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WEATHER CONDITIONS AND BIOSYNTHETICAL PROCESSES IN HOP CONE

POVĚTRNOSTNÍ PODMÍNKY A BIOSYNTETICKÉ PROCESY VE CHMELOVÉ HLÁVCE

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ABSTRACT: An important influence of weather conditions on bitter substances formation is generally accepted. Dynamism of alpha bitter acids formation before harvest in the period 1992 to 1996 showed significant year-to-year differences. It consisted not only in absolute amount of bitter acids, but in composition of hop oils as well. The comparison of meteorological data proved some coherence between alpha bitter acids content and extent of temperature and precipitation deviations from long-term average in the period from July to August. The average air temperatures in the range of 16 to 18 °C seems to be optimal in Saaz growing region from the point of view of bitter acids formation. Increase of temperatures above this optimum to 20 °C can be partly compensated by higher amount of rain precipitation in split portions or by irrigation supplied from the ceiling above hop plants (trickle irrigation). The temperatures above 20 °C with the lack of water lead to the blockage of biosynthetical process.

hops; weather conditions; biosynthesis; hop resins; alpha bitter acids; hop oils

ABSTRAKT: Významný vliv povětrnostních podmínek na tvorbu hořkých látek chmele je všeobecně uznáván. Sledování dynamiky tvorby alfa hořkých kyselin před sklizní v letech 1992 až 1996 ukázalo značné meziroční rozdíly. Spočívaly nejen v absolutním obsahu alfa hořkých kyselin, ale i ve složení chmelových silic. Porovnání meteorologických údajů ukázalo souvislost mezi obsahem alfa hořkých kyselin a mírou teplotních a srážkových odchylek od dlouhodobých průměrů v období červen až srpen daného vegetačního roku. V podmínkách žatecké chmelařské oblasti se z hlediska tvorby hořkých látek ukazují jako nejvhodnější průměrné denní teploty vzduchu v rozmezí 16 až 18 °C. Nárůst teplot nad toto optimum do 20 °C může být do určité míry kompenzován větším množstvím srážek v rozložených dávkách nebo umělou závlahou dodávanou shora (kapková závlaha). Teploty nad hranicí 20 °C při nedostatku vláhy zapříčiňují blokaci biosyntetického procesu.

chmel; povětrnostní podmínky; biosyntéza; chmelové pryskyřice; alfa hořké kyseliny; chmelové silice

INTRODUCTION

The Saaz growing region is the most important hop production region in the Czech Republic. It spreads in the so-called „rain shadow" of the North-Bohemian border mountains. Diversity of soil structure from sandy soils to heavy clay is the characteristic trait of the region. Height overtop represents approximately 300 m from lower location in Ohře river basin to Podlesí. Typical weather conditions, i.e. higher temperatures and lower rain precipitation, are typical traits of the continental climate and significantly influence conditions of hop growing in individual localities. Just the course of weather conditions is generally known as a decisive factor in biosynthesis of bitter acids and hop oils and simultaneously, important yield influencing factor. Hautke (1983) evaluated the influence of weather conditions and other agronomical factors to alpha acids content in the years 1974 to 1979. Temperatures in the period since 3rd August to 10th September, time of harvest, temperature and precipitation are factors which influence yield and content of hop resins. These factors are not closely specified.

Hautke, Petříček (1967) studied quality aspects of bitter substances formation in dependence on weather. Smith (1969) performed extensive investigation of connections between course of temperatures in the period from May to harvest and content of bitter substances. He collected data from several European countries (including former Czechoslovakia). On the basis of processed large data sets he concluded, that average temperatures in the period of 40 to 60 days before harvest had significant influence on the content of alpha bitter acids in hops. He presents that the highest contents of soft resins were reached in the years (in England) when average temperatures in July and August were approximately 16.5 °C. Thomas (1980) found close correlation between alpha bitter acids content and temperature in the period since 24th May to 21st June, i.e. in the period of intensive longitudinal

growth of hop plants. Adverse influence of high temperatures on hop resins formation in the period of hop cones maturation confirmed Zattler, Jehl (1962), Ljašenko (1985) and Fric (1989). Most papers are exclusively aimed at investigation of correlation between temperature and bitter acids contents, only in a few papers the influence of rain precipitation is considered. No paper refers about influence of weather conditions on hop oil composition.

MATERIAL AND METHODS

The course of bitter acids formation in the period of hop cones forming and maturation is performed every year by the Hop Research Institute, Ltd., Žatec. The aim of this is to estimate the level of alpha acids content in forthcoming harvest and determination of its optimum beginning. Samples of hop cones from the middle parts of hop plants are taken in seven day intervals at 15 to 20 chosen localities. First sampling is performed at the beginning of August, when hop cones start their intensive growth. Ten to 15 plants are sampled from each locality. Subsequent sampling is performed approximately at the same place of the hop garden. Last samples are taken about seven days after beginning of the harvest. Maximum comparability of results is ensured in this way.

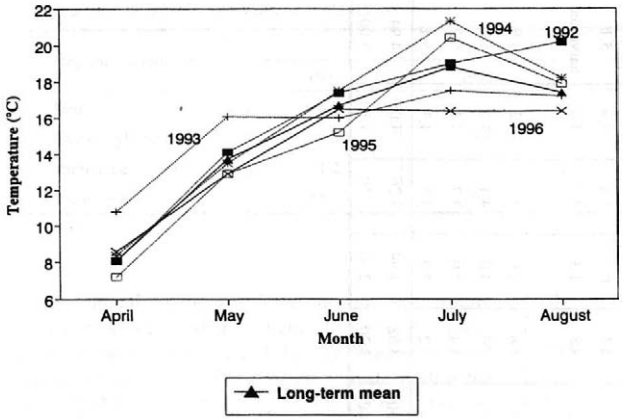
Hop cones samples are dried in laboratory hot-air drier with forced circulation at the temperature 50 to 60 °C. Content of alpha bitter acids is determined by conductometric titration according to ČSN 46 2520-15 method. Hop oil analyses were performed in crop samples by steam distillation/GC chromatography method. Statistical processing of experimental data were performed with the help of computer program Adstat 2.0 (Trilobyte, Pardubice). Meteorological data measured on an automatical computer station located within Hop Research Institute campus. Data were compared with long-term average values (temperatures = 30 year mean, precipitation = 50 year mean).

RESULTS AND DISCUSSION

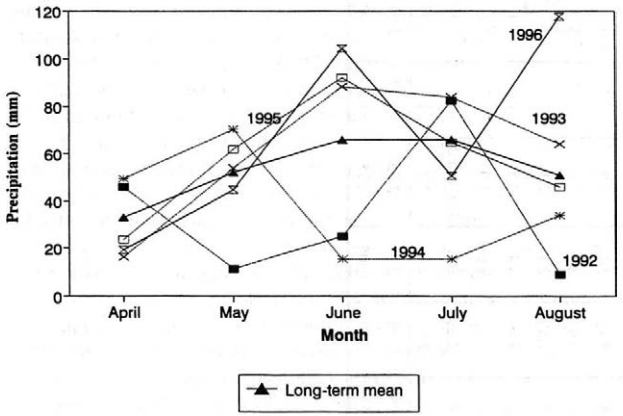
Weather conditions of individual vegetation seasons are significantly different in investigated period. In 1992 the course of temperatures till July corresponded with long-term average, then temperature increased to harvest. The amount of precipitation is characteristic by a high deficit except for July. This vegetation season is considered to be less favourable from the hop growing point of view. In the year 1993 the curve of temperatures and precipitation corresponded with long-term average. Higher precipitation and lower temperatures (15.5 °C) were recorded in the period July to August.

I. Summary of weather parameters (Žatec, 1992–1996)

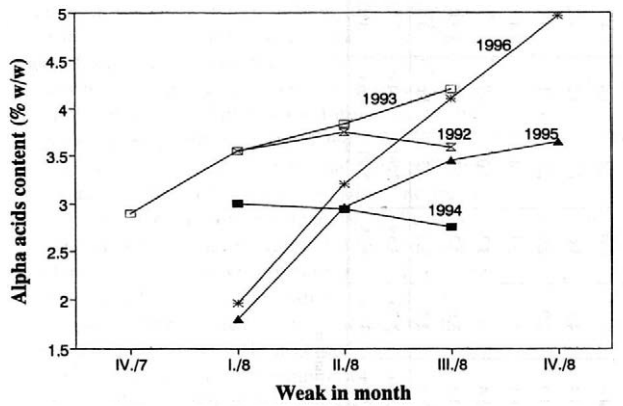
	Year	Month					Mean/Sum
		4	5	6	7	9	
Temperature (°C)	long-term mean	8.1	13.7	16.7	18.8	17.4	14.9
	1992	8.1	14.1	17.4	19.0	20.2	15.8
	deviation	0.0	0.4	0.7	0.2	2.8	0.8
	1993	10.8	16.1	16.0	17.5	17.2	15.5
	deviation	2.7	2.4	−0.73	−1.28	−0.2	0.6
	1994	8.4	13.5	17.5	21.3	18.2	15.8
	deviation	0.3	−0.2	0.8	2.5	0.8	0.8
	1995	7.2	12.9	15.2	20.4	17.9	14.7
	deviation	−0.93	−0.79	−1.49	1.6	0.5	−0.22
	1996	8.6	12.9	16.5	16.4	16.4	14.2
	deviation	0.5	−0.8	−0.2	−2.4	−1.0	−0.78
Precipitation (mm)	long-term mean	33.0	52.0	66.0	66.0	51.0	268.0
	1992	45.9	11.4	28.0	82.2	9.0	176.5
	deviation	12.0	−40.6	−38.0	16.2	−42.0	−91.5
	1993	16.2	53.8	88.3	83.9	64.2	306.4
	deviation	−16.8	1.8	22.3	17.9	13.2	38.4
	1994	49.4	70.4	15.4	15.5	34.0	184.7
	deviation	16.4	18.4	−50.6	−50.5	−17.0	−83.3
	1995	23.6	61.8	92.0	64.6	46.0	288.0
	deviation	−9.4	9.8	26.0	−1.4	−5.0	20.2
	1996	19.2	44.6	104.4	50.6	117.4	336.2
	deviation	−13.8	−7.4	38.4	−15.4	66.4	68.2



1. The course of temperatures (Žatec, 1992–1996)



2. The course of precipitation (Žatec, 1992–1996)



3. Dynamics of alpha acids formation (1992–1996)

Vegetation season of this year was positive from the hop growing and hop quality point of view. The following year 1994, in spite of initial positive course, was characteristic by enormous temperature increase in July and August. The curve of precipitation exhibited deep deficit in the period May to August. Simultaneous effect of both factors caused that the year 1994 is considered as one of the worst years from the brewing substances formation point of view in the history. The course of temperatures in the year 1995 is to a certain

II. Dynamics of alpha acids formation before harvest (1992–1996)

Locality	Lead conductance value (w/w) ČSN 46 2520-15																	
	1992			1993				1994			1995				1996			
	I./8+	II./8	III./8	IV./7	I./8	II./8	III./8	I./8	II./8	III./8	I./8	II./8	III./8	IV./8	I./8	II./8	III./8	IV./8
Pšov	3.9	3.9	3.5	3.1	3.9	4.0	4.3	2.4	1.7	2.3	1.8	2.8	3.3	3.7	1.6	3.0	4.6	5.4
Měcholupy	3.9	3.9	3.9	3.1	3.7	4.3	4.3	3.0	3.0	3.2	1.9	3.2	4.2	3.6	2.0	3.7	4.5	5.4
Blšany	3.6	3.0	3.5	3.7	3.8	3.7	4.4	3.6	3.1	3.0	2.2	3.2	3.7	3.8	2.0	3.1	3.9	4.9
Hořesedly	2.9	3.4	3.4	2.7	2.8	3.5	3.7	2.3	2.3	2.6	*	1.9	3.2	3.2	0.7	2.6	3.6	4.9
Kněževes	3.9	4.1	4.0	2.2	3.1	4.0	4.5	3.0	3.6	2.8	*	2.9	4.2	4.4				
Chrášťany															2.3	4.0	4.1	harvested
Mutějovice	5.4	5.5	4.3	2.7	3.1	3.4	4.7	3.8	3.5	harvested	1.4	3.4	4.2	4.1	1.4	2.6	3.7	4.0
Končtopy	3.6	3.6	3.6	2.8	3.3	3.6	4.1											
Solopysky								4.1	3.9	3.8	1.0	1.9	2.2	2.8	1.7	2.9	3.8	4.4
Ročov	4.0	4.4	5.0	2.4	3.9	4.1	4.5	3.4	3.1	2.9	2.3	3.0	3.3	3.3	0.7	1.8	3.9	5.1
Mšec	3.4	4.1	3.6	2.5	3.2	4.2	4.4	3.0	2.8	2.1	*	2.7	3.2	3.8	1.3	3.0	4.0	harvested
Vrbičany	3.0	2.9	2.9	2.5	3.1	3.1	3.7	1.5	2.1	2.1								
Šlapanice	3.1	3.0	2.7	3.4	3.9	4.1	4.2	3.0	3.4	2.5	1.8	3.4	3.3	3.8	2.6	4.0	4.2	4.9
Počedělice	3.4	3.7	3.7	3.0	3.7	3.9	3.9	3.7	3.6	3.0	1.7	3.6	3.8	4.0	3.0	4.1	4.2	5.0
Postoloprty	3.2	3.8	3.2	3.3	3.7	3.8	3.7	2.5	2.2	harvested	2.2	3.3	3.5	3.4	3.0	3.7	4.2	5.2
Stekník	3.1	3.3	3.0	3.2	3.6	3.8	3.7	2.5	2.8	2.7	1.6	2.9	3.4	3.2	2.9	3.9	4.3	5.1
Arithmetic mean	3.60	3.76	3.59	2.90	3.49	3.82	4.15	2.99	2.94	2.75	1.79	2.94	3.50	3.62	1.94	3.26	4.08	4.94
Median	3.50	3.75	3.55	2.90	3.65	3.85	4.25	3.00	3.05	2.75	1.80	3.00	3.40	3.70	2.00	3.10	4.10	5.00

* beginning of hop cones formation
\+ weak in current month

III. Composition of hop oils (crop means, 1992–1996)

Hop oil component	Year				
	1992	1993	1994	1995	1996
Myrcene	36.8	38.4	51.0	32.4	57.5
Caryophyllene	6.5	6.3	4.8	6.5	4.3
Humulene	21.2	22.9	19.8	21.1	15.6
Farnesene	15.9	18.1	10.6	15.2	9.9

extent identical to the previous one but temperature increase was not so sharp. Owing to sufficient water supply in the decisive period July to August, critical situation of the year 1994 did not repeat. Within the period 1992 to 1996, the year 1996 is the most favourable one from hop growing point of view. Partial lack of precipitation in July was compensated by balanced course of temperatures in June, July and August. Temperatures were lower compared to long-term average, the sum of deviations was –3.6 °C within this period. Simultaneous influence of these factors made a positive impact on biosynthesis of hop resins, hop oils and yield. Contents of alpha bitter acids are comparable with the results recorded in the 1960s and 1970s, i.e. before global decline in last decade.

Factual weather data are collected in the Tab. I and the Figs 1, 2. The course of alpha acids formation (expressed like lead conductance value) during pre-harvest period is summarized in Tab. II and Fig. 3. These data are completed by average crop composition of hop oils in Tab. III. In spite of relatively short evaluated period, it is clear that contents of alpha bitter acids and hop oil composition exhibit considerable year-to-year deviations.

This year's dependence is generally accepted. Temperatures in the initial phase of biosynthetical process at the level of 20 °C in the year 1992 caused fast increase of alpha acids contents, but did not stimulate it till harvest. Further temperature increase in August brought inhibition on bitter acids formation. In 1993 the course of temperatures is stabilized at lower values (16 to 17 °C) and brought the continual growth of alpha bitter acids contents till harvest. Weather and quality extremes are typical for the year 1994. Long-term tropical hot weather at the turn of July and August considerably slowed down not only growth of cones but preferably bitter acids formation as well. Favourable course of temperatures in June and the first decade of July reflected in relatively high alpha acids content in the first sampling at the beginning of August. Subsequent samples unambiguously proved, that this original level of alpha acids content not only increased, but even declined till the beginning of harvest. It can be deduced that the blockade of biosynthetical process occurred. Water supply in the second half of August positively influenced growth of cones, but blockade mentioned above was not completely released. The year 1994 is, in spite of average yield, notable by the lowest

IV. Sum of deviations from long-term means (June, July, August)

Year	Temperature (°C)	Precipitation (mm)	Alpha acids content (% w/w)
1992	+3.7	–63.8	3.6
1993	–2.21	+53.4	4.2
1994	+4.10	–118.1	2.8
1995	+0.62	+19.6	3.7
1996	–3.60	+89.4	5.0

content of alpha bitter acids (LCV = 2.6% w/w) recorded in the Saaz growing region. The temperatures above 20 °C negatively influenced content of alpha acid at the beginning of hop cones forming in the year 1995. Sufficient precipitation supply attenuated to a certain extent adverse effect of the temperatures so that final average level of alpha acids laid at usual value (3.5 to 4.0% w/w). Balanced course of the temperatures at the level ca 16 °C in the period July to August and sufficient water supply in August resulted in relatively steep increase of alpha acids contents in the year 1996. The harvest was postponed by approximately five days this year.

Optimal temperatures for bitter acids formation determined in *in vitro* conditions (F u n g et al., 1996) lies within the interval of 20 to 30 °C with strong decline above the top value of this interval. This relationship seems to be similar in field conditions but shifted to lower temperatures as the results of 1994 documented.

It is interesting that in the years 1994 and 1996 that means the years with entirely different course of weather conditions and dynamics of alpha bitter acids formation (Tab. IV), was the character of hop oils composition very similar. Approximately 75% of total oils weight falls to terpenic fraction. The proportions of sesquiterpenes humulene, caryophyllene, farnesene, selinene and others are considered as dominant for fine hop aroma formation (H a u n o l d , 1988; N a r z i s s , 1992). Myrcene and other monoterpens are thought to be responsible for a penetrant sharp aroma and therefore are unwanted components of hop essential oils. The increase of myrcene contents above 50% rel. in the years 1994 and 1996 exceeds usual values by approximately 20%. At the same time lower contents of caryophyllene, humulene and farnesene are evident. The decline is relevant especially for farnesene content because farnesene is considered to be an important

marker of fine aroma hops in hop oils. Determined values about 10% are approximately by 1/3 lower comparing to usual ones. The changes in hop oils composition reflected in the intensity of hop aroma, but not in its character. In other years of period investigated (1992, 1993, 1995) the composition of hop oils responded to typical values of Saaz hops in spite of lower content level of bitter acids.

CONCLUSIONS

The average day temperatures in the range of 16 to 18 °C within a period July to August seem to be optimum ones for hop resins biosynthesis in conditions of the Saaz growing region. Water supply should be 65 to 70 mm/month. Increase of temperatures above this optimum to 20 °C can be compensated to some extent by higher precipitation in split rates. The influence of hop garden microclimate on hop resin and hop oils formation has not been sufficiently appreciated yet. Additional sources of water, especially trickle irrigation supplied from the irrigator placed in the ceiling of a hop garden, are unambiguously positive factor. A hop plant is not forced to use water received by root system for leaf cooling, but it can be used for biosynthetical processes. Extreme weather conditions in some vegetation seasons help to reveal some coherences between weather conditions and biosynthesis of brewing valuable substances.

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THE EVALUATION OF THE VIRUS-FREE HOP QUALITY AFTER FIVE YEARS GROWING IN THE CZECH REPUBLIC

HODNOCENÍ KVALITY BEZVIRÓZNÍCH CHMELŮ PO PĚTI LETECH PĚSTOVÁNÍ V ČR

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ABSTRACT: Virus-free hops are grown at considerable area at the present time (1996). It represents 13% of total hop gardens area (including new plantings from the year 1996). Virus-free hops still hold (i.e. five years after first planting in the year 1991) considerable quality and quantity advantages comparing with traditional hops. They contain substantially more hop resins, the content of alpha bitter acids is higher by nearly 100% in dependence on the year of harvest. Content of beta bitter acids changes slightly and does not exceed 10 to 15% as a rule. The analyses of hop oils confirm presence of farnesene as the most important marker of the Saaz semi-early red-bine hops in hop oils. Virus-free hops give higher yield. The difference, comparing to traditional hops, is the most significant in the first crop. After achievement of full effectiveness virus-free hops give higher yield by 20 to 25%. No reinfection by ApMV has not been detected yet.

hops; alpha and beta bitter acids; hop oils; gas chromatography; high performance liquid chromatography; Osvald's clone 72; reinfection; ELISA; ApMV

ABSTRAKT: Viruprosté chmele se v současné době (1996) pěstují na významné rozloze. Včetně výsadeb z roku 1996 jsou tyto chmele osázeny na 13 % celkové rozlohy produkčních chmelnic. Ozdravené porosty si nadále (tj. po pěti letech od založení prvních výsazů v roce 1991) udržují značný kvalitativní i kvantitativní náskok v porovnání s tradičními porosty. Meristémové chmele obsahují podstatně více chmelových pryskyřic, obsah alfa hořkých kyselin je vyšší téměř o 100 % v závislosti na ročníku sklizně. Obsah beta hořkých kyselin se mění nepodstatně a nepřevyšuje zpravidla hodnotu 10 až 15 %. Analýzy chmelových silic viruprostých chmelů potvrzují přítomnost farnesenu jako nejdůležitějšího markeru Žateckého poloraného červeňáku ve složení chmelových silic. Další přednosti bezvirózních chmelů je vyšší výnos. Rozdíl v porovnání s kontrolami je nejvýraznější po výsadbě, po dosažení plné výkonnosti porostů dávají ozdravené chmele o 20 až 25 % vyšší výnos. Reinfekce virem mozaiky jabloně ApMV nebyla dosud prokázána ani na prvních vysázených chmelnicích z roku 1991.

chmel; alfa a beta hořké kyseliny; chmelové silice; plynová a kapalinová chromatografie; Osvaldův klon 72; reinfekce; ELISA; ApMV

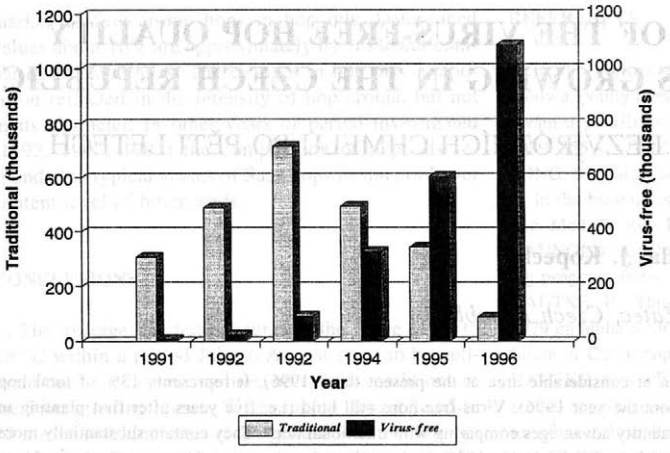
INTRODUCTION

The topic of this paper links-up to the previous one (Krofta, Kroupa, 1995), where quality parameters of virus-free hops from the first three harvests were collated. In this paper the evaluation of hop bitter acids, hop oils analyses of virus-free hops (Osvald's clone 72) from subsequent two harvests 1995, 1996 and overall evaluation of analytical data for the whole period 1992 to 1996 including yield parameters are presented.

In the years 1995, 1996 growing area of virus-free hops Osvald's clone 72 has considerably increased and reached approximately 500 ha at the end of 1996. This progress corresponds with the trend of root stock production in the recent years (Fig. 1). Steep increase of the number of virus-free root stock is apparent. A lot of experience regarding to agronomy technology and pest control have been obtained when growing area of virus-free hops gradually increased (Fric, 1996; Svoboda, Kopecký, 1996).

MATERIAL AND METHODS

Like in the previous period the samples of virus-free and control hops were taken on selected hop gardens in the years 1995, 1996. The samples of hop cones were dried in the hot-air dryer at the temperature of 50 to 60 °C and kept in the dark and cold place until processing. Other hop samples were received directly from hop growers from all growing areas in the Czech Republic. Hop bitter acid were analysed by HPLC method and hop oils by GC method. Hop oils were isolated using steam distillation method (ČSN 46 2520-13). The analyses of hop bitter acids were performed on analytical column Nucleosil 5 µm, 250 x 4.6 mm, RP C_{18} (Macherey Nagel). Mobile phase consisted of the mix-



ture methanol-water-phosphoric acid 850 : 170 : 2.5 (v : v : v). Analyses were performed on liquid chromatograph Schimadzu LC-10A with DAD detector at the wavelength 314 nm. Hop oils were analysed by GC-MS system (Varian 3400 + Finnigan ITD 800) on capillary column DB 5.30 m x 0.25 mm x 0.25 µm, temperature programme 60 to 250 °C, carrier gas helium 4.8.

Statistical evaluation of analytical data had to be frequently made for small data sets (< 10 data). Such results are generally burdened by significant uncertainty. The medians as robust estimates of mean values were used for statistical evaluation of hop bitter acids and hop oil provided that the size of evaluated set is higher than eight data. Using this parameter mean values are not influenced by outliers, which can easily occur if biological samples are evaluated. The mean values of smaller data sets were evaluated according to the Horn method (Meloun, Militký, 1994), that is based on order statistics. In the year 1992 the samples from only three localities were evaluated. The estimates of mean values were calculated like an arithmetic mean from the nearest points. Statistical evaluation of experimental data was performed with the help of statistical programme Adstat 2.0 (TriloByte, Pardubice, ČR).

Virus-free material was obtained by meristem cultures method *in vitro* and thermotherapy (Svoboda, 1993). The valuation of virus-free hop state of health was performed by ELISA method (Clark, Adams, 1977). The occurrence of the most relevant virus, i.e. apple mosaic virus (ApMV), has been investigated (Svoboda, 1993).

RESULTS AND DISCUSSION

Hop resins

In Tab. I the contents of hop bitter acids in virus-free and control hops from the crops 1995, 1996 are summarized. The results confirm the trends from the previous period, i.e. substantial increase of alpha bitter acids contents in virus-free hops by 96% in 1995 and 40% in 1996, respectively, compared to control hops. The increase of beta bitter acids is not so significant (in some samples of virus-free hops is a little bit lower) and does not exceed 10 to 15% as a rule. This fact is practically illustrated on Fig. 2 where bitter acids chromatograms of both hops are superimposed (Brozany, 1996). The increase of alpha/beta ratio from the values of 0.7 to 0.9 for control hops to the values > 1.0 for meristems is the direct consequence of these changes. The differences in cohumulone and colupulone are very small, in the year 1995 not statistically significant, in the year 1996 only difference of cohumulone is significant (critical value 2.12, *t*-statistic 2.28). In spite of this negligible divergency, it can be stated that virus-free hops and control hops are undistinguishable in this quality parameter as virus-free hops preserve this characteristic varietal parameter.

The evaluation of alpha bitter acids contents in the period 1992 to 1996 is given in Fig. 3. Mean crop values of virus-free and control hops for each year are compared. Statistical evaluation of crop mean values of alpha bitter acids in the period 1992 to 1994 using various methods is in Tab. II. The content of alpha bitter acids in virus-free hops is not subject to such significant fluctuations compared to control hops and exhibits appreciable year-to-year stability.

In the year 1994, when the historically lowest content of bitter acids was recorded, virus-free hops coped with adverse weather conditions during vegetation season (long-term hot and dry spell in summer) much better than control hops. Last vegetation season of 1996 was very positive from the biosynthesis of hop resins point of view. It is interesting that this favourable conditions more influenced control hops. While in the years 1994 and 1995 virus-free hops contained more alpha acids by 90 to 100% in the average, in 1996 this difference was only 40%.

I. Evaluation of hop resins, crop 1995, 1996 (virus-free and control hops, HPCL/EBC 7.4.1 method)

		1995					1996				
Locality	Sample	alpha (% w/w)	beta (% w/w)	ratio alpha/beta	cohumulone (% rel.)	colupulone (% rel.)	alpha (% w/w)	beta (% w/w)	ratio alpha/beta	cohumulone (% rel.)	colupulone (% rel.)
Bliževedly	K	4.42	4.22	1.05	24.2	42.3	5.08	5.37	0.95	24.2	41.6
	M	7.57	4.71	1.61	24.5	42.6	6.92	6.18	1.12	25.4	42.3
Steknik	K	3.66	4.31	0.85	23.7	42.0	5.70	6.38	0.89	26.5	40.9
	M	6.27	4.34	1.45	22.7	41.8	8.42	7.98	1.06	25.3	41.4
Brozany	K	3.32	4.10	0.81	25.9	41.5	5.39	5.46	0.99	25.6	42.7
	M	6.55	4.26	1.54	23.7	41.3	7.04	5.44	1.29	23.7	40.7
Kounov	K	2.26	3.68	0.61	28.6	43.8	4.71	5.49	0.86	26.9	42.8
	M	7.09	5.07	1.40	26.6	42.4	6.67	6.07	1.10	22.1	37.8
Holedeč	K	3.86	5.04	0.77	25.1	41.5	5.06	5.75	0.88	24.3	41.5
	M	7.69	4.80	1.61	25.2	41.3	8.40	6.78	1.24	23.4	41.3
Liběšice	K	2.73	4.00	0.68	26.7	42.8	4.43	5.53	0.80	26.5	41.9
	M	5.24	4.00	1.31	23.8	42.1	6.71	5.62	1.19	25.9	43.1
Skytaly	K	3.06	4.10	0.75	27.1	43.0					
	M	6.10	4.11	1.48	27.6	43.5					
Smilovice-Kozár	K						3.96	4.83	0.82	24.5	42.5
	M	7.79	4.94	1.58	24.4	41.9	6.79	6.73	1.01	24.6	42.1
Vědomice	K	3.19	4.58	0.70	25.2	41.0	3.99	4.92	0.81	26.2	40.9
	M	6.44	4.70	1.37	26.0	43.2	5.44	6.38	0.85	23.7	40.1
Tuchořice	K						5.26	5.36	0.98	26.0	42.7
	M						7.34	5.57	1.32	26.0	43.2
Domaželice	K	3.45	4.30	0.80	26.2	42.7	4.33	4.94	0.88	27.0	42.5
	M	5.71	4.75	1.20	25.4	42.2	6.68	6.32	1.06	25.5	41.5
Median	K	3.32	4.22		25.9	42.3	4.89	5.42		26.3	42.2
	M	6.50	4.70		24.9	42.2	6.86	6.25		25.0	41.5
95% confidence interval	K	2.82–3.82	3.96–4.48		24.5–27.3	41.3–43.3	4.15–5.61	5.05–5.78		24.8–27.7	41.2–43.2
	M	5.50–7.49	4.26–4.14		23.4–26.3	41.5–42.9	6.25–7.46	5.64–6.86		23.3–26.6	40.3–42.6

K = control, M = virus-free, alpha acids contents are based on dry matter

Hop oils

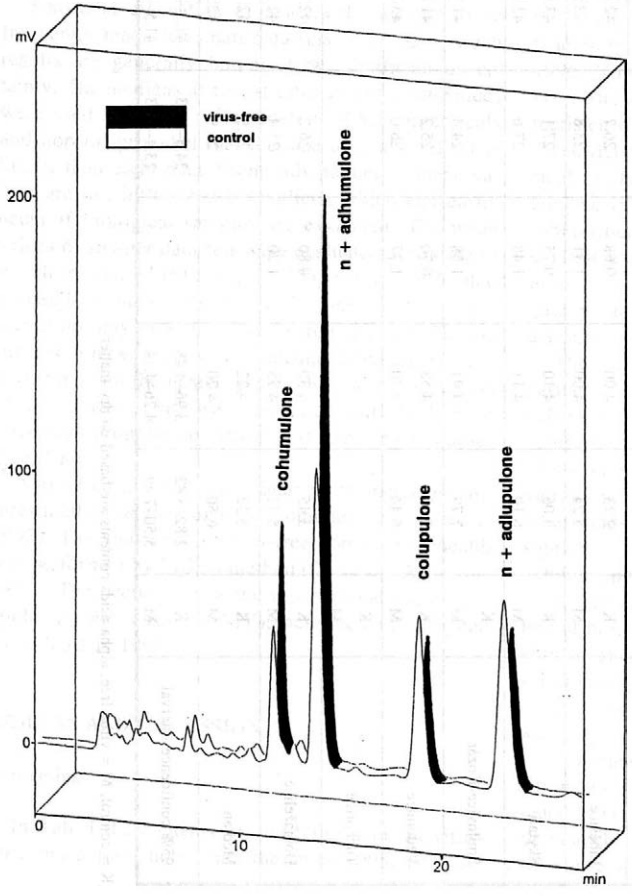
Amounts of the most important mono- and sesquiterpens (myrcene, caryophyllene, humulene and farnesene) were followed in the hop oils composition. Their proportion in virus-free and control hops in the years 1995 and 1996 is collated in Tab. III, corresponding GC chromatogrames (Brozany, 1995) are depicted in Figs 4, 5. The summary of hop oils composition in the 1992 to 1996 period is given in the Tab. IV. Year-to-year variations of terpens proportion seem to be relatively considerable in this period. Especially in the years 1994 and 1996 higher proportion of myrcene is evident. This increase is above all of the detriment to farnesene content. The proportions of humulene and caryophyllene are more balanced. These differences, that comparably occur in virus-free and control hops, are significant so far that influence the intensity of hop aroma in the whole crop. Slight shift in the proportion of individual terpens between virus-free and control hops can be observed. Virus-free hops have lower myrcene content and higher sesquiterpenes content. In the years 1995 and 1996 this shift did not prove to be statistically significant. From the sensoric point of view

II. Statistical evaluation of alpha acids contents (1992–1994)

Locality	Sample	Alpha acids content (% w/w)		
		1992	1993	1994
Steknik	K	4.5	3.7	2.4
	M	5.1	5.8	5.4
Holedeč	K	–	3.3	1.3
	M	–	5.5	4.2
Vědomice	K	1.3	3.3	1.3
	M	3.3	5.5	4.2
Brozany	K	–	5.2	2.4
	M	–	7.0	3.4
Přílepy	K	3.4	3.3	–
	M	6.2	5.6	5.7
Domaželice	K	–	4.4	2.7
	M	–	5.8	4.6

Mean value	1992		1993		1994	
	K	M	K	M	K	M
Arithmetic mean	3.07	4.87	3.93	5.98	2.46	4.77
Median	3.4	5.1	3.7	5.8	2.4	4.95
Horn	3.95*	5.65*	3.85	5.9	2.4	4.8

K = control, M = virus-free *arithmetic mean from 2 nearest values



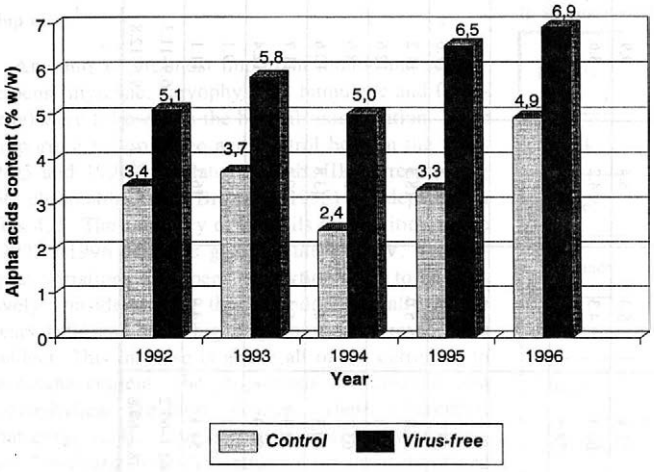
2. Chromatograms of hop bitter acids (virus-free and control hops; Brozany, 1996)

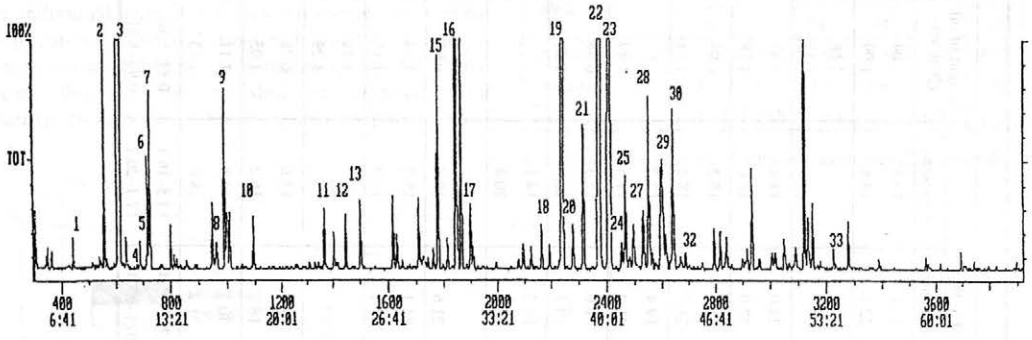
III. Evaluation of hop oils, crop 1995, 1996 (virus-free and control hops, GC/MS method)

Locality	Sample	1995					1996				
		weight of oil (% w/w)	hop oil composition (% rel.)				weight of oil (% w/w)	hop oil composition (% rel.)			
			myrcene	caryophyllene	humulene	farnesene		myrcene	caryophyllene	humulene	farnesene
Blíževedly	K	1.04	41.0	5.1	18.1	15.3	1.04	51.0	5.5	19.3	10.9
	M	1.60	25.9	7.4	22.1	19.8	1.09	53.3	5.3	13.5	10.7
Stekník	K						1.18	67.9	3.2	10.2	8.9
	M						1.33	66.3	2.9	9.8	9.2
Brozany	K	2.19	25.0	6.8	24.0	14.4	1.37	51.8	3.6	11.6	9.3
	M	1.43	23.0	7.1	22.0	18.6	1.28	42.1	5.6	17.9	16.8
Kounov	K	1.00	26.0	8.2	25.5	15.2	0.94	65.1	3.9	13.3	7.8
	M	1.12	27.4	7.6	21.5	18.4	1.11	64.6	3.7	11.9	8.8
Holedeč	K	1.48	36.9	5.5	19.4	12.2					
	M	1.58	29.6	7.4	21.9	17.5	1.49	51.4	5.3	18.4	9.9
Liběšice	K	0.97	36.3	5.9	21.0	15.3	0.88	60.9	4.3	14.2	8.6
	M	0.77	28.1	6.5	19.3	20.4	1.09	49.9	5.7	18.6	11.6
Skytaly	K	0.71	33.2	6.2	19.5	14.1					
	M	1.14	26.6	7.4	22.4	20.4					
Smilovice-Kozár	K										
	M	1.16	28.9	7.0	21.6	19.4	0.93	55.7	4.3	15.8	11.6
Vědomice	K	0.94	32.9	6.5	21.1	15.2	1.24	51.8	5.0	18.3	11.2
	M	0.86	28.7	7.0	21.1	17.9	1.15	51.8	4.5	16.7	12.6
Tuchořice	K						1.18	63.7	3.6	12.8	8.6
	M						1.29	60.3	4.3	13.8	10.6
Domaželice	K	0.68	38.9	5.3	17.4	13.6	0.78	52.4	6.1	20.4	11.7
	M	0.76	32.4	5.8	18.7	16.2	1.05	56.0	5.8	17.3	12.4
Median	K	0.98	34.8	6.1	30.3	14.8	1.11	56.7	4.1	13.8	9.1
	M	1.14	28.1	7.1	21.7	18.6	1.13	54.5	4.9	16.3	11.1
95% confidence interval	K	0.60–1.36	27.7–33.9	5.0–7.1	17.1–23.4	13.5–16.1	0.84–1.38	44.1–69.2	2.75–5.5	8.2–19.3	6.9–11.3
	M	0.76–1.52	26.2–30.0	6.6–7.6	20.1–23.3	17.1–20.1	0.95–1.31	48.2–60.8	3.7–6.1	12.8–19.7	9.5–12.8

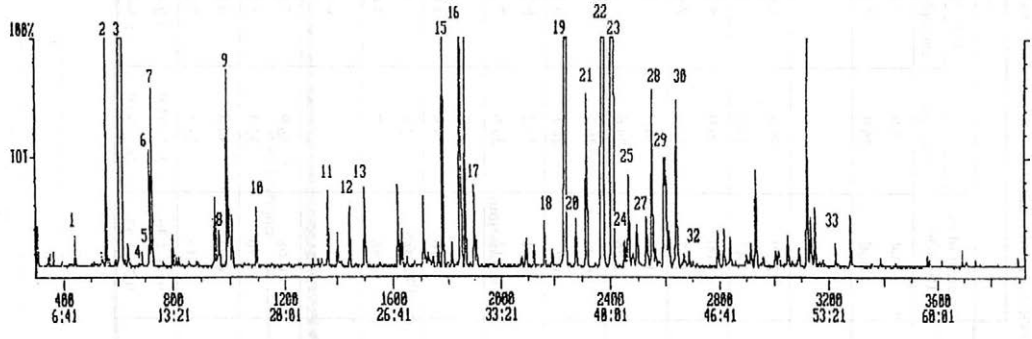
K = control, M = virus-free

3. Crop averages of alpha acids





4. GC chromatogram of the Saaz semi-early red-bine hop plant (Osvald's clone 72)



5. GC chromatogram of the Saaz semi-early red-bine hop plant (Osvald's clone 72/meristeme)

these differences are neglegible as well. More important are year-to-year variations mentioned above.

Yield

Most growers harvest meristem hops separately. Thanks to it relatively reliable data regarding to yield parameters are available. Yields of virus-free and control hops and production of pure alpha acids in kg/ha in the year 1996 are summarized in Tab. V. Yield evaluation in the period 1992 to 1996 expressed like meristem/control ratio ordered according to number of harvests is in Fig. 3. We can see that the most significant yield differences between virus-free and control hops are in the first year's crop. In the further harvests, when hop plants come to full performance, yield con-

IV. Proportion of terpens in hop oils-crop means (1992–1996)

Component	1992		1993		1994		1995		1996	
	K	M	K	M	K	M	K	M	K	M
Myrcene	35.1	34.9	45.1	41.8	55.7	52.1	34.8	28.1	56.7	54.5
Caryophyllene	7.0	6.7	5.2	5.4	4.6	4.5	6.1	7.1	4.1	4.9
Humulene	20.8	21.6	20.0	21.2	17.9	19.7	20.3	21.7	13.8	16.2
Farnesene	18.1	19.3	18.8	18.7	9.5	12.7	14.8	18.6	9.1	11.1

K = control, M = virus-free

V. Comparison of yield parameters, crop 1996 (virus-free and control hops)

Planting (autumn)	Locality	Control	Yield	Alpha acids	Production	Index	
		virus-free	(t/ha)	(% w/w)	(kg alpha/ha)	yield	(kg alpha/ha)
1991	Vědomice	K	1.50	4.00	60	100	100
		M	1.98	5.44	108	132	180
1992	Stekník	K	1.36	5.70	78	100	100
		M	1.63	8.42	137	120	176
	Brozany	K	1.70	5.39	92	100	100
		M	1.75	7.04	123	103	134
	Holedeč	K	1.23	5.06	62	100	100
		M	1.85	8.40	155	150	250
	Domaželice	K	1.88	4.33	81	100	100
		M	2.06	6.68	138	110	170
1993	Bližževedly	K	1.42	5.08	72	100	100
		M	2.11	6.92	146	149	203
	Kounov	K	1.10	4.71	52	100	100
		M	1.74	6.67	116	158	223
	Mlýnce	K	1.16	5.10	59	100	100
		M	1.61	7.40	119	139	202
	Pochválov	K	1.02	4.04	42	100	100
		M	1.25	6.61	83	123	197
	Smilovice	K	1.02	3.96	40	100	100
		M	1.80	6.79	125	176	312
1994	Tuchořice	K	1.11	4.26	58	100	100
		M	1.53	7.34	112	138	193

K = control, M = virus-free

vergence is indicated. Higher yield and simultaneously higher content of alpha bitter acids in virus-free hops result in substantially higher production of pure alpha acids/hectare (by 100% and more).

Reinfection

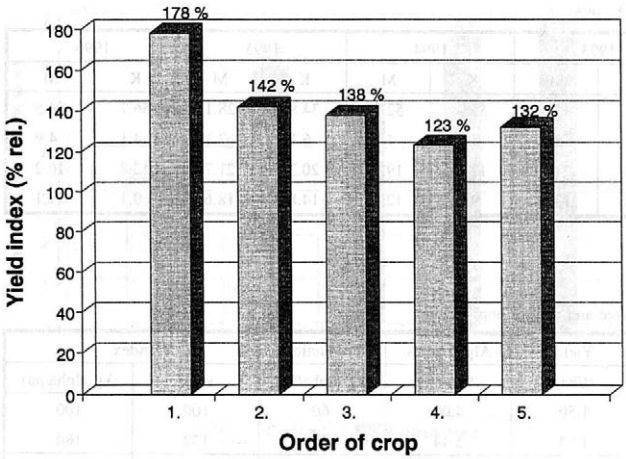
The state of health evaluation was performed systematically at the levels *in vitro*, greenhouse plants, propagation nurseries and virus-free hop gardens. No occurrence of ApMV virus has been detected in taken samples. Virus-free hop gardens established in the year 1991 are not meantime infected by ApMV. It can be supposed that long-term virus-free state will be kept in the conditions of natural infection pressure as well.

CONCLUSIONS

On the basis of hop bitter acids and hop oil analyses results can be stated that virus-free hop of Osvald's clone 72:

- have considerable advantages in comparison with traditional hops all the time;
- hold typical varietal features of the Saaz semi-early red-bine hop.

Virus-free hops have substantially higher content of alpha bitter acids, favourable composition of hop oils and give higher yield. Owing to growth vitality adverse influences of weather conditions on yield and content of brewing substances (hop oils and resins) are attenuated. Planting of virus-free hops (Osvald's clone 72)



has become an important stability factor of Czech hop industry.

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PHENOTYPE VARIABILITY OF THE MAIN CHARACTERISTICS OF HOP VARIETIES AS A SOURCE FOR HOP HYBRIDISATION PROCESS

FENOTYPOVÁ PROMĚNLIVOST HLAVNÍCH ZNAKŮ ODRŮD SLEDOVANÝCH JAKO GENETICKÉ ZDROJE KŘÍŽENÍ CHMELE

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ABSTRACT: The collection of genetic sources of the world assortment of hop varieties contains 289 genotypes, 224 of them are of a foreign origin, they come from 23 countries. They have been cultivated and their characteristics evaluated in the field collection inside the Hop Research Institute, Ltd., Žatec. The possibility of their utilisation as female partners for hybridisation process is studied in dependence on hops yield and bitter acids content. Genotypes of the European origin (Perle, Spalter Select and Hallertau Tradicion) seem to be the most suitable for breeding process of aroma hops. Females of hop varieties Yeoman, Target, Hallertau Magnum, Galena and Chinook proved to be the most suitable ones for hybridisation process of bitter hop varieties.

hops; breeding process; hybridisation process; choice of genotypes

ABSTRAKT: V kolekci genetických zdrojů, představované světovým sortimentem odrůd, je soustředěno 289 genotypů chmele, zahraničního původu je 224 genotypů z 23 zemí. Vysázeny a sledovány jsou v polní kolekci v areálu pracoviště. Podle projevu hlavních znaků (výnos, obsah hořkých látek) je usuzováno na možnost jejich využití jako samičích partnerů pro výchozí křížení. Pro šlechtění aromatického chmele se jako vhodné samičí komponenty projevily genotypy evropského původu (Perle, Spalter Select, Hallertau Tradicion). Pro šlechtění obsažného chmele lze za vhodné samičí komponenty považovat genotypy nejen evropského původu (Yeoman, Target, Hallertau Magnum), ale i odrůdy zámořské (Galena, Chinook).

chmel; šlechtění; křížení; volba genotypů

INTRODUCTION

Development of the world hop market requires some important changes in hop cultivation structure of hop varieties. Together with the extensive and fast spreading of more productive forms of aromatic hops, some new bitter varieties are cultivated as well (Fric, 1996). Besides registered varieties, some new ones are preferred for releasing. World collection of hop varieties is a source for the hybridisation process (Fric et al., 1995; Beránek, 1996). One of the most important assumptions for utilisation of the above mentioned genotypes is steadiness of demanded characteristics in local conditions.

All the legislative regulations restricting the process of hop breeding in CR in the past were cancelled in 1995 and 1996. Good conditions for utilisation of not only typical Czech hops but non-typical Czech aroma varieties as well have been created. More productive bitter hop varieties are requested and it is also one of the very important objectives of hop breeding process at the Hop Research Institute, Ltd., Žatec.

MATERIAL AND METHODS

World collection of hop varieties is placed at the Hop Research Institute, Ltd., Žatec. Spacing of 300 x 100 cm is used. One to three repetitions, each with eight hop plants are cultivated on the total area of 1.5 ha. 289 varieties registered or cancelled from 23 countries of the origin are grown. Only 65 of them come from CR. Steadiness of the yield together with bitter acid content are the two most important factors for determination of their utilisation. Yield of hops is measured after harvest by our picking machine and it is counted over per plant. The weight is expressed in kilograms of fresh hops. Alpha bitter content is evaluated as LCV (according to the Czech state standard – ČSN 46 2520). Data are reviewed in the weight % of dry matter.

Statistical evaluation is carried out by Adstat computer program, 1.25 verse (TriloByte). The value of the characteristic is expressed here as arithmetic mean, its variability is expressed by standard deviation. Va-

riation coefficients are used for comparison of the variability among individual hop varieties. Medium error together with the determination of reliability of the estimate is a base for calculation of conclusive evidence of the difference among the varieties with the help of *t*-test. Statistical significance ensues from the comparison of calculated values with the table ones. Probability of 1% ($P = 0.01$) is commonly considered to be the limit of high significance. If the limit is exceeded, the differences found are considered to be conclusive.

Evaluation data of nine genetic sources of aroma hops and nine of bitter hops are reviewed here for the period 1992 to 1996. Genotypes of foreign origin prevailed.

RESULTS AND DISCUSSION

The collection of genotypes concerning aroma hops breeding is predominantly formed by continental varieties. Yield up to 2 $t.ha^{-1}$ and alpha acids content of 7 to 8% are reached.

If the content of alpha acids is evaluated (Tabs I and II) high rate of variability of all the studied varieties in individual years is evident. It is commonly believed that it is the so-called yearly phenomenon when the yield and the content of alpha acids are strongly influenced by weather conditions in individual years.

Perle, the variety with the highest content of alpha acids (6.06%) is also the one of the most variable ($V = 46.7\%$). It is the only genotype out of the tested collection, where the difference of a found value in comparison with some other ones was statistically significant. But only 10% probability in relation to Fuggle, Hallertau and Hersbrucker was determined. It may be the same for Striesselspalt variety, but these results may be influenced by missing data from 1992. The other tested varieties are under 10% limit of probability in relation to the both Perle and mutually as well. The lowest phenotype variability was determined when Spalter Select variety was studied ($V = 29.07\%$) with the average content of alpha acids 3.99%.

It is obvious from Tabs III and IV that Spalter Select reaches the highest yield (3.33 kg/plant) with phenotype variability $V = 23.12\%$. Low phenotype variability is typical for Perle ($V = 16.21\%$) with good value of average yield. All the tested varieties with the exception of Hersbrucker are better than Striesselspalt.

If the group of aroma hops is evaluated it can be concluded that Spalter Select and Perle are suitable varieties for hybridisation process. Promising results were obtained also when Hallertau Tradicion variety was evaluated.

Collection of genotypes coming predominantly from overseas ones was studied for the possibility of their utilisation in the process of bitter hops hybridisation.

I. Evaluation of aroma genotypes according to the content of alpha acids (Žatec, 1992–1996)

Variety	1992	1993	1994	1995	1996	Mean value	Standard deviation	Variation coefficient	Average error
Perle	5.45	7.10	2.84	4.60	10.31	6.06	2.83	46.70	1.26
Hallertau Tradicion	4.48	2.93	2.60	4.74	5.75	4.16	1.34	32.31	0.60
Spalter Select	5.06	2.93	2.72	4.06	5.20	3.99	1.16	29.07	0.52
Cascade	3.93	4.00	2.03	3.92	5.51	3.88	1.23	31.70	0.55
Willamette	2.73	6.10	1.62	2.03	5.00	3.50	1.96	56.00	0.86
Fuggle	4.36	3.73	1.35	2.30	5.05	3.36	1.51	45.07	0.67
Hallertau	3.08	2.83	1.38	3.12	4.62	3.01	1.15	38.20	0.51
Strisselspalt	–	3.50	1.49	1.89	4.17	2.76	1.28	46.38	0.64
Hersbrucker	–	3.80	0.95	1.76	3.65	2.54	1.41	55.51	0.71

II. Statistical conclusive evidence of the difference of alpha acids content with the help of *t*-test; aroma genotypes (Žatec, 1992–1996)

Perle	Perle								
Hallertau Tradicion	–	Hallertau Tradicion							
Spalter Select	–	–	Spalter Select						
Cascade	–	–	–	Cascade					
Willamette	–	–	–	–	Willamette				
Fuggle	$P = 0.1$	–	–	–	–	Fuggle			
Hallertau	$P = 0.1$	–	–	–	–	–	Hallertau		
Strisselspalt	–	–	–	–	–	–	–	Strisselspalt	
Hersbrucker	$P = 0.1$	–	–	–	–	–	–	–	X

III. Evaluation of aroma genotypes according to the yield of hops (Žatec, 1992–1996)

Variety	1992	1993	1994	1995	1996	Mean value	Standard deviation	Variation coefficient	Average error
Spalter Select	2.01	3.93	3.36	3.58	3.79	3.33	0.77	23.12	0.34
Hallertau Tradicion	1.36	2.95	3.34	3.15	5.04	3.17	1.31	41.32	0.58
Willamette	2.00	3.84	2.87	3.07	3.28	3.01	0.67	22.25	0.30
Perle	2.72	3.50	2.55	2.44	3.31	2.90	0.47	16.21	0.21
Cascade	1.50	2.84	2.41	3.63	4.08	2.89	1.02	35.29	0.46
Hallertau	2.46	3.18	2.79	1.90	3.24	2.71	0.55	20.30	0.20
Fuggle	1.91	2.80	2.56	2.39	3.12	2.56	0.45	17.58	0.20
Hersbrucker	1.48	1.88	2.97	2.43	3.03	2.36	0.68	28.39	0.30
Strisselspalt	1.45	0.96	1.66	0.95	2.09	1.42	0.48	33.80	0.21

IV. Statistical conclusive evidence of the yield difference with the help of *t*-test; aroma genotypes (Žatec, 1992–1996)

Spalter Select	Spalter Select								
Hallertau Tradicion	–	Hallertau Tradicion							
Willamette	–	–	Willamette						
Perle	–	–	–	Perle					
Cascade	–	–	–	–	Cascade				
Hallertau	–	–	–	–	–	Hallertau			
Fuggle	$P = 0.1$	–	–	–	–	–	Fuggle		
Hersbrucker	$P = 0.1$	–	–	–	–	–	–	Hersbrucker	
Strisselspalt	$P = 0.01$	$P = 0.05$	$P = 0.01$	$P = 0.01$	$P = 0.01$	$P = 0.01$	$P = 0.05$	–	X

V. Evaluation of bitter genotypes according to the content of alpha acid (Žatec, 1992–1996)

Variety	1992	1993	1994	1995	1996	Mean value	Standard deviation	Variation coefficient	Average error
Hallertau Magnum	11.03	6.27	6.20	11.78	12.37	9.53	3.05	32.00	1.36
Yeoman	10.30	9.56	7.73	7.76	11.68	9.41	1.70	18.07	0.76
Chinook	7.64	6.93	8.43	5.42	9.50	7.58	1.54	20.32	0.69
Target	8.36	10.20	3.52	7.18	8.34	7.52	2.48	32.98	1.11
Galena	9.12	7.13	6.48	6.60	8.16	7.50	1.12	14.93	0.50
Nugget	7.01	7.46	6.62	6.54	8.03	7.13	0.62	8.70	0.28
Northern Brewer	5.77	6.06	2.81	4.60	8.90	5.63	2.23	39.61	1.00
Brewers Gold	5.98	9.10	3.11	4.74	5.11	5.61	2.21	39.39	0.99
Late Cluster	2.73	2.40	3.11	3.24	5.40	3.38	1.18	34.91	0.53

VI. Statistical conclusive evidence of the difference of alpha acids content with the help of *t*-test; bitter genotypes (Žatec, 1992–1996)

Hallertau Magnum	Hallertau Magnum								
Yeoman	–	Yeoman							
Chinook	–	–	Chinook						
Target	–	–	–	Target					
Galena	–	$P = 0.1$	–	–	Galena				
Nugget	–	$P = 0.05$	–	–	–	Nugget			
Northern Brewer	$P = 0.05$	$P = 0.05$	–	–	–	–	Northern Brewer		
Brewers Gold	$P = 0.05$	$P = 0.05$	–	–	–	–	–	Brewers Gold	
Cluster	$P = 0.01$	$P = 0.01$	$P = 0.01$	$P = 0.01$	$P = 0.01$	$P = 0.01$	$P = 0.1$	$P = 0.1$	X

VII. Evaluation of bitter genotypes according to the yield of hops (Žatec, 1992–1996)

Variety	1992	1993	1994	1995	1996	Mean value	Standard deviation	Variation coefficient	Average error
Galena	2.58	5.93	5.12	3.78	3.78	4.24	1.30	30.66	0.58
Target	3.08	4.58	3.41	4.43	4.80	4.06	0.76	18.72	0.34
Nugget	2.35	4.86	3.94	3.08	5.07	3.86	1.16	30.05	0.52
Late Cluster	1.48	4.64	3.90	3.30	4.54	3.57	1.29	36.13	0.58
Brewers Gold	3.17	4.04	3.66	3.26	3.52	3.53	0.35	9.92	0.16
Hallertau Magnum	1.94	3.08	3.85	3.61	4.53	3.40	0.97	28.53	0.43
Yeoman	3.03	3.48	3.50	3.50	3.40	3.38	0.20	5.92	0.09
Chinook	2.65	4.96	4.18	2.56	2.45	3.36	1.14	33.93	0.51
Northern Brewer	2.34	3.18	2.56	2.74	2.95	2.75	0.33	12.00	0.15

VIII. Statistical conclusive evidence of the yield difference with the help of *t*-test; bitter genotypes (Žatec, 1992–1996)

Galena	Galena								
Target	–	Target							
Nugget	–	–	Nugget						
Late Cluster	–	–	–	Late Cluster					
Brewers Gold	–	–	–	–	Brewers Gold				
Hallertau Magnum	–	–	–	–	–	Hallertau Magnum			
Yeoman	–	–	–	–	–	–	Yeoman		
Chinook	–	–	–	–	–	–	–	Chinook	
Northern Brewer	$P = 0.05$	$P = 0.01$	$P = 0.1$	–	$P = 0.01$	–	$P = 0.01$	–	X

Higher yields than 2 t.ha^{-1} and content of alpha acids higher than 10% were obtained. Similar dependence as in the case of aroma hops (Tabs V and VI) is evident. The most productive variety Hallertau Magnum with the average alpha acid content of 9.53% is also accompanied by higher phenotype variability (V = 32.0%). On the other hand, Yeoman variety has not only high content of alpha acids (9.41%) but low variant coefficient (V = 18.07%) and conclusivity of the difference to nearly all the tested varieties as well.

It is obvious from Tabs VII and VIII that Galena variety has the high yield (4.24 kg/plant) but also higher variability (V = 30.66%). On the other hand, Target variety has slightly lower yield in comparison with Galena, but lower phenotype variability (V = 18.72%). Northern Brewer has also lower phenotype variability but statistical evidence of the yield difference in comparison with other tested varieties is statistically significant. The harvest data concerning Hallertau Magnum were unfortunately influenced by missing 1992 results.

Yeoman and Target seem to be the most suitable varieties for the process of hybridisation of bitter varieties. Hallertau Magnum, Chinook and Galena may be used in this process as well.

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EVALUATION OF IMPORTANT CHARACTERISTICS OF SELECTED VARIETIES OF THE WORLD HOP COLLECTION

HODNOCENÍ VÝZNAMNÝCH ZNAKŮ A VLASTNOSTÍ VYBRANÉHO SOUBORU ODRŮD SVĚTOVÉHO SORTIMENTU CHMELE

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ABSTRACT: The importance of collecting, evaluating and keeping genetic sources is generally acknowledged. The world collection of hop varieties kept in Hop Research Institute in Žatec is a part of hop genofond. It comprises 289 varieties from all the world. Evaluation of selected set of 20 genotypes in 1992 to 1996 proved the important differences in dependence on the characteristic of hop varieties. Those with the highest productivity content of alpha acids (Hallertau Magnum, Yeoman, Galena) usually have a high yield of hops or high content of alpha acids in dry-matter. The lowest rate of variability of these characteristics accompanied by a favourable structure or hop resins was found out in the Yeoman variety. A lack of farnesene and later maturation are the common characteristics of the most productive genotypes. Lower productivity was confirmed together with a favourable structure of bitter acids if aroma and fine-aroma hops were studied. The time of maturation is a common feature which can be characterised as semi-early to semi-late. Another important characteristic is the structure of essential oils, predominantly of farnesene.

hops; breeding process; genofond; world collection of hop varieties; evaluation of characteristics

ABSTRAKT: Význam soustřeďování, hodnocení a uchování genetických zdrojů je všeobecně uznáván. Součástí genofondu chmele na pracovišti v Žatci je kolekce světového sortimentu odrůd o rozsahu 289 genotypů. Hodnocení vybraného souboru 20 genotypů v letech 1992 až 1996 ukázalo významné rozdíly v závislosti na hospodářské charakteristice odrůd. Odrůdy s nejvyšší produkcí alfa hořkých kyselin (Hallertau Magnum, Yeoman, Galena) se vyznačují vysokým výnosem nebo obsahem alfa hořkých kyselin v sušině. Nejnižší míra proměnlivosti těchto znaků, doprovázená příznivou skladbou chmelových pryskyřic, byla zjištěna u odrůdy Yeoman. Společným znakem nevýkonnějších genotypů je nedostatek složky chmelových silic farnesenu a pozdnější doba vyzrání. Genotypy aromatického chmele včetně jemně aromatického potvrdily nižší výkonnost při vhodné skladbě hořkých látek. Společným znakem je doba zrání, charakterizovaná jako poloraná až polopozdní, a zastoupení složky chmelových silic farnesenu.

chmel; šlechtění; genofond; světový sortiment chmelových odrůd; hodnocení znaků

INTRODUCTION

The problem of plant genofond is so important phenomenon that it has become the subject of international agreements. Continuous decrease of intra-species variability of plants under the influence of a man has provoked the necessity of the preservation of their different forms. Collection of genetic sources arose from the both wild and breed forms. Assortments of world plant varieties create an important part of genotype collections.

The beginnings of collecting and study of hop genetic sources are connected with a research activity carrying out at an experimental station which used to be at Deštnice, near a district town Rakovník (approximately 30 km from Žatec). In 1931 36 hop genotypes from six countries were imported and our world collection was established in this way. In the 1950s it was moved to Žatec. The origin mutual structure of domestic and foreign genotypes has been changed. Varieties exported from the world, different from traditional fine-aroma hops, are dominant at present.

Change in the structure of our collection is connected also with the necessity to ensure productive parental material for continuously developing methods of hybridisation (Beránek, 1995). All the forms of hop varieties known in the world hop market are demanded (Barth, 1995). Prevailing share of Czech tradition hops, represented by more productive virus-free forms, is still a typical feature of Czech hop cultivation (Fric, 1996).

MATERIAL AND METHODS

Hop genofond represented by the world collection of hop varieties contains 289 genotypes from 23 countries. The share of foreign varieties amounts to 224 genotypes.

The whole area of the collection (1.5 ha) is divided into two parts. The first part serves for long preservation and the second one for mutual comparison. One to three repetitions are used for each variety. One repetition comprises eight plants. Common cultivation operations are carried out during the vegetation period, hop plants are harvested individually in dependence on their maturation.

Evaluation of genotype characteristics was carried out by a branch Hop classificator (S r p et al., 1978). The yield was found out during harvest by picking machine and expressed in kg of fresh hops per plant. Lead conductance value served as a method for bitter acids analysis, which are expressed in percent of dry matter. Detailed analyses of hop resins were carried out by HPLC (EBC 7.4.1) and essential oils by gas chromatography (ČSN 46 2520).

Classic common elements of statistic characteristic (mean value, disperse, variation coefficient) are used for evaluation of observed features. A computer program Adstat, verse 1.25 (TriloByte, Pardubice) serves for statistic data processing. The collection of 20 genotypes of the most cultivated hop varieties is studied. The values found out in the period 1992 to 1996 at a location in Žatec are included.

RESULTS AND DISCUSSION

Individual varieties are deeply different if we take into account the results obtained. Relevance to an individual hop type was unambiguously demonstrated.

It is obvious from the results showed in Tab. I that Galena variety is the most productive (4.24 kg/plant). Its phenotypic variability is on average (V = 30.66%). Another bitter variety Target is also a productive one (4.06 kg/plant) with a lower phenotype variability (V = 18.72%). Low that means suitable phenotype variability values were determined also in the case of Yeoman (V = 5.92%) and Brewers Gold (V = 9.92%) varieties. Their yields are, however, lower on average by 20%. The average yield of aroma varieties is between 2.5 and 3 kg/plant with the average phenotype variability between 15 and 35%. In the comparison with the geno-

I. Productive characteristics (hop yield, content and productivity of alpha acids) in the studied genotypes (Žatec, 1992–1996)

Variety	Type of hops	Country of origin	Yield (kg.plant^{-1})			Content of alpha acids (%)			Production of alpha acids (kg.ha^{-1})	
			average	dispersion	variation coefficient	average	dispersion	variation coefficient	average	variation coefficient
Chinook	VHC	USA	3.36	1.30	33.93	7.58	2.37	20.32	188	36.31
Galena	VHC		4.24	1.69	30.66	7.50	1.25	14.93	229	24.20
Nugget	VHC		3.86	1.35	30.05	7.13	0.38	8.70	207	37.16
Willamette	A		3.01	0.45	22.25	3.50	3.84	56.00	64	56.46
Fuggle	A	England	2.56	0.20	17.58	3.36	2.28	45.07	61	60.92
Golding	A		2.50	0.77	35.20	2.89	1.12	36.64	56	58.19
Brewers Gold	HC		3.53	0.12	9.92	5.61	4.88	39.39	130	26.85
Northern Brewer	HC		2.75	0.11	12.00	5.63	4.97	39.61	137	68.52
Target	VHC		4.06	0.58	18.72	7.52	6.15	32.98	212	37.02
Yeoman	VHC		3.38	0.04	5.92	9.41	2.89	18.07	235	16.48
Hallertau Tradicion	A	Germany	3.17	1.72	41.32	4.16	1.80	32.31	107	58.47
Hallertau Magnum	VHC		3.40	0.94	28.53	9.53	9.30	32.00	242	49.09
Hersbrucker	A		2.36	0.46	28.39	2.54	1.99	55.51	47	57.41
Perle	A		2.90	0.22	16.21	6.06	8.01	46.70	130	59.45
Spalter Select	A		3.33	0.59	23.12	3.99	1.35	29.07	96	32.71
Tettnang	FA		1.13	0.37	53.98	3.01	2.51	52.63	29	88.21
Aurora	HC	Slovenia	3.02	1.17	35.77	6.98	3.53	26.92	150	43.89
Savinski Golding	A		1.88	0.30	15.89	3.02	2.99	57.21	50	91.91
Vojvodina	HC	Yugoslavia	3.32	1.24	33.58	3.64	1.75	36.38	90	59.78
Osvald's clone 72	FA	CR	1.57	0.15	24.86	3.00	0.93	32.22	36	49.36

Type of hops:
VHC – very high content of bitter acids
HC – high content of bitter acids
A – aroma hops
FA – fine aroma hops

type of fine aroma hops (Osvald's clone 72) are all the tested varieties with the exception of Tettnang better from the point of view of their productivity. Aroma varieties reach a higher yield by 50 to 100% and bitter varieties have the yield up to 165% higher (Galena) than the common aroma variety Osvald's clone 72. The highest content of alpha acids was observed in Hallertau Magnum variety (9.53%) and Yeoman (9.41%). A good feature in the case of Yeoman variety is its low phenotype variability (V = 18.07%). Only Galena and Nugget have lower values of phenotype variability if it is expressed by variation coefficient. Aroma varieties, including fine aroma-genotypes, can be characterised by the values in the range 2.5 to 3.5 alpha acids with a wide range of variation coefficient values (V = 30 to 55%).

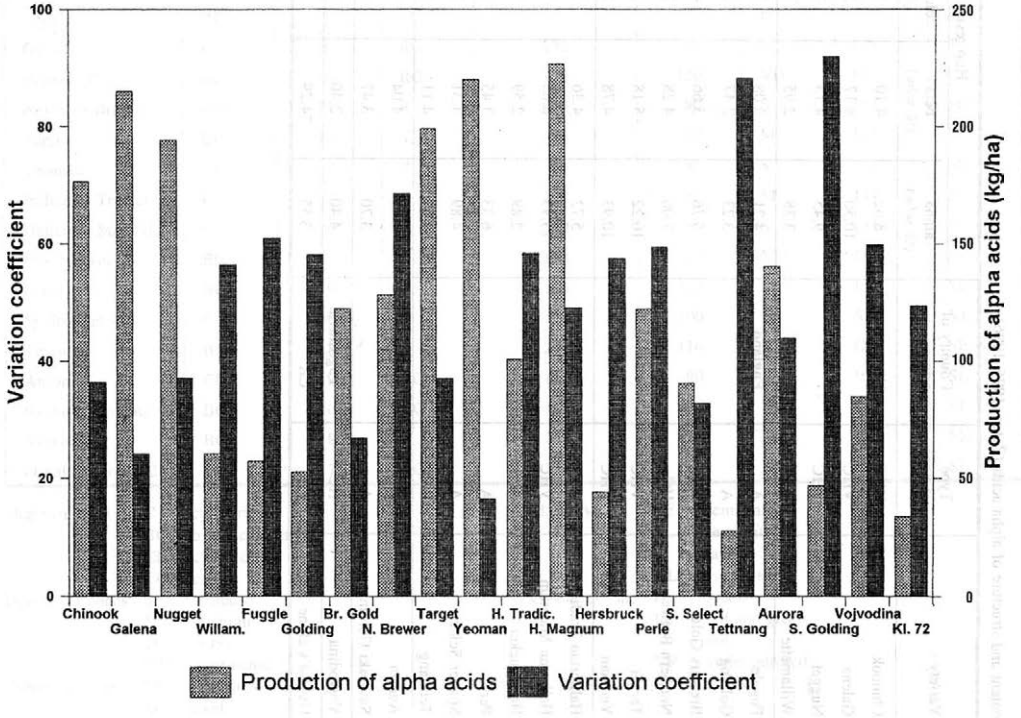
The important characteristic during the process of genotype evaluation is the calculation of alpha acids in kg/ha (Fig. 1). The most productive genotypes (Hallertau Magnum, Yeoman, Galena) have 4 to 5 times higher yield of alpha acids per hectare in comparison with aroma varieties. Even 7 times higher yield of alpha acids per hectare was reached in comparison with fine aroma varieties.

The whole genofond had been biochemically evaluated particularly concerning the content and structure of resins and essential oils since 1995 (Tab. II). As the average data are only short-term, they cannot be used as the final ones.

The results concerning hop resins, particularly alpha acids, can be compared with the data obtained by LCV method. In spite of the fact that different data were obtained, we can state that they are in harmony. The highest value was found out if Hallertau Magnum variety was tested (13.93% of alpha acids). The other four genotypes (Yeoman, Galena, Target and Nugget) had their content of alpha acids by 20% lower, but they are on the level of 10% of alpha acids content. Only 3 to 4.5% were obtained when we tested aroma varieties.

The resin of cohumulone on the level under 25% rel. is being appreciated. Fine aroma hops Osvald's clone 72 and Tettnag reach the values which are the closest to this level (26.5% rel). As an important phenomenon it is possible to consider cohumulone determination in the varieties with high production of alpha acids (Hallertau Magnum – 29.3% rel. and Yeoman – 29.55% rel.).

Myrcene is generally considered to be an undesirable component within essential oil content because of its bad influence on hop aroma. Myrcene content under 40% rel. is appreciated. None of the studied genotypes had the value under this level. Particularly aroma hops reached the level in the range between 40 to 50% rel. Relatively low share of myrcene in bitter hops (Yeoman – 47.7% rel. and Nugget – 47.25% rel.) can be considered as important. The lowest values (41 to 42% rel.) were determined in the varieties Osvald's clone 72 and Tettnang.



1. Rate of variability of alpha acids production

II. Content and structure of alpha acids (Žatec, 1995–1996)

Variety	Type of hops	Country of origin	Hop resins				Hop essential oils				
			alpha (% w/w)	beta (% w/w)	cohumulone (% rel.)	colupulone (% rel.)	weight (g.100 g^{-1})	myrcene (% rel.)	caryophyllene (% rel.)	humulene (% rel.)	farnesene (% rel.)
Chinook	VHC	USA	8.62	4.10	33.10	51.30	1.40	47.10	7.08	12.76	0.34
Galena	VHC		10.50	8.17	35.90	57.70	1.05	51.20	6.11	17.98	0.35
Nugget	VHC		9.45	4.13	35.80	49.35	0.82	47.25	10.62	26.35	0.64
Willamette	A		3.38	2.95	36.65	53.80	1.80	47.20	12.80	34.60	7.01
Fuggle	A	England	4.21	3.08	32.50	50.65	1.43	48.25	8.28	25.05	5.00
Golding	A		3.23	2.47	32.35	50.15	1.05	43.25	10.89	24.55	3.68
Brewers Gold	HC		5.76	3.66	43.30	66.00	1.75	56.10	9.18	17.42	0.17
Northern Brewer	HC		7.06	4.28	29.85	50.60	1.93	53.05	9.04	22.85	0.46
Target	VHC		10.22	5.18	36.30	58.45	1.01	56.80	6.65	14.50	0.16
Yeoman	VHC		10.93	4.78	29.55	47.95	1.61	47.70	9.10	23.05	0.23
Halllertau Tradicion	A	Germany	5.72	4.36	27.05	47.25	1.50	57.50	5.61	17.70	4.54
Hallertau Magnum	VHC		13.93	6.65	29.30	44.20	1.15	60.55	5.70	19.55	0.12
Hersbrucker	A		2.89	2.59	30.75	44.65	0.87	44.85	9.65	26.80	5.08
Perle	A		6.33	3.45	30.90	51.90	1.03	46.35	11.46	31.55	0.19
Spalter Select	A		4.89	4.31	28.20	43.35	1.06	50.90	3.81	9.65	13.30
Tettnang	FA		3.01	4.11	26.85	42.65	1.20	42.40	6.20	19.65	13.35
Aurora	HC	Slovenia	8.45	4.04	34.40	51.45	1.44	53.80	4.94	15.80	5.81
Savinski Golding	A		3.70	3.47	30.50	50.90	1.35	59.20	6.31	21.30	4.16
Vojvodina	HC	Yugoslavia	4.40	2.40	29.45	57.00	0.67	48.30	10.13	30.55	1.15
Osvald's clone 72	FA	CR	3.55	4.29	26.50	42.75	1.10	41.40	5.76	20.60	14.05

Content of farnesene in hop essential oil structure is a typical characteristics in aroma genotypes. Its highest share (13 to 14% rel.) was determined in fine aroma hops. Bitter genotypes show farnesene content approximately 0.5% rel.

The chosen morphological and biological features (Tab. III) complete the genotype characteristic. They were followed for the period of 3 to 5 years and no expressive deviations from the origin characteristic were found out.

It ensues from the determined data that productive genotypes of bitter hops within the studied collection are usually of more vigorous growth and wide cylindrical shape of hop plants. Thickness of the vine is more than 10 mm, fertile laterals are in a smaller distance from the earth and their length exceeded 100 cm. Cones are predominantly of long oval shape, more scarce and of semi-late maturation.

Genotypes of less productive aroma hops have cylindrical or wide cylindrical shape. Thickness of the vine does not exceed 10 mm, fertile laterals are in higher plant levels and their length is as a rule around 100 cm. Cones are usually of oval or long oval shape, medium dense or dense. Their maturation is semi-early or semi-late.

The evaluation data of a selected collection of decisive genotypes in agro-ecological conditions of CR document high yield-level and alpha acids content of bitter hops. The most productive are particularly hop varieties coming from the USA, England and Germany. However, their productive abilities are accompanied by the structure of bitter components which cannot be considered to be suitable ones.

Good productive level of alpha acids together with their corresponding structure show aroma genotypes. They are represented particularly by varieties of the European origin. Of this group, the genotypes of very fine aromatic hops are represented by Osvald's clone 72 and Tettnang varieties. Lower productive value under a favourably evaluated structure of bitter compounds has been proved.

III. Morphological and biological characteristics (Žatec, 1992–1996)

Variety	Shape of plant	Thickness of vine (mm)	Colour of vine	Distance of laterals from earth (cm)	Length of laterals (cm)	Density of cones	Shape of cones	Maturity
Chinook	CL	14	VR	200	120	S	LO	L
Galena	CL	11	R	100	100	S	LO	SL
Nugget	CL	11	G	190	110	S	LO	SL
Willamette	BC–CL	10	G	176	110	D	O	SL
Fuggle	BC	7	RG	154	100	D	O	SL
Golding	C	10	RG	132	100	VD	LO	SL
Brewers Gold	BC	10	RG	170	120	VD	O	L
Northern Brewer	CO	11	R	50	80	S	LO	SE
Target	CO	10	V	82	70	M	LO	SE
Yeoman	CL	9	R	80	80	S	LO	SE
Hallertau Tradicion	C	8	RG	100	80	M	LO	SL
Hallertau Magnum	C	9	G	140	80	S	LO	SL
Hersbrucker	BC	10	RG	168	100	VD	O	SL
Perle	BC	9	G	106	120	M	LO	SL
Spalter Select	C	10	R	150.	100	M	S	VL
Tettnang	BC	8	R	200	110	S	O	SE–SL
Aurora	CO	9	RG	100	90	M	LO	SL
Savinski Golding	BC	10	RG	104	90	VD	LO	SL
Vojvodina	BC	8	V	152	110	D	LO	SL–L
Osvald's clone 72	C	7	RG	170	80	M	O	SE

Shape of plant: C – cylindrical; BC – broad cylindrical; CL – clubbed; CO – conic

Density of cones: S – scarce; M – medium; D – dense; VD – very dense

Shape of cones: LO – long-oval; O – oval; S – square

Maturity: SE – semi-early; SL – semi-late; L – late; VL – very late

Colour of vine: G – green; RG – red and green; R – red; VR – violet and red; V – violet

Brewing industry demand all the types of hop varieties from the both economical and technological point of view. It is certain that also in CR aroma and bitter varieties will find their utilisation.

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IDENTIFICATION OF GENOTYPES IN HOP (*HUMULUS LUPULUS* L.) BY RAPD ANALYSIS USING PROGRAM GEL MANAGER FOR WINDOWS

IDENTIFIKACE GENOTYPŮ CHMELE OTÁČIVÉHO (*HUMULUS LUPULUS* L.) POMOCÍ DNA ANALÝZY METODOU RAPD S VYUŽITÍM PROGRAMU GEL MANAGER FOR WINDOWS

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ABSTRACT: DNA was isolated from the collection of 15 hop varieties. Variability among genotypes was detected by RAPD method (Random Amplified Polymorphic DNA). Set of the 6 primers with the lengths of 8 bp, 10 bp and 13 bp was verified for the amplification. Primer P2 5'ACTTCGCCACGTA3' giving RAPD genotype specific markers with minimal frequency of unspecific amplifications was selected. This marker enabled to identify 15 hop genotypes. RAPD markers were separated on vertical polyacrylamide electrophoresis and visualised by means of Ag^+ ions. Electrophoreograms were evaluated densitometrically after the digitalization, using Gel Manager for Windows program. Genetic similarity among individual genotypes was expressed in the values of Pearson's correlation coefficient, which enabled the creation of dendrogram.

hop; *Humulus lupulus* L.; DNA; RAPD; PCR; fingerprinting

ABSTRAKT: Ze souboru 15 odrůd chmele otáčivého byla izolována DNA. Přehled výtěžností DNA z různých genotypů chmele je uveden na obr. 1. Variabilita mezi genotypy byla detekována metodou RAPD (Random Amplified Polymorphic DNA – náhodná amplifikace polymorfní DNA). Pro amplifikaci byla ověřována sada 6 primerů s délkami 13 bp, 10 bp a 8 bp. Byl vybrán primer P2 5'ACTTCGCCACGTA3' poskytující RAPD genotypově specifické markery s minimální frekvencí náhodných amplifikací. Pomocí tohoto markeru bylo rozlišeno všech 15 genotypů chmele. RAPD markery byly separovány na vertikální polyakrylamidové elektroforéze a vizualizovány pomocí Ag^+ iontů. Elektroforeogram je uveden na obr. 2. Elektroforeogramy byly po digitalizaci denzitometricky vyhodnoceny programem Gel Manger for Windows. Genetická podobnost mezi jednotlivými genotypy byla vyjádřena hodnotami Pearsonova korelačního koeficientu. Přehled korelačních koeficientů je uveden v tab. III. Na základě korelačních koeficientů byla provedena dendrogramová studie, která je uvedena na obr. 3.

chmel; *Humulus lupulus* L.; DNA; RAPD; PCR; fingerprinting

INTRODUCTION

The introduction of RAPD (Random Amplified Polymorphic DNA) enables an objective determination of varietal purity and authenticity. The temporary world trend in propagation and restoring the hop root stock material by means of *in vitro* techniques, represents a further actual area in RAPD varietal markering. Morphological differentiation of hop cultivars *in vitro* cultures is quite impossible.

Biochemical and genetical determination of cultivar fingerprinting is realised by the methods using polymorphism of reserve proteins, isoenzymes and DNA. Vejl, Černý (1994) were engaged in problems of total proteins as hop genetic markers. They characterised *in vitro* mericlones by the spectrum of electrophoretically separated proteins.

Technique of cultivar fingerprinting using DNA polymorphism can be characterized by higher objectivity, accuracy and reliability. RFLP (Restriction Fragment Length Polymorphism) belongs to historically older methods. General knowledge of RFLP is summarized by Botstein et al. (1980). Pillay, Kenny (1994) used RFLP for the determination of the differences in hop cultivars.

PCR (Polymerase Chain Reaction) and RAPD are included in other markering technologies. Both technologies use polymorphism of *in vitro* amplified DNA, as a product of enzymatic reaction *Taq* polymerase or *Tbr* polymerase. The general course of the polymerase chain reaction is described for example by Chein et al. (1976), Williams et al. (1990), Sambrook et al. (1989) and Innis, Gelfand (1990). Samec (1993) described optimal composition and tem-

perature profiles of RAPD reaction in this way: denaturation of template at 90 to 95 °C, annealing of primers at 35 to 40 °C and the extention phase at 72 °C. Jakše et al. (1994) described the use of RAPD markers for distinguishing of 12 Slovenien hop cultivars. He also characterized a set of hop clones by means of amplified microsatellite DNA. Matoušek, Trněná (1996) used polymorphism of genes for 7SL RNA for the identification of hop genotypes. Pillay, Kenny (1994) studied the hop RAPD marker segregation in F_1 generation.

Electrophoretic separation of amplified DNA is necessary for the visualization of RAPD varietal markers polymorphism. Staining of RAPD markers at the separation with horizontal electrophoresis with agarose gel, by means of ethidium bromide described Williams et al. (1990). This course for the RAPD markers in hop used Jakše et al. (1994), Brady et al. (1996) and Pillay, Kenny (1994). Caetano-Anollés, Gresshoff (1994), Vejl (1995) and Vejl, Salava (1995) described the use of vertical electrophoresis with polyacrylamide gel carrier and the following staining by Ag^+ ions.

For the characterization of electrophoreograms it is possible to use REM values (Relative Electrophoretic Mobility). Vejl, Černý (1994) characterized fingerprints of hop mericlones by this method. Densitometric characterising of electrophoreograms using computer analysis of the picture by Gel Manager for Windows program was described by Čurn et al. (1995), Vejl (1995) and Vejl, Salava (1995) in different botanical taxons.

MATERIAL AND METHODS

Plant material

Collection of 15 genotypes (*Humulus lupulus* L.), grown in the hop garden on the experimental field of the Czech University of Agriculture in Prague, was used. The summary of analysed genotypes is shown in Tab. I.

For the DNA isolation young leaves were used, sampled in the second decade of May, from the near to the apex.

DNA isolation

The sampled leaf blades were transferred into sterile polypropylene centrifuge tubes and were fixed by overlaying them by liquid nitrogen. The fixed material was used in the presence of cetyltrimethyl ammonium bromide (CTAB) (Murray, Thompson, 1980; Sahai-Maroof, 1988 – in Tempus, 1993).

Fixed material was mechanically homogenized in the presence of liquid nitrogen and subsequently incubated with 300 µl of extraction buffer (2% CTAB, 0.1M Tris pH = 8, 20mM EDTA, 1.4M NaCl, 2% mercaptoethanol), for 120 minutes in temperate water bath TE8J (Techne, Great Britain) at 60 °C. Subsequently the extraction mixture was complemented by 3000 µl of chloroform-isoamylalcohol (1 : 1) and carefully vertically mixed for 10 minutes. Then the mixture was centrifuged (5000 rpm, 10 minutes, 5 °C) in a table centrifuge Hettich Universal 30RF (Germany). Supernatant was transferred into a sterile polypropylene tubes and carefully mixed with equivalent volume of isopropanol. Precipitation of nucleic acids was realized at –40 °C for 12 hours. Precipitate of nucleic acid--CTAB was obtained by the centrifugation (5000 rpm, 10 minutes, 5 °C). The precipitate was rinsed with 80% ethanol with 0.01M Tris pH = 8 and 0.01M LiCl and subsequently vacuum dried (Chris Alpha 1-4, Germany). Complex of CTAB nucleic acid was diluted in 1000 µl 1 x TE. After adding RN-ase A (100 µg. .1000 μl^{-1}), the solution was incubated in water bath at 37 °C for 30 minutes. The solution was shaken with equivalent volume of phenol-chloroform (1 : 1) and vertically mixed for 10 minutes. Water phase was transferred (after centrifugation 3000 rpm, 10 minutes, 5 °C) into a new tube, where the double volume of 70% ethanol and 0.1 volume 3M natrium acetate, was added to the solution. The solution was subsequently centrifuged (5000 rpm, 10 minutes, 5 °C). After the supernatant removal, the sedimentated DNA was rinsed with 70% ethanol, vacuum dried and dissolved in 50 µl 1 x TE. Isolated DNA was UV-spectrophotometrically quantified (Gene Quant, Pharmacia, Great Britain) and diluted to the constant concentration (0.2 $\mu g.\mu l^{-1}$).

Amplification of DNA

RAPD method was used for the identification of genotypes. The reaction mixture (20 µl) contained: 0.6 U of thermostable polymerase Thermalase *Tbr* (Amresco, USA), 0.24 µg of template DNA, 10 mM of primer (Amresco, USA) and 200 µdNTP (Amresco, USA). The reaction mixture was overlayered by 30 µl PCR oil. Appropriate proteinases in template DNA were heat-inactivated (94 °C, 2 minutes).

These primers were used for the amplification:
P1 5'GCGAAGCGAGCTG3'
P2 5'ACTTCGCCACGTA3'
P3 5'GGTTACCACA3'
P4 5'ACTACGAACG3'
P5 5'ACGTCTGA3'
P6 5'CCTAGGCA3'

Thermocycler Cyclogen (Techne, Great Britain) with thermal and time profile, shown in Tab. II, was used.

Products of amplification were purified with phenol--chloroform (1 : 1) shaken up (10 minutes). After the following centrifugation (5000 rpm, 5 minutes, 5 °C) the water phase was transferred and was used for electrophoretic separation. The products of amplification were stored at –40 °C.

I. Designation and description of analysed genotypes

No.	Cultivar	Country of origin	Description
1	semiearly Žatec red bine hop – clone Blato	Czech Republic	fine aromatic hop, red bine
2	semiearly Žatec red bine hop – Osvald's clone No. 31	Czech Republic	fine aromatic hop, red bine
3	semiearly Žatec red bine hop – Osvald's clone No. 72	Czech Republic	fine aromatic hop, red bine
4	semiearly Žatec red bine hop – Osvald's clone No. 114	Czech Republic	fine aromatic hop, red bine
5	semiearly Žatec red bine hop – clone Aromat	Czech Republic	fine aromatic hop, red bine
6	semiearly Žatec red bine hop – clone Sifem	Czech Republic	fine aromatic hop, red bine
7	semiearly Žatec red bine hop – clone Zlatan	Czech Republic	fine aromatic hop, red bine
8	semiearly Žatec red bine hop – clone Universal	Czech Republic	fine aromatic hop, red bine
9	Bor	Czech Republic	fully aromatic hop, red-violet bine
10	Sládek	Czech Republic	aromatic hop, green bine
11	Northern Brewer	Great Britain	fully aromatic hop, red-green bine
12	Vojvodina	Yugoslavia	fully aromatic hop, violet bine
13	Comet	USA	fully aromatic hop, green-red bine
14	Spalt	Germany	aromatic hop, red bine
15	Tettnang	Germany	aromatic hop, red bine

II. Temperature and time profile of RAPD reaction

Program	PCR phase	Ratio of temperature change (°C.s^{-1})	Temperature (°C)	Time (s)
A: 1 cycle	denaturation	1	94	180
	annealing	1	39.5	100
	extention	1	72	120
B: 40 cycles	denaturation	1	94	60
	annealing	1	39.5	100
	extention	1	72	120
C: 1 cycle	extention	1	72	600

Electrophoresis of RAPD markers

For the electrophoretic separation, vertical electrophoresis Mini Protean (Bio Rad, USA) was used. The separation was performed on 5% polyacrylamide gel with the ratio of acrylamide to N,N'-methylenbisacrylamide 1 : 1 in 1 x TBE buffer. Solution of 5 RAPD product, 5 µl of loading buffer (Sambrook et al., 1989) and 5 µl of deionized H_2O was used for the separation. The separation passed of at the following parameters: constant 40 V, 15 minutes, following constant 60 V, 100 minutes. Molecular weight of amplified fragments was characterized by means of contemporary separated standard Lambda DNA/*Eco* 417(AvaII) (MBI Fermentas, Latvia).

Separated fragments were fixed on the gel carrier by 10 minutes incubation in 10% ethanol with 5% CH_3COOH. Subsequently the gels were saturated by water solution of 0.1% $K_2Cr_2O_7$ with $3.75.10^{-3}$% HNO_3 for 5 minutes. After rinsing in deionized H_2O the gels were developed in 2.5% water solution of waterless Na_2CO_3 with 0.05% formaldehyde. The developing reaction was finished by 5 minutes gel incubation in 5% CH_3COOH. After rinsing in deionized H_2O, the gels were saturated with 5% solution of glycerol (60 minutes) and subsequently dried out.

Evaluation of electrophoreograms by computing picture analysis

Electrophoreograms were digitalized by means of table scanner (Tamarac Art Scan 12000C, Holland) and after the enlargement correction, contrast and background by I Photo Plus version 1.1 program were stored in the form *.TIF files. Gel Manager for Windows (Biosystematika, Great Britain) was used for computer picture analysis. Labelling of profiles of individual RAPD markers, segment and background correction was realized. For the subsequent statistical evaluation of densitometric profiles, the typical polymorphic bands were selected, not influenced by an accidental amplification. Selection of individual profiles and polymorphic bands was realized in the program. Genetical distances or similarities among individual genotypes were determined on the basis of Pearson's

correlation coefficient comparing the position and density of bands (Gel Manager for Windows, 1994).

Pearson's correlation coefficient:

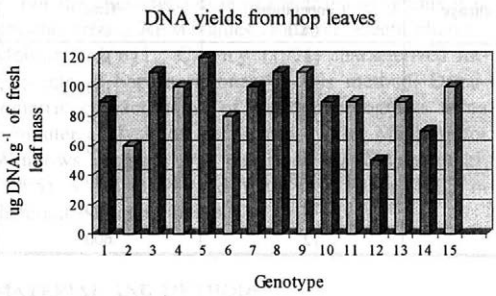
$$r = \frac{\Sigma^n [(Y_{ij} - Y_{j\,av}) \cdot (Y_{ik} - Y_{k\,av})]}{\sqrt{[\Sigma^n (Y_{ij} - Y_{j\,av})^2] \cdot [(Y_{ik} - Y_{k\,av})^2]}}$$

where: r – correlation coefficient
n – number of profiles
i – index of the first profile
j – index of the second profile
Y – absorbance value
Y_{av} – mean absorbance value

RESULTS

DNA isolation

DNA was isolated from the all analysed hop genotypes by means of the method described previously ranged from 0.5 to 1.2 μg DNA.g^{-1} fresh leaf mass. The yield survey is presented in Fig. 1.



1. Graphic survey of DNA yields from hop leaves; for genotype description (No. 1 to 15) see Tab. I

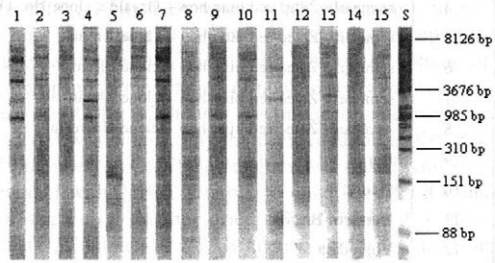
DNA amplification

All of the 6 tested primers yielded products of amplification. Primers P5 and P6 (8 nucleotides) gave products with a high number of bands with high frequency of unspecified amplifications. Unspecified amplifications originated even by the use of ten nucleotide primers (P3, P4). Primer P1 showed the specificity only for minimal number of genotypes.

Products originating by the use of P2 primer (5'ACTTCGCCACGTA3') showed minimal unspecific amplifications and high polymorphism of RAPD markers.

Electrophoretic separation of RAPD markers

The method of vertical electrophoresis with the staining by means of Ag^+ ions showed an excellent ability of amplified DNA separation. The above-mentioned method of gel staining enabled obtaining and following evaluation by densitometric analysis. RAPD products with the primer P2 5'ACTTCGCCACGTA3', separated by vertical polyacrylamide electrophoresis, are shown in Fig. 2.



2. Electrophoreogram of RAPD products – primer P2 5'ACTTCGCCACGTA3' of different hop genotypes (*Humulus lupulus* L.); for genotype description (No. 1 to 15) or electrophoreogram paths, resp. see Tab. I, S = LambdaDNA/Eco471(Avall)

Molecular weight of polymorphic bands ranged from around 80 bp to 8000 bp (Fig. 2). Numbers of bands of RAPD genotype markers are presented in parenthesis behind the name of the analysed cultivars.

Computing analysis of the picture – densitometric evaluation

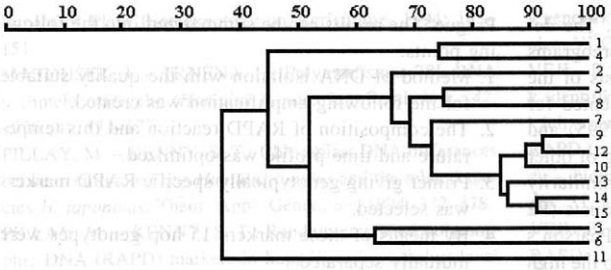
Electrophoreogram of RAPD markers with the primer 5'ACTTCGCCACGTA3' was digitalized. Each of the genotypes was characterized by the position and density of polymorphic bands. The similarities among the densitometric profiles of individual genotypes, expressed by the values of Pearson's correlation coefficient are given in Tab. III.

Correlation coefficients according to Pearson, given in Tab. III, were used for the construction of dendrogram of Fig. 3.

DISCUSSION

The method used for DNA isolation provided sufficient quality of DNA in corresponding quality, suitable for the following amplification. Similar method of DNA isolation (with CTAB) described Pillay, Kenny (1996). The yield of extraction is not presented by these authors.

Tbr polymerase (Amresco, USA) was used for the amplification. All the quoted authors amplified hop DNA by means of *Taq* polymerase. RAPD reaction realized with *Tbr* polymerase showed higher specificity and higher molecular weight of amplified product in comparison with *Taq* polymerase reaction. This information coincide with those presented by the producer of the enzyme. Most of the authors described the removal of proteinase from template DNA by incubation



3. Dendrogram comparison of the genetic distance among hop genotypes (*Humulus lupulus* L.) by means of RAPD markers (primer P2 5'ACTTCGCCACGTA3'); for genotype description (No. 1 to 15) see Tab. I

III. Survey of correlation coefficients according to Pearson, describing genetic resemblance (%) among RAPD products (primer P2 5'ACTTCGCCACGTA3') of different hop genotypes (*Humulus lupulus* L.); for genotype description (No. 1 to 15) see Tab. I

1	100	–	–	–	–	–	–	–	–	–	–	–	–	–	–
2	32	100	–	–	–	–	–	–	–	–	–	–	–	–	–
3	50	65	100	–	–	–	–	–	–	–	–	–	–	–	–
4	74	48	58	100	–	–	–	–	–	–	–	–	–	–	–
5	28	71	53	44	100	–	–	–	–	–	–	–	–	–	–
6	45	49	63	37	54	100	–	–	–	–	–	–	–	–	–
7	71	66	63	70	74	66	100	–	–	–	–	–	–	–	–
8	18	63	41	42	75	40	56	100	–	–	–	–	–	–	–
9	42	69	66	53	77	43	78	70	100	–	–	–	–	–	–
10	44	74	56	58	81	35	81	68	91	100	–	–	–	–	–
11	4	52	38	13	45	55	29	56	37	25	100	–	–	–	–
12	35	68	50	44	75	28	67	70	92	87	33	100	–	–	–
13	40	48	57	41	61	31	64	52	88	77	22	88	100	–	–
14	40	58	56	43	69	40	70	62	87	77	41	90	89	100	–
15	33	71	60	42	71	48	70	63	86	81	50	86	81	91	100
Genotype	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15

at 94 °C for 2 minutes. The $MgCl_2$ concentration used in reactive solution comes out from the optimum composition of reactive buffer, which is a part of the used enzyme.

The temperature and time profile of RAPD reaction corresponds to that one, described by Jakše et al. (1994) and Matoušek, Trněná (1996). The annealing temperature 36 °C, presented by Brady et al. (1996), gave a higher frequency of unspecific amplifications. The used annealing temperature 39.5 °C is lower than the temperature presented by Matoušek, Trněná (1996) (48 °C) and Pillay, Kenny (1996) (42 °C). The frequency of unspecific amplifications was negligible due to the use of suitable primer.

The influence of primer length on the genotype specificity of RAPD markers was studied on the basis of set primer testing of the size 8 bp to 13 bp. The primer length 8 bp and 10 bp, used by Pillay, Kenny (1996), gave a higher frequencies of unspecific amplifications. Only one of the tested primer set (P2 5'ACTTCGCCACGTA3') gave RAPD products, which enabled the differentiation of all 15 analysed hop genotypes. This reality corresponds to the conclusions described by Pillay, Kenny (1996), who distinguished 24 hop genotypes from 60 tested ones. The size of RAPD products by means of 5'ACTTCGCCACGTA3' primer ranged from 80 bp to 8000 bp. The size of genotypically specific RAPD markers ranged in the interval of 50 bp to 5000 bp. Jakše et al. (1994) and Pillay, Kenny (1996) described an analogous size of RAPD genotype markers.

Separation of amplified DNA by means of polyacrylamide electrophoresis is not presented by any of the mentioned authors. The electrophoretic separation by horizontal agarose electrophoresis, described for example by Jakše et al. (1994), Matoušek, Trněná (1996), was replaced by vertical polyacrylamide electrophoresis, which gave, after staining by Ag^+ ions, electrophoreograms with quality suitable for digitalization and following evaluation by Gel Manger for Windows program.

RAPD genotypically specific hop markers are characterized by their size (bp) and number of bands (Jakše et al., 1994; Brady et al., 1996; Pillay, Kenny, 1996). The densitometric evaluation of electrophoregrams by Gel Manager for Windows enables an objective determination of similarities among the

electrophoretic profile in analysed genotypes. The demands on the quality of evaluated electrophoreograms and all the further steps of computing analysis of the picture correspond with the knowledge, presented for example by Čurn et al. (1995), Vejl (1995) and Vejl, Salava (1995), at the fingerprinting of other botanical taxons. For the evaluation of the similarity among electrophoretic profiles of RAPD products (P2 5'ACTTCGCCACGTA3') of hop, only the Pearson's correlation coefficient was used, with regard to the high quality of the electrophoreogram (sharpness of bands, minimum background). For this reason no comparison according to Dice (only the RAPD marker position), which were described by Čurn et al. (1995), Vejl (1995) and Vejl, Salava (1995), was made.

From the dendrogram study the separation groups of Semiearly Žatec red bine hop is evident. The similarity among RAPD marker profiles (P2 5'ACTTCGCCACGTA3') ranged in this group between 65% to 75%. The clones of Semiearly Žatec red bine hop were divided into partial groups – clusters according to the dendrogramatic evaluation: I. Blato and Osvald's clone No. 114, II. Osvald's clone No. 31, III. Aromat and Universal, IV. Zlatan, V. Osvald's clone No. 72 and Sířem. The other analysed genotypes significantly differ for the group of Semiearly Žatec red bine hop. The individual cluster create: VI. Hybrid cultivares Bor and Sládek and the cultivar Vojvodina, VII. Comet, VIII. Spalt and Tettnang and IX. Northern Brewer.

The separation of 15 hop genotypes into 9 clusters significantly corresponded to the country of origin (individual cluster of genotypes from CR, Germany, USA and Great Britain). This fact clearly supports the idea that the used cultivars derived from similar gene resources, are typical for a given area. The determination of relatively big differences (creation of five clusters) in Semiearly Žatec red bine hop is very interesting.

The ability of RAPD method to separate mutually highly relative genotypes (individual clones of Semiearly Žatec red bine hop) shows its high sensitiveness and suitability for the identification of cultivar authenticity of hop plants. Due to the fact, that RAPD represents a random amplification of polymorphic DNA, it is impossible to speak about markering the characters of hop quality by means of RAPD markers.

Studies of RAPD genotypically specific markers create the initial step, which enables mapping and markering of economically important properties of hop (*Humulus lupulus* L.) together with hybridological analyses and other methods of genetic markering.

CONCLUSION

The study represents the introductory results of hop genotype identification realized in the Laboratory of Genetic Analyses at the Department of Genetics and Plant Breeding – Czech University of Agriculture in Prague. The results can be summarized into the following points:

1. Method of DNA isolation with the quality suitable for the following amplification was created.
2. The composition of RAPD reaction and this temperature and time profile was optimized.
3. Primer giving genotypically specific RAPD markers was selected.
4. By means of these markers 15 hop genotypes were mutually separated.
5. Similarities in RAP profiles among single genotypes were characterized by the values of Pearson's correlation coefficient.
6. By means of Gel Manager for Windows the genotypes were separated into groups (clusters) corresponding to a country of origin of hop genotypes.

Abbreviations and terms

Annealing	– annealing of a primer and the template DNA at PCR
Band	– one zone (strip) in electrophoreogram
Cluster	– group of individual with very similar characters
CTAB	– cetyltrimethyl amonium bromide
dNTP	– nucleotide mixture
PCR	– Polymerase Chain Reaction
Primer	– short, one-chain oligonucleotide with the length of 5 to 40 bases
RAPD	– Random Amplified Polymorphic DNA
RFLP	– Restriction Fragment Length Polymorphism

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HOP PROTECTION AGAINST ALFALFA SNOUT WEEVIL (*OTIORHYNCHUS LIGUSTICI* L.) IN CZECH HOP-GARDENS

OCHRANA CHMELE PROTI LALOKONOSCI LIBEČKOVÉMU (*OTIORHYNCHUS LIGUSTICI* L.) V ČR

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ABSTRACT: Alfalfa snout weevil (*Otiorhynchus ligustici* L.) together with damson-hop aphid (*Phorodon humuli* Schrank) and two-spotted spider mite (*Tetranychus urticae* Koch) are the most dangerous pests of Czech hop-gardens in the last three decades. It is connected with the change in his bionomics since that time when the first developmental stages were observed in the hop crowns. Hop protection against this pest is provided by a band spray with the help of a sprayer. High toxic carbofuran which is used for this purpose will be replaced by a new pesticide fipronil, which is more friendly to environment. New non-traditional methods of application, including biological ones are tested in spite of the fact that their utilization in practical conditions seems to be very difficult.

hop; alfalfa snout weevil; hop protection; insecticides; biological control; carbofuran; fipronil

ABSTRAKT: Lalokonosec libečkový (*Otiorhynchus ligustici* L.) patří společně s mšicí chmelovou (*Phorodon humuli* Schrank) a sviluškou chmelovou (*Tetranychus urticae* Koch) k nejvýznamnějším škůdcům chmele v ČR v posledních 30 letech. Tato skutečnost úzce souvisí se změnou bionomie, která spočívá v tom, že se škůdce specializoval na chmel, což bylo potvrzeno zjištěním vývojových stadií ve chmelových babkách v polovině 60. let. Ochrana chmele proti tomuto škůdci je prováděna pásovým postřikem. Toxický carbofuran, který je již řadu let používán pro tento účel, bude postupně nahrazen novým přípravkem ze skupiny fenyl-pyrazolů, fipronilem, který při srovnatelné biologické účinnosti je mnohem příznivější z hlediska ekologického. Jsou ověřovány nové netradiční metody aplikace, včetně metod biologických, a to i přes tu skutečnost, že jejich využití v praktických podmínkách chmelnic se jeví jako velmi obtížné.

chmel; lalokonosec libečkový; ochrana chmele; insekticidy; biologická ochrana; carbofuran; fipronil

INTRODUCTION

Alfalfa snout beetle (*Otiorhynchus ligustici* L.) together with damson-hop aphid and two-spotted spider mite are the most dangerous pests of hop plants in Czech hop-gardens. Higher injury level is connected with the change in the bionomics which arose thirty years ago when some biotypes of *O. ligustici* specialized on hop plants, whereas only adults caused the damage to spring shoots emerging from soil above the earth level before it (Petrlík, Štys, 1986). Developmental stages of *O. ligustici* that live in hop crowns are more detrimental than adults. A contemporary method of hop protection against weevils, band spray, is effective only on adults and all developmental stages under the earth level survive (Vostřel, 1987).

Problem with weevils is not a specific one only in Czech hop-gardens. Similar difficulties with them have also in other countries. Baird et al. (1992) studied migration of *O. sulcatus* in the USA. The bionomics and control measures in Germany are described by Kohlmann, Kastner (1975).

Two main objectives are studied now. The first one is searching for some new pesticides, more friendly to environment than contemporary insecticides (Vostřel et al., 1996) and the second one consists in testing some new non-traditional measures with the help of them we would be able to control developmental stages living in hop crowns (Vostřel, 1988). Not only chemical measurements but biological methods are being tested as well (Vostřel et al., 1996). These methods include also the possibility of using parasitic nematodes to control *O. ligustici* in Germany (Arndt, 1989). Possibilities of utilization of nematodes in biological control against weevils in the world are discussed by Weiser, Mráček (1988). Susceptibility of *O. sulcatus* larvae to *Metarhizium anisopliae* and *M. flavoviridae* (*Deuteromycotina*: *Hyphomycetes*) was studied by Soares et al. (1983).

MATERIAL AND METHODS

SUBSTITUTION OF CARBOFURAN BY A MORE ENVIRONMENTAL FRIENDLY INSECTICIDE

Laboratory tests

Adults of *O. ligustici* were collected in the time of their mass emergence above the earth level in the village Stekník (5 km from Žatec). They were kept in a laboratory under standard abiotic conditions. Sedimentation tower was used for testing biological efficiency. The rate of application liquid corresponded with this one which is commonly used in outdoor conditions. Adults of *O. ligustici* were placed at the bottom of the sedimentation tower and were treated with appropriate concentrations of some insecticides. The pressure of the Potter nozzle was 0.2 Mpa and sedimentation time 10 minutes. The mortality was checked out 72 hours after spraying. Each application was repeated three times. Distilled water was applied as a non-treated control.

Field experiments

Hop-gardens with a medium population density of *O. ligustici* in the villages Stekník (5 km from Žatec) in 1995 and Malá Černoc (15 km from Žatec) in 1996, resp., were chosen for establishment of the field experiment. Hop plants inside experimental plots were treated with insecticides with the help of a motorized-back sprayer (Stihl) with an appropriate rate of application liquid in 1995. A Czech-made sprayer Monzun 1540 pulled by a Czech tractor Zetor was used for application of insecticides in the field experiment in 1996. The mortality of fifty adults of *O. ligustici* was checked inside each experimental plot (variant) three days after application of insecticides.

BIOLOGICAL CONTROL POSSIBILITIES

Besides the field experiment with pesticides the trial with biological control was established at Stekník in 1995. The experimental hop-garden was divided into two parts; the first one for chemical protection and the second one for testing biological methods.

The field experiment with biological control was established on July, 10. The entomophagous fungi *Beauveria bassiana* (Boverol) in 0.3% and 0.5% concentrations delivered by the Czech manufacturer (Fytovita) and entomophagous nematodes *Heterorhabiditis* sp. (*Larvanem*) delivered by Koppert were used for this purpose. An Italien sprayer Tifone was used for application of two thousand litres of applied liquid per hectare. Band spray method was used.

Instructions given by the manufacturers were followed. When Larvanem was used, 50 milion nematodes were applied per 100 sqm in average. The field trial was evaluated by the method of soil diggings in the first decade of May in 1996. Fifteen hop crowns were dug up within each plot.

RESULTS AND DISCUSSION

From the results obtained from laboratory tests and field experiments (Tabs I to IV) it is obvious that a contemporary used pesticide, carbofuran (Furadan 350 F), is still very efficient on adults of *O. ligustici* when applied by a band spray in the time of mass emergence of beetles above the earth level (usually the beginning of May). Unfortunately, it is very toxic to the environment and it was the reason why we were searching for a new insecticide, which would be less detrimental. Fipronil (Regent 70 WG), a new pesticide from a group of phenyl-pyrazols, seems to be an appropriate substitution for carbofuran in future because of its high biological efficiency and better ecological characteristics. Other tested pesticides do not reach such a level of biological efficiency as fipronil or carbofuran.

On the other hand, we have not got any promising results with biological measures. Neither entomophagous fungi, nor nematodes, were effective when applied in field conditions of commercial hop-gardens. However, we would like to continue in testing some biological agents in future as well. As the main problem is the objectiveness of the evaluation of sufficient quantity of larval stages living in hop crowns, which is very problematic if we use a method of hop crown digging up, we decided to obtain an experimental hop-garden where we would be able to dig up by a plough the whole rows of hop crowns to manage to evaluate a big sample of *O. ligustici* pre-imaginal stages. We

I. Biological efficiency of chosen insecticides on adults of alfalfa snout weevil (*O. ligustici* L.) in laboratory tests

Common name	Trade name	Tested concentration	Mortality (%)
Carbosulfan	Marshal 25 EC	0.2	90
Methidathion	Ultracid 40 WP	0.2	83
Imidacloprid	Confidor 70 WG	0.008	95
Fipronil	Regent 70 WG	0.002	98
Fipronil	Regent 70 WG	0.004	100
Carbofuran	Furadan 350 F	0.2	100

II. Biological efficiency of some insecticides on *O. ligustici* adults in the field trial in 1995 (Stekník)

Common name	Trade name	Tested concentration	Mortality (%)
Carbosulfan	Marshal 25 EC	0.2	98
Methidathion	Ultracid 40 WP	0.2	95
Imidacloprid	Confidor 70 WG	0.008	95
Lambda-cyhalothrin	Karate 5 EC	0.06	15
Abamectin	Vertimec 1.8 EC	0.05	13
Pymetrozine	Chess 25 WP	0.08	15
Chlorpyrifos	Dursban 4 E	0.2	10
Carbofuran	Furadan 350 F	0.2	100

III. Biological efficiency of some insecticides on *O. ligustici* adults in the field trial in 1996 (Malá Černoc)

Common name	Trade name	Tested concentration	Mortality (%)
Carbosulfan	Marshal 25 EC	0.2	80
Methidathion	Ultracid 40 WP	0.2	65
Carbofuran	Furadan 350 F	0.2	96
Fipronil	Regent 70 WG	0.002	100
Fipronil	Regent 70 WG	0.004	100

IV. Biological efficiency evaluation (Stekník 1995/1996)

Biopreparate	Number of larvae per 15 hop crowns
Larvanem	9
Boverol in 0.3% conc.	13
Boverol in 0.5% conc.	11
Non-treated plot	10

believe that this is the only way how to find the answer on the question, whether we are able to control *O. ligustici* larvae using the biological measurements.

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INFLUENCE OF TRICKLE IRRIGATION ON THE YIELD STABILITY AND QUALITY OF HOPS IN ŽATEC HOP REGION

VLIV KAPKOVÉ ZÁVLAHY NA STABILITU VÝNOSU A JAKOST CHMELE V ŽATECKÉ OBLASTI

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ABSTRACT: Long-term field trial with trickle irrigation was established in an experimental hop-garden at the Hop Research Institute in Žatec. Higher productivity of hops was proved statistically. Yield of dry hops was higher on the average by 40% during five year observation. Qualitative characteristics of Czech fine aromatic hops were preserved. Irrigation water is delivered to hop plants from an in-line system which is placed on the ceiling of the hop-garden. Its microclimate is influenced favourably and acts as a thermo-regulatory factor. Physiological and biochemical processes in hop plants are favourably influenced as well. It was found that temperature inside a hop-garden, which is treated by trickle irrigation, is lower on the average by 4 °C. This phenomenon is important at first in the time when temperatures during a day are very high.

hop; trickle irrigation; hop yield; hop quality

ABSTRAKT: Na pokusné chmelnici Chmelařského institutu v Žatci byla dlouhodobým pokusem s řízenou kapkovou závlahou prokázána statisticky průkazná produkční účinnost kapkové závlahy. V průměru pětiletého období se zvýšil výnos chmelových hlávek na zavlažovaných plochách o 40 %, přičemž byly zachovány kvalitativní znaky jemného českého aromatického chmele. Závlahová voda dodávaná po kapkách ze stropu konstrukce příznivě ovlivňuje mikroklima v porostu a působí jako termoregulační faktor. Teplota vzduchu v keři se zavlažováním snižuje až o 4 °C, což má při vysokých denních teplotách v průběhu vegetace příznivý vliv na fyziologické a biochemické procesy probíhající v rostlinném organismu.

chmel; kapková závlaha; výnos chmele; jakost chmele

INTRODUCTION

Czech hop-growers' competitiveness not only in the domestic but in the world hop market as well presumes stabilisation of hop yields (Fric, 1994).

All the factors with the help of which it is possible to reach this aim must be mobilized. Climatic conditions in Žatec hop-region are very favourable for hop growing but there is a problem which consists in precipitation deficit which may be in the years with lower rainfall a very important factor. Irrigation systems in this region should cover this deficit and help to keep high level of hop yield.

Hop production can be influenced in a positive way in CR by large and medium-scale irrigation systems on the area larger than 4,700 ha of hop-gardens. Technical facilities of built irrigation systems corresponds to large-scale utilisation. In addition to them new modern irrigation systems will be established. Trickle irrigation system is one of the most convenient methods, which is very suitable for hop irrigation as well (Kochánek et al., 1989).

The piping with irrigators placed on the ceiling of a hop-garden proved to be a very efficient system in our region (Kopecký et al., 1993). The main problem consists in the way of trickle irrigation system control. Objective methods for determination of the need of effective irrigation during the real time are recommended for achievement of demanded yield effect (Sláma, 1980; Slavík, 1980; Sachl, Kopecký, 1984; Sasin, 1993).

Evaluation of trickle irrigation efficiency on hop creation and quality is carried out at Hop Research Institute in Žatec. This experimental work is solved within the program of National Agency for Agricultural Research controlled by the Czech Ministry of Agriculture (no. RE 0950975098).

MATERIAL AND METHODS

Need and efficiency of trickle irrigation was studied by the field experiment established in the experimental hop-garden of Hop Research Institute in Žatec.

The following variants were studied in the experimental hop-garden: 1. trickle irrigation – the piping irrigators in line system is placed on the ceiling of the experimental hop-garden; spacing of irrigators is 1.0 m (type NAAN – integrated Dripline 16; 1.6 l/h); they are placed above each row of hop plants; 2. rain-fed variant.

Uniform cultivation technology was carried out within the both variants in the experimental hop-garden. The only variable factor consists in conditions of water supply – supplementary irrigation.

Irrigational regime of hop is controlled by the method of prognosis of the need of effective irrigation doses (Slavík, 1980, 1990) in the week balanced periods. More than 33% of the whole hop-garden area in CR is under trickle irrigation system.

Influence of supplied irrigation water on yield of hops was evaluated by controlled harvest of the both plots. The method of stray choice of 16 hop plants in four repetitions from the plot of each variant was used for this purpose. Conclusive evidence of the difference was statistically expressed for each year with regard to stochastic character of natural rainfall during experimental years.

Hop quality was evaluated by Wollmer analysis of hop resins from the average harvest samples in laboratory conditions at Hop Research Institute in Žatec.

Influence of trickle irrigation on temperature regime inside the hop-garden was continuously measured with help of the automatic meteorological station (type Mini-Met 1209).

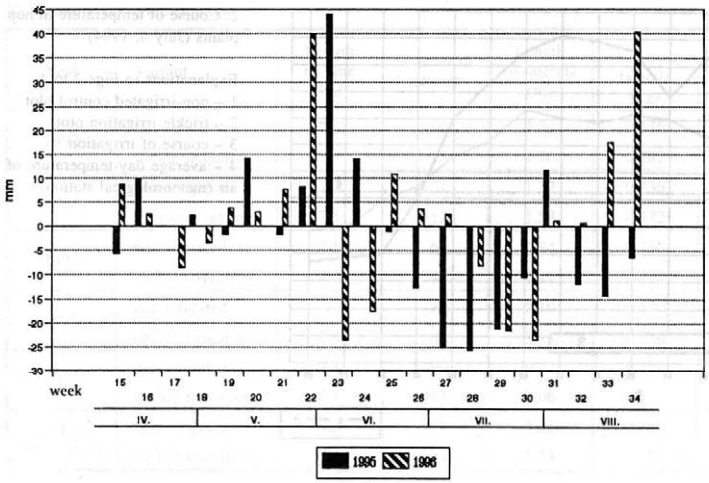
RESULTS AND DISCUSSION

Need of irrigation was recorded in each year during the studied period (1992 to 1996). Meteorological conditions, at first distribution of precipitation during this period, were very unbalanced (Tab. I). That means demands for irrigation were very different for water. We can see in Fig. 1 the development of water need covering in the years 1995 and 1996, resp. These two years were chosen because they differ from each other from this point of view. The survey of the total supply of irrigation water in all the studied years is reviewed in Tab. II. Irrigation doses which were supplied during these years influenced the production of dry hops in a positive way (Tab. III).

Efficiency of supplied irrigation water, resp. need of irrigation in relation to yield of hops is the very important factor during the evaluation of influence of this water. Data contained in Tab. IV proved a very important effect of irrigation doses. The yield contribution of 1 m^3 of water supplied by trickle irrigation is well-balanced in the years with different demands for hop irrigation. Irrigation water supplied by drops from the ceiling of a hop-garden influences microclimate inside it in a positive way. Water is dispersed on hop leaves and

I. Meteorological data during vegetation period of hop (1992–1996); Meteorological station – Hop Research Institute, Žatec, CR

Month	Decade	Air temperature (°C)					Precipitation (mm)				
		1992	1993	1994	1995	1996	1992	1993	1994	1995	1996
IV.	1.	4.9	6.2	5.4	8.7	4.9	4.6	1.4	0.6	2.2	9.8
	2.	7.5	7.5	6.3	4.8	6.9	2.3	1.2	47.6	14.2	5.0
	3.	11.6	16.7	13.5	8.0	13.9	39.0	1.3	1.2	7.2	6.4
	average	8.0	10.1	8.4	7.2	8.6	45.9	3.9	49.4	23.6	21.2
V.	1.	11.4	14.3	11.4	11.7	11.2	4.1	6.4	2.4	5.6	12.8
	2.	15.3	17.2	15.1	10.0	13.5	5.6	4.9	34.2	16.6	17.6
	3.	17.6	17.1	15.1	16.7	13.8	1.7	48.8	33.8	39.6	14.2
	average	14.8	16.2	13.9	12.8	12.8	11.4	60.1	70.4	61.8	44.6
VI.	1.	17.5	19.3	14.9	14.0	19.2	78.4	24.6	8.2	52.6	52.8
	2.	18.9	16.2	15.2	14.4	16.4	8.1	27.8	3.4	35.6	8.6
	3.	19.9	15.3	22.4	17.2	13.9	27.8	37.3	3.6	4.8	43.0
	average	18.8	16.9	17.5	15.2	16.5	114.3	89.7	15.2	93.0	104.4
VII.	1.	19.3	18.4	19.3	19.9	15.7	80.9	15.0	14.3	1.4	30.0
	2.	20.6	16.2	21.2	21.2	21.2	6.1	53.4	30.2	7.2	15.0
	3.	20.6	17.6	23.0	19.9	17.5	12.1	10.0	–	56.0	5.4
	average	20.2	17.4	21.2	20.3	16.4	99.1	78.4	44.5	64.6	50.4
VIII.	1.	22.3	18.9	22.4	19.5	17.0	18.7	22.2	33.2	4.6	15.8
	2.	18.9	18.1	16.1	18.6	16.5	7.9	40.7	34.2	9.0	44.4
	3.	20.5	14.5	16.3	16.0	16.7	7.4	2.0	16.6	32.4	55.4
	average	20.6	17.2	18.3	18.0	16.7	34.0	64.9	84.0	46.0	115.6
Total (mm)							304.7	297.0	263.5	289.0	336.2



1. Review of water need of hop covering week (1995, 1996)

its amount increases in this way. It is evaporated from the surface of leaves, gradually flows on the earth and feeds the soil on a limited earth area. Influence of this water on temperature regime in the place of hop vines is obvious from Fig. 2 to 4, where a day course of air temperature within plots with different water regime in the days with high air temperature during the years 1994 to 1996 is presented. Irrigation system proved to be an effective thermo-regulator element.

The results of growth analysis and quality analysis of hop cones are obvious from Tabs V and VI. Repeated trickle irrigation had a positive influence on hop plant habitus, which was more vigorous with longer laterals. Hop cones were more numerous on hop vines and so the yield was higher. Statistically important share of trickle irrigation was definitely proved. Yield of dry hops was higher in individual years by 17.1; 81.3%. No differences in qualitative characteristics between trickle irrigated and non-irrigated plots were found. Harvested hops had all the typical features of Czech fine aromatic hops. Qualified decision about the correct time of irrigation water supply in optimum doses is necessary for higher yield reaching.

II. Supplied quantity of irrigation water

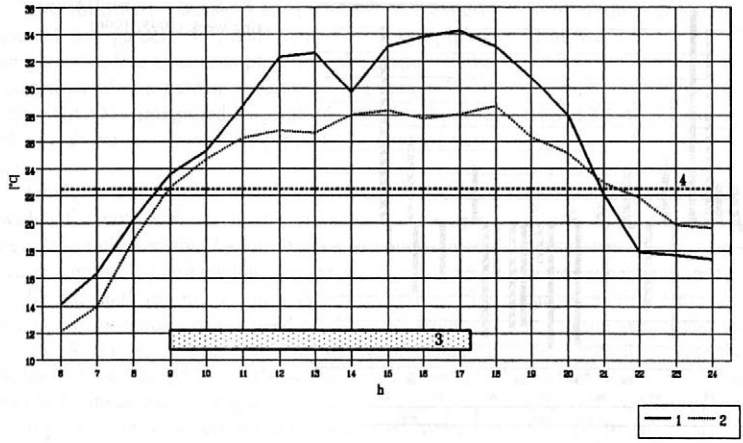
Year	Irrigation water ($m^3.ha^{-1}$)
1992	520
1993	590
1994	1 070
1995	410
1996	320
Average	582

III. The influence of trickle irrigation on yield of dry hops

Year	Yield of dry hops ($t.ha^{-1}$)		Influence of irrigation		Statistically significant difference		
	trickle	rainfed	$t.ha^{-1}$	%	*F*-calculated	*F*-test	significance
1992	1.32	0.96	0.36	27.1	8.14	5.99	> 95%
1993	1.54	1.21	0.33	27.3	7.23	5.99	> 95%
1994	1.45	0.80	0.65	81.3	22.42	5.99	> 95%
1995	1.16	0.72	0.44	61.1	15.54	5.99	> 95%
1996	1.51	1.29	0.22	17.1	13.05	5.99	> 95%
Average	1.40	1.00	0.40	40.0			

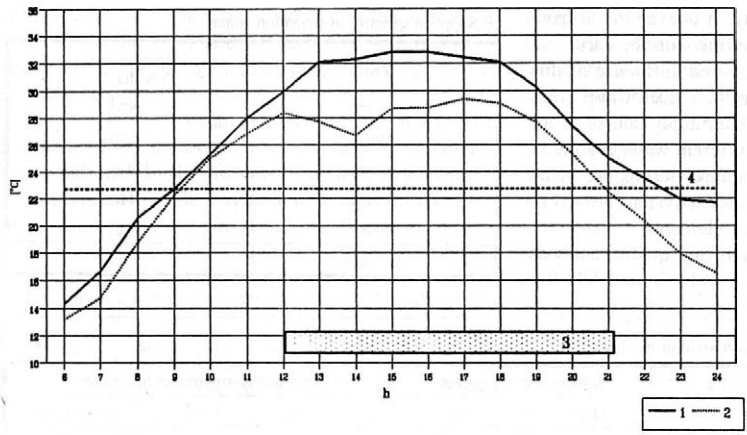
IV. Efficiency of supplied irrigation water

Year	Efficiency of irrigation water ($kg.m^{-3}$)	Need of supplied irrigation water 1 kg
1992	0.69	1.44
1993	0.56	1.78
1994	0.61	1.65
1995	0.93	1.07
1996	0.69	1.45
Average	0.70	1.48

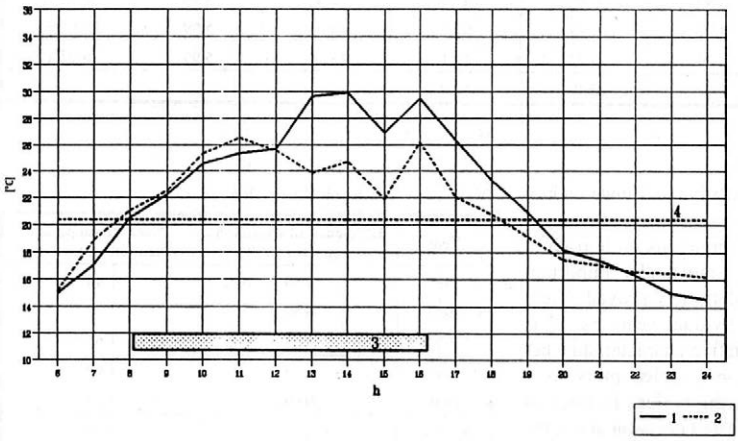


2. Course of temperature in hop plants (July 4, 1994)

Explanations to Figs 2 to 4:
1 – non-irrigated control plot
2 – trickle irrigation plot
3 – course of irrigation
4 – average day-temperature of air (meteorological station)



3. Course of temperature in hop plants (July 20, 1995)



4. Course of temperature in hop plants (July 28, 1996)

V. Values of hop vine creation

Year	Variant	Length of vine (m)	Number of laterals	Length of laterals (m)	Weight of			
					laterals (kg)	leaves (kg)	cones (kg)	vine (kg)
1992	non-irrigated	7.2	92	1.54	0.176	0.831	0.733	0.515
	trickle irrigation	7.4	96	1.76	0.287	0.914	1.114	0.682
1993	non-irrigated	7.3	101	1.32	0.236	1.041	0.986	0.685
	trickle irrigation	7.5	112	1.58	0.372	1.215	1.164	0.793
1994	non-irrigated	7.1	68	0.84	0.156	0.621	0.432	0.435
	trickle irrigation	7.4	82	1.12	0.218	0.845	0.638	0.584
1995	non-irrigated	7.0	82	2.10	0.570	1.420	1.020	0.735
	trickle irrigation	7.3	81	2.18	0.550	1.530	1.080	0.910
1996	non-irrigated	7.3	90	0.77	0.151	0.347	0.403	0.753
	trickle irrigation	7.1	117	1.06	0.183	0.444	0.416	0.810
Average 1992–1996	non-irrigated	7.2	87	1.31	0.260	0.850	0.710	0.643
	trickle irrigation	7.3	98	1.54	0.320	0.990	0.880	0.756

VI. Results of chemical analysis of hop (in % of the content of chemical substances in dry matter of cones)

Year	Variant	Total resins	Soft resins	Humulone	Beta fraction	Hard resins
1992	non-irrigated	12.1	10.5	3.9	6.5	1.6
	trickle irrigation	12.2	10.5	3.7	6.7	1.7
1993	non-irrigated	12.1	10.3	3.5	6.8	1.8
	trickle irrigation	13.1	11.3	3.7	7.6	1.8
1994	non-irrigated	9.3	8.3	1.4	6.9	1.0
	trickle irrigation	9.2	8.1	1.3	6.8	1.1
1995	non-irrigated	11.7	9.6	3.3	6.3	2.1
	trickle irrigation	11.3	9.4	3.2	6.2	1.9
1996	non-irrigated	13.9	12.0	4.3	7.7	1.9
	trickle irrigation	15.6	13.4	4.8	8.6	2.2
Average 1992–1996	non-irrigated	11.8	10.1	3.3	6.8	1.7
	trickle irrigation	12.3	10.5	3.3	7.2	1.7

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EFFECTIVENESS OF SPRAY APPLICATIONS IN HOP PROTECTION

EFEKTIVNOST ROSIČŮ PŘI OCHRANĚ CHMELE

F. Veselý

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ABSTRACT: High quality and effective hop protection measurements depend on harmonisation of the output, setting of nozzles and keeping working regime of a spraying machine. Sprayer Munckhof, produced by a Dutch firm from Horst, type 8010-16 VRVS, was tested for its possibility of utilisation in hop protection against pests and diseases in our hop gardens. The sprayer has application nozzles in front of the air output out of the fan. Better leaf covering by microdrops is reached in this way. Application parameters were compared with a common standard spraying machine Kertitox. Some technical adjustments were made with the aim to achieve excellent covering of hop leaves and cones with application liquid. Nomogram for evaluation of quality of the spray during the process of machine testing has been worked out. There is a possibility of 20 to 30% lower usage of application liquid per hectare if high quality application is carried out. Basic setting and adjustments can be utilised for some other spraying machines with similar technical parameters.

hop; hop protection; spraying machine; setting of sprayers; effectiveness of hop protection

ABSTRAKT: Kvalitní a efektivní ochranné zásahy u chmele jsou závislé na sladění výkonu ventilátoru, nastavení trysek a dodržení pracovního režimu stroje. Testovaným strojem byl rosič Munckhof holandské firmy z Horstu, typ 8010-16 VRVS. Tento rosič má aplikační trysky před výstupem vzduchu od ventilátoru, což zajišťuje větší rovnoměrnost tvorby mikrokapének při postřiku. Aplikační parametry byly porovnány s dosud používaným strojem Kertitox jako referenčním vzorkem. Celkové řešení si vyžádalo i některé technické úpravy k zajištění stejnoměrného pokrytí plochy listů a chmelových hlávek. Při testování strojů byl zároveň zpracován nomogram pro hodnocení kvality postřiku. Výsledky ukazují na možnosti úspory 25 až 30 % postřikové kapaliny na 1 ha při dodržení potřebné kvality postřiku. Základní nastavení a úpravy bude možné využít rovněž u dalších strojů se srovnatelnými technickými parametry.

chmel; ochrana chmele; rosiče; seřízení rosičů; efektivnost ochrany

INTRODUCTION

Introduction of wide spacing in hop cultivation increased demands on qualitative technique for hop protection against pests and diseases. It was the reason why some tests of spraying machines were gradually carried out (Petrlík, Štys, 1963; Balaštík, 1965; Skládal et al., 1970).

After 1970 hop protection was provided at first by Kertitox NA 10 or NA 20 spraying machines which were imported to CR from Hungary. Unfortunately, they were imperfect. High energetic cost and low technical reliability were found out (Vent, 1988). At that time machine testing was already carried out together with determination of the optimal working regime of sprayers (Kříž, 1978, 1984; Chládek, 1988, 1989). Application tests were established to carry out the print method and the method with water-sensitive papers. These methods together with biological ones were used by Petrlík et al. (1985) when working regimes of spraying machines were determined (Petrlík, et al., 1991; Veselý, 1991, 1994).

High intensity of hop protection nowadays brings also negative influence of used pesticides on the environment. High doses of application liquid per hectare are the cause of it. The problem of the efficiency of hop protection measurements is much more difficult than in the case of orchards and vineyards. Quick build-up of the mass of hop plants during vegetation period, considerable change in the height of plants and specificity of pests and diseases are factors playing the decisive role within this complex.

Sprayers used in hop protection must be regularly set up during the growing season. High doses of application liquid are used at present. It is connected with high speed (often higher than 5 $km.h^{-1}$). Quality of the operation is being decreased in this way at first in the time when the wind speed is higher than 3 $m.s^{-1}$.

Many different types of spraying machines are used in hop protection in CR nowadays (Tifone, Holder, Meyers, Hardi, Nobili, Agra, Munckhof, Monzun and some others). Unfortunately, most of them are used without previous testing in hop-gardens and without setting up. In the List of registered pesticides and

sprayers for plant protection for 1996 only five types of licensed machines are reviewed. Other types are without licence and tests in hops. Some technical adjustments are made with the aim to achieve better covering of hop leaves and cones by the application liquid. Sprayers Kertitox and Agra are being reconstructed on Monzun spraying machine (V e s e l ý , 1994).

MATERIAL AND METHODS

Testing was carried out by The Regulations of the Czech Ministry of Agriculture concerning spraying machines testing, issued under No. 1304/04-310 of May 17, 1994. The work was aimed at testing under paragraphs 2, 3 and 5, part II concerning possibilities of spraying machines setting.

Air output

When the fan was tested, air speed was measured by an apparatus equipped by a propeller measuring probe with a digital display (Tab. I).

I. Variants of measurement of air output

Variant	Engine revolutions (r.p.m.)	Transmission of the fan	Installation of side walls
1	1 500	I	no
2	1 800	I	no
3	2 000	I	no
4	1 500	I	yes
5	1 800	I	yes
6	2 000	I	yes
7	1 500	II	no
8	1 800	II	no
9	2 000	II	no
10	1 500	II	yes
11	1 800	II	yes
12	2 000	II	yes

The range of measurement was 0 to 40 $m.s^{-1}$ with precision of 0.1 $m.s^{-1}$. Measured points on the both parts symmetrically identical were distant in five regular distances (0°, 20°, 40°, 60°, 80°) from horizontal connection of the edges of the lower tin of the fan.

Measurement was carried out always at a distance of 1 m from the central axis of the fan and was twice repeated. The result is the average value of the measurements.

Nozzle flow

Nozzle flow was tested under the standard pressure of 1.3 MPa as it was recommended by the producer. The frame bore nozzles of only one type. Flow time was tested with the doses of 100 and 200 l. The average nozzle flow was determined and compared with table data. In the second phase each of the individual nozzles was tested. A hose was conducted into a graduated glass of 2 l. Time of the nozzle flow of 2 l was determined. The difference among individual nozzles was observed.

The test of frame flow was carried out with three frame settings. The time of 200 l flow was measured. Comparison was made with table values and the average value obtained during the first test as well.

All the basic tests of nozzle flow, flow frame setting, steadiness of pump efficiency and pressure keeping were made with the spraying machine full of water (Tab. II).

II. Variants of frame settings (measurement of the flow)

Variant	Nozzle					
	1	2	3	4	5	6
A	ATM	ATM	223	210	210	210
B	ATM	ATM	ATM	230	210	210
C	ATM	230	223	215	210	210

Application tests

The speed during the basic application tests was 3. reduced (according to revolutions of the engine 2.2 to 2.8 km per hour). Revolutions of engine were 1500, 1800 and 200 per minute. Taking of leaves treated by Kuprikol 50 (50% of oxychloride Cu) followed after the application.

Comparison of a spraying machine Munckhof with a common sprayer Kertitox, which was set to the same rate of application liquid per hectare, was performed. The same tractor speed and engine revolutions were kept. Each tractor made five thoroughfares in the experimental hop-garden. Samples were taken from the middle rows. The result of one thoroughfare was evaluated when Munckhof sprayer was tested.

Test 1: Nozzle setting 1–6 of Munckhof sprayer (delivered by the producer) – ATM, 230, 230, 223, 215, 210. Measured flow was 40.52 $l.min^{-1}$. The rate of application liquid per one hectare was 228.6 (55 minutes per hectare). Speed of a tractor 3. reduced, 1800 revolutions per minute, fan setting 2, side walls were installed without extensions.

Test 2: Setting as in the case of test 1; that means 40.52 $l.min^{-1}$, 2228.6 $l.ha^{-1}$, without side walls.

Test 3: ATM, ATM, 230, 215, 215, 210 – flow 45.85 $l.min^{-1}$, 2522 $l.ha^{-1}$, without side walls.

The following variants of application were chosen for tests on the base of the evaluation of previous results (Tab. III).

Frame setting (only with nozzles Albuz), nozzles 1–6: 230, 230, 215, 215, 210, 210. Frame flow

III. Variants of application tests (print method)

Variant	Speed	Engine revolutions (r.p.m.)
1	2nd reduced	1500
2	2nd reduced	1800
3	2nd reduced	2000
4	3rd reduced	1500
5	3rd reduced	1800
6	3rd reduced	2000

V. Measurement of frame flow

Settings of the frame	Measured flow	Table value	Difference (%)
A	40.83	41.62	98.10
B	52.63	58.08	90.61
C	37.73	34.92	108.06

IV. Measurement of nozzle flow

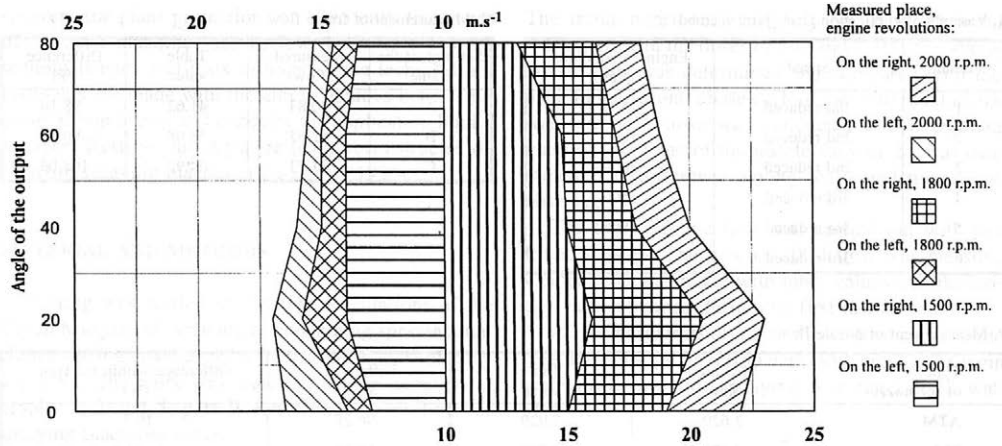
Type of the nozzle	Measured flow	Table value	Difference (%)	Difference within the type (%)
ATM	7.620	7.920	96.21	10.3
230	3.622	3.800	95.35	3.85
223	3.000	2.750	109.09	4.65
215	1.538	1.510	101.85	3.22
210	0.725	0.740	97.97	0.90

VI. Cover index (test papers made by Ciba-Geigy, test 1, 5 thoroughfares)

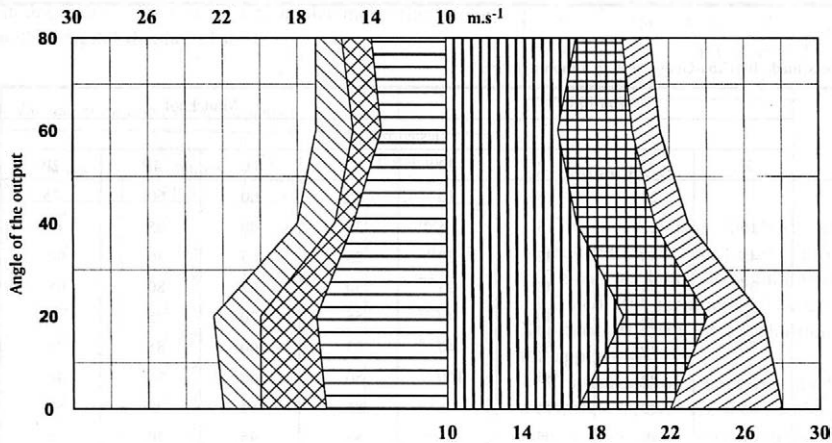
		Kertitox				Munckhof			
		tested rows							
		2L	1L	1P	2P	2L	1L	1P	2P
Height of the plant (cm)	700	15	20	5	10	15	60	60	75
	650	15	25	15	5	30	40	85	40
	600	45	30	35	20	25	65	50	90
	550	35	50	45	25	30	65	80	65
	500	70	80	40	40	55	70	75	55
	450	70	70	60	40	85	100	85	70
	400	95	95	85	100	80	100	75	40
	350	95	50	75	95	85	85	70	50
	300	80	70	95	85	85	95	70	55
	250	95	90	100	55	95	70	45	65
	200	60	95	100	90	95	45	25	75
	150	90	70	75	95	65	50	30	50
	100	50	65	100	90	55	45	15	35
	50	80	75	90	100	40	15	15	15
Average values of the cover index from different height levels									
Height levels (cm)	0–700	63.9	63.2	65.7	60.7	60.0	64.3	55.7	64.3
	0–250	75.0	79.0	93.0	86.0	70.0	45.0	26.0	48.0
	300–500	82.0	91.0	71.0	72.0	78.0	89.0	75.0	54.0
	500–700	36.0	41.0	28.0	20.0	31.0	60.0	70.0	65.0
	600–700	25.0	25.0	18.3	11.7	23.3	55.0	65.0	68.3

2L – 2nd row on the left
1L – 1st row on the left
1P – 1st row on the right
2P – 2nd row on the right

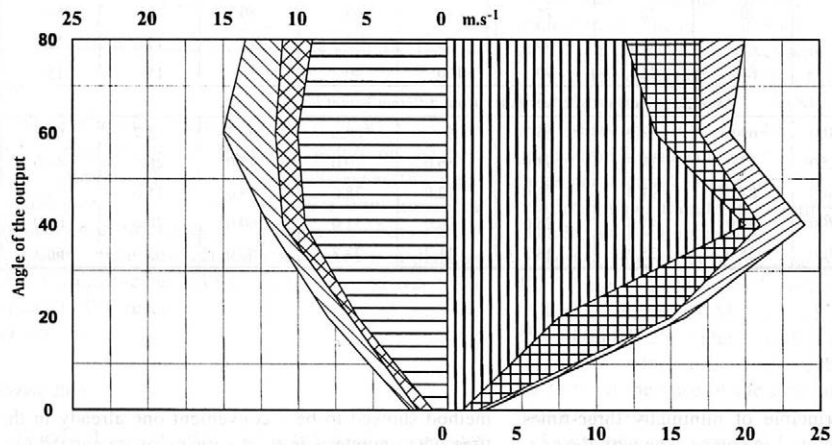
24.2 l.min^{-1}. The principle of minimally three-times higher flow of nozzles 1–3 in comparison with the nozzles 4–6 on each side of the frame was kept. This method showed to be a convenient one already in the time when previous tests of sprayers were carried out. ATM nozzles were not used any more because of their



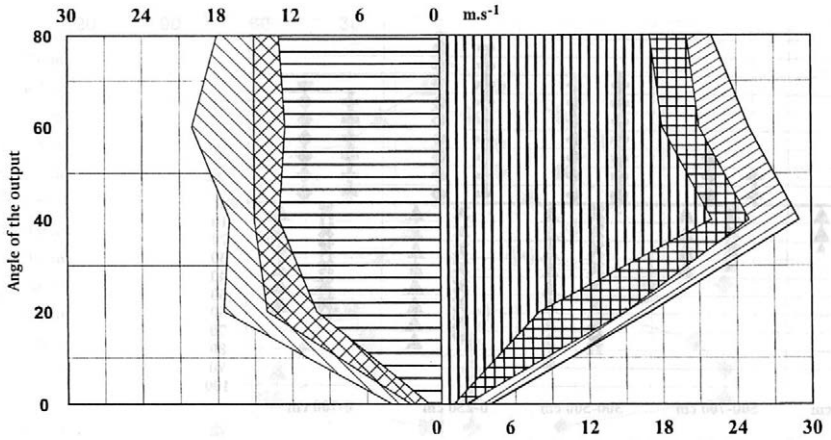
1. Spraying machine type Munckhof (air output of the fan); speed level of the fan 1, without side walls; measured in the distance of 1 m from the centre of the fan ($m.s^{-1}$)



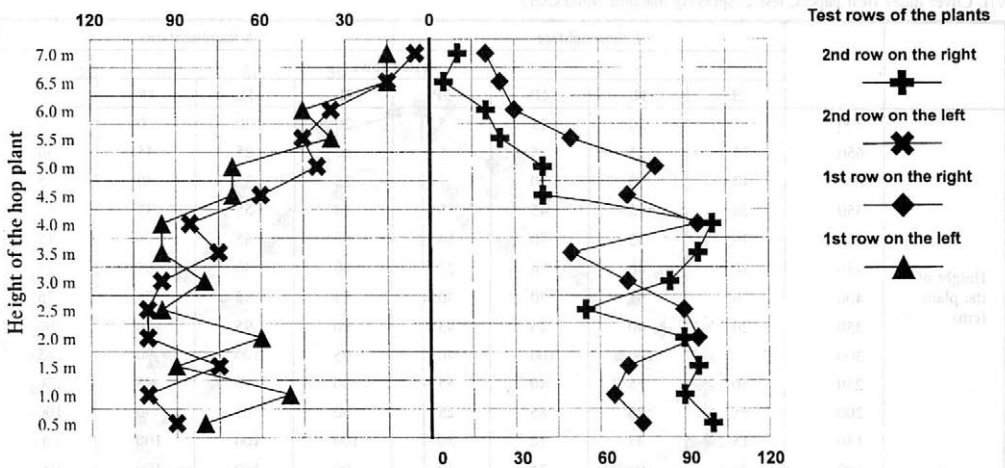
2. Spraying machine type Munckhof (air output of the fan); speed level of the fan 2, without side walls



3. Spraying machine type Munckhof (air output of the fan); speed level of the fan 1, with the installation of side walls



4. Spraying machine type Munckhof (air output of the fan); speed level of the fan 2, with the installation of side walls



5. Spraying machine type Kertitox; cover index – test on sensitive papers made by Ciba-Geigy; settings by Hop Research Institute Žatec, 5 thoroughfares

unsuitable size of droplets. It is necessary for them to have higher pressure than nozzles Albuz.

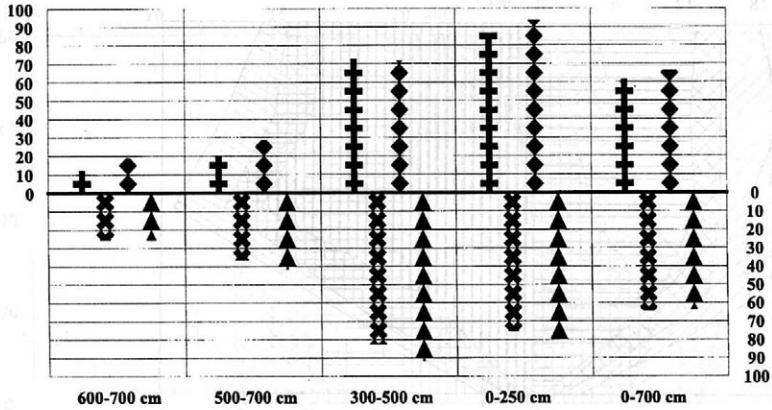
Water sensitive papers were marked and tightened at a distance of 0.5 m between them on a 7 m long nylon cord, which was hung up close to a hop plant on both sides of a thoroughfare on both inclined and removed rows. Taking of leaf samples for the print method was made according to the standard method (Petrlík, Štys, 1965a, b) from the height of 2, 4 and 6 m. Samples of cones were also taken from the height of 4 and 6 m. Covering index corresponds to percentage covering. Possible print omitting is taken into consideration.

RESULTS AND DISCUSSION

Fan testing

Uneven distribution of air output for vertical level is obvious from obtained results. Left side has lower output speed in all the variants than the right one.

Air output is higher to the level of 30° from the zero horizontal level. Decrease of 20% on the left side and 25% on the right side in the variants without side walls followed. With the installation of side walls the situation is much better, air speed is minimal on the level of zero horizontal level. Air speed gradually increased on the left side to the value of 60° if the transmission of the fan is on the 1st grade. If the 2nd grade is turned the value of 20° is determined. The increase of the both



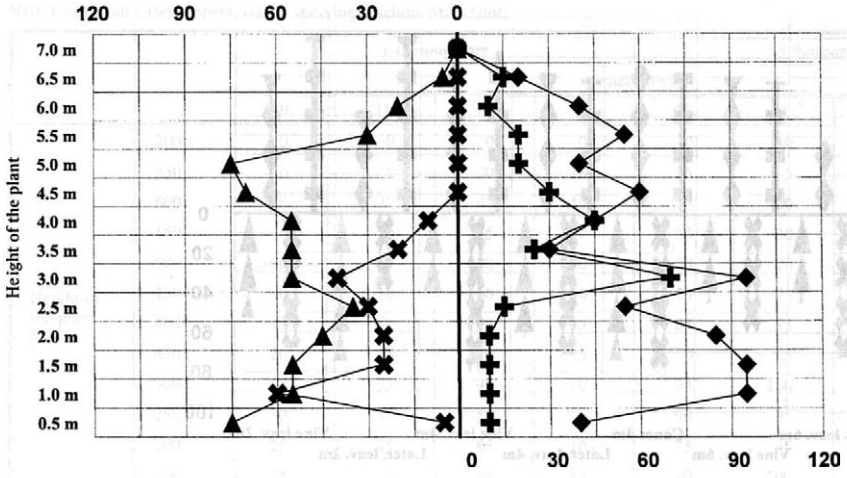
6. Spraying machine type Kertitox; setting by Hop Research Institute Žatec, 2500 l/ha; speed 3 reduced, 1800 r.p.m., 5 thoroughfares, speed level of the fan 3; average values of the cover index from different high levels

VII. Cover index (test papers, test 2, spraying machine Munckhof)

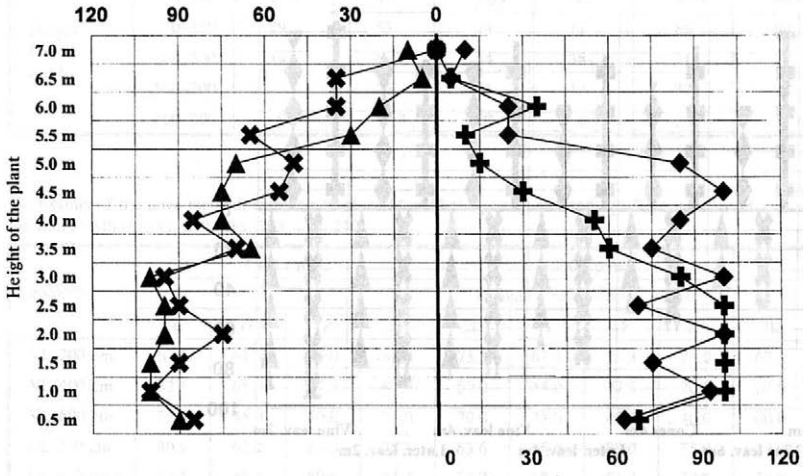
		1 thoroughfare				5 thoroughfares			
		tested rows							
		2L	1L	1P	2P	2L	1L	1P	2P
Height of the plant (cm)	700	15	20	25	5	20	20	0	0
	650	35	35	15	5	50	25	45	50
	600	40	30	30	5	25	35	40	5
	550	20	15	45	25	40	55	35	30
	500	30	55	90	10	65	55	75	15
	450	30	40	70	25	80	30	65	35
	400	20	95	70	30	75	85	75	75
	350	20	40	45	45	90	95	70	100
	300	15	100	100	40	95	100	90	85
	250	50	75	40	55	100	80	85	100
	200	95	75	85	25	90	85	90	100
	150	15	95	70	50	100	100	100	100
	100	20	90	25	40	90	100	100	100
	50	55	95	15	10	90	95	80	45
Average values of the cover index from different height levels									
Height levels (cm)	0–700	32.9	61.4	51.8	26.4	72.1	68.6	67.9	60.0
	0–250	47.0	84.0	47.0	36.0	94.0	92.0	91.0	89.0
	300–500	23.0	66.0	75.0	30.0	81.0	73.0	75.0	62.0
	500–700	28.0	31.0	41.0	10.0	40.0	38.0	39.0	20.0
	600–700	30.0	28.3	23.3	5.0	31.6	26.6	28.3	18.3

transmission grades out to the point of 20° was found on the right side. Mild decrease was followed out later, when the values were > 40°. It is obvious from the results that the figure of air speed is not symmetrical regarding the perpendicular axis of the spraying machine. Deviations are higher than 10%. Munckhof is better than commonly used sprayer Kertitox regarding total efficiency and reached output speed values in the case when side walls are installed and the 2nd transmission grade of the fan is set up.

It ensues from the results that a spraying machine with installed side walls is more suitable for hop protection against pests and diseases, as the air output is limited to the sides directly to the plants. Damage may be caused by air output on condition that air speed is higher than 25 $m.s^{-1}$ according to the physiological condition of a hop plant. If the speed is higher than 30 to 35 $m.s^{-1}$ a serious damage of plants is caused by the sprayer. Strong defoliation is evident to the height of 3 to 3.5 m. Tearing laterals can be also sometimes



7. Spraying machine type Munckhof; cover index – test on sensitive papers made by Ciba-Geigy; setting by the producer, 1 thoroughfare, without side walls (ATM, ATM, 230, 215, 215, 210)



8. Spraying machine type Munckhof; cover index – test on sensitive papers made by Ciba-Geigy; setting by the producer, 5 thoroughfares, without side walls (ATM, ATM, 230, 215, 215, 210), nozzles turning 1–3 by 20° upwards

seen under hop plants on the ground after such an application. A guided stream of air in the direction upwards is more suitable. Lower parts of hop plants must be simultaneously well treated as well. The results of the speed of air output are obvious from Figs 1 to 4.

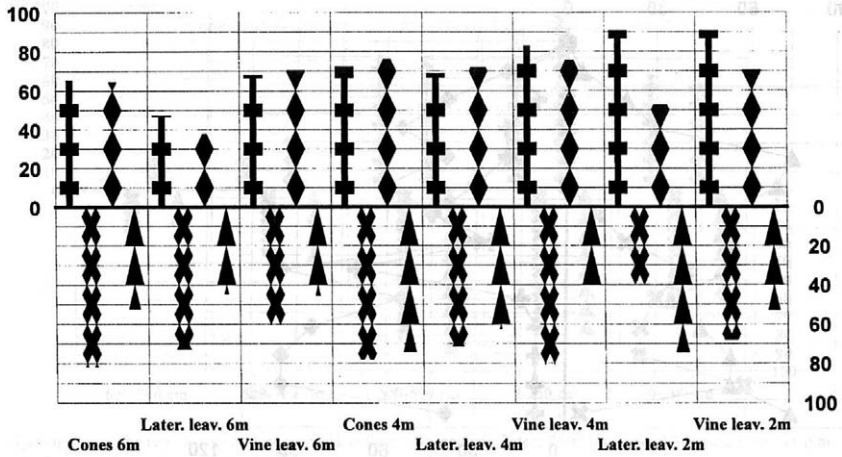
Nozzle testing

The results of these tests are reviewed in Tabs IV and V. ATM nozzles had the biggest difference in the flow whereas Albuz nozzles coped with the demand (max. difference < 5%) in all the tests.

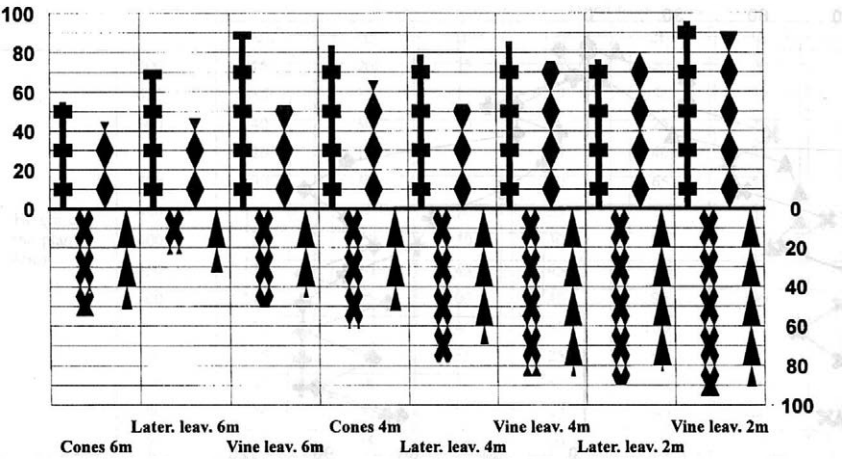
Aplication tests with CG water-sensitive papers (Tabs VI to VIII)

As the results of the test 1 in the profile of 0 to 250 cm and 600 to 700 cm in the case of Munckhof sprayer did not comply with the demands concerning quality of spraying operations, other tests were carried out after dismantling of the side walls. Different frame setting was used in the tests as well.

One thoroughfare for determinations of the working spray range was evaluated. Evaluation of the middle rows after five thoroughfares was done too. The data concerning Kertitox sprayer are surveyed in Figs 5 and



9. Spraying machine type Munckhof – prints of leaves (cover index); frame setting – Albuz (230, 230, 215, 215, 210, 210), side walls + 7.5 cm sheet iron; speed 2 reduced, 2000 r.p.m., 5 thoroughfares



10. Spraying machine type Munckhof – prints of leaves (cover index); frame setting – Albuz (230, 230, 215, 215, 210, 210), side walls + 7.5 cm sheet iron; speed 3 reduced, 2000 r.p.m., 5 thoroughfares

6 and the data concerning Munckhof sprayer are surveyed in Figs 7 and 8.

It is obvious from the results of test 2 that marked improvement was in the height of 0 to 500 cm, where the index evaluation is excellent. On the contrary, a sharp decline of measured values was found out in the height between 550 and 700 cm. It was the reason why we decided in the third variant to set two ATM nozzles in the frame position 1 and 2 with higher minute flow. The pressure was kept on the usual level (1.3 MPa) as it was the most suitable setting for Albuz nozzles. Unfortunately, the results showed another deterioration of the average index in the height between 500 and 700 cm. The highest deterioration was observed in the height of 600 to 700 cm, where the average index value was very poor in all the cases. Therefore we decided to use for the other print tests only Albuz nozzles.

Application tests – the method with leaf prints

The method is more accurate than the method of water-sensitive papers. The data obtained from the tests are reviewed in Tabs IX and X. The results are divided extra for the 2nd and the 3rd reduced speed grade. It is obvious that the efficiency of the application is very good, at first in the case when the speed is slower and revolutions of the engine higher (a variant with reduced speed grade and 2000 revolutions per minute). In spite

VIII. Cover index (test papers, test 3, spraying machine Munckhof)

		1 thoroughfare				5 thoroughfares			
		tested rows							
		2L	1L	1P	2P	2L	1L	1P	2P
Height of the plant (cm)	700	0	0	0	0	0	10	10	0
	650	0	5	20	15	35	5	5	5
	600	0	20	40	10	35	20	25	35
	550	0	30	55	20	65	30	25	10
	500	0	75	40	20	50	70	85	15
	450	0	70	60	30	55	75	100	30
	400	10	55	45	45	85	75	85	55
	350	20	55	30	25	70	65	75	60
	300	40	55	95	70	95	100	100	85
	250	30	35	55	15	90	95	70	100
	200	25	45	85	10	75	95	100	100
	150	25	55	95	10	90	100	75	100
	100	60	55	95	10	100	100	95	100
	50	5	75	40	10	85	90	65	70
Average values of the cover index from different height levels									
Height levels (cm)	0–700	15.4	45	53.9	20.7	66.4	66.4	65.4	54.6
	0–250	29	53	74	11	88	94	81	94
	300–500	14	62	54	38	71	77	89	49
	500–700	0	26	31	13	37	27	30	13
	600–700	0	8.3	20	8.3	23.3	11.6	13.3	13.3

IX. Results of the print method; spraying machine Munckhof, speed 2 reduced, fan 2, installation of side walls plus extension, nozzles on the frame: Albuz (230, 230, 215, 215, 210, 210)

Sample	1500 r.p.m.				1800 r.p.m.				2000 r.p.m.			
	evaluated rows – cover index											
	2L	1L	1P	2P	2L	1L	1P	2P	2L	1L	1P	2P
VL 200 cm	67.5	67.5	65.0	85.0	73.3	67.5	76.3	75.0	67.5	52.5	70.0	90.0
VL 400 cm	82.5	65.0	57.5	87.5	60.0	55.0	77.5	70.0	80.0	40.0	75.0	82.5
VL 600 cm	72.5	45.0	50.0	35.0	70.0	35.0	45.0	40.0	60.0	45.0	70.0	67.5
LL 200 cm	80.8	62.2	83.0	78.3	63.6	75.0	80.0	75.0	39.0	73.9	52.0	90.0
LL 400 cm	62.5	48.1	80.0	83.7	35.7	58.1	56.4	77.8	70.8	62.1	71.4	68.3
LL 600 cm	53.8	28.9	36.5	41.9	46.9	13.8	50.0	24.6	72.2	44.2	37.2	46.6
Con. 400 cm	61.6	58.9	68.0	75.0	57.5	44.4	68.6	59.6	77.5	73.6	75.7	71.7
Con. 600 cm	67.8	47.2	58.0	56.5	38.9	28.8	72.0	41.1	81.2	51.9	63.9	64.7

r.p.m. – revolution per minute (tractor with engine revolution counter)
VL – vine leaves
LL – lateral leaves
Con. – cones

of the fact that covering is slightly lower to the height of 4 m, we can report that it is still in a very good quality. Better data were obtained in the height of 6 m where only one sample of lateral leaves showed lower quality values than are the demanded ones. All the other samples proved excellent quality.

If lower revolutions were tested, poor quality was observed in the both higher and lower hop plant levels. Similar data were obtained if the 3rd reduced speed grade was tested. It may by claimed again that better results were found if the engine revolutions are 2000 per minute. Some data are showed in Figs 9 and 10.

The evaluation of the covering index is divided according to the comparison of biological efficiency of the spraying operations against damson-hop aphid if an insecticide with a very good contact efficiency is used.

Index in the range between 51 and 100 corresponds to high quality application. Index values in the range

X. Results of the print method; spraying machine Munckhof, speed 3 reduced, fan 2, installation of side walls plus extension, nozzles on the frame: Albuz (230, 230, 215, 215, 210, 210)

Sample	1500 r.p.m.				1800 r.p.m.				2000 r.p.m.			
	evaluated rows – cover index											
	2L	1L	1P	2P	2L	1L	1P	2P	2L	1L	1P	2P
VL 200 cm	81.7	52.5	72.5	80.0	82.5	81.7	67.5	75.0	95.0	90.0	90.0	95.0
VL 400 cm	70.0	72.5	87.5	50.0	62.5	67.5	60.0	87.5	85.0	85.0	75.0	85.0
VL 600 cm	40.0	40.0	45.0	20.0	35.0	43.3	40.0	55.0	50.0	45.0	52.5	90.0
LL 200 cm	30.0	60.0	87.5	77.8	82.5	80.5	89.2	91.4	89.1	82.5	80.0	75.6
LL 400 cm	82.0	66.9	82.5	52.5	54.0	66.3	62.2	84.0	78.0	68.8	53.0	78.3
LL 600 cm	28.8	38.3	20.0	30.0	18.3	13.1	15.0	47.8	22.8	32.2	45.8	70.7
Con. 400 cm	71.0	58.0	79.3	61.4	80.8	75.0	62.0	71.0	61.0	51.7	65.0	82.9
Con. 600 cm	31.4	39.5	20.5	61.8	31.1	18.9	44.0	58.4	54.3	51.0	44.0	54.0

of 31 to 50 are only sufficient ones with the danger of non-treated places left and index lower than 30 means the insufficient quality of a spraying operation.

We can state that efficiency of the application is very good and markedly better than in the case of the common sample above all if the engine revolutions are higher, that means 2000 r.p.m.

We can conclude that it is possible to state that spraying machine Munckhof belongs to a very good lower-medium group of spraying machines as its parameters are the following: tank volume is 1000 litres, fan efficiency is 60 000 m^3 of the air per hour, maximum number of nozzles per frame is twelve, maximum frame flow if Albuz nozzles are used is 50 $l.min^{-1}$.

Some smaller construction and technical adjustments are necessary to carry out for the optimal utilisation if this spraying machine is used in hop protection against pests and diseases. We can claim that after these adjustments the machine is able to manage the hop area of 25 to 30 hectares if a demanded repetition of a spraying operation is during 5 to 6 days. It corresponds to daily performance of 5 to 6 hectares.

The average volume of application liquid during the tests was on an average 20 to 33% more economical (maximum 2200 $l.ha^{-1}$) in comparison with current application rates (2800 to 3200 $l.ha^{-1}$). The saving is in fact the main aim of the tests and it means an important economical and ecological contributions in the hop protection process against pests and diseases. The results obtained during the tests work serve as the basis for computer evaluation and graphical processing of nomograms for the evaluation of application quality of spraying operations.

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Original scientific papers, short communications, and selectively reviews, that means papers based on the study of technical literature and reviewing recent knowledge in the given field, are published in this journal. Published papers are in Czech, Slovak or English. Each manuscript must contain a short and a longer summary (including the key words).

The author is fully responsible for the originality of his paper, for its subject and formal correctness. The author shall make a written declaration that his paper has not been published in any other information source.

The board of editors of this journal will decide on paper publication, with respect to expert opinions, scientific importance, contribution and quality of the paper.

The paper extent shall not exceed 15 typescript pages, including tables, figures and graphs.

Manuscript layout shall correspond to the State Standard ČSN 88 0220 (quarto, 30 lines per page, 60 strokes per line, double-spaced typescript). A PC diskette should be provided with the paper, written in an editor program, preferably T602, and with graphical documentation. Tables, figures and photos shall be enclosed separately. The text must contain references to all these annexes.

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In the section **Results** figures and graphs should be used rather than tables for presentation of quantitative values. A statistical analysis of recorded values should be summarized in tables. This section should not contain either theoretical conclusions or deductions, but only factual data should be presented here.

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