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I.H.G.C.

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Dr. Elisabeth Seigner
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Special Thanks to our Sponsors

(logos arranged randomly)

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Foreword

On behalf of the Scientific Commission of the International Hop Growers` Convention (I.H.G.C) it is my pleasure to welcome all of you here in Lublin. With this meeting of the Scientific Commission here in Poland we are continuing the tradition in the name of the I.H.G.C. to bring together hop scientists from all around the globe to discuss their work. As far as my notes go back, this marks the third time - after 1978 and 1999 - that the SC is holding its meeting here in Poland and it is already for the second time that Prof. Dr Ewa Solarska is our perfect host.

Poland is just before taking over the presidency of the EU council on July 1st. Moreover, Poland is preparing for the EU football (soccer) championship next year which will be great entertainment for all. Thus, we have much pleasure in having the opportunity to enjoy the warm hospitality here in Poland already before these events. Our venue Lublin with its five universities is a center of science and our host Prof. Dr Ewa Solarska is connected to the University of Life Sciences in Lublin by her teaching and research activities, thus this is the right atmosphere for an inspiring scientific exchange.

54 participants from 13 hop growing nations are joining this meeting to exchange and discuss latest research on hops. In 22 papers and 22 posters our scientists present their results on hop breeding (classical cross-breeding and biotechnological work as well), management of hop diseases and pests, hop chemistry, physiology, and the improvement of production techniques. During the excursion there will be the opportunity to learn more about Poland and its hop production. The main focus will be on organic hop production.

Our special thanks go to Prof. Dr Ewa Solarska for all her efforts and time she put into the organization of this meeting. She did an excellent job. We highly appreciate the contribution of her team with Marzena Marzec, Eliza Potocka, Adam Kuzdraliński and Marta Muszyńska and of Dr Dominik Szwajgier and Dr Waldemar Gustaw who efficiently supported Prof. Solarska in making this meeting a big success. We would like to express our gratitude to Prof. Dr Marian Wesołowski, rector of the University of Life Sciences in Lublin, for his support to hold this meeting at the university. Many thanks are due to all persons who undertake various tasks and thus help to make this meeting a memorable event. Certainly we are also grateful to the various sponsors who supported the mission of the SC by their financial backing.

Many thanks also to all delegates who are presenting papers or posters for sending me their texts before this meeting. As editor I made only few changes to the manuscripts as submitted by the authors, in order to manage a publication of the Proceedings just in time for this meeting.

In closing, I would like to wish you all the best for a successful conference with a lot of interesting contributions and fruitful discussions. I very much hope that this meeting will also be used to build up and extend various scientific networks for the sake of the hop and brewing industry.

Dr Elisabeth Seigner
Chairwoman, I.H.G.C. Scientific Commission

Vorwort

Im Namen der Wissenschaftlichen Kommission des Internationalen Hopfenbaubüros freue ich mich sehr, Sie hier in Lublin begrüßen zu können. Mit dieser Tagung der Wissenschaftlichen Kommission (WK) hier in Polen führen wir die Tradition fort, alle zwei Jahre die Hopfen-Wissenschaftler aus aller Welt im Namen des IHB zum Informationsaustausch zusammenzuführen. Soweit meine Aufzeichnungen zurückreichen, ist die WK nach 1978 und 1999 bereits zum dritten Mal zu Gast in Polen und Frau Prof. Dr. Ewa Solarska ist bereits zum zweiten Mal nach 1999 unsere perfekte Gastgeberin.

Polen steht kurz vor der Übernahme des Vorsitzes im Rat der Europäischen Union, der Ratspräsidentschaft, am 1. Juli. Darüber hinaus bereitet sich Polen auf die Fussball-Europameisterschaft im nächsten Jahr vor, was sicherlich für uns alle beste Unterhaltung garantiert. Daher freuen wir uns besonders, dass wir bereits im Vorfeld dieser Ereignisse die herzliche Gastfreundschaft hier in Polen genießen dürfen. Unser Tagungsort Lublin ist mit fünf Universitäten Zentrum der Wissenschaft und unsere Gastgeberin Prof. Dr. Ewa Solarska ist durch ihre Lehr- und Forschungstätigkeit mit der Universität of Life Sciences in Lublin verbunden; damit ist dies die richtige Atmosphäre für einen fruchtbaren wissenschaftlichen Austausch.

54 Teilnehmer aus 13 Hopfenbaunationen nehmen an dieser Tagung teil, um neue Forschungsarbeiten rund um den Hopfen auszutauschen und zu diskutieren. In 22 Vorträgen und 22 Postern stellen unsere Wissenschaftler ihre Ergebnisse aus folgenden Bereichen vor: Hopfenzüchtung (konventionelle Züchtung und biotechnologische Methoden), Molekularbiologie, Management von Hopfenkrankheiten und Schädlingen, Hopfeninhaltsstoffe, Physiologie des Hopfens und Verbesserungen in der Produktionstechnik. Bei der Exkursion haben wir die Gelegenheit, mehr über Polen und seinen Hopfenanbau zu erfahren. Ein Schwerpunkt wird der Ökohopfenanbau sein.

Unser besonderer Dank geht an Frau Prof. Dr. Ewa Solarska für all ihre Mühen und die viele Zeit, die sie für die Organisation dieser Tagung aufgewendet hat. Sie hat exzellente Arbeit geleistet. Wir bedanken uns auch bei ihrem Team mit Marzena Marzec, Eliza Potocka, Adam Kuzdraliński und Marta Muszyńska sowie bei Dr. Dominik Szwajgier und Dr. Waldemar Gustaw, die alle Prof. Solarska so wirkungsvoll bei den Vorbereitungen unterstützt haben, damit die Tagung ein großer Erfolg wird. Wir danken Prof. Dr. Marian Wesołowski, dem Rektor der University of Life Scienes in Lublin, für seine Unterstützung, diese Veranstaltung hier abhalten zu können. Wir sagen auch allen Dank, die verschiedenste Aufgaben übernommen haben und damit geholfen haben, dass diese Tagung zu einem unvergeßlichen Ereignis für Sie wird. Selbstverständlich geht unser Dank auch an die Sponsoren, die durch ihre finanzielle Unterstützung die Mission der WK unterstützen.

Vielen Dank auch an alle Teilnehmer, die bei dieser Tagung Vorträge und Poster präsentieren. Als Editor habe ich nur wenig an den Texten geändert, die mir von den Autoren vor der Tagung zugeschickt wurden. So konnten die Proceedings noch rechtzeitig zur Tagung fertiggestellt werden.

Abschließend wünsche ich Ihnen alles Gute für eine erfolgreiche Veranstaltung mit vielen interessanten Beiträgen und wertvollen Diskussionen. Ich hoffe sehr, dass diese Tagung auch zum Auf- und Ausbau verschiedener wissenschaftlicher Netzwerke genutzt wird, zum Wohle der Hopfen- und Brauwirtschaft.

Dr. Elisabeth Seigner
Vorsitzende, IHB, Wissenschaftliche Kommission

Lectures and Posters

I. Session:

HOP BREEDING

NEW HOP (*HUMULUS LUPULUS* L.) AROMA VARIETIES FROM AUSTRALIA

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Abstract

An Australian focus on the development of new hop varieties with distinctive aromas and flavours has resulted in commercialisation of Southern Hallertau, Summer, Southern Saaz and Galaxy. This paper describes their chemical properties in relation to beer quality and flavour, with particular emphasis on the aroma potential of high alpha acid hop varieties Galaxy and Topaz. This work is based on research presented at the 2010 Institute of Brewing and Distilling Convention (Asia Pacific Section), Australia.

Keywords: hop, essential oil, flavour, Galaxy, Summer

Introduction

The development of commercially successful aroma hops in Australia has been underway since the 1980s. Selection was originally conducted by identifying new locally grown varieties with hop acid and essential oil profiles that matched traditional European aroma varieties, such as Saaz, Tettnang and Hallertau Mittelfrueh, as seen in other breeding programmes[1,5]. We have developed Southern Hallertau from Hallertau Mittelfrueh, as well as Southern Saaz and Summer from Saaz. When brewed, Southern Saaz is similar in character to Saaz, providing hoppy and spicy characters, while Summer is much lighter and fruity. Southern Hallertau, Southern Saaz and Summer all strongly outperform (in terms of yield) their parental varieties under Australian conditions, but their yields are not comparable to locally developed high alpha acid cultivars, such as Pride of Ringwood, Super Pride and Topaz. The opportunity therefore exists to use locally developed breeding lines with high yield potential to develop hops with unique aroma qualities with greater yield than varieties originating from a single generation of selection from European parents. Galaxy is the product of direct attempts to introduce high quality aroma characteristics into locally developed breeding lines with high yield potential.

Essential oil profiles have been accepted as a means of defining hop varieties. While gas chromatography-mass spectroscopy (GC-MS) profiles of steam distilled essential oil from hop samples can be used to identify hop varieties[3,8], extrapolating hop flavours from essential oil profiles obtained by GC-MS analysis of steam distilled hop essential oils has never been overly successful. Many highly odour and flavour active compounds present in hop essential oil remain unknown[9] or are not identified in standard essential oil profiles[12]. Differences are detectable between hop oils obtained through traditional steam distillation and hop oils steam distilled under reduced pressure at ambient temperatures or obtained through supercritical CO_2 extraction. The oil profile of samples not exposed to high temperatures in the presence of oxygen will more closely match the hop oils found in the original hop sample[13]. Despite the difficulties associated with extrapolating from an essential oil profile to the potential flavour of a hop in beer, it is clear that the essential oil fraction of hop is closely related to the flavour potential of any variety. This is demonstrated by the ongoing development and commercial success of hop flavour essences derived from different fractions (ie: floral, fruity herbal and spicy) of hop oil[13].

This study compares specific essential oil components from new hop aroma varieties such as Southern Saaz, Summer and Galaxy, as well as other Australian grown varieties.

Methods

The Australian Hop Breeding Programme has a germplasm collection consisting of historical varieties and breeding lines from worldwide hop growing areas[7]. New hop varieties are identified on the basis of picking, yield and hop acid characteristics, essential oil profiles and brewing performance, supported by research in the fields of metabolites[11], polyploidy[10] and molecular genetics[14]. Essential oil profiles (percentage composition data) were obtained by GC-MS analysis of steam distilled essential oils EBC 7.10[4]. Forty selected substances were quantified (only 38 were positively identified) from the steam distilled hop essential oils. The data used in this study came from samples taken during 2003, 2005, 2007, 2008 and 2009. Essential oil profiles from hop pellet samples (the most used commercial product) were used for all varieties except for Stella, for which whole dried cones were used.

Results and Discussion

Compounds quantified using GC-MS included esters (one thioester), ketones, and alcohols (monoterpine and acyclic sesquiterpene alcohols) in the oxygentated group, and mono- and di-terpenes, cyclic monoterpenes, sesquiterpenes and bicyclic sesquiterpenes. The oxygenated compounds present in hop essential oil tend to provide fruity and floral aromas[9]. Esters and ketones are identified with fruity odours, while the alcohols tend to be identified with floral odours. Terpenes (myrcene, humulene, farnesene, caryophyllene) and their oxides (humulene epoxides I and II) are associated with citrus (limonene), herbal and spicy/woody odours[9]. The 40 substances quantified in this study explained a database-wide average of 88% of the essential oil in each sample (data not shown). The percentage composition of essential oil data for 26 compounds identified as having fruity, floral, citrus, herbal or spicy/woody aromas, are presented in Table 1. The amount of essential oil relative to alpha acid (μL.g^{-1} α-acid) is an important metric when determining dosing of aroma hops. Galaxy and Stella have α-acid levels between 13-16%. However, essential oil levels relative to α-acid are similar in Galaxy (184 μL.g^{-1} α-acid), Stella (193 μL.g^{-1} α-acid), Southern Saaz (138 μL.g^{-1} α-acid) and Summer (200 μL.g^{-1} α-acid) (data not shown). Therefore, with careful dosing it should be possible to use high α-acid flavour hops (such as Galaxy and Stella) without unduly affecting the bitterness profiles of the beer being produced.

Compounds with fruity odours make up a higher proportion of the essential oils in Pride of Ringwood, Super Pride and Topaz than in Galaxy, Stella, Southern Saaz and Summer. However, the most odour active compounds investigated are isopentyl isobutyrate and S-methyl hexanethioate (odour threshold of 1 ppb in water). Most thio-esters present in hop do not survive boiling, but can be transmitted to beer in dry hopping[2]. Under normal conditions such compounds are likely to be relevant to the aroma contributed by aroma hops such as Galaxy, Stella, Southern Saaz and Summer. Comparing Southern Saaz and Summer in isolation, it is possible that these compounds may play a role in the distinct difference in aroma provided by these hops. The percentage of these compounds in the essential oil of Summer is nearly double that found in Southern Saaz (Table 1), and Summer produces more oil, and has more oil relative to α-acid than Southern Saaz. Summer is known to provide a pleasant fruity character when used as an aroma hop in beer, while Southern Saaz is more herbal and spicy. Galaxy is known to provide a unique aroma of citrus and fruit when brewed. The levels of compounds quantified in this study that provide fruity aromas were not elevated in Galaxy.

Southern Saaz and Summer have the highest proportion of floral compounds in essential oils. Linalool, considered a positive indicator of hop quality, is included as a compound with a floral aroma, but it is not clear that levels of free linalool in the essential oil of hop have a direct relationship with levels of linalool in beer. There is evidence that free linalool in beer is rapidly degraded, and that glycosidically-bound linalool is responsible for most of the detectable linalool in beer[6]. Whether levels of free linalool in the essential oil of a particular hop are related to the levels of glycosidically-bound linalool in any variety is not understood. With regard to levels of free linalool in the varieties included in this study, lowest levels were observed in Millennium, while highest levels were observed in Galaxy (Table 1).

Limonene was the only compound which was specifically citrus in nature. Other compounds such as linalool, geraniol and farnesene may also contribute to a perception of citrus odour or flavour. Relatively high levels of limonene were observed in Topaz and Galaxy, and very high levels in Stella (Table 1). As Galaxy is noted for the citrus character it imparts to beer, it is likely that other compounds or combinations of compounds are responsible for the citrus character of Galaxy in beer.

High levels of compounds with herbal odours are found in Pride of Ringwood and Super Pride (Table 1). The major contributing components are alpha and beta selinene, high levels of which are characteristic of these varieties. There were moderate levels of compounds with herbal odours in Galaxy hop pellets. Unusually low levels of compounds with herbal odours were seen in Stella (Table 1). Levels of humulene epoxides I and II are approximately 10 times higher in Southern Saaz and Summer, than other varieties included in this study. Humulene epoxides and their hydrolysis products are potentially a component of the herbal characteristic associated with traditional European aroma hop varieties.

It is difficult to see much of a pattern in the distribution between varieties of compounds with spicy or woody odours, due to the dominance of this fraction by myrcene (Table 1). Due to its volatility, myrcene is likely only to be present in beer which has been dry hopped. Within the group of varieties investigated, farnesene only occurs at levels above 1% in Galaxy and Southern Saaz (Table 1). Relatively high levels of spicy/wood odour compounds were seen in Southern Saaz and Summer.

In summary, relatively high levels of compounds which produce fruity aromas seen in the bittering varieties Pride of Ringwood and Super Pride are not likely to be important contributors to flavour due to their being used to provide alpha acid for isomerisation during wort boiling. Galaxy, known to introduce a unique citrus and fruit flavour aroma to beer, had relatively high levels of limonene (citrus aroma) and linalool (floral aroma). When the aroma hop varieties Southern Saaz and Summer are compared, Summer, known to contribute a fruity character to beer, had higher levels of highly odour active floral compounds. Both Southern Saaz and Summer had relatively high levels of compounds with spicy/wood odour characters. Many flavour and odour active compounds in hop essential oil are either present below normal detection limits or not identified. The Australian hop breeding programme will continue to exploit the aroma potential available in hop to develop new varieties that, like Galaxy, contribute truly unique flavours and aromas to beer.

Acknowledgements

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Table 1

Summary of 26 odour active essential oil compounds in 8 Australian grown varieties, and the number of samples from which essential oil profiles were generated.

Year	Threshold# (ppb, water)	Millennium	Pride of Ringwood	Super Pride	Galaxy	Stella	Topaz	Southern Saaz	Southern Summer
Number of Samples									
2003		1		1	1		1		
2005		1	1	2	1		1		
2007						1			
2008		5	5	5	8	1	5	5	5
2009		2	2	4	4		2	5	7
Total number of samples		9	8	12	14	2	9	10	12
Essential oil components									
Fruity									
isobutyl isobutyrate		0.15%	0.10%	0.09%	0.19%	0.15%	0.25%	0.02%	0.05%
isopentyl isobutyrate		0.52%	0.05%	0.23%	0.29%	0.29%	0.39%	0.03%	0.07%
2-nonanone		0.00%	0.12%	0.14%	0.04%	0.10%	0.08%	0.23%	0.13%
S-methyl hexanethioate*	1	0.03%	0.02%	0.04%	0.03%	NA	0.06%	0.02%	0.03%
methyl nonanoate		0.23%	0.36%	0.37%	0.22%	0.39%	0.90%	0.16%	0.11%
2-undecanone	7	0.19%	1.49%	2.07%	0.68%	0.94%	1.13%	1.56%	1.10%
methyl decadienoate	10	1.38%	1.42%	1.29%	1.08%	0.75%	1.39%	0.64%	0.90%
Sum of fruity		**2.51%**	**3.57%**	**4.23%**	**2.54%**	**2.61%**	**4.20%**	**2.66%**	**2.39%**
Floral									
linalool	80	0.20%	0.40%	0.37%	0.66%	0.42%	0.57%	0.43%	0.37%
2-decanone		0.00%	0.14%	0.15%	0.09%	0.19%	0.21%	0.23%	0.15%
geraniol		0.04%	0.21%	0.15%	0.10%	0.13%	0.06%	0.34%	0.24%
farnesol		0.05%	0.23%	0.12%	0.12%	0.24%	0.05%	0.14%	0.26%
Sum of floral		**0.29%**	**0.98%**	**0.78%**	**0.97%**	**0.97%**	**0.89%**	**1.13%**	**1.01%**
Citrus									
Limonene		**0.09%**	**0.10%**	**0.09%**	**0.15%**	**0.35%**	**0.21%**	**0.04%**	**0.05%**
Herbal									
beta pinene	140	0.24%	0.31%	0.28%	0.51%	0.74%	0.41%	0.09%	0.15%
beta phellandrene		0.08%	0.05%	0.07%	0.10%	0.16%	0.17%	0.02%	0.03%
beta selinene		1.42%	10.68%	16.84%	5.07%	0.86%	1.38%	0.83%	1.27%
alpha selinene		1.40%	12.86%	19.23%	6.00%	0.55%	1.70%	0.61%	0.88%
gamma cadinene		1.01%	1.04%	0.44%	1.06%	0.37%	0.48%	0.73%	1.23%
delta cadinene		2.23%	1.95%	0.99%	2.00%	0.63%	1.02%	1.20%	1.97%
humulene expoxide I		0.04%	0.03%	0.00%	0.02%	0.02%	0.02%	0.16%	0.28%
humulene epoxide II		0.28%	0.11%	0.02%	0.14%	0.11%	0.12%	1.54%	2.71%
Sum of herbal		**6.71%**	**27.04%**	**37.87%**	**14.90%**	**3.42%**	**5.30%**	**5.19%**	**8.52%**
Spicy/Woody									
myrcene	13	19.51%	22.61%	24.57%	29.40%	33.17%	31.83%	9.33%	13.19%
alpha copaene		0.40%	0.33%	0.14%	0.36%	0.69%	0.20%	0.22%	0.35%
caryophyllene	64	13.77%	11.94%	5.64%	12.49%	12.68%	11.05%	7.95%	13.56%
beta farnesene		0.04%	0.09%	0.04%	4.03%	0.99%	0.08%	27.09%	0.37%
humulene	120	32.61%	2.28%	1.11%	4.30%	15.49%	13.57%	25.21%	45.47%
caryophyllene oxide		0.13%	0.80%	0.13%	0.47%	0.10%	0.13%	0.59%	0.97%
Sum of spicy/woody		**66.47%**	**38.05%**	**31.62%**	**51.06%**	**63.10%**	**56.86%**	**70.38%**	**73.91%**
% oil accounted for		**76.06%**	**69.74%**	**74.60%**	**69.62%**	**70.44%**	**67.47%**	**79.40%**	**85.88%**

* S-methyl hexanethioate was identified and quantified in samples from 2009 only.
# Odour threshold values in water as given by Briggs et al.[2], with the exception of linalool which is a threshold value in beer.

TRENDS IN HOP BREEDING – NEW AROMA AND BITTER QUALITIES AT THE HOP RESEARCH CENTER HUELL

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LfL Pflanzenbau

Bav. State Research Center for Agriculture

Institute for Crop Science and Plant Breeding
Hop Research Center Huell

Trends in Hop Breeding – new Aroma and Bitter Qualities at the Hop Research Center Huell

A. Lutz, J. Kneidl, S. Seefelder, K. Kammhuber, and E. Seigner

Objective and Results

In current breeding programs we are aiming to develop hop cultivars with specific aroma profiles and high alpha acids, respectively which meet the demands of brewers worldwide. On the other side, by specific hybridization and selection hops should be adapted to alternative non-brewing applications.

Aroma and High Alpha Varieties for Brewing Purposes

In the aroma sector hop cultivars are being developed which represent the classical aroma notes reflecting the noble Hallertauer Mittelfrueher and Tettnanger type and also types with distinctive flavor profiles such as in Saphir, Opal and Smaragd. We also started to breed hops with tropical, fruity or non-hoppy flavor following the trend initiated by US micro-brewers. Moreover, high alpha varieties are available which show alpha acid contents of up to 23.5 %. With this broad spectrum of hops providing various aroma notes or high alpha acids brewers can make their choice for creating special beers of distinct types and flavors.

Key Aspects of Aroma Description

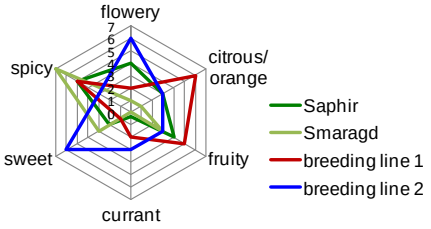
increasing ⟶ decreasing

Purity	pure		inhomogeneous		impure	
Value of Flavor	noble	fine/ mild	medium	un- pleasant	straw-like	muffled
Intensity	maintain- ing/full	long- lasting	medium	weak/ short	strong	pungent
Strange Flavor	smoky, burnt/malty, onion or garlic like					

aroma flavor: 1 - 30 points
land races: 25 - 28 points
aroma selections: 24 - 28 points

bitter varieties with
pleasant aroma: 20 - 23 points
pungent aroma: < 20 points

Aroma Characteristics of Dried Hop Cones



Alternative Non-Brewing Applications of Hops

Recently, breeding lines have been selected with high alpha and beta acids as well. Due to the antimicrobial and bacteriostatic effect of bitter acids these hops pave the way to new applications in the pharmaceutical and medicinal field. Furthermore, beta acids are already used as environmentally-beneficial and health-uncritical substitutes for antibiotics and formaldehyde in the food and ethanol industry. Also several Huell breeding lines showing high contents of Xanthohumol have the potential for alternative usage, since this hop prenylflavonoid has already proven anti-cancerogenic activity in various medicinal studies.

Breeding Line or Cultivar	Alpha Acids (%)	Beta Acids (%)	Bitter Acids Total (%)	Xanthohumol (%)
2003/067/002	9.5 - 14.5	11.0 - 14.0	20 - 27	0.6 - 0.8
2003/067/005	12.0 - 16.5	9.0 - 12.0	21 - 26	0.6 - 0.8
2003/067/044	2.7 - 5.5	15.3 - 21.2	19 - 25	0.9 - 1.5
2001/101/704	10.0 - 15.0	3.2 - 4.7	13 - 19	1.4 - 2.1
2000/109/728	16.5 - 23.5	5.0 - 6.4	21 - 29	0.7 - 1.0
Hall. Taurus	13.0 - 20.0	4.0 - 6.0	17 - 26	0.7 - 1.0

NEW KNOWLEDGE IN CZECH HOP BREEDING

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Abstract

Breeding objectives can be divided into aroma and bitter hops, resistance to stresses, dwarf hops and varieties for pharmaceutical utilization. In 2008 Vital and Kazbek were released, in 2010 Saaz Late and Bohemie were registered. At present we have at our disposal a great number of perspective genotypes with high content of resins as well as those ones with high content of xanthohumol and desmethylxanthohumol (DMX). Breeding aimed at low trellis hops is carried out with the help of a new Eureka project.

Keywords: hop, *Humulus lupulus* L., varieties, genotypes, breeding material, low trellis, dwarf hops, xanthohumol, DMX.

Introduction

Hop breeding in Czech Republic was based on clone selection, whose aim was to get better quality of the original Czech aroma hops. Although hop crossing was carried out in the sixties of the last century its importance used to be neglected. The first Czech varieties, which have had their origin in hop crossing (Bor, Sladek), were registered in 1994. Varieties Premiant (1996), Agnus (2001), Harmonie (2004) and Rubin (2006) followed. Breweries use mostly Sladek, Premiant and Agnus. Higher demands for Harmonie result in extension of its acreage. Hop cones contain many important compounds, which can be utilized within pharmaceutical as well as biomedicine industry (De Keukeliere et al., 2003). Breeding objectives can be divided into aroma and bitter hops, both being resistant to stresses, dwarf varieties for cultivation in low trellis and varieties for pharmaceutical utilization.

Materials and methods

Good genetic resources (breeding material, registered varieties and wild hops) are very important for hop breeding (Nesvadba V., Krofta K., 2005). The breeding process is aimed at crossing of suitable parents. Their progenies are grown on a plot for two years. The best individuals are selected from this collection. These plants are propagated and tested in a breeding nursery. Perspective genotypes are submitted to thorough tests (5-8 years) and after this period they are enrolled to the registration process controlled by Czech government.

Results and discussion

Hop breeding has a long tradition in CR. It is necessary all the perspective genotypes to show good tolerance to fungal diseases (downy and powdery mildew), good agro-technical parameters and last but not least very good brewing characteristics. A pilot brewery is therefore a necessary part of our facilities to test these features. Numerous analyses are carried out in Dept. of Hop Chemistry to characterize new hop genotypes and varieties. Their results are shown in Table 1.

Table 1: New Czech hop varieties

Hop variety	Vital	Kazbek	Saaz Late	Bohemie
Alpha acids (% w/w)	12,0 – 16,0	5,0 – 8,0	3,5 – 6,0	5,0 – 8,0
Beta acids (% w/w)	6,0 – 10,0	4,0 – 6,0	4,0 – 6,5	6,0 – 9,0
Cohumulone (% rel.)	21-26	35 – 40	20 - 25	23 –26
Xanthohumol (% w/w)	0,70 – 1,00	0,30 – 0,45	0,30 – 0,50	0,50 – 0,75
DMX (% w/w)	0,25 – 0,40	0,10 – 0,20	0,07 – 0,12	0,10 – 0,20
Total hop oils (% w/w)	1,5 – 2,5	0,9 – 1,8	0,5 – 1,0	1,0 – 1,5
Myrcene (% rel.)	40 – 55	40 – 50	25 – 35	30 – 40
Caryophylene (% rel.)	5 – 8	10 – 15	6 – 9	7 – 10
Humulene (% rel.)	2 – 5	20 – 35	15 – 20	17 –23
Farnesene (% rel.)	1 – 4	< 1	15 – 20	< 1
Selinens (% rel.)	7 - 15	1 - 3	3 - 4	8 - 12

1. Breeding of aroma hops

Two main ways have been used to provide breeding of aroma hops recently. The first classical one consists in crossing of aroma parental hops and selection of their progenies. In this way we managed to release Sladek, which has good brewing utilization. Nowadays it represents the standard for this group of aroma hops. Within this group Harmonie was registered in 2004 and Bohemie in 2010. This variety has the same qualitative parameters but shprter growing period. The second group is represented by fine aroma hops (Saazer), which are typical for excellent brewing characteristics. Up to now only clone selection has been used within Saazer. Recently it has been used for crossing with suitable male genotypes having their origin in Saazer as well. As the result of this activity we registered Saaz Late in 2010. This variety has the same structure of hop resins and essential oils as Saazer. In 2011 it is grown on the acreage of 3 ha and it will be planted on another 5 ha during this year. The variety is tested in numerous Czech and foreign breweries and it shows very good brewing qualities.

2. Breeding of bitter hops

Breeding of these hops is aimed at high production of alpha acids. New varieties must meet another necessary demands: good tolerance and agro-technical parameters as well as brewing characteristics. In 2001 Agnus was released. It has just above-average production of alpha acids but on the other had its brewing characteristics, including positive influence on beer stability, are excellent. Vital was registered to meet demands of pharmaceutical industry. It also shows higher content of alpha and beta acids but its yield seem to be too low for practical utilization even though brewing tests have proved its very good influence on beer quality. In 2010 we managed to choose five perspective genotypes into governmental registration tests. They show alpha bitter content in the range of 13-17% and yield of hops higher than 3 t/ha.

3. Breeding on resistance

Varieties tolerant to abiotic stresses are not numerous. It is not easy to get a new variety tolerant to drought and hot weather, which would also have good productivity and stability of alpha acids including good brewing parameters. In 2008 we managed to register Kazbek, which shows high level of stability in the content of alpha acids and productivity. It is the first Czech variety to show higher yields than Sladek (> 3.0 t/ha). In 2010 we planted it in a commercial hop garden, which will be used for brewing tests. As it can be obvious from the name, this variety is of Russian origin and it is the evidence that wild hops are mostly utilized within this breeding objective (Patzak et al., 2010).

4. Breeding for pharmaceutical industry

We managed to get 90 new genotypes with xanthohumol content higher than 1%. This seems a very good result in comparison with 2007 when we were able to obtain just seven genotypes with such high content of this precious compound (Figure 1). Even better results were got with DMX. Whereas in 2007 only one genotype had content of DMX higher than 0.25%, three years later 43 new hop genotypes exceeded this value (Figure 2). Planting material for establishment of a field trial was taken from this collection. In 2010 we applied for their incorporation into governmental registration trials. New variety Vital was registered in 2008 within this breeding objective. It shows DMX content at the level of 0.3% w.w. with the range between 0.22 and 0.49% w.w.

Figure 1: Frequency of xanthohumol contents in the progenies bred at its high content

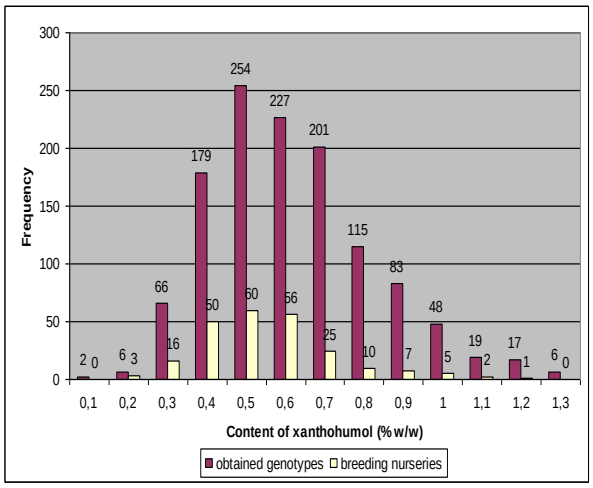
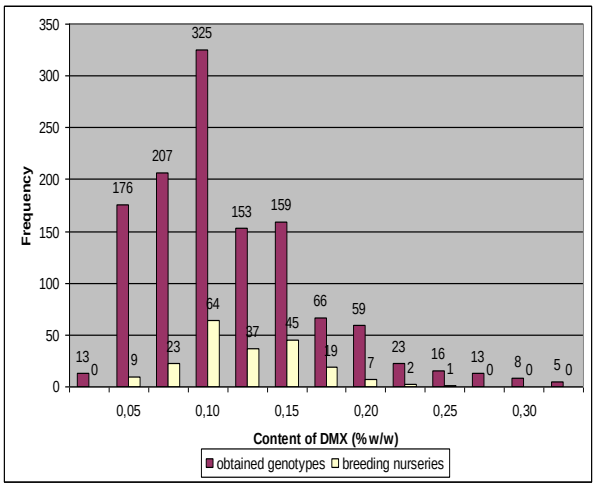


Figure 2: Frequency of DMX contents in the progenies bred at its high content



5. Breeding of dwarf hops

On the base of internode measurement in genotypes for high (Premiant and Sladek) as well as low trellis (First Gold and Czech genotype no. 5021) we determined with the help of t-test statistical significance of the difference with 99% probability between the above-mentioned groups of the genotypes. It is obvious from Table 2 that genotypes for high trellis have the average length of internodes 0.277 m (Premiant) and 0.237 m (Sladek), whereas dwarf genotypes 0.075 m (First Gold) and 0.092 (genotype no 5021). The lowest variability was found out in Premiant (16.59%). On the contrary, the highest variability showed First Gold (24.88%).

Table 2: Variability in the length of internodes in high and dwarf hops

Hop variety	Premiant	Sladek	First Gold	Genotype 5021
Average (m)	0.277	0.237	0.075	0.092
Standard deviation	4.589	5.219	1.876	2.180
Var. coefficient (%)	16.59	21.99	24.88	23.73
Min. length (m)	0.160	0.130	0.025	0.022
Max. length (m)	0.350	0.350	0.112	0.145

Progenies of F_1 generation (H37 and H39) were got from a male UK dwarf genotype. Progeny H7 comes from a parental combination for high trellis. Statistical parameters for the individual progenies are reviewed in Table 3. Dwarf progenies show the both types of genotypes (for low and high trellis), which can be characterized by higher variability (VK = 40.42 – 51.61%). With the help of t-test we determined 99% probability of the difference in the length of internodes within H7 progeny in comparison with the other dwarf progenies.

Table 3: Variability in the length of internodes in high and dwarf hops within the progenies of F_1 generation

Hop variety	H 37	H 38	H 39	H 7
Average (m)	0.067	0.069	0.067	0.168
Standard deviance	32.016	27.986	34.730	37.208
Var. coefficient (%)	47.48	40.42	51.61	22.04
Min. length (m)	0.026	0.026	0.020	0.087
Max. length (m)	0.186	0.154	0.218	0.257

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VITAL – NEW CZECH HOP VARIETY

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In 2008 new variety Vital was registered in Czech Republic. Its name is derived from high contents of biologically active compounds from the group of prenylated flavonoids - xanthohumol and desmethylxanthohumol (DMX). Vital originated from hybrid progenies with majority proportion of Agnus cultivar. Genetically it belongs to the group of high-alpha hops with Euroamerican origin. The crossing of parental components was realized in 1996. Selection from the file of seedlings was carried out on the basis of high contents of alpha and beta acids. Characteristic trait of Vital variety is a long vegetation period at the range of 135-143 days. Harvest maturity comes in the middle of September in climate conditions of Saaz growing region. Vital is medium susceptible to downy mildew (*Pseudoperonospora humuli*) and tolerant to powdery mildew (*Podosphaera macularis*). The plant has sizable regular cylindrical shape. Fruit-bearing laterals are 65–75 cm long. The bine is of green colour. Its diameter on a full-grown plant is 8–9 mm. Hop cone is of a narrow egg shape. Covering bracts are tightly snuggled to the central axis of the cone. Therefore, low losses occur at the crop harvest due to shattering and damage of cones. Mean size of green cones is 35 mm and average weight is 0.8 g. Yield is usually at the level of 1.8–2.5 t/ha. Vital contains 12–16 % w. of alpha acids, cohumulone ratio is in the interval of 21–26 % rel. Contents of beta acids are in the range of 6–10 % w. and, colupulone ratio in the interval of 45–50 % rel. The smell of hops is pronounced hoppy and spicy. Hop oils content is 1.5–2.5 % w. Majority component of hop oil is myrcene (45–60 % rel). Contents of other important sesquiterpens β-caryophyllene, α-humulene, β-farnesene and selinenes are typical for the variety. While caryophylllene content is in usual interval of 5–8 % rel., low content of α-humulene (1–4 % rel.) is very rare. The presence of β-farnesene in the amount of 1–3 % rel. is interesting. Content of selinenes in the range of 10–20 % rel. is very high. Mutual contents of α-humulene, β-farnesene and selinenes are typical for Vital variety and can be used as an important varietal chemotaxonomy trait. Vital contains 2.5–3.5 % of total polyphenols and 0.60–0.80 % of xanthohumol. Unique property of the variety is very high content of DMX. Its amount in green cones is up to 0.40–0.60 % w. Part of the content is lost in the course of drying, therefore only 0.25–0.40 % is found in dry product. It is the amount, which is two times higher at least in comparison with other Czech and foreign hop varieties. High content of prenylflavonoids provides possibilities of utilisation of Vital in other branches of food industry (food supplements) and pharmacy. In breweries it is used in the form of pellets and CO_2-extracts. In brewing trials and beer tasting contests Vital showed good influence on beer taste and smell. Variety Vital was bred by Hop Research Institute in Žatec. Agrotechnical parameters were verified within the scope of research project FR-TI1/012 with financial support of Czech Ministry of Industry and Trade.

VARIABILITY OF WILD HOPS (*HUMULUS LUPULUS* L.)

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Wild hops are very natural important source for hop breeding. Their importance has increased recently because of the utilization within crossing aimed at tolerance and resistance to biotic and abiotic factors. The main objective of this work was to obtain new genotypes showing resistance to fungal diseases, drought, hot weather conditions, etc. Therefore, just the hop plants, which did not show any symptoms of downy and powdery mildew in their natural habitats were chosen. To be sampled they had to show good growing characteristics, productivity, aroma, optimal chemical structures in hop cones, etc. Assessment of those hops has been carried out since 2006. The collection of 103 wild hops was selected in 2010 to extend evaluation of variability. These plants have been planted altogether in a hop garden. Contents and structure of hop resins are reviewed in Table 1.

Table 1: Variability in the contents and structure of hop resins (2006 – 2010)

Parameter	Alpha acids (% w/w)	Beta acids (% w/w)	Ratio alpha/beta	Cohumulone (% rel.)	Colupulone (% rel.)
Minimum	0.10	0.14	0.09	13.6	29.8
Maximum	8.87	8.23	2.07	64.6	82.9
Mean	2.16	3.12	0.74	32.6	49.8
Coef. variability (%)	59.4	41.8	50.1	46.2	36.3

In 2010 we analyzed 18 samples of wild hops taking from the original habitat. One sample was brought from Kyrgyz. It showed alpha acid content 2.21 %, but beta acid content was higher (4.14 %). Four samples were obtained within the international cooperation with University of SS. Cyril and Methodius, Faculty of Natural Sciences in Slovakia. They have their origin from the vicinity of Pezinok and Piestany. Good for the breeding work seems to be a wild hop sampled near the river Váh with good content of alpha acids (3.55 %) and very low percentage (15,40 % rel.) of cohumulone. Czech hops have their origin in North Bohemia. The highest content of alpha acids (4.45%) was found out in a hop plant sampled near the village of Trebusin (Ustek hop region). Six wild hops were obtained within the expedition aimed at the collection of wild hops in North-Osetia. Hop plant sampled near the village of Mozdog had the highest content of alpha acids within this group. (8.87 %). Wild hop from Achsarisar showed also high alpha content (6.67 %). These hops seem to be valuable genetic material.

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RESISTANCE MECHANISMS OF DIFFERENT HOP GENOTYPES TO HOP POWDERY MILDEW

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Abstract

Since resistance of hop cultivars to powdery mildew is in most cases based on a single major gene, it can easily be overcome by the hop powdery mildew fungus Podosphaera macularis (Wallr.) U. Braun & S. Takam. In Germany only the R2 resistance of the cv. Wye Target has remained effective, although this resistance has already been broken in the UK. In order to identify new sources of resistance, 25,000 wild hops and more than 1,800 advanced breeding lines have been screened in a preceding project. Out of 45 wild hops and 52 breeding lines showing powdery mildew resistance against different virulences of fungal races, eight wild hops, two breeding lines, as well as two cultivars have been selected for further investigations on the cellular basis of resistance. For this purpose, histochemical staining techniques to visualize fungal structures and to detect defence reactions of hop cells have been established. Apart from the susceptible control cv. Northern Brewer interaction sites with elongated secondary hyphae (ESH), which are typically for susceptible plants, have also been found in one macroscopically resistant wild hop. In all genotypes examined, resistance is mainly based on a hypersensitive cell death reaction (HR) of the attacked cells. Cell wall appositions (CWAs) and penetration resistance seem to play a minor role, although differences among the genotypes investigated were detected. The percentage of haustorium formation and penetrated CWAs, which were in most cases followed by an HR, also differed in the resistant genotypes. These new insights may support an integrated breeding approach towards durable powdery mildew resistance in hop.

Keywords: *Podosphaera macularis,* resistance, histochemical staining

Abbreviations: CWA, cell wall apposition; dpi, days post inoculation; ESH, elongated secondary hyphae; hpi, hours post inoculation; HR, hypersensitive reaction; WGA-TMR, wheat germ agglutinin-tetramethylrhodamine

Introduction

In the 1970s, infections on hops caused by the powdery mildew fungus appeared for the first time to a significant extent in Germany (Kohlmann and Kastner, 1975). Since that time breeding for powdery mildew resistance has been one of the major objectives at the Hop Research Center Huell.

Powdery mildew susceptibility is characterized by interactions, in which the fungus penetrates the cell wall and nutrient uptake from the host is realized by the formation of a haustorium. In contrast, resistance is achieved when formation of functional haustoria is prevented to a significant extent. This can be observed as penetration resistance, mostly seen as formation of cell wall appositions (CWAs), or by the hypersensitive cell death reaction (HR). CWAs prevent the penetration of the cell wall by the fungus whereas HR of attacked cells stops the development of functional haustoria inside the plant cell (Eichmann and Hückelhoven, 2008). However, there exists also a late, posthaustorial resistance, where macroscopic disease symptoms are missing despite the fact that no HR and CWAs can be detected (Prats et al., 2007).

Currently there is only one active powdery mildew resistance in German hop cultivars, which is based on the R2 resistance derived from the English cv. Wye Target. As this resistance has already been broken in England, new resistance traits should be incorporated into future

cultivars. For this purpose, 25,000 wild hops, 1,800 advanced breeding lines as well as 44 cultivars have been screened in the glass house for powdery mildew resistance after artificial inoculation with hop powdery mildew spores from different virulence types (Seigner et al., 2006). From the genotypes showing a broader spectrum of resistance, eight wild hops, as well as two breeding lines and two cultivars, Merkur and Herkules, have been selected for further investigations. For these genotypes detailed knowledge concerning their resistance reactions is missing. Details of the mechanisms leading to resistance at the cell level may provide important insights, as different resistance mechanisms combined in new cultivars might allow achieving long-term resistance.

Methods

Altogether three independent experiments were performed. Among macroscopically resistant genotypes eight wild hops (WH1-WH8, only data from five wild hops are shown), two advanced breeding lines (BL1 and BL2), two cultivars (Merkur and Herkules) and the susceptible control cv. Northern Brewer were chosen for microscopic studies. Four root cuttings per genotype were potted and grown in a climate chamber. After at least two weeks, leaves from the first unfolded leaf pair were cut off and placed in a petri dish. Inoculation of leaves was carried out with an inoculation tower. Fungal structures were stained with the chitin specific fluorescence dye wheat germ agglutinin-tetramethylrhodamine (WGA-TMR) (Deshmukh et al., 2006). Defence reactions were detected using aniline blue for callose staining and by the autofluorescene of HR cells. For each genotype three leaves from three different plants with at least 60 interaction sites per leaf were evaluated under a fluorescence microscope. The cv. Northern Brewer served as the susceptible control. Results shown are from one experiment.

Results

Histochemical staining techniques have been successfully established. Staining of the hop powdery mildew fungus with WGA-TMR allows the investigation of fungal development on the leaf surface. Detection of callose with aniline blue makes defence reactions such as CWAs or HR cells visible. Autofluorescence was additionally used for the detection of HR cells. Fig. 1 shows the microscopic evaluation of the interaction between phenotypically resistant hop genotypes or the susceptible control Northern Brewer with the powdery mildew fungus.

The percentage of HR cells ranged between 55% in the susceptible control and 94% in one resistant genotype. CWAs appeared in 5% of the interaction sites in the susceptible control and range between 1 and 11% in the resistant genotypes. In one wild hop, 21% of the interactions were penetrated CWAs with a visible haustorium inside the cell, followed in most cases by an HR. These kinds of penetrated CWAs were rarely found in the other genotypes. The percentage of haustoria, which were visible ranged from 1-46%, haustoria at interaction sites, with no ESH were abnormal, i.e. smaller and without haustorial lobes.

In addition, to the susceptible control, in which 35% of interaction sites showed formation of ESH and normally developed haustoria at 24 hours post inoculation (hpi), also on the phenotypically resistant wild hop WH3 11% of interaction sites showed ESH formation at that time. In 32% of the cells containing secondary haustoria an HR was observed at 48 hpi. In 87% of the interactions, the cell below the primary appressorium remained alive. Single sporulation events could be seen 7 days post inoculation (dpi) (data not shown).

Discussion

Among the nine investigated phenotypically resistant genotypes there was none in which penetration resistance was relevant. This raises the question whether in general CWAs play only a minor role in the interaction of hop with the hop powdery mildew fungus. Another explanation might be that resistance in the investigated cultivars is mainly based on single genes with a dominant or semidominant inheritage. In this context it is known that such interactions controlled by single dominant resistance genes in most cases lead to rapid HR of

attacked cells (Glazebrok, 2005; Li et al., 2007). Naturally occurring penetration resistance is described in a few plant species. In barley and tomato, penetration resistance based on a loss of function mutation in an Mlo allele has been described, which is inherited recessively (Piffanelli et al., 2004; Bai at all, 2008). In beet and vine genotypes, penetration resistance based on cell wall appositions was mainly found in partially resistant cultivars (Fernández-Aparicio et al., 2009; Feechan et al., 2011).
Despite the fact that in all genotypes resistance was mainly based on an HR, other investigated parameters varied among the genotypes. For example differences in the formation of haustoria or penetrated CWAs might indicate a diverse genetic basis of resistance in these genotypes. Currently, we are investigating the number of neighbouring cells adjacent to the attacked cells, which are additionally undergoing an HR, since the first experiment gave a hint that there might be differences.

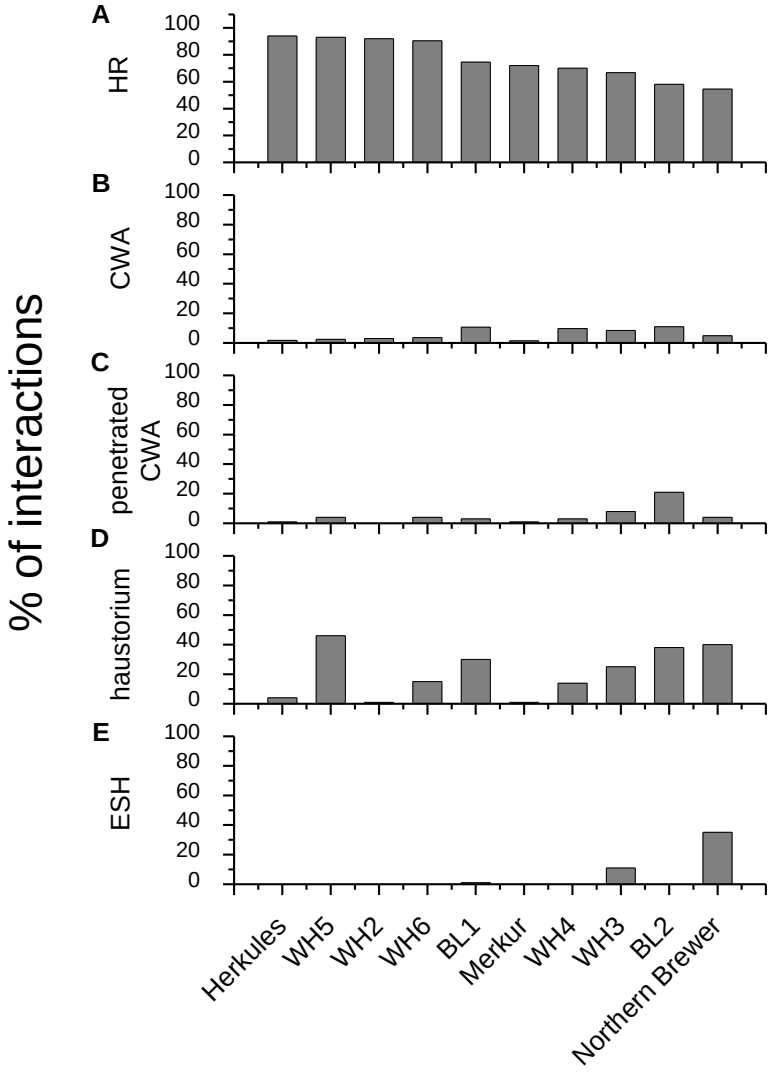

Figure 1: Results of the microscopic evaluation of the interaction of five wild hops, two breeding lines, the cultivars Herkules and Merkur, with the hop powdery mildew fungus. The fully susceptible cultivar Northern Brewer served as control. The time point of evaluation was 24 hpi. For each genotype, three leaves and at least 60 interactions per leaf were evaluated. **A,** The percentage of interactions where a single cell HR was observed. **B,** Percentage of non-penetrated CWAs. **C,** Percentage of penetrated cell wall appositions**,** followed in most cases by an HR. **D,** Percentage of haustorium formation. Haustoria at interaction sites with no ESH were abnormal. **E,** Percentage of interactions where ESH were observed and haustoria developed normally.

Surprisingly, there was one macroscopically resistant wild hop, in which at 24hpi ESH and single sporulation events were observed under the microscope. In this genotype, HR cells occurred below the secondary appressoria 48 hpi, but rarely in cells containing the initial haustorium. Here, maybe investigation of a later time point would be interesting to get some information about the cellular basis of strongly reduced macroscopic symptoms in this genotype. Interestingly, a new pathogen race recently evolved from the race that was used in this study, and the resistance of this particular genotype is now broken (A. Lutz, pers. comm.).
After the analysis of further independent repetitions, detailed information about the cellular reactions of diverse hop genotypes to hop powdery mildew will be available which could then be used for breeding towards resistant cultivars.

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BREEDING FOR RESISTANCE TO HOP POWDERY MILDEW IN POLAND

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Abstract

Powdery mildew caused by *Podosphaera macularis* is one of the most dangerous fungal diseases of hop. The first symptoms are observed on leaves in the form of scattered, isolated, white colonies. Infection of the flowers and young cones causes deformations which result in loss of yield and quality. Studies on the occurrence of powdery mildew on commercial hop gardens in Poland had revealed that disease appeared every year but symptoms severity was different in particular vegetation seasons and hop cultivars. Susceptibility of hop cultivars expressed as infection coefficient, was determined in the technological maturity stage on the basis of the number of infected cones and symptoms severity. The least susceptible Polish cultivar, Lubelski showed a relatively low infection coefficient, ranging from 1.1 to 5.0%, but for the most susceptible Magnum cv. it ranged from 4.8 to 12.8%.

The most effective way to control the powdery mildew is breeding for resistance. The first step of work was the evaluation of effectiveness of currently known sources of resistance on the basis of artificial inoculation tests conducted in controlled conditions using the mixture of powdery mildew isolates occurring in Poland. These analyses showed that only resistances based on R1 and R2 genes from the English cultivars Zenith and Wye Target are still effective. These cultivars exhibited effective resistance to hop powdery mildew also in the field, that is why, they are used in breeding program in Poland. Hop seedlings obtained by hybridization of appropriate parental components were tested for resistance to powdery mildew using artificial inoculation in controlled conditions. Severe disease symptoms were exhibited by 69,9% to 82,2% of individuals, depending on the progenies. These sensitive plants were excluded. The rest of plants were planted to the field where they have been evaluated for resistance under natural infection pressure. Almost 85% of plants recognized as resistant in artificial inoculation test, exhibited resistance to hop powdery mildew also in the field conditions.

Resistances based on action of major genes are easily overcome by new virulent races of pathogen, therefore, we should start looking for new sources of resistance to hop powdery mildew. Wild male accessions collected from different regions of Poland were multiplied by softwood cuttings. Young plants were artificially inoculated by mixture of powdery mildew isolates occurring in Poland. Field tests were also conducted. We identified 7 very promising wild males, which have been used as crossing components in our breeding program.

Keywords: hops, powdery mildew, resistance, breeding

II. Session:

BIOTECHNOLOGY

NEW BIOTECHNOLOGICAL APPROACHES OF GROWING HOP IN THE UKRAINE

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During 2000-2010 we have developed a new technique of clonal *in vitro* micropropagation of 20 Ukrainian hop varieties with the financial and technical support of the hop industry. Since 2005 more than 300 ha of new hop gardens have been created in several regions of Ukraine. The monitoring of the hop infection rate in last period was done from commercial hop gardens in the Zhitomir region, representing 11% of acreage. The regeneration ability of different types of meristems and the efficiency in the elimination of viruses were studied. The modern variation technique of biotechnological virus free material of hop production with using thermotherapy, chemical therapy in combination with the method of apical meristem selection was proposed. The morphogenic potential of hop tissues and organs by selection and modification of culture medium components for the different stages of morphogenesis *in vitro* were investigated. Callus induction was established for the different varieties of Ukraine hops. The influence of different concentrations of phytohormones, mineral and organic compounds of culture medium on induction of roots was studied. The method for the identification of thirteen Ukrainian hops on the base of nine SSR markers was created. All analyzed genotypes have a unique combination of alleles. This allowed us to identify each of them by using nine microsatellite loci. The direct biotechnological adaptation *in vivo* was first done with the hop variety "National" as that had been cloned *in vitro*. As result nearly 100% adapted plants were obtained, without using of hops nursery. This technology foresees the adaptation in phytocontainers, compost and making the direct planting on commercial hop-gardens that allowed to get industrial harvests (1.2-1.5 t/ha) in the next year. The granulation of hop was made on minigranulator Eco-Bio 100 that allowed obtaining high-quality pellets of hops with minimal energy consumption without loss of quality of the ingredients of hops. The long-lived biological investigations of development of hop have shown that productivity of crop on hop gardens is defined in many things of conditions artificial isolation of pistillate plants. We have established the direct dependence between formation of parthenocarpic fruits and development of cone tissue. The histochemical researches have shown that intensive overgrowth of fruits tissue without seeds is promoting to synthesis of proteins in glandular cells of peltate glands and intensify secretory activity of them. The ecological and cytological researches have allowed revealing the agents which initiate the parthenocarpy of hop and promote to increase the quality of the production. We have observed less virus symptoms and good morphological stability of hop plants despite the low levels of agricultural engineering and chemical plant protection in hop growing. During 2003-2010 we have investigated hop gardens in 4 regions of Ukraine where virus free *in vitro* hop clones were adapted. Using the electron microscope and PCR-methods we have shown the presence of hop mosaic virus (HMV) and hop latent virus (HLV) in 1.5% of the samples. On the basis of the results of this complex research we have developed conceptually a new and economically approach of growing hops.

Keywords: hop, micropropagation, *in vitro*, SSR-markers, analysis of genotype, adaptation, direct adaptation, parthenocarpic fruits, histochemical analysis, hop mosaic virus, hop latent virus, virus free hop.

USE OF TISSUE CULTURE TECHNIQUES TO HOP IMPROVEMENT

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Abstract

Plant tissue cultures are considered a powerful tool for improvement of crop plants. We applied different tissue culture techniques in hops to produce virus-free stock plants (meristem culture), vegetatively multiply selected hop mericlones (micropropagation), store hop germplasm (*in vitro* storage), regenerate organogenic shoots for potential use in hop genetic transformation (adventitious shoot regeneration) and to study the chemical composition of hop *in vitro* cultures (callus and cell suspension cultures).

Keywords: meristem culture, micropropagation, *in vitro* storage, organogenesis, callus culture, cell suspension culture

Introduction

Plant tissue culture is a technique, by which small parts of plants excised from intact individuals are cultured in *in vitro* conditions on artificial culture media of different composition. Since the early attempts of Haberlandt (1902) to culture plant cells in a simple nutrient medium for studying physiological and morphological problems, the tissue cultures techniques developed into a powerful tool used in different areas of plant physiology, genetics, cytology and other scientific disciplines. Moreover, many of plant tissue culture techniques have been used currently for commercial production of clonal populations of plants, pathogen-free planting stocks, new breeding materials and genetically modified plants.

The first attempts to culture hop explants in *in vitro* culture date back to the end of 1960's, when Griffin and Coley-Smith (1968) initiated callus cultures from stem internode explants of cv. Eastwell Golding with the aim of study the infection process of hop tissues by *Pseudoperonospora humuli.* Since then, tissue culture techniques in hops have been used for example for production of virus-free stock plants (Vine and Jones, 1969; Adams, 1975; Svoboda, 1992), true-to-type propagation of selected genotypes (Roy et al., 2001; Smýkalová et al., 2001), *in vitro* storage of hop germplasm (Revilla and Martínez, 2002; Reed et al., 2003; Aynalem et al., 2006), regeneration of adventitious shoots from non-meristematic tissues (Rakouský and Matoušek, 1994; Gurriarán et al., 1999), study of plant morphogenesis using organogenic nodule induction (Fortes et al., 2009), and for introduction of foreign genes into hop genome via genetic transformation (Horlemann et al., 2003; Schwekendiek et al., 2007; Aldinger et al., 2009).

In this paper we summarize the use of tissue culture techniques for hop improvement at the Plant Production Research Centre (PPRC) in Piešťany and later on, at the University of Ss. Cyril and Methodius (UCM) in Trnava, Slovak Republic

Tissue cultures of hops

Meristem culture. In agricultural biotechnology, meristem and/or shoot tip culture is used primarily to eliminate viruses from diseased plants and for production of virus-free stocks for field planting. The technique can be used also to prepare source plants for clonal *in vitro* propagation of vegetatively propagated plants and germplasm preservation of both vegeratively and seed propagated plant species. We used meristem culture (Fig. 1A) to produce virus-free stock plants for commercial hop garden establishment in Slovak republic during the years 1994-2000. The technique was successfully applied to ten hop cultivars,

with an efficiency of about 70% of meristems (0.2-0.5 mm of size) developing 1-4 shoots during the 28 day-long culture period. Of the almost three hundred mericlones subjected to thermotherapy in average 65% were viable and ELISA testing confirmed the virus-free status of the *in vitro* regenerants tested (Faragó and Nešťáková, 1998). Recently, the field area of meristem-culture derived hops from this program in Slovakia is cca. 280 ha, which is about 95% of all hop gardens in our country.

Micropropagation. Micropropagation is a true-to-type propagation of plants in *in vitro* culture. *In vitro* micropropagation of hop is feasible starting from different types of explants, such as node cuttings and apical tip explants (Roy et al., 2001; Smýkalová et al., 2001), meristems (Adams, 1975), and shoot or leaf segments (Guarriarán et al., 1999; Peredo et al., 2006). At PPRC in Piešťany, we developed an efficient method of micropropagation for clonal multiplication of meristem culture-derived virus-free plants using a medium devoid of plant growth regulators (Fig. 1B). Both, stage 2 multiplication and stage 3 rooting were achieved on half-strength MS-derived media supplemented with Phytagel instead of agar. The shoot multiplication rates of cultures reached 5-8 new propagules within the 3-week subculture interval, which allowed us to obtain several thousands of clonal plants within three months of multiplication (results not published).

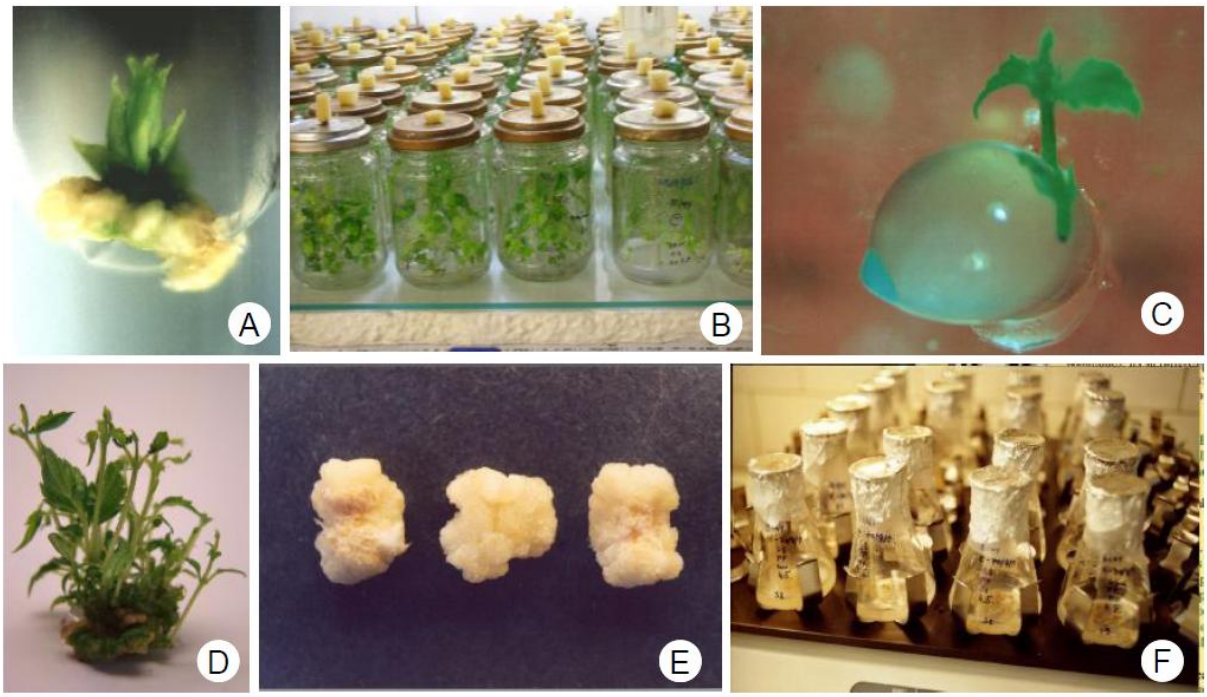

Fig. 1 *In vitro* culture of hop explants using different tissue culture techniques. A) Meristem tip culture for virus eradication and disease-free stock plant production, B) micropropagation of hops via shoot tip and nodal explants culture, C) alginate bead encapsulation technique for *in vitro* storage of hop germplasm, D) adventitious multiple shoot regenaration from internode explants in K-72/6/13 genotype, E) callus culture and F) cell suspension culture for polyphenol and flavonoid content analysis.

In vitro storage. Hop germplasm is traditionally preserved in the form of field collections in areas of cultivation (Revilla and Martínez, 2002). However, conservation of plants in natural conditions is costly in terms of land utilization, labour and risks of losses through environmental hazards and diseases. In addition, virus diseases can accumulate in a field collection and be transferred to additional sites by vegetative propagation (Reed et al., 2003). In vitro techniques have been found to be useful for ex situ conservation of a number of plant species, including meristem culture-derived virus-free germplasms (Fletcher et al., 1998). The storage longevity of in vitro collections depends on many important factors such as

explant type, culture medium composition and culture conditions (Aynalem et al., 2006; Faragó et al., 2008). At PPRC in Piešťany more than 100 accessions of 8 cultivars (Saaz, Bor, Sládek, Siřem, Aromat, Lučan, Zlatan and Premiant) of predominantly meristem culture-derived virus-free hop genotypes is maintained using an optimized *in vitro* storage system. This minimal growth method encompasses the use of full-strength MS-derived medium supplemented with 2% glucose, 7% agar, no plant growth regulators, and the use of Baby-food jars, non-defoliated nodal explants and lower light intensity for culturing single-nodal explants of hops at standard photoperiod and temperature conditions (16h light/8h dark, 25°C light/20°C dark). The subculture interval of cultures maintained in slow growth conditions ranges from 12 to 18 weeks. The cultures are regularly inspected for the potential occurrence of latent endocontaminating bacteria and the genetic stability of *in vitro* stored plant material was studied by molecular analysis of microsatellite markers (FARAGÓ *et al.*, 2008). As an alternative, the use of alginate bead encapsulation technique (Fig. 1C) for cryopreservation of hop germplasm was also attempted (results not published).

Adventitious shoot regeneration. Beside the importance in different tissue culture techniques, regeneration of plants in *in vitro* culture is also an essential prerequisite for generation of transgenic plants. However, only a few reports are available to date on the regeneration of plants from hop tissues other than meristems (Motegi, 1976; Rakouský and Matoušek, 1994; Batista *et al.*, 1996; Gurriarán *et al.*, 1999). We developed a highly efficient *in vitro* system for adventitious shoot regeneration from leaf and internode explants of 12 hop genotypes (Fig. 1D). The highest percentage of plant regeneration (52.7 %) was achieved in genotype Zlatan/1/2T from internode explants cultured on media supplemented with maltose as carbon source, and 2.0 $mg.l^{-1}$ BAP and 2.0 $mg.l^{-1}$ NAA. All the tested genotypes produced adventitious shoots with average frequencies of 1.4-17.4%. The regeneration frequency depended on the explant type (internode segment vs. leaf segment), auxin type (2,4-D vs. NAA), and C-source (glucose vs. maltose).

Callus culture. Culture of plant explants on nutrient media of certain composition results in dedifferentiation of cells of the explant and formation of callus tissue. These calli can be regenerable (organogenic or embryogenic) or non-regenerable. The second type of calli is frequently used for study of secondary metabolites biosynthesis of for production of biologically active secondary metabolites. In hop, callus cultures were attempted to study in aseptic conditions the infection process of the fungus *P. humuli* (Griffin and Coley-Smith, 1968), to regenerate plants through organogenesis (Motegi, 1976; Batista et al., 1996), and to development *in vitro* selection systems for novel sources of resistance to e.g. Verticillium wilt (Connell and Heale, 1986). We are interested in the possibility to establish a convenient *in vitro* system, based on induction of callogenesis and establishment of cell suspension culture in hops for chemical analyses of constituents of *in vitro* cultures and for potential production of interesting flavonoids in *in vitro* culture (Pšenáková et al., 2009). Our system is based on induction of callus formation from two types of explants (internodal segments and leaf segments isolated from *in vitro* grown shoot cultures) on media containing combinations of auxins (NAA or 2,4-D) and a cytokinin (BAP). The content of total polyphenols in this system depends on culture conditions and ranges from 76.6 to 158.5 $mg.l^{-1}$ of gallic acid equivalent (Pšenáková et al., 2009).

Cell suspension culture. The culture of plant cells has long been considered as a convenient tool to study biochemical aspects of plant secondary metabolism and on a large scale it can be a potential source of different secondary products. Suspension cultures are of special interest due to their high growth rate and short cycle of reproduction. The first cell suspension cultures of hops have been established by Robbins and Ratcliffe (1984) to study the intracellular distribution of phosphate in hop cells growing in media with elevated phosphate concentrations. We are interested in the potential of plant cell cultures to produce secondary metabolites, such as polyphenols, flavonoids and prenylated chalcones. As a model system we use cell suspension cultures established from stabilized callus cultures of different hop cultivars in liquid MS media containing different combinations of plant growth regulators. Cell suspension cultures are derived from two types of explants, stem segments (StS) and leaf segments (LS) and are cultured in different culture conditions. Our hop cell

suspensions show relatively high biomass accumulation (FW and DW), depending on the explant type, medium composition and culture conditions. The viability of cells (assessed as % of TTC-positive cells) depends on the concentration of pectinase added to liquid media to liberate cells from cell clumps and ranges from 60.9-90.6 % in media without pectinase to 36.2-65.4 % in media with 1000 μl pectinase.g^{-1} tissue FW. The content of total polyphenols and flavonoids depends mainly on the culture conditions (photoperiod/darkness) and explant source (StS/LS), and less on the culture medium composition and genotype. Using HPLC analysis, we were able to detect also production of xanthohumol in cell suspension cultures of hops. The highest production of xanthohumol was observed in cell suspension cultures established from leaf segment-derived calli in medium containing 1.0 mg.l^{-1} BAP in combination with 1.0 mg.l^{-1} 2,4-D without pectinase and cultured in dark conditions (Pšenáková et al., 2009).

Conclusion

Plant tissue culture is a promising tool to improve hop cultivars using biotechnological methods. A battery of different methods are available for biotechnologist for implementation into standard agricultural approaches, some of them are readily applicable in hop breeding (meristem culture, micropropagation, *in vitro* storage), whereas others need further studies and improvement to full applicability (genetic transformation, callus and cell suspension cultures).

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DEVELOPMENT OF TRIPLOID PLANTS OF HOP (HUMULUS LUPULUS L.)

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Abstract

Production of triploid forms is a method widely used in hop breeding. The studies have shown that triploids are more vigorous, higher yielding and seedless compared to its diploid counterparts. Polish aromatic variety Sybilla is characterized by both a good flavor and high content of alpha acids in cones around 6-8%. Moreover is one of the most promising varieties corresponding to both the demands of the brewing industry and the processors of hop. A major breeding goal has been to produce tetraploid plants as well as triploid aroma-type forms similar to Sybilla in respect to morphological traits.

In the process of breeding triploid plants (2n=3x=30), it is necessary to obtain tetraploids (2n=4x=40). Tetraploids are crossed with diploid (2n=2x=20) male individuals, resulting in triploid forms. In this study tetraploids have been induced by culturing apical buds of diploid cultivar Sybilla on media supplemented with the colchicine. Shoot apices isolated from *in vitro*-grown cultures, were placed in liquid medium MS containing different colchicine concentrations: 0.1%; 0,05%; 0,1% and incubated on an orbital shaker for 24h or 48h (Roy et al. 2001). After colchicine treatment, shoot apices were transferred to shoot multiplication medium (Kremheller 1989). After 14 days of post-treatment growth, the viability of plants was estimated. Four week old plants were placed on rooting medium. Flow cytometry was performed to detect ploidy level of plants. Colchicine-treated plants KG15/2010 and KG16/2010 which were proved to be tetraploid were crossed under controlled conditions with two diploid male parents D 8 and D 11 that were previously found in Poland during the Kaszuby district expedition. The obtained seeds were sown and plantlets were evaluated for the ploidy level by flow cytometry as well as mitotic chromosome count in somatic cells.

The negative effect of colchicine treatment on buds was reflected in their lethality. The highest lethality was observed after a 72h exposure with 0.1% colchicine. According to the results from flow cytometry eight of nine applied colchicine treatments generated tetraploids. The highest induction of tetraploids (28.57%) was achieved with the 0,1% colchicine for 48h while the lowest with 0,01% for 24h. Apart from generating tetraploids, this technique generated mixoploid plants (2n=2x=20/2n=4x=40). All crosses of tetraploid plants with diploid parent generated seeds. Flow cytometry revealed that from the total of 29 plants 27 were triploids. Cytological examination of chromosome number confirmed ploidy level of plants. The obtained triploids will be evaluated for their agronomic performance.

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PLOIDY AND SEX EXPRESSION IN HOP PLANTS

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In dioecious hop (*Humulus lupulus* L.) two sex chromosomes are responsible for the tentative XY mechanism (Shephard et al., 2000). Occasionally, hermaphroditic hop plants, carrying both flower types on the same plant, spontaneously occur. They are often of predominantly male phenotype, but may also be predominantly female plants or plants with an approximately 50:50 ratio of male and female flowers (Neve, 1961; Shephard, 2000). Monoecious expression of sex in hop is most likely due to chromosomal number disorders of either triploid, tetraploid or aneuploid origin (Haunold, 1971; Shephard et al., 2000). In our study, 58 monoecious hop plants, progenies of various crosses of diploid hop parents, were classified into six categories according to their level of expression of intersexuality and analyzed by flow cytometry to estimate ploidy level.

Young green leaves of field-grown monoecious, diploid (cvs. Savinjski golding, Wye Target, Magnum and male 2/1), triploid (cv. Celeia) and tetraploid (cv. Apolon) dioecious hop plants were used for flow cytometry analysis. During flowering, monoecious plants were categorized into six classes, according to their level of expression of female and/or male flowers (Neve, 1961).

In total, 41% of monoecious plants were triploids, while the remainder had a diploid chromosome number. Since triploid monoecious plants originate from diploid parents, an effect of unreduced gametes from either male or female parent is suspected. The only one plant with just male inflorescences was diploid. All of the plants (10) with only female inflorescences had a diploid chromosome number. Twenty-two morphologically predominantly female plants (Fm phenotype) were diploids, whereas all of the 24 predominantly male plants (Mf phenotype) were triploids and all of the triploids observed were of Mf phenotype. The only plant with an approximately equal share of male and female flowers (FM phenotype) was a diploid. Not a single almost entirely male plant with some hermaphrodite terminal flowers (Mh phenotype) was detected.

The predominantly male phenotype with a few female cones was observed to be connected with a triploid chromosome number in monoecious hop plants. On the other hand, predominantly female plants with some male flowers were diploids.

Keywords: hop, sex expression, monoecious plant

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III. Session:

MOLECULAR INVESTIGATIONS ON HOPS

A DIVERSITY ARRAYS TECHNOLOGY (DART) PLATFORM FOR HIGH-THROUGHPUT GENOTYPING OF HOP (*HUMULUS LUPULUS* L.)

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Abstract

Molecular marker technology has an important role in the future of hop breeding. This study examined the applicability of diversity arrays technology (DArT) for high-throughput, cost-effective genotyping of hop. A total of 730 high quality polymorphic markers were identified from 92 hop accessions. A genetic diversity analysis was conducted to validate the robustness of DArT in a hop system. The hop accessions separated into genetically distinct North American and European groupings, with hybrids between the two groups clearly distinguishable. Levels of genetic diversity were similar in the North American and European groups, but higher in the hybrid group, with a total of 0.317 for all accessions. This analysis concurred with the current understanding of hop phylogenetics and diversity and demonstrates DArT to be a highly effective marker technology for hop.

Keywords: hops, diversity arrays technology (DArT), molecular markers, phylogenetics

Introduction

The development of superior hop cultivars relies on the effective utilisation of genetic diversity. It is, therefore, necessary to understand the scope of hop genetic diversity available for breeding. Studies indicate that the major hop cultivars are of narrow genetic origin and have limited genetic variability between them (Jakše et al. 2001; Murakami et al. 2006). Morphological studies offered the earliest assessments of genetic variation in hop (Davis 1957), later followed by biochemical markers (Stevens et al. 2000). In recent years molecular technologies have allowed a more direct and sophisticated approach. The use of molecular markers has greatly improved the understanding of hop genetic variation, however these technologies remain costly and low-throughput due to intensive and laborious process. Diversity arrays technology (DArT) is a recent marker technology, invented specifically to overcome these barriers (Kilian et al. 2005). Here we present the effectiveness of DArT in a hop system, in terms of the capacity to generate quality polymorphic markers; and the accuracy and resolution of a genetic diversity analysis, in comparison with the current understanding of molecular variation and phylogenetics.

Methods

A total of 92 hop accessions were used in this study. These accessions comprised 32 wild and 60 cultivated accessions, both historical and current commercial cultivars, as well as examples of four of the five taxonomic varieties of *H. lupulus*: var. *lupulus*, var. *lupuloides*,

var. *pubescens* and var. *neomexicanus*. Samples of the accessions were sourced from Europe, North America and Australia from collections held by Wye Hops, John I. Haas Inc, USDA-ARS National Genetic Resources Programme Germplasm Resources Information Network (GRIN), Hop Products Australia and the Slovenian Institute of Hop Research and Brewing (see author affiliations for locations).

DNA was extracted using the CTAB extraction protocol to obtain 15 µL of DNA at a concentration of 100 ng/µL for each accession, as described in Howard et al. (2011). DArT markers were developed using the standard DArT protocol, as described in Howard et al. (2011), using the *PstI/BstNI* restriction enzyme combination. A library of 6,144 clones was constructed. Only high quality markers (*P*-value>77%, call rate>85% and 100% allele-calling consistency across replicated accessions) were selected for analysis. Polymorphism information content (PIC) was also calculated.

An analysis of phylogenetics and genetic diversity was performed as described in Howard et al. (2011). A pairwise genetic distance matrix was computed, and used to produce an unrooted Unweighted Pair Group Method with Algorithmic Mean (UPGMA) dendrogram. Partitioning of accessions into genetic groups was investigated by Principal Co-ordinates Analysis (PCoA). Analysis of Molecular Variance (AMOVA) was used to examine the distribution of genetic variation within the accessions included in the study. Average genetic diversity over loci (π_n) and pairwise genetic distances among groups (*F*-statistics) were calculated. Population substructuring of the accessions was investigated using STRUCTURE version 2.3.1, a model-based clustering method employing a Bayesian algorithm, with the Δ*K* statistic used to determine the most likely number of groups (*K*).

Results

DArT identified 730 high quality polymorphic markers from the 92 hop accessions, at a polymorphism rate of 11.9%. These markers were identified with highly stringent quality criteria, including PIC (0.335), scoring reproducibility (99.97%), call rate (97.58%) and *P*-value (89.90%).

In an analysis of phylogenetics and genetic diversity, three distinct groups amongst the hop accessions were identified: (1) wild North American hops and all accessions of taxonomic varieties *lupuloides*, *pubescens* and *neomexicanus;* (2) wild European hops and cultivars solely of European genetic origin; and (3) cultivars derived from hybridisation between European and North American hops. The North American group was widely separated from the European and hybrid groups in both the PCoA and UPGMA dendrogram. The hybrid group was genetically intermediate between the North American and European groups, but more genetically similar to the European group. The PCoA indicated that the primary factor separating all accessions was the major disjunction between the North American and European groups (*C1 and C2* explaining 87% of total variance). Confirming this, significant partitioning of genetic variation was detected between the groups (AMOVA), with 75.2% of variation existing between the groups. Pairwise F_{st} values provided further validation that groups were significantly differentiated (P<0.001), and reflected patterns observed in the PCoA and UPGMA dendrogram, with the greatest genetic differentiation detected between North American and European accessions (F_{st}=0.903); less differentiation between North American and hybrid accessions (F_{st}=0.770); and least genetic differentiation between European and hybrid accessions (F_{st}=0.485). Population substructuring of hop accessions was modelled using STRUCTURE. Maximum Δ*K* was found to be *K*=2, indicating two groups making genetic contributions. A plot of membership coefficients determined by STRUCTURE established the two genetic sources as the North American and European groups. Hybrids had a combination of North American and European genetic ancestry, but greater European contribution. Group partitioning was analogous to the PCoA and UPGMA dendrogram. The level of genetic diversity was determined through π_n, and had a total of 0.317 for the selected hop accessions. Levels of genetic diversity did not differ significantly between North American and European groups (π_n=0.081$\pm$0.012 and

π_n=0.069±0.006, respectively), but were significantly higher in the hybrid group (π_n=0.168±0.016, P<0.001).

Discussion

This study utilised DArT for hop genotyping. A total of 730 polymorphic markers were identified from 92 hop accessions. The markers identified were found to be of high quality, and comparable with DArT markers identified in other species, as assessed through polymorphism rate and the quality parameters PIC, reproducibility, call-rate and *P*-value (Table 1). However, both polymorphism rate and PIC are comparatively lower to values determined in hop using other marker systems (Table 1). This could be attributed to the tendency of DArT to target low-copy genic sequences (Heller-Uszynska et al. 2010), which are found in much less abundance in the genome compared to the repetitive, non-genic fractions of the genome from which the alternate markers are derived. Alternately, the lower polymorphism rate and PIC values could be attributed to the genotypes selected for the study, as the identification of polymorphic markers is dependent upon the scope of diversity of genotypes used to develop the DArT array.

Table 1. Mean scores for quality parameters for the 730 polymorphic DArT markers identified in this study compared with scores for other species employing DArT and compared with other hop studies employing alternate marker systems.

Quality parameter	This study	Examples from other DArT studies	Examples from other hop studies
Polymorphism rate	11.90%	10.40% wheat (Akbari et al. 2006) 14.60% cassava (Xia et al. 2005) 7.00% sugarcane (Heller-Uszynska et al. 2010)	59.50% AFLP (Townsend and Henning 2009) 43.50% AFLP (Seefelder et al. 2000) 57.60% AFLP (Patzak 2001) 57.90% ISSR (Danilova et al. 2003) 32.60% ISSR (Patzak 2001) 38.60% RAPD (Šuštar-Vozlič and Javornik 1999) 42.30% RAPD (Patzak 2001) 71.00% STS (Patzak 2001)
PIC	0.34	0.34 pigeonpea (Yang et al. 2006) 0.31 wheat (Akbari et al. 2006) 0.38 barley (Wenzl et al. 2004)	0.61 SSR (Stajner et al. 2008) 0.64 Microsatellite (Jakše et al. 2004) 0.38 Microsatellite (Jakše et al. 2010)
Reproducibility	99.97%	99.80% barley (Wenzl et al. 2004) 97.71% *Asplenium* (James et al. 2008)	
Call-rate	97.58%	92.50% sugarcane (Heller-Uszynska et al. 2010) 95.00% barley (Wenzl et al. 2004) 99.20% wheat (Akbari et al. 2006)	
P-value	89.90%	81.40% banana (Risterucci et al. 2009) 80.68% sugarcane (Heller-Uszynska et al. 2010)	

An analysis of phylogenetics and genetic diversity was conducted to validate the accuracy and resolution of DArT in a hop system. The genetic relationships determined through DArT analysis concurred with the current understanding gained using alternate marker systems, such as AFLP, RAPD, microsatellites, ISSR and STS (see Howard et al. 2011 for a comprehensive list of studies). The consensus amongst these previous phylogenetic investigations is that there are two primary genetic groups of hop: European (including wild and cultivated material) and North American (wild material only). All statistical analyses conducted in this study, including PCoA, UPGMA dendrogram, AMOVA and STRUCTURE, separated the hop accessions into genetically differentiated North American and European groupings, with hybrids between the two groups clearly detectable. Some previous studies, with a more comprehensive selection of genotypes, were able to resolve genetic relationships in hops in greater detail, with emergent groups corresponding to factors such as geographical origin, breeding history and chemical content. This study was a test of the robustness of DArT, rather than a comprehensive analysis of hop genetic structure and diversity, and so did not capture the full scope of genotypes required for such an analysis. The consistency across the results obtained in this study, and with the results obtained in previous studies does, however, demonstrate the utility of DArT markers for analysis of genetic structure and diversity in hop. Levels of genetic diversity were found to be similar in the North American and European groupings, but higher in the hybrid group, with a total value of 0.317 amongst all accessions. This value is similar to the levels of genetic diversity found in other hop studies (Jakše et al. 2004; Peredo et al. 2010), determined through alternate means.

In conclusion DArT can be considered an effective marker technology for hop, with the capacity to detect and score hundreds of polymorphisms. It was found to be both time and cost-efficient, producing markers of high quality. It is anticipated that these markers will be a valuable resource for numerous applications in hop breeding and genetics studies, such as mapping, marker-assisted selection, genetic identity testing, guidance in the maintenance of genetic diversity and the directed breeding of superior cultivars.

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MOLECULAR MAPPING OF QTLS FOR XANTHOHUMOL AND DMX CONTENTS IN HOP

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Abstract

Recently, a medicinal usage of hop metabolic compounds is in raising interest, mainly prenylflavonoids: desmethylxanthohumol (DMX) and xanthohumol (X). Quantitative trait loci (QTL) mapping is good tool for founding of reliable molecular markers for prospective breeding. We used STS, EST-SSR and AFLP methods for analysis of 116 individual female F1 hop genotypes. A total of 117 markers were used to construct the genetic linkage map for female parent Taurus. In QTL analysis, 18 putative main QTLs for xanthohumol and 12 putative main QTLs for DMX were detected on all seven LGs. Our analysis confirmed negative correlation between xanthohumol and DMX caused by O-methyltransferase (OMT1) methylation.

Keywords: hop, *Humulus lupulus* L., AFLP, EST-SSR, STS, SSR, linkage analysis, QTL mapping, polyphenols

Introduction

Hop (*Humulus lupulus* L.) is a dioecious perennial climbing plant. Only female plants are cultivated for commercial use, mainly in the brewing industry and to a smaller extent for pharmaceutical purposes. Female inflorescences, referred to as cones, contain hop bitter resins, essential oils, polyphenols and tannins (Neve 1991). Hop bitter acids are the most important beer bittering agents, but they are also interesting from a medicinal perspective in view of their wide variety of reported biological activities, including antimicrobial, anti-inflammatory, and cancer chemopreventive activities (Van Cleemput et al, 2009).

Next medicinal perspective compound are prenylflavonoids: desmethylxanthohumol (DMX) and xanthohumol (X), the most abundant in fresh and properly preserved hops (Stevens et al., 1999). Hop prenylchalcones show a broad spectrum of inhibition mechanisms at the initiation, promotion and progression stages of carcinogenesis (Gerhäuser et al., 2002) and they are isomerized to isoxanthohumol (IX) and a mixture of the prenylflavanones, 8-prenylnaringenin (8-PN), and 6-prenylnaringenin (6-PN), respectively, in the brew kettle (Milligan et al, 1999).

There are known several structural genes of prenylflavonoids biosynthesis (Matoušek et al., 2002; Tsurumaru et al., 2010), when hop O-methyltransferase (OMT1) performs the final reaction step (DMX is methylated to X) in lupulin glands (Nagel et al., 2008). The expression of structural genes is specifically regulated by several transcription factors: MYB, bHLH and bZIP (Matoušek et al., 2007; 2010).The content of prenylflavonoids is controlled by many genes and it is typical representative of quantitative traits. The developed molecular markers associated with prenylflavonoids accumulation trait could help in the selection of superior progenies in breeding programs. Early stage selection is highly desirable, especially in perennial species, and quantitative trait loci (QTL) mapping is good tool for this purpose. This method has already been used in hop to identify molecular markers for several quantitative traits (Seefelder et al. 2000; Koie et al. 2005; Čerenak et al. 2006)

In our present study, we constructed female genetic linkage map and detected QTLs of desmethylxanthohumol and xanthohumol contents, which could be used for marker-assisted selection in future.

Materials and Methods

Plant material and DNA isolation

A total of 116 individual female hop genotypes from F1 progeny of crossing: Taurus x Sm06 H14 11/167 were used for experiments. Hop plants were cultivated in nursery of the Hop Research Institute in Žatec two years. Hop resins and polyphenols were estimated from dry cones according to the EBC 7.7. method (1997) by liquid chromatography (HPLC) on the column Nucleosil RP C_{18} (Macherey-Nagel, Germany, 5 μm, 250 x 4 mm) using a SHIMADZU LC 20A chromatograph (Shimadzu, Japan) with diode array detectors (DAD) according to Krofta (2003). DNA was isolated from the young leaves of all samples according to Patzak (2001) and it was used for molecular genetic analyses.

Molecular DNA analyses

For molecular analyses, we used sixteen SSR (Hadonou et al., 2004; Jakse et al., 2002; 2008; Stajner et al., 2005), thirty-two STS and EST-SSR (Patzak et al., 2007; Patzak and Matoušek, 2011) loci and seven AFLP primer combinations (Patzak, 2001; 2003). In a typical PCR reaction (*Taq* PCR master mix kit, Qiagen, Hilden, FRG) we used the following amplification conditions: 2 min at 94 °C, 35 cycles (30 s at 94 °C; 60 s at 54 °C, 90 s at 72 °C); 10 min at 72 °C. PCR was performed on TGradient thermocycler (Biometra, Goettingen, FRG). AFLP reactions were carried out according to Patzak (2001; 2003). Amplification products were resolved via 5% denaturing (8M urea) polyacrylamide gel vertical electrophoresis and visualized by silver-staining (Patzak, 2001). The products were scored for the presence or absence in each sample, based on size measured with 20 bp DNA Marker (Bio-Rad, Hercules, CA, USA).

Linkage and QTL analysis

STATISTICA 8.0 CZ (StatSoft, Tulsa, OK, USA) was used for evaluation of chemical analyses data by basic statistic functions. Dominant polymorphic molecular markers were used to construct the genetic linkage map for female parent, using RIL Sib-mating strategy. Linkage analysis was performed with Multipoint v2.1 (MultiQTL, Haifa, Israel). The Kosambi mapping function was used to transform the recombination frequency to genetic distances (cM). MultiQTL v2.6 (MultiQTL, Haifa, Israel) was used to identify and locate QTLs associated with xanthohumol, desmethylxanthohumol (DMX) and other bitter acids compounds. The association between phenotype and genotype was investigated by using marker analysis. The percent of variance explained by each QTL was calculated.

Results

In our work, we analysed 116 individual female hop genotypes of F1 progeny by SSR, STS, EST-SSR and AFLP methods. From these analyses, 59 polymorphic STS and EST-SSR markers, 35 polymorphic SSR markers and 32 polymorphic AFLP markers were scored with segregation ratios similar to 3:1 or 1:1 in F1 progeny. A total of 117 markers were used to construct the genetic linkage map for female parent Taurus. Constructed genetic map forming 25 linkage groups (LGs) for recombination frequency lower than 0.3. We merged these linkage groups to seven more consistent LGs with 2924.1 cM of total map distance for QTL analysis (not shown).

The bitter acids and polyphenols contents in hop cones were measured by chemical HPLC analyses in all used F1 progeny plants (not shown). The frequencies of observed polyphenols (X and DMX) contents showed a normal distribution. The frequencies of observed bitter acids contents showed a population distortion with 82 high bitter acids plants and 34 low acids plants (2.33:1 ratio). Good correlations, with correlation coefficients 0.969 for alpha – beta bitter acids and 0.731 for cohumulone - colupulone, were obtained. None correlations for bitter acids – polyphenols and X – DMX were obtained.

In QTL analysis, 18 putative main QTLs for xanthohumol (10 positive and 8 negative) and 12 putative main QTLs for DMX (7 positive and 5 negative) were detected on seven LGs by single locus analysis (Table 1). Six from all QTLs were common to both polyphenols

contents. All these QTLs were detected based on the LOD scores. Maximum explained phenotypic variation of QTL marker was 28.035% for X and 34.034% for DMX, respectively (Table 1).

Discussion

The different marker systems (SSR, STS, EST-SSR and AFLP) were used for construction of a meaningful map. Even though this coverage, we didn‘t achieve identical number of linkage groups with number of hop chromosomes (n=10). Nevertheless, this map was successfully used for QTL analysis. The mapping hard depends on parent genotypes, when we used cultivar Taurus as mother component. Previous published hop female maps were done on different genotypes: Wye Target (Seefelder et al. 2000), Chinook (Koie et al. 2005) and Magnum (Čerenak et al. 2006). Three chalcone synthase-like genes (*vps*, *chs3*, and *chs4*) were mapped together on one linkage group (LG2) similar to Magnum female map (Čerenak et al. 2006). Several identical SSR and AFLP molecular markers were mapped similarly, but several weren't. In our previous work (Patzak et al., 2007), we also found some crossovers between German cultivars Magnum and Taurus for SSR and STS markers.

QTL analysis confirmed that content of prenylflavonoids is controlled by many genes with regulation network and no major genes. Phenotypic effect of 24 putative QTL molecular markers, identified on all seven LGs, varied from -9.506 to -22.5 for content decreasing and from 10.897 to 34.043 for content increasing, respectively. Similar phenotypic variation was accounted for alpha-acid content in hop (Čerenak et al. 2006; Koie et al. 2005). For bitter acids content, we found two most reliable QTL markers: allele for geranylpyrophosphate synthase - small subunit (GPPS-SSU) with 35% positive phenotypic effect on LG3 and allele for 2-C-methyl-D-erythritol 2,4-cyclodiphosphate synthase (CMPS_220) with -35% negative phenotypic effect on LG5 (not shown). *gpps* is involved in metabolic pathway of terpene synthesis (Wang and Dixon, 2009), but it can be also involved in bitter acids synthesis as prenyl transferase role same as aIPT (Tsurumaru et al., 2010). *cmps* is involved in non-mevalonate (MVA) pathway of isopentenyl diphosphates synthesis, which is a main substrate of all biosynthesis in hop cones (Nagel et al., 2008). The substrate competition and joined branched biosynthetic pathways influence the content of secondary metabolites in hop cone and lupulin glands. Negative correlation between xanthohumol and DMX due to *omt*1 (Nagel et al., 2008) was confirmed by two close QTL markers for allele of *omt*1 (OMT1_300) and unknown gene (CTT3_380). Unfortunately, these markers were a typical for Taurus genome, when we found OMT1_300 in 14 and CTT3 380 only in four genotypes from 80 actual world cultivars. The most potential AFLP marker EACGMCAT_254 for both prenylflavonoids (Table 1) will be sequence characterized in the future. We hope, that reported QTL markers are promising for further use in marker-assisted selection breeding.

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Table 1. QTL analysis of statistically significant molecular markers for **a)** xanthohumol and **b)** DMX contents by MultiQTL v2.6 (MultiQTL, Haifa, Israel).

a)

Linkage group	Marker	Type	LOD	Average content	Phenotypic effect	%
LG7	EACCMCTC_155	AFLP	0.726	0.716	0.158	22.067
	CTT3_370	EST-SSR	1.786	0.841	-0.161	-19.144
	EACGMTCA_160	AFLP	1.695	0.789	-0.114	-14.449
	EM19_120	SSR	0.774	0.809	-0.091	-11.249
	HLGA3_140	SSR	1.246	0.795	-0.1	-12.579
	CMPS_230	EST-SSR	1.355	0.745	0.13	17.45
	HLAGA6_194	SSR	0.914	0.763	0.095	12.451
LG1	LAR1_160	EST-SSR	1.057	0.765	0.099	12.941
	EACCMCTC_100	AFLP	0.838	0.764	0.091	11.911
LG2	CGG2_250	EST-SSR	1.626	0.798	0.114	14.286
LG3	HLAGA6_162	SSR	1.491	0.784	-0.107	-13.648
LG4	CTT3_380	EST-SSR	7.163	0.799	0.224	28.035
	OMT1_300	STS	2.757	0.772	0.146	18.912
LG5	EACGMCAT_254	AFLP	1.051	0.887	0.222	25.029
	TAA3_310	EST-SSR	1.937	0.777	0.123	15.83
LG6	GA2_250	EST-SSR	1.883	0.795	-0.121	-15.22
	NBDP_220	STS	1.261	0.796	-0.101	-12.688
	CGG2_260	EST-SSR	1.734	0.799	-0.118	-14.769

b)

Linkage group	Marker	Type	LOD	Average content	Phenotypic effect	%
LG7	EACGMTCA_160	AFLP	2.673	0.08	-0.018	-22.5
	EACCMCAT_240	AFLP	1.346	0.076	0.015	19.737
	HLGT4_195	SSR	1.116	0.076	0.014	18.421
	EM30_210	SSR	0.694	0.077	0.011	14.286
LG1	EACCMCTC_100	AFLP	0.454	0.078	0.0085	10.897
LG2	WRKY75_205	EST-SSR	0.819	0.078	-0.01	-12.821
LG3	GPPS_SSU	STS	0.844	0.08	0.01	12.5
LG4	CTT3_380	EST-SSR	0.811	0.079	-0.01	-12.658
	OMT1_300	STS	1.118	0.081	-0.012	-14.815
LG5	EACGMCAT_254	AFLP	1.937	0.094	0.032	34.043
	TAA3_310	EST-SSR	1.274	0.079	0.013	16.456
LG6	HLACA3_215	SSR	0.357	0.081	-0.0077	-9.506

QTL MAPPING OF VERTICILLIUM RESISTANCE AND YIELD TRAITS IN HOP

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Abstract

The effects of quantitative trait loci (QTLs) on quality traits (yield, alpha-acid content) and verticillium wilt resistance in hop (*Humulus lupulus* L.) were studied using amplified fragment length polymorphism (AFLP) and microsatellite markers (SSRs). A genetic linkage map was constructed from a mapping population consisting of 144 progeny from a double pseudo-testcross. A total of 205 markers were located on the 11 linkage groups (LGs), with 2 LGs located separately on the maternal and paternal maps, covering a total map length of 615 cM. The crossing family segregated quantitatively for alpha-acid content, yield traits and verticillium resistance in 2008 and 2009. Seven putative QTLs were identified on both maps.

The reported genetic map provides a good tool for the further development of a marker-assisted selection programme in hop.

Keywords: *Humulus lupulus* L., alpha acid content, yield, verticillium resistance, genetic mapping, quantitative trait loci, QTLs

Introduction

The hop breeding program at the Slovenian Institute of Hop Research and Brewing (SIHRB) has a tradition of more than 50 years, and 12 hop varieties have been released. Today, 95 % of Slovene hop fields are planted with Slovene varieties, of which Aurora represents more than 60 %. The main purpose is to develop hop varieties with improved quantity and quality of yield and resistance against fungal diseases and pests, combining classical and molecular approaches. Since 1997, when an outbreak of the lethal form of Verticillium wilt (*Verticillium albo-atrum* Reinke & Berthold) occurred (Radišek et al., 2006), breeding for resistance to wilt disease has been included as a new goal and is today one of the most intensive parts of the breeding program. As a result, the majority of new breeding lines that are currently subject to official variety tests have inbuilt high verticillium wilt resistance.

The aim of the research was to identify locations in the genome involved in the response of hop plant to verticillium wilt infection. Identification of new QTLs involved in expressing alpha acid content and yield was correlated with previously reported QTLs determined on a different population (Cerenak et al., 2009).

Methods

The F_1 full-sib family was used for genetic analysis correlated especially with verticillium wilt resistance. Our mapping population was obtained by crossing the resistant English variety Wye Target with the Slovene breeding line 2/1, which is susceptible to verticillium wilt. The analysis included 144 full-sib genotypes and the two parents. The progenies displayed clear morphological variations in many traits, including vigour, alpha-acid content and verticillium resistance.

Total genomic DNA was extracted from young leaves using a modified CTAB method according to Kump and Javornik (1996). The AFLP protocol was performed according to Vos et al. (1995), with modifications of the method adjusted for fluorescent analysis (Cerenak et al., 2006). The progenies of the family were selectively amplified with 18 *Eco*RI/*Mse*I and 18 *Pst*I/*Mse*I primer pair combinations. A variety of microsatellite and STS markers used for the

map construction has been developed by our group and are partly published (Jakše et al., 2002, Štajner et al., 2005, Jakše et al., 2008, Jakše et al., 2010); some were also taken from the literature (Brady et al., 1996, Hadanou et al., 2004). Altogether, segregation data for 128 co-dominant markers and 171 AFLP markers were used in the map construction.

The content of alpha-acids was determined by the lead conductance value (LCV) method (Analytica EBC 1998) and the yield was measured in 2008 and 2009.

Verticillium wilt resistance was determined by artificial infections under the controlled conditions of a growing chamber between 2005 and 2009. Twelve plants per genotype were inoculated by inoculum of *V. albo-atrum* (lethal pathotype PV1, genotype PG2). Plants were assessed at weekly intervals on a 0–5 scale according to the proportion of foliage affected by wilt symptoms. At the end of assessment of external symptoms, the vascular tissue of plants was examined in cross-sections of stems and roots. For pathogen re-isolation and infection conformation, the vascular tissue was placed on potato dextrose agar (PDA) and species identification of the pathogen was checked by light microscopy.

Linkage analysis was carried out using the JoinMap® 4.0 program (Van Ooijen, 2006). The map was constructed using a LOD of 6.0 for the grouping of markers. The Kosambi mapping function was used to convert recombination data to map distances. MapQTL® 5 (Van Ooijen, 2004) was used to identify and locate QTLs associated with each phenotypic trait assessed in each environment by performing the Kruskal-Wallis non-parametric test, as well as both interval mapping (Lander and Botstein 1989) and multiple-QTL mapping (MQM; Jansen and Stam 1994). In the regions of putative QTLs, the markers with the highest LOD values were taken as co-factors. A backward elimination procedure was used to select co-factors significantly associated with each trait at $P < 0.02$ and used in the MQM. Genome-wide threshold values ($P < 0.05$) for declaring the presence of QTL were estimated from 1,000 permutations of each phenotypic trait (Churchill and Doerge, 1994). Individual parental effects and the interaction effect of each putative QTL were calculated according to Knott et al. (1997). The 1-LOD and 2-LOD support intervals were determined for each LOD peak (Conneally et al. 1985). Maps were drawn using MapChart version 2.2 software.

Results and Discussion

Both types of polymorphic markers (AFLPs, SSRs) were tested for their inheritance pattern in the mapping population. Segregation ratios were tested using the chi-square test and, as suspected, a large number of markers did not fit the expected Mendelian ratios ($p<0.01$). Nevertheless, 205 out of 299 markers were successfully mapped on an integral map. Eighty-two markers were common to both parents.

A total of 205 markers were placed on the integral map, forming 12 major linkage groups and 3 doublets, which are assumed to be part of other groups, defining 615 cM of the total map distance.

QTL analyses were carried out in 2008 and 2009 using data for *Verticillium* resistance, alpha acid content, harvest index and dry cone weight. A total of seven putative QTLs were detected on both female and male maps with LOD values larger than the threshold obtained by permutations. The QTL for *Verticillium* resistance was detected on LG03 of both maps. In 2009, the QTL for alpha acid content was identified on LG01 using both maps, while the QTL associated with harvest index was localized in 2008 on LG03 of both maps. The putative QTL controlling dry cone weight was detected in 2008 on LG03 of the female map.

Due to the number of common SSR markers, correspodence was established between the major linkage groups obtained in the current study and those published by Cerenak et al. (2009) for the cross between Hallertauer Magnum and 2/1. Wye-3 thus corresponds to Magnum-1, on which QTLs for harvest index (*hi1-02, hi1-03, hi3-03, hi1-04, hi1-05, hi1-06*) and dry cone weight (*dcw1-02, dcw1-03, dcw1-04, dcw-2-04, dcw1-05, dcw1-06*) were previously reported (Cerenak et al., 2009). Similarly, Wye-1 corresponds to Magnum-3, in which the QTLs for alpha-acid content were detected (*α1-03, α3-04, α1-05, α1-06*).

With the development of the reported hop map, QTLs associated with verticillium wilt resistance were detected for the first time and homology between the previously reported genetic map (Cerenak et al., 2009) was established, providing a practical tool for further research.

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ISOLATION AND CHARACTERIZATION OF *VERTICILLIUM* RESISTANCE GENE HOMOLOGS IN HOP

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Abstract

Verticillium wilt is an important disease of hops, often resulting in major yield losses in regions in which it occurs. In addition, the disease exhibits different levels of severity, being described as mild or lethal. Genetic sources of *Verticillium* tolerance are known for several plant species, but the mechanism underlying plant resistance to the fungus has been best described in tomato, in which resistance is conferred by the *Ve* locus. In hop, the most *Verticillium* resistant cultivar is the British 'Wye Target', presumably an inherited trait from a wild US progenitor. A short part of the hop *Ve*-like sequence was amplified by degenerate primers based on the tomato sequence. Using a TAIL-PCR approach, we managed to recover complete sequences of two similar groups. Both sequences exhibit structural properties of *Ve* genes from tomato. Efforts are under way to isolate the functional genes from resistant cultivars and wild sources and conduct functional tests of the isolated genes in a model system.

Keywords: Verticillium, plant resistance genes, TAIL-PCR, gene isolation

Introduction

Verticillium wilt is an important fungal disease of hop, often resulting in major yield losses in regions in which it occurs. In addition, the disease exhibits different levels of severity, described as mild or lethal (Radisek et. al, 2006). *Verticillium* species also form resilient resting structures, making them difficult to eradicate from infested soil, which means that planting resistant cultivars is the most viable strategy to sustain production in affected areas. Genetic sources of *Verticillium* resistance are known for several plant species, but the mechanism underlying plant resistance to the fungus has been described only in tomato (*Solanum lycopersicum*), in which resistance is conferred by the *Ve* locus (Fradin et al., 2009). This locus contains two similar genes, SlVe1 and SlVe2, which code for cell surface receptors.

The most *Verticillium* resistant hop cultivar is the British 'Wye Target', its resistance presumably inherited from wild germplasm originating from a US progenitor (Darby, 2001). This cultivar exhibits a high degree of resistance even to the lethal strain of *Verticillium albo-atrum* (Radisek et al., 2006).

Southern hybridization analysis using the tomato Ve sequence as a probe suggested a low copy number of putative Ve gene homologs in hop genome (Jakse, 2009). The aim of the present study was to employ available hop EST sequences, deposited in PlantGDB, to identify and sequence complete coding regions of putative hop homologs of the tomato Ve1 gene. A similar approach has recently been used to retrieve the sequence of a SlVe homolog in mint (Vining and Davis, 2009).

Methods

A tBLASTx search of hop ESTs was performed with SlVe1 as the query sequence, which yielded a single hit with 50% amino acid similarity to SlVe1. A tBLASTx search of the entire nucleotide sequence database was then employed to retrieve various plant homologs of the sequence and an alignment of their nucleotide sequences was performed with ClustalX.

Several degenerate primers based on the alignment and several gene specific primers based on the EST sequence were constructed. Following successful PCR amplification with DNA from 'Wye Target', cloning of products yielded a sequence with a high degree of similarity to both SlVe1 and the hop EST.

A number of rounds of Thermal Assymetric Interlaced PCR (TAIL-PCR; Liu et. al, 1995) based on the sequence of the cloned PCR product was conducted. After each TAIL-PCR, PCR products from various bands cut from gel were cloned. Several clones originating from each band were sequenced. Sequence analyses were performed in the CodonCode Aligner, after which new primers based on the sequences retrieved in the previous round of TAIL-PCR were constructed, followed by another round of TAIL-PCR. This cycle was repeated until the entire coding regions of the genes and a short stretch of both upstream and downstream regions had been retrieved.

Primers based on HlVe2 upstream and downstream regions allowed successful amplification of a PCR product of expected size, which was cloned. Several resulting clones were sequenced.

Results and discussion

Two distinct consensus sequences of putative hop Ve-like genes were retrieved and named HlVe1 and HlVe2. The consensus sequences of HlVe1 and HlVe2 coding regions show 82% nucleotide sequence identity and 73% amino acid identity (82% positives), with gaps accounting for 3% of the sequence. Both HlVe1 and HlVe2 share roughly 50% protein sequence identity (70% positives) with SlVe1.

We managed to clone the entire coding regions and some upstream and downstream sequence of 3 variants of HlVe2. Cloning different variants of HlVe1 has as yet proved unsuccessful. However, the distribution of SNPs/indels in various cloned sequences derived from single rounds of TAIL-PCR allow us to infer that at least three distinct variants of this group of sequences exist.

This was our first effort to identify and clone candidate resistance genes putatively involved in hop resistance to *Verticillium*. Current activities include cloning the complete coding regions of HlVe1 variants and developing gene markers for mapping the Ve-like loci onto an existing genetic map of 'Wye Target'. We also intend to assess the genetic variability of HlVe1 and HlVe2 between tolerant and susceptible cultivars and in wild hop germplasm.

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TRANSCRIPTION FACTOR *PAP1/ATMYB75* REGULATES FLAVONOID PRODUCTION IN TRANSGENIC HOP (*HUMULUS LUPULUS* L.)

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Abstract

Biologically active flavonoids, like xanthohumol, are produced in hop female inflorescences. Metabolic engineering by modifying biosynthetic pathways would increase the yield in hop. For this purpose, the regulatory factor *pap1*/*Atmyb75* from *Arabidopsis thaliana* L. was introduced into hop plants cv. Tettnanger by *Agrobacterium*-mediated genetic transformation. Twenty kanamycin-resistant transgenic plants were obtained. The *pap1*/*Atmyb75* gene was stably incorporated and expressed in the hop genome. In comparison to wild type plants the color of female cones of transgenic plants was reddish to pink. By quantitative PCR it was demonstrated that in transgenic hop plants the rate of expression of chalcone synthase (*chs*), chalcone isomerase (*chi*), flavonoid 3´hydroxylase (*f3'h*) and valerophenone synthase (*vps*) was modified. Chemical analysis using LC-MS and UV measurements of transgenic hop plants revealed higher levels of anthocyanins, rutin and isoquercitin in cones in comparison to wild type plants.

Keywords: Flavonoid biosynthesis, *Humulus lupulus*, plant transcriptional factor, *pap1*/*Atmyb75*, anthocyanins, quercitin

Introduction

More than 1000 compounds have been identified in hop, including volatile oils, α-acids, β-acids and prenylated flavonoids (Chadwick et al. 2006). Some flavonoids like alpha and beta acids play an important role as flavoring compounds in beer brewing. Others like xanthohumol have cytostatic characteristics useful for cancer treatment. However, yield and/or extractability from plants have often been poor. Metabolic engineering of plants by modifying biosynthetic pathways would overcome this problem. Biosynthesis of flavonoids is highly regulated. Therefore, constitutively over-expressing genes of this pathway may not be a successful approach. Unlike the former, regulating the expression of genes in transgenic hop by heterologous and/or homologous regulatory elements would be a feasible approach. It was shown that the *pap1*/*Atmyb75* gene transferred into heterologous systems enhanced gene expression in the phenylpropanoid and flavonoid pathways (Borewitz et al., 2000; Matousek et al., 2006). In the present study, we introduced the regulatory factor *pap1/Atmyb75* from *Arabidopsis thaliana* L. into hop by genetic transformation. Furthermore, by quantitative PCR it was determined to which extend the transcription of genes in the flavonoids biosynthetic pathway was modified. Finally, it was analyzed if the chemical composition and quantity of flavonoids in transgenic hop was altered.

Methods

Genetic transformation of hop (*Humulus lupulus* L. cv. Tettnanger) was performed with *Agrobacterium tumefaciens* EHA 101 harboring the plasmid pLV-65. The T-DNA contained the regulatory gene *pap1/Atmyb75* (GenBank accession: AT1G56650.1) and the selection marker *nptII* (Matoušek et al*.* 2006). Putative transgenic plants were screened by Triplex PCR according to Horlemann et al*.* 2003Transgenic plants were transferred from the greenhouse to an outdoor containment facility. Genomic DNA was extracted from leaves of wild type and transgenic plants and the integration of the transgene was verified by PCR. Total RNA was isolated from leaves and cones of wild type and transgenic plants and the expression of the gene *pap1/Atmyb75* was analyzed by RT-PCR. The rate of transcription of genes involved in the flavonoid biosynthesis pathway (*chs, chi, f3'h* and *vps*) was determined by quantitative PCR. Moreover, the content of anthocyanins, rutin and isoquercitrin was determined in cones of wild type and transgenic plants using High performance liquid chromatography.

Results

The gene *pap1/Atmyb75* from *Arabidopsis thaliana* L*.* was successfully introduced into the hop genome via *A. tumefaciens*. A total of 20 kanamycin-resistant plants were identify by PCR. Only the *nptII* positive transgenic plants showed a 385 bp band, which correspond to the gene *pap1/Atmyb75* (Fig 1).

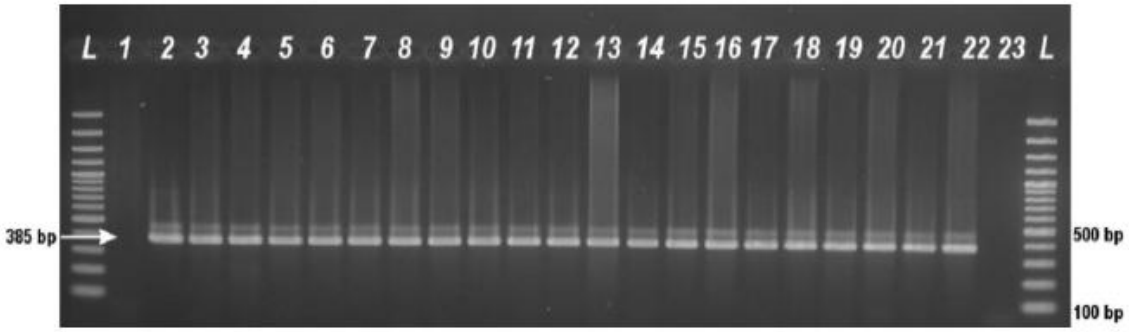

Figure 1: PCR analysis for the integration of the *pap1/Atmyb75* gene in transgenic plants. L: DNA size 100 bp ladder, 1: non-transformed hop (WT), 2: plasmid DNA, 3-22: transgenic hop plants (9, 10, 11, 14, 15, 16, 22, 24, 25, 27, 28, 29, 31, 32, 34, 41, 43, 54, 56, and 203), 23: PCR reaction mix without template.

RT-PCR analysis was performed with seven transgenic plants (10, 11, 14, 15, 24, 29 and 56) as well as with wild type plants. A *pap1/Atmyb75* specific band with an expected size of 163 bp was amplified from leaves (Fig. 2a) and cones (Fig. 2b) of transgenic plants. In wild type plants no signal was detected. As a positive control a DNA sample from a transgenic plant was used. In all samples a quality control for RNA was performed with a RT-PCR using 18S primer (481 bp) (data not shown).

The flowers and cones of *pap1/Atmyb75* transgenic plants showed a reddish to pink pigmentation (Fig. 3a). The profile of secondary metabolites in both wild type and transgenic *pap1/Atmyb75* hop cones was determined by HPLC. Extracts prepared from hop cones revealed a significant increase in anthocyanin content in transgenic plants compared to WT as revealed by UV absorbance of extracts at 550 nm (Fig. 3a).

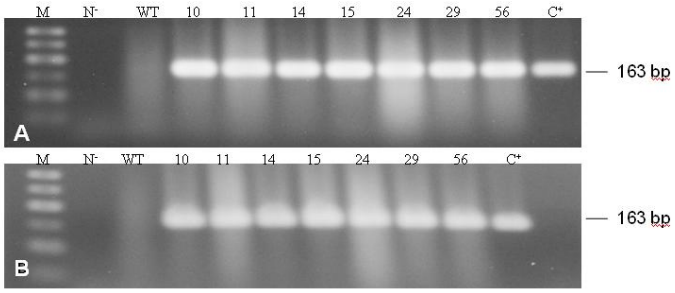

Figure 2. RT-PCR analysis demonstrating *pap1/Atmyb75* expression in leaves (**a**) and (**b**) in cones of transgenic plants 10, 11, 14, 15, 24, 29 and 56. Lanes WT: wild type; C^+: positive control (DNA WT); N^-: negative control (PCR reaction mix without template); M: molecular weight marker (50 bp DNA Ladder).

Furthermore, a significant increase was observed in the quantity of flavonol glycosides especially rutin (quercetin-3-O-β-D-rutinoside) and isoquercetin (quercetin-3-O-β-D-glucoside) in all transgenic *pap1/Atmyb75* plants relative to the wild type (Fig. 3b).

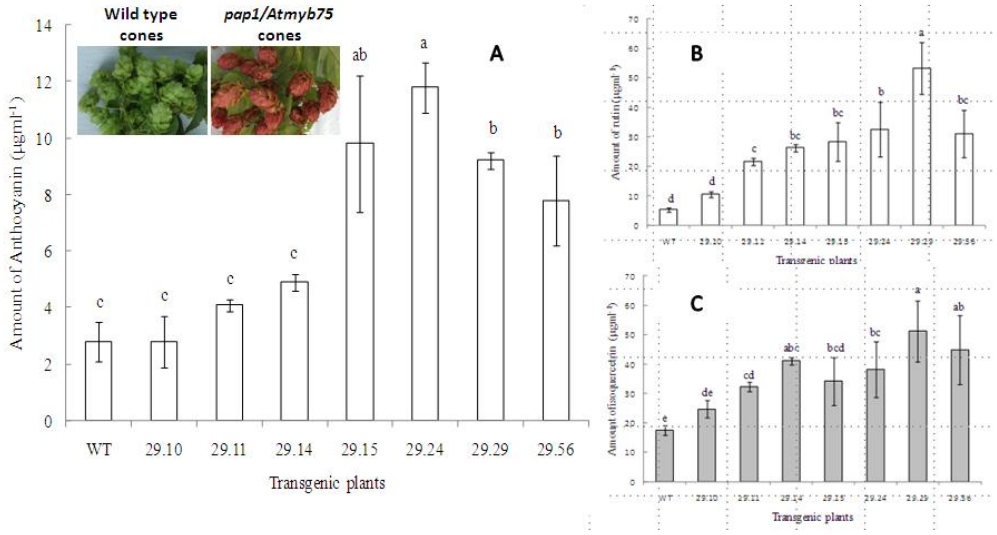

Figure 3. Content of anthocyanin (a) (μgml^{-1}), rutin (b) and isoquercitrin (c) (μgml^{-1}) in cones of wild type and transgenic plants. The phenotype of wild type and transgenic *pap1/Atmyb75* cones is shown.

After verifying the expression of *pap1/Atmyb75* in transgenic hop clones it was interesting to learn how the transcription rate of genes of the biosynthesis of flavonoids was affected. Three transgenic clones were chosen to compare the transcription rate of their genes to that of wild type plants. It was shown that in transgenic plants constitutively expressing *pap1/Atmyb75* had a significant influence on the expression of *chs*, *chi*, *f3´h* and *vps*. Overall *chs*, *chi* and *f3´h* were up-regulated.

Discussion

For the first time genetic engineering of hop (*Humulus lupulus* L. cv. Tettnanger) with a heterologous transcription factor *pap1/myb75* from *Arabidopsis thaliana* L. was successfully accomplished. Previously, this regulatory element has been introduced by genetic transformation in *Arabidopsis thaliana, Nicotiana benthamiania, Nicotiana tabacum, Petunia hybrida* and *Solanum lycopersicum* (Borevitz et al. 2000; Tohge et al. 2005; Matoušek et al. 2006; Xie et al. 2006; Zhou et al. 2008; Zuluaga et al. 2008).

In this study, the *pap1/Atmyb75* gene was instrumental for the red color of hop flowers and cones. Over-expression of *pap1* resulted in *A. thaliana* L. in red colored leaves and pink roots and flowers (Borevitz et al. 2000). Moreover, transgenic tomato plants expressing *pap1*

showed a red coloration in the veins, rachis, and petioles, as well as a reddish-purple pigmentation in stems, flowers, and fruits (Zuluaga et al. 2008). It was interesting to note that in the *pap1/Atmyb75* transgenic plants in addition to anthocyanins, the quantity of other flavonoids was elevated. Similar observations were made in other species like *A. thaliana* L. (Borevitz et al. 2000) and tobacco (Zhou et al. 2008). For the first time we have shown that a heterologous transcription factor (*pap1/Atmyb75*) from *A. thaliana* L. was able to modify the flavonoid biosynthesis resulting in a increase production of flavonoids in hop. Under the control of a 35S promoter, *pap1/Atmyb75* was capable of increasing the rate of expression of key enzymes of this pathway. With these encouraging results other heterologous and/or homologous regulatory elements may be used to elevate the production of pharmacologically important flavonoids in hop.

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Acknowledgements

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FUNCTIONAL ANALYSES OF LUPULIN GLAND-SPECIFIC REGULATORY FACTORS FROM WD40, BHLH AND MYB FAMILIES OF HOP (*HUMULUS LUPULUS* L.) SHOW FORMATION OF CRUCIAL COMPLEXES ACTIVATING *CHS*_H1 GENES.

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Abstract

Complex ($3{,}5.10^6$ pfu) lupulin gland-specific cDNA library from Osvald's 72 hop was constructed. From this cDNA library we isolated first hop-specific allelic isoforms of transcription factors (TF) bHLH (*Hl*bHLH2, AC:FR751553) and WD40 (*Hl*WD40_1, AC:NM_122360), which share high homology with well described TT8 and TTG1 TFs of *A. thaliana*, respectively, as well as with corresponding orthologues AN1 and AN11 from *Petunia hybrida.* All these TFs are involved in combinatorial control of light-responsive and tissue-specific activation of phenylpropanoid pathway. *Hl*bHLH2 and *Hl*WD40_1 are quite lupulin gland-specific and, according to our transient expression experiments, they form specific complexes by interacting with previously isolated lupulin gland-specific *Hl*Myb2 (AC:FN646081) and *Hl*Myb3 (AC:AM501509) TFs. The complex formation leads to strong activation of *chs*_H1 promoter. This activation is dependent on the presence of PMyb-like a box CCWACC positioned on 5' end of the cloned promoter. The interplay and regulation of expression of these crucial TF complexes could co-determine the rate of accumulation of valuable metabolites of lupulin.

Keywords: transcription factors, protein complexes, transient expression assay, promoter elements, lupulin metabolome, *Humulus lupulus* L., *N. benthamiana*

Introduction

Hop glandular trichomes contain specific part of hop metabolome including α and β bitter acids valuable for the brewing process and other compounds like prenylated chalcones (e.g. xanthohumol) that are of particular recent interest in their view of significant bioactivity and anticancerogenic properties. An important role in their biosynthesis is attributed to true chalcone synthase-CHS_H1 (EC 2.3.1.74) (Matoušek, et al., 2002) encoded by the oligofamily of *chs*_H1 genes that we previously characterized including promoter elements (Matoušek et al., 2006).

During last years we described several lupulin-gland specific transcription factors (TF) from Myb and bZip families putatively involved in hop metabolome regulation (Matoušek et al., 2005; 2007; 2010). Especially a number of Myb TFs participate in so-called combinatorial TFs action (Singh et al. 1998) that provides new phenotypic variability valuable for the breeding and selection process. From various data obtained from research of *A. thaliana* (Riechmann and Ratcliffe, 2000) and other plant species, one can deduce that especially R2R3 MYBs, basic helix-loop-helix (bHLH), and WDR TFs are involved in one of the "combinatorial" regulatory pathways responsible for the co-determination of plant secondary mebolome, cell fate and some processes of plant morphogenesis (e.g. Ramsay and Glower, 2005; Feller et al., 2011). Myb, bHLH and WDR TFs form complexes by protein:protein interaction that specifies their function. In these interactions the Myb R3 domain and N-terminal region of bHLH are involved (Grotewold et al. 2000).

In the present work, we cloned newly the lupulin-specific TFs *Hl*bHLH2, *Hl*WD40_1 and *Hl*Myb2 and showed that together with previously described *Hl*Myb3 (Matoušek et al., 2007) these TFs form crucial complexes having an ability to activate *chs*_H1 promoter strongly.

Materials and Methods

TFs *HlMyb1*, l-*HlMyb3*, s-*HlMyb3*, *Hl*bHLH1 were described previously (Matoušek et al., 2005, 2006, 2007 and 2009). These genes were cloned from cDNA library of hop clone Osvald 72 using probes generated from conserved motifs or based on cDNA ESTs selected using cDNA AFLP method (De Keukeleire et al., 2005). For cloning of *Hl*bHLH2 and *Hl*WD40_1 we combined screening of EST and trichome databases (GenBank, http://www.ncbi.nlm.nih.gov/ and TrichOME (http://trichome.noble.org/trichomedb/) for specific motifs with PCR amplification of cDNA fragments from lupulin-specific cDNA library of Osvald's 72 hop. In addition, 3'RACE and inverse PCR approach was used to complete *Hl*WD40_1 cDNA. Other genes like *Hl*JMyb1, *Hl*Myb2, MIXTA genes and sequences from *A. thaliana* were amplified using sequence information from EMBL database. Based on cloned cDNA sequences, plant expression vectors pLV07 were constructed and *A. tumefaciens* strains were prepared as described previously (Matoušek et al., 2010). Promoter activity was evaluated using GUS activity assay (Matoušek et al., 2010) and Real-time PCR mRNA quantifications were performed using specific primers essentially as described previously (Matoušek et al., 2010).

Results and Discussion

In the present work we cloned newly several TFs from the cDNA library derived from lupulin glands of Czech hop, Osvald's 72. This cDNA library was more specific and complex ($3{,}5.10^6$ pfu) than previously constructed cDNA library from hop flowers and cones (Matoušek et al., 2007, 2010). The high complexity and specificity of the library enabled us to amplify full-length clones of *Hl*bHLH2, *Hl*WD40_1 and *Hl*Myb2 TFs from hop using pre-selections in trichome-specific library (Table 1). Homology comparisons showed that *Hl*bHLH2 having predicted MW about 77.1 kDa and pI 5.1 shares significant homology with AN1 TF from *P. hybrida* and its homologue TT8 from *A. thaliana* (Fig.1). *Hl*WD40_1 having predicted MW about 38 kDa and pI 4.79 is significantly homologous to AN11 from *P. hybrida* and to *A. thaliana* TTG1 TF (Fig.1). Newly cloned hop TFs together with previously cloned HlMyb3 (Matoušek et al., 2007) showed high specificity for glandular tissue (Fig. 2, A-D) suggesting an important role(s) of these TFs in the lupulin metabolome biosynthesis. Based on these assumptions functional analyses were performed using "combinatorial" transient expression assay that we developed previously (Matoušek et al., 2010). We used system of infiltration of *A. tumefaciens* strains containing TF vectors and GUS reporter/promoter containing vector into *N. benthamiana* leaves. Transient expression system revealed that in the principle two activation TF complexes are formed after co-infiltration of *Hl*bHLH2, *Hl*WD40_1 and either *Hl*Myb2 or *Hl*Myb3, while individual TFs did not show any significant P*chs*_H1 activation (Fig.2 E). Activation by *Hl*M3W1H2 usually reached about 30% of *Hl*M2W1H2 activity (Fig.2, Table 1). It was found (Table 1) that individual complex components can be partly substituted by other TFs from hop or *A. thaliana*. For instance, Myb components can be partly substituted by *At*Myb12 and *At*Myb23; WD40_1 component can be substituted by *ttg1* so that the heterologous complex reached 40% of activity of homologous *Hl*M2W1H2(Table 1). Our results show that homologous hop complexes are quite *Hl*Myb2 and *Hl*Myb3-specific, as no activity was observed with other hop Myb or Myb-like TFs. In addition, according to our unpublished results *Hl*Jmyb1 is a strong competitive inhibitor of both hop TF complexes. Simultaneously, our results revealed that there are clear differences between *Hl*M2W1H2 and *Hl*M3W1H2 complexes in dynamics of action (not shown), as well as in their dependency on various binding boxes within P*chs*_H1. It was found that the function of both complexes depends on the complexity of PMyb-like CCWACC box positioned on the 5' end of cloned P*chs*_H1 (not shown). While complex *Hl*M2W1H2 is quite specific for P*chs*_H1, *Hl*M3W1H2 has the ability to co-activate P*chs*4. Non of these complexes has the ability to activate another promoters of genes involved in lupulin

biosynthesis like *omt*1 or *vps* promoters. The expression balance among various promoter activating complexes and complex inhibitors, as has been shown also in other studies (e.g. Feller et al., 2011), influences final mRNA steady state levels. Our study indicates that this system is valid also for *chs*_H1 and it could co-determine the rate of accumulation of valuable metabolites of lupulin.

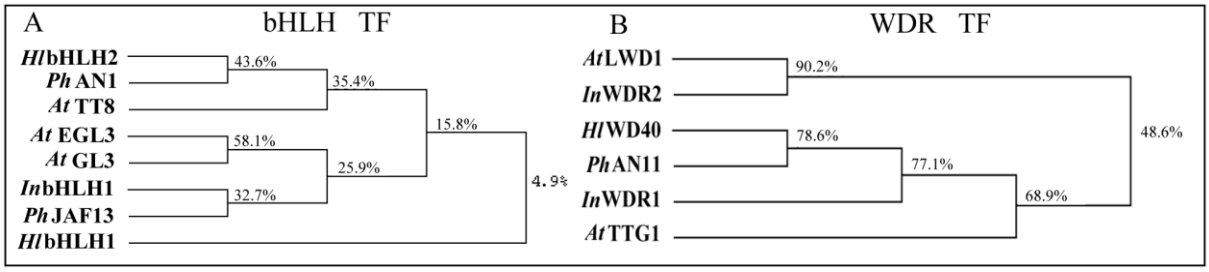

Fig. 1 Homology of cloned hops TFs bHLH (A) and WDR (B) to known regulators of phenylpropanoid pathways (aminoacid identity % is shown).

Table 1 Substitution of activating complexes with hop and arabidopsis TFs

Substituting component			MWH complex activity %**
TF type	Gene symbol	GenBank	
Myb (M)[1]	*Hl*Myb2	FN646081	100.0
	Hl s-Myb3	AM501509	33.7
	Hl l-Myb3	AM501509	15.5
	Hl Myb1	AJ876882	1.4
	Hl JMyb1	AB292244	0.3
	*At*Myb23	AT5G40330	23.3
	*At*Myb12	AT2G47460	35.3
Myb like[1]	*Hl*Mixta3	-	2.2
	*Hl*Mixta4	-	1.0
WD repeat[2] (W)	*ttg1*	AT5G24520	40.8
MW[3]	*Hl* s-Myb3 + *ttg1*	-	15.8
	*At*Myb23+ *ttg1*	-	10.8
	*At*Myb12+ *ttg1*	-	25.7
bHLH (H)[4]	*Hl*bHLH1	FN646080	1.8
WH[5]	*ttg1*+ *Hl*bHLH1	-	6.7
MH[6]	*Hl*bHLH1+ *Hl* s-Myb3	-	6.4
	*Hl*bHLH1+ *Hl* Myb1	-	3.4

The following TFs formed the basis: [1]*Hl*bHLH2 and *Hl*WD40_1; [2]*Hl*Myb2 and *Hl*bHLH2; [3]*Hl*bHLH2; [4]*Hl*Myb2 and *Hl*WD40_1; [5]*Hl*Myb2; [6]*Hl*WD40_1. **activity of *Hl*M2W1H2 with P*chs*_H1 is taken as 100%; samples were collected at optimal interval post infiltration.

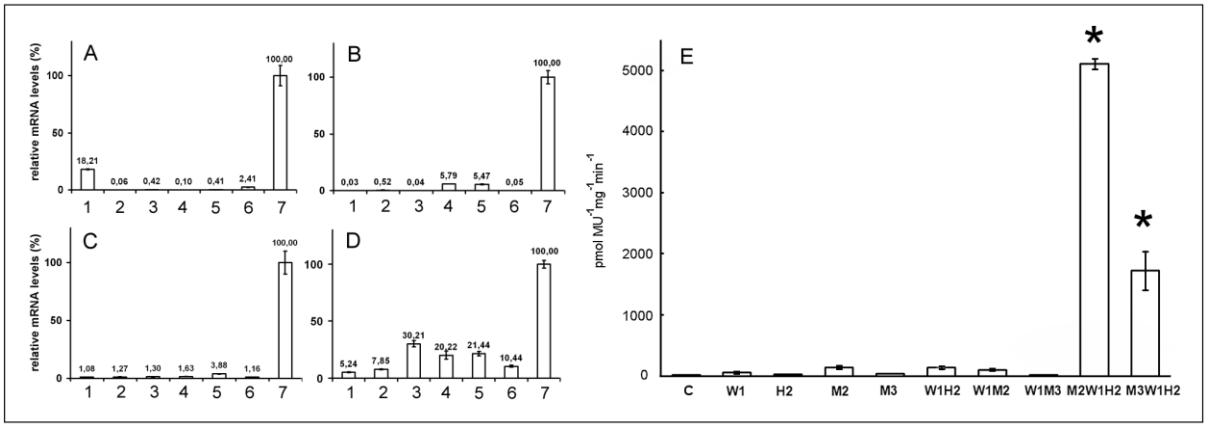

Fig.2 Real Time RT PCR analysis of expression of hop HlMyb3 (A), HlMyb2 (B), HlbHLH2 (C) and HlWD40_1 (D) in roots (1); young leaves (2); petioles (3); flowers (4); young cones

(5); immature pollen (6) and lupulin glands (7). GAPDH was used as internal reference transcript. (E) activation of P*chs*_H1 by hop TF and TFs complexes. Activation by full *Hl*M2W1H2 and *Hl*M3W1H2 complexes is marked by asterisks.

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COMPLEMENTATION ANALYSIS OF HOP TRANSCRIPTION FACTORS USING *ARABIDOPSIS THALIANA* GENES IN TRANSIENT SYSTEM AND IN TRANSGENOTES

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Several hop genes which are involved in the phenylpropanoid pathway responsible for the production of prenylated chalcones (e.g. xanthohumol) with significant bioactivity and anticancerogenic properties have been cloned so far. Some of them are intensively studied in our lab. These include genes encoding for the crucial enzymes of this pathway, like chalcone synthases (CHS_H1) (Matoušek et al., 2006) or o-methyltransferase (OMT) (Nagel et al., 2008) and putative genes encoding for their regulators, transcription factors (TFs). The genes for TFs belong to the families of Myb (*Hl*Myb1, *Hl*JMyb1, *Hl*Myb2, *Hl*Myb3), bZip (*Hl*bZip1, *Hl*bZip2), bHLH (*Hl*bHLH1, *Hl*bHLH2) and WDR (*Hl*WD40_1) classes. Three basic strategies to understand their role in the regulation of the phenylpropanoid pathway have been approached. First, TF genes were cloned under control of constitutive promoter 35S into *Agrobacterium tumefaciens* vectors and their overexpression in transgenotes was studied. Because the hop transformation is labour intensive and time consuming (still in progress to get transgenic cones with lupulin glands), we performed such analysis also on model plants *Arabidopsis thaliana*, *Nicotiana benthamiana* and *Petunia hybrida*. Overexpression of the hop TF genes showed that in some cases (*Hl*Myb1, *Hl*Myb2, *Hl*Myb3, *Hl*WD40_1) they are able to affect plant morphology (i.e. enhanced branching, dwarfism or gigantism). It demonstrates that our cloned hop TFs are active and able to bind to promoter targets also in heterologous genomes. The second approach is the study of possible complementation of known *Arabidopsis* mutant genes either with significant homology or involved in similar pathways as hop genes. This study is still in progress, but preliminary results show that in some cases the complementation can occur (*AtbHLH42* complemented with *Hl*bHLH1; *AtMyb91* complemented with s-*Hl*Myb3). The third strategy is using agrobacterium vectors with the *chs*_H1 or *omt* gene promoter sequences fused with GUS marker gene. After infiltration of such promoter/reporter construct together with complex of hop TFs (*Hl*WD40_1 with *Hl*bHLH2 and *Hl*Myb2 or 3) into *N. benthamiana* leaves considerable activation of *chs*_H1 promoter (P*chs*_H1) has been achieved. This combinatorial control of phenylpropanoid pathway seems to be light-responsive. Whereas TF complexes with *Hl*Myb2 and *Hl*Myb3 activate the promoter up to 500 fold times, *Hl*JMyb1 acts as suppressor of both complexes. Individual TFs did not show any significant P*chs*_H1 activation.

This work was supported by the Czech Science Foundation GACR 521/08/0740, by the National Agency for Agricultural Research of the Ministry of Agriculture of CR, QH81052 and by GA ASCR AV0Z50510513. The authors would like to thank Mrs. H. Matoušková, Ing. O. Horáková and Ing. L. Orctová for their excellent technical assistance.

Keywords: chalcone synthase, transcription factors, transgenosis, transient expression

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HOP STUNT VIROID (HSVD) DISEASE CAUSES ALTERATION OF EXPRESSION OF HOP TRANSCRIPTION FACTORS FROM MYB, BHLH AND WRKY FAMILIES

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Abstract

A serious pathogen that has been emerging as a threat for hop-gardens in Japan, USA and China, hop stunt viroid (HSVd) is remarkable for being simple in structure, but complex in induction of symptoms. The pathogenesis of this non-coding, circular and highly complementary ssRNA molecule has not been clearly explained, though involvement of viroid-derived small RNAs is considered to be plausible. By further disbalancing the expression of various target transcription factors (TFs), the symptoms in our model hop *cv*. Admiral could be induced. In addition to stunted growth and leaf rigidity of plants infected by HSVd-g (AC: E01844.1), we observed shifts in the levels of secondary metabolites and changes in petiole colouration (unpublished). Consequently, we examined several hop TFs recently cloned in our laboratory, which are cone-specific and putatively connected with lupulin metabolites biosynthesis, for possibly altered expression as a response to viroid infection. Using quantitative RT-PCR approaches we found several of these TFs to be expressed differentially in infected and control plants. Most notably, we observed 8-fold decrease of *HlbHLH2* (AC:FR751553) mRNA in both leaves and petioles. On the contrary, we found an increase of *HlMyb5* to 15-fold / 7-fold expression, and *HlWRKY75* to 4,5-fold / 7-fold expression in symptomatic leaves and petioles, respectively, as compared to healthy control. In addition, previously described TFs from MYB family were observed to have increased mRNA levels, namely *HlMyb1* (Matoušek *et al*., 2005) having 2-fold higher and *HlMyb3* (Matoušek *et al*., 2007) having 5-fold higher expression in symptomatic petioles and leaves, respectively. Functional analyses of novel hop TFs mentioned above are in progress to provide deeper insight on regulation of lupulin metabolome during HSVd infection, as well as in healthy plants.

Keywords: *Humulus lupulus* L., hop stunt viroid, transcription factors, lupulin metabolome regulation, quantitative RT-PCR

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This work was supported by projects GACR 521/08/0740, GACR P501/10/J018, and AV0Z50510513. The authors would like to thank Mrs. H. Matoušková, Ing. O. Horáková and Ing. L. Orctová for their excellent technical assistance.

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IV. Session:

CHEMICAL ANALYSIS OF HOP COMPOUNDS

DIFFERENTIATION OF THE WORLD HOP COLLECTION BY MEANS OF THE LOW MOLECULAR POLYPHENOLS

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Abstract

The composition of the quercetin and kaempferol glycosides of hops is genetically determined and therefore depending on the variety. The analysis of the world hop collection showed that some varieties are well distinguishable, however other varieties like the land races are very similar. Similarities and differences can be presented by means of a principal component analysis. A formation of groups is not observable. The composition of the flavonoids is definitely a suitable tool for the differentiation of hop varieties besides the bitter substances and essential oils.

Keywords: hop varieties, polyphenols, flavonoids, UHPLC

Introduction

Up to now the composition of the bitter substances and essential oils is used to distinguish hop varieties. But also the low molecular polyphenols are genetically determined and thus they are a characteristic feature for a variety. Especially the composition of the quercetin- and kaempferolglycosides is suitable for the differentiation of varieties. In this project first convenient methods for sample preparation and UHPLC-analysis had been worked out and then the whole world hop collection (160 samples) was analysed. A Principal component analysis was made with the gained data to get a visual presentation of the similarities and differences and another target was to find out formations of groups.

Methods

For sample preparation 5 g ground hops are extracted with 50 ml acetone/water (3:1) in an ultrasonic bath for 15 minutes. Then the solution is filtered through a pleated filter. The filtrate is transferred into a separating funnel and shaken with 50 ml hexane. The no polar substances go into the hexane-phase and the flavanoids remain in the acetone/water phase. As an internal standard a solution of 1 ml flavanone in acetone (250 mg flavones in 25 ml acetone) is added. Flavanone is no natural ingredient of hops and delimits the polar flavonoids from the no polar bitter substances, xanthohumol and the prenylated naringenines. All these substances elute after flavanone. For HPLC-analysis it is filtered once again with a syringe filter. The UHPLC-analyses are performed by an UHPLC-system.

UHPLC-Conditions: oven temperature: 40°C
column: EC 125/2 NUCLEODURSphinx RP, 3 µm from Macherey & Nagel
eluent A: 100 ml methanol, 3 ml H_3PO_4 filled up to 1 l with water
eluent B: 700 ml methanol, 3 ml H_3PO_4 filled up to 1 l with water
eluent C: methanol

gradient:	detection wavelength:	
0 Min.: 100 % A	benzoic acid-derivates:	250 nm
5 Min.: 100 % A	cinnamic acid-derivates:	
30 Min.: 70 % A, 30 % B	catechines:	280 nm
55 Min.: 10 % A, 90 % B	quercetin-,	
56 Min.: 100 % C	kaempferolglycosides:	350 nm
60 Min.: 100 % C	multifidolglucoside:	280 nm
61 Min.: 100 % A		

Results

The figure 1 shows on the left side UHPLC-chromatograms of the flavonoids from the varieties Opal, Hersbrucker Spät and Herkules as examples with great differences. On the right side you can see the chemical structures of identified substances. Further substances will be clarified at the Technical University Munich by means of a mass spectrometer.

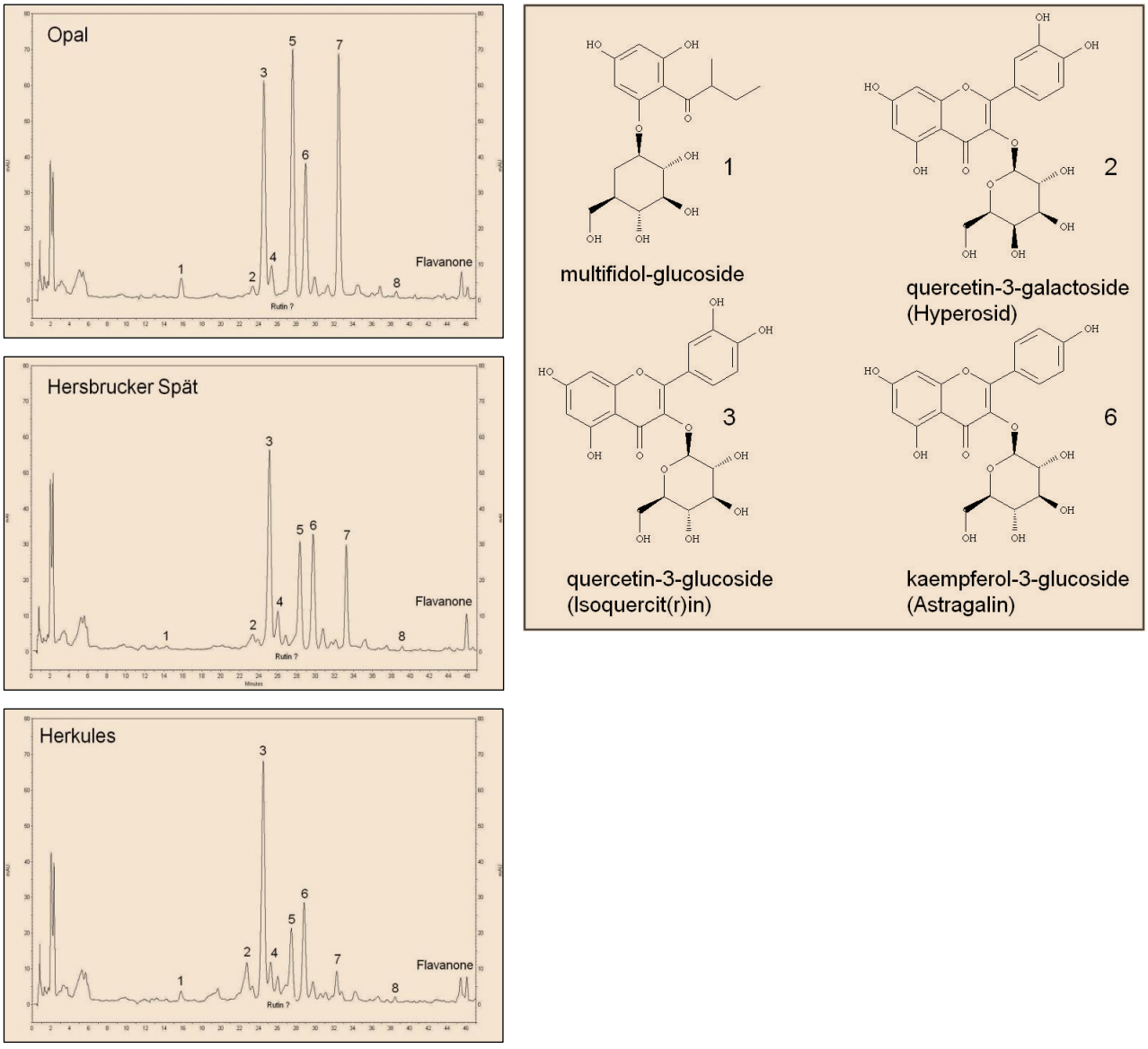

Figure 1: UHPLC-chromatograms of the flavonoids from the varieties Opal, Hersbrucker Spät, Herkules and the chemical structures of identified substances

The whole in Hüll available world hop collection (160 samples) was analysed with this method and then a principal component analysis (PCA) (Figure 2) was done wit the gained data. The eight labeled peaks of the chromatograms were taken into consideration for the PCA.

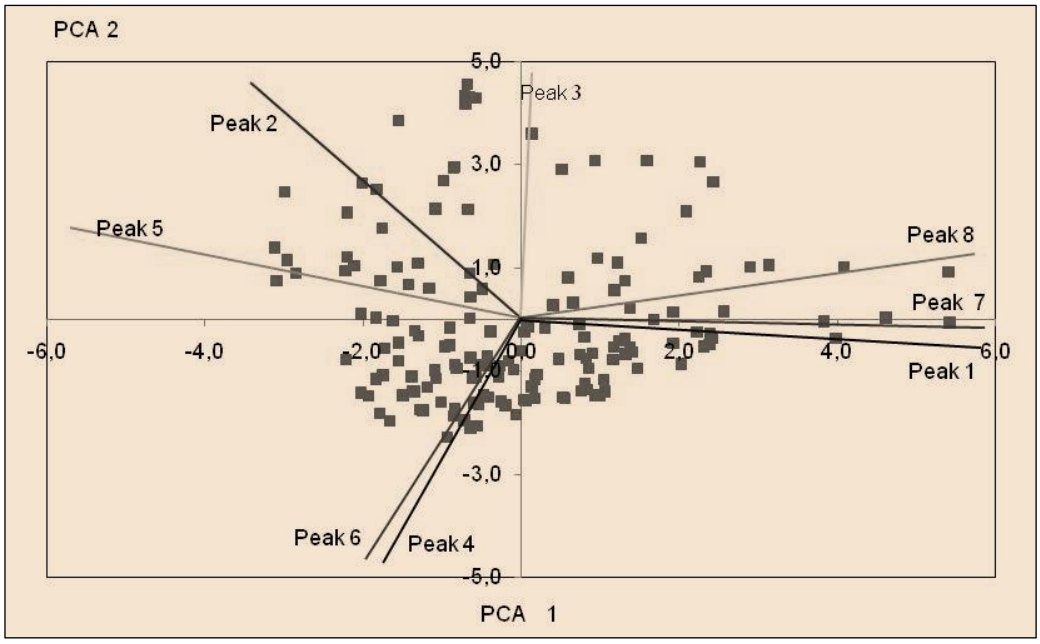

Figure 2: Principal component analysis of the world hop collection

As shown in the figure 2 some varieties are very well distinguishable and others have a very similar flavonoid composition. A group formation is not observable.

Perspectives

For this project investigations were made in the harvest of the year 2009. The harvest years 2010 and 2011 will be included in the work. It is expected that the TUM can elucidate the structures of further flavonoids, especially the peaks 5 and 7 would be important. Then a final publication is planned. An additional target would be to find out which role the flavonoids in the resistance mechanisms of hops play. Plants produce surely polyphenols to defend themselves against diseases and pests. But these are perspectives for the future.

Acknowledgement

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STUDY OF THE PRODUCTION OF SECONDARY METABOLITES IN SHOOT AND CALLUS CULTURES AND FIELD GROWN PLANTS OF HOP

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Introduction

The *in vitro* plant cultures are able to produce and accumulate a lot of secondary metabolites. Polyphenols, alkaloids, terpenoids, and steroids can be attractive for the use in practice. Secondary metabolites produced by the plant organ cultures are very similar to the secondary metabolites of mother's plants. The content of biologically active substances in hop extracts is a prerequisite for the potential use of hop crops and explant cultures to produce these substances for pharmaceutical, food, or agricultural uses.

Results

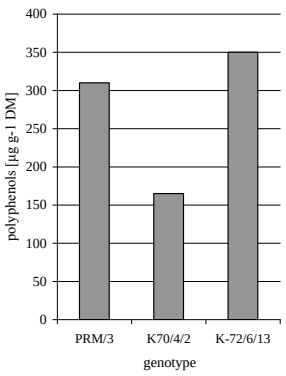

Fig. 1 Polyphenols content in callus cultures

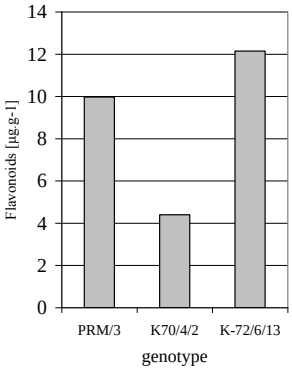

Fig. 2 Flavonoids content in callus cultures

Tab. 1 Polyphenols and flavonoids content in shoot and wild hop

Cultivars	Polyphenols [mg.g^{-1} DM]	Flavonoids [mg.g^{-1} DM]
Premiant	2,27±0,09	1,43±0,08
Bor (K-70)	2,96±0,11	1,51±0,08
K-72	3,04±0,09	1,44±0,09
Wild Hop	4,00±0,14	0,50±0,02

Conclusion

The content of polyphenols in the shoot cultures was comparable to its amount in extracts from cones of wild hops and almost a third lower than in the leaves of the field grown cultivars stored in the gene bank. The content of flavonoids was six to eight times higher in the shoot cultures than in the leaves of the field grown plants at the beginning of the growing season. The possible reason for this was the unfavourable weather condition for the formation of these metabolites in the field grown plants, because in the year in which the climatic conditions were favourable (2007), the content of flavonoids in shoot cultures was three to six times lower. The amount of secondary metabolites in callus extracts was of one order of magnitude and of two to three orders of magnitude lower than in extracts from shoot cultures, regarding polyphenols and flavonoids, respectively. Optimization of cultivation of hop tissue cultures gives the possibility to use these experimental models for studying overproduction of polyphenols or other bioactive substances.

Keywords: polyphenols, flavonoids, hop, shoot, callus cultures

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EFFECT OF GENOTYPE AND GROWING PERIOD ON ANTIOXIDANT ACTIVITIES OF HOP LEAF EXTRACTS

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Abstract

Many of secondary metabolites present in hops (*Humulus lupulus* L.*)* exhibit interesting bioactive properties, among others antioxidant and chemoprotective effects. Chemical constituents of hops are of considerable importance not only in the brewing industry, but they can serve as sources of biologically active substances such as polyphenols, flavonoids and prenylated chalcones also for potential pharmaceutical applications.

Keywords: antioxidant activity, *Humulus lupulus* L., DPPH, BCLM, polyphenols, flavonoids

Introduction

Hops contain a large number of biologically active compounds such as bitter and polyphenolic compounds and essential oils. Currently, much attention has been focused on hop polyphenols and flavonoids, which exhibit a wide range of biological activities, such as antioxidant, antiviral, antimicrobial, and anticancer properties. The aims of our study were i) to compare the antioxidant activities (AA) of hop extracts prepared from leaf samples collected from plants during the vegetative growth period and from cones during the generative growth stage in hydrophilic and lipophilic conditions, and ii) to analyze the correlative relationships between AAs of extracts from hop leaves and/or cones.

Methods

The analyzed samples were extracted from hop plants growing in the *ex vitro* virus-free hop germplasm collection maintained in the Gene bank of Slovak republic at PPRC in Piešťany (SR). The antioxidant activities were determinated using the DPPH and BCLM methods.

Results

In hydrophilic conditions, statistically significant correlation was detected between the cultivar/clone and AA in leaves collected in May ($P<0.001$), July ($P<0.01$), and September 2010 ($P<0.001$). In lipophilic conditions, similar trends were observed in AAs using both the BCLM and DPPH methods. The effect of hop cultivar/clone on the level of AAs was statistically significant for all dates of sample collection ($P<0.001$). Differences in AAs of leaf extracts between the individual sample collection dates, i.e. between May and July and between July and September, respectively, were also statistically significant ($P<0.001$). The correlation between the average values of AAs measured in extracts from leaves and AAs of extracts from cones was statistically significant ($P<0.01$) only if the BCLM method was used for AA determination.

Acknowledgement

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BIOACTIVE COMPOUNDS IN HOP CULTIVARS GROWING IN POLAND

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Hop (*Humulus lupulus* L.) is one of the basic raw materials employed in brewing, but this plant is also interesting for its bioactive compounds which could be used in medicine. In present work the content of some bioactive substances in hop were investigated. Five of Polish hop cultivars (dry hop cones) from the harvest of 2010 were sampled from some hop-gardens near Lublin in Poland. Two aroma (Sybilla, Lubelski) and three bitter (Magnum, Marynka, Junga) hop cultivars were analysed in this study for content of alpha-acids, tannin, xanthohumol, total phenolic, flavan-3-ols and proanthocyanidins.

The alpha-acids and tannins were analysed by spectrophotometric methods and the results were expressed as % of dry weight [1]. The xanthohumol content was determined by high-performance liquid chromatography with diode array detection and the results were described as % of dry weight [2] The total phenolic content was measured using modified Folin-Ciocalteau method and the results were expressed as mg of gallic acid equivalent (GAE) per gram of dry weight [3]. The flavan-3-ols and proanthocyanidins content was analysed with vanilin reagent and the results are expressed as mg of catechin equivalent (CE) per gram of dry weight [3].

It was found that cultivar differed in content of bioactive compounds. The highest content of tannin, total phenolic and flavan-3-ols were found in Marynka cultivar and they amounted 18.52 % dry wt, 7.25 mg GAE/g dry wt and 39.83 mg CE/g dry wt, respectively. The concentrations of xanthohumol in hop cultivars amounted in the ranges of 0.06 to 051 % (w/w). The highest amount of the xanthohumol was found in Sybilla cultivar, but on the other hand the lowest content of tannin and flavan-3-ols (9,66 % dry wt and 0,96 mg CE/g dry wt, respectively) were found in that cultivar. Magnum cultivar had the highest amount of alpha-acids (12.9 % dry wt).

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ANTICHOLINESTERASE ACTIVITY OF HOPS

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The hop plant (*Humulus lupulus* L.) has been widely used in the pharmaceutical and in the brewing industry (formation of protein-polyphenol deposits in worts as well as flavour, aroma, bitterness and the microbial stability of beer).

Based on works published in the past only the indirect positive connection between beer consumption and the risk of Alzheimer's disease (AD) can be established. Hop components exhibited antiinflammatory and antiproliferative activity, scavenged the reactive oxygen species (peroxyl and hydroxyl radicals), inhibited the production of superoxide anion radical and nitric oxide. Xanthohumol effectively chelated Fe^{2+} and Fe^{3+}. At the present moment, the only effective strategy for AD treatment is the use of acetylcholinesterase (AChE) and butyrylcholinesterase (BChE) inhibitors**.**

In present work, the inhibition of AChE and BChE by hop extracts was evaluated. Three bittering ("Magnum", "Marynka", "NB") and two aromatic ("Sybilla", "Lubelski") hop varieties were used in this study in the fresh frozen form (-30^{o}C). Inhibition of AChE and BChE was measured using modified method of Ellman using acetylthiocholine (or butyrylthiocholine) and 5,5′-dithiobis(2-nitrobenzoic acid) (405 nm). Thermostability of cholinesterase inhibitors from hops was also measured after hops boiling typically performed in the mashing house.

It can be stated that three hop varieties ("Lubelski","Marynka","NB") exhibited the highest anti-AChE and anti-BChE activities. The differences in the inhibition of both enzymes were seen in the case of the individual hop varieties what suggests the specificity of the inhibitors. The anticholinesterase activity exhibited by three samples of Marynka hop strongly depended on the growing location and conditions. The anti-AChE activities of extracts from "Lubelski", "Marynka" and "NB" and anti-BChE (from "NB") increased after hop boiling. The anti-BChE activities of extracts from "Lubelski" and "Marynka" decreased in comparison to non-boiled hop samples.

In conclusion it can be stated that hop can be a valuable source of anticholinesterase inhibitors for the treatment of AD.

INFLUENCE OF ISOMERISATION ON THE COMPOSITION OF HOP RESINS AND ESSENTIAL OILS.

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Abstract

The current economic situation has caused interest in the brewery products that offer higher efficiency of alpha acids. One way to increase the efficiency of alpha acids is their isomerisation in hop pellets. Alpha acids contain in the traditional pellets come into isomerised form during the boiling of wort. Approximately 35% of alpha acids are utilized.

Isomerization of alpha acids in hop pellets leads to the changes in composition of hop resins and essential oils. Studies of hop oils and resins composition before and after isomerisation were performed on Magnum cultivar. Standard pellets contain a simple alpha acids, that are comprised of iso alpha acids, cohumulene, n+ad humulene and beta acids (colupulone and n+ad lupulone). Isomerisation of pellets is devided into two stages: first – adding of magnesium oxide to the hops powder before granulation, second - heating the pellets. Durind the heating, magnesium hydroxide is formed and above 90% of components of alpha acids are chemically bonded. Easily soluble iso alpha acids are formed, while beta acids are not altered (Tab. 1).

Table 1. The content of alpha acids and iso alpha acids before and after isomerisation.

Pellets of Magnum	Before isomerisation	After isomerisation
alpha acids	12,4 %	0,0 %
co humulone	3,4 %	0,0 %
n+ad humulone	9,0 %	0,0 %
iso alpha acids	0,6 %	12,6 %
iso co humulone	0,0 %	3,5 %
iso n humulone	0,6 %	7,8 %

Analysing the changes in the composition of hop oils before and after isomerisation, we noticed that the positive essential oils ingredients (eg. humulene, linalool, farnesene, caryophyllene) were sable. Adverse myrcene decreased from 55% to about 20%.

Iso-pellets require a shorter contact with the wort during the boiling. Utilization of iso alpha acids is 20-30% higher comparing to the simple alpha acids. The advantages of using iso-pellets are: increasing the effectiveness of utilization of alpha acids during wort boiling, maintaining the composition of positive ingredients of hop oils, decreasing of adverse myrcene, shortening the boiling wort, increasing product stability, reducing energy costs reducing hop consumption and less restrictive conditions of pellets storage.

Keywords: Hop product, isomerisation, hop resins, alpha acids, hop oils.

V. Session:

MANAGEMENT OF HOP DISEASES AND PESTS

PROBIOTIC MICROORGANISMS WITH FERMENTED PLANT EXTRACTS IN PROTECTION OF ORGANIC HOPS

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Abstract

In 2010 field studies were carried out to estimate the plant protection against diseases and pests for organic hop production by probiotic microorganisms with fermented plant extracts in comparison with protection by using Quassia, whey and Myco-Sin. The efficacy of probiotic microorganisms with fermented plant extracts in control of downy and powdery mildews and also main pests i.e. aphids and spider mites was similar to efficacy quassia in control *Phorodon humuli*, Myco-Sin in diseases control and whey in spider mites control.

Keywords: hops, organic farming, diseases and pests control, probiotic microorganisms with fermented plant extracts

Introduction

Organic control methods do not pollute the environment and are not harmful to beneficial insects and animals, or to the people using them. Insecticides of plant origin are used in the organic production of many plant species, as well as vegetable oils, powders and insecticidal soaps, acaricidal natural product as whey that are selective, with a narrow range of effects and of lower toxicity, as well as biological products (Sivčev 2010). As a rule, such plant protection products require a more frequent application. Copper-based and sulphur-based fungicides as Myco-Sin are still leading products in suppressing diseases of cultivated organically plants (Žežlina 2010). Quassia extract is used in organic hop growing in Germany to control *Phorodon humuli* (Weihrauch at al 2007). The impact of probiotic microorganisms called also EM in promoting plant growth by controlling or suppressing pests and diseases has been reported from many countries. Wang et al (2000) highlight the control of Phytopthora with EM derivatives in China and Australia. Wood et al (1999) also states the control of pickleworm in cucumber with EM. Effective Microorganisms (EM) defended wheat most efficiently against Septoria disease (*Septoria nodorum*) and brown leaf blight (*Drechslera tritici-repentis*). The EM with added winter rape seeds inhibited the development of brown rust (*Puccinia recondita*) on leaves (Boligłowa, Gleń 2008).

In the last years, probiotic microorganisms are used more and more often in organic farming. Mixture of microorganisms consisting mainly of lactic acid bacteria, photosynthetic bacteria, yeasts and *Actinomycetes* which co-exist for the benefit of whichever environment they are introduced. The microorganisms are effective and disease-suppressing. Some of which are known to produce bioactive substances such as vitamins, hormones, enzymes, antioxidants and antibiotics that can directly, or indirectly enhance plant growth and protection. The fermented plant extracts is also used to enhance efficacy of the microorganisms in plant protection against diseases and pests (Daly, *et al.* 2000, Kyan *et al.* 1999).

The aim of this study was the comparison of plant protection against diseases and pests for organic hop production based on quasia, MycoSin and whey with protection based on probiotic microorganisms enhanced fermented plant extracts.

Methods

The study on organic hop protection was carried out in 2010 in a private hop garden at Jastków near Lublin. Efficacy of probiotic microorganisms in control of powdery and downy

mildews, Damson-hop aphids and two spotted spider mites was evaluated on hop cultivar Marynka. Probiotic microorganisms applications were performed on the ground of two active preparation EMa and EMa5 obtained from ProBio Original™ mother material. The following sprayings were made in season:

one time in April	20 l EMa 5 and PM fermented tancy in 650 l water/ ha for spraying of soil
two times in May	20 l EMa + 3 l EMa5 in 1000 l of water/1 ha
two times in June	20 l EMa + 6 l EMa5 in 2000 l of water/ ha+ 50 l of PM fermented nettle in 2000 l of water/ha
three times in July	20 l EMa + 6 l EMa5+ 60 l of PM fermented common sow- thistle and common dandelion extracts in 2000 l of water/ ha
three times in Aug	40 l EMa + 6 l EMa5 + 60 l of PM fermented common sow- thistle and common dandelion extracts in 2000 l of water/ ha

PM fermented plant extract was made from fresh weeds and PM according to procedure described by Kyan *et al*. (1999).

Quasia extract was used two times in July in dose 20 g in 1000 l water. Whey proteins powder was used 3 times in July and 1 time in August in dose 12 kg in 1000 l water. MycoSin was used 7 times in dose 10 kg in 1000 l water.

Diseases incidence on plants was estimated according to method described by Solarska (1999). The numbers of cones injured by Damson-hop aphids and two spotted spider mites were counted and efficacy was calculated according to Abbott formula.

Table 1. Weather conditions at Jastków in 2010

month	**aver. temp. in C degree**	**aver. max. temp. in C degree**	**aver. min. temp. in C degree**	**Rainfall in mm**
April	8.8	14.2	4.1	30.5
May	13.8	18.3	9.9	162.1
June	17.3	22.3	12.5	69.6
July	20.8	26.2	15.6	79.2
August	19.3	25.1	14.5	70.9
	aver.=**16.0**	aver.=21.2	aver.=11.3	∑= **412.3**

Results

In 2010 Damson hop-aphids appeared in July and its population was low. The efficacy of tested preparations in reduction of the pest was very good, while quasia and PM fermented plant extracts were most effective against the pest (tab.2).

The two spotted spider mites also was reduced by tested preparations on very good level, while whey proteins powder and PM fermented plant extracts were most effective against the pest (tab.3).

Table 2. Injuries of hop cones cv. Marynka in result of aphids feeding and efficacy of treatment against the pest

Object	Number of damaged cones in scale degree:					Efficacy in %
	1	2	3	4	5	
EMa with plant extracts	499	1	0	0	0	96
Quassia	500	0	0	0	0	98
Plant extracts	497	3	0	0	0	90
EMa	493	7	0	0	0	85
Control	478	20	2	0	0	-

Injuries of cones according scalei: 1- lack of injuries, 2- injuries to 20% of cone, 3- injuries from 21% to 50% of cone, 4- injuries from 51% to 80% of cone, 5- injuries above 81% of cone

Table 3. Injuries of hop cones cv. Marynka in result of spider mites feeding and efficacy of treatment against the pest

Object	Number of damaged cones in scale degree:					Efficacy in %
	1	2	3	4	5	
EMa with plant extracts	500	0	0	0	0	98
Whey	500	0	0	0	0	98
Plant extracts	500	0	0	0	0	96
EMa	498	2	0	0	0	92
Control	478	20	2	0	0	-

Injuries of cones according scalei: 1- lack of injuries, 2- injuries to 20% of cone, 3- injuries from 21% to 50% of cone, 4- injuries from 51% to 80% of cone, 5- injuries above 81% of cone

Table 4. Efficacy of used natural products in protection of hop against downy and powdery mildews

Object	Efficacy against downy mildew (%)	Cones infected by Pseudoperonospora humuli (%)	Efficacy against powdery mildew (%)	Cones infected by Spherotheca humuli (%)
EMa with plant extracts	60	35	79	4,5
MycoSin	53	46	72	6,0
Plant extracts, MycoSin	59	38	74	5,5
EMa, MycoSin	50	47	63	8,6
Control	-	91	-	23,6

In 2010 downy mildew was very dangerous for hops because of very often rainfall especially in May (Tab.1). The symptoms of primary and secondary infection caused by fungus *Pseudoperonospora humuli* were observed and all treatments with using MycoSin and probiotic microorganisms with fermented plant extracts were too little effective (Tab. 4). The occurrence of powdery mildew was weaker than downy mildew in season 2010 and efficacy of tested preparations for disease control was sufficient. Amongst the tested natural products against powdery mildew PM fermented plant extracts were the most effective.

Discussion

The achieved results during studies show that protection of hops by organic methods such as use of probiotic microorganisms fermented with plant extracts is effective and this way of hop protection has similar efficacy to quasia in aphids control, to whey in two spotted spider mites control and to downy and powdery mildews control. Similar results were obtained by other authors who found that use of probiotic microorganisms in mixture with PM plant extracts defended some plants most effectively against diseases and pests (Kyan *et al.* 1999). In the study, two preparations formulated on the ground ProBio Original™ mother material used in mixture with PM fermented common sow-thistle and common dandelion extracts were very effective against hop aphids. From 2011 hop garden in Jastków is certificated as organic.

Acknowledgement

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DOWNY MILDEW CONTROL IN ORGANIC HOPS: HOW MUCH COPPER IS ACTUALLY NEEDED?

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Abstract

For the control of hop downy mildew, in an organic hop garden in the Hallertau two new copper hydroxide formulations were tested during 2010 with reduced amounts of copper (2 and 3 kg Cu/ha per year), compared to the currently permitted 4 kg Cu/ha. In addition, the effect of three plant strengtheners ('Biplantol', 'Frutogard' and 'Herbagreen') applied with the copper hydroxides was assessed. Compared to the untreated control plot (67.5 % cone infection on 18 August), all treatments provided distinct, highly significant better control (0.7 – 4.7 % cone infection). The controlling effect in the plots with 3 kg Cu/ha was always better than in the 2 kg Cu/ha plots. The addition of plant strengtheners always led to an even better control, with the best results with 'Frutogard'. The 'Frutogard' plot with 2 kg Cu/ha had still a lower infection rate than the current organic standard, copper oxychloride applied at 4 kg/ha, but it has to be taken into account that the use of 'Frutogard', a product that contains phosphonate, is currently discussed highly controversial in organic farming. However, according to these preliminary results from the first of three project years, it is most likely that with novel copper hydroxide formulations the use of copper for the control of fungal diseases can be reduced by almost 50 % in future.

Keywords: Organic hops, Downy Mildew, *Pseudoperonospora humuli*, control, copper hydroxide, plant strengtheners

Introduction

The control of fungal diseases is a crucial problem in most organic crops worldwide. In organic hop production, especially an ample control of downy mildew *Pseudoperonospora humuli* raises difficulties for the growers. As the use of synthetic pesticides is prohibited for organic growers, they are completely dependent on compounds that do also occur in nature. Hence, currently copper compounds like copper oxychloride or copper hydroxide are a key factor of downy mildew control and are permitted in organic hops in Germany at an application rate of up to 4 kg/ha elementary copper per year (Bioland, 2011). On the other hand, the use of copper as a heavy metal in plant protection is regarded as most critical by European environmental protection agencies, especially in Germany, where there are strong tendencies to ban the use of copper compounds in agriculture in general. To solve this dilemma, there are lots of ongoing research activities in German organic crops like fruit, grapes or hops, in order to to find alternatives to copper or, at least, to reduce the amount of copper used, for the control of fungal diseases. In this study, we introduce a three years' research project on the reduction of copper use in organic hops, and report on preliminary results from the first project year 2010.

Methods

The trial was carried out in an organic hop garden (cv. Perle) in Haushausen near Wolnzach in the Hallertau growing region of Bavaria. The entire field with a size of almost 1.3 ha was used for the trial. In the center of the field, a Burkard spore trap was set up to monitor the actual disease pressure (numbers of sporangia) in the garden from 4 May until harvest.

The trial comprised 13 treatments that were laid out in two replications, respectively, with two sub-plots to each replication. Treatments comprised an untreated control, copper oxychloride (4 kg Cu/ha) as organic standard, two new copper hydroxide formulations (SPU-02720-F, powdery, and SPU-02700-F, liquid) at application rates of 2 and 3 kg copper/ha, respectively, and three different plant strengtheners ('Herbagreen', 'Biplantol' and 'Frutogard'), each applied together with SPU-02700-F at 2 and 3 kg copper/ha, respectively. To each spraying a customary farm mixture of sulphur and fine mineral powders was added.

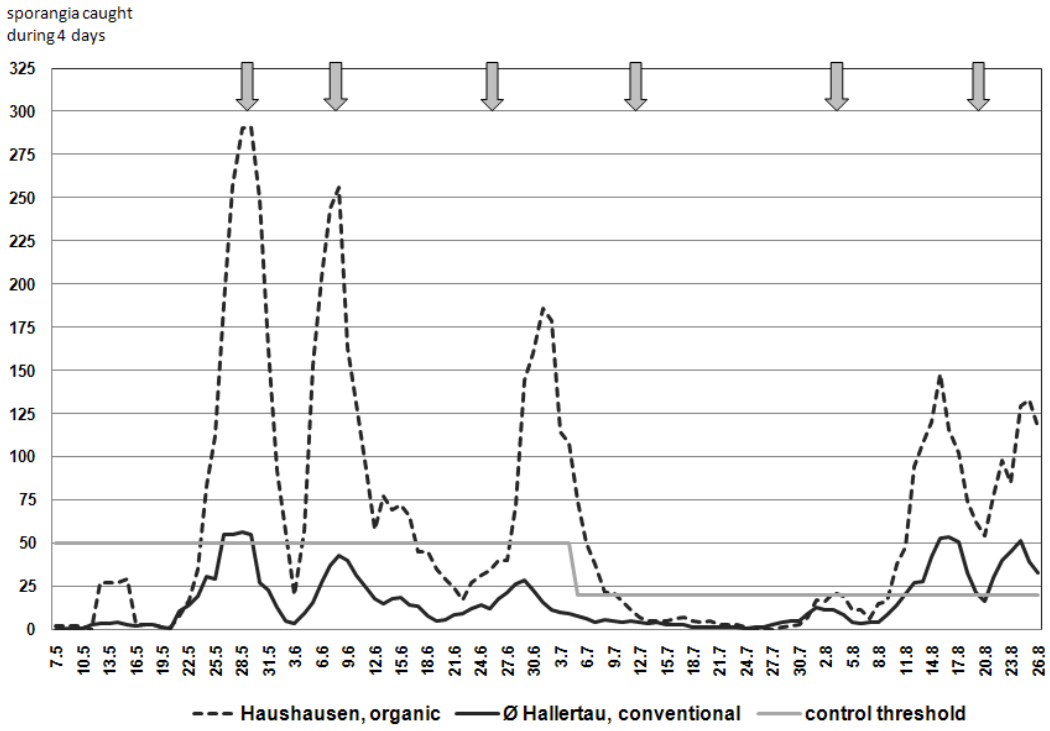

Figure 1: Downy mildew sporangia numbers caught in four days, respectively, in a Burkard spore trap in an organic hop garden in Haushausen, compared to the average number from four other traps in conventional gardens in the Hallertau, Germany, during 2010. The arrows indicate spraying dates in Haushausen.

Primary infection of Downy Mildew was controlled manually, by removing infected shoots ('spikes') from the plants in mid-May. The sprayings to control secondary infection were usually applied after a call for spraying was released by the regional disease forecasting system. Altogether six sprayings were applied in all plots (28 May, 6 June, 24 June, 12 July, 4 August, 20 August). 'Biplantol' and 'Herbagreen' were added to the spray mixtures only for the first five applications, and 'Frutogard' only for the first three applications.

The monitoring scheme comprised four assessments of primary infection (3 May, 17 May, 4 June, 23 June) and six monitorings of secondary infection (17 May, 4 June, 23 June, 8 July, 11 August, 18 August), split into infection of leaves, flowers, and cones, where appropriate. Finally, cones of all plots samples were taken during harvest (1 September) and the level of infestation was assessed on dried cones.

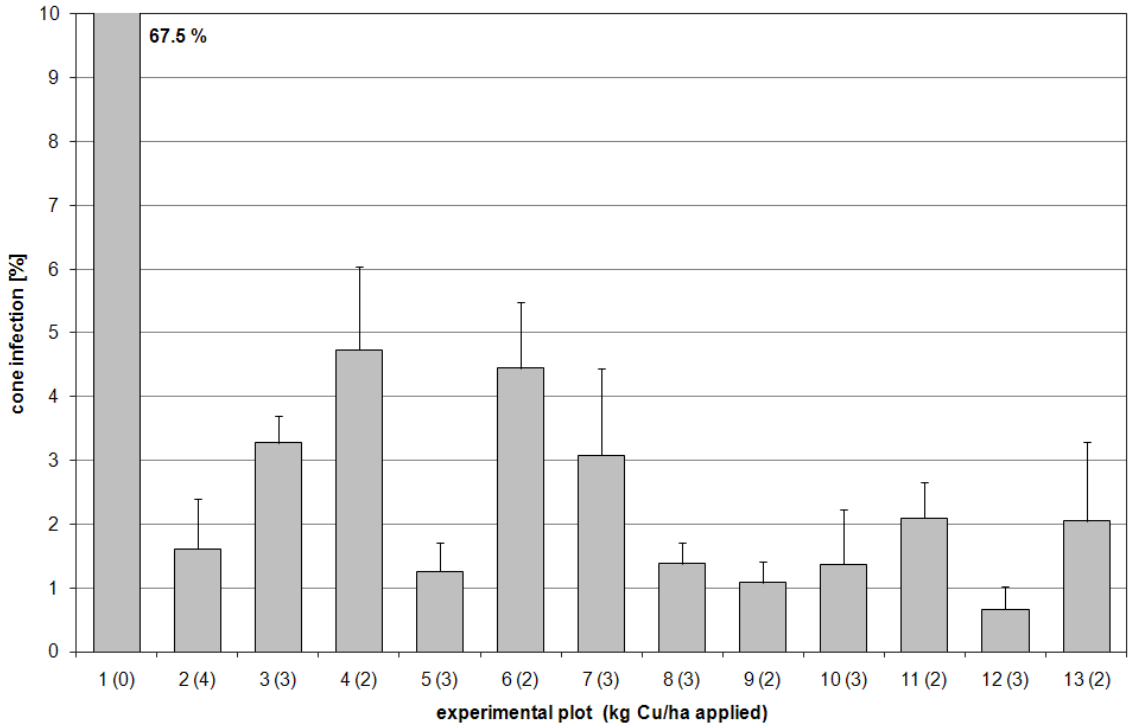

Figure 2: Downy mildew cone infection [%] in an organic hop garden in Haushausen, Hallertau, Germany, on 18 August 2010. Means ± s.e. of four replications, respectively. Experimental plots: 1 control; 2 Cu oxychloride (4 kg Cu/ha); 3 SPU-02700-F (3 kg Cu/ha); 4 SPU-02700-F (2 kg Cu/ha); 5 SPU-02720-F (3 kg Cu/ha); 6 SPU-02720-F (2 kg Cu/ha); 7 as 5; 8 SPU-02700-F (3 kg Cu/ha) + 'Herbagreen'; 9 SPU-02700-F (2 kg Cu/ha) + 'Frutogard'; 10 SPU-02700-F (3 kg Cu/ha) + 'Biplantol'; 11 SPU-02700-F (2 kg Cu/ha) + 'Biplantol'; 12 SPU-02700-F (3 kg Cu/ha) + 'Frutogard'; 13 SPU-02700-F (2 kg Cu/ha) + 'Herbagreen'.

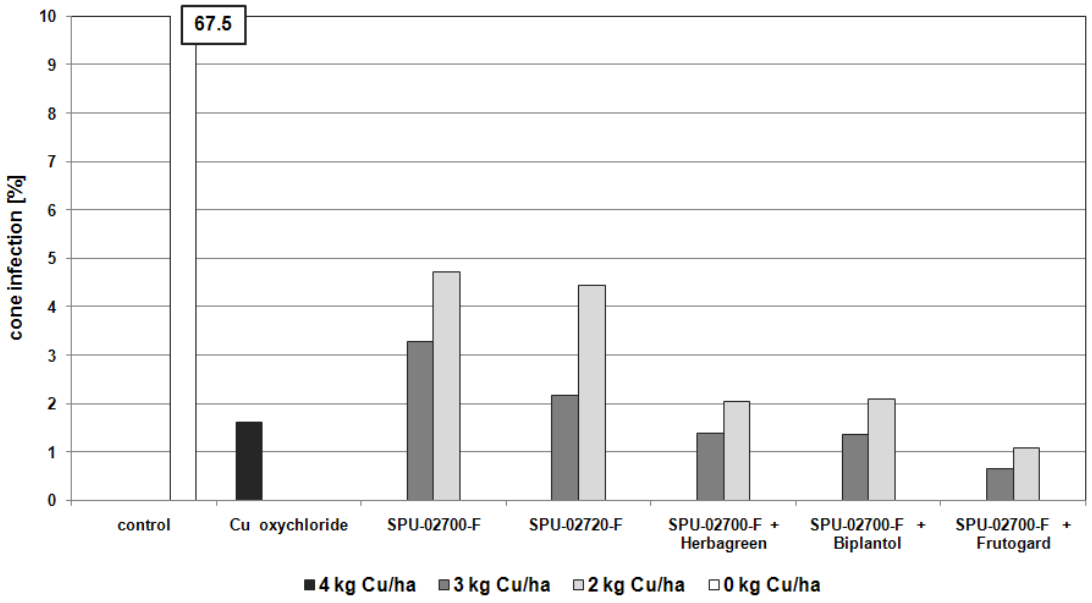

Figure 3: Downy mildew cone infection [%] in an organic hop garden in Haushausen, Hallertau, Germany, on 18 August 2010. Means ± s.e. of four replications, respectively. Comparison of the different treatments under consideration of the amount of copper applied.

Results

The year 2010 was characterised by an extremely high downy mildew primary infection, with up to 0.7 spikes per training on 4 June. This led to a very heavy disease pressure, which was mirrored by according numbers of sporangia caught in the spore trap in the experimental organic field, and which were up to six times higher than the average values in conventionally managed fields (Fig. 1). The disease pressure led to in 67.5 % infected cones in the untreated control plot on 18 August and 86.1 % infected cones in the harvest samples of the same plot. Nevertheless, all plots with copper treatments achieved a satisfactory efficacy of downy mildew control, however with distinct differences between the plots (Fig. 2).

The comparison of particular treatments with 2 or 3 kg Cu/ha and year shows that in all treatments the cone infection rate of the 3 kg Cu/ha plots was distinctly lower. Furthermore, the addition of plant strengtheners always led to an even better control, with the best results with 'Frutogard'. The 'Frutogard' plot with 2 kg Cu/ha had still a lower infection rate than the current organic standard, copper oxychloride applied at 4 kg/ha (Fig. 3).

Discussion

Most notable was the extreme disease pressure in the Haushausen organic garden, compared to the average disease pressure in conventional gardens. The reason is probably the crucial problem of organic hop growing, because, other than in conventional hop growing, there is no registered compound for the control of downy mildew primary infection. This leads later to a drastcially increased secondary disease spread, as clearly visible in Fig. 1.

The 2010 results of the project are very promising. Obviously the new formulations of copper hydroxide will be able to reduce the amount of copper used for the control of fungal diseases. Consequently, the liquid formulation 'SPU-02700-F' was registered in Germany only in February 2011 as a fungicide in hops and other crops under the trade name 'Cuprozin progress' (copper content 250 g/l), and the registration of the powdery formulation 'SPU-02720-F' is planned for mid-2011 under the trade name 'Funguran progress' (copper content 350 g/kg).

The addition of plant strengtheners even increased the control effect in all cases, but it has to be taken into account that the use of 'Frutogard', a product that contains phosphonate, is currently discussed highly controversial in organic farming. However, according to these preliminary results from the first of three project years, it is most likely that with novel copper hydroxide formulations the use of copper for the control of fungal diseases can be reduced by almost 50 % in future. On the other hand, the results also substantiate the statement of Engelhard et al. (2007) that for the control of fungal diseases in organic hops or any other organic crop a complete substitution of copper is absolutely not in sight, and that growers still have to rely on this compound and its future availability.

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THE EFFECT OF APPLICATION OF COPPER FUNGICIDES ON PHOTOSYNTHESIS PARAMETERS AND LEVEL OF ELEMENTARY COPPER IN HOPS

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Abstract

Photosynthesis and transpiration rates in the interval of 30 minutes before and 30 minutes after copper fungicide application show increase from the level of 5.0 to 7.0 μmol $CO_2.m^{-2}.s^{-1}$ and 0.75 to 1.00 mmol $H_20.m^{-2}.s^{-1}$. Long-term measurements show that increase of photo-synthesis rate after copper application is temporal and fades away after 10-14 days. Contents of elementary copper in hop cones are up to 500 mg/kg if total amount of applied copper does not exceed 15 kg/ha. Contents of copper on leaves are 2-5 times higher at the same application dose. Application of 5 kg copper per one hectare of vigorous growth of Agnus increases content of copper on hop leaves by 1000 mg/kg at least. The same amout of copper increases its content in hop cones by 300 mg/kg at the ripening period. Tight correlation between the amount of copper applied and its content in hops does not exist. Elementary copper from leaves is brought into harvested hops in the form of biological admixtures. Copper content in hop cones shows decreasing trend, which is given by gradual increase of cones size at the ripening period. Similar trend on hop leaves shows that washing and dissolving of copper compounds by atmospheric water can participate in this process as well. Common content of copper in hop cones and leaves is up to 20-25 mg/kg.

Keywords: hops, downy mildew, copper, photosynthesis rate, transpiration rate

Introduction

Downy mildew (*Pseudoperonospora humuli* L.) is the most important fungal disease of hop. It can cause high losses in yield and quality of harvested hops. First symptoms of infestation appear on young sprouts in early spring. Leaves are of green-yellow colour, stunt and due to shortening of internodia become accumulated. They are commonly call “spikes“. Spikes are the main source for further spreading of the disease in the course of hop maturation /Vostřel 2010/. An important regulation factor of downy mildew occurence in the phase of flowering and cone forming is application of copper fungicides. Disease control consists in disrupting the function of the cellular proteins of fungi and bacteria /Marsh, 1937/. Copper is an essential microelement needed by plants /Sommer, 1931/. Appr. 70 % of copper is localised in chloroplasts where it acts as chlorophyll stabilizer. Copper acts as acceptor or electron donor and becomes a component of many oxidation-reduction systems. It participates on lignin biosynthesis that stabilises cell walls. Copper as protein component plays important role in photosynthesis processes and cell respiration. Plant requirement for this chemical element is small and moves in the range of 2 to 25 ppm. Portner /2006/ presents total copper take-off 4054 mg per hectare of hop garden, from which falls 581 g/ha to cones, the rest for bine and leaves. There are few references describing the measurement of photosynthesis in hop. Peat /1974/ showed that mature cones had measurable photosynthetic activity, which rarely exceed respiratory loss. Hnilickova /2007/ determined saturation irradiance and photosynthetic capacity for several Czech hop varieties by gasometric method. Photosynthesis is affected by many environmental stresses that limit the potential growth of canopies. Many stresses are caused directly or indirectly by water shortages, mineral requirements, particularly nitrogen. Fungal dideases and pests may also induce stresses by reducing the green leaf area, the sap flow, or by altering plant metabolism /Baret, 2007/. Theoretically, application of pesticides by spraying on leaf surface can bring about stress conditions as well.

Hop is an important commodity used in food industry. Therefore, hygienic limits of pollutants (heavy metals) have to be followed and respected (Public Notice of Ministry of Health Nr. 298/1997). It is necessary to deal with this topic particularly for metals, which are contained in pesticides used for chemical control of hop. Maximum residue level (MRL) of elementary copper in hops and hop products valid in EU is 1000 mg/kg. Within the scope of research project „Integrated hop production" repeated tests of multiple copper fungicides application were performed with the aim of determination its impact on photosyntesis rate and on the content of elementary copper in hops in the course of 2008–2010 vegetation seasons.

Methods

Kuprikol 50, Kuprikol 250 SC, Cuproxat SC and Curzate K were used for the tests in the compliance with methodical hints. Copper fungicides were applied on Agnus variety with the help of common mistblowers. The experimental hop garden was divided into 3 parts, each of 0.3 ha acreage. The first treatment was applied on the whole plot, the second one on 2/3 of the experimental area and the third one on 1/3 of the area. Initial intention of three applications was realised only in 2008. During subsequent years only two applications were carried out due to early harvest (2009) or muddy terrain (2010). Kuprikol 50 was used in 2008, fungcides Kuprikol 250 SC and Cuproxat SC were used in 2009. In 2010 Kuprikol 250 SC was applied. With respect to high infection pressure of downy mildew Curzate K was applied out of plan on July 13th 2010. The exceptional application of copper increased its contents on leaves (109 mg/kg) at the start of the test. Application terms and amounts of elementary copper after each treatment expressed in kg/ha are summarized in Table 1. In 2008 totally 15 kg of elementary copper was applied in three treatments, in 2009 7.9 kg and 12.8 kg of copper in 2010. Photosynthesis and transpiration rates were measured in intact leaves with the commercial handheld infrared analyser LCpro+ (ADC Bio Scientific Ltd., UK) with a leaf chamber that enables measurements at an irradiance PHAR (400−700 mn) in the range of 0−2000 μmol.m^{-2}.s^{-1} and at the temperature of –5 to +50 °C. Measurements were made in regular intervals allowing the conditions to become fixed in the measuring chamber (23 °C, 600 nm of irradiation).

Table 1: Application terms of fungicides and dosage of elementary copper (kg Cu/ha)

Application	2008		2009		2010	
	Date	Cu (kg/ha)	Date	Cu (kg/ha)	Date	Cu (kg/ha)
I.	22.7.	5.0	27.7.	5.0	13.7.	2.8
II.	12.8.	5.0	14.8.	2.9	1.8.	5.0
III.	27.8.	5.0	-	-	22.8.	5.0
Cu total	*(kg/ha)*	*15.0*		*7.9*		*12.8*

Tests were started in the second half of July and lasted till the first decade of September. Samples of hop leaves and hop cones were taken after each treatment randomly from 10 plants at height of 2–6 meters in appr. one week intervals. Time dependence of copper contents on leaves and in cones were obtained for each application. Drying of plant material was peformed at ambient temperature in a dark room. Dry samples of cones and leaves were analysed in an authorised laboratory for the content of elementary copper by ICP-AES method. Detection limit was 1.5 mg Cu/kg.

Results and Discussion

Photosynthesis and transpiration rates in the interval of 30 minutes before and 30 minutes after copper fungicide application are shown on Figures 1 and 2. Determined data show that photosynthesis and transpiration rates increased after copper treatment from the level of 5.0 to 7.0 μmol CO_2.m^{-2}.s^{-1} and 0.75 to 1.00 mmol H_20.m^{-2}.s^{-1} respectively. It can be explained by suplement of copper ion to the photosynthetic system of leaf on the supposition that leaves of hop plant are able to accept copper cation after foliar application. Repeated measurements of photosynthesis rates in the course of vegetation season ranged in the interval of 4.5-8.0 μmol CO_2.m^{-2}.s^{-1} in the period 2008–2010. Long-term measurements show

that increase of photosynthesis rate after copper application is temporal and fades away after 10-14 days. Changes in photosynthesis rates reflect current weather conditions and development stage of hop plants.

Copper contents in hop cones are summarized in Table 2. Contents of elementary copper in cones after the first application dose moved in the interval of 300 to 400 mg/kg. The second copper treatment increased its content to the level of 500 mg/kg. The highest content of copper in cones 834 mg/kg was found in 2008 immediately after the third copper application. Final copper content in cones after all treatment was in the interval of 323 mg/kg in 2010 and 471 mg/kg in 2008. The amount of elementary copper on hop leaves is 2–5 times higher compared to cones (data not shown). It is given by lower ratio surface/weight, which is 15-20 cm^2/g for cones and 45-60 cm^2/g for leaves. The experiments were carried out in the period when development of leaf area was finished. On the contrary experiment period of hop cones comprised the whole development cycle from the stage of flowering to the harvest ripeness. Application of 5 kg copper per one hectare of vigorous growth of Agnus increases content of copper in hop cones by 300 mg/kg at the ripening period. The same amout of copper increases its content on hop leaves by 1000 mg/kg at least. Tight correla-tion between the amount of copper applied and its content in hops does not exist. Time series of the copper content on leaves and in cones show perceptible, though irregular, decreasing trend. It can be explained by gradual increase of hop cones size from the stage of flowering at the last decade of July to the mature size in the last decade of August. The same trend on hop leaves is probably caused by washing off, or dissolving of copper compounds by atmospheric water (rain, dew). Small copper demands of plants under the level of 20-25 mg/kg confirm contents of copper in untreated cones in 2009 (Table 2) and organic hops from 2010 crop harvest (22 mg/kg, Table 3). High contents of copper on hop leaves should not be underestimated. Elementary copper from leaves is brought into harvested hops in the form of biological admixtures. Potential contribution of biological admixtures shoud not exceed 60 mg/kg if hops contain up to 3 % of admixtures (leaves) and copper contents on hop leaves is max. 2000 mg/kg. Contents of elementary copper in harvested samples of Czech, German and Polish hops from the 2010 crop harvest are shown in Table 3. Most of values in Czech hops are in the interval of 20–300 mg/kg. The highest determined value in Premiant variety (633 mg/kg) is still far from the limit 1000 mg/kg valid for EU countries. Comparable data were obtained in Czech hops from the crop harvests 1990 and 1992. Individual values were mostly in the range of 250–350 mg/kg and just rarely contents above 1000 mg/kg were determined. Extremely high contents of copper in hops can be caused by technology mistakes - insufficient homogenisation of powdery preparatives before application. Most of values in German hops are in the interval of 100 až 400 mg/kg. There are also two limit values, high content in Taurus 807 mg/kg and low value of 19 mg/kg (Hallertauer Tradition) at the level of natural background as well. Copper contents in Polish hops are substantialy lower. Hop growing region is located nearby of Pulawy-Lublin in south-east Poland with different climate and lower infection pressure of fungal diseases.

Acknowledgements

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Figure 1, 2: The course of photosynthesis (left) and transpiration (right) rates of hop plants treated with copper fungicide before and after application in 2010

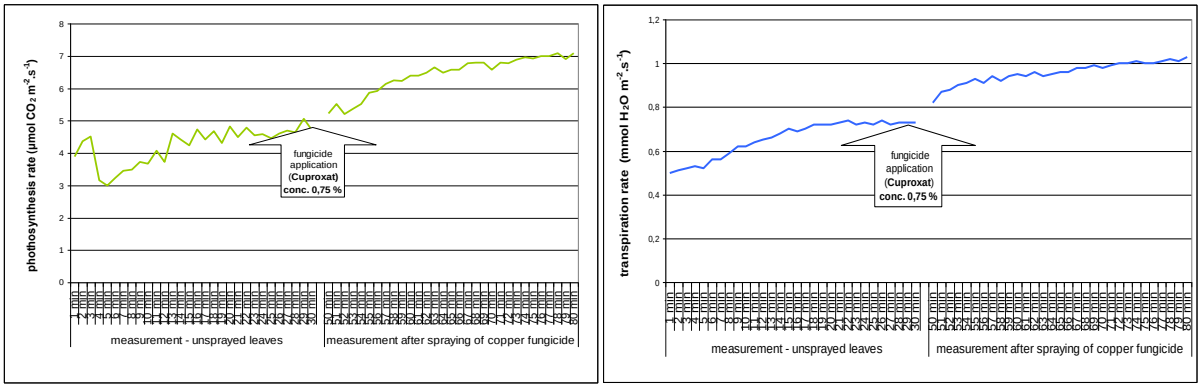

Table 2: Contents of elementary copper in hop cones after fungicide applications

2008							2009					2010				
Date	I. Application		II. Application		III. Application		Date	I. Application		II. Application		Date	I. Application		II. Application	
	days	content	days	content	days	content		days	content	days	content		days	content	days	content
21.7.	control	-	-	-	-	-	25.7.	control	6	-	-	29.7.	control	109	-	-
22.7.	2 hours	-	-	-	-	-	27.7.	2 hours	371	-	-	2.8.	1	312	-	-
24.7.	2	-	-	-	-	-	28.7.	1	302	-	-	4.8.	3	316	-	-
28.7.	6	-	-	-	-	-	31.7.	4	286	-	-	8.8.	7	219	-	-
31.7.	9	-	-	-	-	-	3.8.	7	395	-	-	16.8.	14	176	-	-
8.8.	17	405	-	-	-	-	6.8.	10	417	-	-	23.8.	22	208	1	482
13.8.	23	-	1	-	-	-	11.8.	15	335	-	-	30.8.	29	175	8	505
14.8.	24	201	2	504	-	-	14.8.	-	-	2 hours	489	6.9.	36	153	15	491
18.8.	28	133	6	487	-	-	17.8.	21	415	3	501	10.9.	40	144	19	323
25.8.	35	122	13	309	-	-	24.8.	28	429	10	556					
28.8.	38	93	16	247	1	834	28.8.	32	350	14	456					
1.9.	42	86	20	143	5	705										
5.9.	46	108	24	201	9	471										

Table 3: Copper contents in Czech, German and Polish hop varieties, crop 2010

Variety	Copper content (mg/kg)	Variety	Copper content (mg/kg)
Czech Republic			
Agnus	106	Saaz	148
Agnus	257	Saaz	270
Premiant	74	Saaz	214
Premiant	166	Saaz	145
Premiant	633	Saaz	179
Premiant	278	Saaz	99
Sladek	234	Saaz	192
Sladek	439	Saaz-organic	22
Germany			
Hall. Tradition	19	Herkules	328
Northern Brewer	206	Taurus	807
Perle	170	Spalter Select	249
Saphir	344	Opal	421
Poland			
Sybilla	44	Magnum	30
Lomik	33	Lubelski	153

MOVENTO - NEW INSECTICIDE FOR APHIDS CONTROL ON HOPS

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Abstract

Movento 100 SC belongs to the tetramic acids, a completely new chemical class of insecticides. Spirotertamat, the fully systemic active ingredient of Movento, shows very good efficacy against *Phorodon humuli.* Due to this mode of action, Movento 100 SC shows excellent activity, mainly by ingestion, against immature pest life stages. Product is very effective when is applied by spraying in dose 1.5 l/ha in 2000 l of water. Movento 100 SC is new, valuable tool in antiresistance strategy of *Phorodon humuli* control on hops.

Keywords: hops, damson hop aphid, control, Movento 100 SC

Introduction

Damson-hop aphid is one of the plant –eating pests that every year infests hop plants and cause damage of economic importance. Therefore hop plants must be carefully protected against the pest (Jastrzebski 2004). The majority of aphicides applied to control *Phorodon humuli* lose their efficacy a short time as its resistance to the successive pesticide groups becomes greater (Loxdale et. al 1998). Hop protection against *Phorodon humuli* in Poland for more than last ten years is based mainly on foliar application of imidacloprid (Confidor 200 SL). Generally Confidor 200 SL provides good efficacy, when is applied in line with label recommendation. Nevertheless in last few seasons was observed weaker efficacy of Confidor 200 SL, mainly due to dry application period and partly due to too late applications on plants strongly attacked by aphids. Some samples of *Phorodon humuli* from Region Lublin were tested in Bayer CropScience Lab Monheim, results confirm low level of resistance of *Phorodon humuli* in Poland. Even if the current biological effectiveness of imidacloprid is sufficient there is urgent need to develop new efficient aphicide (Vostrel, 2007).

Movento 100 SC is developed in priority for the control of sucking insects such as aphids, whiteflies, scales and mealy bugs on a wide range of crops and contains 100 g as/L. The tetramic acid derivative spirotetramat belongs to a completely new chemical class of insecticides. As spiromesifen or spirodiclofen, it inhibits the lipid biosynthesis (LBI). Studies to determine cross-resistance with Movento 100 SC with other commercial insecticides have demonstrated no cross-resistance.

After foliar application, spirotetramat shows full systemic behaviour. Since the contact activity of spirotetramat is only limited a sufficient leaf uptake is needed for a high efficacy.

Due to this mode of action, Movento 100 SC shows excellent activity, mainly by ingestion, against immature pest life stages providing extended residual activity.

Spirotetramat, is a fully systemic insecticide with a xylem as well as a phloem mobility into the plant. Following application to plant foliage, Movento 100 SC moves to all plant tissues including new shoot, leaf and root growth.

Movento 100 SC has a very low toxicity against beneficial arthropods.

Movento 100 SC must be applied onto the foliage of the crop using appropriate spray equipment. The volume of the spray to be used must be sufficient to give a correct coverage but due to its full systemic properties some poor level of canopy covering can be balanced.

There are no specific recommendations concerning the spray volume. Common practice volumes used in the trials presented generally followed rules related to the canopy height:

For hop, more than 2000L/ha is normally used.

Existing or ongoing registrations: Movento is under development worldwide. The main formulations are the SC100 gai/L, 240 SC and OD 150 gai/L. Various formulations of Movento are registered for hop protection against *Phorodon humuli*:

- Movento 240 SC in USA, Canada,
- Movento 150 OD in Austria, Czech Republic, Germany
- Movento 100 SC is registered in Switzerland and in Poland label approval expected end of 2011.

Material and methods

In the growing seasons of 2009- 2010 five efficacy trials with Movento 100 SC in control of *Phorodon humuli* on hop were conducted according EPPO guidelines PP 1/22 (3). Studies were performed at the Hop Experimental Stations at Jastków and Kępa and on private farm in Piotrowice, region Lublin. In all trials standard product was Confidor 200 SL applied in recommended dose 0,54 l of product/ha. In two trials in season 2010 Teppeki 50 WG was applied as additional standard in dose 0,18 kg/ha. Tested dose of Movento 100 SC was 1,5 l of product/ha, water volume for all products: 2000 l/ha.

Treatments were conducted in the July when an average of 11- 35 aphids were found per leaf. Average numbers of aphids on leaves were defined prior to treatments and 3, 7, 14 and 21 days after treatment. Aphids were counted on 50 leaves sampled randomly from 25 bines in the middle of each plot. By 25 leaves sampled from the upper, 13 leaves form middle and 12 leaves from lower parts of bines. The degree of damaged cones was estimated and the presence of aphids in cones was determined during harvest of hops. From each plot, 10 randomly chosen bines were harvested and 10 cones were sampled from each bine. Total number of estimated cones from one plot amounted 100. Injury of cones was estimated according scale: 1 – lack of injury, 2 – to 20% of damaged cones, 3 – 21-50% of damaged cones, 4 – 51 – 80% of damaged cones, 5 – above 81% of damaged cones (Solarska, Jastrzebski, 1998).

Potential effect of phytotoxicity was assessed during each efficacy assessment.

Efficacy of chemical action was calculated according to the Abbott formula.

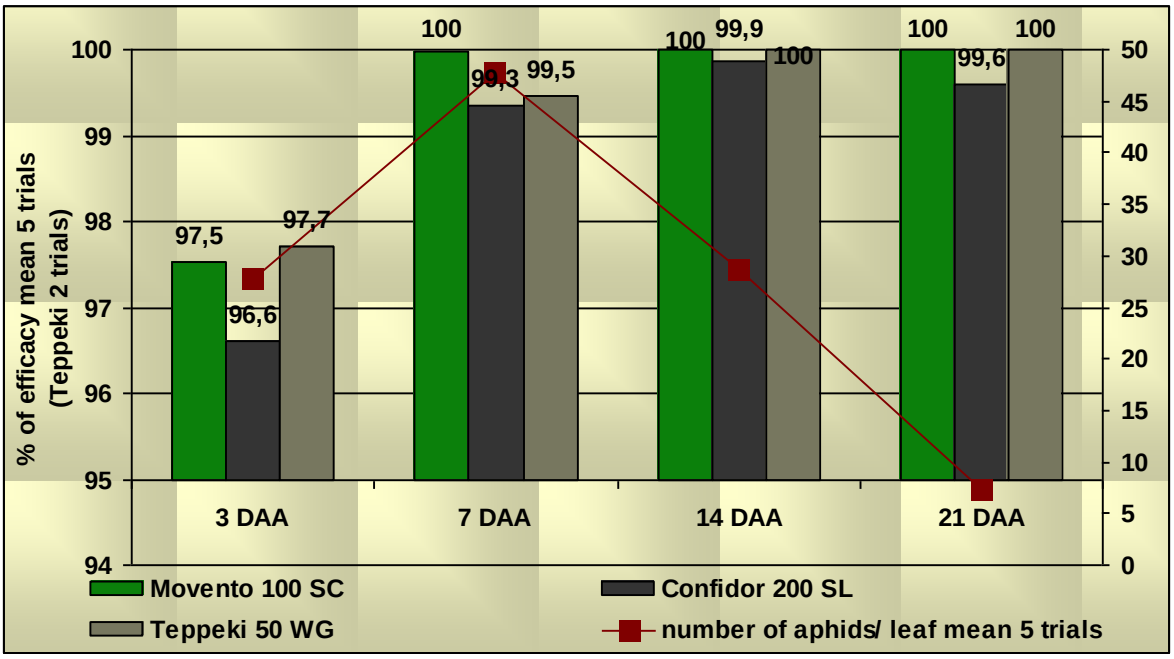
Results and discussion

Results of 5 trials conducted in seasons 2009-2010 confirmed very good efficacy of tested insecticide Movento 100 SC applied in dose 1,5 l/ha in control of *Phorodon humuli* on hop (fig.1). In each trial level of infestation was enough to carry out good trials but very often from 14 days after treatment natural slow reduction of aphids population in control object was observed. Efficacy results confirm good initial and lasting efficacy of Movento 100 SC equivalent or superior to standards Confidor 200 SL and Teppeki 50 WG.

The degree of damaged cones and the presence of aphids in cones, determined during harvest of hops confirmed positive effect of application tested product on quality of cones, no injury of cones was observed. No phytotoxicity was observed during efficacy assessments. Mentioned above natural reduction of aphids population shortly after treatment occurs probably in result of Confidor application. Development of *P. humuli* population depends mainly on weather conditions, of which air temperature as well as sum and distribution of rainfall in the summer have the most significant influence on the course and rate of pest development (Miciński&Ruszkiewicz, 1974).

Confidor has been applied in Poland to control *P.humuli* on hops since 1993. Since 1994 the population has been found to develop rapidly on untreated plants at the beginning of hop development, and then sometimes to decline rapidly from early July or late June. The changes in populations can not be caused by weather conditions. The changes in the dynamics of *P.humuli* populations not treated with Confidor may result from its sublethal detrimental effect that continues to affect –subsequent generations (Jastrzebski 2004).

Fig. 1. Efficacy of Movento 100 SC in control of *Phorodon humuli* on hop



DAA- days after application

Conclusions

In 5 trials carried out in Poland, results with Movento 100 SC used at 150 g as./ha against *Phorodon humuli* were quite consistent and homogeneous: at that tested dose rate, Movento 100 SC provided excellent control of aphid population, even in severe and quite curative circumstances, from 3 to 21 days after the application. Results were equivalent to or superior, for the initial efficacy and lasting effect, to the standard CONFIDOR 200 SL in all trials and Teppeki 50 WG in two trials. No phytotoxity was observed during efficacy trials conducted in seasons 2009-2010.

Movento 100 SC is highly effective tool as a part of antiresistance strategy in control of *Phorodon humuli* on hop. Further trials in high infested by aphids locations are planned for seasons 2011 and 2012.

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HEXYTHIAZOX, THE MITICIDE FOR SPIDER MITE (*TETRANYCHUS URTICAE* KOCH) CONTROL IN CZECH HOPS

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Abstract

Hexythiazox, as a perspective miticide from the group of thiazolidine mite growth regulators has been used to control two-spotted spider mites (*Tetranychus urticae* Koch) on hops in Czech Republic since 1996, when it was registered there under the commercial name Nissorun 10 WP. To investigate the phenomenon of *T. urticae* resistance to this compound, samples of field populations of this pest were taken within the individual hop growing CR regions in 2004, 2007 and 2010. After their transfer into laboratory rearing in an air-conditioned bio laboratory they were subjected to laboratory tests in a Potter tower. Values of C 100 M and LD 90 (100, resp. 90% mortality) were determined to find out if this miticide was still good enough to control spider mites in Czech and Moravian hop gardens within an anti-resistant strategy.

Keywords: two-spotted spider mite (*Tetranychus urticae* Koch), hop (*Humulus lupulus* L.), hop protection, miticide, hexythiazox, resistance, LC 50, C 100 M, anti-resistant strategy.

Introduction

Spider mites are a pest threatening many crops all over the world. Because of their great reproductive capacity, they are able to destroy plants within a short space of time. Population density of spider mites depends on the temperature and relative humidity. A rather short development time ensures six to a maximum of nine generations during a season, which increases the danger of resistance (MALAIS & RAVENSBERG 1992). Two-spotted spider mite (*Tetranychus urticae* Koch) is second only to the damson-hop aphid [*Phorodon humuli* (Schrank)] as the most dangerous pest of hop in all hop-growing regions in the world. Severe infestation can cause complete defoliation. Most economic damage is associated with cones. Spider mites not only contaminate the cones by their presence, but feeding on cones results in desiccated, brittle and discolored cones. Cones damaged by spider mites tend to shatter so that both quality and quantity of yield is reduced. Oxidation of damaged cones is accelerated and storability is reduced (MAHAFFEE ET AL. 2009). Under unusually hot and dry weather conditions during June and July of 1976 outbreaks of two-spotted spider mites occurred, and it was found that organophosphorous insecticides had become totally ineffective against field strains of *T. urticae* in Czech hop-yards. Since that time a series of miticides have been used against this pest and less efficient pesticides had to be gradually replaced by more effective ones (VOSTŘEL 1996).

For more than 20 years, hop protection against two-spotted spider mite in Czech Republic has been nearly entirely based on the treatment made by hexythiazox (Nissorun, Ordoval, Savey) applied prior to adult mite buildup, up to burr formation in hop vines, whereas propargite is used to control spider mites in the time of hop cone formation (VOSTŘEL 2009). Fenpyroximate is recommended to apply in July if population density of spider mites does not exceed the level of five mobile stages of *T. urticae* per leaf. In the years with lower population densities of *T. urticae*, one application of fenpyroximate or hexythiazox was usually sufficient to control this dangerous pest (VOSTŘEL 1999). Despite its good effectiveness in some cases spider mites have survived after propargite application and therefore there is the need to replace it. Therefore, bifenezate will extend the spectrum of registered miticides for efficient control of spider mites in hops with EU. Nevertheless, to ensure high efficacy of these specific zoocides good application practice is needed. This consists of maintaining the recommended rate of application together with a rotation of these

efficient miticides (VOSTŘEL 2010), which should be applied only if it is actually necessary to reduce costs for farmers and support integrated pest management measures (WEIHRAUCH 2001, 2005).

Materials and Methods

To investigate the phenomenon of *T. urticae* resistance to hexythiazox, samples of field populations of this pest were taken within individual hop growing regions. Field samples of *T. urticae* were collected during harvest time in the third decade of August and culture set up in the laboratory. Their offspring were used in the laboratory tests. Spider mites were placed in an air-conditioned room at a temperature of 20-22°C, 16-hours photoperiod and 60-70% RH. Hop seedlings were used as host plants. These plants were grown in a glasshouse throughout the year. Hop leaves were taken from untreated or residue-free hop plants.

The spraying method (HŮRKOVA & GESNER 1981) required discs of host-plant leaves placed on moist filter paper in Petri dishes in order to prevent tested spider mites from escaping. Petri dishes were placed at the bottom of a Potter tower (30 cm in diameter and 96 cm high) and sprayed with 1.0 ml of a solution of hexythiazox (trade name: Nissorun 10 WP) using the Potter's nozzle under a pressure of 0.2 MPa. A geometric series of four concentrations (0.05; 0.025; 0.0125 and 0.00625%) was tested. Treated leaf discs were removed from the tower after a sedimentation time of 10 minutes.

Twenty-four hours before spraying 25 females were transferred to each of four discs cut from hop leaves so as to reach a total of 100 mites in each experimental unit and to deposit eggs. The leaf discs were placed in a 9 cm diameter Petri dish. Moist filter paper was placed at the bottom of the Petri dishes to prevent spider mites from escaping. Shortly before hexythiazox application females were removed using a fine, slightly moistened brush so as just eggs stayed on the discs. The tested miticide was applied in the following sequence: non-treated (control) leaves and treated leaves in order from the lowest (0.00625%) to the highest (0.05%) tested concentration. Emerging larvae were counted each day for the period of 4 days after spray so as to determine mortality of the tested spider mites. Each experiment was replicated three times; hence 300 spider mites were tested under each concentration of hexythiazox. Resistance factors (RF) could not be established because the LD 50 values needed for their determination were exceeded in all four concentrations that were tested. Values of C 100M, LD 90 and percentage of *T. urticae* mortality within the individually tested concentrations for the resistant field strains were determined as average values for the individual regions: Žatec (Louny and Rakovnik districts), Úštěk and Tršice. The results from 2004, 2007 and 2010 are reviewed in Table 1

Results

It is obvious from Table 1 that in terms of average values for each tested concentration, there were no large differences in mortality of T. urticae strains sampled from the individual hop growing regions nor were there differences between the individual years. No larvae emerged from eggs if hexythiazox was applied in the highest tested concentration under which it is registered and methodically recommended for practical hop protection against two spotted spider mites in Czech and Moravian hop gardens. Mortality between the individual years did not change much even if the miticide was sprayed in 0.025%. The lowest concentration was used the bigger differences between the seasons were found out. The most severe decline in the mortality of eggs was observed in the lowest tested concentration in Žatec region, Louny district (89.3% biological efficiency in 2004 and 67.4% efficacy in 2010). On the contrary, in Úštěk region the mortality dropped just by 9.3% if we compare these years. The same trend can be observed in C 100 M and LD 90 values, which are derived from T. urticae mortality.

Discussion

As miticide hexythiazox has been used to protect hops against resistant strains of two-spotted spider mites in Czech and Moravian hop-yards for fifteen years, we can expect

lower efficiency of this compound due to the development of *T. urticae* resistance. To prevent the outbreak of spider mites it is necessary to carry out regular laboratory tests to find out biological efficacy, C 100 M and LD 90 in field strains of two-spotted spider mite sampled before harvest every year (VOSTŘEL 2009). Its efficacy is determined in other crops as well. In experiments carried out in Florida strawberry plantations hexythiazox belonged together with bifenezate and abamectin to the most efficient miticides and provided significant reduction of this pest (PRICE & NAGLE 2009). The spectrum of the registered miticides in CR will be extended by another compound, bifenezate (VOSTŘEL 2010), which is not only fairly efficient on resistant spider mites but its great potential as a selective pesticide makes bifenazate a very good prospect for an IPM hop protection system (JAMES 2002).

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Table 1: Biological efficiency of hexythiazox (Nissorun 10 WP) on two-spotted spider mite (*Tetranychus urticae* Koch) in laboratory tests in 2004, 2007 and 2010

Tested Strain	Concentration of Nissorun 10 WP/ Mortality [%]					
	0.05%	0.025%	0.0125%	0.00625%	C100M	LD90
Žatec Region						
Louny District						
2004	100.0%	100.0%	95.9%	89.3%	0.025%	0.00691%
2007	100.0%	99.1%	95.1%	82.3%	0.05%	0.01001%
2010	100.0%	98.2%	86.3%	67.4%	0.05%	0.01638%
Rakovník District						
2004	100,0%	100.0%	96.7%	87.1%	0.025%	0.00814%
2007	100.0%	98.8%	93.8%	80.8%	0.05%	0.01067%
2010	100.0%	97.7%	90.0%	70.8 %	0.05%	0.01250%
Úštěk Region						
2004	100.0%	100.0%	96.3%	90.0%	0.025%	0.00625%
2007	100.0%	99.3%	97.5%	87.5%	0.05%	0.00781%
2010	100.0%	99.3%	91.2%	80.7%	0.05%	0.01178%
Tršice Region						
2004	100.0%	100.0%	97.5%	90.5%	0.025%	%
2007	100.0%	100.0%	97.0%	78.5%	0.025%	0.01014%
2010	100.0%	98.7%	89.7%	77.3%	0.05%	0.01292%

PESTICIDE SCREENING PROGRAMME OF HOPS BY V.F. HUMULUS

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Abstract

Since September 1, 2008, new unified standards of MRL (Maximum Residue Limit) have been introduced throughout the EU. The regulation covers approximately 1100 pesticides used in agriculture, either within the EU or in other countries. In 2010, there were 26 registered active substances for the protection of hops allowed for use in Czech Republic. On the basis of brewers' increasing requirements for health safety of hop products (such as regulations valid in Japan - Positive List System for Agricultural Chemical Residues in Foods (May 2006)), we have decided to extend our research and business activity and begin an assessment of pesticide residue in hops. During the growing season 2010, we monitored content and decomposition curves of active substances (*eg*. Fosetyl-Al, Azoxystrobin, Fenpyroximate, Flonicamid) for selected pesticides. Samples for GC-MS and LC-MS analysis were prepared by a modified multiresidue method QuEChERS. We tested samples of hop cones collected during the harvest for all substances that had been used during the growing period. Last year, we analyzed a total of 22 types of pesticides. In the future, we plan to broaden the list of analyzed pesticides (including for example Cymoxanil, Mandipropamid, Spirotetramat, MCPA, *etc.*) and work on reducing LOD (Limit of Detection) for selected pesticides. Until now, the performed analysis of hop products has not shown any which exceed MRL standards.

Keywords: pesticide, LC-MS, GC-MS, QuECheRS, hops

HOP SNOUT WEEVIL (*NEOPLINTHUS TIGRATUS PORCATUS* PANZER) IS THE IMPORTANT INSECT PEST OF HOP (*HUMULUS LUPULUS* L.) IN SLOVENIA

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Abstract

Hop snout weevil (Neoplinthus tigratus porcatus Panzer) with another hop pest (hop damson aphid, spider mite, European corn borer, hop flea beetle) is important pest of Slovenian hop gardens in last ten years. The increasing appearance is connected with changing of climatic conditions and agrotechnical measures (reduction use of contact insecticides and giving up phytosanitary hygienic measures). The hop snout weevil is present only in Savinjska valley, and absent in Koroška and Ptuj-Ormož region. In spring 2011 hop plants were damaged from 10 to 70 % with hop snout weevil in hop fields in Savinja valley. The infestation level in hop fields with hop snout weevil wasn't in correlation with the variety of hops or age. Protection against hop snout weevil in hop field is difficult because we don't have registered any insecticides in Slovenia yet. High toxic insecticides which were used for this purpose will be replaced by a new insecticide, which will be friendly to environment. New non-traditional methods for control weevils, including biological ones (used entomopathogenic nematodes) are tested in spite of the fact that their utilization in practical conditions seems to be very difficult.

Keywords: hop snout weevil, *Neoplinthus tigratus porcatus,* hop, *Humulus lupulus*

Introduction

Snout weevil attack not only vegetables, ornamental plants, viticulture and fruit productions, but they cause a lot of problems also in hop fields. In other European countries, the most damage is caused by alfalfa snout weevil (*Otiorhynchus ligustici* L.). Till now it wasn't common pest of hop, but lately the increase of population is mentioned in all hop producing countries in Europe (Czech Republic, Germany and France) (Vostrel, 1997; Engelhard, 2007). In America the damage in hop is not caused by alfalfa snout weevil (*O. ligustici* L.), but other species of snout weevil (*O. ovatus, O. sulcatus, O. rugosotriatus*), that causes similar damage as alfalfa snout weevil (Barbour, 2009).

In Slovenia, the more common species of snout weevil which causes problems in hop fields is hop snout weevil (*Neoplinthus tigratus porcatus* Panzer). It was first found in Savinjska valley in 1893 and it is most likely that he immigrated from wild hop to grown hop (Kač, 1957). It's one of the oldest pests of hop, and the most damage he caused before world first war. In last 50 years hop snout weevil didn't caused a lot of damage, but now, after year 2000, his population has increase (Rak Cizej and Radišek, 2009).

The most damage of hop snout weevil (*Neoplinthus tigratus porcatus* Panzer) it's not caused by adults. Economic losses can results from larvae feeding which make curve corridors into the roots and too stems of hop plants. Root damage by larvae reduces nutrient uptake and plant growth and increases water stress. Consequence of this is can reduce quality and quantity of hop yield. Heavy infestations may cause that individual plants destroy (Rak Cizej and Žolnir, 2002).

Materials and methods

Monitoring of hop snout weevil in hop fields

During the years 2008 and 2011 were checking hop fields to monitoring hop snout weevil (*Neoplinthus tigratus porcatus* Panzer) in spring time, by hop cutting. Hop fields were randomly chosen in all hop production regions in Slovenian (Savinjska valley, Koroška region and Ptujsko-Ormoška area.). In every field randomly choose 100 plants of hop, counted the number of underground stem in one plant and checked them for larvae (we checked approximately 500 underground stem in one hop field).

Results and Discussion

Presence larvae of hop snout weevil in hop fields

In underground hop stems we found larvae of hop snout weevil (*Neoplinthus tigratus porcatus*) and no larvae of alfalfa snout weevil (*Otiorhynchus ligustici*). Between years 2008 and 2011 we checked 71 hop fields of all different ages and variety. There were 18 fields between 2-5 years, 20 fields 6-9 years, 21 between 10-13 years, 8 fields of age between 14-17 and 4 more than 18 years old. We checked hop gardens with different varieties (Aurora, Dana, Savinjski golding, Celeia and Bobek), with most frequent examination in hop gardens planted with Slovenian widespread variety Aurora.

In the beginning we expected in the older plantations greater damage plants. We have found exactly the opposite; young fields (2-5 years)have big percent of damages (in average they have 25 % damaged underground stems), while the oldest fields have the lowest percent of damages (only 13 %) (Fig. 1). Somewhere in the middle (18 % damage in average per field) were fields from 6-13 years old. Age of hop field didn't play any role in number of damages which caused hop snout weevil.

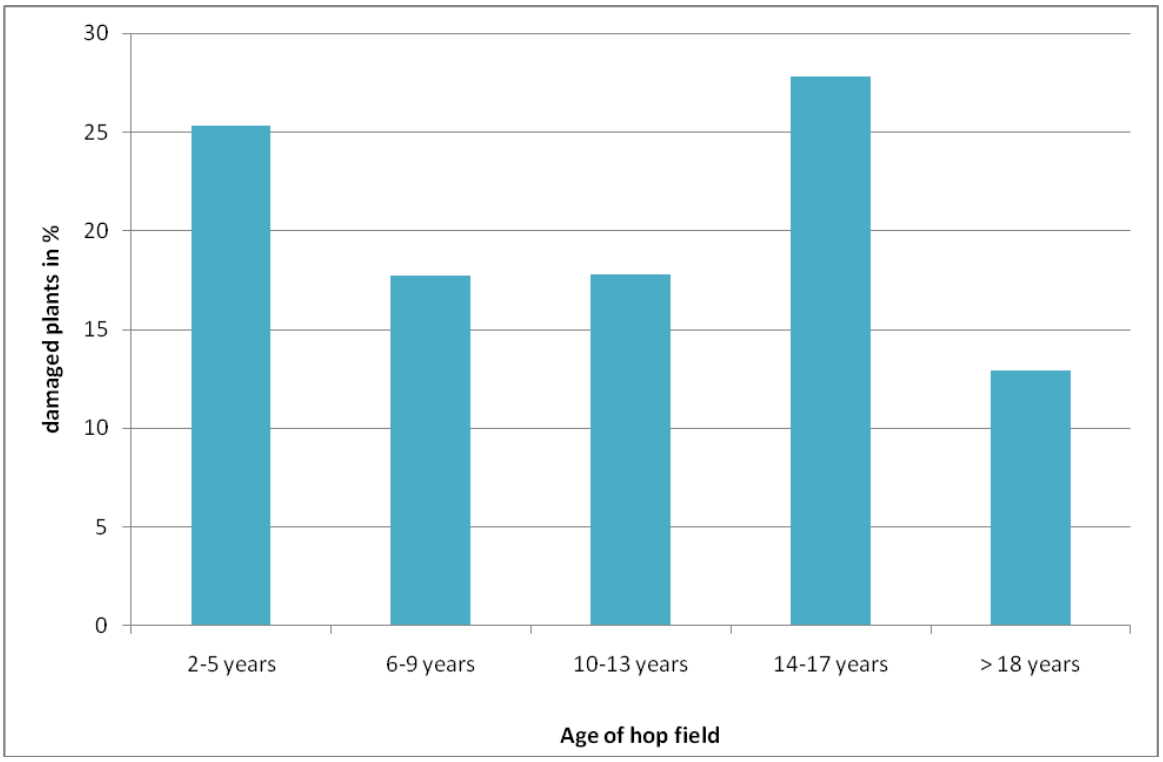

Fig. 1: Damaged plants in hop fields caused by hop snout weevil (*Neoplinthus tigratus porcatus*), April 2008-2011

In all the years of monitoring, we found out that the biggest population of hop snout weevil is in Savinjska valley. In all examined fields in Savinjska region we find damage from hop snout weevil. Hop snout weevil was not found on Koroška region and Ptuj-Ormož area. In Fig. 2, we can see percent of damages caused by hop snout weevil on hop plants depended from location, variety and age of plantation.

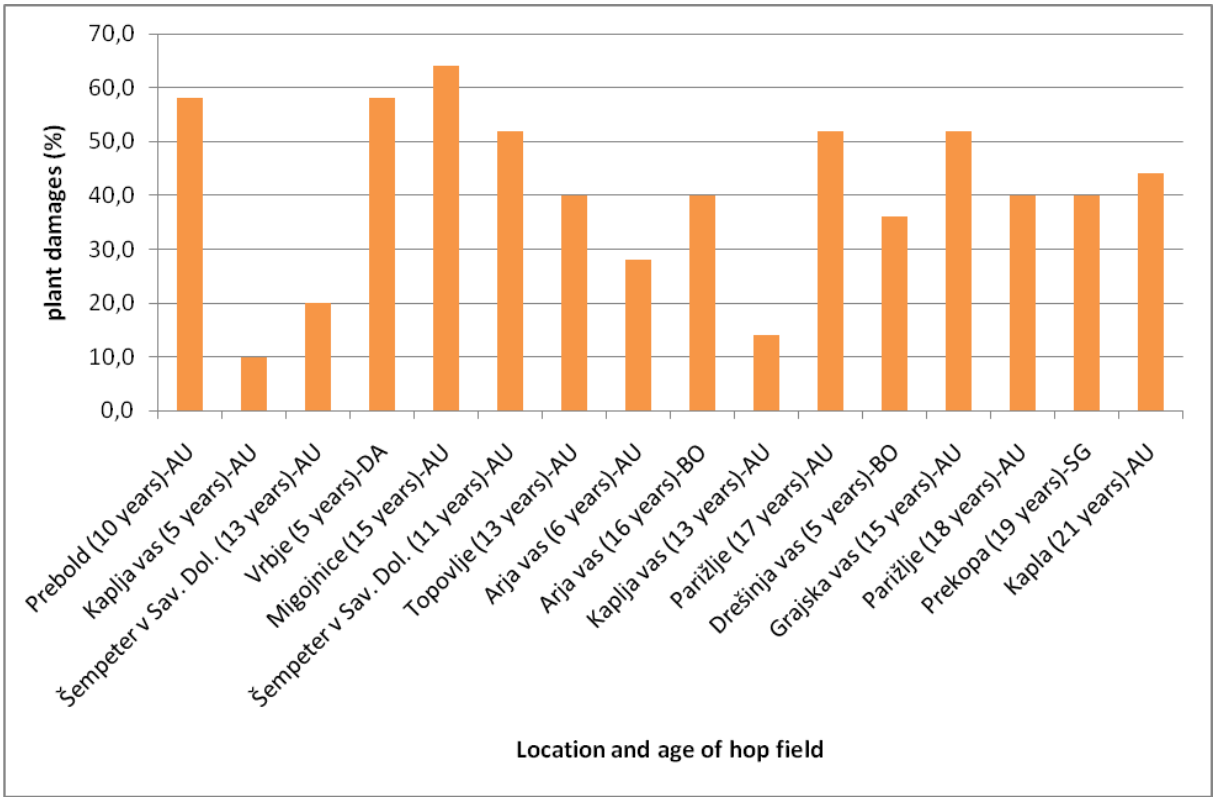

Fig. 2: Damaged plants in hop field of hop snout weevil (*Neoplinthus tigratus porcatus* Panzer) in Savinjska valley, April 2011

Legend: AU-aurora, DA-dana, BO-bobek, SG-savinjski golding

The damages on hop plants were between 10 and 70 %. We can also see, that age of hop field and variety of hop don't play important role of occurrence hop snout weevil. The only thing that we can do against it, is the consistent use of phytosanitary measures. One of those measures, which is the most important, is that after we cut hop plants, we clean all the remains and destroy them properly (with burning). Till now, we don't have any registered insecticide, that would kill larvae of hop snout weevil and repression of adults is impossible, because they don't feed with leaves of hop.

Conclusions

- The biggest population of hop snout weevil is in Savinjska valley. It didn't appear in Ptuj-Ormož area and Koroška region.
- The damage is caused by larvae and not by an adult. They make curve corridors in stems and roots.
- The population of hop snout weevil is increasing every year. The reasons for that are probably because don't carry out phytosanitary hygienic measures, changes in weather conditions, reduced use of insecticides with contact mode of action.
- To reduce the population requires a careful implementation of phytosanitary hygienic measures. The most important measure is that after cutting hop plants we clean all the remains and destroy them properly. The burning is the best way to destroy attack plants.

- Use of insecticides, from the aspect of protecting the environment as well as their poor performance, is unacceptable. In the future it will be necessary to study the possibility of biological control of larvae of hop snout weevil.

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HOP PROTECTION AGAINST ALFALFA SNOUT BEETLE (*OTIORHYNCHUS LIGUSTICI* L.) WITH THE HELP OF METEOROLOGICAL DATA IN BOHEMIAN AND MORAVIAN HOP GARDENS

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Numerous species of root weevil, *Otiorhynhcus* spp. (*Coleoptera: Curculionidae*), infest hop (*Humulus lupulus* L.). Whereas black vine weevil (*O. sulcatus* F.) is dominant species infesting hop in Washington and Oregon hop yards (MAHAFFEE, ET AL., 2009), alfalfa snout beetle (*Otiorhynhus ligustici* L.) is the most important pest from this family in Czech and Moravian hop gardens (VOSTREL, 1999). This family contains more than 40.000 species, all of which have a head that is extended into a long snout or rostrum. The jaws are on the end of the rostrum and the antennae are implanted in the middle (MALAIS & RAVENSBERG, 1992).

The wingless beetles are about 9-13 mm long of black to black-brown color. They usually shelter under clods of earth during the day where they are difficult to see since their color blends with the soil. They are active in the spring when they lay eggs in the soil from which the legless larvae develop and feed on the roots before pupating (NEVE, 1991). Severe larval infestation can significantly shorten the life of a hop yard. The larvae of this beetle are not easily controlled chemically because they live in the soil in hop crowns. In trials carried out to try to control larvae in a nontraditional way good results were obtained neither from the pesticides applied in form of watering in the autumn nor from the granular insecticides applied onto soil surface to the hop plants and by injection into the rootstock. (VOSTREL, 1988). The only efficient way to control *O. ligustici* is to apply an efficient insecticide in the spring when newly born adults emerged from soil and damage young hop shoots. In the past treatment was recommended when 100 beetles per 100 plants were found out (PETRLIK & STYS, 1988). Nevertheless, population density of weevils in Czech a Moravian hop gardens has decreased in comparison with the past and therefore it has been necessary to overestimate the economic threshold, which is now 10 beetles per 100 plants. Besides counting beetles at the soil surface on hop shoots and under clods of earth, soil temperature in the depth of 50 cm is measured with the help of soil probe. It was found out that beetles began to emerge from soil when temperature in 50 cm reaches the value of 8 °C. Nevertheless, 13-15 °C in the above-mentioned depth is necessary for mass emergence of beetles. It usually happens in the second half of April, which is also the recommended time for treatment of young shoots to control this pest and to prevent oviposition by females.

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SPECIES OF HOP FLEA BEETLES (*CHRYSOMELIDAE, ALTICINAE*) ATTACKING HOP PLANTS IN CZECH HOP GARDENS

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The screening of *Alticinae* beetles was carried out in the village of Oploty belonging to Zatec (Saaz) hop region in 2003-2004. It showed that besides *P. attenuatus* the following other species of this subfamily were found and determined in Czech hop gardens: black flea beetle *(Phyllotreta atra),* cabbage flea beetle *(Phyllotreta striolata=vittata)*, flea beetle *(Phyllotreta nigripes),* cabbage stem flea beetle *(Psylliodes chrysocephala),* barley flea beetle *(Phyllotreta vittula)*, small stripped flea beetle (*Phyllotreta undulata*) and large stripped flea beetle *Phyllotreta nemorum*.

Population dynamics of spring generation of *P. attenuatus* was monitored with the help of yellow dishes filled with water changed every day and placed in the rows near hop plants. In 2003 the first beetles were trapped in the end of March. Population density was the heaviest in the end of April. *P. attenuatus* was the most frequent species (75%), followed by *P. nigripes* (8.5%), *P. atra* (8.5%) and *P. nigra* (6.0%). In 2004 we found first hop flea beetles at the beginning of April. Nevertheless, population density culminated in the third decade of this month. Sex ration (male/female) moved since 1:2 (April 16) to 1:9 (April 27). Hop flea beetle *P.attenuatus* was again the dominant species (90.2%), followed by *P. nigripes* (8.7%) and *P. atra* (1.1%). Females full of eggs were found out on April 23. Four days later (April 27) part of females deposited eggs and on April 29 all the eggs were deposited. Population density of flea beetles on the experimental hop garden was 11x higher in 2004 than in 2003.

P. nigripes and *P. atra* were also the only ones to accompany *P. attenuatus* during harvest, when flea beetles were sampled with the help of exhauster. At that time these species formed 8.5 % of the trapped beetles from the subfamily *Alticinae.* They are known as the pests of rape and other crops from the family *Brassicaceae*. Population density of these species is therefore dependant on the vicinity of rape field as well as on the occurrence of plants from the family *Brassicaceae* inside a hop garden.

We can conclude that *P. attenuatus* is entirely the most dominant species not only at the beginning of the season but during the whole vegetation period and harvest as well. It is also the only one from this subfamily to cause damage on hop plants on the contrary to the other above-mentioned species. Despite we can see them sometimes on hop plants they are not harmful there as they do not eat and develop on *Humulus lupulus* L. A part of *P. attenuatus* population was parasited by microsporidia (Protozoa), genus *Gregarina*.

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STUDIES OF *VERTICILLIUM* WILT IN HOPS

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Abstract

In Germany hop growing is an export oriented agricultural business producing 30 % of the world hop crop including fine aroma and high alpha cultivars. Since 2005 an outbreak of more severe hop wilt has resulted in massive yield losses in some regions of the Hallertau. The occurrence of lethal *Verticillium* strains in England in 1924 and Slovenia in 1997 showed how dangerous this soilborne fungus is, especially for the perennial hop plant without crop rotation. Once the *Verticillium* fungus has infected the root system of the plant, there are up to now no effective chemical treatments for this disease. In order to guarantee the amount and the high quality of this raw material for brewing beer, it was necessary to study *Verticillium* more extensively in order to control this fungal disease.

A preventive way to safe the hop gardens from spreading this dangerous disease has to be found. Due to the fact that the procedure of cultivating the *Verticillium* fungus is very laborious and time-consuming, a rapid and effective diagnostic method has to be established to proof the occurrence of the fungus directly from bines which is crucial for breeding and for the propagation of healthy root cuttings from different varieties.

First of all, a collection of various *Verticillium* strains has been established. After the isolation and cultivation of the fungus from infected bines, *Verticillium albo-atrum* was identified in all samples collected from several regions of the Hallertau. A molecular approach using the AFLP method has been applied to genotype the single-spore isolates of the *Verticillium* strains. References with known virulence from England and Slovenia were included in these investigations. With new generated AFLP primer combinations single-spore isolates from highly infected hops inside the Hallertau showed a very close genetic relationship to lethal *Verticillium* isolates from England and Slovenia. In the dendrogram they are grouped in a separate cluster. Although mild *Verticillium* strains from Germany, England and Slovenia are geographically separated, they form an own group in this diversity study similar to the lethal ones. In addition, an artificial infection test in the greenhouse has proven the occurrence of lethal strains in Germany. Lethal English and Slovenian isolates showed the same pathogenicity like the isolates from highly infected Hallertauer hop gardens.

Thus, it can be concluded that inside the Hallertau new lethal strains have developed. One focus of our work is to continue this monitoring of the Hallertau hop growing region with an improved rapid molecular test directly from the bines. Furthermore, specific microorganisms should be tested as possible bio-antagonists against the Verticillium fungus - first in the laboratory, later in highly infected hop gardens. Several studies have already shown that specific bacteria, fungi and plant extracts can used as excellent biological control agents against soil-borne pathogens. Currently, breeding lines and wildhops are being tested for wilt-resistance in highly infected hop fields. Also tests with agronomical measurements like fertilization and usage of hop waste are being performed.

Keywords: Hop wilt, genetic diversity, molecular detection, bio-antagonists

DEVELOPMENT OF A RAPID MOLECULAR IN-PLANTA TEST FOR THE DETECTION OF *VERTICILLIUM* PATHOTYPES IN HOPS AND STRATEGIES FOR PREVENTION OF WILT

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Abstract

Verticillium wilt is a devastating disease in hops causing high yield losses. A rapid identification would allow taking measures in time to contain and defeat the fungus. Therefore, a rapid molecular *in-planta* test on the basis of multiplex quantitative real-time polymerase chain reaction (PCR) will be developed. A rapid molecular testing of the soil can help to prevent the spreading of *Verticillium* and select fields without *Verticillium* contaminations. Biological control is a strategy for controlling and preventing of wilt. We selected five biological control agents to analyze their effects on hop plants. Preliminary experiments have proven the colonization of *Burkholderia terricola* on hop roots.

Keywords: *Verticillium*, hop, rapid molecular *in-planta* test, biological control

Introduction

Verticillium wilt, caused by *Verticilllium* spp., is a dangerous disease in hop (*Humulus lupulus* L.). The fungus causes considerable economic crop failure (OEPP/EPPO 2007). *V. albo-atrum* Reinke & Berthold is the most widespread *Verticillium* species in hops, whereas *V. dahliae* Klebahn is rarely found. These species belong to the group of soilborne pathogens. *Verticillium* can survive several years in the soil by producing resting structures (OEPP/EPPO 2007). After the infection of the roots by the fungus the vascular system of the plant is colonized (Engelhard 1957).

Verticillium wilt was documented in England in 1924 for the first time by Harris (1927). Later the fluctuating (mild) and progressive (lethal) pathotypes could be differentiated (Keyworth 1942). In 1952 the mild pathogen type has been described in the Hallertau, Germany, for the first time (Kohlmann and Kastner 1974). Since 2005 *Verticillium* wilt has increased in Germany, especially in some regions of the Hallertau. The fact that former wilt resistant varieties e.g. 'Northern Brewer' or 'Perle' are showing symptoms, is a hint for the change of the virulence of the pathogen (Seefelder et al. 2009). The procedure to isolate and detect the *Verticillium* in hop bines is time-consuming and laborious. Therefore, the intention is to develop a rapid molecular *in-planta* PCR test to detect different pathotypes directly in bines. Furthermore, a quantitative real-time PCR based test should be developed to determine the concentration of *Verticillium* in soil.

Up to now, there are no effective chemical treatments available (OEPP/EPPO 2007). To prevent *Verticillium* wilt, there are only few measures, like the planting of resistant or tolerant plants and phytosanitary arrangements (Radišek et al. 2004). Biological control is an environmentally friendly way to suppress *Verticillium* wilt. The interaction between biocontrol agents, host plants, pathogen and microbial community had been described in detail by Handelsman and Stabb (1996). Berg (2009) reported about some plant-associated

microorganismens which showed positive effects on plants and could prevent disease symptoms.

Methods

Rapid molecular *in-planta* PCR test. A new method to isolate the DNA directly from the bine is developed. First the interior part of the hop bine was milled with a homogenizer (MP FastPrep). Therefore different parameters such as speed, time, different bead size and bead material were tested. The DNA extraction was done by several commercial isolation kits. For the quantitative real-time PCR detection of *V. albo-atrum* and *V. dahliae* special probes were designed using Beacon Designer 7. For this step available sequences were used (Nazar et al. 1991).

Biological Control. To analyze the influence of beneficial microorganisms on hop five different bacteria and different varieties of hops were chosen. The experiments to analyze the colonization of the bacteria on roots are arranged according to Berg et al. with some modifications (2000). The roots of the plants were washed with tap water and were dipped in the bacterial suspension. Spontaneous mutants resistant to Rifampicin were used. After four weeks roots were sampled and washed in NaCl. This solution was plated on nutrient agar containing Rifampicin. After seven days the bacterial colonies were counted. The plant growth effect was analyzed. Currently, one bacterial species of bacteria *Burkholderia terricola* ZR2-12 (Grasser et al. 2011) and two cultivars of hops ‘Herkules’ and ‘Perle’ are being tested.

Results

Rapid molecular *in-planta* PCR test. The homogenization was done using specific beads. For the isolation of the DNA several tested commercial kits were effective. To detect *V. albo-atrum* and *V. dahliae* in one step a multiplex real-time PCR protocol and specific probes were developed. Results obtained by the newly developed and the original method are identical.

Biological Control. First preliminary experiments were done. *Burkholderia terricola* ZR2-12 could be re-isolated from the hop roots. The tests will be expanded using other hop varieties and beneficial bacteria and a sufficiently high number of replications.

Discussion

To develop a rapid test to detect *Verticillium albo-atrum* and *V. dahliae* is very important for the farmers. The classical method comprising the isolation and cultivation of *Verticillium* from the hop bine (Seefelder et al. 2009) and the subsequent isolation of the DNA according to a modified standard protocol (Doyle and Doyle 1990) takes four to six weeks. The new method for the DNA isolation directly from the bine to identify *V. albo-atrum* and *V. dahliae* takes only one to two days which is a significant improvement. Further work will focus on the detection of the mild and lethal pathotypes of *V. albo-atrum* using multiplex real-time PCR. Furthermore, we plan to develop a rapid molecular test to detect *Verticillium* in soil. We tested several commercial kits for the detection of DNA in the soil. Only soil samples artificially infected with *Verticillium* were tested positive. Soil samples below 1 g and inhibiting substances in the soil matrix may cause this failure to detect the pathogen by the PCR reaction. This test should be applied for preliminary investigations, whether the pathogen is already in the soil, before planting a hop garden. Similar tests already exist to determine *V. dahliae*, e.g. in strawberry fields (Neubauer and Heitmann 2011).Thereby complex tests are used, where the resting structure of *V. dahliae* (microsclerotia) are sieved and cultivated. These tests take about four weeks. The problem to detect *V. albo-atrum* in soil is that there are no resting structure can be sieved. The development of a rapid molecular test from the soil is the only way to detect *V. albo-atrum*.

Another aim of this work is to develop strategies for containment and prevention of wilt using biological control. The experiments will be done with several hop varieties and five beneficial bacteria. Successful colonization of roots with bacteria will be analyzed using confocal laser

scanning microscopy. Furthermore, the interaction between the beneficial bacteria and *V. albo-atrum* should be analyzed. For this purpose the plants will be dipped into a bacterial suspension and planted in soil infected with *Verticillium* in order to test for health promoting effects of the beneficial bacteria on the plants. The objective is to find biological plant protecting agents to contain and prevent hop infection with *Verticillium*. *Serratia plymuthica* HRO-C48 and *Pseudomonas fluorescens* PICF7 represent successful applications of beneficial bacteria against *V. dahliae* (Müller et al. 2007; Prieto and Mercado-Blanco 2008). This approach will identify species of bacteria which show positive effects on hops.

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STRATEGIES FOR MANAGEMENT OF POWDERY MILDEW ON HOP CONES

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Abstract

In this research, the timing of initial fungicide applications and mode of action directed at various crop growth stages was evaluated. Infection of leaves in July and cones near harvest was determined in replicated small plot trials conducted in a research hop yard during two growing seasons. The preliminary results point to a critical period of cone susceptibility in late July to early August. Application of highly effective fungicides during this time appears critical for minimizing powdery mildew on hop cones.

Keywords: *Podosphaera macularis, Humulus lupulus*, ontogenic resistance

Introduction

Since hop powdery mildew, caused by *Podosphaera macularis,* was first confirmed in the United States Pacific Northwest in 1997 (Ocamb et al, 1999) at least 8 effective synthetic fungicides with five different modes of action have been registered for management of this disease. Although numerous trials have been conducted to support registration of these products little is known regarding timing applications of specific modes of action to various crop growth stages, or when applications should be initiated and terminated. Previous studies (Seigner et al., 2003) and modeling research has raised questions about the susceptibility of hop cones at different developmental stages. Ontogenic (age-related) resistance to powdery mildew is common in many hosts and plant tissues, but hop cones have been presumed to be susceptible to infection until harvest. Late season cone infection is assumed to be associated with cone browning and a condition referred to as 'cone early maturity', where cones appear to prematurely ripen (Coley-Smith, 1964; Gent et al., 2008). Understanding these associations and their impact on disease development, yield, and quality is important for growers to objectively determine how long to continue fungicide applications. The purpose of this research was to evaluate the timing of initial fungicide actions, and mode of action directed at various crop growth stages.

Methods

Trials involving various rotations and application timings of quinoxyfen (Quintec 250 SC), trifloxystrobin (Flint 50 WG), myclobutanil (Rally 40W), spiroxamine (Accrue 2.5 FC), and triflumizole (Procure 480SC) were conducted during 2007 and 2008 in a research hop yard (cultivar Galena) located at the Washington State University Irrigated Agriculture Research and Extension Center near Prosser, Washington. Each treatment consisted of four replications arranged in a randomized complete block design with nine plant plots in a single row with 0.9 x 3 m (plant x row) spacing under a 3 m trellis with one string per plant. To encourage uniform emergence of subsequent shoots, plants were desiccated by applying paraquat (2.4 L/ha Gramoxone Inteon) + 1% Hasten Modified Vegetable Oil on 2 May, and 8 May in 2007 and 2008, respectively. Fungicide spray applications were made with a Stihl backpack mist blower (model no. SR420). To reduce impacts of potential spray drift onto adjacent plots, only the middle seven plants in each plot were evaluated for disease.

2007 trials: The purpose of this trial was to evaluate timing and fungicide modes of action for initiation of management programs, as well as timing of subsequent applications. Two calendar-based treatments received fungicide applications on approximately two-week

intervals beginning 18 May (two weeks after desiccation and ending 6 August for a total of seven applications. Two treatments initiated at 1000 (base 6^{o} C) growing degree-days (GGD; calculated as actual GDD with 15 minute temperature data) after desiccation were applied on approximately two-week intervals beginning 25 Jun and ending 6 Aug for a total of 4 applications. Two model-based treatments were initiated 18 May (two-weeks after desiccation) with subsequent applications based on infection risk as predicted by the current hop powdery mildew infection risk index (Mahaffee et al., 2003) (available on AgWeatherNet; http://weather.wsu.edu) and ending 13 August for a total of nine applications. Applications for the risk index-based treatments were on approximately one-, two-, or three-week intervals based on predictions of high, moderate, or low infection risk, respectively. All treatments except the non-treated control consisted of single applications of quinoxyfen in rotation with single applications of trifloxystrobin with one of each pair of treatments starting with quinoxyfen and the other with trifloxystrobin (Table 1). On 11 July five leaves from both the east and west side of each plant (10 leaves total per plant) were arbitrarily selected and examined for the presence of powdery mildew. On 28 August one lateral branch was arbitrarily selected from the east side of each plant. The hop cones on those branches (average 177 cones per plot) were visually evaluated for signs of powdery mildew.

2008 trials: The purpose of this trial was to evaluate application timings of four different fungicide modes of action. Ten treatments received blocked applications (two sequential applications of the same product) of quinoxyfen in rotation with blocked applications of trifloxystrobin, myclobutanil, and/or spiroxamine (Table 1). Fungicide applications began 24 May in all treatments except the non-treated control, and subsequent applications were made on approximately two-week intervals. On 9 July disease incidence (eight leaves per plant) was evaluated as described above. On 3 September disease incidence on cones (average 176 cones per plot) was determined as described above.

Results

Environmental conditions were very favorable for hop powdery mildew in trials both seasons as indicated by the current hop powdery mildew infection risk index (Figure 1) resulting in high inoculum pressure and high disease incidence on cones at harvest.

2007 trials: All treatments significantly reduced both leaf and cone infection compared to the non-treated control (Table 1). The calendar-based treatment that received applications of quinoxyfen on 6 August resulted in significantly less cone infection (P = 0.05) than all other treatments except the GGD treatment that also received quinoxyfen on 6 August. That GGD treatment was different from all remaining treatments at P = 0.15, even though it only received four applications compared to seven and nine for the calendar- and risk index-based treatments, respectively. Treatment initiation date appeared to significantly reduce mid-season leaf infection but not cone infection in late August.

2008 trials: All treatments significantly reduced leaf disease incidence compared to the non-treated control. All four treatments that received quinoxyfen applications on 18 July and 1 August significantly reduced cone infection compared to all other treatments. Significant reductions of cone infection relative to the non-treated control were observed with both treatments that received applications of trifloxystobin on 18 July and 1 August. No significant reductions in cone infection relative to the non-treated control were observed in treatments with myclobutanil or spiroxamine applied on 18 July and 1 August (Table 1).

Discussion

These trials were conducted under very high disease pressure resulting in cone incidence considerably higher than is commercially acceptable. Such disease levels can effectively illuminate relative efficacy of various management strategies if impacts are measured fairly soon after implementation. Other research conducted in commercial hop yards has demonstrated a relationship between early to mid-season disease management and cone infection at harvest (Mahaffee et al, 2003; Gent et al., 2008). The lack of differences in cone infection associated with starting date in this trial may be due to progressively higher

inoculum pressure from non-treated areas of the research yard onto the small plots as the season progressed. This may have overwhelmed and masked differences due to early season inoculum control associated with starting date.

These trials clearly showed there is a period during late July to early August (mid-summer in the Northern Hemisphere) when application of highly effective fungicides is critical for minimizing powdery mildew incidence on hop cones. This reduction was observed even if mid-season leaf infection incidence was high in those same treatments. Later applications of the same highly effective fungicide did little to reduce incidence of cone infection. It is likely that this critical period varies among seasons. Additional research is underway to better define when this critical period occurs, quantify the susceptibility of hop cones at various development stages and determine the effect of late season fungicide applications on cone yield and quality.

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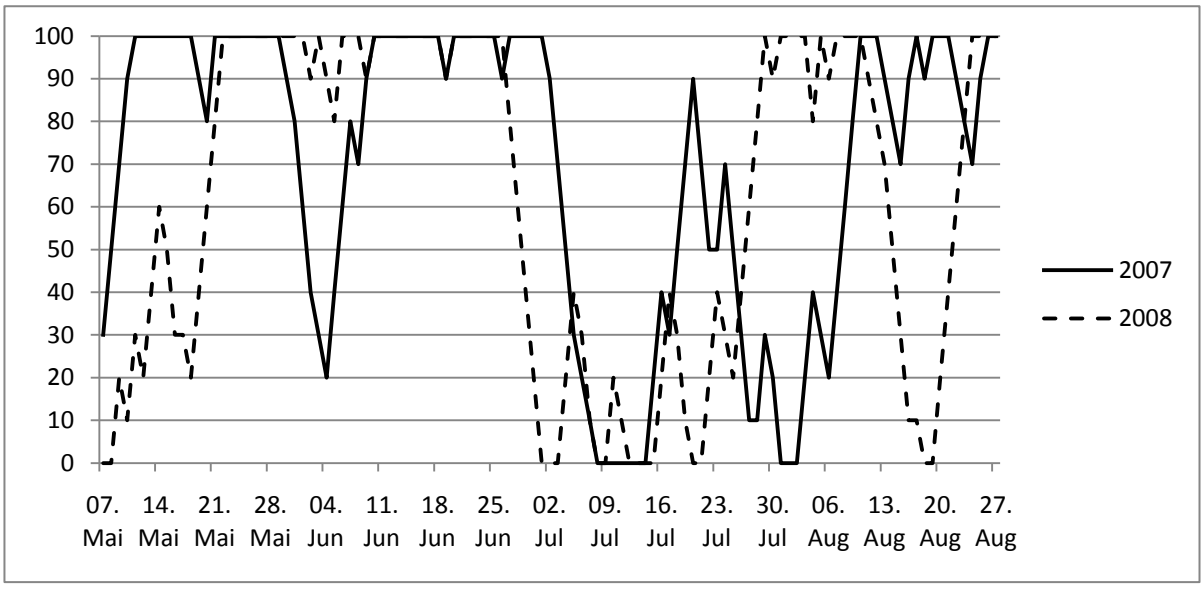

Figure 1. Infection risk during the 2007 through 2010 growing seasons at Washington State University, Irrigated Agriculture Research and Extension Center, Prosser, WA as predicted by the current hop powdery mildew infection risk model available at AgWeatherNet (http://weather.wsu.edu).

Table 1. Effect of fungicide application timing on hop powdery mildew.

2007					2008			
Treatment[1]	Spray Timing[2]	Spray initiation/ intervals[4]	% leaf infection 11 Jul[5]	% cone infection[5]	Treatment[1]	Spray Timing[3]	% leaf infection 9 Jul[5]	% cone Infection[5]
Non-treated			85.7d	99.0d	Non-treated		77.7e	100.0e
Quinoxyfen Trifloxystrobin	1,6,10,12 3,8,11	A	42.5b	30.8a	Quinoxyfen Trifloxystrobin	1,2,5,6 3,4,7,8	15.2ab	51.3ab
Trifloxystrobin Quinoxyfen	1,6,10,12 3,8,11	A	30.0a	54.8c	Trifloxystrobin Quinoxyfen	1,2,5,6 3,4,7,8	48.7d	76.0c
Quinoxyfen Trifloxystrobin	8,11 10,12	B	58.6c	55.4c	Quinoxyfen Myclobutani	1,2,5,6 3,4,7,8	21.4a	38.1a
Trifloxystrobin Quinoxyfen	8,11 10,12	B	60.0c	35.1ab	Myclobutanil Quinoxyfen	1,2,5,6 3,4,7,8	23.7b	92.1de
Quinoxyfen Trifloxystrobin	1,4,7,9,13 25,8,11	C	21.8a	48.6abc	Quinoxyfen Spiroxamine	1,2,5,6 3,4,7,8	19.2ab	40.0ab
Trifloxystrobin Quinoxyfen	1,4,7,9,13 2,5,8,11	C	31.1ab	49.7bc	Spiroxamine Quinoxyfen	1,2,5,6 3,4,7,8	37.5c	87.3cde
					Trifloxystrobin Spiroxamine Myclobutanil Quinoxyfen	1,2 3,4 5,6 7,8	42.0c	90.8cde
					Quinoxyfen Trifloxystrobin Spiroxamine Myclobutanil	1,2 3,4 5,6 7,8	51.3d	98.5de
					Myclobutanil Quinoxyfen Trifloxystrobin Spiroxamine	1, 2 3,4 5,6 7,8	47.8c	84.1cd
					Spiroxamine Myclobutanil Quinoxyfen Trifloxystrobin	1,2 3,4 5,6 7,8	12.1a	53.1b
FPLSD[6]			12.1	18.7			10.9	14.7

[1] Quinoxyfen (Quintec 250 SC; 0.3 L/ha pre-training, 0.45 L/ha training to wire, and 0.6 L/ha wire to 14 day pre-harvest); trifloxystrobin (Flint 50 WG; 140 g in 293-567 L/ha, 210 g in 568-850 L/ha, and 280 g in 851-1890 L/ha); myclobutanil (Rally 40W; 420 g/ha); spiroxamine (Accrue 2.5 FC; 1.35 L/ha); and triflumizole (Procure 480SC; 0.9 L/ha). Rates are formulated product per ha, per volume spray solution, or percent spray solution.

[2] 2007 Application dates: 1 = 18 May, 2 = 25 May, 3 = 29 May, 4 = 1 June, 5 = 8 June, 6 = 11 June, 7 = 15 June, 8 = 25 June, 9 = 2 July, 10 = 9 July, 11 = 23 July, 12 = 6 August, 13 = 13 August. Application 1 - 2 in 473 L water/ha, 3 - 4 in 662 L water/ha, 5 - 6 in 945 L water/ha, 7 - 13 in 1040 L water/ha.

[3] 2008 application dates: 1 = 24 May, 2 = 6 June, 3 = 20 June, 4 = 4 July, 5 = 18 July, 6 = 1 August, 7 = 15 August, 8 = 30 August. Applications 1 - 3 in 709 L water/ha, 4 in 992 L water/ha, and 5 - 8 in 1087 L water /ha.

[4] Treatment initiation/subsequent spray intervals: A = 16 days post-desiccation/two-week intervals; B= 1000 Base 6^{o} C growing-degree-days post- desiccation/two-week intervals; C = 16 days post- desiccation/risk index (based on current hop powdery mildew risk index: online: http://weather.wsu.edu).

[5] Numbers within each column followed by the same letter are not significantly different.

[6] FPLSD = Fisher's protected least significant difference, $P = 0.05$.

Acknowledgements

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MANAGEMENT OF CERCOSPORA AND PHOMA LEAF SPOT ON HOPS IN SLOVENIA

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Abstract

In the last 5 years, increased infection by cercospora (*Cercospora cantuariensis*) and phoma (*Phoma exigua*) leaf spot on hop have been observed in Slovenian hop gardens. As a response to the outbreaks of these new and not well studied diseases, extensive monitoring in production and natural hop habitats of Slovenia was performed. By using artificial infections, the pathogenicity and aggressiveness were determined for both fungi and the resistance of hop varieties was tested. Fungicide efficacy was evaluated by »*in vitro*« testing and epidemiological characters were studied. Based on all the findings, an integrated management strategy was developed for controlling these diseases.

Keywords: fungi, plant diseases, *Humulus lupulus*, climate change

Introduction

The appearance of new diseases in plant production is a permanent process, because of constant adaptations of pathogens, changes in the environment or technology and introduction from already infected areas. Cercospora (*Cercospora cantuariensis*) and phoma (*Phoma exigua*) leaf spot are new fungal diseases which have caused extensive outbreaks in Slovenia and Austria in the years 2005 and 2007, affecting leaves and cones (Radisek et al., 2008, 2009). Disease damage assessments revealed 7 to 35 % of infected cones, which is comparable with severe outbreaks of the most important diseases on hops, such as downy mildew (*Pseudoperonospora humuli*) or powdery mildew (*Sphaerotheca macularis*).

Until these outbreaks *C. cantuariensis* and *P. exigua* have been described as unimportant and rare hop pathogens. The first finding of cercospora leaf spot was in 1922, near Canterbury in England. The disease affected only leaves of the variety Canterbury Golding and, in the following year,s appeared in the form of small leaf infections (Wormald, 1928). The outbreaks of *P. exigua* in Slovenia are the first reports on hop, however similar disease symptoms on leaves have been previously described in England in 1926, when the causal agent was described as *Ascochyta humuli* (Wormald 1946). With recent taxonomic rearrangement, many species of the genus *Ascochyta* now belong to the genus *Phoma,* so there is high possibility that *P. exigua* and *A. humuli* represent the same pathogen. There is also a record of *A. humuli* mild leaf infections from former republics of the Soviet Union (Pidopličko, 1978).

Severe outbreaks of cercospora and phoma leaf spot in such a short period with a high aggression level are extremely rare. As response to the appearance of these new and not well studied diseases, we performed several activities, which are presented in this paper.

Materials and Methods

Monitoring survey and pathogen isolation

In the period of 2008-2010, systematic monitoring was carried out in active and abandoned hop production areas and in natural hop habitats of Slovenia. In all locations, samples of leaves and cones were taken in September, when there is a higher probability of finding cercospora and phoma infections. All samples were microscopically analysed and, in the event of finding fungal infection, pathogen isolation was done from infected tissue. Isolates of *C. cantuariensis* and *P. exigua* were maintained for further analysis as cultures on potato dextrose agar (PDA), V8 and oatmeal agar (OA) at 4°C in the dark.

Plant inoculations

A plant inoculation technique was used to determine pathogenicity and aggressiveness for both fungi and the resistance of hop varieties. Artificial inoculations were performed by spraying leaves and mature cones of detached lateral shoots with a pathogen inoculum. Controls were sprayed with sterile distilled water. Four bunches, each containing 2-3 lateral shots, were used for each treatment. Bunches were covered with plastic bags and incubated in a growth chamber (Kambič, RK-13300) with relative humidity at 80% under a 12-h photoperiod of fluorescent light (L 58W/77; Fluora, Osram). Temperatures were 20 ºC during the light period and 15 ºC during the dark period. Disease assessments were performed on a 0–5 scale (0 = no symptoms, 1 = 1 to 20 %; 2 = 21 to 40 %; 3 = 41 to 60 %; 4 = 61 to 80 %; and 5 = 81 to 100 %). Pathogen re-isolations were done from lesions on the leaves and cones.

»In vitro« tests of fungicide efficacy

Fifteen fungicides (Table 1) were tested *in vitro* for their inhibitory effect on mycelia growth of 2 representative isolates of each fungus. Fungicides were dissolved in sterile water and sterilised in 70% ethanol. For each fungicide, 7 different concentrations of 0, 1, 10, 100, 1000, 1500, 2000 µg a.i./ml of medium (V8- *C. cantuariensis*; PDA- *P.exigua*) were used. For strobilurin fungicides, SHAM treatments (100 µg/ml) were done to prevent an alternative fungal oxidase respiratory pathway and concentrations of 0,001, 0,01, 0,1, 1, 10 in 100 µg a.i./ml of medium were used.

Table 1: List of fungicides and active ingredients used in trials

Product	**Active ingredient**	**a.i content**	**Supplier**
Folpan 80 WDG	folpet	80 % ± 25 g/kg	Makhteshim-Agan, Israel
Quadris SC	azoxystrobin	250 g/l	Syngenta Limited, VB
Zato 50 WG	trifloxystrobin	50%	Bayer CropScience
Bravo 500 SC	chlorothalonil	515 g/l	Syngenta Crop Protection AG, CH
Folicur EW 250	tebuconazole	250 g/l	Bayer CropScience, Nem
Systhane 12-E	miclobutanil	125 g/l ± 6 %	Dow AgroSciences Gmbh, Av
Aliette Flash	fosetil-Al	80%	Bayer CropScience AG, Nem
Fonganil Gold SL	metalaxyl-M	494,85 g/l	Syngenta Crop Protection AG, CH
Delan 700 WG	dithianon	70%	BASF SE
Dithane M-45	mancozeb	80 %	Dow AgroSciences Gmbh, Av
Cantus WG	boscalid	50%	BASF SE
Topsin-M WG	thiophanate methyl	70%	NISSO Chemical Europe Gmbh
Champion 50 WP	Copper oxychloride	50 ± 5 %	Agrtol International
Pepelin (WG)	sulphur	80 ± 4 %	BASF AG
Antracol WG 70	propineb	70 % ± 4 %	Bayer AG, Nem

Three replicates of each plate were inoculated with a 5mm diameter plug taken from the edge of actively growing *C. cercospora* or *P. exigua* isolates. Colony diameters were measured at 3-day intervals until 21 days after plate inoculation. For each concentration of each fungicide, the inhibition of colony growth of each isolate was calculated: % INH = [(no fungicide treated –-fungicide treated)/no fungicide treated] x 100. The significance of interactions between fungicides and isolates was assessed by analysis of variance using Statgraphics Plus 6.0.

Epidemiological analysis of the pathogens

Disease appearance was monitored by a volumetric spore sampler (Burkard) in hop gardens in which severe outbreaks of cercospora and phoma leaf spot had been detected in previous years. In addition, an agro-meteorological station (Adcon A733 GSM/GPRS) was established in the vicinity of hop gardens to determine the influence of meteorological conditions on the appearance of infection. Pilot analysis of meteorological data for the period 2000-2009 was done to assess the possible influence of climatic changes on the appearance of new disease outbreaks.

Results and Discussion

The monitoring survey in the period from 2008-2010 included 57 different locations in which hop samples were taken. Based on microscopic examination of samples, a general spread of *P. exigua* was found, since it was confirmed in all locations. Such results were expected because of the polyphagic and facultative nature of this fungus. *Cercospora cantuariensis* was found in only 14 locations, in the northern and eastern part of Slovenia. This result showing that this fungus is not yet established in all production and natural hop habitats of Slovenia and is probably spreading from northern areas towards the south. For more accurate conclusions and to determine the spreading status of *C. cantuariensis,* monitoring should be extended to more locations including other hop growing regions.

Isolates obtained during monitoring were used for testing the resistance of 15 hop varieties that are included in the Slovenian national variety catalogue. Plant inoculations revealed different levels of resistance among varieties and a higher aggressiveness of *C. cantuariensis* than *P. exigua*. To determine other potential hosts of these fungi, pathogenicity tests were also done on 17 different weed species. Artificial inoculations revealed that *P. exigua* could infect potato weed (*Galinsoga parviflora*), redshank (*Polygonum persicaria*) and Japanese knotweed (*Reynoutria japonica*), while successful infection in *C. cantuariensis* was found only on hop related hemp (*Cannabis sativa).*

Fungicide *in vitro* testing showed a high inhibition index of the fungicides Topsin-M WG, Systhane 12-E, Dithane M45, Folicur EW250 and Quadris against *C. cantuariensis*, while fungicides based on copper oxychloride (Champion 50 WP) and sulphur (Pepelin) showed low efficacy. In the fungus *P. exigua,* similar results with a high inhibition index were obtained with the fungicides Folicur EW250, Topsin-M WG, Systhane 12-E, Dithane M45 and Aliette Flash. In contrast to *C. cantuariensis,* the copper oxychloride fungicide Champion 50WG revealed 50% higher efficacy to *P. exigua* (Figure 1). The same effect was obtained in strobilurin based fungicides, with which trifloxystrobin (Zato 50 WG) showed higher efficacy against *P. exigua* than the azoxystrobin fungicide Quadris.

Epidemiological analysis in infected hop gardens revealed that volumetric spore samplers are appropriate for detection of *C. cantuarienis* conidia, but not for *P. exigua*. Pilot analysis of meteorological data for the period 2000-2009 revealed a connection of severe outbreaks with intensive and frequent rainfall. In the years of the outbreaks 2005 and 2007, average rainfall in August and September exceeded 400mm, whih is 200mm more than the long term average rainfall in the same period.

Based on all the results, a control strategy for cercospora and phoma leaf spot diseases was incorporated in integrated plant protection management for hops in Slovenia.

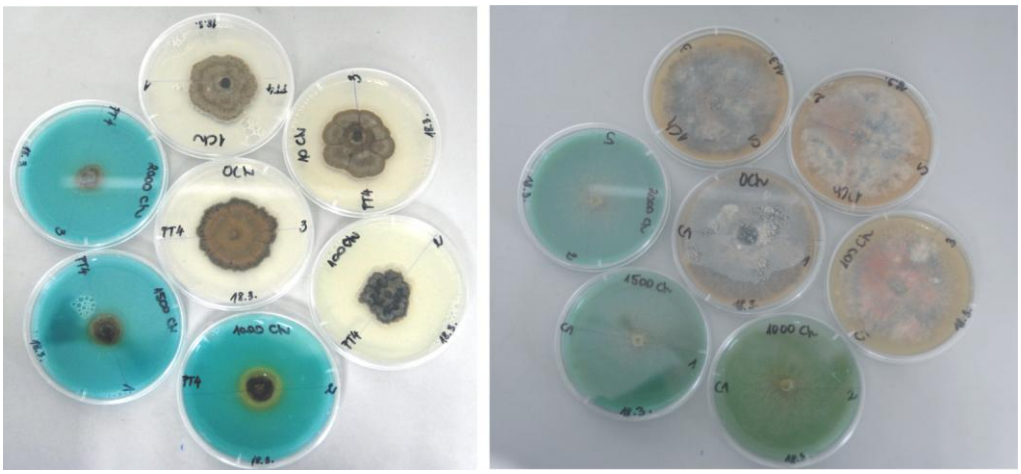

Figure 1: In vitro test of copper oxychloride fungicide Champion 50WG in different concentrations (0, 1, 10, 100, 1000, 1500, 2000 µg a.i./ml of medium) against *Phoma exigua* (left) and *Cercospora cantuariensis* (right).

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EVALUATION OF HEALTH STATUS IN HOP VARIETIES

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Abstract

State of health was evaluated with the help of DAS–ELISA in 43 hop varieties belonging to the collection of gene resources keeping in Hop Research Institute, Co., Ltd. in Zatec. The tests were carried out in 2005 and 2006. The presence of Apple mosaic virus (ApMV) from the genus *Ilarvirus* as well as Hop mosaic virus (HpMV) from the genus *Carlavirus* were found out. The varieties originate from eight countries (Czech Republic, England, Germany, Japan, Poland, Slovenia, the Ukraine and the USA). Totally 235 hop plants were evaluated. We have found that 28 (65.1 %) of the varieties were infected with ApMV and 17 (39.1 %) of them with HpMV and 12 (27.9 %) were infected by both viruses.

Keywords: hop, *Humulus lupulus* L., virus, ApMV, HpMV, ELISA, varieties

Introduction

Many viruses and viroid were determined in hop plants [3]. Among the most important viruses belongs the Apple mosaic virus (ApMV) from the genus *Ilarvirus*, Hop mosaic virus (HpMV), Hop latent virus (HLV) from the genus *Carlavirus* and Arabis mosaic virus (ArMV) from the genus *Nepovirus*. Hop latent viroid (HLVd) from the group *Pospiviroid* is the most frequent viroid found in hops.

Knowledge of health state in the gene resources is very important. High rate of infection was determined in hop varieties grown in various countries: in Czech Republic (Polák, Svoboda 1989, Svoboda, 1993), in Poland (Skomra, 2004) in the USA (Postman et al, 2004) and in Australia (Pethbridge et al., 2008). The field collection of hop gene resources in Hop Research Institute, Co., Ltd. in Žatec involves a wide spectrum of gene materials, which are necessary for breeding work. They belong to the biggest ones in the world.

Apple mosaic virus (ApMV) and Hop mosaic virus (HpMV) are commonly contained in hop plants (Svoboda, 1993). The original assessment was therefore aimed at finding these viruses in the studied hop varieties.

Material and methods

Plant material

Hop Research Institute, Co., Ltd. has kept since 1974 a field collection of hop varieties, which is regularly enriched. At present 325 accessions from 21 countries are incorporated into the collection. Hop plants are cultivated in the spacing of 3.0 x 1.0 m in 4 - 8 repetitions for each variety. Agricultural technology is carried out in a common way.

Detection of viruses

Young upper leaves were taken from hop plants in the collection of gene resources. Virus free hop plants of Osvald clone no. 72 placed in the isolation plot inside a glasshouse along with negative and positive controls served as a negative control in the tests. Antibodies against ApMV and HpMV from Löewe firm (Germany) marked by alkaline phosphatase as well as other chemical compounds were used in the tests. Determination with a help of DAS - ELISA was carried out by using methods by Clark and Adams (1997) as modified by Svoboda (2006). Plant extracts were prepared from pulverized young upper leaves and mixed with extraction buffer in the ratio 1:10 (w/v). Samples were pipetted in 200 µl in two repetitions and incubated in microtitrate desks over night in a refrigerator at 4 °C. The results were expressed quantitatively by the measurement of absorbance at 405 nm with a help of

photometer MRX II (ThermoLabSystems). The level of a positive reaction was determined as a double of the average values used as a negative control.

Results and discussion

With a help of DAS-ELISA the evaluation of health state in 43 hop varieties of gene resources collection was carried out (tab. 1). The varieties have their origin in eight countries (Czech Republic, England, Germany, Poland, Slovenia, Ukraine and USA). Totally 235 hop plants were assessed. It was found that 28 varieties (65.1 %) and 81 plants (52.2 %) were infected by ApMV and 17 varieties (39.1 %) and 24 plants (30.0 %) by HpMV. Simultaneous presence of both viruses was determined in 12 varieties (27.9 %). The results confirmed a high occurrence level of HpMV and ApMV is in agreement with the results obtained in other hop growing countries. In former Czechoslovakia, a high infection by ApMV and HMV Polák, Svoboda, 1989, Svoboda, 1993) was found in foreign and domestic varieties. In Poland 88 % of the tested hop varieties were infected by ApMV and HpMV (Skomra, 2004) as compared with the situation in USA (Postman et al., 2004) where 67 % infection by HpMV and 29 % infection caused by ApMV were reported only.

Conclusion

Gene resources are very important for breeding, serve also for preservation biodiversity of hop and for phytopathology research. Evaluation health state of 43 varieties from field collection of hop varieties keeping in Hop Research Institute, Co., Ltd. by DAS–ELISA, showed a high infection rate by ApMV and HMV. These results extend our knowledge about spread these viruses in gene resources of hop. Assessment of health state in other varieties (contamination by viruses and viroid) will be studied further research.

Acknowledgement

Thanks for excellent laboratory work to Ing. Ivana Malirova

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Table 1: Evaluation health statut in hop varieties

Assessment/year			2005				2006			
No	**Variety**	**Origine**	**Plants**	**Samples**	**Tests**	**ApMV**	**Plants**	**Samples**	**Tests**	**HMV**
1	Early Prolific	England	4	4	4	4	2	2	2	2
2	Petham Golding	England	3	3	3	3	2	2	2	0
3	WGV	England		1	1	1	1	1	1	0
4	Northern Brewer	England	4	4	4	3	2	2	2	1
5	Density	England	4	4	4	4	2	2	2	0
6	Wyoming	England	4	4	4	4	1	1	1	0
7	Golding	England	4	4	4	4	2	2	2	0
8	Cascade	USA	4	4	4	4	2	2	2	1
9	Humulus neomexicanus	USA	1	1	1	1	2	2	2	1
10	Willamette	USA	4	4	4	4	2	2	2	0
11	White Bine	USA	4	4	4	4	2	2	2	0
12	Ultra	USA	4	4	4	0	2	2	2	0
13	Aquila	USA	4	4	4	0	1	1	1	0
14	Liberty	USA	3	3	3	0	2	2	2	0
15	Centennial	USA	3	3	3	1	2	2	2	2
16	Crystal	USA	4	4	4	3	2	2	2	1
17	Sacramento English Cluster	USA	1	1	1	1	1	1	1	1
18	Oregon Cluster	USA	1	1	1	1	1	1	1	0
19	Late z Austrálie	Slovenia	2	2	2	1	2	2	2	2
20	Atlas	Slovenia	4	4	4	0	2	2	2	0
21	Osv.kl.126	Czech R.	4	4	4	3	2	2	2	0
22	M 2/27	Czech R.	4	4	4	4	2	2	2	1
23	45c	Czech R.	4	4	4	4	2	2	2	0
24	25	Czech R.	4	4	4	2	2	2	2	1
25	310B	Czech R.	4	4	4	2	2	2	2	0
26	Marynka	Poland	4	4	4	0	2	2	2	0
27	C-966	Japan	4	4	4	1	2	2	2	0
28	Hersbrucker Pure	Germany	4	4	4	0	1	1	1	0
29	Hallertauer Magnum	Germany	4	4	4	4	2	2	2	2
30	Hallertauer Tradition	Germany	4	4	4	0	2	2	2	1
31	2879	Ukraine	4	4	4	3	2	2	2	0
32	2887	Ukraine	4	4	4	4	2	2	2	2

Assessment/year			2005				2006			
33	Avans	Ukraine	4	4	4	0	2	2	2	2
34	Smena	Ukraine	4	4	4	0	2	2	2	0
35	Slavutič	Ukraine	4	4	4	0	2	2	2	2
36	Kumir	Ukraine	4	4	4	0	2	2	2	1
37	Zaklad	Ukraine	4	4	4	0	2	2	2	0
38	Slavjanka	Ukraine	4	4	4	0	2	2	2	0
39	Alma	Ukraine	4	4	4	0	2	2	2	0
40	Granit	Ukraine	4	4	4	0	2	2	2	1
41	Stimul	Ukraine	4	4	4	4	2	2	2	0
42	Aromat Polesja	Ukraine	4	4	4	3	2	2	2	0
43	Regent	Ukraine	4	4	4	4	2	2	2	0
	Totally	**43**	**154**	**155**	**155**	**81**	**80**	**80**	**80**	**24**

VI. Session:

PHYSIOLOGY OF HOP

THE PHYSIOLOGICAL PARAMETERS OF HOP PLANT (*HUMULUS LUPULUS* L.)

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Abstract

This paper evaluates the influence of genotype on the photosynthetic and transpiration activity of hops. The three years measurements of photosynthesis and transpiration showed that there was a statistically significant effect of year. Significantly, hop genotypes (Premiant, Nb. 4784, Nb. 4788, Nb. 4837, Nb. 4237) managed to receive solar radiation on the site Rybnany the best.The Rybnany location would be the universal site for the planting and cultivation of selected genotypes of hops on average. All the new breedings of hop were photosynthetically more efficient than a control variety Premiant. The highest rate of photosynthesis and transpiration has been statistically significant for genotype Nb. 4237. The highest results of the photosynthesis and transpiration rate achieved significantly in the period BBCH 32. The lowest values were achieved in the last term measurements BBCH 69-81, where we attribute the reduction rate of photosynthesis by the transition of plant to the phase of maturation.

Keywords: hop, photosynthesis, transpiration, genotypes

Introduction

Photosynthesis and transpiration is the basic physiological process in plants and one of the important factors in the formation of crop yield. Many studies have examined the relationship between photosynthetic indicators and external natural factors (Yu *et al.* 2001). These depend on many external factors and their changes during the development of plants (Kakani *et al.* 2004). Examples include temperature (Traore *et al.* 2000), the concentration of CO_2 (Coviella *et al.* 2002), the watering system (Matzke *et al.* 1990), the methods of fertilizer application and cultivation of the soil (Bruns, Abel 2003). All hop plant aboveground organs other than fruit are ready for the intensive course of photosynthesis (Rybacek, 1980). To study the photosynthesis production of higher plants, gasometry methods are mainly used (Sestak, Catsky 1966). Kenny (2005) reported that older hop leaves have a significantly lower photosynthetic efficiency. This author examined the average rate of photosynthesis in forty genotypes originating from North America and the countries of the former Yugoslavia. Kenny (2005) stated that the American variety of Willamette at the saturation irradiance of 2000 mol $CO_2.m^{-2}.s^{-1}$ demonstrated an average photosynthetic efficiency of 17.5 μmol CO_2 $m^{-2}.s^{-1}$. Transpiration is the water output in the form of water vapor from the surface of the plant into the surrounding atmosphere. Hnilickova, Hnilicka (2008) studied the transpiration of variety Agnus, Premiant, Harmonie and newbreeding 4257. The lowest transpiration rates were found in the technical maturity of hop cones. Hnilickova, Hnilicka (2009) studied the transpiration of variety Saaz - Osvald clone 72 according to water stress. The transpiration rate was measured at 0.987 mmol H_2O $m^{-2}.s^{-1}$ after nine days without watering**.**

Methods

This paper aims to assess the photosynthetic and transpiration capacity of selected genotypes of hop plants in the period of vegetation in 2007, 2008 and 2009. The experiment monitored Premiant variety (as control variety) and newbreedings with the numerical designations 4237, 4837, 4784 and 4788, of which the first two are genotypes that rank amongst aromatic hops (4237 and 4837). The newbreeding numbered 4237 is a genotype

with higher resistance to drought and high temperatures. It has a longer growing season, which is very important for division of the peak workload over the hop harvest period. The newbreeding denoted as 4837 displays the same content and composition of hop resins as found in Saaz. Its main advantage is high yield (up to 3 t ha^{-1}). The sites of the experiment – Suchdol, Rybňany, Radovesice and Očihov - located in Saaz and Auscha hop growing region. The main cultivation operations in 2007, 2008 and 2009 were the same as in normal production hop gardens. To measure the rate of photosynthesis and transpiration, an LC pro+ device was used. LC pro+ (an infrared leaf analyzer) can measure basic physiological processes in a leaf without separating it from the plant. This device tracks the physiology of the leaf, which is inserted into the measuring chamber under controlled temperatures and lighting. The differences in gas concentration and air flow levels inside the chamber form the basis for calculating rates of assimilation and transpiration every 20 seconds. Measurement is carried out on an infrared CO_2 gas analyzer (IRGA). The water content in the air is evaluated by moisture sensors. For this experiment, measurements were conducted at a constant temperature of 23°C and 600 nm of irradiation. From each genotype of hops were selected an average plant and photosynthetic and transpiration rate measured on the given date. This were measured in the period of ontogensis of hop BBCH 32, BBCH 37, BBCH 39, BBCH 61, BBCH 65, BBCH 69-81 in 2007, 2008 and 2009.

Results

The three years measurements of photosynthesis and transpiration rate showed that there was a statistically significant effect of year. The highest values were achieved in 2007. In 2007 the average rate of photosynthesis and transpiration was 7.53 μmol CO_2 $m^{-2}.s^{-1}$ a 1,16 mmol H_2O $m^{-2}.s^{-1}$. Statistically significant lowest rate of photosynthesis and transpiration was reached in 2009 (see Table 1)

Tab.1 : The effect of year on rate of photosynthesis and transpiration in 2007-2009

Year	photosynthesis rate (μmol CO_2 $m^{-2}.s^{-1}$)	transpiration rate (mmol H_2O $m^{-2}.s^{-1}$)
2007	7.53 a	1.16 a
2008	7.16 b	1.11 b
2009	6.78 c	1.07 c
Minimal significant difference	**0.0072**	**0.0036**

In 2007-2009 there was a statistically significant the effect of growing locations on the rate of photosynthesis and transpiration. Significantly, hop genotypes (Premiant, Nb. 4784, Nb. 4788, Nb. 4837, Nb. 4237) managed to receive solar radiation on the location Rybnany the best. There was the average rate of photosynthesis about 7.44 μmol CO_2 $m^{-2}.s^{-1}$. Significantly lowest rate of photosynthesis was reached at the site Suchdol 6.78 μmol CO_2 $m^{-2}.s^{-1}$. On the contrary, the transpiration rate was significantly highest in the locality Suchdol (1.19 mmol H_2O $m^{-2}.s^{-1}$). Statistically lowest transpiration rate over the monitored period was achieved at the locality Očihov (1.07 mmol H_2O $m^{-2}.s^{-1}$). (see Table 2)

Tab. 2: The effect of location on rate of photosynthesis and transpiration in 2007-2009

location	photosynthesis rate (μmol CO_2 $m^{-2}.s^{-1}$)	transpiration rate (mmol H_2O $m^{-2}.s^{-1}$)
Suchdol	6.78 d	1.19 a
Rybňany	7.44 a	1.09 c
Radovesice	7.17 c	1.11 b
Očihov	7.23 b	1.07 d
Minimal significant difference	**0.009**	**0.005**

In the monitored period 2007-2009 there was a statistically significant effect of the hop genotype on the rate of photosynthesis and transpiration. In general, the newbreedings of hop plants were photosynthetically more efficient than a control variety Premiant. The highest rate of photosynthesis and transpiration has been statistically significant for genotype 4237, in 2011 it has registered as a variety Saaz late amethyst (7.39 μmol CO_2 $m^{-2}.s^{-1}$ and 1.15 mmol H_2O $m^{-2}.s^{-1}$). Significantly lowest photosynthetic rates were achieved in the control variety Premiant (6.80 μmol CO_2 $m^{-2}.s^{-1}$). Significantly lowest rate of transpirations were achieved in the Nb. 4837, in 2011 it has registered as a variety Bohemie. (see Table 3)

Tab. 3: The effect of genotype on rate of photosynthesis and transpiration in 2007-2009

genotyp	photosynthesis rate (μmol CO_2 $m^{-2}.s^{-1}$)	transpiration rate (mmol H_2O $m^{-2}.s^{-1}$)
Premiant	6.80 e	1.10 d
Nš. 4784	7.24 b	1.12 c
Nš. 4788	7.20 c	1.14 b
Nš. 4837 (Bohemie)	7.17 d	1.09 e
Nš. 4237 (Saaz podní ametyst)	7.39 a	1.15 a
Minimal significant difference	**0.011**	**0.005**

In the monitored period 2007-2009 was also a statistically significant the effect of term measurements on the rate of photosynthesis and transpiration. Significantly, the highest rate of photosynthesis and transpiration was the first time measurements BBCH 32 (7.82 μmol CO_2 $m^{-2}.s^{-1}$ and 1.21 mmol H_2O $m^{-2}.s^{-1}$). The lowest values were reached significantly in the last term measurements BBCH 69-81 (6.00 μmol CO_2 $m^{-2}.s^{-1}$ and 1.02 mmol H_2O $m^{-2}.s^{-1}$). The transpiration rate gradually decreases with age of the plant. Interestingly, there is the increase of the photosynthesis rate in term BBCH 65. (see Table 4)

Tab. 4 : The effect of term measurements on rate of photosynthesis and transpiration in 2007-2009

term of measurement	photosynthesis rate (μmol CO_2 $m^{-2}.s^{-1}$)	transpiration rate (mmol H_2O $m^{-2}.s^{-1}$)
BBCH 32	7.82 a	1.21 a
BBCH 37	7.58 b	1.18 b
BBCH 39	7.16 c	1.13 c
BBCH 61	6.81 d	1.13 d
BBCH 65	7.59 b	1.04 e
BBCH 69-81	6.00 e	1.02 f
Minimal significant difference	**0.012**	**0.006**

Discussion

According to the results see Table 1, the rate of photosynthesis and transpiration were highest in 2007. In this case the result we attribute to the influence of weather conditions in 2007 for the hops less favorable compared with years 2008 and 2009. Weather conditions during the first seven months of 2007 were above average temperatures with strong sunshine duration, which showed a higher average rate of photosynthesis. As regards the amount of fallen precipitation, the first half of year was normal. In the first half of August were noticed the intense thunderstorm accompanied by torrential rain. Significantly, the hop genotypes managed to receive solar radiation on the site Rybňany the best. The experimental hop garden is at an altitude of 215 m in the Saaz hop growing region. Located in warm, dry region, the sum of temperatures above 10 ° C is 2600-2800 ° C per year. Average annual temperatures range from 8-9 ° C. The average annual rainfall is estimated at less than 500 mm.

From these results we can conclude that on average the location Rybnany would be universal for planting and cultivation of selected genotypes (Premiant, Nb. 4784, Nb. 4788, Nb. 4837, Nb. 4237) of hop. All genotypes are reaching the highest results in the rate of photosynthesis in this location. Significantly lowest rate of photosynthesis was reached at the site Suchdol. On the contrary, the rate of transpiration was significantly the highest at this site. This was caused due to weather conditions and water-supply of hop plants.

The Table 3 show significantly, the effect of genotypes on measured physiological parameters. All the newbreedings were photosynthetically more efficient than a control variety Premiant. This result we attribute a more massive plant habit with dense foliage. The larger plant assimilation area, the higher photosynthetic activity. It also shows that the genotype 4237 (Saaz late amethyst) are photosynthetically very efficient variety. This fact is reflected in the yield of dry harvested cones. Genotype 4237, according to zoning, seems to be plastic.

Table 4 describes the effect of term measurement on the rate of photosynthesis and transpiration. The highest results achieved significantly in time BBCH 32, which represents the developmental stage of plant height 2-3 m. The plant is in full elongation growth. In other terms the photosynthetic rate decreased slightly in response to the current state of vegetation and condition of plants, up to date BBCH 65, which represents the flowering stage. Larcher (1995) describes that the transition from vegetative to generative phase of hop is the photosynthesis particularly important and high. In this case, we confirm the author. The lowest values we were achieved in the last term measurements BBCH 69-81. The transpiration rate had a downward trend in time series toward the technical maturity of hops.

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VII. Session:

HOP PRODUCTION

DEVELOPMENT OF LOW TRELLIS IN CZECH REPUBLIC

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Abstract

In CR hop is traditionally cultivated on high trellises. The growing technology has been improved recently. Nevertheless, it is still based on vine ability to turn round a wire and reach the height of 7-8 m. This technology can hardly become automated because spring operations (fixing wire at the top of a wirework and in the soil as well as young shoot training) must be carried out by labours regardless unfavourable weather conditions, which often occurs during the spring.

Higher inputs into hop growing and unstable prizes for hops have been typical for recent years. Hop growers must decrease inputs to be profitable. Low trellis system of hop growing may be one of the ways to solve this situation. Lack of good labours and decreasing interest in such work is another problem. In the past secondary school and university students made up the essential part of temporary workers as these activities used to be compulsory for them. Nowadays, people from Slovakia, Ukraine and Rumania represent the main source of this labour.

Hop growing on low trellis seem to be a quick and pragmatic alternative. This type of hop cultivation is not entirely new for Czech hop research as some trials were carried out as early as the beginning of the nineties (1992-1996). Nevertheless, at that time there used to be no such need and therefore no research continued.

In 2008 the first hop growers in Žatec (Saaz) hop region began to establish low trellises. Availability of a mobile picking machine produced by Chmelařství was the most important hint for them to start this type of hop growing. According to Central Institute for Supervising and Testing in Agriculture (CISTA), which register hop gardens in CR, the total acreage has increased recently from 17.4 ha in 2009 to 36.8 ha in 2010.

Hop Research Institute in cooperation with Czech University of Life Sciences of Prague (Faculty of Engineering, Dept. of Agricultural machines) finished some machines necessary for agro-technical operations in low trellises. Practical models of seeding machines as well as machines for mechanical cutting and chemical regulation of hop growth, for building up the shape of hop plant habits, fertilizer applicators, machines for low trellis building, spraying machines for hop protection against pests and diseases as well as shallow and deep soil treatment have been realized within the project.

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DEVELOPMENT OF A MECHATRONIC DEVICE FOR FULLY AUTOMATIC WIRE STRINGING IN HOPS

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With support from the Bavarian State Research Center for Agriculture, Soller GmbH has developed a device for a fully automatic wire stringing in hops. This equipment is attached to the front-end loader of the tractor and controlled by sensors. While driving it automatically fixes the wire in a height of 7 m in predefined intervals to the wire trellis. The huge advantage of this automatisation is that labour on the front-end loader platform can be saved. Furthermore, the risk of accident can be reduced and the work can be done regardless of weather conditions.

SENSOR CONTROLLED INDIVIDUAL PLANT TREATMENT IN THE PESTICIDE APPLICATION

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When the hop shoots begin to grow in the development stage 07 – 15 soil pests such as the alfalfa weevil (*Otiorrhynchus ligustici*), the flea beetle (*Phyllotreta* and *Psylliodes*), the grey caterpillar (*Cnephasia alticolana*) as well as the wire worm (Agriotes lineatus l.) damage the hop plants and shoots so badly that it can result in the dying off of plants. At the same time the control of the downy mildew primary infection (*Pseudoperonospora humuli*) is carried out on approx. 50 % of the areas.

The plant protection products needed for this spraying are applied in the form of a single plant treatment. This application method is called “watering”. To date it is carried out by two workers on a tractor where the spray mixture is applied with manually activated spraying lances or watering sticks on the hop plant. In order to achieve a correct positioning and dosage and to protect the user and facilitate the work a technique was developed which enables the hop plant to be recognized with the aid of sensor technology and which distributes the plant protective absolutely accurately.

To locate the hop plant an optical sensor is mounted in front of the tractor on both sides, which when passing by can recognize the hidden training wire and therefore the position of the hop plant. The jet unit developed by the company “Agrotop” for individual plant treatment was attached behind the sensors and watered a certain amount of spay mixture onto the shoots. The working speed is 4-6 km/h and the jet output can be varied by changing the pressure from 2.5 up to 5 bar between 280 – 800 l/ha. So you can water 2- 3 ha per hour.

PESTICIDE REDUCTION THROUGH SENSOR IMPLEMENTATION

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Before and after the stripping of the lower portion of the vines and the training of the hop bines (BBCH 11 – 19) pesticides are applied on the hop shoots in so-called „row-treatments“ using 1-3 nozzles per side to control downy mildew primary infections or pests such as flea beetle (*Psylliodes attenuata*) and alfalfa snout weevil (*Otiorhynchus ligustici*). The amount of water used in row treatments is 300- 400 l/ha. Due to the wide distance between the plants (1.4-1.6 m) and the sparse soil coverage of the just sprouted or trained shoots approx. 80 - 90 % of the spray liquid is spread on the soil at the full-length row treatment. By switching off the spraying fan between the hop plants the amount of spraying liquid could be reduced significantly at the same efficacy while protecting the environment.

For this purpose the plant protection device has been modified by replacing the nozzle unit for the watering procedure by 2-3 fan nozzles to be used for spraying. Arranging the nozzle vertically (for usage after the training) the trained hop can be treated up to a height of 1.5 m.

While driving the optical sensor recognizes the training wires or the hop bines and opens the nozzles via pneumatic valves. Depending on the driving speed the time lag as well as the opening time of the nozzles can be adjusted at the control module. At the Hop Reseach Center Hüll in two precise trials before and after the stripping and training the amount of pesticides could be reduced by 61 and 55 %, respectively.