

# ***Results of Experiments Involving Chosen Stimulating Agents in Hop Growing***

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**EXECUTIVE SUMMARY:** The paper deals with the effects of the preparation Lexin (auxins + fulvic and humic acids), brassinosteroids and the preparation Pentakeep (5-amino-levullinic acid) upon the physiological conditions and yield of hops in the arid region of the North-West Bohemia. The monitored preparations appeared to have positive impact upon the formation of  $\alpha$ -acrid acids in hop cones. Treated plants showed generally better physiological condition resulting into a higher yield of cones compared to untreated reference sample.

**Key words:** Pentakeep-S, brassinosteroids, Lexin, hops, yield,  $\alpha$ -acrid acids, photosynthesis rate, transpiration rate

## **INTRODUCTION**

The hop growing has a long and far-famed tradition in the Czech Republic as well as the product it is used for – beer.

Given the premium quality and grade of the Czech hops, more specifically of the specie called “Žatecký poloranný červěňák” (Žatec’s semi-early red stalk hops), the Czech hop growing industry enjoys a unique and irreplaceable position both in domestic and global agriculture.

Czech hops were and still are being exported to approximately 70 countries on 5 continents (CHALOUPSKÝ, ŠTRANC 2006).

The hop growing in the Czech Republic is concentrated in three renamed hop growing regions (Žatec region, Ústěk region, Tršice region) out of which the largest, best-known and most important is the Žatec region. However, an essential part of this region is situated in the rain shadow of the Ore Mountains and Doupovské Hills. Therefore, the growth and development of hop plants is adversely influenced by droughts the number of which is significantly rising due to the ongoing global climatic changes (ŠTRANC et al. 2007a). This reduces the productivity of hop plants, the hop cone yield fluctuates and are generally lower and the content of acrid substance in cones keeps dropping, too. The final consequence of the previous is an unsatisfactory efficiency of the hops production.

What plays a key role in the mitigation of stress to which hop plants are exposed due to the precipitation deficit is the implementation of artificial irrigation. However, results of some of our experiments (ŠTRANC et al. 2003) indicate that the precipitation deficit related and low/high temperature related stress may be limited to a certain extent by the application of suitable stimulating agents respectively plant growth regulators. What has appeared for the time being as the most promising preparations are brassinosteroids, preparations Lexin and Pentakeep-S.

Brassinosteroids are quite recently discovered steroid-type phyto-hormones. Similarly to gibberelins, abscisic acid and some other steroid hormones, brassinosteroids belong to the family of terpenoids. For the first time, they were found in 1970 (in the U.S.) in the pollen of rape (*Brassica napus*) the Latin name of which gave the name to brassinosteroids (ŠTRANC et al. 2006).

The physiological effects of brassinosteroids reside first of all in the stimulation of the long growth of plants and improving their resistance against stress including but not limited to high and low temperatures, soil and atmospheric dryness and salination (SAKURAI et al. 1999, SAM et al. 2001, UPRETI et al. 2004, VIDYA et al. 1999).

Lexin is a liquid concentrate of high-molecular humic acids and low-molecular fulvic acids that, among other, play the role of the donor of its main component – a synthetic analogue of auxin.

Lexin functions as a plant stimulator and as a soil conditioner. It stimulates both long growth of cells and their division. It also supports their differentiation and formation of vascular bundles. It positively influences the permeability of cell membranes stimulating so not only the long growth of plants but also their recovery, growth and development of roots, intake and utilisation of nutrients. In addition to the above, it enhances the content of chlorophyll in plants, the productivity of photosynthesis and transportation of assimilates to seeds and fruits (HRADECKÁ et al. 2006).

Plants treated by Lexin are generally more vital, healthier and more resistant to various stress effects. Lexin has positive effects on soil by improving its physical, biological and chemical properties enhancing so its production capacity (ŠTRANC et al. 2007b).

The preparation Pentakeep-S is sometimes described as a fertiliser the key component of which is the 5-amino-levullic acid (ALA) derived from levullose (currently called D-fructose). ALA stimulates the formation of chlorophyll, it supports enzymatic activity including but not limited to the transmission of substances, transformation of nitrate ions to ammonia ions, intake and utilisation of nutrients, photosynthesis, it controls plant transpiration (in dark) and opening of stomata. Moreover, it enhances the utilisation of sunshine radiation and the resistance of plants against stress. Combined with nutrients contained in Pentakeep-S, ALA positively influences the relations between macro and micro nutrients in plants, it improves their physiological and health condition and, consequently, the productivity, yield and quality of the production (TANAKA 2005).

## **MATERIALS AND METHODS**

In 2007, we verified stimulators at hop-gardens of ZEVOS Čínov and ZS Slatina.

The objective was to assess the effects of chosen growth stimulating agents upon the ontogenesis, physiological condition including but not limited the yield and quality of the hop specie “Žatecký poloraný červeňák” (ŽPČ) under the agro-environmental conditions of the Žatec and Ústěck hop growing areas. We included to the experiments brassinosteroids and the auxin-type preparation Lexin at the experimental stations Siřejovice (ZS Slatina) and Čínov (ZEVOS Čínov). At the station Siřejovice, we monitored the effects of the Japanese preparation Pentakeep-S and the fungicide Horizon 250 EW that controls the growth of the plants.

**The locality Čínov** belongs to the Žatecká basin, in terms of geo-morphology. The hop-garden itself is on a flat ground, 212 meters above the level of sea (Žitná, area 1.6 ha). In terms of pedology, it is a fluvisoil on carbonate-free fluvial sediments with light to medium-heavy grain size, with light to medium humous soil admixture of medium quality. The agro-chemical analysis of the soil brought the following results: 368 ppm P, 845 ppm K, 416 ppm Mg, 5514 ppm Ca and pH = 7.3. In terms of climate, the locality belongs to the region B2. The experimental specie of hop as used in this locality was ŽPČ – Osvald’s clone 72 M, planted in 1998, plant spacing 300 x 100 cm, plant row being oriented from north to south. The condition of the plant was very good. The size of one experimental plot was 0.4 ha. The experiments included in total four variants (see the tab. 1).

**The Locality Siřejovice** belongs to the Dolnoohárecká plateau, in terms of geo-morphology. The hop-garden is a flat accumulation plot with the elevation of 175 m above

sea level (U Vrbičan, area 4 ha). In terms of pedology, it is typical black earth on loess - a carbonate variety with heavy grain size, very high content of humous soil of medium to very high quality (4.6 %). An agro-chemical analysis of the soil showed the following results: 281 ppm P, 829 ppm K, 404 ppm Mg, 8961 ppm Ca, 24.1 ppm S, 17.1 ppm Cu, 8.6 ppm Zn, 11.3 ppm Mn, 19 ppm Fe, 1,6 ppm B a pH = 7.2. In terms of climate, the locality belongs to the region A2.

The experimental hop specie used in this locality was ŽPČ – Osvald’s clone 31, planted in 1988, plant spacing 300 x 100 cm, orientation of the plant row from east to west. The condition of the plants was good. The size of one experimental plot was 0.3 ha. The experiments monitored in total ten variants (see the tab. 2).

*Overview of variants included to the experiments at the locality Čínov*

Tab. 1

Variant		Application date – hop phase	Dose	Area
K	Reference sample + Cuproxat SC	prior to reaching the top of the hop garden structure + in full flowering phase	Fungicide only	0,4 ha
BR1	1. Brassinosteroid 2x + Cuproxat SC	prior to reaching the top of the hop garden structure + in full flowering phase (21.6. a 14.7.2007)	according to ÚOCHB (in mg/ha)	0,4 ha
BR5	5. Brassinosteroid 2x + Cuproxat SC	prior to reaching the top of the hop garden structure + in full flowering phase (21.6. a 14.7.2007)	according to ÚOCHB (in mg/ha)	0,4 ha
L	Lexin 2x + Cuproxat SC	prior to reaching the top of the hop garden structure + in full flowering phase (21.6. a 14.7.2007)	0,25 l/ha + 0,25 l/ha	0,4 ha

*Overview of variants included to the experiments at the locality Šiřejovice*

Tab. 2

Variant		Application date – hop phase	Dose	Area
KC0	Reference sample	locally, specifically: at the locality Čínov	2000 l/ha	0,3 ha
BC1	1. Brassinosteroid 2x	All agrotechnical and protective interventions on a chosen standard and uniform formation of cones (29.6. a 5.8.2007)	according to ÚOCHB (in mg/ha)	0,3 ha
BC5	5. Brassinosteroid 2x	when the plant reached the top of the hop garden structure + upon the formation of cones (29.6. a 5.8.2007)	according to ÚOCHB (in mg/ha)	0,3 ha
PC1	Pentakeep-S 1x	when the plant reached the top of the hop garden structure (29.6.2007)	0.5 l/ha	0,3 ha
PC2	Pentakeep-S 1x	In full flowering phase to the beginning of the formation of cones (28.7.2007)	0.5 l/ha	0,3 ha
PC3	Pentakeep-S 1x	upon the formation of cones (5.8.2007)	0.5 l/ha	0,3 ha
PC4	Pentakeep-S 2x	when the plant reached the top of the hop garden structure + upon the formation of cones (29.6. a 5.8.2007)	0.5 l/ha + 0.5 l/ha	0,3 ha
LC0	Lexin 2x	when the plant reached the top of the hop garden structure + upon the formation of cones (29.6. a 5.8.2007)	0.33 l/ha + 0.33 l/ha	0,3 ha
LCS	Lexin + sucrose 1x	during the rapping phase (12.8.2007)	0.2 l/ha + 8.0 kg/ha	0,3 ha
HC0	Horizon 250 EW 1x	During the rapping phase (12.8.2007)	0.15%	0,3 ha

The hop plants of all variants were checked in the course of their vegetation for the rate of photosynthesis and transpiration using a commercial portable infra-red analyser LC pro+ (ADC Bio Scientific Ltd.) that makes it possible to carry out metering at the density of radiation exposure FAR (400-700 nm) within the scope 0-2000  $\mu\text{mol.m}^{-2}.\text{s}^{-1}$  and the temperature within the range -5 to +50 °C.

After cutting of the plants and delivery to the harvest centre, all hop plants of individual variants, cones were picked in a standard way, dried and weighed. Chemical analyses of soil and the determination of the conductometric value of the cones (KH – i.e. the content of  $\alpha$ -acrid acids) were executed by the regional farming Laboratory at Postoloprty.

## RESULTS

### ***Results of the Vegetation Monitoring***

At the time of the launch of the experiments, the hop plants of all variants on both experimental stations had a balanced height (habitus) and no economically significant diseases were detected (peronospora, mildew) and vermin (plant louse, red spider mite). However, later on during the vegetation period, the experimental blocks of the hop-gardens experienced the occurrence of peronospora, plant louse, and red spider mite. The referred-to vermin was timely and successfully eliminated. It could not have any adverse impact upon the yield and quality of hops.

We noticed darker green colour of leaves 5 to 6 days after the application of the preparations as under verification at both experimental stations.

### ***Results of the Examination of the Physiological Conditions of Hop***

The physiological condition of the hop plants (content of chlorophyll, photosynthesis rate and transpiration rate) were monitored at the station Sirejovice, only. As well, the informative chemical analysis of hop leaves was done at this station, only.

#### *Content of Chlorophyll in Leaves*

The content of chlorophyll in the leaves of hop plant stalk and young sprouts was checked at the station Sirejovice on July 3, 2007, i.e. after the first application of biologically active substance on the monitored variants. We compared the results to values established in the untreated plant variant sample (KC0).

*Content of chlorophyll in stalk and young sprout leaves of hop plants at the locality Sirejovice*  
Tab. 3

Variant		Mean value in stalk leaves	Rel. in % stalk leaves	Mean value in young sprout leaves	Rel. in % young sprout leaves
KC0	<i>Reference sample</i>	40.4	100.0	33.2	100.0
PC1	Pentakeep-S 1x	43.3	107.1	35.1	105.8
PC2	<i>Pentakeep-S 1x</i>	40.6	100.6	33.0	99.3
PC3	<i>Pentakeep-S 1x</i>	40.4	100.0	33.7	101.7
PC4	Pentakeep-S 2x	42.4	105.0	35.8	108.0
BC1	1. Brassinosteroid 2x	42.5	105.1	37.0	111.5
BC5	5. Brassinosteroid 2x	45.0	111.4	38.8	116.9
LC0	Lexin 2x	46.2	114.3	39.9	120.2

The results of the experiment indicated that almost all preparations stimulated formation of chlorophyll in leaves first of all those on young sprouts that are younger compared to leaves on the stalk of the plant. This finding matches the results of the vegetation monitoring. The most efficient items in this meaning were brassinosteroids and Lexin. What

has appeared is that the occurrence of premature drying of cones is in a negative correlation with the content of chlorophyll. It is likely to be related to a better utilisation of sun radiation in the lower (more shadowed) height profile of the hop plants and a higher intensity of the photosynthesis.

#### Photosynthesis Rate

The photosynthesis rate was determined on July 3, July 17 and July 24. For the results see the table 4 and the diagram 1.

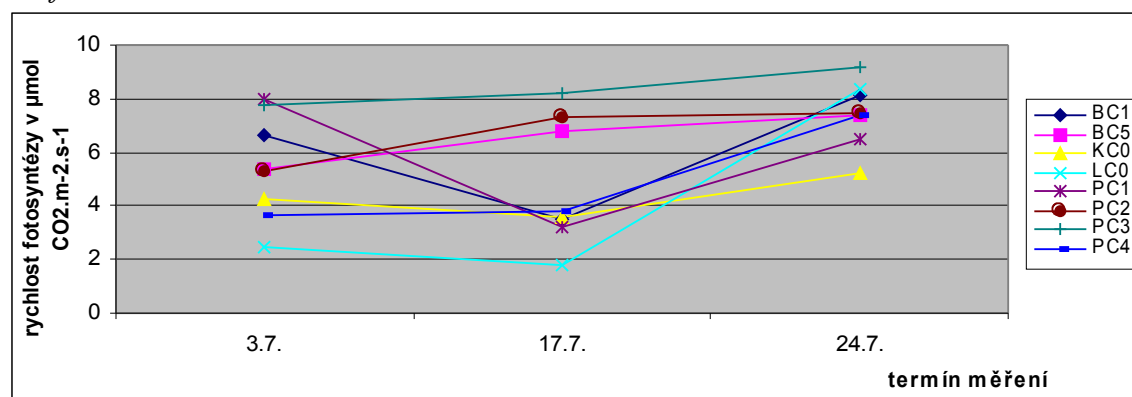
#### Average photosynthesis rate ( $\mu\text{mol CO}_2\cdot\text{m}^{-2}\cdot\text{s}^{-1}$ ) at the locality Siřejovice

Tab. 4

Metering date	July 3	July 17	July 24	Mean value for the monitored period
BC1	6.65	3.49	8.17	6.10
BC5	5.38	6.79	7.37	6.52
KC0	4.25	3.59	5.26	4.37
LC0	2.48	1.79	8.35	4.21
PC1	8.02	3.19	6.46	5.89
PC2	5.27	7.33	7.43	6.68
PC3	7.78	8.18	9.17	8.38
PC4	3.68	3.77	7.41	4.95

#### Average photosynthesis rate at the locality Siřejovice

Graf 1



A generally considerable rise in the intensity of the photosynthesis (in all variants) was recorded by the last metering (July 24) and it is in compliance with the hop ontogenesis. The highest average photosynthesis rate and its lowest fluctuations throughout the monitored period were showed by the hop plants of the var. BC5 ( $6,52 \mu\text{mol CO}_2\cdot\text{m}^{-2}\cdot\text{s}^{-1}$ ), PC2 ( $6,68 \mu\text{mol CO}_2\cdot\text{m}^{-2}\cdot\text{s}^{-1}$ ) and PC3 ( $8,38 \mu\text{mol CO}_2\cdot\text{m}^{-2}\cdot\text{s}^{-1}$ ).

#### Transpiration Rate

What we checked at the same dates as the photosynthesis rate of the hop plants was the transpiration rate using the tool LC pro+ (ADC Bio Scientific Ltd.), too. For an overview of the results see the tables 5 and the diagram 2.

#### Average transpiration rate ( $\text{mmol H}_2\text{O}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$ ) at the locality Siřejovice

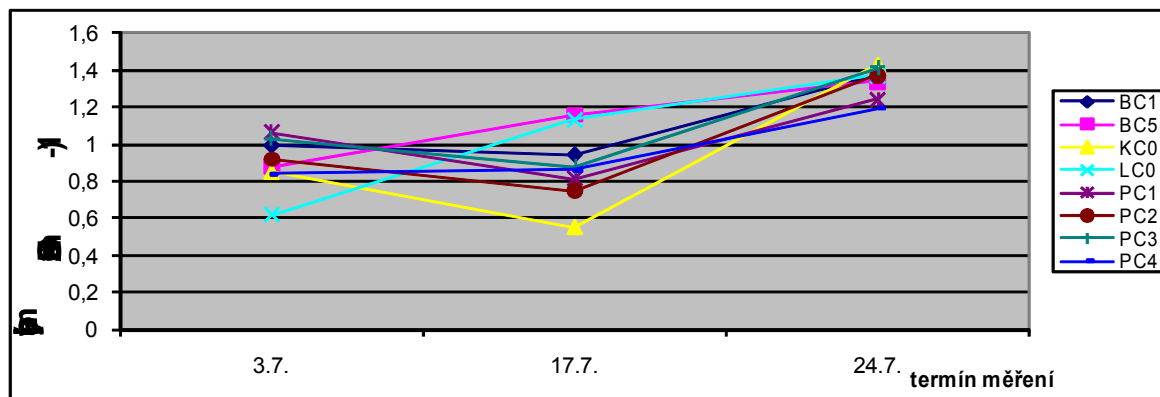
Tab. 5

Metering date	July 3	July 17	July 24	Mean value for monitored period
BC1	0.99	0.94	1.37	1.10
BC5	0.88	1.16	1.34	1.13

KC0	0.85	0.55	1.43	0.94
LC0	0.62	1.13	1.37	1.04
PC1	1.06	0.81	1.24	1.04
PC2	0.92	0.75	1.37	1.01
PC3	1.03	0.88	1.41	1.11
PC4	0.84	0.86	1.19	0.96

Mean transpiration rate at the locality Sirejovice

Graf 2



What can be deduced from the relation of the photosynthesis rate and transpiration rate is the efficiency of the utilisation of water in the formation of biomass respective dry matter produced by hop plants of individual experimental variants. It appears from this point of view that hop plants treated with Pentakeep-S and brassinosteroids (variants PC3, PC2, BC5 and PC1 – applied at the moment when the plant reached the top of the hop-garden structure up to formation of cones) showed the best water management.

We note that the above-referred to metering has to take into consideration weather conditions and the growth or development phase of hop plants as the physiological effects of the various preparations verified by us are quite different and the period of their efficiency is not the same.

#### Chemical Analysis of Leaves Checking for Chosen Elements

Results of the informative analysis of hop leaves for the content of chosen elements (Sirejovice 2007) Tab. 6

Variant		Cu (mg/kg)	Zn (mg/kg)	Mn (mg/kg)	Fe (mg/kg)	B (mg/kg)	S (%)
KC0	Reference sample	1280.0	46.0	205.0	327.0	43.2	0.26
PC4	Pentakeep-S 2x	1160.0	45.2	246.0	386.0	54.0	0.30
BC5	5. Brassinosteroid 2x	781.0	113.0	160.0	727.0	102.0	0.31

The results of the informative chemical analysis of hop leaves indicate that treatment of plants with the monitored preparations resulted in most cases to an increase of the content of nutrients in them. Therefore, such a fact indicates that there is a more intensive intake respectively higher sorption activity of the roots systems of hop plants.

The finding that plants treated by brassinosteroids and Pentakeep-S are subject to relatively high concentration of mineral nutrients in leaves and they show quite a high efficiency of the utilisation of water documents more efficient use of the soil moisture by hop plants after a repeated application of the above preparations.

### Harvest Results

The experimental variant hop plants at the station Čínov were harvested on August 26, 2007, and those at the station Sirejovice on September 2- 4, 2007, by harvesting systems installed at the respective harvest centres.

Dry hops yield at the station Čínov (at 10 % humidity)

Tab. 7

Variant		Yield t/ha	Yield rel. v %
KC0	Reference sample	<b>1.325</b>	100.0
BC1	1. Brassinosteroid 2x	<b>1.550</b>	117.0
BC5	5. Brassinosteroid 2x	<b>1.555</b>	117.4
LC0	Lexin 2x	<b>1.668</b>	125.9

The harvest results (tab.7) indicate that the application of brassinosteroids and Lexin to the hop at the station Čínov had positive effects on the development of cones and yield. Both verified types of brassinosteroids, both BR 1 (var. BC1) and BR 5 (var. BC5) increased the yield compared to the reference by approx. 17 %. The application of Lexin increased the yield practically by 26% and it is very significant.

Yield of dry hops (at 10 % humidity) and % KH at the station Sirejovice

Tab. 8

Variant		Yield t/ha	Yield rel. v %	% KH in dry matter Aug 24, 2007	% KH in dry matter Aug 30, 2007
KC0	Reference sample	<b>1,155</b>	100,0	2,4	<b>2,5</b>
PC1	Pentakeep-S 1x	<b>1,108</b>	95,9	2,8	<b>2,8</b>
PC2	Pentakeep-S 1x	<b>1,328</b>	115,0	2,7	<b>3,0</b>
PC3	Pentakeep-S 1x	<b>1,311</b>	113,5	3,0	<b>3,0</b>
PC4	Pentakeep-S 2x	<b>1,552</b>	134,4	2,4	<b>2,5</b>
BC1	1. Brassinosteroid 2x	<b>1,145</b>	99,1	3,6	<b>3,0</b>
BC5	5. Brassinosteroid 2x	<b>1,437</b>	124,4	2,8	<b>2,8</b>
LC0	Lexin 2x	<b>1,405</b>	121,6	2,7	<b>3,7</b>
LCS	Lexin + sucrose 1x	<b>1,439</b>	124,6	3,0	<b>3,7</b>
HC0	Horizon 250 EW 1x	<b>1,281</b>	110,9	3,1	<b>3,3</b>

KH – conductometric value of cones (content of  $\alpha$ -acrid acids)

The yields as found out at the station Sirejovice indicate that the application of brassinosteroid BR 1 (var. BC1) produced the same yield results as the reference sample meanwhile the application of brassinosteroid BR 5 (var. BC5) on the plants increased the yield by 282 kg/ha, i.e.24.4% compared to the reference sample. The application of Lexin showed a similar effect. What has very positive effects was a single application of Lexin combined with sucrose. The absolutely highest increment in terms of the cone yield (34.4 %) was recorded in the variant PC4 after the application of Pentakeep-S in the phase when the hop plants reached the top of the hop-garden structure (29.6.) and upon the formation of cones (5.8.). The application of the fungicide with growth control effects (Horizon) improved the yield by 10.9%.

Hop cones of variants treated with brassinosteroids were the most rape compared to other variants (in particular the variant BC1). This fact was documented by the results of the chemical analyses of the cones checking them for the content of  $\alpha$ -acrid acids ( $\alpha$ -acids).

The results of respective analyses indicate that a relatively high (absolutely the highest) content of  $\alpha$ -acids, namely 3.6 % as early as in the pre-harvest phase (24.8.) in the cones of plants treated with brassinosteroid BR 1 (var. BC1). Such a content gradually dropped starting

from the second decade of August and its value found at the end August (30.8.) was 3.0 %. Despite such a decline, the referred to value of the content of  $\alpha$ -acids is higher by 20 % compared to the reference sample cones.

The content of  $\alpha$ -acids in the cones of plants treated by brassinosteroid BR 5 (var. BC5) in the pre-harvest phase stagnated. Their content upon the last sampling (30.8.) and follow up chemical analysis was higher by 12 % than the content in the cones taken from the reference plants.

The above results indicate that the formation of  $\alpha$ -acids in the cones of plants treated by brassinosteroids (var. BC1 a BC5) culminated in the first half of the third decade of August, that means earlier than in the cones of the reference variant and other variant plants. The application of Pentakeep-S positively stimulated the formation of  $\alpha$ -acids in most variant hop cones. Depending on the time and number of applications of this preparation, we recorded a rise in the content of  $\alpha$ -acid by up to 20%.

The Horizon fungicide preparation enhanced the formation of  $\alpha$ -acids by 32% and Lexin even by 48%. The application of Lexin combined with sucrose showed similar effects.

Concerning the content of  $\alpha$ -acids in hop cones, we note that their formation was adversely affected in later August 2007 (among other) by the instant weather conditions and lack of sunshine.

## CONCLUSIONS

- 1) The experiments suggest that the treatment of hop by Pentakeep-S and brassinosteroids, including but not limited to BR 5, enhances not only the rate of photosynthesis but it apparently positively stimulates the transportation of assimilates to the sink respectively cones. We believe that the referred to preparations accelerate enzymatic reactions, they stimulate decomposition of starch mainly in the skotoperiod to sucrose that may be intensively transported to the sink.
- 2) The application of Pentakeep-S, however, particularly of brassinosteroids (BR 5 again most of all) increased the content of chlorophyll in leaves and most likely it improved the utilisation of sun radiation at the lower shadowed levels of the higher hop plants. This fact is likely to be behind a lower occurrence of undesirable so called premature drying of generative organs (cones). A somewhat higher occurrence of lesions on the top level leaves of the hop plants following intensive sun radiation is apparently related to the utilisation of sunshine by the hop plants after the application of brassinosteroids in particular in interaction with applied pesticides. However, we found out the absolutely highest content of chlorophyll in leaves after the application of Lexin.
- 3) A proper application of brassinosteroids, Pentakeep-S and Lexin (and in fact the fungicide Horizon 250 EW) in relation to hop ontogenesis, even if applied repeatedly, significantly increased the cone yield.

The results achieved also indicate that the formation of  $\alpha$ -acids in cones of plants treated with brassinosteroids culminated in the first decade of August, i.e. earlier than in the cones of plants of the reference variant and plants as included to other variants. We also note that all preparations as under verification showed positive effects upon the formation of  $\alpha$ -acids. However, the highest rise in the content of  $\alpha$ -acids was recorded after the application of Lexin and after the application of Lexin in combination with sucrose. In both cases, the increase amounted to incredible 48 %.

- 4) The finding that the high-yield variants (var. PC4 and BC5) are subject to a relatively high concentration of mineral nutrients indicates there is a more effective use of soil humidity by the hop plants after their repeated treatment with Pentakeep-S and brassinosteroids.

Should this positive finding be confirmed, the application of the above-mentioned preparations would have a significant economic effect for hop growing in arid conditions of the Czech Republic in particular in the Žatec region (the efficiency of Lexin in this aspect was not the subject-matter of the experimental verification).

- 5) Pentakeep-S effected the morpho-genesis of hop plants by weakening the apical domination of vegetation tops supporting their branching respectively formation of sprouts. Therefore, it appeared that its application will be less suitable during the period of the long growth of hop plants.

The monitoring results also indicate that Pentakeep-S is typical of a considerably fast efficiency. That indicates that its active substance easily incorporates the substance metabolism of plants and, apparently therefore, the effects fade away quite soon, too. In line with the above, the experiments proved a great efficiency of its repeated application.

- 6) We noted that the variant treated with Lexin in combination with sucrose has a very positive cone yield and the content of  $\alpha$ -acids (at acceptable price). We believe that the

additionally intended application of the stimulating agent Lexin combined with Pentakeep-S would even more significantly improve the quantity and quality of the final product – hop cones (unfortunately, this variant could not be implemented with regard to a limited quantity of Pentakeep-S available for our experiments. Therefore, “mere” sucrose was used instead as easier available to us).

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