, BBCH 61, BBCH 71-75).

5 = No fertilization with fertilizers that include P, K and N, watering with 50 l/ha '*Mineral green'* at the end of May, reduced spraying with PPP (only with products based on Al-fosetyl on 15 and 25 May to suppress downy mildew primary infection), spraying with '*Mineral yellow'* every 14 days, starting straight after winding up sprouts on strings (BBCH 25).

The rest of the agrotechnique was the same for all plots and performed in accordance with the good agricultural practice. '*Mineral yellow'* was used separately, not mixed with PPP.

In comparison with the treatments in 2009, the differences in 2008 season were:

- In the autumn of 2007 plots of treatments 2, 3, 4 and 5 were watered with '*Mineral green'* in the quantity of 320 l/ha.
- Treatments 3 and 4 were done without the use of PPP during the whole season.
- Each spraying with '*Mineral yellow'* was performed a day after the spraying with PPP (treatments 2 and 4).

Fifteen plots of hops were mechanically harvested one after another at the time of technologic maturity (11 September 2009). Before the harvest outer rows were removed and two inner rows were evaluated. Final plots were measured and the number of plants and strings per plot was counted. The yield was weighed plot by plot. Samples of cones were taken from each plot for analysis of alpha acid, nitrate and moisture content. Moisture content in hop cones was measured according to Analytica-EBC (1998) [1], alpha acid content according to Analytica-EBC (2000) [2], nitrate content according to DIN/EN (1998) [5].

Table 1: Dates of applied plant protection products and '*Mineral'* used in the experiment in 2009 with regard to treatment
Preglednica 1: Datumi uporabe fitofarmacevtskih sredstev in Minerala v poskusu v letu 2009 glede na obravnavanje

Application time	Performed at treatments	Product/active ingredient	Dose per ha**
12.5.09	2, 3, 4, 5	Mineral green	1:100
15.5.09	1, 2, 3, 4, 5	Aliette flash (Al-fosetyl)	2.5 kg/ha
25.5.09	1, 2, 3, 4, 5	Aliette flash (Al-fosetyl)	5 kg/ha
1.6.09	3, 5	Mineral yellow	1:100
12.6.09	1, 2, 4	Teppeki (flonicamid), Vertimec 1.8 % EC (abamectin), Cuprablau – Z ultra (copper hydroxide)	0.18 kg/ha 1.25 l/ha 3 kg/ha
15.6.09	2, 3, 4, 5	Mineral yellow	1:100 (Mineral:water)
3.7.09	1, 2, 4	Delan 700 WG (dithianon)	1.2 kg/ha
4.7.09	2, 3, 4, 5	Mineral yellow	1:100
20.7.09	1, 2, 4	Folpan 80 WDG (folpet), Nissorun 10 WP (hexythiazox), Pepelin (sulphur)	3 kg/ha 1 kg/ha 3 kg/ha
20.7.09	2, 3, 4, 5	Mineral yellow	1:100
30.7.09	1, 2, 4	Cuprablau – Z ultra (copper hydroxide), Pepelin (sulphur), Systhane 12 E (myclobutanil), Ortus 5 SC (fenpyroximate), Karate CS (lambda- cyhalothrin)	6 kg/ha 3 kg/ha 1.2 l/ha 2.4 l/ha 0.25 l/ha
30.7.09	3, 5	Mineral yellow	1:100
17.8.09	1, 2, 4	Zato 50 WG (trifloxystrobin), Vertimec 1.8 % EC (abamectin), Cuprablau – Z ultra (copper hydroxide), Pepelin (sulphur)	0.6 kg/ha 1.25 l/ha 6 kg/ha 3 kg/ha
17.8.09	3, 5	Mineral yellow	1:100

**Water consumption is 1000 l/ha. ** Poraba vode je 1000 l/ha

2.3 Soil analysis and Nmin content in soil

After the harvest in 2008 the soil was supplied with excessive amounts of phosphorus and with adequate to medium amounts of potassium (Al method) (Table 2). After the harvest in 2009 the soil was analysed for plant available nitrogen (Nmin) in the upper layer of soil (0-25 cm) with regard to treatment.

Table 2: Plant available P and K quantity and soil pH after harvest in 2008 with regard to treatment
Preglednica 2: Rastlinam dostopni P in K v tleh ter vrednost pH po obiranju v letu 2008 glede na obravnavanje

	pH in KCl	P_2O_5 (mg/100 g soil)	K_2O (mg/100 g soil)	Organic matter (%)
1	5,4	32 D	22 C	2,1
2	5,3	30 D	18 B	2,2
3	5,3	33 D	20 C	2,2
4	5,0	29 D	18 B	2,1
5	5,4	28 D	19 B	2,1

*Letters next to the numbers indicate nutrient supply class; C = adequate supply, D = excessive supply [6]
*Črke ob številkah označujejo razred oskrbljenosti z določenim hranilom; C = dobro preskrbljena, D = pretirano oskrbljena [6]

2.4 Weather conditions

In 2009 the temperatures were relatively high in May and dropped suddenly at the end of the month. Compared to the long term average, more precipitation occurred in June 2009 (174 mm) and at the beginning of July. At the beginning of August the temperatures were relatively high (Figure 1).

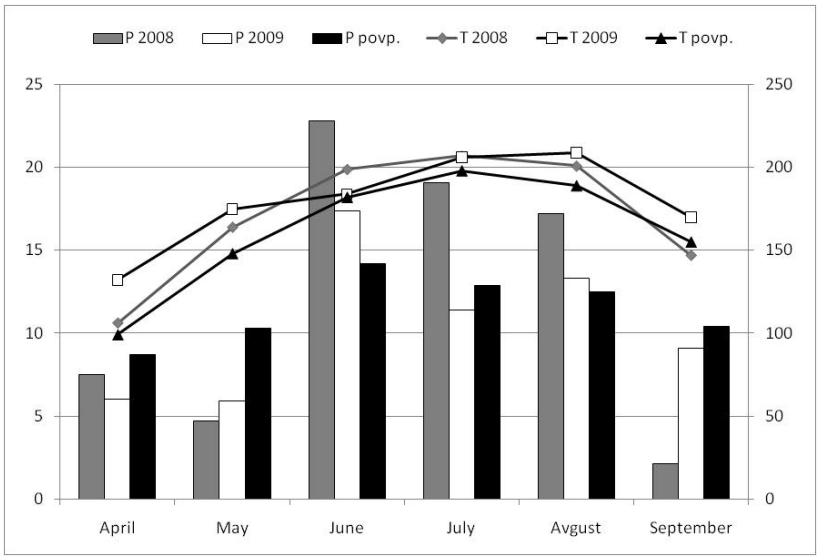

Figure 1: Weather conditions in the hop growth season in 2008 and 2009 compared to the long term average; P = precipitation amount (mm), T = decade average temperature (^{o}C)
Slika 1: Vremenske razmere v rastni sezoni hmelja v letih 2008 in 2009 v primerjavi z dolgoletnim povprečjem; P = količina padavin (mm), T = povprečna temperatura dekade

2.5 Plant growth, growth stages and assessment of pests and diseases

Plant growth was measured once to twice weekly with regard to fertilization variant. At the same time the growth stages were recorded. On each plot leaves were randomly selected at three different heights of hop plants in the same amount: leaves from the bottom of plant (0-2 m), leaves from the middle part (2-4 m) and from the upper part (4-6 m) on 11 June 2009, 3 July 2009 and 20 August 2009. Leaves were examined under a stereoscope. Spider mites (*Tetranychus urticae*) - separately mobile ones and eggs - and hop damson aphids (*Phorodon humuli*) were counted.

After the harvest 400 cones were randomly selected for each sample (plot) and infection was determined in the laboratory. Estimation of infection with downy mildew (*Pseudoperonospora humuli*), powdery mildew (*Sphaerotheca humuli* (*macularis*)), gray mould (*Botrytis cinerea*) and spider mite (*Tetranychus urticae*) was made for each cone. Downy mildew and powdery mildew were assessed on a scale of 0-4 (0 = no presence of disease, 1 = infection up to 1 %, 2 = infection from 1-5 %, 3= infection from 5-20 % and 4 = more than 20 % of infected cones). The infection percentage index was calculated using the formula of Townsend-Heuberger [7] .

3 RESULTS AND DISCUSSION

3.1 Plant growth and growth stages

There were no significant differences in plant growth among treatments in 2009 (Figure 2). There were no differences among treatments at the beginning of growth stages. At the beginning of June there were longer side sprouts at treatment 4 compared to other treatments.

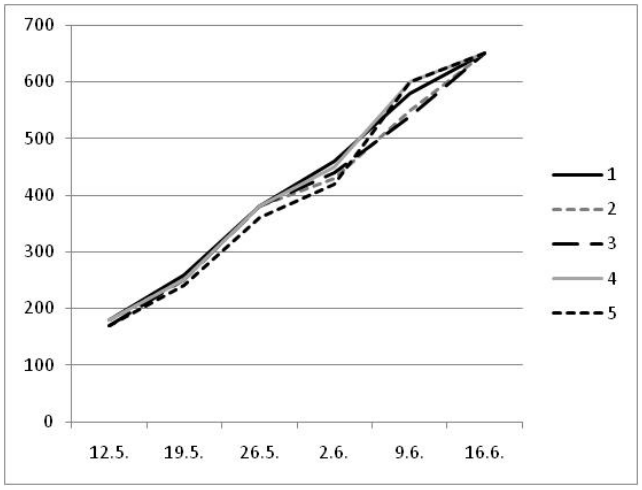

Figure 2: Plant growth with regard to treatment (1, 2, 3, 4, 5) in 2009
Slika 2: Rast rastlin glede na obravnavanje (1, 2, 3, 4, 5) v letu 2009

3.2 Nmin content in soil after harvest

There was low content of plant available N in the upper layer of the soil after the harvest (Table 3), which was probably due to the fact that the experiment was carried out on shallow soil and high precipitation quantity in June, at the time of fast growth and development of hop plants.

Table 3: Nmin content in soil (0-25 cm) after harvest in 2009 with regard to treatment
Preglednica 3: Rezultati Nmin analize zgornjega sloja tal (0-25 cm) glede na obravnavanje v letu 2009

Treatment	NO_3 – N (kg/ha)	NH_4 – N (kg/ha)	Total plant available N (kg/ha)
1	4	5	9
2	0	4	4
3	0	3	3
4	0	4	4
5	0	3	3

3.3 Plant health

3.3.1 Pests on hop leaves

At the beginning of the season, on 11 June, aphid population was similar on the parcels with treatment 1 (conventional production) and treatment 5 (reduced PPP use) (Table 4, Figure 3).

Later, after spraying with systemic insecticide (active ingredients imidacloprid and abamectin) in treatment 1, aphid population on these parcels was reduced (3 July).

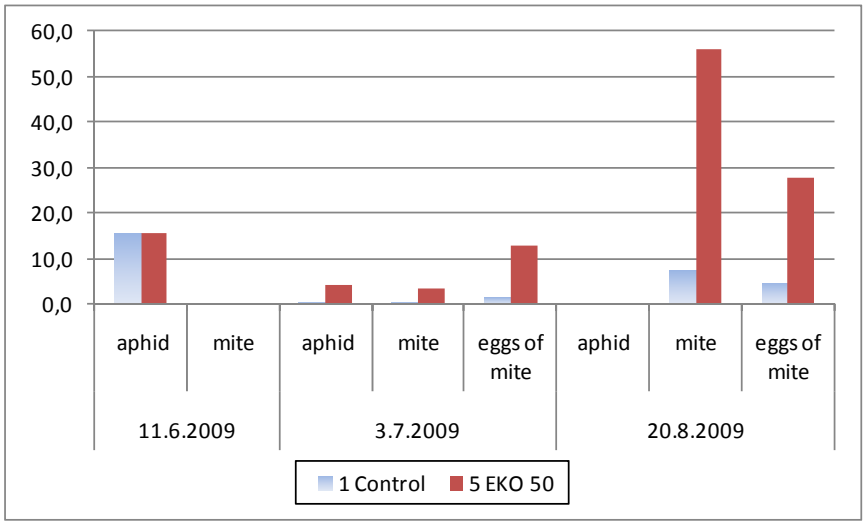

Figure 3: Average number of hop damson aphids, mobile spider mites and their eggs per leaf on different dates of assessment at treatments 1 and 5
Slika 3: Povprečno število hmeljeve listne uši, navadne pršice in jajčec navadne pršice na listih hmelja v različnih ocenjevalnih obdobjih pri obravnavanjih 1 in 5

On plots with reduced PPP use (treatment 5) spider mite (mobile stages and eggs) was present in large number (Table 4, Figure 3) and caused damage to leaves and cones. Spider mite pressure was also high on the parcels with treatment 1 (average 7.3 mites per leaf) where acaricides were used (Table 4, Figure 3). But, the difference in spider mite population among treatments where acaricides were used and not used according to the spraying program was high.

Table 4: The average number of hop damson aphids and mobile spider mites with their eggs per leaf on different dates of assessment
Preglednica 4: Povprečno število hmeljeve listne uši, gibljivih stadijev in jajčec navadne pršice na listu hmelja ocenjeno v različnih terminih

Treat. No.	11.6.2009		3.7.2009			20.8.2009		
	Aphid	Spider mite	Aphid	Spider mite	Eggs of mite	Aphid	Spider mite	Eggs of mite
1	15,64 a	0,00	0,02 a	0,42 a	1,54 a	0,00	7,30 a	4,50 a
5	15,75 a	0,00	4,04 b	3,54 b	12,72 b	0,00	56,00 b	27,70 b

[a,b] Identical letters indicate no significant difference between group means with regard to Duncan multiple test (p=0.05)
[a,b] Skupine z enako črko v indeksu znotraj stolpcev pri posameznih obravnavanjih se med seboj statistično značilno ne razlikujejo (p=0,05)

3.3.2 Disease presence on cones at harvest

At harvest, infection with downy mildew was detected at all treatments, slightly higher at treatments 2 and 5. Powdery mildew was the least present on plots with treatment 1 and the most with treatment 5 (Table 5, Figure 4).

Table 5: The share of cones attacked by diseases (in %) at harvest, 11 September 2009
Preglednica 5: Odstotek napadenosti storžkov z boleznimi v času tehnološke zrelosti hmelja (11.9.2009)

Treatment	Downy mildew*	Powdery mildew*
1	0.50[a]	0.06[a]
2	2.16[b]	0.56[b]
3	0.56[a]	0.50[b]
4	0.13[a]	0.75[b]
5	1.13[a]	0.94[b]

*Identical letter indicates that there is no significant difference between group means with regard to Duncan multiple test (p=0.05)
*Skupine z enako črko v indeksu znotraj stolpcev pri posameznih obravnavanjih se med seboj statistično značilno ne razlikujejo (Duncanov test mnogoterih primerjav, p=0.05)

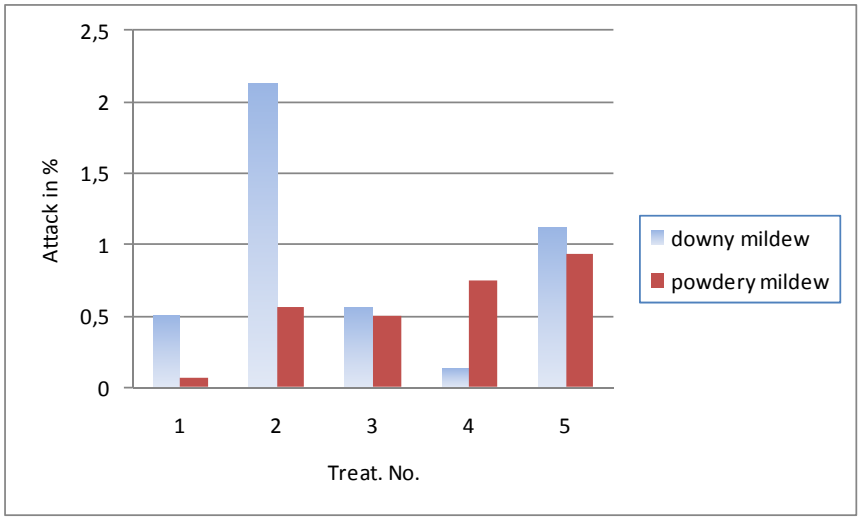

Figure 4: Cones attacked by disease at harvest, 11 September 2009
Slika 4: Odstotek napada storžkov hmelja z boleznimi v času tehnološke zrelosti, 11. september 2009

3.4 Yield, alpha acid and nitrate content in hop cones and alpha acid yield

At treatments where no mineral N was used (treatments 2, 3 and 5), cone yield and alpha acid yield were significantly lower compared to treatments 1 and 4. At treatment 4 where Mineral was included in conventional production higher yield was recorded compared to control (conventional production), but the differences in yield could not be statistically confirmed (Table 6, Figure 5).

If treatments 2 and 5 are compared (in both cases no N fertilization was included, 50 l/ha Mineral at the end of May was applied, the difference was in the use of PPP and '*Mineral'* spraying) it was observed that if PPP were not used (except Al-fosetyl at the beginning of the season) the yield decreased by 171 kg/ha although the difference could not be statistically confirmed.

Table 6: Cone yield, alpha acid content and alpha acid yield in the experiment in 2009
Preglednica 6: Pridelek storžkov (kg suhe snovi), vsebnost alfa kislin v storžkih ter pridelek alfa kislin v poskusu v letu 2009

	Yield (kg/ha DM)	Yield (kg/plant DM)	Yield (kg/string DM)	Alpha acid content (% DM)	Alpha acid yield (kg/ha)	Alpha acid yield (kg/string)	Alpha acid yield (kg/ plant)	Nitrate content in hop cones (mg/100 g DM)
1	1883 a*	0.69 a	0.29 a	3.7 a	70 a	0.011 a	0.025 a	523 a
2	1248 b	0.39 b	0.17 b	3.4 a	42 c	0.006 c	0.013 c	18 b
3	1292 b	0.46 b	0.18 b	3.5 a	47 bc	0.007 bc	0.016 bc	9 b
4	1971 a	0.69 a	0.30 a	3.2 a	64 ab	0.010 ab	0.023 ab	527 a
5	1077 b	0.35 b	0.16 b	3.5 a	38 c	0.006 c	0.012 c	25 b

*The same letter in a column indicates that there is no significant difference between treatments with regard to Duncan multiple test (p=0.05)
*Enaka črka v stolpcu pomeni, da med obravnavanjema ni statistično značilne razlike (Duncanov test mnogoterih primerjav, p=0,05)

When we compared treatments 3 and 5, we observed that additional 25 l/ha of Mineral compared to 50 l/ha had a positive effect on the hop yield (by 215 kg/ha) in the production where the use of mineral N fertilization and PPP tried to be avoided although the differences in the yield could not be statistically confirmed (Table 6).

There were no significant differences among treatments in alpha acid content in hop cones (Table 6). There were also no significant differences in nitrate content among treatments where fertilization with N was performed (treatments 1 and 4) and where fertilization without N was performed (treatments 2, 3 and 5; Table 6).

4 CONCLUSIONS

It was found that if we want to achieve the expected yield in the season with high precipitation in June, which is the time of the fastest growth, nitrogen fertilization is obligatory on the investigated type of the soil. A significantly lower yield was achieved with treatments where no mineral N was applied during the growth season. In spite of watering and spraying with '*Mineral'* the yield decreased by 635 kg/ha in the absence of N fertilization, namely. The same was established for the previous season (2008) with high precipitation in July [4] although not at such extent.

While in 2008 no positive effect of '*Mineral'* inclusion (watering and spraying) on the hop yield and alpha acid yield was recorded in conventional production of Celeia cultivar, it was established in 2009 that '*Mineral'* inclusion had a positive effect on the yield (88 kg/ha dry matter of hop cones), but the differences could not be statistically confirmed.

Production of hops only with '*Mineral'* watering and spraying, without PPP and mineral fertilizers failed in 2009 because of the outbreak of hop downy mildew – primary infection was strong at the start of the growing season. Lots of spikes appeared at the beginning of May, so it was estimated that at the time further production would probably not be possible without PPP use. After Al-fosetyl spraying there was no such need any more and finally hop was produced with reduced PPP application on a large scale, but as a result the yield was also

lower. Further investigations into the reduced PPP use and its effect on plants will continue in the next season.

If pest pressure is too high (depending on weather conditions and other factors in a particular year), only the use of '*Mineral'* products is not enough. When the use of PPP is tried to be avoided, observations should be performed on a regular basis and swift actions (application of PPP) should be taken in case of higher pest pressure.

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POTASSIUM AND PROLINE CONTENT IN HOP LEAVES AS BIOCHEMICAL MARKER FOR DROUGHT STRESS TOLERANCE

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ABSTRACT

In the investigation biochemical markers which could serve as drought stress tolerance pointers were measured. Potassium and proline content in hop leaves of different hop varieties (four Slovenian, two German and one South African hop varieties, wild hop labelled JUG2 and hybrid 279/122) at different water regimes were observed. The investigation showed biochemical markers that will contribute to faster selection of drought stress tolerant hybrids and the method of testing. Hop plants had higher potassium content in leaves under the drought stress conditions compared to adequate water supply, but the values differed from variety to variety. From July to August proline content in leaves decreased by more than 80 % when plants were watered regularly while it increased in the leaves of plants that were exposed to water stress. Preliminary results can be a basis for further investigations since no clear boundary was detected among the varieties.

Key words: hop, *Humulus lupulus* L., potassium content, proline content, drought stress

VSEBNOST KALIJA IN PROLINA V LISTIH HMELJA KOT BIOKEMIČNIH MARKERJEV TOLERANTNOSTI NA SUŠNI STRES

IZVLEČEK

V raziskavi smo preučevali biokemične markerje, ki bi lahko služili kot pokazatelji odpornosti oziroma občutljivosti hmelja na sušni stres. Analizirali smo vsebnost kalija in prolina v listih različnih sort hmelja pri različnih vodnih režimih. Vključene bo bile štiri slovenske sorte hmelja, dve nemški, ena južnoafriška, divji hmelj z oznako JUG2 in križanec 279/122. Raziskava je nakazala biokemične markerje, ki bi lahko pripomogli k hitrejši selekciji na sušo bolj tolerantnih križancev in razvoju metode testiranja. Hmelj je imel namreč večjo vsebnost kalija v listih v razmerah sušnega stresa v primerjavi z ustrezno preskrbo z vodo, vendar so bile vrednosti različne glede na sorto. Od julija do avgusta se je vsebnost prolina v listih zmanjšala za več kot 80 % pri redno zalivanih rastlinah, medtem ko se je v listih rastlin, izpostavljenih sušnemu stresu, povečala. Predstavljeni preliminarni rezultati služijo kot osnova za nadaljnje raziskave, saj niso bile nakazane jasne razlike med sortami.

Ključne besede: hmelj, *Humulus lupulus* L., vsebnost kalija, vsebnost prolina, sušni stres

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1 INTRODUCTION

Hop presents an important export material for Slovenia as about 95 % of the yield is sold on the global market. In the changing climatic conditions with frequent drought it is important to plant drough tolerant varieties which give yields with solid quality in the years with different water supply.

Environmental stresses present the most limiting factors in agricultural productivity. Apart from biotic stress caused by plant pathogens, there are a number of abiotic stresses such as extreme temperatures, drought, salinity, heavy metals and radiation which all have detrimental effects on plant growth and yield. However, certain plant species and ecotypes have developed various mechanisms to adapt to such stress conditions. For sustainable agriculture development, future crops should have abiotic stress resistant traits and a mechanism for stress tolerance.

Many plants accumulate organic osmolytes in response to the imposition of environmental stresses that cause cellular dehydration. Proline, which increases proportionately faster than other amino acids in plants under water stress, has been suggested as an evaluating parameter for irrigation scheduling and for selection of drought-resistant varieties of different species [1,2,4,5,6]. Potassium uptake as an index for screening cultivars on drought resistance was also reported [10].

Harmful effect of drought stress can also be alleviated with potassium fertilization. In plant cells potassium has an affect on osmosis and probably changes pH of the stroma in chloroplast. Increased potassium content in cells increases their osmotic potential, which leads to increased acceptance of water from the companion cells and to changes of turgor [9].

Despite the fact that each hop producing country has its own breeding program, so far tolerance to abiotic stress as a main breeding goal has not been included to such an extent as tolerances to biotic stresses (diseases, pests). Some studies in the field of hop plant response to unpleasant climatic conditions have been reported lately [3,12]. It was agreed previously that the depth of active roots and the leaf mass quantity are of extreme importance under drought conditions [7]. The concentration of cell sap, the stomas response and net photosynthesis have already been partly studied in physiological studies [11].

Tolerance of Slovenian hop varieties to drought has mainly been established from practical experiences. Considerable influence of weather conditions on the growth and yield of Savinjski golding variety has been recorded [9], while Atlas variety has shown quite good drought tolerance also in extreme years (own observations). Aurora variety, which is widely grown in Slovenian hop gardens, is well known for its plasticity under different environmental conditions.

Biochemical markers which could serve as drought stress tolerance pointers were determined in the research. Potassium and proline contents were analyzed in the leaves of different hop varieties under different water regimes.

2 MATERIAL AND METHODS

2.1 Material

Hop cultivars included in the research were:
- four Slovenian hop varieties: Aurora, Celeia, Cicero, Dana,
- two German varieties: Hallertauer Merkur, Hallertauer Taurus,
- one South African variety: Southern Star,
- wild hop labelled JUG2 and
- perspective hybrid 279/122.

The varieties were divided into two categories based on preliminary investigations: more tolerant to drought stress (Aurora, Celeia, Southern Star, JUG2) and more sensitive to drought stress (Hallertauer Merkur, Cicero).

2.2 Field and pot trials

Field trial
Five plants of each included hop variety were planted in the field in 2004. They were cultivated according to the good agricultural practice and exposed to natural weather conditions.

Pot trial
Pot trial was conducted in a glasshouse in 2004. Five pots (plants) of each variety listed above were included in the trial. Pots were outside during winter and displaced into the glasshouse in spring. Three different treatments were carried out:
- drought stress simulation (DS): in the middle of July plants were watered for the last time and left without water for a month,
- plants were watered regularly (WR) and
- plants were watered by natural rainfall (WP).

2.3 Sampling

Samples were taken in 2005 and 2006. Hop plant leaves were sampled at three different heights of the plant – lower, middle and upper part (each in the same quantity) until one litre sample was collected. Leaves were sampled in the middle of July (when the plants were last watered at DS treatment – 18 July), in the middle of August (18 August) and in October of 2005. In 2006 sampling was performed based on the experiences from the previous season: in mid-July (18 July), beginning of August (3 August) and mid-August (18 August).

2.4 Chemical analyses

In leaf samples moisture content was determined in general, proline content was analyzed spectrofotometricaly (MKH 14; in-house method) while total amount of potassium was determined with MKH 22 method (in-house method).

2.5 Weather conditions

In 2005 season drought stress was not recorded in the field due to favourable weather conditions (Figure 1). Spring was relatively cold and rainy which retarded hop growth. In May temperatures rose and hop plants grew fast. In the middle of May a sudden cooling with a large amount of precipitation occurred. In spite of a short period of warm weather with enough precipitation in June, cold weather which continued throughout July caused slower growth of hop plants. In August relatively cold and wet weather continued which resulted in lower quality of yield.

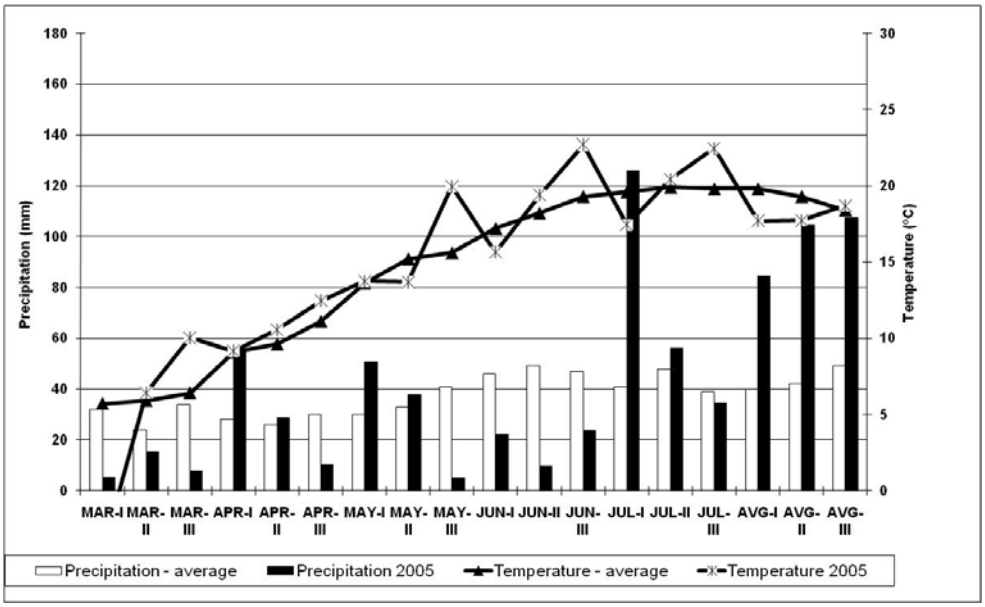

Figure 1: Precipitation amount and average decade's temperatures in growth season of hop in 2005 compared to long term average
Slika 1: Padavine in povprečne dekadne temperature v rastni sezoni hmelja v letu 2005 v primerjavi z dolgoletnim povprečjem

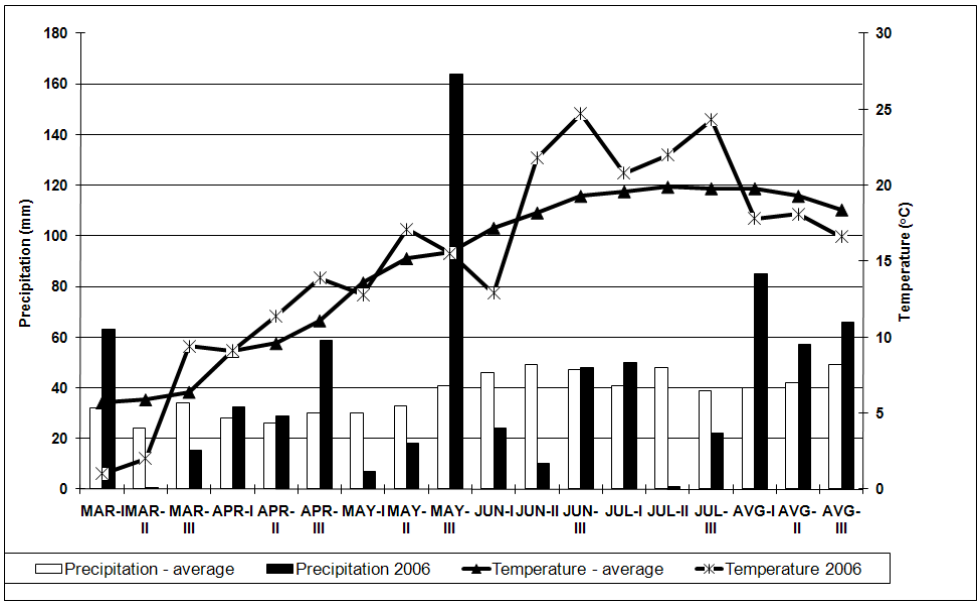

Figure 2: Precipitation amount and average decade's temperatures in growth season of hop in 2006 compared to long term average
Slika 2: Padavine in povprečne dekadne temperature v rastni sezoni hmelja v letu 2006 v primerjavi z dolgoletnim povprečjem

In 2006 season spring was cold and wet (Figure 2). There was a lot of precipitation at the beginning of June. After the warming, hop plants grew faster, but above-average temperatures in the second half of June and in July accompanied by a deficiency of precipitation slowed plant growth again. Plants went faster through the growth stages, photosynthesis was slowed down. After hot and dry July, a relatively cold and wet beginning of August followed.

3 RESULTS AND DISCUSSION

3.1 Proline content in hop leaves

3.1.1 Proline content in hop leaves – pot experiment

At the start of the experiment, in the middle of July 2005, proline content in hop leaves was from 0.90 mg/g dry matter (DM) in wild hop JUG2 to 1.77 mg/g in Southern Star. Hop varieties more sensitive to drought stress (Hallertauer Merkur and Cicero) had medium high proline content in leaves (1.45 mg/g and 1.01 mg/g DM respectively) (Table 1).

Table 1: Proline content in hop leaves (mg/g DM) in pot experiment in 2005
Preglednica 1: Vsebnost prolina v listih hmelja (mg/g suhe snovi) v lončnem poskusu v letu 2005

	18.7.2005	19.8.2005			20.10.2005		
	Initial value	WR*	WP	DS	WR	WP	DS
TOLERANT							
Aurora	1.06	0.24	0.29	8.32	0.22	0.90	0.27
Celeia	1.24	0.18	0.27	4.96	0.48	0.27	0.28
Taurus	1.43	0.09	0.29	4.97	0.26	0.39	0.19
Southern Star	1.77	0.25	0.27	2.77	0.03	0.70	0.11
JUG2	0.90	0.37	0.23	1.66	0.12	0.32	0.13
SENSITIVE							
Merkur	1.54	0.22	0.12	2.60	0.15	0.28	0.17
Cicero	1.01	0.30	0.26	3.30	0.25	0.46	0.21
NEW VARIETY AND HYBRID							
Dana	1.17	0.21	0.24	0.54	0.07	0.29	0.22
279/122	1.53	0.27	0.33	4.75	0.50	0.26	0.25

*WR = watered regularly, WP = watered with regard to precipitation outdoors, DS = drought stress simulation

In one month proline content in leaves decreased by more than 80 % if plants were watered and increased if plants were exposed to drought stress. In varieties more tolerant to drought it increased from 1.6 times in Southern Star to 7.8 times in Aurora. In drought more sensitive varieties Hallertauer Merkur and Cicero it increased by 1.7 and 3.2 times respectively. In hybrid 279/122 proline content increased 3.1-fold. The only exception was Dana variety (Table 1).

In the middle of August the highest proline content in hop leaves was detected in Aurora hop plants which were not watered since mid-July (8.32 mg/g DM). The value was also high in Celeia (4.96 mg/g) and Hallertauer Taurus (4.97 mg/g). Strong response to drought was also recorded in hybrid 279/122.

From mid-August (after we started watering again) to October proline content in the leaves drastically decreased in plants that were exposed to drought for one month in the summer (DS

treatment) in all varieties. As a result of this the values in October were even lower compared to the leaves of plants that were watered in the summer. On the other hand, from mid-August to October proline content in the leaves of hop plants watered regularly in the summer (WR treatment) changed differently from variety to variety.

Next year, in July 2006, the highest proline content in the leaves of the majority of varieties was recorded in plants that were not watered for a month in the previous season – the content was from 0.47mg/g DM in Southern Star leaves to 2.88 mg/g DM in Hallertauer Taurus leaves. After two weeks of experiment the initial value of proline content in hop leaves decreased if plants were watered while in the leaves of non-watered plants proline content varied from variety to variety. There were no detectable differences between tolerant and sensitive varieties (no data provided).

3.1.2 Proline content in hop leaves – field experiment

At the beginning of experiment in July 2005 proline content in hop leaves of different varieties was relatively similar – it varied from 0.23 mg/g to 0.41 mg/g (Table 2). From mid-July to the end of August proline content in hop leaves increased mainly in Aurora (2.5-fold) and Hallertauer Taurus (1.6-fold).

Table 2: Proline content in hop leaves (mg/g DM) in field experiment in 2005 and 2006
Preglednica 2: Vsebnost prolina v listih hmelja (mg/g suhe snovi) v poljskem poskusu v 2005 in 2006

	19.7.05	2.8.05	23.8.05	18.10.05	18.7.06	2.8.06	18.8.06	9.10.06
TOLERANT								
Aurora	0.26	0.64	0.35	0.14	0.35	0.35	0.37	0.16
Celeia	0.37	0.55	0.38	0.24	0.58	0.25	0.24	0.37
Taurus	0.41	0.66	0.58	0.61	0.56	0.46	0.70	0.33
Southern Star	0.32	0.47	0.36	0.38	0.41	0.38	0.39	0.34
JUG2	0.32	0.51	0.40	0.51	0.60	0.57	0.43	0.27
SENSITIVE								
Merkur	0.35	0.44	0.43	0.27	1.25	0.42	0.42	0.40
Cicero	0.31	0.38	0.36	0.28	0.37	0.32	0.33	0.33
NEW VARIETY AND HYBRID								
Dana	0.23	0.38	0.36	0.15	0.34	0.24	0.53	0.20
279/122	0.36	0.53	0.29	0.20	0.56	0.18	0.47	0.14

From the beginning of August to mid August proline content decreased in the leaves of drought more tolerant varieties but remained unchanged in sensitive varieties. Proline content in hybrid 279/122 under the 2005 field conditions was comparable to drought more tolerant varieties, and in Dana more to less tolerant varieties (Table 2).

Mid-July 2006 was hot and dry. Proline content in hop leaves of all varieties was higher compared to the same period in 2005, which was rainy and colder. From mid-July to the beginning of August proline content in the leaves decreased or remained unchanged; no obvious differences among drought more or less tolerant varieties were detected. From the beginning of August to mid-August proline content in leaves remained unchanged in drought less tolerant varieties as in the previous season, but by contrast proline content in drought more tolerant varieties was not the same (Table 2). The response to dry conditions in the field was not as strong as in the pot experiment, or not detectable at all because the roots of hop

plants can grow deep in the soil and absorb water from the lower soil layers therefore water deficits were not that high.

3.2 Potassium content in hop leaves

3.2.1 Potassium content in hop leaves – pot experiment

At the beginning of pot experiment, in mid-July 2005, the lowest potassium content in hop leaves was recorded in Hallertauer Merkur and Cicero, and in drought sensitive varieties (0.50 % and 0.61 % respectively). In drought more tolerant varieties potassium content in leaves was from 0.70 % in Hallertauer Taurus to 1.34 % in Aurora and Southern Star. In hybrid 279/122 it was in the same rank as in drought more tolerant varieties (0.84 %), in a new Slovenian Dana variety it was more similar to drought less tolerant varieties (0.56 %) (Table 3).

Table 3: Potassium content in hop leaves (% of DM) in pot experiment in 2005
Preglednica 3: Vsebnost kalija v listih hmelja (% v suhi snovi) v lončnem poskusu v letu 2005

	18.7.05	19.8.05		20.10.05	
	Initial value	WR*	DS	WR	DS
TOLERANT					
Aurora	1.34	0.57	1.18	0.84	1.55
Celeia	1.06	0.37	1.98	0.96	2.30
Taurus	0.70	0.67	1.48	0.61	2.06
Southern Star	1.34	0.70	1.06	0.77	1.15
JUG2	0.82	0.73	0.53	0.67	0.99
SENSITIVE					
Merkur	0.50	0.78	1.27	0.74	1.44
Cicero	0.61	0.75	1.22	0.86	2.49
NEW VARIETY AND HYBRID					
Dana	0.84	0.48	1.20	0.49	1.45
279/122	0.56	0.65	1.30	1.35	1.42

*WR = watered regularly, DS = drought stress simulation

If plants were watered, potassium content decreased in leaves of drought more tolerant varieties after a month, but increased a bit in drought less tolerant ones (Table 3). The response of Dana cultivar was comparable to tolerant varieties, and the response of 279/122 to drought less tolerant. When plants were left without water, potassium content increased more in leaves of drought less tolerant varieties compared to the plants of the same varieties that were watered. The response of drought more tolerant varieties to no watering conditions was different; in some drought more tolerant varieties (Aurora, Southern Star, JUG2) proline content in leaves decreased, but increased in Celeia and Hallertauer Taurus.

In mid-August the value was higher in the leaves of non-watered plants compared to the watered ones (with exception of JUG2) (Table 3). In drought sensitive varieties Hallertauer Merkur and Cicero potassium content was 1.6-fold higher in the leaves of non-watered plants compared to the leaves of watered plants, and in drought more tolerant varieties from 2.0-fold to 2.6-fold higher. This trend was not recorded in wild JUG2 and Southern Star which showed similar response as drought less tolerant varieties (Table 3).

In the time of regular watering which started in mid-August potassium content in the leaves of plants that were not watered for a whole month increased or remained unchanged until October. Potassium content in leaves of watered plants remained almost unchanged in the majority of varieties and increased in some (Aurora, Celeia and 279/122) (Table 3).

In mid-July of 2006, potassium content in the leaves of plants that were not watered for a month in the previous season was similar or higher compared to the watered ones. The only exception was Southern Star where the value was similar. At that time the experiment started again; we stopped or continued watering the same plants as in the previous season. After two weeks potassium content in the leaves of plants that were not watered increased from 1.1-fold in Hallertauer Taurus to 2.4-fold in Celeia. The only exception was Aurora where potassium content remained unchanged. Among drought less or more tolerant varieties no differences in response of plants regarding potassium content in the leaves were recorded. In the leaves of watered plants potassium content remained unchanged or decreased by more than a half in Southern Star and JUG2. From the beginning to mid-August potassium content in leaves decreased by half in all varieties (data not provided).

3.2.2 Potassium content in hop leaves – field experiment

There was no water deficiency in the field in the summer of 2005. The season was wet with not very high temperatures. In mid-July potassium content in the leaves of drought sensitive varieties was higher (from 1.05 % in Hallertauer Merkur to 1.23 % in Cicero) compared to drought more tolerant varieties (from 0.51 % in Southern Star to 0.93 % in JUG2) (Table 4), which was just the opposite to the pot experiment results (Table 3). The values increased by the beginning of August and exceeded the values of drought sensitive varieties.

Table 4: Potassium content in hop leaves (% in DM) in field experiment in 2005 and 2006
Preglednica 4: Vsebnost kalija v listih hmelja (mg/g suhe snovi) v poljskem poskusu v 2005 in 2006

	19.7.05	2.8.05	23.8.05	18.10.05	18.7.06	2.8.06	18.8.06	9.10.06
TOLERANT								
Aurora	0.60	1.62	0.89	0.33	1.07	1.06	0.69	0.28
Celeia	0.89	1.07	0.72	0.20	1.35	1.08	0.94	0.45
Taurus	0.52	1.78	0.88	0.77	0.69	0.50	0.93	0.36
Southern Star	0.51	1.59	0.87	0.91	0.70	0.46	0.53	0.41
JUG2	0.93	1.01	0.85	0.77	0.63	0.49	0.43	0.38
SENSITIVE								
Merkur	1.05	0.99	1.00	0.72	0.46	0.35	0.56	0.34
Cicero	1.23	0.96	0.96	0.26	1.03	1.07	0.41	0.42
NEW VARIETY AND HYBRID								
Dana	1.22	1.62	0.75	0.47	0.40	0.73	0.52	0.44
279/122	1.41	1.90	0.80	0.96	0.71	0.41	0.53	0.38

From mid-July to the beginning of August potassium content increased most in the leaves of Hallertauer Taurus (to 1.78 %), Southern Star (to 1.59 %) and Aurora (to 1.62 %). From the beginning to mid-August potassium content decreased in drought tolerant varieties and remained unchanged in drought sensitive ones. Genotypes 279/122 and Dana variety showed increased potassium content in leaves as it was detected in drought more tolerant varieties

while initial values (in mid-July) were more comparable to drought sensitive varieties. From mid-August to October the values remained unchanged, or they decreased (Table 4).

Year 2006 was dry with very high temperatures in the second half of July and relatively cold and rainy in the first half of August. Potassium content in the leaves of drought more tolerant varieties was higher compared to the same period of the previous year but lower in drought sensitive varieties. In two weeks when dry weather finally prevailed (mid-July to the beginning of August) no obvious differences were recorded between more or less drought tolerant varieties.

From the beginning of August by October potassium content decreased in the majority of varieties (Table 4).

4 CONCLUSIONS

Higher potassium content in leaves was recorded in hop plants under drought stress conditions compared to adequate water supply, but the values differed from variety to variety. In July, potassium content in the leaves of drought more tolerant varieties was higher in drier season compared to the season with enough precipitation but lower in drought sensitive varieties.

From July to August proline content in leaves decreased by more than 80 % if plants were watered regularly and increased in the leaves of plants that were exposed to water stress. In varieties that are considered to be tolerant to drought stress it increased from 1.6-fold in Southern Star to 7.8-fold in Aurora. In sensitive varieties proline content increased from 1.7-fold in Hallertauer Merkur to 3.2-fold in Cicero (to 2.6 mg/g and to 3.3 mg/g respectively).

Preliminary results can be a basis for further investigations since no clear boundary was detected among the varieties.

The investigation showed biochemical markers that could contribute to faster selection of drought stress tolerant hybrids and the development of selection method, but investigations will continue in the future.

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NUŽNOST RESTRUKTURIRANJA PROIZVODNJE HMELJA KORIŠTENJEM NJEGOVIH LJEKOVITIH OSOBINA U SUVREMENOM PIVARSTVU I FARMAKOLOGIJI

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SAŽETAK

U razdoblju od 1993. do 2008. godine svjetska proizvodnja piva bilježi stalni porast, dok utrošak α-kiselina u istom razdoblju opada. Razlog tome je smanjenje utroška α-kiselina po 1 hL pivske sladovine i smanjenje gorčine piva. Posljedično, smanjenjem količine dodanih α-kiselina po hL pivske sladovine smanjen je i unos ostalih kemijskih spojeva korisnih za ljudsko zdravlje kao što su polifenoli i bioflavonidi, a poglavito unos ksantohumola. Pozitivni učinak ksantohumola i izo-ksantohumola na ljudsko zdravlje dokazan je kliničkim testovima, stoga bi jedan od pravaca restrukturiranja svjetske industrije hmelja mogao ići prema snabdijevanj farmaceutskih tvrtki. Drugi pravac restrukturiranja je postojeći lanac snabdijevanja pivarske industrije. Svjetska i europska pivarska industrija, suočena s aktivnostima anti-alkoholnog lobija trebala bi poboljšati marketinški imidž piva, pri čemu hmeljni ljekoviti sastojci kao što su bioflavonidi povećavaju njegovu dodanu vrijednost. Time se može ostvariti povećanje utroška hmelja i hmeljnih pripravaka.

Ključne riječi: hmelj, alfa-kiseline, bioflavonidi, restrukturiranje proizvodnje hmelja, funkcionalna hrana

NEEDS OF HOP PRODUCTION RESTRUCTURING BY USING MEDICAL CHARACTERISTICS OF HOP IN MODERN BREWING AND PHARMACOLOGY

ABSTRACT

Between 1993 and 2008 world beer production increased constantly. At the same time consumption of α-acids decreased. The reason is manly in decrease of α-acids consumption per 1 hL of beer worth which caused decrease of beer bitterness. Consequently, decrease of α-acids consumption leaded to lower input of other chemical compounds of hops, polyphenols and bioflavonoids, mainly xanthohumol and isoxanthohumol, which positive effects on human health were proven by clinical tests. One direction of world hop restructuring could be established by new supply chain to pharmacy while the second direction is in present supply chain of beer industry. World, particularly European beer industry is faced with major activities of anti-alcoholic lobby so it is necessary to improve the marketing image of beer. However, some medical and chemical characteristics hop such as bioflovonoids could contribute to additional value of beer.

Key words: hops, alpha-acids, bioflavonoids, restructuring of hop production, functional food

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1 UVOD

Iako u današnje vrijeme latinska izreka *„Historia est magistra vitae“* izaziva vrlo često podsmjeh, ostaje činjenica da su korisne osobine hmelja na ljudsko zdravlje prvi puta opisane u knjizi „Phisika“ redovnice Abbess Hildegard od Bingena *(1098 - 1179)* [27,30]. Poznato je da je pivo najstarije alkoholno piće koje datira još od vremena Sumerske civilizacije i Babilona *(4000 g. pr. Kr.)*, a Babilonski kralj Hamurabi *(1729 – 1686. pr. Kr.)* u svojem zakoniku po prvi put u povijesti civilizacija navodi pojam „rok trajnosti piva“, koji je ujedno bio i prvi nutricionistički standard [3,21,22].

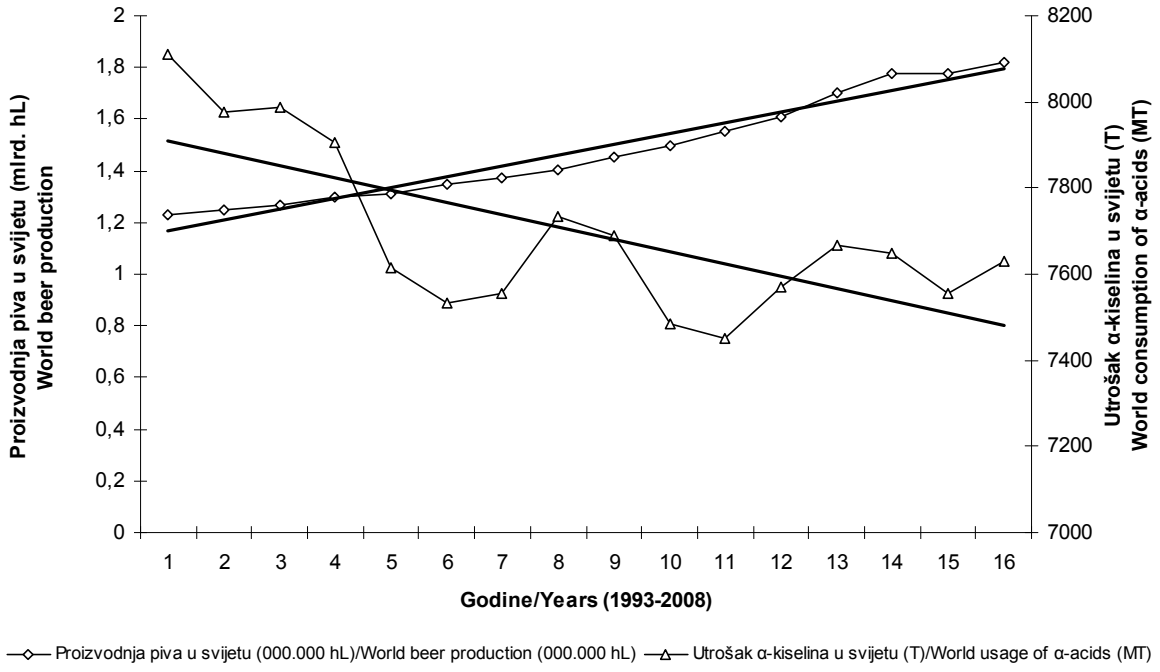
Postupak hmeljenja pivske sladovine otkriven je tek u srednjem vijeku [3], ali prava veza između pivarstva i hmeljarstva uspostavljena je točno 23. 4. 1516. godine, kada je u Ingolstadtu na Godišnjem skupu trgovaca objavljen Zakon o čistoći piva kojeg je donio Bavarski knez Wilhelm IV. U tom zakonu između ostalog stoji slijedeće: „Posebno želimo biti sigurni u to da se u našim gradovima, na našim tržnicama i širom naše zemlje, ništa neće upotrebljavati kao dodatak pivu osim ječma, hmelja i vode“ [27]. Iako je proizvodnja hmelja na području Njemačke bila organizirana još od 1028. godine [5], nakon proglašenja Zakona o čistoći piva razvoj hmeljarstva i pivarstva bio je usko i neraskidivo vezan. Pivo je od tada postalo nezaobilazno piće ne samo u gostionicama i pivnicama, a također je tadašnje pivo zbog svojeg digestivnog i diuretskog djelovanja postalo i nezaobilazno piće "svakodnevne" farmakopeje.

Sve to doprinijelo je da pivo u mnogim zemljama dobije status prehrambenog proizvoda, a ne samo alkoholnog pića. Danas, 493 godine nakon proglašenja Zakona o čistoći piva, predsjednik najmoćnijeg pivarskog svjetskog udruženja, Brewers of Europe, govori o stvaranju tzv. "beer friendly" atmosfere, kao i potrebi jačanja imidža piva (?!) [11]. Znači li to da pivo, u današnjem značenju te riječi, nema više marketinški imidž dobrog pića i prehrambenog proizvoda? Ako je tako, što je tome doprinijelo?

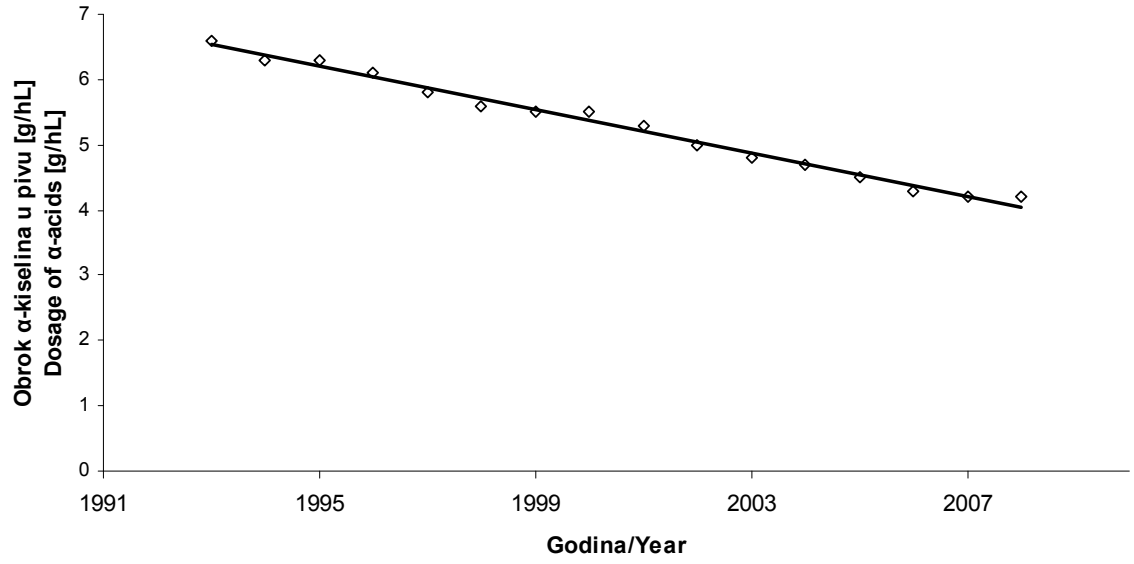
U ovom radu iznijeti će se neki trendovi u proizvodnji hmelja i piva, a vezani uz ljekovite ali i s nutritivno korisne osobine hmelja, koje predstavljaju polazište u promišljanju bilo kakvog restrukturiranja proizvodnje hmelja (ali i piva).

2 TRENDOVI U PROIZVODNJI HMELJA I PIVA U SVIJETU

Iako svi autori u suvremenoj pivarskoj literaturi navode brojne korisne osobine hmelja, kao što su gorčina, antiseptička moć α- i ß-kiselina i aroma piva [4,8,21,22,23] svi ekonomski pokazatelji proizvodnje hmelja govore o padu proizvodnje hmelja [1,2]. Premda u razdoblju od 1993. do 2008. proizvodnja piva bilježi stalni rast, u istom razdoblju opada potrošnja hmelja, izražena kroz utrošak čistih α-kiselina (Slika 1). Pad potrošnje hmelja posljedica je stalnog smanjenja gorčine piva odnosno smanjenja utroška čistih α-kiselina u hmeljnom obroku za hmeljenje 1 hL pivske sladovine (Slika 2).

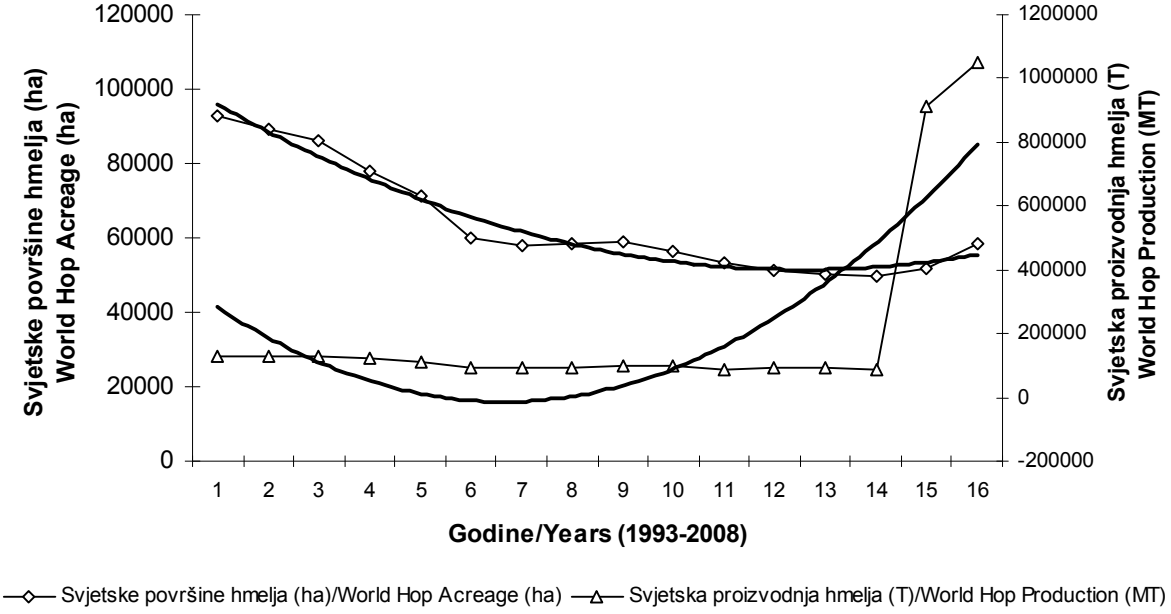


Slika 1: Rast svjetske proizvodnje piva i smanjenje utroška α-kiselina u razdoblju od 1993 do 2008
Figure 1: Increase of beer production and decrease of α-acids consumption in the world from 1993 to 2008



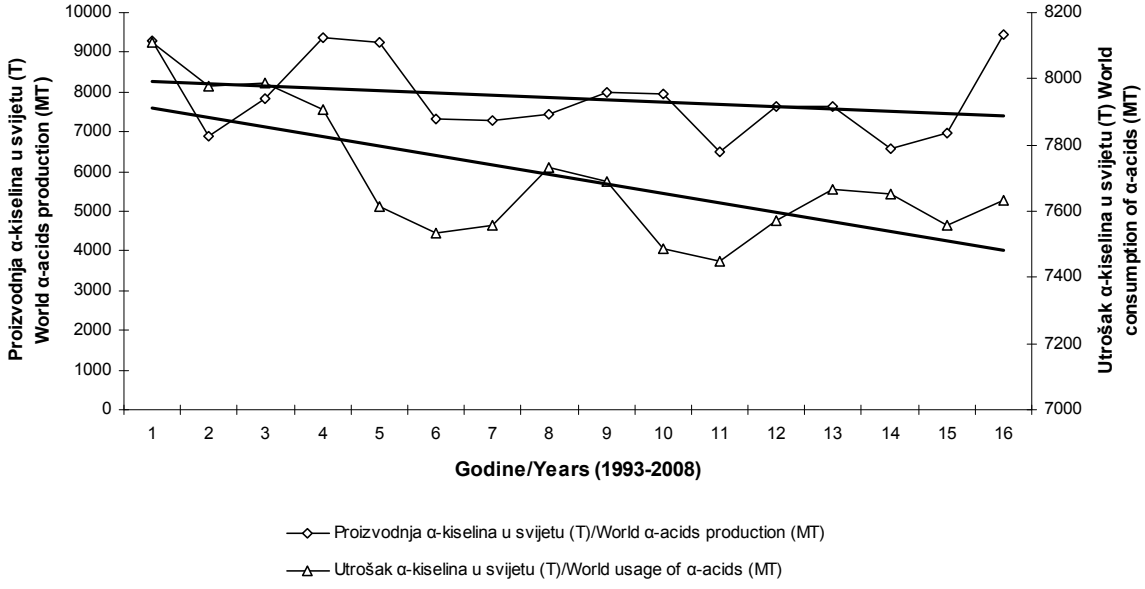
Slika 2: Obrok α-kiselina u pivu [g/hL]
Figure 2: Dosage of α-acids in beer [g/ hL]

Posljedično, svjetske površine pod hmeljem se smanjuju ali zbog povećanja razine agrotehnike ne dolazi do statistički opravdanog smanjenja proizvodnje hmelja (Slika 3).



Slika 3: Svjetske površine hmelja (ha) i proizvodnja hmelja (T) u razdoblju od 1993 do 2008
Figure 3: World hop areas (ha) and world hop production (MT) form 1993 to 2008

U petnaestogodišnjem razdoblju smanjuje se količina proizvedenih α-kiselina, što nije statistički opravdano, dok smanjenje utroška α-kiselina u proizvodnji piva bilježi statistički opravdan osrednji negativni trend (Slika 4).



Slika 4: Usporedba smanjenja proizvodnje α-kiselina u svijetu sa smanjenjem njihovog utroška u razdoblju od 1993 do 2008
Figure 4: Comparison between decrease of world α-acids production and their consumption in increment period of 1993 till 2008

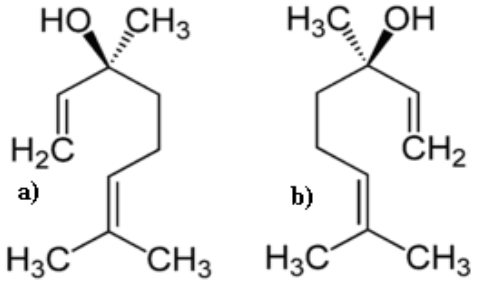
Iz ovih grafikona razvidno je da je glavi uzrok hmeljarskih kriza prevelika ponuda hmelja. No, takav zaključak moguće je donijeti tek površno analizirajući brojke bez dublje analize i

poznavanja svih osobitosti biljne proizvodnje, kao što su: visok stupanj rizika, biološkog karakter. Uzrok su također i teško predvidivi trendovi u pivarskoj proizvodnji piva, koji su sve do globalne financijske recesije imali isključivo uzlazni tok.

Zanimljivo je da u pivarstvu sve više dolazi do pojave „gushing-a“ što je izraženije sa smanjenjem gorčine piva (BU - engl. Bitterness units; 10 BU piva = 1 g α-kiselina · hL^{-1}). Stoga se samo po sebi nameće logično pitanje: - Plaćaju li možda svjetske pivovare previsoku cijenu marketinškog dodvoravanja "Coca-Cola" generaciji potrošača?

3 TEHNOLOŠKI PROBLEMI U SUVREMENOM PIVARSTVU

Fenomen "gushing-a" ili nekontroliranog pjenjenja piva u boci, opisan je prvi puta početkom 20. stoljeća [13]. "Gushing" je uzrokovan djelovanjem hidrofobnih čestica koji su po svojoj prirodi proteinski kompleksi poznati pod nazivom hidrofobini a luče ih gljivice iz roda *Fusarium spp.*, poznatih pod nazivom hidrofobini. Stoga je intenzitet "gushing-a" doveden u vezu s higijenskim ili fitosanitarnim stanjem ječma, odnosno stupnjem infekcije gljivicama iz roda *Fusarium s.pp.*. Istraživanja "gushing-a" usmjeravana su ranije isključivo na pivarski ječam, dok je utjecaj hmelja bio potpuno zanemaren. Međutim, novija istraživanja pokazuju da povećanje obroka hmelja smanjuje "gushing" pri čemu u usporedbi s α- i izo-α-kiselinama veći učinak imaju hmeljna ulja i linalol. To ukazuje da pivo veće gorčine i ohmeljena tzv. "suhim" postupkom može imati manji "gushing" u usporedbi sa pivima niže gorčine [13]. Linalol (Slika 5) je terpenski alkohol koji se u prirodi nalazi samo u cvjetovima aromatskih biljaka, u koje spada i hmelj. Ovaj spoj se stereokemijski pojavljuje u lijevoj i desnoj projekciji i ima visoku sposobnost vezanja hidrofobina.



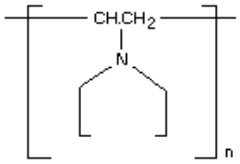
Slika 5: Stereokemijske projekcije linalola a) L(+) – Linalol i b) D(-) – Linalol

Slijedeći fenomen je nestabilnost pjene piva, što je suprotno "gushing-u". Nestabilnost pjene piva uzrokuje difuznost mjehurića pjene, a izo-α-kiseline, koje nastaju izomerizacijom α-kiselina tijekom kuhanja pivske sladovine s hmeljem ili se pak dodaju izomeriziranim hmeljnim pripravcima, primjerice termički obrađeni hmeljni ekstrakti, smanjuju osrednju difuznost mjehurića nastalih djelovanjem hidrofobnih proteina i za 50 % smanjuju visoku difuznost mjehurića nastalih djelovanjem hidrofilnih proteina [15].

Primjena hmelja je također važna i s aspekta mikrobiologije piva što postaje izraženije sa smanjenjem gorčine. Danas je jedan od najvećih problema suvremenog pivarstva kontaminiranost piva bakterijama mliječno-kiselog vrenja [4,8,21,22,23,30,31]. Poznato je da u okolišu nepovoljnom za razvoj mikroorganizama opstaju samo najprilagodljiviji koji svoje zaštitne osobine prenose na iduće generacije rezistentnih mikroorganizama [22,23]. Neki autori [32] pak navode i povijesni razlog toj pojavi, jer budući se pivo proizvodi od 7000. g.

pr. Kr. kontaminanti su se prilagodili okolišu. Međutim, postavlja se pitanje, ne bi li proces prilagodbe mikroorganizama bio kudikamo sporiji da se nije smanjivo utrošak α-kiselina u posljednjih petnaestak godina (Sl. 2). Kako to da je ta pojava učestalija upravo u vrijeme smanjenja utroška α-kiselina, ako se pivo proizvodi 9000 godina?

Stabilnost i bistrenje piva se osim uobičajenim postupcima filtracije kroz kiselgur rješava i primjenom poliamidnih adsorbansa kao što je polivinilpolipirolidon (internacionalna kratica: PVPP) [10] (Slika 6).



Slika 6: Polivinilpolipirrolidon (PVPP)

α-kiseline imaju i izuzetno važnu antiseptičku ulogu pa su stoga važne za očuvanje piva od mikrobiološke kontaminacije. Antiseptička moć α-kiselina opada sa povećanjem stupnja izomerizacije, što je veća izomerizacija α-kiselina u izo-α-kiseline i što je veće njihovo zasićenje vodikovim ionima opada antiseptička im opada. Najveću antiseptičku moć imaju prirodne α-kiseline, nešto slabiju dihidro-izo-α-kiseline, osrednju tetrahidro-izo-α-kiseline, a najslabiju heksa-hidro-izo-α-kiseline [24,30,31]. S tog aspekta "suho" hmeljenje piva ima svoju veliku opravdanost. Naime, postupak "suhog" hmeljenja djeluje dvojako, s jedne strane dovodi do povećavanja mikrobiološke stabilnosti piva, a s druge strane zbog djelovanja hmeljnih ulja i linalola smanjuje "gushing" [13].

4 POLIFENOLI I BIOFLAVONIDI HMELJA U SUVREMENOJ NUTRICIONISTICI I FARMAKOLOGIJI

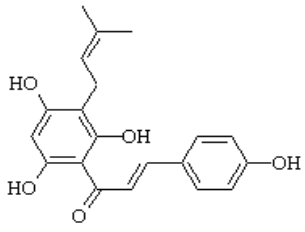
Količina i sastav polifenola nasljedna je osobina kultivara. Međutim, na količinu ukupnih polifenola u hmelju djeluje i način čuvanja hmelja jer u nepovoljnim uvjetima dolazi do brže oksidacije polifenola i ukupnih α-kiselina. Oksidacijski procesi se najbrže odvijaju u prirodnom hmelju (prirodne šišarice stiješnjene u hmeljne vreće) uskladištenim u nepovoljnim uvjetima kao što su: neadekvatna skladišta u kojima se čuvaju hmeljne bale, slabo sabijene šišarice hmelja u hmeljnim vrećama, prirodno osvjetljavanje skladišta i visoke temperature. Najvažniji pokazatelj stupnja oksidiranosti polifenola je polimerizacijski indeks, a to je kvocijent ukupnih polifenola i antocijanogena (proantocijana). Poželjno je da polimerizacijski indeks bude što niži, a u pravilu je niži u aromatskih kultivara u usporedbi s gorkim i visokogorkim kultivarima. Ta osobina je u uskoj vezi s morfološkim osobinama šišarica, što podrazumijeva oblik brakteja i udjel centralnog vretena ili rachisa šišarice u odnosu na brakteje. Kultivari koji formiraju šišarice finih brakteja nazivaju se ″plemeniti¨ i redovito imaju niži udjel polifenola u usporedbi s onima koji formiraju grube šišarice. Poželjno je da udjel centralnog vretena (ili rachisa šišarice) u odnosu na brakteje bude što manji. Na količinu ukupnih polifenola utječe i zaraženost nasada biljnim virusima. Nepovoljni agroekološki uvjeti, kao što je vodeni stres ne djeluju na povećanje količina polifenola [9]. Količina ukupnih polofenola istraživana u cv. Saaz veća je u šišaricama zaraženih biljaka biljnim virusima, u odnosu na šišarice nezaraženih biljaka [16,17,30]. Svojevremeno, zbog izrazito negativnog stava o utjecaju polifenola na fizikalne osobine piva, prerađivači i

distrubiteri hmelja razvili su izomerizirane hmeljne ekstrakte bez polifenolnih sastojaka. No, antioksidativno djelovanje takvih hmeljnih pripravaka, nažalost posve nestaje [17,30]. Od konca devedesetih velika pozornost posvećuje se utjecaju polifenola na kvalitetu piva. O njihovom utjecaju mišljenja su podjeljena. Naime, polifenoli koji se nalaze u sladu i hmelju mogu negativno djelovati na fizikalne osobine piva. Točnije, određene grupe polifenola mogu uzrokovati nepovratno zamućenje piva i nazivaju se turbidno aktivni polifenoli. Zbog toga neke pivovare teže njihovom smanjenju ili potpunom uklanjanju. To se može učiniti korištenjem slada proizvedenog od kultivara ječma koji ne sadrže proantocijane kao i pročišćenih hmeljnih ekstrakta. Prisustvo turbidno aktivnih polifenola može se smanjiti njihovom adsorpcijom na polivinilpolipirolidon (PVPP) za vrijeme filtracije piva.

Suprotno negativnom stavu, postoji i pozitivni nutricionistički stav prema nazočnosti polifenola u hrani. Poznato je da polifenoli djeluju kao prirodni antioksidans s dvostrukim djelovanjem. Polifenoli štite hranu, naročito masti u hrani, od kvarenja pod utjecajem kisika, a u tijelu čovjeka djeluju kao "hvatači" slobodnih radikala pa im se pripisuje antikarcinogeno djelovanje. Hmelj je biljka s razmjerno visokim udjelom polifenola. Udio polifenola u šišaricama hmelja po nekim istraživačima iznosi 2-5%, a po drugima 4-6% [30].

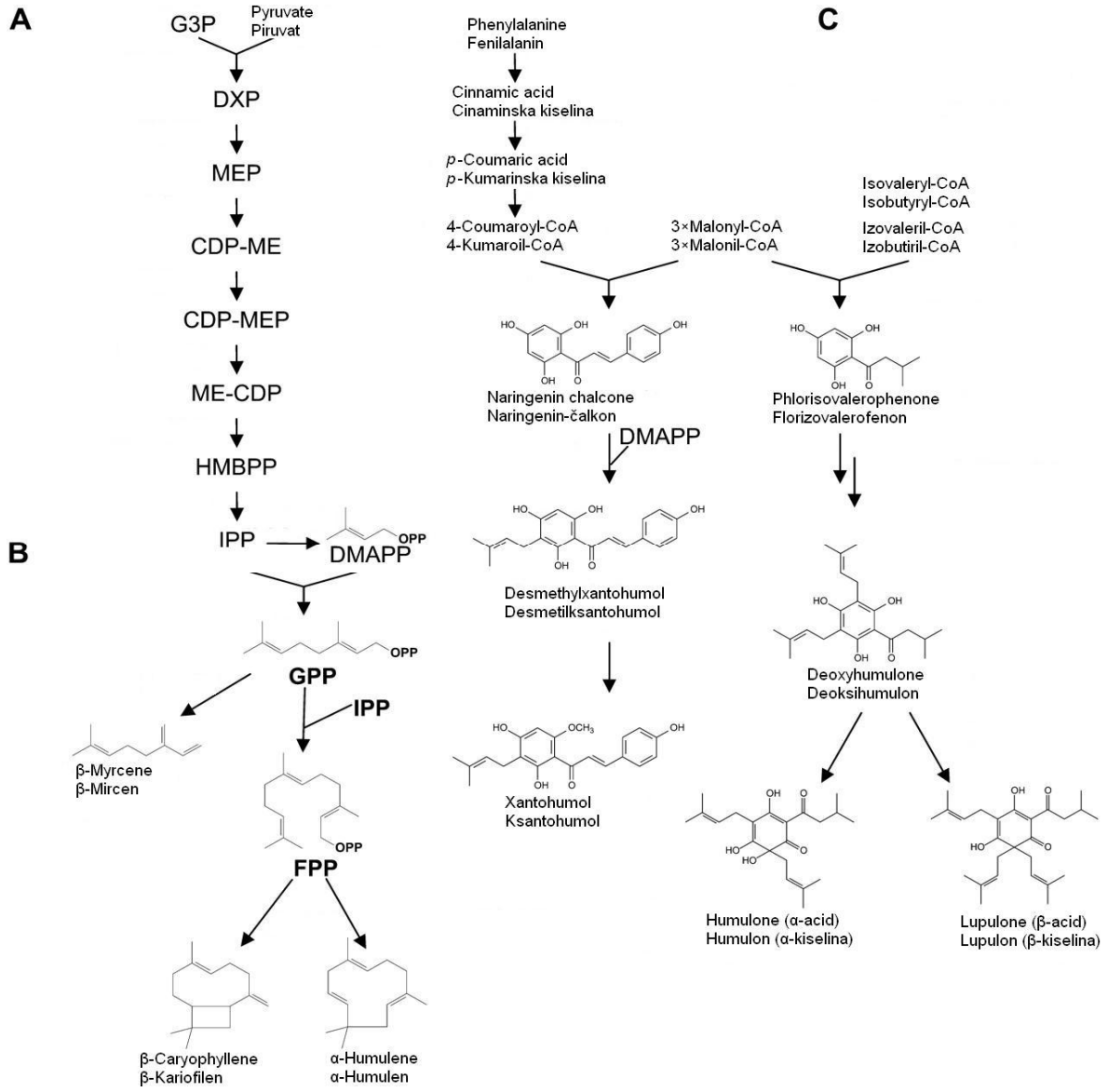
Međutim, problem turbidno aktivnih polifenola postaje izražen samo u slučaju velike oksidiranosti hmelja i visokih vrijednosti polimerizacijskog indeksa, dakle onda kada se upotrebljava stari hmelj [30, 31], pri čemu vrijeme ili datum berbe hmelja ne utječe na stabilnost piva, čak i onog koje je ohmeljeno "suhim" postupkom [12], već isključivo način i uvjeti skladištenja hmelja i hmeljnih pripravaka [17,30,31]. Prema tome turbidno aktivni polifenoli ne predstavljaju potencijalnu opasnost u svježem hmelju niskog HSI indeksa [31]. No, neosporna je činjenica da je udjel ukupnih polifenola u pivarskom ječmu puno veći u usporedbi s hmeljem, pogotovo ako se uzme u obzir činjenica da je količina slada za proizvodnju 1 hL sladovine višekratno veća od količine hmelja potrebne za hmeljenje 1 hL sladovine.

Ksantohumol (Slika 7) spada u skupinu bioflavonida. Kao što je poznato bioflavonidi su skupina spojeva koji se zbog svoje složene biosinteze (Slika 8.) nastaju i akumuliraju se isključivo u generativnim i vegetativnim dijelovima biljaka, dakle u cvjetovima i lišću i gotovo da ih je nemoguće sintetizirati u laboratorijskim uvjetima.



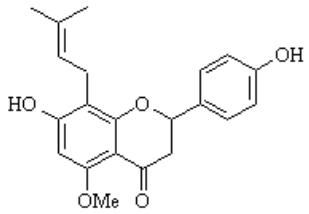
Slika 7: Strukturna formula ksantohumola
Figure 7: Structural formula of xanthohumol

Tvorba ksantohumola odvija se isključivo u lupulinskim žlijezdama hmelja u ciklusu biosinteze terpenskih spojeva, koji je s molekularnog stanovišta detaljno opisan tek 2008. godine [33]. Sam postupak biosinteze terpena izuzetno je složen i objedinjuje sintezu svih gorkih i aromatskih spojeva hmelja, kao i bioflavonida, a time i ksantohumola (Sl. 8) [33].



Slika 8: Ciklus biosinteze terpenskih spojeva u lupulinskim žlijezdama prema Wangu i sur., 2008 [*orig.* 33]
Figure 8: Biosynthetic pathways for terpene-derived products in the lupulin glands according to Wang et al. (2008) [33]

Udjel ksantohumola ovisi o prvenstveno nasljednim osobinama kultivara hmelja, a ne toliko o stresnim čimbenicima, konkretno vodeni stres ne djeluje na povećanje količine ksantohumola [9]. Ksanthumol tijekom kuhanja pivske sladovine s hmeljem izomerizira u izoksanthumol (Slika 9).



Slika 9: Strukturna formula izoksantohumola
Figure 9: Strucutural formula of isoxanthohumol

U osnovi, riječ je o endotermnoj kemijskoj reakciji koja dovodi do izomerizacije slično kao i kod izomerizacije α-kiselina tijekom koje 80 – 90 % ukupne količine ksantohumola se dijelom gubi a djelom prelazi u izoksantohumol [18].

Do danas su potvrđeni brojni pozitivni farmakološki, a i terapeutski učinci ksantohumola i izo-ksantohumola na mnoga karcinogena oboljenja, zbog čega je ksantohumol uvršten antikarcinogene preventivne tvari [6]. Osim toga, potvrđeno je i njihovo pozitivno djelovanje na regulaciju metabolizma jetre, što je naročito važno kod bolesnika s teškim oštećenjima jetre [25], a također poznato je i pozitivno estrogeno djelovanje [30]. Pri kliničkim ispitivanjima, niti u jednom slučaju kod kliničkih ispitanika nisu utvrđene nikakve kontraindikacije (*op.* za razliku od sintetskih farmakoloških preparata).

Zbog takvih ljekovitih osobina ksanthumol doprinosi jačanju tržišnog imidža piva ako ga u pivu ima u zadovoljavajućim količinama, ali to se na žalost ne odnosi na većinu pivskih brandova [29].

Iako prva istraživanja o prisustvu ksantohumola, izoksantohumola i hmeljnih polifenola datiraju još od konca devedesetih godina [17,18] trebalo je čekati dosta vremena da se pojave prvi hmeljni pripravci koji su sadržavali ksantohumol u zadovoljavajućim količinama. Naime, uobičajeni i najraširenij postupak ekstrakcije hmeljnih sastojaka je CO_2 superkritična ekstracija [19,20]. Međutim, ekstrakt dobiven etanolskom ekstrakcijom ima puno veći kapacitet uklanjanja slobodnih radikala u usporedbi s CO_2 ekstraktom [14]. Danas postoji i hmeljni pripravak trgovačkog imena Xantho-Flav™, čiji kapacitet uklanjanja slobodnih radikala stoji u potpunoj pozitivnoj korelaciji s koncentracijom ksantohumola u pripravku ($r = 0,99$ za hidroksilne radikale, odnosno $r = 0,98$ za perkosilne radikale) [14].

5 KAKO ISKORISTITI LJEKOVITA SVOJSTVA HMELJA U CILJU RESTRUKTURIRANJA PROIZVODNJE HMELJA?

Da bi se odgovorilo na to pitanje potrebno je izvesti jednostavnu računicu. Naime, količina ksantohumola u različitih hmeljnih kultivara varira u granicama od 0,1 – 1,5 % [9, 18]. U kultivaru Aurora ona iznosi 0,4 % u suhoj tvari šišarica [9]. Što znači da se u 1 kg suhe tvari šišarica kultivara Aurora nalazi aproksimativno 0,4 kg (ili 400 grama) ksantohumola. Prosječni udjel vode u šišaricama hmelja nakon njihovog sušenja i kondicioniranja iznosi 10 %. To znači da je za proizvodnju 400 grama ksantohumola potrebno 110 kg hmeljnih šišarica s aproksimativnim udjelom vode od 10 %, što je u praksi nemoguće, jer ne postoji niti jedan tehnološki proces koji bi imao 100 %-tno iskorištenje ulazne sirovine [21]. Međutim, podaci o iskorištenju ulazne hmeljne sirovine za ekstrakciju 1 kg ksantohumola nisu poznati, no realno je pretpostaviti da iskorištenje tehnološkog postupka etanolske ekstrakcije ksantohumola aproksimativno iznosi 30 – 40 %. Na žalost, stvari glede proizvodnje ksantohumola u farmaceutske svrhe nisu tako jednostavne kako se čine na prvi pogled. Činjenica je da se na WEB portalu tvrtke Axxora (www.axxora.com) mogu pronaći ponude za oba bioflavonida, ksantohumol i izoksantohumol, a cijena 5 mg ksantohumola 99 %-tne čistoće, u konzistenciji narančastog praha iznosi i do 25 €. To ukazuje na nisko iskorištenje tehnološkog postupka ekstrakcije ksantohumola, a posljedično na visoki utrošak hmeljne sirovine. Što predstavlja bitan element povećanja potražnje za hmeljem [26].

Premda postoji mogućnost proizvodnje farmakoloških pripravaka ksantohumola i izo-ksantohumola i njihovog uvođenja u terapiju bolesnika oboljelih od malignih oboljenja ili pak

u preventivne primjene u onih osoba koje imaju genetičke predispozicije prema malignim oboljenjima (*op.* za što se autori ovog članka nadaju da neće trebati proteći puno vremena), ostaje i dalje činjenica da se najveći učinak na povećanje potražnje za hmeljem može se postići povećanjem utroška α-kiselina po hektolitru piva, odnosno pivske sladovine. Naravno, to je lako reći, ali postavlja se pitanje kako? Da bi se odgovorilo na to pitanje potrebno se potrebno se vratiti na početak priče. Naime, na ovogodišnjem 32. Kongresu EBC-a održanom u Hamburgu, 10 – 14.5. Alberto Da Ponte, predsjednik krovne Europske pivarske asocijacije Brewers of Europe (akronim: BoE) unutar koje EBC (European Brewery Convention) djeluje kao stručna i znanstvena logistika, u svojem plenarnom izlaganju iznio je temeljni cilj BoE i EBC-a, a to je stvaranje cit. "beer friendly" poslovnog okruženja [11]. O čemu se ovdje zapravo radi? Naime, u Skandinavskim zemljama jača tzv. Anti-alkoholni lobi čiji je osnovni argument da pivo nema korisni učinak za zdravlje ljudi a pivarske kompanije poradi stvaranja što većeg profita u oglašavanju svojih brand-ova orijentirane su isključivo na mladu generaciju potrošača, čime se otvara put mladima prema alkoholizmu. Stoga, po njima pivu treba skinuti status prehrambenog proizvoda i dodatno ga oporezivati kako bi se njegova potrošnja smanjila.

Koliko god to na prvi pogled čudno izgledalo u strateškom smislu hmelj bi, zbog svojih pozitivnih nutritivnih osobina i ljekovitosti navedenih kemijskih sastojaka, uvelike mogao poboljšati marketinški imidž suvremenih pivskih brand-ova, dajući pivu novu dodanu vrijednost [28]. Veliku ulogu u promicanju te dodane vrijednosti ima i IHGC (International Hop Growers' Convention) kao krovna svjetska asocijacija proizvođača hmelja. Čini se da je došlo vrijeme novog dijaloga između europskih pivara i proizvođača hmelja na temu doprinosa hmeljne sirovine ne samo gorčini i aromi piva, kako se na hmelj isključivo do sada gledalo [7,15], već i zdravlju potrošača [29]. S obzirom na navedene sastojke hmelja koji prelaze u pivo, a potencijalno su korisni za ljudsko zdravlje, potrebno je razmatrati pivo i s aspekta funkcionalne hrane. Naravno, uvjet je da spomenuti biološki aktivni spojevi budu prisutni u dovoljno velikim koncentracijama koje mogu postići zamjetljiv učinak na ljudski organizam, pri čemu treba uzeti u obzir i faktor resorpcije u organizam.

6 ZAKLJUČAK

Proizvodnja hmelja u svijetu od početka devedesetih godina ima obilježja stalne krize, koja je najvećim djelom uvjetovana smanjenjem utroška hmelja odnosno smanjenjem utroška α-kiselina/hL pivske sladovine u cilju smanjenja gorčine piva. Pri tome, osim smanjenja gorčine smanjio se je u pivu i udjel bioflavonida i polifenola koji imaju povoljno djelovanje na zdravlje ljudi. Restrukturiranje svjetske industrije hmelja nema alternativu, međutim proces restrukturiranja treba ići u dva pravca, jedan je otvaranje novog distribucijskog kanala prema farmaceutskim kućama u cilju proizvodnje nove generacije prirodnih lijekova, a drugi podrazumijeva povećanje utroška hmelja od strane pivarske industrije kako bi se održala osnovna karakteristika piva, a to je piće korisno za zdravlje potrošača, odnosno funkcionalna hrana.

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MODELIRANJE ZGODNJE NAPOVEDI STOPNJE ALFA-KISLIN PRI HMELJU (*Humulus lupulus* L.)

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IZVLEČEK

Analiza soodvisnosti med meteorološkimi spremenljivkami in stopnjo alfa-kislin v kultivarjih hmelja je v obdobju 1994-2008 pokazala, da se slovenski kultivarji zelo podobno odzivajo na vremenske vplive. Testirali smo vplive temperature zraka, količine padavin in dolžine sončevega obsevanja na količino alfa-kislin v storžkih. Vse proučevane meteorološke spremenljivke kažejo v določenih fenoloških fazah hmeljne rastline od najmanj zmerne do visoke stopnje soodvisnosti s stopnjami alfa-kislin testiranih kultivarjev hmelja. Te lahko združimo glede na meteorološke odzive v dve skupini. Aurora, Celeia in Savinjski golding kažejo zelo visoko pozitivno medsebojno korelacijo ($r=0,9$; $p<0,001$). Na osnovi analiz soodvisnosti med stopnjami alfa-kislin v kultivarjih hmelja in meteorološkimi spremenljivkami je oblikovan osnutek modela za zgodnje napovedi alfa-kislin za kultivarje hmelja na območju Slovenije. Rezultati aplikacije modela potrjujejo njegovo uporabnost za nadaljnje raziskave.

Ključne besede: hmelj, alfa-kisline, vremenski podatki, modeliranje, simulacija

MODELLING OF ALPHA-ACID CONTENT EARLY PREDICTION BY HOPS (*Humulus lupulus* L.)

ABSTRACT

Correlation analysis between meteorological variables and alpha-acid contents in hop varieties in time period 1994-2008 showed, that Slovenian varieties react with resemblance on weather circumstances. Tests of air temperature, rainfall and length of sunshine impacts on alpha-acid content in hop cones were carried out. The included meteorological variables point out in certain phenological phases of hop plants from moderate to high level of dependency with alpha-acid values of varieties analysed. Related to weather situation these hop varieties could be sort out into 2 groups. Aurora, Celeia and Savinjski golding show a very high positive interacting correlation ($r=0,9$; $p<0,001$). Based on correlation analysis between alpha-acid values in hop varieties and meteorological variables, a model concept for early prediction of alpha-acid values for Slovenian hop varieties was formulated. The results validate the application of the model for further research.

Key words: hops, alpha-acids, weather data, modelling, simulation

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1 UVOD

V procesu kmetijske proizvodnje vplivajo na pridelek številne spremenljivke, čeprav neposrednih vzročno posledičnih povezav ni vedno moč opaziti na prvi pogled. Z veliko stopnjo gotovosti lahko predvidevamo, da je kmetijska pridelava poljščin odvisna od vremenskih oziroma podnebnih razmer kot so temperatura zraka, sončno obsevanje, zračna vlaga in količina padavin ter pogostnosti in intenzivnosti mejnih dogodkov kot so suše, poplave in viharji. Proučevanje vpliva spreminjanja vremenskih razmer in drugih dejavnikov na kmetijsko pridelavo nam omogoča, da pri načrtovanju čim bolj izkoristimo podnebne razmere in tako dosežemo najboljšo možno količino in kakovost pridelka. V zadnjih desetletjih smo priča izraziti spremenljivosti podnebja, ki ji v veliki meri botruje človek [6].

Pri pridelavi hmelja velja, da so pomembni dejavniki, ki vplivajo na količino alfa-kislin v hmelju svetloba, temperatura zraka in voda. Temperatura je pomemben ekološki dejavnik, od katerega je odvisna tudi dinamika rasti in razvoj rastline hmelja. Biološki procesi se pri hmelju začnejo spomladi z dnem, ko je povprečna dnevna temperatura zraka več dni zaporedoma višja od 4 do 5 °C. Te temperaturne vrednosti pa določajo tudi spomladanski temperaturni prag za hmelj. Optimalno rast hmelja omogočajo temperature med 15 in 18 °C. Spodnja temperatura za normalno rast hmelja med rastno dobo je 10 °C. Hmelj potrebuje v obdobju od rezi do nastopa tehnološke zrelosti vsoto efektivnih temperatur od 2700 do 2900 °C. Glede preskrbljenosti z vodo je zahtevna rastlina in jo zato uvrščamo med rastline humidnih območij. Ocenjeno je, da potrebuje v rastni dobi od 500 do 600 mm padavin. Oskrba rastlin z vodo pa ni odvisna le od količine padavin, ampak tudi od njihove razporeditve. Prav tako je hmelj ena izmed občutljivejših rastlin glede pomanjkanja svetlobe. Dolžina dneva vpliva na rastline na več načinov, najbolj pa je znana njihova reakcija na začetek cvetenja. Hmelj je rastlina kratkega dne, začetek cvetenja pa je odvisen tudi od ostalih pogojev [1].

Vsebnost alfa-kislin v storžkih hmelja predstavlja enega pomembnejših tržnih parametrov v okviru globalnega povpraševanja po hmelju. O izraziti koncentraciji pivovarske industrije in s tem vedno bolj nadzorovanega nakupa hmeljskih proizvodov pričajo podatki, da so v letu 2008 vsega štiri pivovarska združenja AB InBev (21,4 %), SABMiller (9,6 %), Heineken (8,9 %) in Carlsberg (6,0 %) proizvedla nad 50 % svetovnih količin piva, ki so znašale 1,815 mrd hl [2]. Pri večini odkupljenega hmelja je kot najpomembnejši kriterij izpostavljena vsebnost grenčičnih snovi (alfa-kislin). Vse manj pa je tradicionalnih pivovarn, ki upoštevajo pri nakupu hmelja njegov izvor ter kakovost eteričnih olj [7,9]. Vsebnost alfa-kislin se skozi leta spreminja v odvisnosti od meteoroloških razmer v času rasti hmeljne rastline. Avtorji si niso povsem enotni [7,8,12] v oceni, kateri parametri in v katerem obdobju vegetacije rastline hmelja imajo večji vpliv na stopnjo alfa-kislin. Zattler [14] na osnovi svoje študije sklepa, da je visoka vsebnost alfa-kislin povezana z deževnim poletjem, podpovprečnimi temperaturami in povprečno stopnjo sončevega obsevanja. Thompson [13] sklepa, da je letno nihanje vsebnosti alfa-kislin povezano s temperaturnimi nihanji v razdobju 40 do 60 dni pred obiranjem in da hmeljarji na ta nihanja nimajo vpliva.

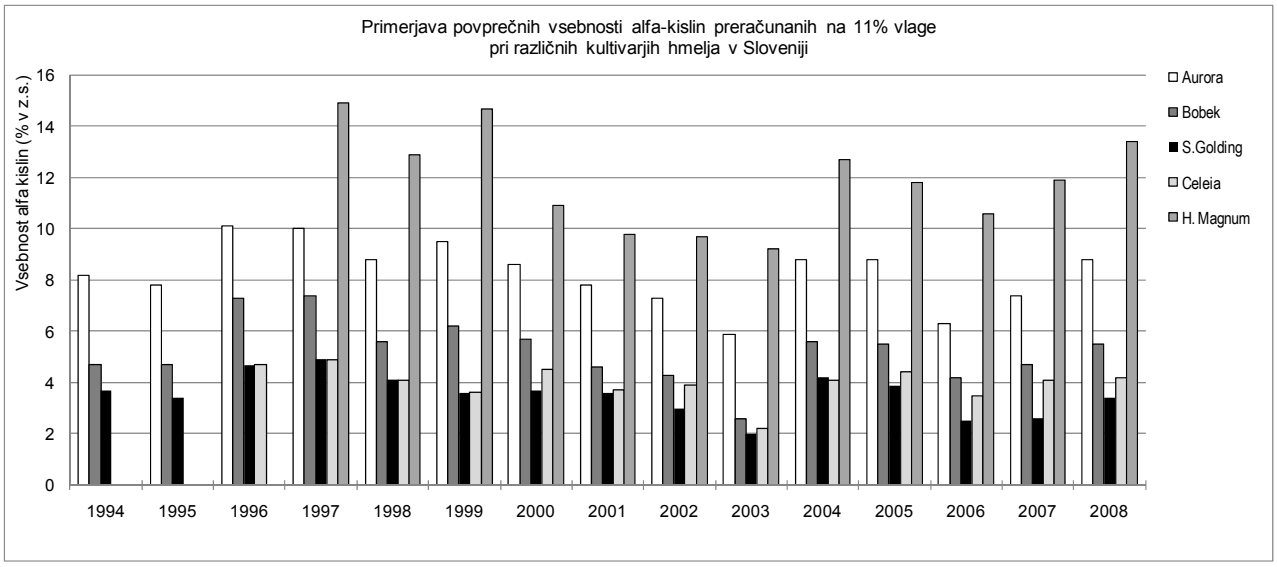
Na osnovi preliminarne študije smo za naš testni model zajeli meteorološke podatke delov fenoloških faz od F_0 do F_7 za hmelj [1]. Kasnejša obdobja za napovedi pridelka niso več zanimiva, saj lahko vsebnost alfa-kislin določimo s kemičnimi analizami.

2 MATERIALI IN METODE

Analizo vpliva vremenskih razmer na pridelek grenčičnih snovi smo izvedli na kultivarjih Aurora, Bobek, Celeia, Savinjski golding in Hallertauer Magnum, ki zastopajo nad 95 % površin hmeljišč v Sloveniji.

2.1 Vzorčenje

Analizirani vzorci predstavljajo povprečno vrednost alfa-kislin posameznega kultivarja celotnega območja pridelave v Sloveniji. Vzorčenje in analize so bile narejene v laboratoriju za agrokemijo na Inštitutu za hmeljarstvo in pivovarstvo Slovenije (IHPS) v Žalcu. Vsebnost alfa-kislin je bila določena z akreditirano metodo Konduktometrična vrednost hmelja s toluensko ekstrakcijo, standardizirana metoda po Analytici EBC 1998, revizija 2000, 7.4. V raziskavi so zajeti podatki 2000 kemičnih analiz vseh kultivarjev. Povprečne vrednosti kemičnih analiz alfa-kislin kultivarjev so prikazane na sliki 1.



Slika 1: Povprečne vsebnosti alfa-kislin za zračno suh hmelj z 11 % vlage (A-K v z.s.) v obdobju 1994 do 2008
Figure 1: The average values of alpha-acid content in dry hop with 11 % of moisture in the period 1994 - 2008

2.2 Meteorološki podatki

Meteorološki podatki Agencije Republike Slovenije za okolje, Urada za meteorologijo, se nanašajo na merilna mesta Celje, Šmartno pri Slovenj Gradcu, Velenje in Starše pri Mariboru. Podatki o sončevem obsevanju niso bili na voljo za postajo Starše pri Mariboru in Velenje. Podatki merilne postaje Velenje pa so bili dosegljivi le do leta 2006, oz. do prenehanja njenega delovanja. Povprečne vrednosti meteoroloških spremenljivk smo izračunavali ob enakovrednem upoštevanju podatkov vseh štirih in od leta 2006 treh merilnih postaj. To povprečje predstavlja dober približek, saj se tudi rastišča hmelja razprostirajo znotraj tega geografskega območja. Potrebo rastline po toploti smo zajeli v spremenljivki skupne vsote povprečnih dnevnih temperatur v določenem časovnem obdobju. Potreba po vodi se izkazuje v spremenljivki skupne količine padavin ob predpostavki odsotnosti namakanja pridelka. Potreba po svetlobi je zajeta v spremenljivki vsote ur obsevanja sonca na obravnavanem geografskem območju. Pri modeliranju smo upoštevali tudi povprečno relativno zračno vlago v proučevanem obdobju.

Preglednica 1: Vrednosti meteoroloških spremenljivk uporabljenih pri izgradnji modela
Table 1: Values of meteorological variables used in development of the model

LETO	SUM VEGETACIJA				LETNO POVPREČJE			
	T	S	D	RH*	T	S	D	RH
1994	1.859,400	814,200	492,400	72,336	10,90	5,33	3,13	77,18
1995	1.770,525	835,950	347,400	71,750	9,63	4,96	3,13	77,63
1996	1.751,250	895,350	507,900	71,585	8,79	4,76	3,32	78,04
1997	1.699,950	891,200	387,850	68,965	9,53	5,57	2,81	76,12
1998	1.809,625	918,000	451,550	70,728	9,88	5,65	3,21	74,69
1999	1.882,750	845,200	576,400	72,591	9,95	5,14	3,24	76,62
2000	1.961,225	1.028,350	342,700	67,800	11,07	6,12	2,71	73,76
2001	1.851,650	933,400	443,500	68,166	10,30	5,55	2,85	73,24
2002	1.952,575	942,450	347,125	70,410	10,93	5,41	2,81	76,64
2003	2.036,100	1.029,100	206,250	65,361	10,36	5,96	2,29	73,19
2004	1.731,150	726,550	534,500	74,942	9,63	4,61	3,26	78,40
2005	1.855,600	851,300	499,300	66,892	9,33	5,07	3,26	73,65
2006	1.901,500	891,400	359,800	65,135	9,11	5,13	2,58	72,51
2007	2.023,467	978,250	364,967	65,271	10,67	5,55	3,15	73,77
2008	1.847,933	826,700	548,733	70,668	10,55	4,94	3,10	75,84

SUM VEGETACIJA	Integralni podatki neodvisnih meteoroloških spremenljivk, izračunani za čas vegetacije rastline hmelja. *Podatek za relativno vlago predstavlja povprečje v istem obdobju
T	temperatura (^oC)
S	število ur sončevega obsevanja (ur)
D	količina padavin (mm/m^2)
RH	relativna zračna vlaga (%)

2.3 Oblikovanje modela

Na osnovi predhodnih rezultatov analize soodvisnosti med proučevanimi spremenljivkami smo pripravili testni model za zgodnje napovedi alfa-kislin v slovenskih kultivarjih hmelja. Za odvisno spremenljivko smo izračunali povprečne utežne vrednosti stopnje alfa-kislin kultivarjev prve skupine (Aurora, Bobek in Savinjski golding) in odvisno spremenljivko poimenovali »VIRTUAL«. S tem smo testni model posplošili za napoved povprečne vsebnosti alfa-kislin vseh pomembnejših kultivarjev v Sloveniji. Model smo gradili ob predpostavki, da imajo različni meteorološki parametri v posameznih fenoloških fazah različne vplive. Zato smo kot neodvisne spremenljivke izbrali segmentirane meteorološke spremenljivke in jih v testnem modelu različno utežili.

Uporabili smo metodo multiple linearne regresije in na osnovi izkušenj izbrali neodvisne spremenljivke. Točnost modela smo testirali z metodo prečnega preverjanja, »izpusti enega« (ang. Leave-one-out) [5].

3 REZULTATI

Medsebojno podobnost odzivov kultivarjev hmelja na meteorološke pogoje smo proučevali s korelacijsko analizo. Uvodne analize kažejo, da se vsi proučevani kultivarji v veliki meri podobno odzivajo na zunanje vplive. Na osnovi moči medsebojne povezave lahko kultivarje razdelimo v dve skupini. V prvi skupini so Aurora, Bobek in Savinjski golding, ki kažejo zelo močne soodvisnosti v stopnji alfa-kislin pri stopnji tveganja $\alpha<0,001$ (pregl. 2). Zelo visoke

in statistično značilne vrednosti Pearsonovega korelacijskega koeficienta nakazujejo na možnost, da na količino alfa-kislin pri teh treh kultivarjih v veliki meri vplivajo enake spremenljivke v istem časovnem obdobju. Prav tako lahko sklepamo, da bodo analize vpliva meteoroloških spremenljivk na en kultivar veljale tudi za ostala dva. To bi lahko olajšalo napovedovanje stopnje alfa-kislin v kultivarjih na osnovi le enega modela, saj bi lahko napovedi za posamezne kultivarje korigirali s t.i. »konstanto značilnosti kultivarja«. Celeia in Hallertauer Magnum kažeta s to skupino nekoliko nižjo stopnjo soodvisnosti pri izbranih meteoroloških pogojih.

Preglednica 2: Matrika soodvisnosti preučevanih kultivarjev hmelja
Table 2: Register of co-independency for investigated hop varieties

		AURORA	BOBEK	S_GOLDING	CELEIA	MAGNUM
AURORA	Pearson Correlation	1	,956(**)	,921(**)	,784(**)	,845(**)
	Sig. (1-tailed)		,000	,000	,001	,000
	N	15	15	15	13	12
BOBEK	Pearson Correlation	,956(**)	1	,895(**)	,854(**)	,860(**)
	Sig. (1-tailed)	,000		,000	,000	,000
	N	15	15	15	13	12
S_GOLDING	Pearson Correlation	,921(**)	,895(**)	1	,800(**)	,671(**)
	Sig. (1-tailed)	,000	,000		,001	,008
	N	15	15	15	13	12
CELEIA	Pearson Correlation	,784(**)	,854(**)	,800(**)	1	,557(*)
	Sig. (1-tailed)	,001	,000	,001		,030
	N	13	13	13	13	12
MAGNUM	Pearson Correlation	,845(**)	,860(**)	,671(**)	,557(*)	1
	Sig. (1-tailed)	,000	,000	,008	,030	
	N	12	12	12	12	12

** Pearson Correlation is significant at the 0.01 level (1-tailed)
* Pearson Correlation is significant at the 0.05 level (1-tailed)

Uvodne analize soodvisnosti med preučevanimi spremenljivkami nakazujejo, da povečanje vsote efektivnih temperatur vpliva na znižanje vsebnosti alfa-kislin pri proučevanih kultivarjih. Tak sklep lahko sprejmemo pri stopnji tveganja $\alpha<0,05$ pri vseh kultivarjih hmelja. Za prvo skupino (Aurora, Bobek in Savinjski golding) lahko to trdimo celo z 99,9 % gotovostjo. Do podobnih ugotovitev so prišli tudi drugi avtorji [7,12].

Vpliv skupnega števila ur sončevega obsevanja kaže zmerne negativne korelacije s stopnjo vsebnosti alfa-kislin. Trditev, da povečanje števila sončnih ur zmanjšuje količino alfa-kislin v kultivarjih hmelja, je identična ugotovitvam drugih raziskovalcev [7,12], vendar pa je potrebna še presoje, saj delne korelacije kažejo pozitivno soodvisnost med stopnjo alfa-kislin in proučevanim parametrom. Pojav bi lahko razložili z interakcijo med vsebnostjo vode, številom ur sončevega obsevanja in stopnjo alfa-kislin. Povečanje števila ur sončevega sevanja vpliva na evaporacijo vode iz tal. Količina padavin pa je pozitivno korelirana s stopnjo alfa-kislin (Aurora $r=0,86$; $p<0,001$). Pri tem je lahko učinek sončevega obsevanja na izhlapevanje vode večji, kot je pozitivni učinek sončevega obsevanja na razvoj alfa-kislin. V kolikor bodo nadaljnje raziskave to potrdile, bo moč negativni vpliv kompenzirati z natančnim terminskim namakanjem hmeljišč.

Edini parameter, ki izkazuje pozitivno soodvisnost s stopnjo alfa-kislin pri vseh preučevanih kultivarjih je količina padavin. Trditev, da povečana količina padavin pozitivno vpliva na stopnjo alfa-kislin pri kultivarjih hmelja lahko povzamemo pri najmanj 99 % stopnji zaupanja za vse proučevane kultivarje prve skupine. Do podobnih ugotovitev so prišli tudi drugi avtorji [7,8,14], pri tem pa naša raziskava kaže na visoko stopnjo povezave med kultivarji in količino padavin v proučevanem časovnem razdobju. Tudi ta trditev zahteva tehten razmislek in dopolnilo. Natančnejše analize vpliva količine padavin nam kažejo, da je bolj kot absolutna količina padavin pomembna čim bolj enakomerna porazdelitev v času vegetacije, še posebej pa to velja za F_6 in F_7 fenološki fazi hmeljne rastline.

S tako oblikovanim testnim modelom smo napovedali povprečne letne vrednosti alfa-kislin vseh treh testiranih kultivarjev. Za nas so bile zanimive predvsem napovedi vsebnosti alfa-kislin vsakega kultivarja posebej, zato smo z navzkrižnim polnjenjem modela s podatki izračunali koeficiente, ki so lastni vsaki sorti in posameznim, ožjim pridelovalnim območjem. Vrednosti korekcijskih konstant so zbrane v preglednici 3. Iz napovedi testnega modela lahko izračunamo vrednosti stopnje alfa-kislin za določen kultivar na ožjem pridelovalnem območju po enačbi:

$$\text{vrednost alfa-kislin za kultivar na ožjem območju} = \text{napoved modela} \times K_{sorte} \times K_{kraja} \qquad /1/$$

Tako lahko iz povprečnih meteoroloških podatkov za Slovenijo napovemo stopnjo alfa-kislin za določen kultivar hmelja neodvisno od pridelovalnega območja.

Preglednica 3: Korekcijske konstante značilne za kultivarje hmelja in pridelovalna območja
Table 3: Correction constants that characterise hop varieties and growing regions

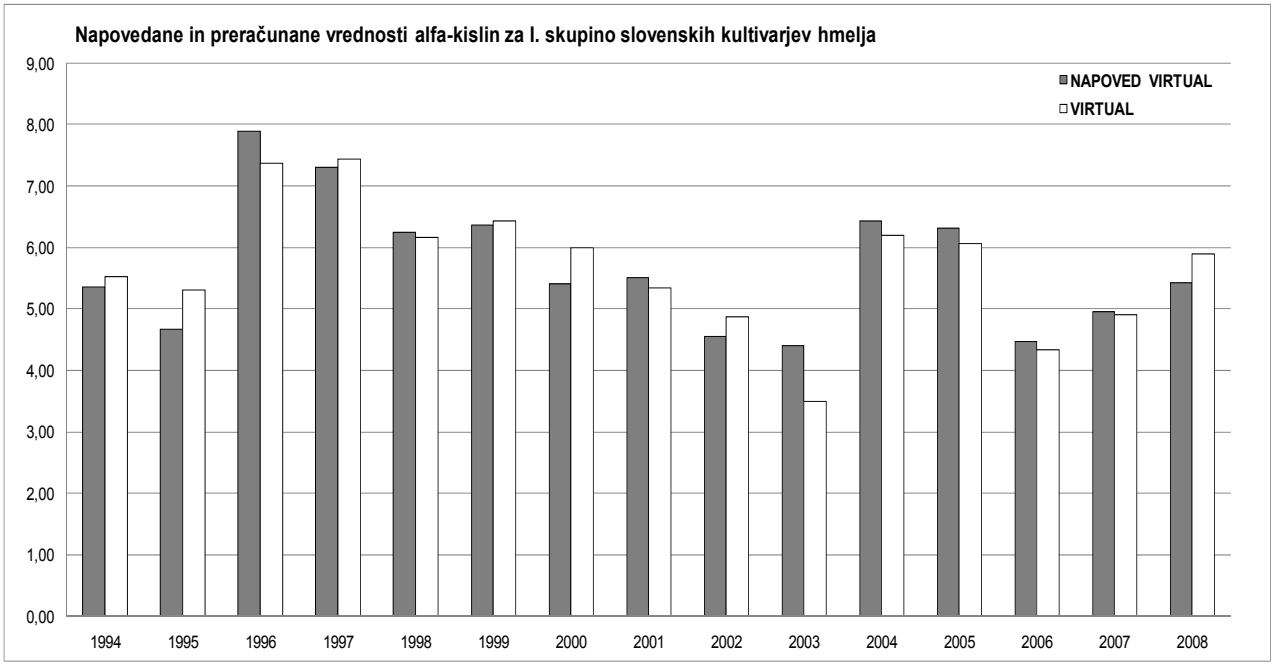
VREDNOSTI KOREKCIJSKE KONSTANTE ZA:	PRIDELOVALNO OBMOČJE SLOVENJ GRADEC			PRIDELOVALNO OBMOČJE CELJE			PRIDELOVALNO OBMOČJE SAVINJSKA DOLINA		
	AURORA	BOBEK	GOLDING	AURORA	BOBEK	GOLDING	AURORA	BOBEK	GOLDING
KULTIVAR	1,44	0,90	0,61	1,44	0,90	0,61	1,44	0,90	0,61
PRIDELOVALNO OBMOČJE	1,37	1,37	1,37	1,10	1,10	1,10	1,05	1,05	1,05

Testni regresijski model zgodnje napovedi stopnje alfa-kislin v kultivarjih hmelja, ki smo ga zgradili na osnovi meteoroloških podatkov, nam je napoved izračunal že v sredini julija. Analiza prečnega preverjanja točnosti modela je pokazala, da model napoveduje vrednosti ob 7 % povprečni napaki. Podatki so zbrani v preglednici 4. Na sliki 2 pa so prikazane napovedane in izmerjene vrednosti vsebnosti alfa-kislin za odvisno spremenljivko »Virtualni kultivar«.

Vrednosti korelacijskih koeficientov med napovedanimi in izmerjenimi vrednostmi vsebnosti alfa-kislin so zelo visoke ($r=0,9$ pri $p<0,001$), kar kaže na visoko stopnjo zanesljivosti napovedi. Manj zanesljiv rezultat je model podal le v ekstremnih meteoroloških razmerah leta 2003, ko je napovedal vrednost stopnje alfa-kislin 4,4, izračunana vrednost pa je bila 3,5. Predhodna ocena povprečne vrednosti alfa-kislin obravnavanih kultivarjev hmelja je z ekonomskega stališča zelo pomembna, saj nudi tako hmeljarjem, kot tudi trgovcem s hmeljem že vsaj 6 tednov pred obiranjem precej natančnejšo oceno potenciala skupne pridelave grenčic.

Preglednica 4: Napovedane in izpeljane vrednosti alfa-kislin v primerjavi z izmerjenimi vrednostmi pri analiziranih kultivarjih hmelja
Table 4: Predicted and derived alpha-acid values in comparison to measured ones for hop varieties analysed

LETO	NAPOVEDANE IN KORIGIRANE NAPOVEDANE VREDNOSTI STOPNJE ALFA-KISLIN (% v z.s.)				STOPNJA ALFA-KISLIN DOLOČENA S KEMIJSKO ANALIZO (% v z.s.)		
	MODEL	AURORA	BOBEK	S. GOLDING	AURORA	BOBEK	S. GOLDING
1994	5,4	7,7	5,1	3,3	8,2	4,7	3,7
1995	4,7	6,5	4,4	2,9	7,8	4,7	3,4
1996	7,9	11,0	7,4	4,8	10,1	7,3	4,7
1997	7,3	10,2	6,8	4,5	10,0	7,4	4,9
1998	6,2	8,7	5,8	3,8	8,8	5,6	4,1
1999	6,4	9,9	6,0	3,9	9,5	6,2	3,6
2000	5,4	7,6	5,1	3,3	8,6	5,7	3,7
2001	5,5	7,7	5,1	3,4	7,8	4,6	3,6
2002	4,6	6,4	4,3	2,8	7,3	4,3	3,0
2003	4,4	6,2	4,1	2,7	5,9	2,6	2,0
2004	6,4	9,0	6,0	3,9	8,8	5,6	4,2
2005	6,3	8,8	5,9	3,8	8,8	5,5	3,9
2006	4,5	6,3	4,2	2,7	6,3	4,2	2,5
2007	4,9	6,9	4,6	3,0	7,4	4,7	2,6
2008	5,4	7,6	5,9	3,3	8,8	5,5	3,4



Slika 2: Napovedane in izračunane vrednosti neodvisne spremenljivke vsebnosti alfa-kislin za prvo skupino kultivarjev hmelja
Figure 2: Predicted and calculated values of independent variable alpha-acid content for the first group of hop varieties

4 ZAKLJUČEK

Primerjava vrednosti koeficientov korelacije med testiranimi meteorološkimi spremenljivkami in stopnjami alfa-kislin slovenskih kultivarjev hmelja nam je pokazala, da se kultivarji Aurora, Bobek in Savinjski golding odzivajo na meteorološke vplive precej enotno. To nam je omogočilo pripravo univerzalnega modela za zgodnjo napoved stopnje alfa-kislin. V testnem modelu smo kot neodvisne spremenljivke uporabili različno utežene meteorološke podatke.

Količina padavin v času cvetenja in storžkanja hmelja je ključnega pomena za količino alfa-kislin. Pri tem pa morajo biti padavine čim bolj enakomerno porazdeljene. Sončevo obsevanje ima vsaj dvojen vpliv na hmeljno rastlino. V pozitivni smeri deluje pri nastanku alfa-kislin, hkrati pa lahko povzroča preveliko izsušitev tal in s tem negativno vpliva na rast in razvoj rastline. Spremenljivka povprečnih dnevnih temperatur, ki pojasni znaten del variance pri Aurori, je v korelaciji z vsemi testiranimi kultivarji. Aurora, Bobek in Savinjski golding kažejo korelacijo na stopnji statistične značilnosti $p<0,001$. Celeia in Hallertauer Magnum pa na še sprejemljivi stopnji tveganja $\alpha<0,05$.

Z matematičnim modelom lahko napovemo stopnjo alfa-kislin pri naših treh najpomembnejših kultivarjih. Model izračunava vrednosti na osnovi povprečnih meteoroloških podatkov. S pomočjo korekcijskih konstant pa lahko napovemo stopnjo alfa-kislin tudi za posamezen kultivar na ožjem pridelovalnem območju. Zgodnje napovedi stopnje alfa-kislin v storžkih hmelja so ključnega pomena tako za hmeljarje, kot tudi za trgovce s hmeljem – predvsem tam, kjer so tako pogodbene količine, kot tudi cene hmelja pogojene z vsebnostjo alfa-kislin pridelanih kultivarjev. Cena hmelja je lahko vezana na odbitke oz. pribitke glede na odstopanja od pogodbeno dogovorjenih vsebnosti alfa-kislin. Podobno pa so lahko tudi pogodbene količine hmelja pridelovalca pogojene z vsebnostjo alfa-kislin. S tem menimo, da bodo natančnejše zgodnje ocene kakovosti pridelka hmelja s pomočjo modela še dodatno vplivale na kakovostnejše podjetniško odločanje v proizvodno-prodajni verigi hmeljarstva.

V nadaljevanju bo moč model razširiti s korekcijskimi konstantami še za nove sorte hmelja. Na osnovi raziskave lahko zaključimo, da bi lahko napovedali stopnjo alfa-kislin v kultivarjih hmelja še v času razvoja storžkov. V nadaljevanju bo potrebno model še dodatno validirati in razširiti na osnovi analize ekstremnih meteoroloških dogodkov.

ZAHVALA

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PRISOTNOST RILČKARJEV V SLOVENSKIH HMELJIŠČIH

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IZVLEČEK

Hmeljev rilčkar (*Neoplinthus tigratus porcatus* Panzer) v zadnjih desetih letih ponovno postaja pomemben škodljivec v slovenskih hmeljiščih. To je povezano predvsem s spremembo podnebnih razmer kot tudi z ostalimi agrotehničnimi ukrepi (predvsem z zmanjšano uporabo kontaktnih insekticidov in neizvajanjem higienskih ukrepov). Lucernin rilčkar (*Otiorhynchus ligustici* L.) v slovenskih hmeljiščih ni pogosto prisoten. Za zatiranje hmeljevega in lucerninega rilčarja v hmelju trenutno nimamo na razpolago nobenega insekticida. Zelo toksične insekticide za zatiranje rilčarjev, katerih uporaba sedaj v hmeljiščih ni več dovoljena, bi bilo potrebno nadomestiti z novimi ukrepi, ki bodo okolju bolj prijazni. Nove metode zatiranja rilčarjev vključujejo tudi biotične pristope (entomopatogene ogorčice in glive), katerih praktično delovanje je potrebno preveriti v praksi.

Ključne besede: hmelj, *Humulus lupulus* L.*,* škodljivci hmelja, hmeljev rilčkar, *Neoplinthus tigratus porcatus*, lucernin rilčkar, *Otiorhynchus ligustici*

PRESENCE OF SNOUT WEEVILS IN SLOVENIAN HOP GARDENS

ABSTRACT

The hop snout weevil (*Neoplinthus tigratus porcatus* Panzer) becoming again important pest in Slovenian hop gardens in the last ten years. The increasing appearance is connected with changing of climatic conditions and agrotechnical measures (reduction use of contact insecticides and non-performig hygienic measures). Alfalfa snout weevil (*Otiorhynchus ligustici* L.) isn't frequently present in Slovenia hop gardens. There is no insecticide available momentarily for control of hop and alfalfa snout weevils in hops. High toxic insecticides which were use for this purpose should be replaced with new measures, which are environmental friendly. New methods for control of weevils are includes biological accessions (entomopathogenic nematodes and fungi) which should be tested in practical conditions.

Key words: hop, *Humulus lupulus* L., hop pests, hop snout weevil, *Neoplinthus tigratus porcatus*, alfalfa snout weevil, *Otiorhynchus ligustici*

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1 UVOD

Rilčkarji so v večini primerov nadležni in trdovratni škodljivci, tako pri pridelavi zelenjave, okrasnih rastlin, kot tudi v vinogradništvu in sadjarstvu. Tudi v hmeljarstvu niso izjema, kjer so znani že vrsto let. Pri pridelavi hmelja v drugih evropskih državah običajno omenjajo lucerninega rilčkarja (*Otiorhynchus ligustici* L.) [5,7,12], kateri do sedaj ni veljal kot pomemben škodljivec hmelja. V mnogih pomembnih pridelovalkah hmelja v Evropi je v zadnjih letih njegova populacija zelo narasla. Z njim imajo težave na Češkem [11], v Nemčiji [2] kot tudi v Franciji. V Ameriki imajo na hmelju težave z drugimi vrstami rilčkarjev, ki povzročajo podobno škodo kot lucernin rilčkar [1]. V Sloveniji lucerninega rilčkarja redko najdemo v hmeljiščih, temveč se pogosto soočamo z drugo vrsto rilčkarja, tako imenovanega hmeljevega rilčkarja (njegov sinonim je tudi hmeljev hrošč), *Neoplinthus tigratus porcatus* Panzer = *Plinthus porcatus* [4,6]. Hmeljev rilčkar ni neznan škodljivec hmeljišč, saj se je prvič pojavil v večjem obsegu že leta 1893 v hmeljiščih v Savinjski dolini [3]. Najverjetneje se je preselil iz divjega hmelja na gojenega [3]. Hmeljev rilčkar je eden izmed najstarejših znanih škodljivcev hmelja, ki je v preteklosti na hmelju povzročal veliko škode, še posebej pred prvo svetovno vojno [4,6]. V zadnjih 50 letih se je pojavljal sicer vsako leto, vendar ne v (pre)velikem številu in na hmelju ni povzročal škode [13]. Nekoliko pogosteje in številčnejše se je hmeljev rilčkar pojavljal v hmeljiščih na Kozjanskem, kjer je konec šestdesetih in osemdesetih let, predvsem v letu 1988, povzročil znatno zmanjšanje pridelka ter propadanje številnih hmeljnih rastlin ter celih nasadov [6,13]. Po letu 2000 hmeljev rilčkar ponovno pridobiva na pomenu, saj ga zelo pogosto najdemo v hmeljiščih, predvsem v Savinjski dolini [8,9,13].

Odrasel hrošč lucerninega rilčkarja na hmelju izjeda luknje v mladih poganjkih, ki se ob vetru pogosto prelomijo. Njihove ličinke objedajo korenine hmelja oziroma povzročajo črvivost trt [12]. Pri hmeljevem rilčkarju povzročajo največjo škodo ličinke, ki se zarijejo v trte hmelja in rijejo proti koreniki ter s tem povzročajo črvivost trt [3,4,6,10]. Objedena korenika in trta ima za posledico slabši in manj kakovosten pridelek hmelja, pri močnem napadu pa posamezne rastline lahko tudi propadejo [4,6].

2 MATERIAL IN METODE

2.1 Spremljanje hmeljevega in lucerninega rilčkarja v hmeljiščih ob rezi hmelja

V letu 2008 smo v začetku aprila, v času rezi hmelja, v naključno izbranih hmeljiščih v Savinjski dolini, Ptujsko-Ormoškem območju ter na Koroškem ugotavljali prisotnost ličink hmeljevega in lucerninega rilčkarja. V vsakem hmeljišču, ne glede na velikost, smo naključno pregledali 50 rastlin. Pri vsaki rastlini smo prešteli število poganjkov, ugotavljali število poškodovanih trt ter prisotnost ličink v trtah. Določili smo vrsto ličink ter njihov razvojni stadij.

Pri pregledu sadik hmelja B certifikata (CS_B) smo spomladi leta 2009 ugotavljali poškodovanost sadik (podzemnih trt), ki so jih poškodovale ličinke rilčkarjev. Sadike so bile nabrane v certificiranih matičnih hmeljišč (CMH). Iz vsakega hmeljišča smo naključno pregledali 3 % narezanih sadik. Zabeležili smo starost nasada, sorto hmelja in način izvajanja fitosanitarnih-higienskih ukrepov.

2.2 Spremljanje hmeljevega in lucerninega rilčkarja med vegetacijo

V letu 2009 smo konec aprila in v maju, v času čiščenja in navijanja hmelja, vizualno opazovali nasade hmelja in ugotavljali prisotnost odraslih rilčkarjev. Opazovanja smo opravljali zgodaj zjutraj in v oblačnem vremenu, saj je predvsem za hmeljevega rilčkarja znano, da ne mara svetlobe. Opazovanja so bila zahtevna še posebej zato, ker imajo hrošči na pokrovkah ostanke zemlje in jih je velikokrat težko opaziti. Opazovanja smo izvedli po naključju v 10 hmeljiščih v Savinjski dolini, v dveh hmeljiščih v Dornavi, enem v Vidmu pri Ptuju ter v dveh hmeljiščih v Trgovišču.

V enem hmeljišču v Trgovišču, kjer smo našli večje število odraslih hroščev lucerninega rilčkarja, smo ocenjevali njihovo prisotnost in povzročeno škodo na mladih hmeljnih poganjkih. Populacijo lucerninega rilčkarja smo ugotavljali na 100 rastlinah hmelja, na površini zemlje okrog rastlin, kot tudi v zemlji (5 cm globoko), kjer se rilčkarji pogosto zadržujejo preko dneva. Prav tako smo na 100 rastlinah ugotavljali delež napadenih poganjkov hmelja. Ocenjevanja smo v omenjenem hmeljišču izvedli 3-krat.

3 REZULTATI IN DISKUSIJA

3.1 Prisotnost ličink hmeljevega in lucerninega rilčkarja ob rezi hmelja

Pri pregledu podzemnih trt (ostanki po rezi hmelja) smo našli le ličinke hmeljevega rilčkarja. V nobeni trti nismo našli ličink lucerninega rilčkarja. Ličinki teh dveh rilčkarjev se poleg velikosti najbolj zanesljivo ločita po dlakavosti. Ličinka lucerninega rilčkarja je bolj dlakava kot ličinka hmeljevega rilčkarja. Drugače sta obe vrsti ličink snežno beli z rjavo glavo. V nasadih je bila različna napadenost podzemnih trt. Prva tako tudi vsaka poškodovana trta, ki je dajala izgled črvivosti, ni vsebovala ličink hmeljevega rilčkarja. Odstotek poškodovanih trt se je gibal od 0,3 do 60,0 in le 27,3 % poškodovanih trt je vsebovalo ličinke (preglednica 1). Ličinke hmeljevega rilčkarja so bile v različnih larvarnih (razvojnih) fazah in sicer od L1 do L4, najpogosteje pa sta bili zastopani L2 in L3 faza. Prisotnost ličink v nasadih hmelja je bila različna po območjih kot tudi glede na starost nasada in vrsto tal. Trenutno še ni mogoče najti povezave med poškodovanostjo hmelja z ličinkami hmeljevega rilčkarja, starostjo nasada ter vrsto tal (lastnost tal).

Preglednica 1: Prisotnost ličnik hmeljevega rilčkarja (*Neoplinthus tigratus porcatus*) v sadikah hmelja, v različnih hmeljiščih, spomladi leta 2008
Table 1: Presence of larvae's hop snout weevil, *Neoplinthus tigratus porcatus*, in cutting hop plants, on different hop gardens, in spring 2008

Lokacija hmeljišča	Sorta hmelja	Starost nasada (leta)	Velikost nasada (ha)	Lastnosti tal	Število pregledanih trt	Poškodova-ne trte (%)	Trte, ki vsebujejo ličinke (%)
Šentrupert	AU	20	3,30	srednje težka	260	60,0	27,3
Arja vas	SG	5	1,50	lahka peščena	234	33,3	17,1
Orla vas	CEL	4	1,10	srednje težka	268	4,4	2,2
Poljče	AU	20	1,25	lahka peščena	254	20,4	2,8
Žalec	AU	7	5,00	srednje težka	322	0,3	0,0
Trgovišče	AU	20	2,80	peščena	274	1,8	1,5

Legenda: AU-Aurora; SG-Savinjski golding; CEL-Celeia

Spomladi leta 2009 smo pregledali sadike hmelja B certifikata (CS_B) in ugotavljali vrsto rilčkarjev in odstotek poškodovanih sadik. Pri pregledih smo ugotovili prisotnost ličink le hmeljevega rilčkarja. Ličink lucerninega rilčkarja nismo našli v nobenem nasadu, tudi na Ptujsko-Ormoškem območju, kjer obstaja večja verjetnost pojava lucerninega rilčkarja. Poškodovanost sadik od hmeljevega rilčkarja je bila različna glede na območje, sorto hmelja kot tudi starost nasada. Rezultate monitoringa ličink hmeljevega rilčkarja podajamo v preglednici 2. V bodoče bomo z monitoringom nadaljevali, saj želimo ugotoviti interakcije z napadenostjo in prisotnostjo/odsotnostjo ličink hmeljevega rilčkarja glede na lokacijo pridelave, vrsto tal, sorto hmelja, starost nasada, izvajanje/neizvajanje fitosanitarnih-higienskih ukrepov hmeljarjev, ipd.

Preglednica 2: Prisotnost ličink hmeljevega rilčkarja (*Neoplinthus tigratus porcatus*) v certificiranih sadikah hmelja (CS_B), april 2009
Table 2: Presence of larvae of hop snout weevil (*Neoplinthus tigratus porcatus*) in certified hop materials (CS_B), April 2009

Območje	Lokacija	Sorta hmelja	Starost nasada (leta)	Poškodova-nost sadik (%)	Opomba
Savinjska dolina	Dolenja vas	Aurora	7	20-30	
	Drešinja vas	Bobek	1	0	
	Drešinja vas	Aurora	7	0	
	Drešinja vas	Celeia	5	80-100	ostanke po rezi odstranjujejo že več let
	Gomilsko	Aurora	10	0	
	Gotovlje	Aurora	9	80-100	ostanke po rezi redno odstranjujejo
	Gotovlje	Celeia	6	0	ostanke po rezi odstranjujejo že več let
	Grajska vas	Aurora	8	0	
	Migojnice	Dana	3	40-50	ostanke po rezi odstranjujejo že več let
	Migojnice	Aurora	12	40-50	ostanke po rezi odstranjujejo že več let
	Parižlje	Aurora	14	80-100	ne odstranjuje ostankov po rezi
	Parižlje	Aurora	10	20-30	
	Poljče	Aurora	13	0	
	Prekopa	Aurora	10	0	
	Prebold	Aurora	15	0	
	Šempeter	Aurora	10	0	
	Šempeter	Aurora	8	0	
	Topovlje	Aurora	10	80-100	
	Topovlje	Aurora	8	100	
	Topovlje	Savinjski golding	9	80-100	
	Vransko	Celeia	12	30-40	ostanke po rezi odstranjujejo
Koroška	Radlje ob Dravi	Savinjski golding	6	0	
	Radlje ob Dravi	Aurora	7	0	
	Radlje ob Dravi	Celeia	7	0	
	Šmartno pri Slov. Gradcu	Aurora	11	0	
Ptujsko-Ormoško	Polskava pri Pragerskem	Bobek	2	0	
	Videm ob Ptuju	Aurora	5	20-30	

Iz preglednice 2 je razvidno, da ličink hmeljevega rilčkarja nismo našli na Koroškem pridelovalnem območju, ne v Slovenj Gradcu kot tudi ne v Radljah. Prisotnost hmeljevega rilčkarja je največja na območju Savinjske doline, kjer je tudi največja intenziteta hmeljišč, najmanjša pa na Ptujsko-Ormoškem območju. V več kot polovici pregledanih hmeljišč so bile prisotne ličinke hmeljevega rilčkarja, od tega pa je bila v več kot 44 % hmeljišč stopnja napadenosti sadik večja od 30 odstotkov. V nekaterih hmeljiščih je bil odstotek poškodovanih sadik tudi do 100 % (preglednica 2). V 60 letih prejšnjega stoletja je bilo na območju Savinjske doline poškodovanih do največ 25 % trt [4]. Populacija hmeljevega rilčkarja je tako v Savinjski dolini skokovito narasla.

Na podlagi podatkov monitoringa ni mogoče opaziti razlik v preferenci hmeljevega rilčkarja glede na sorto hmelja. Sprva smo domnevali, da je prisotnost hmeljevega rilčkarja pogojena s starostjo nasada, vendar je to bolj izjema kot pravilo. Če je na določeni lokaciji oziroma območju rilčkar prisoten, potem je lahko napadeno tudi mlajše hmeljišče (npr. sorta Dana stara 3 leta). Na prisotnost hmeljevega rilčkarja vpliva hmeljar z rednim in doslednim izvajanjem fitosanitarnih-higienskih ukrepov, kamor sodi pobiranje ostankov po rezi hmelja, odvoz ostankov iz nasada ter uničenje le-teh na primernem mestu. Pri uničenju mislimo na sežig, saj ličinka hmeljevega rilčkarja preživi v trti, tudi če je ta izpostavljena soncu [6, 10]. Za zatiranje hmeljevega rilčkarja, predvsem njenih ličink, trenutno ni registriran noben insekticid. Sicer gre za talnega škodljivca, katerega ličinke so v notranjosti rastlin in v takih primerih je uporaba insekticidov z vidika varovanja okolja kot tudi njihove učinkovitosti, zelo sporna. Zatiranje odraslih hroščev hmeljevega rilčkarja je skoraj nemogoče, saj jih ne najdemo na listih hmelja. Za razliko od lucerninega rilčkarja, ki se prehranjuje s poganjki hmelja.

3.2 Ugotavljanje populacije rilčkarjev v hmeljiščih med vegetacijo

Ko smo opravljali vizualne preglede hmeljišč v Savinjski dolini smo na nekaterih lokacijah našli le posamezne hrošče hmeljevega rilčkarja. Nikjer nismo opazili poškodb na listih hmeljnih rastlin. Opazovanja so sicer zelo nezanesljiva, ker je hmeljev rilčkar nočni škodljivec in se preko dneva zadržuje tik pod površjem zemlje ali v zemlji. Prav tako velja tudi za lucerninega rilčkarja, vendar smo ga kljub temu preko dneva zasledili na spodnjih delih rastline.

V Trgovišču pri Ormožu smo v letošnjem letu v enem hmeljišču, kjer je bil posajen hmelj sorte Aurora prvo leto, našli večje število odraslih hroščev lucerninega rilčkarja. V tem nasadu je lucernin rilčkar povzročil poškodbe na mladih poganjkih hmelja. Konec aprila in v začetku maja smo v povprečju na rastlino našli 1,8 odraslega hrošča lucerninega rilčkarja (preglednica 3). Hmeljišče je obdano z gozdom in po pripovedovanjih pridelovalca je bil lucernin rilčkar v tem hmeljišču prisoten tudi v preteklosti. Njegova populacija je verjetno velika zaradi sosednjih njiv, kjer so v preteklosti gojili sladkorno peso, ki je tudi gostiteljica lucerninega rilčkarja. Na starejših hmeljnih rastlinah lucernin rilčkar ni povzročal posebne škode, za razliko od prvoletnih rastlin, kjer je bila v povprečju poškodovana skoraj polovica poganjkov na rastlino (preglednica 3).

Preglednica 3: Povprečno število odraslih hroščev lucerninega rilčkarja (*Otiorhynchus ligustici*) in odstotek poškodovanih poganjkov hmelja (*Humulus lupulus*) na rastlino v različnih obdobjih ocenjevanja v letu 2009

Table 3: Average No. of alfalfa weevil adults (*Otiorhynchus ligustici*) and percentage damaged hop sprouts (*Humulus lupulus*) on plant in different dates of assessment in 2009

Datumi ocenjevanj	povp. število rilčkarjev/rastlino	% poškodovanih poganjkov/rastlino
17. april 2009	1,8	54,0
20. april 2009	1,2	55,2
4. maj 2009	1,3	38,6

Populacija lucerninega rilčkarja je bila v hmeljišču zelo različna. Ob robu hmeljišča, ki meji z gozdom je bilo rilčkarja zaslediti v večjem številu, kar je posledica gojenja sladkorne pese v preteklosti.

4 ZAKLJUČKI

S pridobljeni podatki spremljanja hmeljevega rilčkarja (*Neoplinthus tigratus porcatus*) in lucerninega rilčkarja (*Otiorhynchus ligustici*) lahko zaključimo:

- Hmeljev rilčkar je v velikem številu zastopan na območju Savinjske doline, v manjšem na Ptujsko-Ormoškem območju, nismo pa ga zasledili na Koroškem.

- Lucerninega rilčkarja smo našli v večjem obsegu le na eni lokaciji in sicer v Trgovišču, kjer so odrasli hrošči povzročali škodo na mladih poganjkih hmelja.

- V podzemnih delih stebla (sadikah) hmelja smo našli le ličinke hmeljevega rilčkarja.

- Pri hmeljevem rilčkarju lahko rečemo, da ne delajo škode odrasli hrošči, temveč samo ličinke, ki delajo krivuljaste hodnike v notranjosti stebel in tudi v koreniki ter tako povzročajo t.i. »črvivost trt«.

- Populacija hmeljevega rilčkarja je po vsej verjetnosti narasla zaradi neupoštevanja fitosanitarnih-higienskih ukrepov, zmanjšane uporabe insekticidov s kontaktnim načinom delovanja, spremenjenih podnebnih razmer, idr.

- Za zmanjšanje populacije je potrebno dosledno izvajanje fitosanitarnih-higienskih ukrepov, kamor sodi redno pobiranje ostankov po rezi hmelja in odvoz ter uničenje ostankov izven hmeljišč. Najbolj zanesljiv ukrep je sežiganje le-teh, ali zasip z najmanj 50 cm tal na primernih deponijah.

- Uporaba insekticidov je z vidika varovanja okolja kot tudi njihove slabe učinkovitosti nesprejemljiva, zato bo potrebno v bodoče preveriti možnost in učinkovitost uporabe biotičnega zatiranja ličink hmeljevega rilčkarja.

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UPORABA DAZOMETA ZA RAZKUŽEVANJE HMELJNEGA KOMPOSTA

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IZVLEČEK

Pridelovanje hmelja ustvarja veliko organske mase v obliki hmeljevine, ki se po obiranju pridelka pogosto odlaga na različnih deponijah. Na deponijah, kjer se je hmeljevina nalagala več let, so po letih razgradnje nastale velike količine preperelega komposta. Z odstranitvijo ostankov vodil in razkuževanjem, ki je potrebno zaradi prisotnosti škodljivih organizmov, bi lahko takšen kompost uporabili kot vir rastnih substratov. V prispevku predstavljamo poskus v katerem smo določali učinkovitost razkuževanja preperelega hmeljnega komposta s fitofarmacevtskim pripravkom Basamid Granulat, ki vsebuje aktivno snov dazomet. Učinkovitost razkuževanja smo določali s spremljanjem kaljenja plevelov, populacije prostoživečih ogorčic in vpliva na infekcijski potencial gliv *Verticillium dahliae* in *Fusarium solani*.

Ključne besede: hmelj, *Humulus lupulus* L., hmeljevina, kompostiranje

APPLICATION OF DAZOMET FOR DESINFECTION OF HOP COMPOST

ABSTRACT

Hop growing made a lot of organic mass in a form of hop waste which is often put to different dumps after harvest. On dumps where hop waste was put for many years a large quantities of fully decayed compost was formed. By the removal of remains of strings and disinfection, which is necessary to eliminate harmful organisms, such compost could be used as a source of different growing substrates. The manuscript presents efficacy trial of disinfection of hop compost by using phytopharmaceutical product Basamid Granulat, which contains active ingredient dazomet. The disinfection efficacy was evaluated by analysis of germination rate of weeds seeds, nematode population and influence on *Verticillium dahliae* and *Fusarium solani* infection potential.

Key words: Hop, *Humulus lupulus* L., hop waste, composting

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1 UVOD

Pridelovanje hmelja ustvarja veliko organskih odpadkov v obliki hmeljevine, ki se po obiranju pridelka pogosto odlaga na različnih deponijah. Zaradi obremenjevanja okolja s smradom, ostanki vodil in kurjenja, prenašanja bolezni ter neprimernih lokacij odlagališč, hmeljevina še vedno predstavlja problem, ki je aktualen že nekaj desetletij. Ker hmeljevina po drugi strani predstavlja bogat vir hranil in organske mase je zelo zanimiva za nadaljnjo uporabo, s čimer se je v preteklosti ukvarjalo več raziskovalcev [6]. Možne rešitve se ponujajo v proizvodnji organskih gnojil in substratov, proizvodnji bioplina, uporabi v obliki kuriva in celo živinske krme.

V praksi je najpogostejša rešitev nekaj mesečno kompostiranje hmeljevine in kasnejše zaoravanje na poljedelske površine. Obstajajo pa tudi deponije, kamor se je hmeljevina nalagala več let, kjer so po letih razgradnje nastale velike količine popolnoma preperelega komposta. Z odstranitvijo ostankov vodil, kar omogočajo sodobni ločevalni stroji, ter izpostavitvi razkuževalnim postopkom, bi se takšen kompost lahko nadalje uporabili kot vir rastnih substratov. Prav tako bi lahko hmeljevina v prihodnosti postala pomemben vir organske mase v komercialnih kompostarnah.

Kompost je lahko vir različnih bolezni, škodljivcev in plevelov, seveda v odvisnosti od okuženosti primarnega substrata in načina kompostiranja. Med povzročitelji rastlinskih bolezni se lahko s kompostom prenašajo bakterije (*Erwinia carotovora, Xanthomonas campestris, Ralstonia solanacearum, Clavibacter michiganensis*), virusi (tobacco mosaic virus, tomato mosaic virus), oomicete (*Pytium* spp., *Phythophthora* spp.) in glive, med katerimi največ okužb povzročajo vrste *Fusarium oxysporum, F. solani, Verticillium albo-atrum, V. dahliae in Sclerotinia sclerotiorum* [7], prenašajo pa se lahko tudi nekateri rastlinski škodljivci, na primer rastlinsko parazitske ogorčice. V nekaterih kompostih pa lahko odkrijemo tudi živalske in humane patogene organizme [4].

Pri kompostiranju večjih kupov organske mase je termofilna faza prvi proces pri katerem zaradi visokih temperatur (40-70°C) prihaja do izrazitega odmiranja patogenih organizmov. Če ta proces ni nadzorovan ali pravilno izveden lahko določen del organizmov preživi, zato je v nadaljnjih korakih potrebno izvesti še dodatne postopke, ki vključujejo fizično (para, UV in gamma žarki) ali kemično razkuževanje, kjer se največkrat uporabljajo pripravki na osnovi aktivnih snovi kot so metil-bromid, metam-natrij, kloropikrin in dazomet.

V letu 2009 smo na IHPS postavili poskus, v katerem smo kemično razkuževali večje količine preperelega hmeljnega komposta s fitofarmacevtskim pripravkom Basamid Granulat, ki vsebuje aktivno snov dazomet. Učinkovitost razkuževanja smo določali s spremljanjem kaljenja plevelov, populacije prostoživečih ogorčic in vpliva na infekcijski potencial gliv *Verticillium dahliae* in *Fusarium solani*.

2 MATERIAL IN METODE

2.1 Kompost in postopek razkuževanja

V poskusu, ki je potekal na deponiji IHPS, smo razkuževali 80 m^3 preperelega komposta, ki je nastal z večletno razgradnjo hmeljevine. Pred razkuževanjem je bil kompost mehansko premešan in presejan s premičnim bobenskim sitom (Primus; Komptech GmbH Frohnleiten,

Avstrija), ki je odstranil ostanke vodil. Opravljena je bila tudi kemična analiza, katere rezultati so predstavljeni v preglednici 1. Površino širine 8 m in dolžine 20 m (160m^2) namenjeno razkuževanju komposta smo najprej prekrili s PVC folijo. Sledil je enakomeren nasip 25 cm plasti komposta, na katero smo posuli pripravek Basamid Granulat (98% aktivne snovi dazomet; Kanesho soil treatment SPRL/BVBA) v odmerku 62,5g/m^2. Po uporabi pripravka smo nasuli še 25 cm komposta tako, da je debelina kupa znašala 50 cm. Celotno površino kupa smo dobro zalili, prekrili in zatesnili s PVC folijo. Poskus je potekal od 1. do 31. avgusta, ko so bile povprečne dnevne temperature 20,9ºC (min. 14,8ºC; max. 27,8ºC) (meritve avtomatske meteorološke postaje ADCON; lokacija IHPS).

Preglednica 1: Rezultati kemične analize hmeljnega komposta v času postavitve poskusa
Table 1: Results of chemical analysis of hop compost in the time of setting experiment

Parameter	**Enota**	**V sveži snovi**	**V zračno suhi snovi**	**V suhi snovi**
Vlaga v sveži snovi	%	49,8	-	-
Vlaga v zračno suhi snovi	%	-	6,0	-
pH v KCl	-	-	7,5	-
P_2O_5 - lahkodostopni	mg/100g	337,5	632,0	672,3
K_2O - lahkodostopni	mg/100g	84,6	158,5	168,6
CaO - lahkodostopni	%	5,11	8,57	10,18
Mg – lahkodostopni	mg/100g	211,9	396,8	422,1
P - celokupni	%	0,23	0,42	0,45
K - celokupni	%	0,12	0,23	0,24
Ca - celokupni	%	5,47	10,24	10,89
Mg - celokupni	%	0,72	1,35	1,44
N - celokupni	%	1,01	1,90	2,02

2.2 Določanje fungicidne aktivnosti

Usmerili smo se v kvantitativno določanje aktivnosti biocida dazomet na glivi *Verticillium dahliae* in *Fusarium solani* s pomočjo mikrobioloških sond. Osnovo sond so predstavljale 150 ml vreče, ki smo jih izdelali iz štirih slojev visoko kakovostne poliestrske tkanine (velikost por 16 µm; Sefar), v katerih se je nahajal inokulum posamezne glive. Inokulum smo pripravili iz posušenih delov 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 izolatov obeh gliv, ki smo jih predhodno namnožili v tekočem umetnem gojišču (General fungal medium) z eno (1) tedensko inkubacijo na rotacijskem stresalniku. Sledila je štiri (4) tedenska inkubacija v temi pri sobni temperaturi s tedenskim mešanjem, da smo zagotovili enakomerno razraščanje glive po trtah. V vsako sondo smo vnesli 10, z micelijem dobro preraščenih 5 cm trt. V času razkuževanja smo na petih različnih mestih kompostnega kupa v razdalji 2 m vnesli po 1 sondo vsake glive na višino 0 cm, 25 cm in 50 cm. Sondam smo pred in po 10 dnevnem razkuževanjem določili infekcijski potencial (CFU/g) s tehniko serijskih redčitev na modificiranem Komada gojišču [1]. Pri tem smo posamezno sondo razdeli na dva podvzorca (a/b) in ju ločeno analizirali.

2.3 Določanje herbicidne aktivnosti v obliki uničevanja semena

Pred razkuževanjem smo na šestih različnih mestih še neurejenega kompostnega kupa odvzeli vzorce, s katerimi smo napolnili šest lončkov prostornine 4 L. Enako vzorčenje smo naredili

20 dni po razkuževanju, vendar smo po 2 vzorca na različnih mestih kompostnega kupa odvzeli na višini 0 cm, 25 cm in 50 cm. Vse lončke smo v izogib zunanje semenske kontaminacije prenesli v rastno komoro (Kambič, RK-13300) in jih vzdrževali pri naslednjih pogojih: 12-urna fotoperioda fluorescentne svetlobe (L 58W/77; Fluora, Osram); 70% relativna zračna vlaga; dnevna temperatura 20º C, nočna temperatura 15º C. V vsakem lončku smo po dvo mesečnem gojenju v rastni komori prešteli in s herbološkimi ključi determinirali vzkaljene rastline.

2.4 Določanje nematocidne aktivnosti

Pred razkuževanjem smo na dveh različnih mestih še neurejenega kompostnega kupa odvzeli vzorce za kvantifikacijo in determinacijo začetne populacije ogorčic. Sledilo je vzorčenje dvajset dni po razkuževanju. Na treh različnih mestih kompostnega kupa smo odvzeli vzorce na višinah 0 cm, 25 cm in 50 cm. Odvzete vzorce komposta smo v laboratoriju premešali in odvzeli reprezentativen podvzorec prostornine 100 cm^3, katerega smo analizirali. Izločanje celokupne populacije ogorčic iz podvzorcev komposta smo opravili z metodo vrtinčenja [5]. Izločene ogorčice smo zbrali v 1 ml vode in jih za 20 sekund izpostavili temperaturi 65°C v vodni kopeli. Dodali smo 1 ml fiksativa TAF (raztopina trietanolamin-formalin) in ohladili na 4°C. Fiksiranim ogorčicam smo določili redovni in podredovni sestav s pomočjo determinacijskih ključev in jih kvantificirali z uporabo mikroskopske lupe. Namen ločevanja ogorčic na osnovi redu in podredu je bil ugotoviti delež prostoživečih ogorčic, v katere uvrščamo rastlinske parazite ter rastlinam neškodljive - saprofitske ogorčice.

3 REZULTATI IN DISKUSIJA

3.1 Fungicidno delovanje

V poskus smo vključili glivi *V. dahliae* in *F. solani*, ki spadata talne fitopatogene organizme s širokim spektrom gostiteljskih rastlin. Infekcije z omenjenima glivama opazimo kot odmiranje in venenje rastlin, pri čemer gliva *V. dahliae* močno prizadene in kolonizira rastlinski prevodni sistem, pri vrsti *F. solani* pa prihaja do gnitja korenin in koreninskega vratu. Glivi sta fakultativna organizma in se lahko prenašata z odmrlimi ostanki rastlin, prav tako pa tudi s kompostom, katerega vir so bile okužene rastline. V primeru glive *V. dahliae* so vir širjenja in ohranjanja trajni organi mikrosklerociji, ki omogočajo glivi preživeti tudi več kot 10 let v odsotnosti gostiteljskih rastlin, medtem ko *Fusarium solani* preživi neugodne razmere v obliki klamidospor [8,9].

Pred razkuževanjem kompostnega kupa smo v 4 ponovitvah izmerili začetno stanje infekcijskega potenciala v sondah za vsako glivo. Pri glivi *V. dahliae* smo določili povprečni infekcijski potencial sond 63,6 x 10^4 CFU/g, medtem ko je pri glivi *F. solani* znašal 306 x 10^4 CFU/g. V času razkuževanja smo na petih različnih mestih in višinah (0 cm, 25 cm in 50 cm) kompostnega kupa vnesli sonde in jih ponovno analizirali po 10 dneh razkuževanja.

Iz preglednice 2 je razvidno, da je pripravek Basamid Granulat v vseh 5-ih sondah uničil obe glivi na višini kompostnega kupa 25 cm in 50 cm. Na višini 0 cm sta glivi preživeli z delnim padcem infekcijskega potencila, pri čemer smo določili 83,7% učinkovitost (Abbott) pripravka za zatiranje glive *V. dahliae* in 50,4% za zatiranje glive *F. solani*.

Preglednica 2**:** Infekcijski potencial gliv *Verticillium dahliae* in *Fusarium solani* v sondah po 10 dnevnem razkuževanjem hmeljnega komposta z dazometom
Table 2: Infection potential of *Verticillium dahliae* and *Fusarium solani* in probes; 10 days after dazomet application

Gliva	**Višina komposta**	**Infekcijski potencial (CFU/g x 10^4)**					
		Sonda					**Povprečje**
		1	2	3	4	5	
Verticillium dahliae	0cm	27,6	0,06	2,16	16,4	5,9	10,4
	25cm	0	0	0	0	0	0
	50cm	0	0	0	0	0	0
Fusarium solani	0cm	50,4	188,0	140,0	216,0	164,0	151,7
	25cm	0	0	0	0	0	0
	50cm	0	0	0	0	0	0

3.2 Vpliv na kaljenje in uničevanje semena plevelov

Raziskave so pokazale, da ima dazomet negativen vpliv na kaljenje različnih plevelnih vrst, pri čemer je učinkovitost močno odvisna od odmerka in metod zadrževanja plinske faze, ki je toksična večini organizmov [2]. V našem poskusu smo določali učinkovitost pripravka Basamid Granulat na osnovi vzorcev odvzetih pred in 20 dni po razkuževanju hmeljnega komposta. Vse vzorce smo v izogib zunanje semenske kontaminacije prenesli v rastno komoro, kjer smo po 2 mesečnem gojenju določili vrsto in število plevelov na vzorec (preglednica 3).

Preglednica 3**:** Prisotnost plevelnih rastlin v hmeljnem kompostu pred in po 20 dnevnem razkuževanjem z dazometom
Table 3: Presents of weeds in hop compost before and after 20 days of dazomet desinfection

[x]Vzorec/ obravnavanje	**navadni plešec (CAPBP)**	**navadna zvezdica (STEME)**	**toga zajčja deteljica (OXAEU)**	**velika kopriva (URTDI)**	**bela metlika (CHEAL)**	**perzijski jetičnik (VERPE)**	**drobnocvetni rogovilček (GASPA)**	**Skupno št. plevelov/ vzorec**
1. nerazkuženo[a]	1	3	0	5	3	1	0	13
2. nerazkuženo[a]	0	1	0	5	6	1	0	13
3. nerazkuženo[a]	0	0	3	6	0	0	0	9
4. nerazkuženo[a]	0	2	0	2	1	0	1	6
5. nerazkuženo[a]	0	3	0	7	4	0	0	14
6. nerazkuženo[a]	0	0	0	4	4	0	0	8
1. razkuženo 0 cm[a]	2	1	1	6	0	0	0	10
2. razkuženo 0 cm[a]	0	0	0	3	2	0	0	5
3. razkuženo 0 cm[a]	0	0	2	2	1	0	0	5
4. razkuženo 25 cm[b]	0	0	0	3	0	0	0	3
5. razkuženo 25 cm[b]	0	0	0	10	1	0	0	11
6. razkuženo 25 cm[b]	0	0	0	4	0	0	0	4
7. razkuženo 50 cm[a]	0	0	0	5	1	0	1	7
8. razkuženo 50 cm[a]	0	1	0	4	2	0	2	9
9. razkuženo 50 cm[a]	0	0	0	7	1	0	0	8

[x]Skupine z enako črko pri posameznem obravnavanju se med seboj statistično značilno ne razlikujejo (Duncan-ov test mnogoterih primerjav, α = 5 %)
[x]Identical letter indicate no significant difference between group means (P>0.05) on test of Duncan

V kompostu smo potrdili prisotnost 7 različnih plevelnih vrst, med katerimi sta bili najbolj zastopani velika kopriva in bela metlika. Primerjava skupnega števila vzniklih plevelov na

posamezen vzorec pred in po razkuževanju ni pokazala statističnih razlik (ANOVA). Razlike v primerjavi raznovrstnosti plevelov na vzorec smo ugotovili med nerazkuženimi in razkuženimi vzorci na višini 25 cm, kjer je večino vzniklih rastlin predstavljala velika kopriva. Rezultati kažejo na inhibicijo pripravka Basamid Granulat na seme plevelov samo na višini 25 cm, kjer je bila nanesena plast pripravka, pri tem pa je nakazana neučinkovitost za uničevanje semena velike koprive.

3.3 Nematicidno delovanje

Začetna populacija (pred razkuževanjem) prostoživečih ogorčic je bila sestavljena iz naslednjih redov in podredov: Rhabditina, Cephalobina, Tylenchina, Aphelenchina, Enoplida, Dorylaimida ter Monchisterida. Rastlinsko parazitske ogorčice so uvrščene v redova Tylenchina in Aphelenchina ter v podred Dorylamida, katerih skupen delež v začetni populaciji je bil 38,4 %, medtem ko je bil delež saprofitskih ogorčic 57,9 % (preglednica 4).

Preglednica 4**:** Redovni in podredovni sestav populacije ogorčic v vzorcih komposta pred in po razkuževanju hmeljenega komposta z dazometom
Table 4: Order and sub-order population structure of nematodes in samples of compost before and after dazomet application

	Rhabditina	Cephalobina	Tylenchina	Aphelenchina	Enoplida	Dorylaimida	Monhysterida	Skupno število ogorčic	% fitoparazitov	% saprofitov
nerazkuženo – zač. populacija	39	14	23	4	2	10	3	93	38,4	57,9
razkuženo 0 cm	156	16	0	0	0	1	0	173	0,4	99,6
razkuženo 25 cm	119	37	2	0	0	0	1	158	1,1	98,9
razkuženo 50 cm	517	430	7	13	0	2	3	972	2,2	97,8

Nematološke analize vzorcev razkuženega komosta so pokazale, da se je število saprofitskih ogorčic znatno povečalo in sicer iz 56 osebkov v 100 g komposta na 172 osebkov na 0 cm, 329 na 25 cm in 950 na 50 cm. Število fitoparazitskih ogorčic pa se je zmanjšalo z začetnih 37 na le en osebek pri 0 cm, na 2 osebka pri 25 cm ter na 22 osebkov pri 50 cm.

Ugotovili smo, da je razkuževanje komposta z dazometom le delno učinkovalo na prostoživeče ogorčice. Neposrednega učinka dazometa na saprofitske vrste ne moremo ugotoviti, ker je pri večini teh vrst značilno zelo hitro razmnoževanje v ugodnih pogojih (razvojni krog pri posameznih vrstah lahko traja le 3 – 4 dni). Porast števila osebkov saprofitskih vrst lahko pripišemo ugodnim razmeram (večja vlažnost in temperatura v kompostnem kupu) po končanem delovanju aktivne snovi. Na rastlinsko parazitske ogorčice je imelo razkuževanje najboljši učinek na višini 0 in 25 cm, medtem ko je bil učinek dazometa na višini 50 cm slabši, kjer je bila smrtnost rastlinsko parazitskih ogorčic približno polovična.

4 ZAKLJUČEK

Dazomet (tetrahidro-3,5-dimetil-1,3,5-tiadiazin-2-tion) je široko uporabljena aktivna snov, ki se v kmetijstvu uporablja predvsem za razkuževanje tal že od leta 1970 naprej, najdemo pa ga tudi v industriji pri proizvodnji kartona, izdelkov iz gume in različnih strojnih transmisijskih elementov. Z razgradnjo (hidrolizo) dazometa nastane ditio-karbaminska kislina in plin metil-izotiocianat, ki sta toksična za večino organizmov. Iz omenjenega je učinkovitost dazometa močno povezana s talno vlago, temperaturo in tipom tal. Pri uporabi pripravka Basamid Granulat, ki vsebuje 98% dazometa, se priporočeni odmerki za razkuževanje tal gibljejo med 40-60 g/m^2 in zagotavljajo učinkovanje do globine 20-25 cm. V primerih razkuževanja tal ali substratov v kupih se na folijo nasuje plast debeline 20-25 cm, po njej pa se enakomerno posuje 50-70 g/m^2 pripravka. Sledi nova plast debeline 20-25 cm, pri čemer postopek ponavljamo do višine kupa enega (1) m in ga nato nepredušno zapremo s folijo. Skupno se tako na 1 m^3 porabi 200-300 g pripravka [3].

V poskusu, ki smo ga izvedli na IHPS, smo po opisanem postopku razkuževali preperel hmeljni kompost, pri čemer smo uporabili pripravek Basamid Granulat v odmerku 62,5 g/m^2, ki smo ga aplicirali med dve 25 cm plasti komposta. Pri tem smo določali učinkovitost pripravka s spremljanjem vpliva na talni glivi *V. dahliae* in *F. solani*, semena plevelov in vpliva na populacijo prostoživečih ogorčic.

Rezultati so pokazali 100 % učinkovitost pripravka oz. popolno uničenje kultur izolatov gliv *V. dahliae* in *F. solani* na višini kupa 25 in 50 cm, medtem ko so kulture v sondah na višini 0 cm preživele in je bila učinkovitost pripravka na osnovi padca infekcijskega potencila pri glivi *V. dahliae* 83,7 %, pri glivi *F. solani* pa 50,4 %. Pri uničevanju semena plevelov nismo ugotovili razlik med številom vzniklih plevelov na posamezen vzorec pred in po razkuževanju. Učinkovitost dazometa smo zaznali samo pri primerjavi raznovrstnosti vzklilih plevelov med nerazkuženimi in razkuženimi vzorci na višini kupa 25 cm, kjer so preživela le semena velike koprive.

Analize ogorčic so pokazale, da dazomet vpliva na pestrost nematopopulacije v tleh, pri čemer se je v našem primeru močno zmanjšal delež rastlinsko parazitskih ogorčic. Ugotovljeno je bilo tudi, da je delovanje dazometa z ozirom na globino oziroma višino sproščanja precej neenakomerno. Določen delež ogorčic preživi in v ugodnih okoljskih razmerah pride do precejšnjega porasta populacije saprofitskih vrst.

Na osnovi analiz lahko zaključimo, da v poskusu uporabljeni odmerek 62,5 g pripravka/m^2 v z raztrosom na višini 25 cm ni dosegel želenega učinka v vseh plasteh kompostnega kupa. Slabše delovanje razlagamo z dejstvom, da smo razkuževali preperel kompost, ki je vseboval skoraj 100 % vsebnost organskih snovi z visokim deležem humusa, kar močno vpliva na slabše delovanje dazometa. Prav tako je v primeru uničevanja semena plevelov učinkovitost mnogo višja, ko uničujemo že klijoča semena plevelov, kar dosežemo z nekaj dnevnim predhodnim navlaževanjem, česar v našem poskusu nismo izvedli.

Iz omenjenega sklepamo, da je za učinkovito razkuževanje tovrstnih kompostov potrebno uporabiti višje odmerke pripravka v več plasteh, s čimer bi dosegli sproščanje toksičnih substanc v višjih koncentracijah z enakomernejšo razporeditvijo. Prav tako bi bilo potrebno opraviti predhodno navlaževanje komposta. Za popolno uničevanje fitopatogenih gliv bi v našem poskusu zadostovali že dve plasti pripravka v priporočenem odmerku 60-70 g/m^2 na višini kupa 0 cm in 25 cm, medtem ko bi za učinkovitejše zatiranje semena plevelov in

ogorčic bilo potrebno aplicirati pripravek na vseh treh višinah (0, 25 in 50 cm) v odmerku tudi do 80 g/m^2. Dejansko preverbo učinkovitosti priporočenega postopka bo potrebno ovrednotiti z nadaljevanjem poskusov in analiz.

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SPREMLJANJE JABOLČNEGA ZAVIJAČA (*Cydia pomonella* L.) NA OBMOČJU CELJSKE REGIJE OD LETA 2004 DO 2009

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IZVLEČEK

Spremljanje in napoved pojava ter razvoja škodljivih organizmov v sadjarstvu temelji na spremljanju in vrednotenju tako biotičnih kot abiotičnih dejavnikov in deluje v okviru Opazovalno napovedovalne službe Slovenije, ki je organizirana v pet regijskih centrov. Jabolčni zavijač je gospodarsko najpomembnejši stalni škodljivec jablan, ki ga je potrebno zatirati. Dinamiko razvoja jabolčnega zavijača spremljamo s pomočjo biotehničnih pripomočkov, vrednotenja meteoroloških spremenljivk in opazovanj v naravi. Omenjene metode spremljanja nam omogočajo dokaj natančno določitev optimalnega časa zatiranja jabolčnega zavijača. Ta pristop je edina pot k zmanjševanju ostankov fitofarmacevtskih sredstev v samih pridelkih kot tudi v tleh in okolju. V članku je podrobnejše predstavljen način spremljanja jabolčnega zavijača na območju Celjske regije.

Ključne besede: jabolčni zavijač, *Cydia pomonella* L., feromonska vaba, insektarij, sadjarstvo

MONITORING OF CODLING MOTH (*Cydia pomonella* L.) IN CELJE REGION IN YEARS 2004 TO 2009

ABSTRACT

Monitoring and forecasting about occurrence and development of harmful organisms in fruit growing is based on evaluation of biotic and abiotic factors and take an active part in the Observation and forecasting service of Slovenia, which is organized in five regional centers. Codling moth, the most important permanent pest of apple orchards, can cause high yield losses, so it needs to be suppressed. Development of codling moth is observed using biotechnical instruments, evaluation of meteorological data and field observations. These modes of monitoring help us to predict the need and optimal time for control of codling moth in apple orchards. By means of a good forecasting system this is the only way to reduce the use of plant protection products (PPP) and also to reduce the accumulation of PPP in fruits, soil and environment. In this article the modes of codling moth monitoring in Celje region are presented.

Key words: codling moth, *Cydia pomonella* L., pheromon trap, insectarium, fruit growing

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1 UVOD

Na območju Celjske regije, kjer jabolka predstavljajo večino sadjarske proizvodnje, predstavlja jabolčni zavijač gospodarsko najpomembnejšega škodljivca, zato so tudi metode opazovanja in napovedovanja obsežne in natančne. Spremljanje in zagotavljanje meteoroloških podatkov, podatkov o pojavu in razširjenosti škodljivega organizma, izdelava in izdaja priporočil ter ukrepov za preprečevanje njihovega širjenja je namenjeno tržnim pridelovalcem sadja in drugim ljubiteljskim sadjarjem.

Podnebne spremembe močno vplivajo na pojav posameznih gospodarsko pomembnih škodljivcev v sadjarstvu. V spremenjenih podnebnih razmerah se bo pri številnih škodljivcih pojavljalo večje število generacij na leto. Pričakujemo lahko predvsem zgodnejše pojavljanje škodljivih vrst, ki jih bomo sledili še v pozno jesen. Znano je, da ima jabolčni zavijač pri nas dva rodova letno [7] vendar v zadnjih letih, ko so se povprečne temperature precej dvignile, strokovnjaki domnevajo, da naj bi se pojavil tudi tretji rod, kar pa še ni dokazano [5].

Jabolčni zavijač (*Cydia pomonella* L.) sodi v družino zavijačev in sukačev (Tortricidae), uvrščamo ga med zavijače plodov [7]. Sodi med najbolj razširjene in znane škodljivce jabolk ter hrušk in orehov, včasih napade tudi breskve, marelice in slive. Škodo, ki jo povzroča iz leta v leto niha, doseže tudi 20 % ali več črvivih plodov.

Metuljček meri čez krila 15 do 22 mm, prednja krila so pepelnato sive barve z več prečnimi rjavimi črtami. Ob zunanjem robu prednjih kril je značilna rjava pega z dvema kovinsko svetlečima lisama v obliki oklepajev po katerih ga ločimo od drugih vrst zavijačev. Komaj izlegla gosenica, je belkasta s črno glavo in meri v dolžino 1,4 mm. V razvoju preide 5 razvojnih stadijev in ko doraste doseže velikost med 18 do 20 mm. Ko se gosenica zabubi je dolga približno 1 cm in se nahaja v belem zapredku [7].

Prezimijo gosenice v belih zapredkih v razpokah na lubju na deblu in debelejših vejah, včasih pa tudi na opornih stebrih. V aprilu in maju se zabubijo, prvi metuljčki pa se običajno pojavijo v prvi ali drugi dekadi maja, ko temperature dosežejo več kot 15 °C [7]. Njihov pojav se v povprečju ujema s fenološko fazo BBCH 67 - 69 (intenzivno odpadanje venčnih listov). Kako dolgo živijo je odvisno od temperature. Živijo teden ali dva, če je hladneje tudi 23 do 30 dni [1]. Metuljčki postanejo aktivni zvečer, ko letajo in se parijo, samice pa odlagajo jajčeca. Samica prične odlagati jajčeca, ko znaša vsota efektivnih temperatur pri pragu 10 °C okrog 200 do 210 °C. Embrionalni razvoj traja v odvisnosti od toplote 8 do 18 dni. Gosenica se zavrta v plod in v njem preživi tri do štiri tedne. Nato zapusti plod in si poišče skrivališče v razpokah skorje, kjer se zaprede. Gosenice, ki sklenejo razvoj do konca julija, se v belih zapredkih zabubijo in iz njih izletijo metuljčki drugega rodu. Tiste, ki pa niso sklenile razvoja do takrat v zapredkih prezimijo skupaj z gosenicami drugega rodu [7].

2 MATERIAL IN METODE DELA

Spremljanje in napoved pojava ter razvoja škodljivih organizmov v sadjarstvu temelji na spremljanju in vrednotenju tako biotičnih kot abiotskih dejavnikov. Dinamiko razvoja jabolčnega zavijača spremljamo s pomočjo biotehničnih pripomočkov, vrednotenja agrometeoroloških spremenljivk in vizualnega opazovanja. Omenjene metode spremljanja nam omogočajo dokaj natančno določitev optimalnega časa zatiranja jabolčnega zavijača.

2.1 Spremljanje pojava jabolčnega zavijača z biotehničnimi pripomočki

2.1.1 Metoda zbiranja zapredkov z valovito lepenko in spremljanje izleta metuljčkov iz insektarija

Zapredke gosenic jabolčnega zavijača zbiramo s pomočjo valovite lepenke s katero ovijemo debla neškropljenih jablan in sicer za prvi rod v prvi dekadi junija, za drugi rod pa v začetku avgusta. Svitke lepenke, kamor se skrijejo in zabubijo gosenice, prenesemo v insektarij. Po pojavu prvega metuljčka v insektariju (običajno, ko je dosežena vsota 100 °C pri pragu 10 °C) vsakodnevno spremljamo izlet metuljčkov. Iz podatkov izdelamo krivuljo leta metuljčkov prve in druge generacije. Podatki nam v prvi vrsti služijo kot informacija o začetku in koncu leta metuljev posamezne generacije. S krivuljo leta metuljčkov se določi tudi vrh leta in v povezavi z ostalimi metodami spremljanja napove optimalen čas zatiranja tega škodljivca.

2.1.2 Metoda spremljanja pojava jabolčnega zavijača s feromonskimi (seksualnimi) vabami

Enostavna in najbolj razširjena metoda spremljanja pojava jabolčnega zavijača je lov metuljčkov s feromonskimi vabami (proizvajalec Pherocon, Serbios). Podatki ulovov se beležijo na 3 dni oziroma dvakrat na teden. Na podlagi ulovov metuljčkov na feromonskih vabah je določen tudi orientacijski prag škodljivosti. V dobrih pogojih za let metuljev je prag škodljivosti 7 do 10 metuljčkov na vabo na teden za prvi in 5 do 7 metuljčkov na vabo na teden za drugi rod. Poleg jabolčnega zavijača na ta način spremljamo tudi breskovega in češpljevega zavijača ter ostale zavijače lupine sadja, listne in lesne zavrtače, zapredkarje in steklokrilke.

V biološki pridelavi sadja postaja uporaba feromonov oziroma metoda zbeganja ali konfuzija ena izmed vodilnih metod zatiranja jabolčnega in breskovega zavijača. Omenjena metoda se uveljavlja tudi v integrirani pridelavi in se uporablja kot del strategije varstva proti omenjenim škodljivcem.

2.2 Spremljanje in vrednotenje agrometeoroloških spremenljivk

V Sloveniji je pet regijskih centrov. Centri so opremljeni z enotnim agrometeorološkim sistemom postaj Adcon Telemetry. Regijski center Celjske in Koroške regije ima 13 agrometeoroloških postaj. Za potrebe sadjarske proizvodnje so postaje postavljene na sadjarskih lokacijah: Črnova, Braslovče, Kasaze, Ponikva, Frankolovo in Bistrica ob Sotli. Vse postaje so opremljene s senzorji za spremljanje osnovnih vremenskih podatkov kot so: temperatura zraka, relativna zračna vlaga, padavine in trajanje omočenosti listne mase. Ti podatki predstavljajo vhodne spremenljivke za delovanje modelov za napoved škodljivih organizmov. Podatki nam prav tako omogočajo spremljanje vsote temperaturnih pragov in so eden izmed orodij za lažje in bolj natančno izdajanje napovedi pojavnosti škodljivih organizmov. Pri spremljanju in napovedovanju zatiranja jabolčnega zavijača se poslužujemo podatkov o temperaturni vsoti pri pragu 10 °C. Do neke mere lahko spremljamo let metuljčkov s pomočjo spremljanja večernih temperatur, kader te ne dosegajo 15 °C, ni nevarnosti odlaganja jajčec.

2.3 Vizualna opazovanja

Vizualno opazujemo fenološki razvoj jablan in pojav posameznih razvojnih stadijev škodljivcev in poškodb, ki jih le-ti povzročajo. Na območju Celjske regije imamo vzpostavljeno mrežo fenoloških opazovanj po različnih lokacijah in posameznih sortah jablan. V mrežo opazovanj so vključene tudi vse sadjarske lokacije, kjer imamo postavljene agrometeorološke postaje. Opazovanja, nabiranje in pregledovanje naključnih vzorcev v nasadih so osnova za določanje preseganja praga gospodarske škode in izdelave strategije zatiranja jabolčnega zavijača.

3 REZULTATI IN DISKUSIJA

Na območju Celjske regije beležimo v zadnjih šestih letih začetek leta prve generacije jabolčnega zavijača v naravi od konec aprila do začetka prve dekade meseca maja. V tem času so jablane običajno že dosegle vrh cvetenja (BBCH 65) in prehajajo v fenološko fazo (FF) intenzivnega odpadanja listja (BBCH 67) ali pa se je cvetenje ravno zaključilo (BBCH 69). Vsota efektivnih temperatur pri pragu 10 °C ($\Sigma T_{10\ °C}$) je tedaj okoli 80 °C ali pa se tej vrednosti približuje, kar je razvidno tudi iz podatkov v preglednici 1.

Preglednica 1: Datumi pojava prvih metuljčkov jabolčnega zavijača na feromonskih vabah ter prvih in zadnjih metuljčkov v insektariju, Žalec, 2004 – 2009 [2,3,4,8,9]
Table 1: Dates of occurrence of first adult codling moths trapped into pheromone traps and dates of first and last trapped adult moths in insectarium, Žalec, 2004 – 2009 [2,3,4,8,9]

leto	pojav 1. metulja na feromonski vabi	$\Sigma T_{10°C} = 80$ °C	FF ob pojavu 1. metulja v naravi	ulov 1. metulja v insektariju	ulov zadnjega metulja v insektariju
2009	27. april	25. april	67 - 69	20. maj	21. julij
2008	5. maj	11. maj	69	14. .maj	7. julij
2007	26. april	27. april	69	8. maj	17. julij
2006	04. maj	03. maj	65	16. maj	8. julij
2005	02. maj	01. maj	65 - 67	16. maj	27. julij
2004	06. maj	03. maj	67	21. maj	21. julij

Literatura navaja, da se let prve generacije jabolčnega zavijača začne pri vsoti 100 °C efektivnih temperatur. Po naših podatkih se na območju Celjske regije let metuljčkov prvega rodu v naravi začne nekoliko prej, v povprečju pri vsoti 80 °C kot nam za lokacijo Žalec kažejo ulovi na feromonskih vabah. Pri vsoti efektivnih temperatur 100 °C se po naših podatkih začnejo loviti metuljčki prvega rodu jabolčnega zavijača v insektariju. Znano je, da se samci pojavijo prej kot samice (protandrija) [7], zato se tudi na feromonske vabe ujamejo prej, kot se pojavijo prvi metuljčki v insektariju. Čas trajanja leta prve generacije je odvisen od vremenskih razmer. V ugodnih vremenskih razmerah je vrh leta hitro dosežen in se tudi hitro zaključi. V primeru hladnega in deževnega vremena v mesecu juniju pa se let prve generacije zavleče, kar kažejo tudi podatki za lokacijo Žalec (preglednica 1). Na območju Celjske regije se let prve generacije jabolčnega zavijača v povprečju zaključi sredi meseca julija. Let druge generacije se po naših podatkih začne, ko je dosežena vsota 650 °C (preglednica 2 in 3) in se zaključi konec meseca avgusta ali pa se zavleče v prvo dekado meseca septembra.

Preglednica 2: Datumi pojava prvih metuljčkov jabolčnega zavijača drugega rodu v insektariju, Žalec, 2006 – 2009 [2,3,4]
Table 2: Dates of occurrence of first adult codling moths of second generation trapped in insectarium, Žalec, 2006 – 2009 [2,3,4]

leto	pojav 1. metulja v insektariju 2. rod
2009	19. 7.
2008	15. 7.
2007	5. 7.
2006	17. 7.

Preglednica 3: Datumi doseženih temperaturnih vsot pri pragu 10 °C ($\Sigma T_{10°C}$) na sadjarskih lokacijah: Kasaze, Ponikva, Črnova, Braslovče, Frankolovo, kjer so postavljene agrometeorološke postaje Adcon Telemetry, 2004 - 2009 [2,3,4,8,9]
Table 3: Dates of sums of effective temperature at threshold 10°C ($\Sigma T_{10°C}$) on fruit growing locations: Kasaze, Ponikva, Črnova, Braslovče, Frankolovo, where meteorological stations Adcon Telemetry are placed, 2004 – 2009 [2,3,4,8,9]

$\Sigma T_{10°C}$	80 °C	250 °C	300 °C	350 °C	600 °C	650 °C
2009	25.4.–26. 4.	22. 5.–24. 5.	27. 5.–2. 6.	6. 6.–10. 6.	4. 7.–12. 7.	10. 7.–16. 7.
2008	11. 5.–14. 5.	1. 6.–4. 6.	7. 6.–10. 6.	13. 6.–17. 6.	6. 7.–9. 7.	10. 7.–12. 7.
2007	27.4.–30. 4.	22. 5.– 4. 5.	30. 5.– 3. 6.	5. 6.–7. 6.	25. 6.–30. 6.	3. 7.–5. 7.
2006	3. 5.–5. 5.	7. 6.–9. 6.	14. 6.–16. 6.	18. 6.–19. 6.	7. 7.–8. 7.	12. 7.–13. 7.
2005	30. 4.–1. 5.	29. 5.– 30. 5.	3. 6.– 7. 6.	13. 6.–15. 6.	6. 7.–10. 7.	13. 7.–14. 7.
2004	1.5.– 3. 5.	2. 6..–9. 6.	12. 6.–15. 6.	19. 6.–21. 6.	15. 7.–18. 7.	18. 7.–22. 7.

V preglednici 3 so podani podatki o pomembnih vsotah efektivnih temperatur (prag 10°C) za razvoj jabolčnega zavijača. Pri tem je zelo pomemben podatek o natančnem razvojnem stadiju škodljivca, saj so skupine insekticidov (predvsem zaviralci razvoja insektov in insekticidov MAC skupine) za zatiranje jabolčnega zavijača zelo selektivne in delujejo na točno določene razvojne stadije.

Orientacijske vsote efektivnih temperatur za napoved uporabe posameznih skupin insekticidov [5, 7] za zatiranje so:

- 230-250 °C uporaba zaviralcev razvoja insektov,
- 260 °C uporaba insekticidov MAC skupine ali klornikotinilne skupine,
- 300 °C uporaba kontaktnih insekticidov.

Glede na naše rezultate spremljanja pojava jabolčnega zavijača, fenološkega razvoja jablan in meteoroloških podatkov ugotavljamo, da je strategija zatiranja v zadnjih letih šla v smeri povečanja števila škropljenj. Tako je iz treh prešla na povprečno pet do šest škropljenj na leto, kar ugotavljajo tudi strokovnjaki KGZS – Zavod Maribor [5]. Vzroke gre pripisati predvsem višjim temperaturam in posledično segrevanju ozračja. Temperature so na letnem nivoju za več kot 1 °C višje od dolgoletnega povprečja.

4 ZAKLJUČKI

Jabolčni zavijač je gospodarsko najpomembnejši stalni škodljivec jablan. Pri strategiji zatiranja jabolčnega zavijača je pomembno upoštevati vse omenjene metode spremljanja.

Napoved optimalnega časa zatiranja obeh rodov jabolčnega zavijača je izdelana na podlagi korelacije med pojavom metuljev v insektariju, ulovih na feromonskih vabah, vsoto efektivnih temperatur pri pragu 10 °C in spremljanju razvoja škodljivca v naravi.

Na območju Celjske regije se začne prvi rod jabolčnega zavijača pojavljati konec meseca aprila ali v začetku prve dekade meseca maja. Po naših podatkih se prvi metuljček pojavi, ko je dosežena vsota efektivnih temperatur 80 °C pri pragu 10 °C. Dolžina leta je odvisna od vremenskih razmer v času leta in v povprečju traja do sredine meseca julija, kar sovpada z začetkom leta druge generacije, ki se zaključi konec avgusta ali prve dni septembra.

Strategija zatiranja jabolčnega zavijača je v zadnjih letih prešla iz treh na povprečno pet do šest škropljenj na leto, kar je vplivalo tudi na popuščanje delovanja določenih pripravkov.

Glede na zmanjšanje števila pripravkov za zatiranje jabolčnega zavijača in zagotavljanja antirezistentne strategije bo v prihodnosti potrebno omejiti število škropljenj na največ 4 škropljenja na leto. Strategija zatiranja mora poleg neposredne uporabe insekticidov vključevati tudi druge načina varovanja jablan kot so: metoda zbeganja (konfuzija), uporaba pripravkov na osnovi virusa granuloze ter biotičnih pripravkov (entomopatogene ogorčice …).

5 LITERATURA

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MOLEKULSKI MARKERJI V DIAGNOSTIKI IN GENETSKIH ANALIZAH FITOPATOGENIH GLIV

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IZVLEČEK

Molekulski markerji so z razvojem hibridizacijskih tehnik in polimerazne verižne reakcije (PCR) postali osnovno orodje v diagnostiki in genetskih analizah različnih organizmov. Uporabnost molekulskih markerjev je podana s predstavitvijo AFLP (amplified fragment length polymorphism) markerske tehnike, ki v zadnjem desetletju predstavlja najpogostejšo tehniko za vrednotenje genetske variabilnosti fitopatogenih gliv, ter SCAR (sequence characterized amplified region) markerjev, ki so namenjeni predvsem diagnostičnim analizam. Prispevek je usmerjen tudi podrobnejši predstavitvi molekulskih analiz gliv *Verticillium albo-atrum* in *V. dahliae*, ki so omogočile razjasnitev odnosov med izolati iz različnih gostiteljskih rastlin, ter razvoj novih diagnostičnih analiz.

Ključne besede: AFLP, SCAR, *Verticillium* spp.

MOLECULAR MARKERS IN DIAGNOSTICS AND GENETIC ANALYSIS OF PHYTOPATHOGENIC FUNGI

ABSTRACT

With the development of hybridisation techniques and polymerase chain reaction (PCR), molecular markers became primary tool in diagnostics and genetic analysis of different organisms. The usefulness of molecular markers is presented through the novel molecular technique AFLP (amplified fragment length polymorphism), which is the most frequently used technique for assessing the genetic variability of phytopathogenic fungi in the last decade, and SCAR (sequence characterized amplified region) markers, which are used in diagnostics analysis. The manuscript also presents a detailed review of the molecular analysis of *Verticillium albo-atrum* and *V. dahliae*, which has managed to clarify the relationships among different isolates and to develop new diagnostic analysis.

Key words: AFLP, SCAR, *Verticillium* spp.

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1 UVOD

Molekulski markerji so z razvojem hibridizacijskih tehnik in *in vitro* namnoževanja DNA v polimerazni verižni reakciji (PCR) postali osnovna tehnika v diagnostiki in proučevanju variabilnosti organizmov. Veliko taksonomskih in filogenetskih študij pri glivah je bilo usmerjenih predvsem v analizo specifičnih lokusov ali posameznih delov genoma. Tako pomemben delež raziskav predstavljajo analize ribosomske DNA (rDNA), saj ta kompleks genov vsebuje variabilne in ohranjene regije, prav tako pa je prisoten v več ponovitvah in zato lahko določljiv. Kodirajoča 18S podenota ribosomskih genov spada med evolucijsko ohranjene regije, ki se je pri glivah predvsem proučevala pri iskanju razlik med različnimi rodovi, medtem ko so se pri analizi razlik med vrstami analizirale ITS[1] in IGS[2] regije, ki so lahko zelo variabilne v DNA zaporedju [2]. Pri ločevanju posameznih vrst je bilo veliko raziskav usmerjenih tudi v analizo mitohondrijske DNA, za katero je znano, da ima višjo evolucijsko stopnjo v primerjavi z jedrno DNA [4]. Naslednje specifično tarčno mesto proučevanja predstavljajo ponovljiva DNA zaporedja, kot so mikrosateliti, za katere je značilna visoka stopnja mutacij in so primerni za vrednotenje genetske variabilnosti. Tako so bili različni oligonukleotidi z mikrosatelitno ali minisatelitno ponovitvijo uporabljeni kot multilokusne RFLP sonde pri proučevanju več kot 70 vrst gliv iz taksonomskih razredov Ascomycetes, Zygomycetes in Deuteromycetes [37]. Pomembno je omeniti tudi raziskave iskanja variabilnosti med glivami s ponovljivimi DNA zaporedji pridobljenimi iz bakteriofaga M13 bakterije *Esherichia coli* in ponovljivimi zaporedji REP[3] in ERIC[4], ki predstavljata znaten delež nekodirajoče DNA pri bakterijah [33].

V zadnjem desetletju pri identifikaciji in vrednotenju variabilnosti gliv ter ostalih organizmov prevladujejo predvsem RAPD in AFLP markerji, katerih osnovo predstavlja polimerazna verižna reakcija (PCR) in katerih vrednost se izraža v hkratni analizi več lokusov v posamezni reakciji. RAPD tehnika temelji na uporabi enega samega začetnega oligonukleotida s poljubnim za proučevan organizem nespecifičnim zaporedjem, ki ob prileganju na bližnja nasproti si orientirana mesta na DNA molekulah sproži namnoževanje v PCR reakciji. RAPD markerji so izkazali primernost predvsem pri vrednotenju genetske variabilnosti med ozko sorodnimi organizmi, lahko pa jih uporabimo tudi pri taksonomskih in filogenetskih študijah [8]. AFLP predstavlja novejšo molekulsko tehniko in je podrobneje predstavljena v naslednjem poglavju.

2 AFLP MARKERJI PRI ANALIZAH FITOPATOGENIH GLIV

AFLP tehniko so razvili in patentirali leta 1993 ter dve leti kasneje tudi objavili [36]. Metoda temelji na selektivnem PCR namnoževanju restrikcijskih fragmentov, ki so produkt razreza genomske DNA z restrikcijskimi endonukleazami. Stopnja variabilnosti med organizmi je določena z odkrivanjem razlik v prepoznavnih mestih restrikcijskih encimov, ki nastajajo s spremembami v zaporedju nukleotidov. AFLP tehnika tako združuje lastnosti hibridizacijskih tehnik kot je RFLP in možnosti *in vitro* namnoževanja DNA v PCR reakciji. Prednost AFLP tehnike se izraža v občutljivosti odkrivanja polimorfizma in analizah organizmov ne glede na njihov izvor, kompleksnost ali velikost genoma. AFLP zajame celoten genom organizma in omogoča hkratno analizo večih lokusov v eni analizi. Prav tako omogoča povečanje števila

[1] angl. Internal Transcribed Spacer
[2] angl. Intergenic Spacer
[3] angl. Repetitive extragenic palindromic sequence
[4] angl. Enterobacterial repetative intergenic consensus

proučevanih lokusov, saj lahko v analizi spreminjamo restrikcijske encime in povečujemo število kombinacij začetnih oligonukleotidov [36].

Možnost odkrivanja genetske variabilnosti med ozko sorodnimi organizmi, ki jo ponuja AFLP analiza se pri proučevanju fitopatogenih gliv uporablja predvsem pri identifikaciji različno virulentnih izolatov ali patotipov ter populacijskih študijah izolatov določene vrste. Majer in sod. [19] so prvi uporabili AFLP tehniko pri proučevanju gliv, ki povzročajo rastlinske bolezni. V analizi so proučevali izolate glive *Cladosporium fulvum* in *Pyrenopeziza brassicae* iz različnih geografskih območij. Pri tem so identificirali visok nivo polimorfizma med izolati obeh gliv, vendar ne poročajo o povezavah polimorfizma z virulenco ali izvorom izolatov. AFLP analiza se je izkazala za zelo učinkovito pri proučevanju sorodnosti izolatov glive *Cercospora zeae-maydis* iz Združenih držav Amerike in Afrike. Omenjena gliva parazitira koruzo in je že več desetletij razširjena v ZDA, kjer se pojavlja v obliki patotipa I in II, v Afriki pa se je prvič pojavila leta 1988. Z AFLP so primerjali izolate iz obeh kontinentov in v analizi uporabili restrikcijska encima *Eco*RI in *Mse*I ter radioaktivno vizualizacijo namnoženih produktov. Ugotovili so razlike med obema patotipoma iz ZDA in visok nivo sorodnosti afriških izolatov s patotipom II [10].

V zadnjih letih se je število raziskav fitopatogenih gliv z AFLP markerji precej povečalo in po zadnjih podatkih (Web of Science®) dosega število več kot 150 znanstvenih člankov v revijah s faktorjem vpliva SCI (Science Citation Index). Kot pregled uporabnosti v preglednici 1 navajamo nekaj vzorčnih primerov prvih raziskav fitopatogenih gliv z AFLP markerji.

Preglednica 1: Pregled nekaterih prvih raziskav fitopatogenih gliv z AFLP markerji
Table 1: Review of some of the first AFLP analysis of phytopathogenic fungi

Gliva	Namen analize	Restrikcijski encimi	Vir
Cochliobolus sativus	Določanje razlik med različno virulentnimi izolati	*Eco*RI/*Mse*I	[39]
Colletotrichum lindemuthianum	Določanje razlik med različno virulentnimi izolati	*Eco*RI/*Mse*I	[11]
Diaporthe/Phomosis helianthi	Določanje razlik med izolati iz različnih geografskih območij	*Eco*RI/*Mse*I	[32]
Eutypa lata *Eutypa armeniaceae*	Določanje razlik med izolati iz različnih geografskih območij	*Eco*RI/*Mse*I	[9]
Fusarium oxysporum	Določanje razlik med različnimi specializiranimi formami (*formae specialis*)	*Eco*RI/*Msp*I	[1]
Macrophomina phaseolina	Določanje razlik med izolati iz različnih geografskih območij	*Eco*RI/*Mse*I	[34]

3 SCAR MARKERJI

AFLP in RAPD tehniki se uporabljata predvsem za analize genomov, vendar metodološko nista primerni za izvajanje rutinskih analiz. Pretvorba specifičnih RAPD ali AFLP markerjev v SCAR markerje pa lahko zelo poveča njihovo uporabnost [38]. SCAR marker (Sequence characterized amplified region) predstavlja DNA fragment določenega lokusa v genomu, ki se lahko identificira s PCR namnoževanjem s specifičnimi začetnimi oligonukleotidi. Razvoj SCAR markerjev temelji na določitvi nukleotidnih zaporedjih specifičnih RAPD ali AFLP markerjev, katera so nato osnova za izdelavo SCAR začetnih oligonukleotidov. Bistvena prednost pretvorbe RAPD ali AFLP markerjev v SCAR markerje se izraža v identifikaciji samo enega lokusa, manjši občutljivosti PCR namnoževanja na reakcijske pogoje, hitrosti in enostavnosti analize ter potencialu, da postanejo kodominatni markerji [27].

V diagnostiki rastlinskih patogenov SCAR markerji predstavljajo nove možnosti pri identifikaciji povzročiteljev bolezni, saj relativna enostavnost analize omogoča hitro in natančno detekcijo mikroorganizmov v različnih medijih kot so tla, voda in rastline, kar postavlja nove okvire pri proučevanju rastlinskih bolezni. Tako so Manzanares-Dauleux in sod. [20] opravili pomembno delo pri določanju patotipov glive *Plasmodiophora brassicae*, ki okužujejo križnice in povzročajo bolezen golšavost kapusnic. Omenjena skupina je odkrila RAPD marker, ki je specifičen za zelo virulenten patotip P1 te glive. Odkrit marker so klonirali ter mu določili nukleotidno zaporedje na osnovi katerega so razvili začetne oligonukleotide. Pri testiranju velikega števila izolatov so ugotovili namnoževanje SCAR markerja samo pri izolatih patotipa P1, kar potrjuje njegovo vrednost pri identifikaciji omenjenega patotipa. Razvit SCAR marker so nato optimizirali za identifikacijo v okuženem rastlinskem tkivu. Podobno so Hermosa in sod. [13] na osnovi RAPD analize razvili SCAR marker za določanje patotipa 11 glive *Trichoderma atroviride*, ki je patentiran za biotično zatiranje različnih talnih gliv. Omenjen marker sedaj omogoča lažje spremljanje dinamike patentiranega patotipa na poskusnih poljih.

4 MOLEKULSKE ANALIZE GLIV *VERTICILLIUM ALBO-ATRUM* IN *V. DAHLIAE*

Angleška raziskovalca Carder in Barbara [5] sta leta 1991 med prvimi uporabila molekulske tehnike pri določanju genetske variabilnosti med različnimi izolati vrst *V. albo-atrum*, *V. dahliae*, *V. tricorpus*, *V. nubilum*, *V. nigrescens* in *V. lecanii*. Pri tem sta v analizo vključila razrez genomske DNA z *Eco*RI restrikcijskim encimom in RFLP metodo, kjer sta za iskanje polimofizma uporabila hibridizacijo s sondami rDNA iz konoplje, mtDNA iz *V. albo-atrum*, ter naključnimi genomskimi sondami iz glive *V. albo-atrum*. Z neposredno vizualizacijo razreza genomske DNA na agaroznem gelu sta opazila razlike med *V. albo-atrum* in *V. dahliae* ter med ostalimi vrstami, vendar analiza zaradi prisotnosti velikega števila fragmentov ni bila vedno jasna. Glavno odkritje te raziskave pa predstavljajo rezultati RFLP analize z genomskimi sondami, s katerimi sta pri vrsti *V. albo-atrum* ugotovila razlike med izolati iz lucerne (skupina L) in izolati iz ostalih gostiteljskih rastlin (skupina NL), kar je potrjevalo predhodne analize o fiziološki specializaciji izolatov iz lucerne [12].

Barbara in njegova skupina so nato nadaljevali raziskave z RFLP metodo v smeri določanja genetskih razlik med izolati glive *V. dahliae*. Pri tem so v analizo vključili širok spekter izolatov, ki je vključeval tudi fiziološko specializirane izolate iz mete in diploidne izolate *V. dahliae* var. *longisporium*, ki večinoma parazitirajo križnice. Hibridizacijo po Southernu so izvedli z 71 naključnimi genomski sondami pridobljenimi iz izolata *V. dahliae* in ostalimi sondami iz predhodnih raziskav. Rezultati analize so potrdili specifičnost izolatov iz mete (skupina M) in diploidnih izolatov (skupina D). Ostali izolati *V. dahliae* so se razvrstili v dve osnovni skupini A in B ter v vmesno skupino I (intermediate), ki pa se za razliko od skupin M niso D ujemale s patogenostjo izolatov, njihovim geografskim izvorom in ostalimi lastnostmi [25,26].

Pri določanju genetske variabilnosti je bilo veliko analiz usmerjenih k proučevanju nekodogenih variabilnih regij med rDNA geni. Tako so Nazar in sod. [24] z določitvijo nukleotidnega zaporedja ITS regij med izolatom *V. albo-atrum* iz lucerne in izolatom *V. dahliae* iz sončnic odkrili nehomologijo v ITS 1 in ITS 2 regijah. Na osnovi razlik so nato razvili specifični hibridizacijski sondi in PCR začetne oligonukleotide, ki se uporabljajo v diagnostiki obeh vrst. Ista skupina raziskovalcev je uporabila specifične začetne

oligonukleotide pri testiranju kanadskih izolatov gliv *V. albo-atrum*, *V. dahliae* in *V. tricorpus* iz krompirja. Pri tem so odkrili izolat *V. albo-atrum*, ki ni dal pozitivnega signala po PCR reakciji. Omenjenemu izolatu so določili nukleotidno zaporedje ITS regij in ugotovili razliko v 17 baznih parih v primerjavi z ostalimi izolati *V. albo-atrum*. Na osnovi teh razlik so nato razvili specifične začetne oligonukleotide in testirali še izolate *V. albo-atrum* iz Anglije in Nizozemske. Tudi med temi izolati so našli dve skupini izolatov *V. albo-atrum* iz krompirja, kar je dokazovalo odkritje nove podskupine te glive, ki se sedaj pojavlja pod oznako Grp2[5], medtem ko skupino Grp1 predstavljajo izolati ostalih gostiteljskih rastlin [31].

Morton in sod., [23] so v analizo ITS regij vključili izolate iz predhodno določenih RFLP skupin gliv *V. albo-atrum* (L in NL) in *V. dahliae* (A, B, I, M, D) [5,24,25] ter novo odkrito Grp2 skupino glive *V. albo-atrum*. Pri tem so z določitvijo nukleotidnega zaporedja ITS regij potrdili razliko med skupino izolatov *V. albo-atrum* iz lucerne (L) in izolati ostalih gostiteljskih rastlin (NL). Izolati *V. albo-atrum* so se razlikovali od *V. dahliae* v 5-6 nukleotidih. Slednji so se na osnovi razlike 6 nukleotidov razdelili na diploidne (D) in haploidne izolate (A, B, I, M). Pri skupini Grp2 *V. albo-atrum* so potrdili razliko 17 nukleotidov od ostalih izolatov te vrste in presenetljivo ugotovili podobnost z ITS zaporedjem glive *V. tricorpus* manj pomembne fitopatogene vrste.

Pomembne so tudi raziskave izolatov z RAPD markerji. Barasubiye in sod. [3] so prvi uporabili omenjeno metodo za vrednotenje genetske variabilnosti izolatov *V. albo-atrum*. V raziskavo so vključili 15 izolatov iz krompirja in 20 lucerne, ki so jih analizirali s petimi začetnimi oligonukleotidi. RAPD analiza je jasno ločila obe patogeni skupini izolatov in potrdila predhodne ugotovitve o različnosti izolatov iz lucerne. Do podobnih rezultatov sta z RAPD analizo prišla tudi Koike in Fujita [15] pri proučevanju japonskih izolatov glive *V. dahliae* in *V. albo-atrum* iz različnih gostiteljskih rastlin.

Obsežnejšo raziskavo Grp2 skupine sta opravila Mahuku in Platt [17], ki sta z RAPD metodo in razrezom IGS regije (IGS-RFLP) analizirala 21 izolatov *V. tricorpus* in 64 različnih izolatov *V. albo-atrum*, od katerih jih je 21 predstavljalo skupino Grp2. RAPD analiza je jasno določila razlike med vsemi tremi skupinami izolatov in določitev 34% povprečnega koeficienta sorodnosti med Grp2 in *V. tricorpus* ter 35% med Grp2 in ostalimi izolati *V. albo-atrum* (Grp1). Variabilnost je izrazila tudi analiza IGS regije, ki je prav tako jasno določila razlike med omenjenimi skupinami. Raziskovalca na osnovi rezultatov navajata možnost potrditve Grp2 skupine kot nove vrste rodu *Verticillium*, ki je najverjetneje nastala z hibridizacijo izolata *V. albo-atrum* iz skupine Grp1 in *V. tricorpus*.

Collins in sod. [7] so z AFLP metodo in analizo ITS regij proučevali izolate *V. dahliae*, ki parazitirajo križnice. Rezultati so določili dve podskupini diploidnih izolatov *V. dahliae* var. *longisporium*, od katerih jih večina predstavlja skupino imenovano α, ostali izolati pa skupino β. Določeni skupini nimata povezave s patogenostjo ali geografskim izvorom. Molekulska analiza je prav tako ločila haploidne izolate *V. dahliae* iz križnic od izolatov ostalih gostiteljskih rastlin, kar skupaj kaže na tri skupine izolatov, ki so prilagojeni parazitiranju križnic. Avtorji navajajo, da zaradi visoke variabilnosti skupin α in β izolatov *V. dahliae* var. *longisporium* predlagano ime za vse diploidne *V. longisporium* ni primerno.

Molekulski markerji so se izkazali tudi pri določanju razlik med različno virulentnimi izolati, ki so največkrat produkt vpliva monokulturnega gojenja poljščin. Tako so španski

[5] Grp: kratica angleške besede group (skupina)

raziskovalci z RAPD metodo ugotovili razlike med bolj (*D*) in manj (*ND*) virulentnima patotipoma glive *V. dahliae*, ki parazitirata bombaž in oljke. Identificiranim specifičnim markerjem so določili nukleotidno zaporedje na osnovi katerega so razvili za vsak patotip specifične začetne oligonukleotide (SCAR), s katerimi so nato analizirali več izolatov iz različnih geografskih območij. Analiza s SCAR markerji je identificirala virulentne izolate iz Kalifornije in Kitajske, kar potrjuje hipotezo, da je bil patotip *D* v Španijo prenesen najverjetneje iz ene od teh dveh držav [28]. Podobno raziskava je bila opravljena tudi v Sloveniji, kjer smo z optimizirano AFLP tehniko odkrili DNA fragmente, ki so specifični različno virulentnima hmeljnima patotipoma PG1 in PG2 glive *V. albo-atrum*, kar je potrjevalo ugotovitve testiranja virulence o prisotnosti dveh hmeljnih patotipov v slovenskih hmeljiščih in predstavlja prvo tovrstno poročilo o identifikaciji DNA fragmentov, ki so v povezavi z virulenco pri izolatih *V. albo-atrum* iz hmelja [30].

Z dosedanjimi raziskavami so raziskovalci določili razlike med fitopatogenimi vrstami gliv iz rodu *Verticillium* in razlike med nekaterimi izolati iz različnih gostiteljev. Na osnovi opisanih raziskav z molekulskimi metodami se tako pri *V. albo-atrum* priznavata dve osnovni skupini:

- Grp1, ki vključuje podskupino L (izolati iz lucerne) in NL (izolate ostalih gostiteljskih rastlin, kamor uvršamo tudi izolate iz hmelja) in
- Grp2, ki predstavlja posebno skupino izolatov iz krompirja.

Izolati glive *V. dahliae* kažejo višji nivo variabilnosti, ki pa razen diploidnih izolatov *V. dahliae* var. *longisporium* in haploidnih izolatov iz mete ter bombaža ne kažejo jasnih fizioloških skupin oz. prilagoditve na določeno skupino gostiteljskih rastlin.

4.1 Uporaba molekulskih metod v diagnostiki gliv *V. albo-atrum* in *V. dahliae*

Hitra in zanesljiva identifikacija povzročiteljev bolezni ima velik pomen pri preprečevanju nadaljnjega širjenja bolezni. Klasična diagnostika temelji predvsem na osnovi morfoloških lastnosti, uporabi selektivnih gojišč, patogenih testih, analizah vegetativne kompatibilnosti in razlikah v biokemičnih lastnostih. Omenjene metode so večinoma delovno zahtevne, dolgotrajne in podvržene vplivom okolja, kar otežuje zanesljivost identifikacije. Za določitev vrste na osnovi morfoloških lastnosti je pri glivah *V. albo-atrum* in *V. dahliae* potrebna vsaj 2-3 tedenska inkubacija. Prav tako določanje patotipov s patogenimi testi časovno zahteva najmanj 2-3 mesečno analizo. Vpeljava molekulskih metod, s katerimi lahko neposredno analiziramo genom proučevanega organizma in PCR tehnologije, pomeni pomembno dopolnitev obstoječih analitičnih metod.

Prva uporaba PCR metode za identifikacijo izolatov *V. albo-atrum* in *V. dahliae* je temeljila na izdelavi specifičnih začetnih oligonukleotidov, ki zaznajo razlike v ITS regijah rDNA genov [24]. Razviti začetni oligonukleotidi so omogočili širši razvoj diagnostičnih metod predvsem pri določanju prisotnosti omenjenih gliv v rastlinah in tleh. Tako so Hu in sod. [14] s pomočjo različnih standardov DNA razvili kvantitativno metodo določanja prisotnosti *V. albo-atrum* in *V. dahliae* v okuženih rastlinah lucerne in sončnic. Volossiouk in sod., [35] so prvi objavili neposredno PCR detekcijo glive *V. dahliae* v tleh. Pri tem so z namenom povečanja občutljivosti analize uporabili nested-PCR metodo, ki temelji na dveh zaporednih PCR reakcijah. Prvo namnoževanje so izvedli z začetnimi oligonukleotidi, ki so jih razvili iz nukleotidnih zaporedij ITS regij specifičnih za rod *Verticillium*, analizo pa nato nadaljevali s specifičnimi ITS začetnimi oligonukleotidi za glivo *V. dahliae*. Enako metodologijo so

Mahuku in sod. [18] uporabili pri določanju krompirjevih izolatov skupin Grp1 in Grp2 glive *V. albo-atrum* v rastlinah in tleh.

Carder in sod. [6] so razvili drugo skupino specifičnih začetnih oligonukleotidov, ki so temeljili na nukleotidnih zaporedjih naključnih genomskih sond, katere so uporabljali pri RFLP analizah izolatov *V. albo-atrum* in *V. dahliae* [5,24,25]. Pri tem so uspešno razvili specifične začetne oligonukleotide za določanje izolatov glive *V. dahliae* in izolatov skupine NL glive *V. albo-atrum*.

Pri *V. dahliae* so znani tudi začetni oligonukleotidi, ki so razviti na osnovi RAPD analiz. Li in sod. [16] so pri proučevanju izolatov gliv *V. albo-atrum*, *V. dahliae* in *V. tricorpus* odkrili RAPD marker, ki je specifičen samo izolatom *V. dahliae*. Na osnovi nukleotidnega zaporedja polimorfnega markerja so razvili začetne oligonukleotide, ki so ohranili svojo specifičnost pri testiranju širokega spektra različnih izolatov *V. dahliae* in nekaterih sorodnih gliv. Znani so tudi začetni oligonukleotidi, ki so specifični za določanje različno virulentnih patotipov D in ND na bombažu in oljki [28]. Omenjene začetne oligonukleotide so Mercado-Blanco in sod. [21,22] uporabili pri razvoju nested-PCR metode za določanje omenjenih patotipov v obeh gostiteljskih vrstah. V povezavi s prej omenjeno AFLP raziskavo različno virulentnih izolatov glive *V. albo-atrum* iz hmelja (poglavje 4), so bili v Sloveniji na osnovi patotipsko specifičnih AFLP fragmentov izdelani SCAR markerji za določanje zelo viruletnega patotipa PG2. SCAR markerji so omogočili nadaljnji razvoj multipleks in nested PCR diagnostične analize [29], vključeni pa so tudi v diagnostični protokol za določanje gliv *V. albo-atrum* in *V. dahliae* na hmelju, ki ga pripravlja EPPO (European Plant Protection Organisation).

Preglednica 2: Najpogosteje uporabljeni začetni oligonukleotidi v diagnostiki gliv *V. albo-atrum* in *V. dahliae*
Table 2: The most common primers used in diagnostic of *V. albo-atrum* and *V. dahliae*

Nukleotidno zaporedje (5'-3')	Specifičnost namnoževanja	Referenca
CCG GTA CAT CAG TCT CTT TA ACT CCG ATG CGA GCT GTA AT	*V. albo-atrum* Grp1 (ITS regija)	[24]
CCG GTA CAT CAG TCT CTA TA CAA CCG TTG CCG TACGAG AC	*V. albo-atrum* Grp2 (ITS regija)	[31]
CCG GTC CAT CAG TCT CTC TG ACT CCG ATG CGA GCT GTA AC	*V. dahliae* (ITS regija)	[24]
CTC ATA ACC CTT TGT GAA CC CCG AGG TCA ACC GTT GCC G	*Verticillium* spp. (ITS regija)	[35]
CAT GGA TAA CCG TGG TAA TT CCA TTC AAT CGG TAG TAG CG	*Verticillium* spp. (RFLP sonda)	[6]
ATG GAC CGA ACA GCT AGG TA TCT CAG ATA TAT GCT GCT GC	*V. albo-atrum* skupina NL (RFLP sonda)	[6]
CGG TGA CAT AAT ACT GAG AG GAC GAT GCG GAT TGA ACG AA	*V. dahliae* (RFLP sonda)	[6]
CAC ATT CAG TTC AGG AGA CGG A CCT TCT ACT GGA GTA TTT CGG	*V. dahliae* (RAPD marker)	[16]
CAT GTT GCT CTG TTG ACT GG GAC ACG GTA TCT TTT GCT GAA	*V. dahliae* patotip D (RAPD marker)	[28]
CAG GGG ATA CTG GTA CGA GAC G ATG AGT ATT GCC GAT AAG AAC A	*V. dahliae* patotip ND (RAPD marker)	[28]
GGTAAGACTCCTTACCGATGCTG ATTCACACGCTACATATCAAACA	*V.albo-atrum* hmeljni patotip PV1,genotip PG2 (AFLP marker)	[29]

5 ZAKLJUČEK

Pričetek uporabe molekulskih tehnik v genetskih analizah fitopatogenih gliv predstavlja prelomni korak, ki je omogočil pridobiti nova spoznanja pri razumevanju taksonomije, filogenije, razvoja virulence, patogenosti in interakcij med glivami ter rastlinami. Ob tem je prišlo do razvoja novih diagnostičnih analiz, ki so omogočile občutljivejšo, zanesljivejšo in hitrejšo identifikacijo fitopatogenih gliv. Ta napredek je močno obogatil tudi epidemiološke študije, saj sodobne diagnostične analize omogočajo detekcijo v različnih medijih kot so tla, voda in rastline, kar postavlja nove okvire pri proučevanju rastlinskih bolezni. Uporaba molekulskih tehnik bo po eni strani v prihodnosti bolj in bolj omogočala odkrivanje temeljnih spoznanj, po drugi strani pa bo na področju diagnostike vpeljevala nove standarde, ki bodo usmerjeni predvsem v visoko občutljivost, hitrost, enostavnost in zanesljivost analiz.

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