ISSN 1814-2206

International Hop Growers` Convention

PROCEEDINGS OF THE SCIENTIFIC COMMISSION

KIEV, UKRAINE, 04 - 09 JUNE 2013

Impressum:

Scientific Commission
International Hop Growers` Convention (I.H.G.C.)
Hop Research Center Hüll – Bavarian State Research Center for Agriculture (LfL),
Hüll 5 1/3, 85283 Wolnzach, Germany
Internet: http://www.lfl.bayern.de/ipz/hopfen/10585/index.php
Dr. Elisabeth Seigner
June 2013

Special Thanks to our Sponsors

OFFICIAL PARTNERS:

OBOLON
www.obolon.ua

ZEMAN
www.pat-lutskkij-pivzavo.uaprom.net

JSB "NATIONAL INVESTMENTS
www.jsbni.kiev.ua/

HOTEL "KYIV"
www.htl.kiev.ua

RESTORAUNT "PROKHODIMETS"
www.prokhodymets.ua

TM "SMACHNE KACHENYA"
www.www.duck.com.ua

TM "VYSOKY POSOL"

AGRICULTURAL CORPORATION "SKVIRA"

"APEKS" LTD
www.apeks-plant.com.ua

THE PRIME EXCURSION BUREAU
www.primetour.ua

Contents

Foreword

As chairperson and secretary of the Scientific Commission (SC) of the International Hop Growers` Convention (I.H.G.C.) it is my pleasure to welcome all of you here in Kiev. This is the very first time for the Scientific Commission that a meeting will be held in the Ukraine. With its very impressive buildings and monuments our venue Kiev reflects the full splendor and glory of a rich past and presence. Kiev is not only a very important traffic junction, it is the economic, cultural and also the scientific center of the Ukraine. We are guests of the National University of Life & Environmental Sciences of Ukraine (NULES) and of the vice-rector for scientific, innovative and international activities of this university Prof. Dr Maksym Melnychuk. We have much pleasure in having the opportunity to enjoy the warm hospitality of our host Prof. Dr Maksym Melnychuk and his team that arranged the perfect surrounding for our meeting.

In the focus of interest are the hop research activities in the various fields.Scientists and experts from 12 hop growing nations present their work in papers and posters comprising the following topics: hop breeding (classical cross-breeding and biotechnological work), studies on hop diseases and pests as well as integrated plant protection strategies, hop chemistry, improvement of production techniques, the physiology of hop and a brewing topic with hop-derived beer aroma notes.

Many thanks to all participants who provided the manuscripts of their papers or posters for the Proceedings. As editor I compiled the texts as submitted by the authors in order to manage the publication of the Proceedings just in time for this meeting.

In addition to this scientific program, an excursion arranged by Prof. Melnychuk will give you the opportunity to learn more about the Ukraine and in particular about the Kiev surroundings as agricultural region and as hop production area.

Our special thanks go to Prof. Dr Maksym Melnychuk for all his efforts and time he put into the organization of this meeting. He did an excellent job in creating a perfect conference atmosphere and in arranging an interesting and great social program for you.

We highly appreciate the contribution of his team with Mr Eugene Kanarsky, Mr Igor Antipov, Mr Andriy Kliyvadenko, Mr Artur Likhanov, Mr Vitaliy Overchenko and Ms Lili Nedobiichyk that efficiently supported Prof. Melnychuk in all preparations. We say thank you to Prof. Dr Valeriy Dubrovin for his valuable contribution to the success of this meeting. We would like to express our gratitude to the rector of NULES Prof. Dr Dmytro O. Melnychuk for his support to hold this meeting at his university. Many thanks are due to all persons with their various tasks who helped to make this meeting a great event.

Certainly we are also grateful to the various sponsors who are supporting the mission of the SC by their financial backing.

In closing, I would like to wish you all a pleasant and fruitful conference. Take in all interesting presentations and 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
Chairperson, I.H.G.C. Scientific Commission

Vorwort

Als Vorsitzende und Sekretärin der Wissenschaftlichen Kommission des Internationalen Hopfenbaubüros (IHB) freue ich mich sehr, Sie in Kiew willkommen zu heißen. Es ist das erste Mal, dass die Wissenschaftliche Kommission eine Tagung in der Ukraine abhält. Mit ihren beeindruckenden Gebäuden und Monumenten zeigt unser Tagungsort Kiew den ganzen Glanz seiner reichen Vergangenheit und Gegenwart. Kiew ist nicht nur ein Verkehrsknotenpunkt, sondern auch das wirtschaftliche, kulturelle und zudem das wissenschaftliche Zentrum der Ukraine. Wir sind Gäste der National University of Life & Environmental Sciences of Ukraine (NULES) und des Vize-Rektors für wissenschaftliche, innovative und internationale Aktivitäten dieser Universität Prof. Dr. Maksym Melnychuk. Wir dürfen die herzliche Gastfreundschaft unseres Gastgebers Prof. Dr. Melnychuk und seines Teams genießen, die das perfekte Ambiente für unsere Tagung arrangiert haben.

Im Mittelpunkt stehen die Forschungsaktivitäten in den verschiedenen Bereichen der Hopfenforschung. Wissenschaftler und Experten aus 12 Hopfenbaunationen stellen ihre Arbeiten in Vorträgen und Postern vor, die folgende Themen umfassen: Hopfenzüchtung (klassische Züchtung und biotechnologische Arbeiten), Studien zu Hopfenkrankheiten und -schädlingen ebenso wie Strategien zum Integrierten Pflanzenschutz, Hopfenchemie, Verbesserungen in den Produktionstechniken, die Physiologie des Hopfens und auch ein Brauthema zu den Aromastoffen des Hopfens im Bier.

Vielen Dank allen Teilnehmern, die Manuskripte ihrer Vorträge und Poster für die Veröffentlichung in den Proceedings zur Verfügung gestellt haben. Als Editor habe ich die Texte so, wie ich sie von den Autoren übermittelt bekommen habe, für die Proceedings zusammengestellt, damit der Tagungsband rechtzeitig bis zum Beginn der Tagung veröffentlicht werden konnte.

Zusätzlich zum wissenschaftlichen Programm wird eine Exkursion angeboten, die von Prof. Melnychuk organisiert wurde. Damit möchten wir Ihnen die Gelegenheit bieten, die Ukraine und insbesondere die Umgebung von Kiew als Landwirtschaftsregion und Hopfenbaugebiet kennen zu lernen.

Mein besonderer Dank geht an Prof. Dr. Maksym Melnychuk für all seine Bemühungen und seine Zeit, die er in die Organisation dieser Tagung gesteckt hat. Er hat ausgezeichnete Arbeit geleistet, indem er für uns eine perfekte Konferenzatmosphäre geschaffen hat und ein interessantes und großartiges Rahmenprogramm zusammengestellt hat.

Wir bedanken uns auch bei seinem Team mit Eugene Kanarsky, Igor Antipov, Andriy Kliyvadenko, Artur Likhanov, Vitaliy Overchenko und Frau Lili Nedobiichyk, das Prof. Melnychuk so effektiv bei den Vorbereitungen unterstützt hat. Prof. Dr Valeriy Dubrovin sagen wir Dank für seinen wertvollen Beitrag zum Erfolg der Tagung. Wir danken Prof. Dr. Dmytro O. Melnychuk, dem Rektor der NULES, 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 uns alle wird.

Selbstverständlich geht unser Dank auch an die verschiedenen Sponsoren, die mit ihrer finanziellen Unterstützung die Mission der Wissenschaftlichen Kommission vorwärts getragen haben.

Am Schluss darf ich Ihnen noch eine angenehme und erfolgreiche Tagung wünschen. Nutzen Sie all die interessanten Beiträge und Diskussionen. Außerdem hoffe ich sehr, dass Sie dieses Meeting auch zum Anlass nehmen, verschiedene wissenschaftliche Netze aufzubauen und zu erweitern zum Wohle der Hopfen- und Brauwirtschaft.

Dr. Elisabeth Seigner
Vorsitzende, IHB, Wissenschaftliche Kommission

Lectures and Posters

I. Session:

Hop Breeding

RECENT ADVANCES IN HOP BREEDING IN AUSTRALIA

Koutoulis, A.[1], McAdam, E.[1], Price, A.[1], Shellie, R.[2], Whittock, S[1,3]

[1]School of Plant Science, Private Bag 55, University of Tasmania, Hobart TAS 7001, Australia;
[2]Australian Centre for Research on Separation Science, Private Bag 75, University of Tasmania, Hobart TAS 7001, Australia;
[3]Hop Products Australia, 26 Cambridge Road, Bellerive, TAS, 7018, Australia;
E-mail: Anthony.Koutoulis@utas.edu.au

Abstract

Hop production in Australia relies upon varieties bred locally. The Australian hop industry has survived due to a continual commitment to R&D dating back to the 1950s. Since 1994, collaborative research between Hop Products Australia and the University of Tasmania has focussed on understanding hop germplasm with the view of gaining insight into the organoleptic potential of hop to meet existing and emerging market demand. A major thrust of this research has been to develop technology that supports breeding of seedless varieties; however, more recently there has been an increased focus on discovering information about the inheritance, genetic control and genetic architecture of hop using molecular markers as well as dissecting the complex chemical composition of hop using a suite of sophisticated separation science technologies to allow clear inferences about how measurable hop chemistry influences beer flavour. This successful collaborative approach has provided the necessary tools to advance hop breeding efforts in Australia to develop high yielding, seedless varieties with diverse and exciting flavour potential, and provides the level of underpinning information required in order to respond flexibly to any future changes in breeding objectives.

Keywords: genetic variation, hop, molecular markers, separation science

HOP BREEDING IN CZECH REPUBLIC

Nesvadba, V., Krofta, K., Polončíková, Z., Henychová, A.

Hop Research Institute, Žatec, Czech Republic
E-mail: nesvadba@chizatec.cz

Abstract

Genetic resources containing 370 hop varieties from all over the world and 348 wild hops are used for hop breeding. Eleven hop cultivars are registered in CR. Hop breeding process is aimed at high alpha acids contents, dwarf and flavor hops. Brewing tests are necessary for the development and further utilization of new hop varieties. Nowadays 17 perspective genotypes are under the registration process. Czech hops begin to be commonly used also for cold hopping, where tests are aimed at optimal needs of hops and optimal time of maceration in lager tanks.

Keywords: hop, *Humulus lupulus* L., genetic resources, hop breeding, varieties, flavors, dwarf hops, perspective genotypes, cold hopping.

Introduction

Saaz hops have been growing at the territory of Czech Republic for more than 1000 years. In the twentieth of the last century dr. Osvald began breeding aimed at positive selection. The first registered clones of Saazer were the result of his work. Hop crossing as a new method started to be used in the middle of the 20th century. Nevertheless, the first hybrid varieties (Bor, Sládek, Premiant) were not released until the nineties by dr. Beránek. At that time crossing was aimed entirely at quality of hops and their influence at beer. Hop quality included tolerance to diseases, agro technical operations, pick-ability, good balance of cones for drying, etc. New demanded features issue from the changes in technology (e.g. low trellises). In the past stress was put on cones structure, e.g. shape of bracts and cones, lupuline color, etc. Nowadays, such characteristics are loosing their importance, as they have no effect on quality of hops from the customer's point of view. Nevertheless, many breeding criteria have been kept from brewing point of view, especially character of bitterness, impression after having a drink, taste and aroma of beer, sensorial intensity of bitterness, etc. Recently, new characteristics are getting more important from both pharmacy and specific beer flavors. Research has been aimed at contents of polyphenols such as xanthohumol and DMX (Colgate, E. C. et al., 2007; Pšenáková et al., 2010) as well as at beer aroma and special flavors (Nesvadba, 2012). The main objective of contemporary breeding is to gain ground not only in hop growing practice but also in brewing industry. Many breweries are conservative and therefore it is not easy to persuade them to try new varieties, especially within aroma ones. In bitter hops it is easier because their prize is mainly emphasized. Therefore, these cultivars have just two criteria – yield and content of alpha acids.

Materials and methods

Contents and composition of hop resins were determined by liquid chromatography (EBC 7.7) from dry cones. Content of essential oils is commonly determined with the help of distilled method as a share, which vaporize with steam vapor from 100 g of hops during boiling. Composition of hops is determined by gas chromatography (Krofta, 2008). More than 50 compounds were identified in this way (monoterpenes, sesquiterpenes, alcohols, esters, ketones, etc.). Selected compounds of essential oils were according to sensorial character classified into five groups with dominant aroma character (Table 1). Hop aroma is evaluated in a sensorial way in dry cones immediately after harvest. Perspective genotypes are tested from the brewing point of view. Experimental batches are brewed in the pilot brewery in Hop Research Institute in Žatec. The volume of one batch amounts to 50 liters. The same process is kept when experimental batches are produced. Just the type of hopping or a hop variety (a perspective genotype) is changed. Beer is produced by the traditional technology

of bottom fermentation (mash room, fermentation room, storage cellar). Standard raw materials are commonly used (barley malt, water, yeasts). Only sample of hops is changeable so as to be able to determine the influence of the tested hops on qualitative parameters of beer.

Table 1: Sensorial character of the essential oil compounds

Fruity	*Floral*	*Citrusy*	*Herbal*	*Spicy*
isobutylisobutyrate	linalool	limonene	beta pinene	myrcene
2+3 methylbutylisobutyrate	geraniol		beta phellandrene	alpha copaene
2-nonanone	farnesol		beta selinene	caryophyllene
S-methylthiohexanoate	2-decanone		alpha selinene	farnesene
methylnonanoate			gamma cadinene	humulene
2-undecanone			delta cadinene	caryophyllene epoxide
methyldecadienoate			humulene epoxide I	
			humulene epoxide II	

Results and Discussion

Genetic sources contained in the special collection are the base of hop breeding. The collection includes 370 genotypes, particularly cultivars grown all over the world (field collection) as well as other 236 wild hop genotypes brought from expeditions (Table 2), which have been planted inside a working collection. Other 112 wild genotypes are observed at the original localities within Czech and Slovak Republics. Wild hops are typical by their high variability (Nesvadba et al., 2011).

Table 2: Number of tested wild hops

Origin	Europe	Caucasus	North America	Asia
Number	98	64	67	7

Since 2001 aroma varieties (Harmonie, Kazbek, Bohemie and Saaz Late) as well as bitter cultivars (Agnus, Rubín, Vital) have been released in Czech Republic. They have been finding their use in Czech and foreign breweries. Saazer and Saaz Late show that their individual aroma characteristics are in a good balance, only herbal aroma is slighter (Figure 1). Harmonie and Rubín have stable aroma composition with slightly higher share of herbal flavor (Figure 2), which is missing in Saazer and Saze Late. These cultivars must be harvested in technological ripeness because ageing of cones may negatively change the aroma.

Fruity flavor has no negative effect if the ratios of the other aromas are well balanced. Higher ratio of this flavor is typical for Premiant and especially for a new bitter variety Vital (Figure 3). Very distinctive flavor is citrusy as it is a very intensive aroma, which is substantially different from hoppy flavor. Just Kazbek is typical by its very pleasant citrusy aroma, which makes it of a great demand in breweries specialized at new specific types of beer (Figure 4).

Figure 1: Character of flavor in Saazer

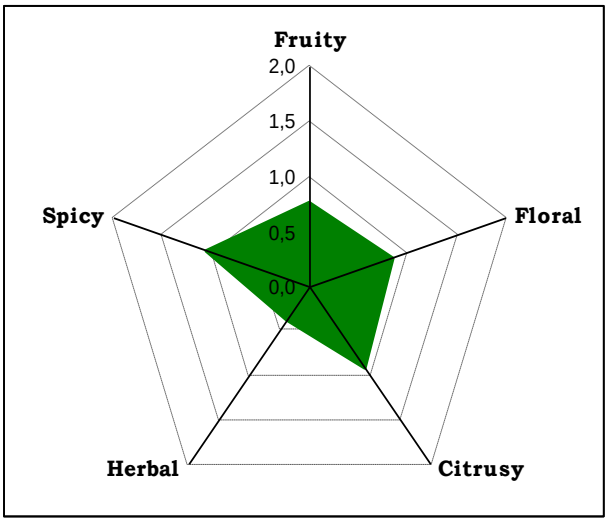


Figure 2: Character of flavor in Rubín

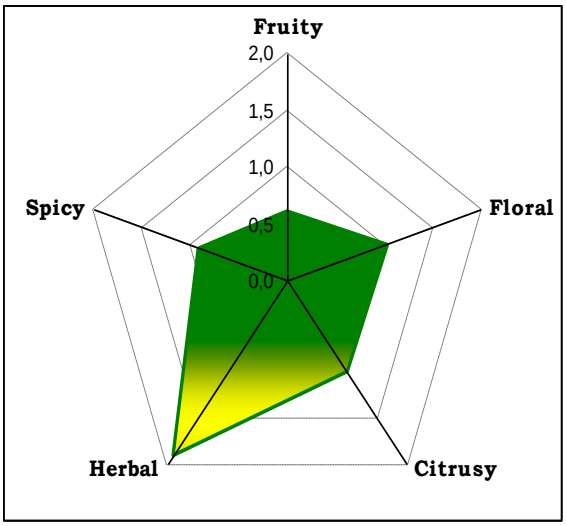


Figure 3: Character of flavor in Vital

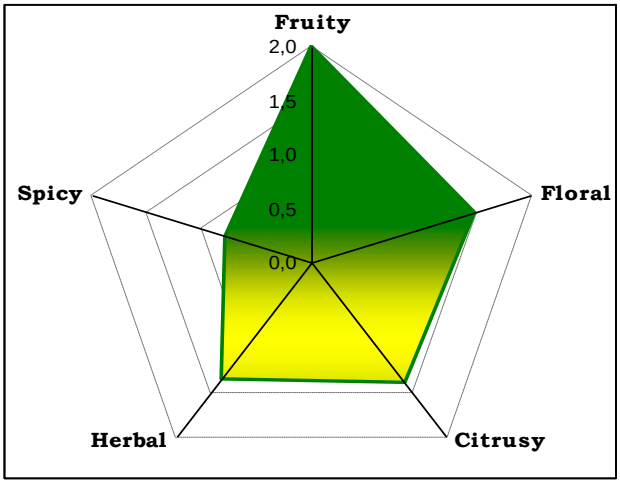
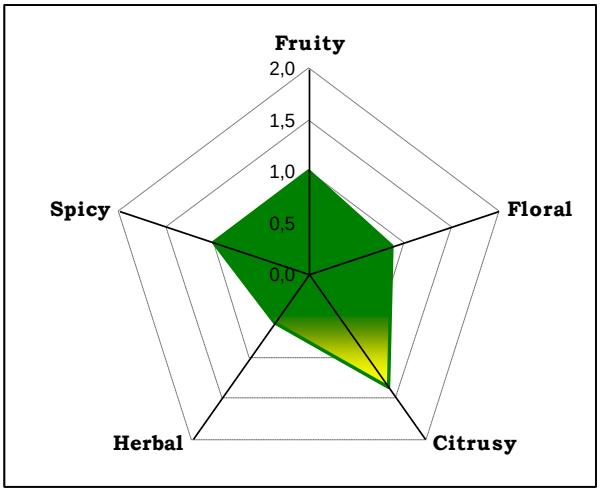


Figure 4: Character of flavor in Kazbek



Within perspective genotypes those ones with specific flavors belong to needed ones. Many genotypes with orange, tropical fruit, strawberry, walnut, peach, pear, onion, coconut or watermelon have been developed. Breweries are becoming still more and more interested in brewing tests with these flavor hops. Besides these special hops traditional aroma cultivars as well as bitter and dwarf genotypes are being developed. All the perspective genotypes must show good stability, agro-technical aspects, storage ability and brewing characteristics.

New varieties and perspective genotypes are tested on their brewing characteristics in a pilot brewery in Hop Research Institute in Žatec as well as in Czech commercial breweries. Besides above mentioned specific flavor hops cold hopping is tested, which should lead to the increase in need of hops generally as the demand of hops is much higher than if classic hopping is carried out.

Hop oils are subjected to significant changes during wort boiling. Most of them evaporate without utilization with water steam. If genuine hoppy aroma should be supply to the beer hops have to be added at cold period, i.e. to lager tanks. Extraction of hops components into the beer is promoted by ethanol. Cold hopping can affect not only aroma but also bitterness, colloidal, flavor and foam stability of beer, polyphenol and nitrates content. A lot of experimental conditions have to be tested during cold hopping introduction (variety, beer composition, time, temperature, static/dynamic system, yeasts, filtration etc.). Dry hopping

aroma is sensitive to experimental procedures (Table 3). It offers huge opportunities for innovations at beer market.

Table 3: Sensorial assessments of beers dry hopped by Sládek and Premiant after 1 and 3 weeks of lagering

Variety	Lagering time	Assessor										Σ Preferences
		1	2	3	4	5	6	7	8	9	10	
Sládek	1 week	-	+	+	-	-	-	-	-	-	+	3
Premiant	1 week	+	-	-	+	+	+	+	+	+	-	7
Sládek	3 weeks	+	-	+	+	-	+	+	-	-	+	6
Premiant	3 weeks	-	+	-	-	+	-	-	+	+	-	4

Conclusion

Many genotypes with various flavor characters have been developed. Nowadays, Kazbek has become the more demanded as it issues from the interest coming from many Czech as well as foreign breweries. New perspective genotypes include some other demanded flavors: citrusy, strawberry, mentholy, soapy, etc. Many of these genotypes are tested within experimental batches in various types of beers. Their specific flavor becomes more distinctive in cold hopping during secondary fermentation. Therefore, we expect new flavor varieties to be released besides Kazbek.

New aroma as well as bitter varieties are tested within brewing tests. Up to now results show that not only aroma but also bitter hops are suitable for cold hopping. It is necessary to test the time of maceration in lager tanks because varieties with higher contents of hop resins and essential oils may change their aroma, which may become unpleasant. Very good for this purpose are also hop cultivars with high contents of alpha acids because of high contents of essential oils they have no negative effect at the bitterness (Rubín, Columbus, Chinook).

References

Colgate, E. C., Miranda, C. L., Stevens, J. F., Bray, T. M., Ho, E., 2007. Xantohumol, a prenylflavonoid derived from hops induces apoptosis and inhibits NF-*kappa*B activation in prostate epithelial cells. In: *Cancer Letters*. I

KROFTA, K., 2008: Assessment of hop quality (in Czech), 4/08, Guideliness for practice. 4/2008, Chmelařský institut Žatec.

NESVADBA V., KROFTA K., MARZOEV A., PŠENÁKOVÁ I., FARAGÓ J., MURSALIEV M., KYRDALIEV K., POLONČÍKOVÁ Z., HENYCHOVÁ A.: Variability of wild hops (*Humulus lupulus L*.). In Proc. Scient. Comm. I.H.G.C., Lublin, Poland: 20.

NESVADBA V., 2012: Breeding programs and flavor hops in the Czech Republic. Czech Hops 2012, Ministry of Agriculture of the Czech Republic: 25-26.

PŠENÁKOVÁ, I., HETEŠOVÁ, L., NEMEČEK, P., FARAGÓ, J., KRAIC, J., 2010: Genotype and seasonal variation in antioxidant activity of hop extracts. Agriculture (Poľnohospodárstvo), 56, 2010: 106–113.

Acknowledgements

This work was supported by Czech Ministry of Education within the Research Project Eureca no. LF 11008. Breeding project 3.d and Genetic resources, is a part of “National Program of Conservation and Utilization of Genetic Resources in Plants and Biodiversity” (MZe 33083/03-300 6.2.1) issued by Czech Ministry of Agriculture.

KAZBEK - FLAVOR HOPS

Nesvadba, V., Polončíková, Z., Henychová, A.

Hop Research Institute, Žatec, Czech Republic
E-mail: nesvadba@chizatec.cz

All Czech hop varieties that have recently been bred have a good brewing value, which is due to a genetic share of Saaz in their origin. One of the major selection criteria for breeding is hop aroma, which is hoppy in Czech varieties, with a different intensity depending on the content of hop oils. The long-standing breeding tradition has been particularly focused on the selection of mild aroma hops. However, in the recent years some breweries have become increasingly interested in varieties with a specific aroma, which are referred to as “flavor hops”. Aromas of flavor hops are not typical hoppy but citrusy, fruity, spicy, floral herbal, etc. As a result, the breeders have started aiming their attention at the selection of hops with a such specific aromas as well.

In 2008, Kazbek was registered as a variety with a specific aroma increasing the diversity of Czech hop varieties. It was developed by hybridization of selected descendents of hybrid materials originating from Russian wild hops. It is a late aroma variety and is suitable for the second as well as for cold hopping. Its typical characteristics include a huge habitat and a cylindrical or even bludgeon-like shape. Laterals are very long (up to two meters), growing at the low or medium level of the plant. Hop cones are elongated with a medium or thick density. Tips of covering bracts lean away from the hop cone. Aroma of the hop cones is spicy-citrusy. Bines have a red-green color and a thickness of 12–15 mm. The yield ranges from 2.1 to 3.0 tons per hectare.

Table 1: Contents of hop resins in Kazbek

Kazbek – Hop Resins	
Total resins (% w/w)	17 – 22
Alpha acids (% w/w)	5.0 – 8.0
Beta acids (% w/w)	4.0 – 6.0
Ratio alpha/beta	0.9 – 1.5
Cohumulone (rel.)	35 – 40
Colupulone (% rel.)	57 – 62

Currently, many promising genotypes with specific aromas are the subject of breeding. They include both aroma hops and bitter hops, as well as dwarf hops conventient for cultivation in low trellises. Many of these genotypes have been submitted to brewing tests. Their specific aroma becomes most apparent during the secondary fermentation. We expect more hop varieties with specific aromas to be registered in the near future, following the Kazbek variety.

Acknowledgements

Ministry of Agriculture of CR institutional support for the development of research organization RO1486434704.

BREEDING NEW LOW TRELLIS HOPS IN CZECH REPUBLIC

Nesvadba, V., Henychová, A., Polončíková, Z.,

Hop Research Institute, Žatec, Czech Republic
E-mail: nesvadba@chizatec.cz

Significant changes in hop growing technology have been recorded in Czech Republic recently. They consist in the beginning of hop cultivation on low trellises. On the base of the cooperation with the UK it is possible to speed up considerably the development of new Czech dwarf hop varieties suitable for low trellises under Czech climatic conditions. Thanks to the crossings (H19, 20, 21, 22) carried out in 2010 we have managed to get 19,000 seeds. Czech fine aroma hop Saazer (Osvald clone no. 31) was chosen as a mother plant. Male plants from the gene-fond of dwarf hops were used for their pollination. The first assessments of the progeny were made in 2011. Qualitative characteristics were observed in all the plants within the progenies. They served as criteria for the subsequent selection aimed at obtaining perspective genotypes convenient for cultivation in low trellises. Totally 114 chosen genotypes will be submitted to detail assessments in 2012.

The best genotypes were harvested so as to get their yields in kilograms of fresh hops per plant. Chemical analyses of hop cones and sensory perception evaluations were carried out as well. Perspective genotypes are shown in Table 1. The best genotype PG/2/8 reached the yield of 3.52 kg of fresh hops per plant. If we take into consideration the spacing of 0.75 x 3.0 m and conversional coefficient of dry matter 4, we can conclude that potentially it is possible to get a crop of 3.85 t of dry hops per hectare. It is by far the best yield as the other perspective genotypes show the highest yield at the level of 2.4 t/ha, and 2.2 t/ha respectively. Many genotypes show also higher contents of alpha acids. The highest ones (> 15%) have only two of them, which on the other hand show only a low yield. Therefore, they can not be considered perspective. Very important in aroma genotypes is the keep low ratio between alpha and beta acids. Such genotypes have generally wide variability in the percentage of cohumulone. Many genotypes show interesting specific aromas.

Table 1: Perspective hop genotypes suitable for low trellis cultivation

Genotype			Yield	Alpha acids	Beta acids	*Ratio*	Cohumulone
H	R	P	(kg/plant)	(% ww.)	(% ww.)	*alpha/beta*	(% rel.)
PG	2	8	**3.52**	6.47	3.89	*1.7*	23.3
MA	2	21	**2.20**	7.70	4.24	*1.8*	29.7
38	16	1	**2.00**	10.81	5.49	*2.0*	30.6
37	22	1	0.52	**15.48**	4.83	*3.2*	22.7
37	22	2	0.89	**15.06**	5.01	*3.0*	30.0
37	23	4	0.80	**13.48**	4.78	*2.8*	37.8
PG	3	7	1.20	5.90	5.98	***1.0***	26.2
38	9	1	1.02	8.09	7.45	***1.1***	28.8
39	30	1	1.53	7.03	6.37	***1.1***	26.0
39	33	6	0.92	7.29	6.52	***1.1***	18.9

Acknowledgements

The work was supported by Czech Ministry of Education and Science within the Research Project EUREKA No. 11008.

COULD THERE BE A DOMINANT GENE FOR SUSCEPTIBILITY TO HOP POWDERY MILDEW?

Darby, P.

Wye Hops Ltd., China Farm Office, Upper Harbledown, Canterbury, Kent CT2 9AR, UK.
E-mail: Peter.Darby@wyehops.co.uk

Abstract

Seedling hop plants inoculated with *Podosphaera macularis* ssp. *humuli* in glasshouse tests usually show Mendelian segregation for major genes conferring resistance. However, distorted segregation is sometimes observed, often with a higher proportion of susceptible phenotypes than expected. A few specific breeding lines and their close relatives consistently exhibit such an effect suggesting a genetic explanation. This paper hypothesises the presence of a gene for susceptibility which is dominant over the R_2 resistance gene. Segregation data over several generations from the recent Wye Hops Ltd programme is presented in support of this hypothesis. There is an indication that the expression of the dominant gene may be specific to seedling tissue and a glasshouse environment.

Keywords: Resistance, powdery mildew, hop

Introduction

Resistance shown by the cultivated hop plant (*Humulus lupulus* L.) to infection by *Podosphaera macularis* ssp. *humuli (Wallr.) Braun and Tak.* (formerly *Sphaerotheca macularis* or *S. humuli*) was first recorded by Salmon (1917) who distinguished two classes of reaction: complete immunity and, under glasshouse conditions, a hypertrophic leaf reaction at infection sites which he termed “blister”. Based on differential host reactions, Liyanage *et al* (1973) proposed three resistance genes (B, I_1 and I_2) while Royle (1978) indicated that a further two resistance genes could be differentiated, designating them by R to acknowledge that all these genes provided resistance rather than immunity. The segregation of progeny in glasshouse tests indicated the action of major genes for resistance for most of these interactions although differential host reactions remained the only means of distinguishing R_1 and R_3 until a suitable pathotype had been developed. A further resistance gene, R_6, was later discovered in progeny of *cv.* ‘Nugget’. Test crosses using appropriate isolates confirmed the presence of at least seven major genes for resistance (Darby, 1998a, 1998b, 2001) and that the expression of resistance followed a gene-for-gene interaction. Godwin *et al* (1987) showed that the R_2 gene conferred resistance through a hypersensitive reaction.

Screening of seedlings for segregation to powdery mildew is carried out in several breeding programmes. All report Mendelian segregation of resistance conforming to the gene-for-gene model outlined by Darby (2001). However, occasionally the segregation ratios do not conform and this has been observed in many of the programmes throughout the world (E. Seigner, A. Čerenak *pers. comm*; P. Matthews, *pers. comm*.). Henning *et al* (2011) have reported a QTL for dominant susceptibility to powdery mildew in a population thought to be segregating for R_6 resistance. In this paper, data is presented to suggest that some of these anomalous results may be explained by the additional presence of a dominant gene, or genes, for susceptibility.

Methods

All progeny were screened under glasshouse conditions according to the methods described by Darby (2005). Until 1981, the reactions could be unambiguously classified as resistant or susceptible. However, since 1981 an aggressive isolate obtained from *cv.* ‘Zenith’ has been used and it produced a discontinuous graded series of reactions (Table 1). Within a family, seedlings were ranked according to the intensity and frequency of sporulation.

Table 1. Classification of seedling reactions in glasshouse tests.

Reaction classes	*Observed symptoms*
1 Immune	*No sporulation*
2 Resistant	*Very occasional small sporulating spots*
3 Poor resistance	*Many sporulating spots but weak sporulation*
4 Partial susceptibility	*Strong sporulation from a limited number of sites*
5 Full susceptibility	*Strong sporulation over much of leaf*

Results and Discussion

Distortion of expected segregation ratios for resistance

Scrutiny of the segregation patterns for progeny families since 1981 revealed many where the ratio of resistant to susceptible phenotypes did not agree with expectation from known resistance genes in the parents. In most instances, such anomalous results were inconsistent for the same parent in different crosses. However, two male parents were identified which consistently gave distorted segregation ratios in their progeny (Table 2).

Table 2. Segregation of progeny in crosses between R_2 male and susceptible females**.**

	Male 18/84/36			*Male 24/87/7*		
	12/91	*47/95*	*57/98*	*41/96*	*42/96*	*43/96*
Resistant	39	67	30	22	19	32
Susceptible	61	94	54	62	56	56
$\chi_{1:1}$	4.84 *	4.53 *	6.86 **	19.05 ***	18.25 ***	6.55 *
$\chi_{1:3}$	10.45 **	23.70 ***	5.14 *	0.06 ns	0.00 ns	6.06 *
$\chi_{3:5}$	0.10 ns	1.16 ns	0.11 ns	4.58 *	4.74 *	0.05 ns

*ns non-significant deviation from expected, * significant deviation at p= 5%, ** at p=1%, *** at p=0.1%*

In all crosses, these parents produced a much higher proportion of susceptible progeny than expected. If it is hypothesised that some of the R_2 progeny are failing to express resistance due to the action of a dominant gene for susceptibility, then the ratio in crosses with a susceptible female should tend towards 1 Resistant : 3 Susceptible. If it is assumed that the penetrance of the gene is incomplete and that only a proportion of the progeny will fail to express resistance then the ratio is likely to be approx. 3 Resistant : 5 Susceptible. Testing these anomalous families against these ratios indicated that they were segregating according to such expectations. A similar result was obtained for crosses between these males and heterozygous R_2 resistant females.

Identification of plants which could carry a gene for susceptibility

In 2008 a seedling of *cv.* 'Boadicea' was observed which showed chimerical susceptibility to powdery mildew (Figure 1). 'Boadicea' is homozygous for R_2 resistance and, therefore, all progeny carry a copy of the gene from their mother. This observation indicated that expression of R_2 resistance can be silenced. It also prompted the suggestion that progeny carrying the hypothetical susceptibility gene might be identified in a resistant population, such as progeny from homozygous R_2 parents. Up until 2012, Wye Hops Ltd have identified 27 such parents but examination of the segregation results indicated that only 6 of these have produced progeny with an unexpectedly high proportion of susceptible phenotypes in at least one cross in the glasshouse tests (Table 3). All are closely related to 18/84/36, one of the male parents producing anomalous segregation results. This strongly suggests a genetic effect.

Figure 1. Chimerical susceptibility shown by a seedling of *cv.* 'Boadicea'

A pedigree linkage to female parent 37/91/22 is notable. This is the mother of family 50/95 from which *cv.* 'Sovereign' was selected. 'Sovereign' was granted EU PVR in 2010 and, in 2012, was grown on 59 ha in the UK. Being from a homozygous mother, it was known to carry the R_2 resistance gene. However, in the powdery mildew screen carried out in 1996 it showed sporulation at many infection sites and was ranked only 33rd in the assessment of the family of 44 individuals. Despite such symptoms in the glasshouse as a seedling, it subsequently showed no powdery mildew infection in the field until 2000 when the v_2 pathotype appeared in the hop fields at Wye College. In commercial production now in the UK it is infected by powdery mildew but exhibits strong tolerance of the disease showing very little cone discoloration.

Table 3. Homozygous parents showing unexpected susceptibility in progeny 1981-2012.

No. crosses made	*No. crosses with susceptibility*	*Parent*	*Sex*	*Comment*
10	*1*	*Boadicea*	*F*	*Only in cross with male 18/84/36*
5	*1*	*35/91/18*	*M*	*s. 18/84/7*
3	*2*	*37/91/22*	*F*	*From open pollination of 18/84/32*
3	*1*	*50/95/23*	*F*	*s. 37/91/22*
3	*1*	*37/96/4*	*M*	*s. 37/91/22*
6	*2*	*37/96/24*	*F*	*s. 37/91/22*

If within family 50/95 there was segregation for a dominant gene for susceptibility which could silence R_2 resistance in a proportion of the population, then it should be found in the lowest ranked half of the progeny which would include *cv*. 'Sovereign'.

'Sovereign' has been used as a parent in six crosses with either susceptible parents or parents heterozygous for R_2. Several of these show distorted segregation ratios for resistance amongst progeny (Table 4). However, if the reaction classes likely to result from the action of a dominant gene for susceptibility (S) are excluded from the analysis, most crosses tested against a Chi-square give non-significant deviation from expectation. Thus, it would seem that where there is distortion in the segregation ratios, it may arise from inclusion of reaction classes where a dominant gene for susceptibility may be effective.

Table 4. Segregation for powdery mildew resistance in progeny of cv. 'Sovereign'.

		Sovereign x susceptible male			*Sovereign x resistant male*		
Expected genotype	*Reaction class*	*28/06*	*30/06*	*21/07*	*48/05*	*50/05*	*24/07*
$R_2 s$	*1*	0	1	3	6	5	15
	2	7	6	14	16	6	10
$R_2 S$	*3*	5	5	5	14	26	5
	4	10	8	1	4	0	5
$r_2 S$	*5*	18	17	21	23	19	24
$r_2 s$							
χ *all classes*		6.40 *	4.57 *	0.00 ns	10.71 **	2.38 ns	18.36 ***
χ *R2S excluded*		0.28 ns	0.16 ns	2.37 ns	2.31 ns	6.81 *	1.65 ns

*ns non-significant deviation from expected, * significant deviation at p= 5%, ** at p=1%, *** at p=0.1%*

These results indicate that there is an effect which is exhibited over several generations and which is most simply explained by the presence of a dominant gene for susceptibility. However, there may be other explanations such as the action of several closely linked polygenes giving a gene dosage effect. Furthermore, the effect appears specific in expression to certain breeding lines and to seedling tissues in a glasshouse environment. If the hypothetical gene exists, it may not express in mature hosts or in the field.

Although further work is need to confirm, or otherwise, the presence of a dominant major gene for susceptibility, these results give a first indication that it may exist in a few specific breeding lines within the Wye Hops Ltd germplasm collection.

References

Darby, P. 1998a. The symptoms and biology of hop powdery mildew. In: Hop Powdery Mildew Electronic Symposium, 1998 US Hop Industry Joint Meeting, Yakima, USA, http://www.scisoc.org/hpmes/darby.htm

Darby, P. 1998b. UK researchers offer advice on hop powdery mildew. Brewers Digest, 1998 (2): 24-26.

Darby, P. 2001. Single gene traits in hop breeding. Proceedings of the Scientific Commission of the International Hop Growers Convention, Canterbury, UK., 2001. Ed. E.Seigner. 76-80.

Darby, P. 2005. The assessment of resistance to diseases in the UK hop breeding programme. Proceedings of the Scientific Commission of the International Hop Growers Convention, George, South Africa, 2005 (ISSN 1814-2192). Ed. E.Seigner. 7-11.

Godwin J.R., Mansfield, J.W. and Darby, P. 1987. Microscopical studies of resistance to powdery mildew in the hop cultivar Wye Target. Pl. Path. 36: 21-32.

Henning, J.A., Townsend, M.S., Gent, D.H., Bassil, N., Matthews, P., Buck, E. and Beatson, R. 2011. QTL mapping of powdery mildew susceptibility in hop (*Humulus lupulus* L.). Euphytica 180: 411-420.

Liyanage, A. de S., Neve, R.A.N. and Royle, D.J. 1973. Resistance to hop powdery mildew (*Sphaerotheca humuli* (DC) Burr). Report of the Department of Hop Research, Wye College, for 1972: 49-50.

Royle, D.J. 1978. Powdery mildew of the hop. In: D.M. Spencer (Ed), The powdery mildews, Academic Press, New York: 381-409.

Salmon, E.S. 1917. On forms of the hop (*Humulus lupulus* L.) resistant to mildew (*Sphaerotheca humuli* (DC) Burr). J. Agric Sci. 5: 455-460.

BREEDING OF SPECIAL FLAVOR HOPS TO PAVE THE WAY TO THE CRAFT BREWERS

Lutz, A.[1], Kneidl, J.[1], Kammhuber, K.[2], and Seigner, E.[1]

Bavarian State Research Center for Agriculture, Institute for Crop Science and Plant Breeding, Hop Research Center Huell, Hop Breeding Research[1] and Hop Analytics[2], Huell 5 1/3, Wolnzach, Germany
E-mail: Elisabeth.Seigner@LfL.bayern.de

Abstract

The development of “Special Flavor” Hops represents a completely new direction in the breeding history of the Hop Research Center Huell. Without neglecting the classical breeding objectives, new hop varieties with fruity, citrusy and floral aroma compositions have been bred which should satisfy the demand of craft brewers for strong differentiating hop-derived aroma and flavor notes. Experimental lines from this program with sensory descriptions comprising the full aroma richness of an orchard attracted great interest by brewers worldwide. In a lot of brewing trials using traditional as well as dry hopping techniques the various top and bottom fermented beers revealed unique aroma and flavor impressions imparted by these new Huell breeds. Since 2012 the four Special Flavor Hops named “Mandarina Bavaria”, “Huell Melon”, “Hallertau Blanc” and “Polaris” have been grown commercially on 48 ha with increasing acreage in 2013.

Keywords: breeding, differentiating aroma characteristics, aroma and flavor in beer

Introduction

In recent years, the US craft brewer scene has set new records in constantly increasing its beer production, beer sales and its hop consumption by using 46 % of the US hops although having only a 7 % share of the US beer market. Thus, the craft brewers are a highly interesting clientele for hop producers. So far, in particular the US growers and to some extent also hop growers from Australia and New Zealand benefited from this increasing hop demand of the craft brewers` sector by providing hop varieties with strong differentiating aroma notes such as Cascade, Centennial, Chinook, Citra, Simcoe, Amarillo, Topas, Galaxy and Nelson Sauvin, to name only the most important “Flavo(u)r Hops”. Especially US hop growers already adapted their production to these craft brewers` demands by producing “Flavor” hops on one third of the US hop acreage in 2012.

Until recently, all breeding efforts at the Hop Research Center (HRC) Huell have been focused to develop aroma varieties representing the classical European noble aroma type and high alpha varieties with traditional hoppy aroma characteristics. Inspired by US craft brewers and other innovative brewers worldwide already in 2006 a new breeding program was started at Huell to develop hops with clearly distinctive fruity, citrusy, floral and exotic aroma impressions. In addition to specific crosses, seedlings from former high alpha breeding programs were screened for typically “unhoppy” fruity and exotic aroma notes.

These new Huell breeds with their multifaceted aroma compositions should pave the way of German hops to this profitable craft brewers` market.

Material and Methods

First, crosses were conducted between the US cultivar Cascade and Huell male hops, while later on also Huell breeding lines with fruity aroma impressions on both parental sides were used. Seedlings pre-selected for disease resistance/tolerance, vigor, sex and cone shape were grown in the hop breeding yard in Huell and later on in Rohrbach providing the option to conduct selection under different soil conditions. Based on organoleptic aroma assessment promising seedlings revealing fruity, citrusy and floral aroma notes were harvested. Bitter compounds of the dried cones were chemically analyzed using HPLC (ANALYTICA-EBC

7.7). Based on EBC 7.10 the oil content was determined, while the aroma composition was estimated via head space chromatography. Details are given by Lutz et al., 2012. The total polyphenol content was analyzed according to EBC 9.11. In addition to specific crosses, seedlings from former high alpha breeding programs were screened in the same way for differentiating fruity, citrusy and exotic aroma notes. Numerous brewing trials were conducted by interested brewers worldwide including US craft brewers based on their own recipes using traditional hop addition and/ or the dry hopping procedure. Beer tastings were conducted by experienced and by specially trained testing panels.

Results and Discussion

Pursuing the objective to create hops with unique distinguishing aroma impressions crosses were conducted between the US cv. Cascade and male Huell breeding lines to combine fruity, citrusy aroma compounds (key compounds of some wild hops and cultivars with North American genetic background) with traditional herbal, woody and spicy aroma impressions of European genetic origin. In addition, the Huell germplasm should bring into the progeny disease resistance/ tolerance and good agronomic performance under German growing conditions (soil, weather, etc). In addition, seedlings from former high alpha crossing programs, where North American hop germplasm was introduced into Huell material to increase the alpha acid content and to bring in specific disease resistances, were now screened for novel aroma characteristics which were excluded in former times.

Based on organoleptic evaluation hops revealing these new “unhoppy” aroma impressions were harvested, if they also showed good agronomic performance and disease resistance/tolerance. Chemical analyses of these hops confirmed their distinctive composition of the essential oil compounds, especially when compared to the landrace Hallertauer Mittelfrueh with European noble aroma style and to the US cultivar Cascade as reference for a typical “Flavor” hop.

After the harvest in 2011, several promising experimental lines were presented to the members of the Society of Hop Research including German hop growers, hop traders, national as well as international brewers and also US craft brewers which showed great interest in using the Huell breeding lines in brewing trials. A lot of brewing trials conducted in Germany, Belgium, Austria and the USA proved the unique flavoring potential of these new Huell Special Flavor Hops.

Four hop breeding lines with excellent performance in various brewing trials by imparting unique complex fruity, citrusy and floral aroma notes combined with traditional hoppy aroma features were submitted to registration for Plant Variety Rights (PVR) to the EU PVR office by the Society of Hop Research (GfH) in 2012. Following also the great interest of hop growers and hop traders, these four hop varieties (cultivars) named “Mandarina Bavaria”, “Huell Melon”, “Hallertau Blanc” and “Polaris” were propagated and grown on 48 ha under specific license agreements. Harvest of these very young stands was still limited in 2012 and thus, only small amounts of hops are currently commercially available for brewers.

„Mandarina Bavaria“ (MB) shows fruity aroma with pronounced mandarin and citrus notes combined with traditionally hoppy nuances which is a quite new aroma composition in the Huell hop portfolio showing similarities to the US cultivars Cascade and Centennial. „Huell Melon“(HN) arouses associations of ripe honeydew melon accompanied by sweet apricot and strawberry nuances. With this aroma signature this new Huell Special Flavor Hop is unique in the international hop variety spectrum. Aroma impressions which remind to green fruits prevail in „Hallertau Blanc“ (HC) with a floral-fruity basic note reflecting the typical bouquet of a fine white wine.

In contrast to the above described Huell Special Flavor Hops which derived from crosses with the US cv. Cascade as mother, the forth cultivar „Polaris“ (PA) derived from a cross based on Huell germplasm on both sides with the US cv. Nugget and a Japanese breeding line in its pedigree. This cross was still conducted by our former breeder Herbert Ehrmaier following the sole objective to increase the alpha acid content. One seedling of this progeny attracted attention due to its special fresh aroma reminding to mint and glacier sweets.

Furthermore, it showed an extremely high alpha acid content of up to 24 % and a total oil content of 4.4 – 4.8 ml per 100 g cones. With these data Polaris proved to be frontrunner within the international spectrum of hop cultivars.

Table 1: *Chemical data from the Huell Special Flavor Hops. Data are based on the results obtained from 3-5 harvest years; the value for the total polyphenol content was determined only in samples harvested in 2012; [1]in % (w/w); [2]relative in % alpha acids; [3]ml/100 g of dried cones.*

	EBC 7.7				EBC 9.11	EBC 7.10
Cultivar	α-acids[1]	β-acids[1]	cohum-ulone[2]	xantho-humol[1]	poly-phenols[1]	total oil[3]
Mandarina Bav. (2007/018/013)	7.0 - 10.0	4.0 - 7.0	28 - 35	0.5 - 0.7	2.3 - 2.7	1.5 - 2.2
Huell Melon (2009/002/706)	7.0 - 8.0	6.0 - 8.0	25 - 28	0.4 - 0.7	3.0	0.8 - 2.1
Hallertau Blanc (2007/019/008)	9.0 - 11.0	4.0 - 7.0	19 - 25	0.2 - 0.5	3.1	1.5 - 1.8
Polaris (2000/109/728)	18.0 - 24.0	5.0 - 6.5	22 - 29	0.9 - 1.0	2.6 - 2.7	4.4 - 4.8

The sensorically perceived aroma characteristics of these four hop cultivars could be confirmed by the headspace GC analyses which also approved the novelty of these new breeds. Based on a total of 76 GC peaks which could be identified as specific chemical substances, 39 essential oil compounds could be assigned to six specific odor categories: fruity (with 7 compounds), citrusy (4), floral (4), spicy/resinous (3), woody (10) and herbal (9). When comparing the GC peak areas of these six aroma categories we got a rough idea about the aroma potential of the new experimental lines compared to that of Hallertau Mfr and of Cascade as references for classical and "flavor" aroma, respectively. Further details are given by Kammhuber in this edition.

Recent studies back up that also in these Huell Special Flavor cultivars (Kammhuber et al., unpublished) soil, climate conditions and in particular, the harvest time have significant impact on the aroma composition (qualitatively and quantitatively) as already shown in other hops (Bailey et al., 2009).

Brewing trials

Sensory impressions and chemical data of dried hop cones are insufficient to estimate the potential of a specific hop variety in beers, especially since hop-derived key aroma compounds which really contribute to the aroma and flavor in beer are just under investigation (Steinhaus and Schieberle, 2007; Gros et al., 2012; Van Opstaele et al., 2012). Moreover, taking into account the endless variations in the whole brewing process (various beer styles, different amounts of hop, exposure time of hop, influence of yeast strains etc.) brewing trials to assess the brewing quality of these Huell Special Flavor hops were urgently needed.

Using "Mandarina Bavaria" as sole hop addition top as well as bottom fermented beers with traditional late hopping and/ or dry hopping showed a hoppy basic note with strong fruity-citrusy potential. In general, a pronounced mandarin orange aroma and flavor could be perceived in these beers with excellent drinkability. "Hallertau Blanc" proved to introduce aroma notes of mango and in particular of green fruits and gooseberry to beers which gave the impression of the bouquet of a white wine. Comparable brewing trial conditions with

“Huell Melon” as exclusive hop addition revealed intriguing unique sweet aroma notes with clearly perceptible honeydew melon aroma, slight apricot notes and floral nuances. Huell Melon is the youngest of these Huell breeds and thus, only limited amounts of hops were available for brewing trials. Tasters highlighted the excellent aroma and bitter quality of “Polaris” in single hopped beers. Multiple fruity, citrusy and minty flavor notes could be perceived in these beers.

It is expected that new studies on the essential oil compositions of these new Huell Special Flavor Hops will cast light on the issue whether special compounds such as thiols which are assumed to determine the specific aroma of Cascade and Nelson Sauvin (Gros et al., 2012) also contribute to unique aroma and flavor impressions imparted by these hops to beers.

Although, at current no further Huell Special Flavor Hop should be released, breeding efforts are going on and selections with other extraordinary aroma signatures reminding to apple, mint, apricot and cassis are already in the pipeline. For some brewers even hops with celery and lovage nuances drummed up enthusiasm for experimentation.

Acknowledgements

We wish to give special thanks to C. Petzina, B. Wyschkon, M. Hainzlmaier and S. Weihrauch for their excellent technical assistance. Moreover, our thanks go to the team of the hop breeding group. Thanks are also due to the Society of Hop Research and the German Hop Growers Association in getting us into contact with experts from the hop and brewing sector. We highly appreciate the support of the hop trading firms and of all brewers who conducted brewing trial with Huell breeds. Great thanks are due to the Hop Producer Group HVG for funding part of this work.

References

Bailey, B.; Schönberger , Ch.; Drexler, G.; Gahr, A.; Newman, R.; Pöschl, M. and Geiger, E.: The Influence of Hop Harvest Date on Hop Aroma in Dry-Hopped Beers, MBAA TQ, (2009), doi:10.1094/TQ-46-2-0409-01.

EBC Analytical Committee 1998: Analytica EBC, EBC 7.7, 7.10, and 9.11. European Brewery Convention, Fachverlag Hans Carl, Nürnberg.

Gros, J., Peeters, F., and Collin, S. 2012. Occurrence of Odorant Polyfunctional Thiols in Beers Hopped with Different Cultivars. First Evidence of an S-Cysteine Conjugate in Hop (*Humulus lupulus* L.). J. Agric. Food Chem. 60, 7805-7816.

Lutz, A., Kammhuber, K., and E. Seigner, E. 2012. New Trend in Hop Breeding at the Hop Research Center Huell. BrewingScience 65 (March/April 2012), 24-32.

Steinhaus, M.; Schieberle, P. 2007. Transfer of the potent hop odorants linalool, geraniol and 4-methyl-4-sulfanyl-2-pentanone from hops into beer. In: European Brewery Convention, Proceedings of the 31st EBC Congress, Venice. Fachverlag Hans Carl, Nuremberg, Germany, ISBN: 798-90-70143-24-4.

Van Opstaele, F., De Causmaecker, B., Aerts, G., De Cooman, L. 2012. Characterization of novel varietal floral hop aromas by headspace solid phase microextraction and gas chromatography mass spectrometry/olfactometry. J. Agric. Food Chem. 60, 12270-12281.

INTERSEXES IN HOP – MORPHOLOGICAL, CYTOLOGICAL AND GENETIC VIEW

Škof, S.[1], Čerenak, A.[2], Jakše, J.[1], Bohanec, B.[1], Javornik B.[1]

[1]University of Ljubljana, Biotechnical Faculty, Agronomy Department, Jamnikarjeva 101, Ljubljana SI-1000, Slovenia
[2]Slovenian Institute of Hop Research and Brewing, Cesta Žalskega tabora 2, Žalec SI-3310, Slovenia
E-mail: branka.javornik@bf.uni-lj.si

Humulus lupulus L. is a dioecious perennial plant, in which sexes differ only in generative organs, and only female hops are cultivated. Following hybridisation among dioecious plants, different proportions of intersexes can arise. To reveal possible correlations between sex expression and ploidy level, we analysed 58 hop intersexes, progenies of various crosses of diploid parents, to provide additional data on hop monoeciosity. Flow cytometric analysis revealed that a high percentage of intersexes were of triploid genotype (41.4%). In order to determine whether the cause of triploid formation is unreduced pollen grains, the ploidy level of pollen nuclei of five pollinating lines was studied by flow cytometry using a newly established procedure based on disruption of pollen grains by chopping in frozen buffer. Since ploidy screening showed normal pollen nuclei in analysed males, inheritance analysis of parental alleles in triploid progeny was carried out using six codominant microsatellite markers and, in most cases (84.2%), it demonstrated the involvement of unreduced male gametes. A predominantly male phenotype, with a few female cones, was found among triploids. Mainly diploid intersexes were confirmed, with one exception, in which an aneuploidic number of chromosomes was found (21). No clear correlations between nuclear DNA content and type of sex expression in diploid hops were found. The lower DNA content in males was measured by AT-specific DAPI and intercalating PI dye. The estimated AT frequency placed hop among species with a high AT content, which was slightly higher in male than in female plants (63.0% vs. 62.5%).

Reference:

ŠKOF, Suzana, ČERENAK, Andreja, JAKŠE, Jernej, BOHANEC, Borut, JAVORNIK, Branka. Ploidy and sex expression in monoecious hop (Humulus lupulus). *Botany*. 2012, vol. 90, no. 7, str. 617-626. http://dx.doi.org/10.1139/b2012-037.

BIOCHEMICAL AND GENETIC DIVERSITY OF WILD HOPS IN THE SLOVAK REPUBLIC

Farago, J.[1], Nesvadba, V.[2], Pšenáková, I.[1], Krofta, K.[2], Henychová, A.[2], Patzak, J.[2]

[1]Department of Biotechnology, Faculty of Natural Sciences, University of SS. Cyril and Methodius in Trnava, Slovak Republic; E-mail: juraj.farago@ucm.sk
[2]Hop Research Institute, Co., Ltd., Kadaňská 2525, Žatec, Czech Republic
E-mail: patzak@chizatec.cz

Abstract

During the year 2012, samples from 43 wild grown hops from Western and Middle Slovakia were collected and subjected to molecular (SSR, STS), and biochemical (polyphenol and flavonoid content) analyses. Spectrophotometric analyses confirmed high variability in polyphenol and flavonoid contents, especially in plant mature leaves (38 genotypes) in comparison to ripe cones (12 genotypes). The molecular analyses, contrary to previously published results that wild hop genotypes have been divided into two major groups, showed subdivision of wild hop genotypes into several distinct groups: the North American wild hops, the old European cultivars, and the European wild hops including the Caucasus region-, Slovak wild hop-, old European cultivar's- and other continental European wild hop subgroups.

Keywords: wild species, polyphenols, flavonoids, SSR analysis, STS analysis, diversity

Introduction

The hop plant, *Humulus lupulus* L., is commercially cultivated for brewing purposes in a number of countries worldwide. Even though there are more than some 200 cultivars grown in hopyards all over the world, many analyses, for example those of microsatellite loci showed a very limited genetic variation present in modern hop cultivars (Jakše et al., 2001). This narrow range of genetic variation has been mainly attributed to the use of restricted number of varieties in hop breeding progammes (Jakše et al., 2001, Patzak et al., 2007). It is generally accepted that wild germplasm could provide new genetic resources for breeding to broaden the genetic variation of hop varieties. Currently, also some novel breeding aims, such as increasing the content of phytomedicinally important compounds in hop cones, highlights the increasing role of wild species in genetic improvement of the crop. Wild hops may also be a direct source of interesting biologically active phytochemicals as well as biopesticidal compounds (Hampton et al., 2003). Slovak Republic belongs to the traditional hop growing countries with the earliest record of hop cultivation dating back to the year 1113, when mentioned in the so called Zobor act. Contrary to this long-lasting tradition and to the situation in neighbouring countries, e.g. Poland, Czech Republic, Hungary and Austria (Brudzinsky and Baranovski, 2003; Patzak et al., 2010b; Murakami et al., 2006b), up to date no study is known on the occurence, distribution, and genetic structure of wild hops grown in Slovakia. Therefore, we have initiated a bilateral project with a primary aim to fill in this gap, and to monitor the distribution of wild growing hop plants throughout the country and to characterize their genetic and biochemical structures with reference to potential use in breeding programmes, or for alternative applications as sources of interesting secondary metabolites.In this paper, we report on the biochemical and genetic diversity of wild hops from different localities in the Western and a part of Middle Slovakia.

Materials and Methods

Plant material

In total, 43 wild hops (*Humulus lupulus* var. *lupulus* L., SK_n, n=1-43) were collected in 2012 from the western and middle part of the Slovak Republic. During two collection trips young leaves from shoot tips for molecular analyses (May 2012), and mature leaves and ripe cones

for biochemical analyses (September 2012) were sampled from selected plants. For comparison of molecular data, a total of 192 individual wild hop genotypes from Czech Republic (CZ), France (FR), Switzerland (SW), the Caucasus region of Russia (RU), Canada (CA) and USA (US) and five commercial hop cultivars according to our previous works (Patzak et al. 2010a,b) were used.

Biochemical analyses

Total polyphenol content analysis

The total content of polyphenols in plant extracts was determined spectrophotometrically using the method of Singleton and Rossi (1965). Gallic acid was used as the standard. Total content of polyphenols was expressed as equivalents of gallic acid in one gram of dry hop leaves or cones.

Total flavonoid content analysis

Contents of flavonoids in hop extracts were determined by the spectrophotometer method according to Rakotoarison et al. (1997). Quercetin was used as the standard. Total content of flavonoids was expressed as equivalents of quercetin in one gram of dry plant material (leaves or cones).

DNA analyses

DNA isolation and polymerase chain reaction (PCR)

Total genomic DNA was isolated from young leaves according to Patzak (2001). Nine SSR (Hadonou et al., 2004; Jakše et al., 2002) and three STS (Patzak et al., 2007) loci were used for molecular analyses. In a typical PCR reaction (*Taq* PCR master mix kit, Qiagen, FRG) the following amplification conditions were used: 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 a TGradient thermocycler (Biometra, FRG). Amplification products were resolved via 5% denaturing (8M urea) polyacrylamide gel vertical electrophoresis and visualized by silver-staining (Patzak, 2001).

Genetic diversity analysis

The presence or absence of the PCR products was coded by 1 or 0, respectively, to generate the raw data matrix. Genetic similarity/dissimilarity among accessions was estimated using Jaccard's (1908) similarity coefficients by DARwin v. 5.0.155 (Dissimilarity Analysis and Representation for Windows, http://darwin.cirad.fr/darwin). For hierarchical clustering, we used the neighbor-joining (NJ) cluster analysis based on unweighted pair group method with arithmetic mean (UPGMA) by DARwin v. 5.0.155. The generated dendrogram was visualised by Geneious Pro 4.8.2 (Biomatters Ltd., Auckland, New Zealand).

Results and Discussion

Biochemical diversity analysis

Total polyfenol and flavonoid content in mature leaves was determined using standardized spectrophotometric assays in 38 wild hops collected from Western and Middle Slovakia. The concentration of polyphenols in samples varied significantly from 1.66 to 10.28 mg/g DW with coefficient of variation (CV) of 44,7%. Similarly high variability was detected in the flavonoid content of leaves, ranging from 0.58 to 3.88 mg/g DW (CV = 44,4%) (Fig. 1). The polyphenol and flavonoid content in cones of 12 wild grown hops was about 2- to 12-times higher than in mature leaves reaching 37.2 mg/g DW in the case of polyphenols and 7.6 mg/g in the case of flavonoids, respectively (Fig. 2). However, the CVs for polyphenol and flavonoid content in the generative part of plants were much lower, 15.3 for polyphenol content, and 8.1 for the flavonoid content, respectively, than in leaves. When compared with our previous studies on commercial hop cultivars (Pšenáková et al., 2010), these results show that polyphenol and flavonoid contents in wild grown hops may be similar or even higher, than in commercially grown cultivars.

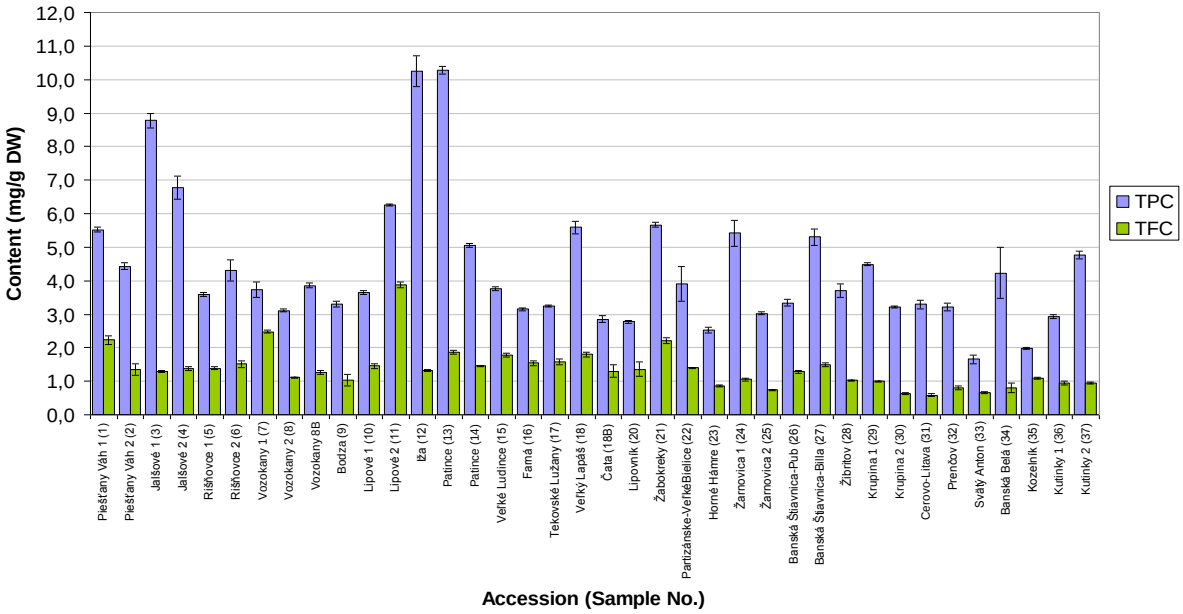


Figure 1: Comparison of the total polyphenol (TPC) and flavonoid contents (TFC) in mature leaves of 38 wild hops from different localities of Western and Middle Slovakia.

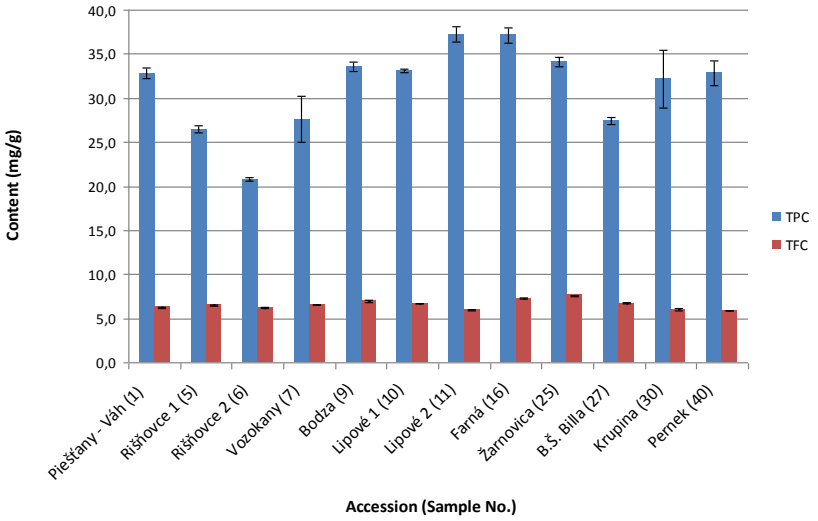


Figure 2: Comparison of the total polyphenol (TPC) and flavonoid contents (TFC) in cones of 12 wild hops from different localities of Western and Middle Slovakia.

Genetic diversity analysis

Using molecular analyses of nine SSR and three STS loci we analyzed the genetic diversity of 43 Slovak wild hops, in comparison to 192 wild hop genotypes and five commercial hop cultivars from the collection of hop germplasm at the HRI, Žatec (Czech republic). The resulting dendrogram (Fig. 3) showed that the wild hop genotypes were divided into several distinct groups. The most distant group included North American wild hops (blue). Hybrid hop cultivars (Galena, Brewers Gold and Kirin) separated this group from European wild hops and old European cultivars (Osvald's clone 72 and Fuggle). The European wild hop group included the Caucasus region subgroup (violet), Slovak wild hop subgroup (red), old European cultivar's subgroup and other continental European wild hop subgroup. Some Slovak wild hops, SK_16, SK_36 and SK_37, were grouped into the European cultivar's subgroup and they were probably wildened forms of cultivated Saazer hops grown historically in this region. Generally, the close genetic relationship within continental

European wild hops has been caused by an introgression of cultivated hops into the natural populations during the historical cultivation period in Europe. The most of Slovak wild hops were divided into two groups: the first inside the other continental European wild hop subgroup and the second separated the Slovak wild hop subgroup. The wild hops from detached Slovak subgroup occurred together with other Slovak wild hops in all localities, but more in eastern part of studied region. This distribution of genetic diversity can be due to recombinations with wild hops from the east parts of Europe, while Slovak wild hop subgroup was more closely to the Caucasus region subgroup.

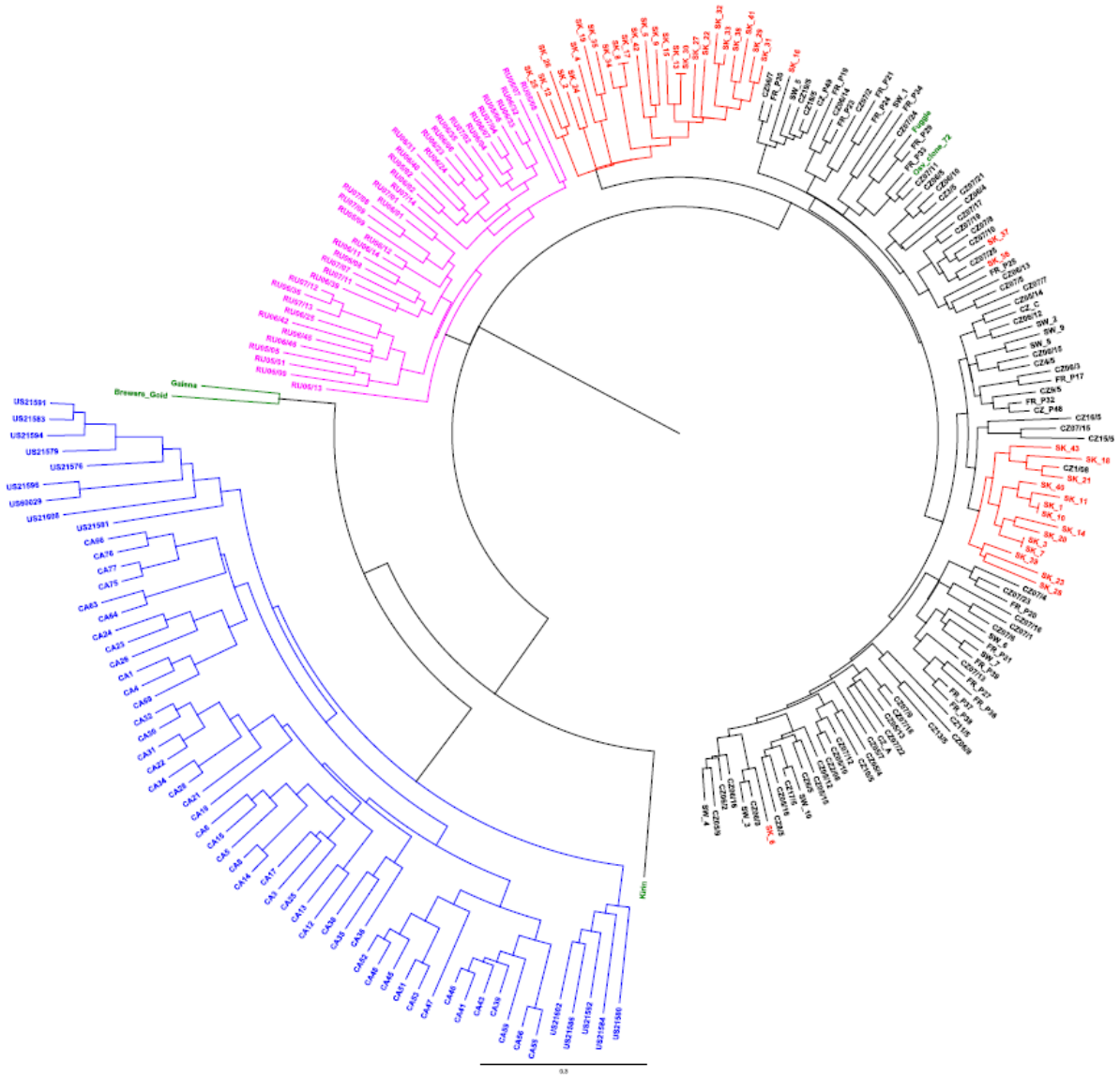


Figure 3: Dendrogram showing the genetic similarity/dissimilarity among accessions of 43 Slovak wild hops (red colour), in comparison to 192 wild hop genotypes (blue, violet, and black) and five commercial hop cultivars (green) constructed using molecular analyses of nine SSR and three STS loci.

Our genetic diversity analysis concurred with previously published results that wild hop genotypes have been divided into two major groups of North American and Eurasian wild hops, divided into two subgroups of continental Europe and Caucasus region (Murakami et al. 2006a,b, Patzak et al. 2010a,b).

Acknowledgement

This work was financially supported by the Agency for Support of Research and Development (APVV, Agentúra pre podporu výskumu a vývoja) in Slovakia and Ministry of Education, Youth and Sport (MŠMT, Ministerstvo školstva, mládeže a tělovýchovy) in Czech Republic within the Bilateral Project SK-CZ-0087-11.

References

Brudzynski, A., Baranowski, K. 2003: Laboratory and industrial scale brewing trials with Lubelski, Marynka, Oktawia, and other Polish hop varieties. J. Inst. Brew., 109: 154-156.

Hadonou, A.M., Walden, R., and Darby, P. 2004. Isolation and characterization of polymorphic microsatellites for assessment of genetic variation of hops (*Humulus lupulus* L.). Mol. Ecol. Notes 4: 280-282.

Hampton R., Nickerson G., Whitney P., Haunold A. 2002: Comparative chemical attributes of native North American hop, *Humulus lupulus* var. *lupuloides* E. Small. Phytochemistry, 61: 855-862.

Jaccard, P. 1908. Nouvelles rescherches sur la distribution florale. Bull. Soc. Vaud. Sci. Nat. 44: 223-270.

Jakše J, Kindlhofer K, Javornik B (2001). Assessment of genetic variation and differentiation of hop genotypes by microsatellite and AFLP markers. Genome 44: 773–782.

Jakše, J., Bandelj, D., and Javornik, B. 2002. Eleven new microsatellites for hop (*Humulus lupulus* L.). Mol. Ecol. Notes 2: 544-546.

Murakami, A., Darby, P., Javorník, B., Pais, M.S., Seigner, E., Lutz, A., and Svoboda, P. 2006*a*. Microsatellite DNA analysis of wild hops, *Humulus lupulus* L. Genet. Resources Crop Evol. 53:1553-1562.

Murakami, A., Darby, P., Javorník, B., Pais, M.S., Seigner, E., Lutz, A., and Svoboda, P. 2006*b*. Molecular phylogeny of wild hops, *Humulus lupulus* L. Heredity 97: 66-74.

Patzak, J. 2001. Comparison of RAPD, STS, ISSR and AFLP molecular methods used for assessment of genetic diversity in hop (*Humulus lupulus* L.). Euphytica 121: 9-18.

Patzak, J., Nesvadba, V., Krofta, K., Henychová, A., Marzoev, A.I., Richards, K. 2010b. Evaluation of genetic variability of wild hops (*Humulus lupulus* L.) in Canada and the Caucasus region by chemical and molecular methods. Genome 53: 545-557.

Patzak, J., Nesvadba, V., Henychová, A., and Krofta, K. 2010a. Assessment of the genetic diversity of wild hops (*Humulus lupulus* L.) in Europe using chemical and molecular analyses. Biochem. Sys. Evol. 38: 136-145.

Patzak, J., Vrba, L., and Matousek, J. 2007. New STS molecular markers for assessment of genetic diversity and DNA fingerprinting in hop (*Humulus lupulus* L.). Genome 50: 15-25.

Pšenáková, I., Hetešová, L., Nemeček, P., Faragó, J., Kraic, J. 2010: Genotype and seasonal variation in antioxidant activity of hop extracts. Agriculture 56: 106−113.

Rakotoarison, D., Gressier, B., Trotin, F., Brunet, C., Dine, T., Luyckx, M., Vasseur, J., Cazin, M., Cazin, J.C., Pinkas, M. 1997. Antioxidant activites of polyphenolic extracts from flowers, *in vitro* callus and cell suspension cultures of *Crataegus monogyna*. Pharmazia, 52: 60−63.

Singelton, V.L., Rossi, J.A. 1965. Colorimetry of total phenolics with phosphomolybdic-phosphotungstic acid reagents. Am. J. Enol. Viticult., 16: 144–158.

II. Session:
Biotechnology

BIOTECHNOLOGICAL APPROACH FOR IN VITRO CLONING OF VIRUS-FREE HOPS (HUMULUS LUPULUS L.) AND FORMATION OF ORGANIC HOP GARDENS IN THE UKRAINE

Melnychuk, M., Boyko, A., Grygoruk, I., Dubrovin, V., Makarenko, N., Golub, G., Overchenko, V., Likhanov, A., Kliuvadenko, A., Pilipchuk, E., Shulga, V.

National University of Life and Environmental Sciences of Ukraine,
13 Geroiv Oborony str., 03041 Kiev, Ukraine,
E-mail: virlab@nauu.kiev.ua

Keywords: Hop (Humulus Lupulus L.), clonal micropropagation, culture in vitro, viral pathogens, polymerase chain reaction, direct adaptation, formation of organic hop gardens

The primary prerequisite for obtaining high yields of hops (*Humulus lupulus* L.) is receiption and introduction of high-quality planting material of hops with a high specific varieties and hops free of pathogens, including viruses, as well as using the latest technology of growing of organic and a high quality of hop's raw.

In Ukraine hop production is concentrated in farmsteads of woodlands and forest areas where soil and climatic conditions are most optimal match for biological characteristics and to get a high-quality hop's raw of aromatic and bitter hops varieties for the needs of the domestic brewing industry. Our country with a strong base for growing hops, unfortunately, feels a sharp reduction of its production due to the lack of means of hardware tillage, keeping, processing and alteration of hop's raw, inadequate of growing technology and a limited number of high-quality, competitive planting material of hops. So it's necessary to improve an existing and create a new biotechnology for cultivation of varieties and hybrids for update and improve the processing of plantation of hop cones.

There are a number of negative factors, before laying the plantation of hops, which are affecting for yield and quality of organic hop garden, including a viral pathogens, varietal specificity, quality of planting material, preparation of soil of organic hop garden. Ignoring only one factor is not possible to correct the mistake in the future, even using the latest technology and growing technology, advanced system of protection and fertilization of organic hop garden.

Viral pathogens are spread on plants of hop, affects them in different ways. Most virulent strains of viruses affecting hop in Ukraine belong to groups of *Ilarvirus*, *Nepovirus* and *Carlavirus*. External symptoms are manifest affected hops is very diverse and can be clearly expressed and very weak. However, some plants and even varieties are asymptomatic carriers of viruses, which are localized in a latent form. Such features of viruses are often not considered by breeders and farms which are massively propagated hops. Therefore, there is need to develop and implement methods for diagnosis, identification of viruses, and most importantly, methods of obtaining of virus-free planting material of hops. One of the most effective methods of struggle with viral infections is the method of clonal micropropagation. The method of clonal micropropagation is mainly seen as an opportunity of vegetative propagation of plants and their improvement *in vitro*. Existing methods of clonal micropropagation of plants can not be used for hop culture without careful study. Breeding of a new species and varieties of hops *in vitro* occurs a significant number of challenges at all stages of micropropagation, starting with the selection of initial explants, a sterile culture, recovery, selection of components and nutrient concentrations at different stages of morphogenesis. Especially important step in growing technology is rooting and adaptation to the conditions of organic hop garden (*in vivo*). In the case of an effective biotechnology of breeding may speed up the breeding process, get healed and genetically uniform planting

material, reduce the duration of juvenile phase and losses of crop hops. The next step of biotechnological process is a control of varietal purity of all stages of hop's culture *in vitro*.

Differentiation and identification of varieties, lines and hybrids of agricultural plants is an important element of breeding, seed production and it's relevant in the copyright protection of varieties. The introduction of molecular techniques of applied virology and biotechnology, along with the existing methods of identification of plant varieties, can significantly add it and reduce it to the terms of identification of varieties for needs a few plant material (leaves, roots, stems, etc.) at any stage of ontogeny, having the test of minimum number of extracted DNA of plants. Therefore, research towards the development of molecular-diagnostics for viral infection and for tested methods of SSR-identifying of genotypes of hop plant is important for hop industry to enhance its competitiveness.

The transition from conditions of cultivation *in vitro* to *in vivo* conditions is the most important stage of agricultural biotechnology for getting high-quality products of hop. At this stage, lost the largest number of seedlings in a quantitative sense and it take place in re-contamination of pathogens. Scholars of our university designed and successfully implemented a method of direct adaptation of hops. This method makes it possible to reduce the cost of rearing seedlings and get a yield of 0,8-1,2 t/ha of hop cones in the first year of vegetation, depending on variety and growing in agricultural and climatic conditions.

The aim of our research is developing of technologies of landing of organic hop garden by planting material after culture *in vitro* on the virus-free basis by the direct adaptation to *in vivo* for organic production of hops.

Results and Discussion

For landing of organic hop garden we're used varieties of hops, which have high quality and technological indicators, and competitive in the market of hop's raw. In Ukrainian market one of the most competitive variety of hop is National (authors Prof. Maksym Melnychuk and Dr. Maya Zagrafova). National – is hop variety established by selection with hybrid populations of aromatic parent form and precocious male plant of Ukrainian origin, type of cultivar - aroma. The growing period – 120-140 days. Resistant to pests and diseases, especially viral pathogens. Bush like cylindrical form, quantity of leaves is average. Stem is green, length of lateral branches - 80-100 cm, cones like oval-elongated form with high density. Sort is intensive type. Harvest of hop cones - 2.5 - 3.0 t/ha, content of alpha acids about 7,4 – 9 %. Technology of cultivation requires a high level of farming and full of nutrients. Raw of variety is suitable for producing high quality brand of premium beer by extraction and granulation. It has DNA-identification and DNA-profiling like relatively common European varieties of hops (Czech Republic, Germany, Ukraine).

We have chosen the method of allocating the apical meristem for receiving virus-free plant material, which subsequently used to clonal micropropagation. In particular, we've request the using of three types of meristematic explants that were distribute by size: type I (meristem with 2-3 puff primordium) – ~250 to 400 micrometers, type II (meristem with primordium and partly meristematic zone) – ~400 to 550 micrometers, type III (meristematic zone) – from 550 to 800 micrometers (Fig. 8).

In our opinion optimal should be considered using of explants of the apical zone of hop plants of II type, because these dimensions make the best using of regenerative capacity of tissues (64-76%) and free them of viral pathogen (56-60%). To increase the coefficient of obtaining virus-free plant material we've proposed using the method of thermotherapy. We've seen a similar picture of the display of morphogenetic ability of apexes of both varieties of hops, in imposing of conditions of thermotherapy method of apical meristems. Our results showed that a combination of thermotherapy (exposure time – 20 days at a temperature of 37 ± 1^0C) and the allocation of apical meristem (meristem with primordium and the partly meristematic zone in the 400-550 micrometers) allows to get 75 - 90% of healed plant material.

The best period for the selection of vegetative buds of above ground plant parts in agricultural communities is period of May to mid-July. The selection of wintering buds of rhizomes for using them as primary explants when injected into *in vitro* culture, so period – April month. The highest morphogenic potential observed a vegetative buds of tops and shoots hops.

We've obtaining the aseptic culture of vegetative buds and segments of stem of varieties of hops using 0.1% solution of mercuric chloride ($HgCl_2$) with exposure times of 7 minutes, which allows to obtain sterile material with high regenerative ability of about 90% samples. The results of numerical experiments to study the morphogenetic potential of tissues and organs hops *in vitro* brought about the feasibility of using as a base, the culture medium MS. The optimum ratio of growth regulators to enhance an axillary meristems and growth of microstems is BAP at a concentration of 1.5 mg / L and CC in concentrations of 1.0 mg / L for a variety of hops National. During the research of induction of rhizogenesis shows the impact of different concentrations of phytohormones, mineral and organic nutrient compounds on the formation of the root system. It is proved that the best breeding ground for rooting microstems of hops is medium containing of 0.1 mg / L of BAP + 1.5 mg / L IBA, the maximum percentage of rooting plants reached to 100%. Specified the dependence of frequency root of light intensity. The highest percentage of rooting occurred in light intensity of 2500 lux - 95%, which sharply decreased with increasing light intensity.

Checking of hop's varietal purity in culture *in vitro* was performed by using of microsatellite DNA sequences that are thermocyclering in the following combinations: (1 - reaction HlG-A3, 11a-59, HlG-T2, 2 - reaction HlG-A9, HlG-T1, HlG-T5, 3 - reaction 3a-88, 5-2, HlG-A4, 7a-82). For the polymerase chain reaction (PCR) prepared the reaction rate of 23.8 mcl of PCR-mixture - added 0.2 mcl of Taq-polymerase per sample. To 24 mcl of final reaction mixture placed in a clean test tube and added to 1 ml of DNA samples in test tubes with reaction mixture. Amplified DNA prepared for analysis at ABI Prism genetic analyzer. In the process of conduct of capillary electrophoresis samples a computer program Genescan determined the size of fluorescent labeled PCR-products and retain the data. Then, the stored data are exported to a computer program Genotyper or Genemapper for genotyping.

The resulting genotype of varieties of hop – National, which is represented as a binary code 11110101010001100010000000010011010000110010 is compared with codes by known varieties and the original DNA plants that served as a donor for the introduction in culture *in vitro*. So the samples corresponded to the variety of hop – National and it's identical to the original plant.

Our previous studies and researches of diagnosis and identification of hop's viruses made it possible decided of the most common viruses in agricultural communities of Ukraine. These include, traditionally hop's viruses, hop mosaic virus (HpMV), hop latent virus (HpLV), apple mosaic virus (ApMV), and the virus that was first identified on the hop in Ukraine – cucumber mosaic virus (CMV).

To create a design of primers like specific to nucleotide sequences of latent virus hops (Hop latent virus), cucumber mosaic virus (Cucumber mosaic virus), apple mosaic virus (Apple Mosaic Virus), conducted the bioinformatic analysis, the first step of which was screening of conservative gene sequences, encoding a membrane protein of related viruses using genetic data bank (GenBank). Based on aggregate data for known nucleotide sequences of viral genomes revealed specific conserved nucleotide sequences, which are further used as the matrix for oligonucleotide primers in the synthesis of virus-specific nucleic acid fragments. The analysis was performed using the software «MultAline» (Multiple sequence alignment).

Using consensus nucleotide sequences of genes encoding the coat protein of these viruses, using software «Primer3» has created design of primers. Analyzing the primer pair for GC-composition, found that they are in an optimal range, and amplification products have sizes for latent virus hops - 427 b.p., tobacco mosaic virus - 347 b.p., cucumber mosaic virus - 157 b.p., and apple mosaic virus - 109 b.p. As a result, researches of cloned hop plants on virus carrier by methods of molecular and biological (RT PCR), ELISA, electron microscopy, all plants after recovery in culture *in vitro* were virus-free.

After working out the required quantity and rearing to size plant hops carried an adaptation. The first stage of adaptation is before adaptation stage – training in culture *in vitro*, the second stage - the adaptation in a greenhouse and hardening of plants to conditions *in vivo*, the third stage involves a direct adaptation of an industrial organic hop garden.

During a planting of industrial plantations of hop by material that received by technology *in vitro*, conduct science-based before planting tillage, which has a critical to any technology of planting or landing plantations. By this event lays the basic of framework for obtaining high yields for existence of organic hop garden. Hop has a number of biological characteristics that must be considered when laying an organic hop garden.

National hop variety besides high quality biochemical and technological parameters proved to be the most labile sort that easily adapts to changing conditions of growing. This sort showed 100% yield at all stages of the adaptation process. In the first year of vegetation gives yield – 1.0 - 1.2 t / ha. It has a high resistance to pathogens especially viral. Our research has shown that the variety of hop National after culture *in vitro* more resistant to viruses compared to other varieties also after culture *in vitro* for 15-22%, and 35-69% in comparison with varieties that reproduce by the traditional way for 1-3 year of vegetation.

For obtained an organic produce of hops we use an integrated system such an agricultural biotechnological alternatives:

- clonal micropropagation *in vitro* of hop and effective rehabilitation and obtaining genetically uniform of plant material;
- direct adaptation of *in vivo* of healing plants, which allows planting and laying of organic hop garden without stage of manifold;
- three breeding schemes, which allow in expressive mode to obtain resistant plants to pathogens and fight with bacterial diseases of plants in the national farm;
- production and application of complex of microbiological and entomological plant protection products;
- the production of biologically active organic fertilizers and nutrient solutions on the basis of microbiological fermentation of cattle and edged of phytomass.

Adaptive agricultural bioengineering system contains the technical components:

- a set of hardware and machinery for tillage, adding of biologicals and bio-fertilizers;
- the system of preparing and filing the nutrient of solution through drip irrigation;
- machines for harvesting;
- technological equipment for drying and granulation.

Conclusions

The technology of clonal micropropagation *in vitro* of hop has developed, it is recommended for mass reproduction for effective rehabilitation and obtaining a genetically uniform plant material.DNA-identification and differentiation of hop's varieties of Ukrainian selection on base of 10 SSR-markers opens new opportunities and enables to make a varieties identification of received virus-free regeneration on stage of cloning micropropagation and also allows to significantly reduce the time of the determination of any varieties of hops.

Also molecular test systems for the most virulent viruses of hop were developed. Analyzing of pairs of primers for GC-composition, determined that they are in the optimal range, and amplification products with sizes for latent virus hops - 427 b.p., tobacco mosaic virus - 347 b.p., cucumber mosaic virus - 157 b.p., and apple mosaic virus - 109 b.p. 4.5 thousand regeneration-plants for planting at organic hop garden with American varieties Newport, Nugget, and Ukrainian variety National on the basis of non-virus were obtained and planted in 2012. Direct adaptation *in vivo* of cloned *in vitro* hop's plant varieties Newport, Nugget and National allowed us to have 100% adaptation. In 2013 year it is expecting the first yield and developing special hop granules combination for organic beer production.

III. Session:
Hop Chemistry and Beer

ANALYTICAL AROMA CHARACTERIZATION OF THE NEW HÜLLER "SPECIAL-FLAVOR-HOPS"

Kammhuber, K.

Bavarian State Research Center for Agriculture, Institute for Crop Production and Plant Breeding, Hop Research Center Hüll, Hüll 5 1/3, D-85283 Wolnzach, Germany, E-Mail: Klaus.Kammhuber@LfL.bayern.de

Abstract

Hop aroma is very complex, but it makes sense to define indicator substances, which are correlated with sensorial impressions. Therefore aroma analytics is an important tool to support hop breeding. The oil compositions of the new Hüller "Special-Flavor-Hops" are quite different from conventional hops. The total oil contents as well as the oil compositions depend heavily on the date of harvest and on the growing locations. Mass spectrometry, GC sniffing and investigations about sulfur containing compounds will certainly result in new insights about hop aroma.

Keywords: Special-Flavor-Hops, essential oils, aroma, biogenesis

Introduction

Hop has three groups of valuable ingredients (α-acids, essential oils and polyphenols). But recently the focus is more on the essential oils (aroma components) than on other ingredients, because the craft brewer scene is getting larger and larger and the craft brewers are especially interested in special, unusual aromas. Aroma analytics is an important tool to support hop breeding. Sensory aroma evaluation is subjective, aroma analytics is objective, but the data must be interpreted. Hop aroma is very complex and can not even be satisfying reproduced by a large number of compounds (Lit.1), but there are indicator components, which are responsible for the typical aroma characteristics of hops (Lit. 2,3,4) (Table 1).

Table 1: aroma active compounds of hop

fruity (esters)	**floral**	**citrus**	**herbal/vegetables**
2-methylbutyl-2-methylbutyrate	linalool	citronellal	α-pinene
2-methylbutyl-isobutyrate	2-decanone	citronellol	α-selinene
2-nonanone	2-undecanone	limonene	cadinene
4,8-decadiene-acid-methylester	farnesol		selinadiene
4-decene-acid-methylester	geraniol		ß-farnesene
capryl-acid-methylester	geranyl-acetate		ß-phellandrene
enanthic-acid-methylester	nerol		ß-pinene
isobutyl-isobutyrate	pentadecanone		ß-selinene
methyl-6-methylheptanoate	tridecanone		
spicy/woody	**grass/hay**	**sulphuric**	
myrcene	hexanal	dimethylsulfide	
α-copaene		4-mercapto-4-methyl-pentane-2-one (4-MMP)	
ß-caryophyllene			
humulene			
caryophylleneoxide			
eudesmol			

This table doesn't claim to be complete. In the future certainly many other aroma active compounds will be found. But these components enable a first estimation of the hop aroma characteristic.

Methods

In Hüll for the aroma components headspace gaschromatography and steam distillation followed by gaschromatography are carried out. The headspace technique allows a qualitative determination, which is suitable to compare varieties. Quantitative determinations are performed by steam distillation and gaschromatography according to EBC 7.10 and 7.12. But a steam distillate represents not what you really smell.

Results

The table 2 shows some selected components, which are typical for a hop variety.

Table 2: oil composition of the new Hüller "Special-Flavor-Hops"

	Polaris	Mandarina Bavaria	Hallertau Blanc	Huell Melon
total oils	4.4 – 4.8	1.5 – 2.2	1.5 – 1.8	0.8 – 2.1
myrcene	51.4	63.3	57.0	49.9
limonene	0.4	0.2	0.5	0.2
2-methyl-butyl-isobutyrate	1.0	1.4	1.2	3.1
enanthic-acid-methylester	1.3	0.4	0.3	0.2
capryl-acid-methylester	1.2	0.6	0.2	0.4
linalool	0.2	0.3	0.4	0.1
ß-caryophyllene	8.8	2.0	0.7	0.6
humulene	21.2	6.3	1.4	1.2
ß-farnesene	0.0	0.0	0.0	0.0
ß-selinene	0.5	2.2	6.5	2.3
α-selinene	0.3	2.4	8.2	2.5
unknown	0.0	5.1	0.0	0.0
geranyl-acetate	0.5	0.0	0.0	0.0
geraniol	0.2	0.4	0.1	0.1

Total oil in ml/100g hops, components in % of the total oil

The variety Polaris has as a special feature a very high content of enanthic-acid-methylester and capryl-acid-methylester. Mandarina Bavaria has an high peak of an unknown substance after the ß- and α- selinenes, which must be identified by a mass spectrometer. The varieties Hallertau Blanc and Huell Melon have a very unconventional oil composition. ß-Caryophyllene and humulene are present only in very low concentrations, but the ß- and α-selinene peaks are very high.

The α- and ß-acids, xanthohumol as well as the polyphenols are represented in table 3 in addition.

Table 3: α-, ß-acids, xanthohumol and polyphenols of the new Hüller "Special-Flavor-Hops"

	Polaris	Mandarina Bavaria	Hallertau Blanc	Huell Melon
α-acids	18.0 – 24.0	7.0 – 10.0	9.0 – 11.0	7.0 – 8.0
ß-acids	5.0 – 6.5	4.0 – 7.0	4.0 – 7.0	6.0 – 8.0
cohumulone	22 – 29	28 – 35	19 – 25	25 – 28
xanthohumol	0.9 – 1.0	0.5 – 0.7	0.2 – 0.5	0.4 – 0.7
polyphenols	2.6 – 2.7	2.3 – 2.7	3.1	3.0

α-, ß-acids, xanthohumol, polyphenols in % of dried hops, cohumulone in % of the α-acids

The total oil content as well as the oil composition depend also highly on the time of harvest (figure 1).

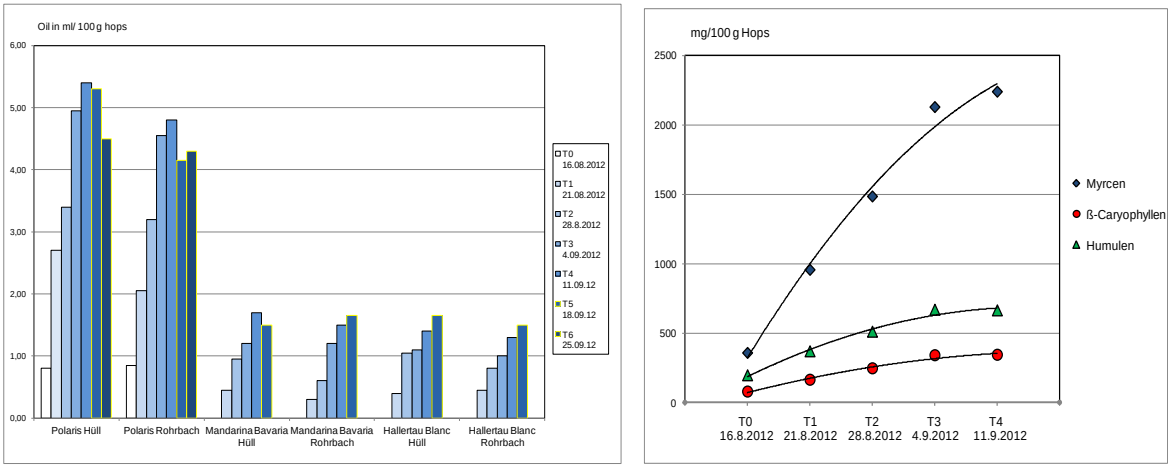


Figure 1: Biogenesis of the total oil content of the varieties Polaris, Mandarina Bavaria, Hallertau Blanc and of single compounds of the variety Polaris

The content of myrcene increases more than the other oil components.Therefore the correct date of harvest is very important for the aroma characteristic of the special flavor hops.

Perspectives

In a research project, which is carried out in cooperation with the TUM (Prof. Dr. Coelhan), we try to find out which components of the oils are aroma active by GC sniffing. Unknown substances for example in Mandarina Bavaria shall be identified by a mass spectrometer and first investigations about sulfur containing compounds shall be done. We are exspecting a lot of new insights about the hop aroma by this project.

References

1) Belitz, H.-D., Grosch, W.: Lehrbuch der Lebensmittelchemie, zweite Auflage, Springer Verlag, 1985, 265

2) Whittock, S.; Koutoulis, A.: New hop (Humulus lupulus L.) aroma varieties from Australia, Proceedings of the Scientific Commission, International Hop Growers` Convention, Lublin, Poland, ISSN 1814-2192, (2011), pp. 10-13

3) Lutz, A., Kammhuber, K., Seigner, E.: New trend in hop breeding at thr hop research center in Huell, Brewing Science, March/April 2012 (Vol.65) 24-32

4) Steinhaus, M.; Schieberle, P.: Transfer of potent hop odorants linalool, geraniol and 4-methyl-4-sulfanyl-2-pentanone from hops into beer. International 31st European Brewery' Convention Congress. Venedig, Hans Carl, 2007, S. 1004–1011

Acknowledgement

The author would like to thank the German HVG Hop Growers Association (Erzeuger-gemeinschaft Hopfen HVG e.G.) for their financial support.

DRY HOPPING TECHNIQUES - HOW TO EXTRACT THE BEST HOP FLAVOURS

Schönberger, Ch.[1], Gahr, A.[2], Drexler, G.[1], Wiesen, E.[1], Zunkel, M.[1]

[1]Barth Innovations, Freiligrathstr. 7/9, 90482 Nürnberg
[2]Hopfenveredlung St. Johann, Mainburger Str. 15, 95538 St. Johann-Train
E-mail: Christina.schoenberger@johbarth.de

Abstract

With the success of craft beers in various countries the technique of dry hopping is today receiving a lot of attention. Dry hopping means to add the hops in the more or less final beer in order to extract very distinct hop flavours. This technique was developed in the UK in the beginning of the 19th century. Due to the enormous success of dry hopped beer also Brewing Science is currently looking into the scientific principles of dry hopping. This talk will give an overview about established dry hopping procedures and about how much is known about the aroma contribution by dry hopping.

Keywords: dry hopping, hop aroma, extraction, hop varieties

Introduction

Dry hopping is a cold extraction in a low alcoholic solution. With increasing alcohol content more substances are being extracted into the beer. Each hop variety and each combination of hop varieties is suitable for dry hopping. The most important parameters that influence the extraction process are: contact area and contact time, temperature, hop product, amount of hops, technique of dry hopping. These parameters will be discussed and examples will be giving.

References

Eyres, G.; Dufour, J.-P. Hop Essential Oil: Analysis, Chemical Composition and Odor Characteristics. In Beer in Health and Disease Prevention; Preedy, V. R., Ed.; Academic Press: San Diego, 2009; pp. 239–254.

Takoi, K.; Koie, K.; Itoga, Y.; Katayama, Y.; Shimase, M.; Nakayama, Y.; Watari, J. Biotransformation of Hop-Derived Monoterpene Alcohols by Lager Yeast and Their Contribution to the Flavor of Hopped Beer. Journal of Agricultural and Food Chemistry 2010, 58, 5050–5058.

Krottenthaler, M; Hanke, S; Kappler, S; Becker, T Influences to the Transfer Rate of Hop Aroma Compounds During Dry-Hopping of Lager Beers. In Proceedings of the 33rd European Brewing Congress; Glasgow, UK, 2011.

Wolfe, P., Shellhammer, T., A Study of Factors Affecting the Extraction of Flavor When Dry Hopping Beer, Masther These of Oregon State University, http://ir.library.oregonstate.edu/xmlui/bitstream/handle/1957/34093/Wolfe_thesis.pdf

COHUMULONE AND BEER BITTERNESS

Krofta, K.

Hop Research institute Co., Ltd, Žatec, Czech Republic,
E-mail: krofta@chizatec.cz

Introduction

Cohumulone, an alpha acid component, was considered to be the cause of unpleasant bitterness of beer for a long time. In 1972, Rigby published a paper presenting the theory claiming that a higher proportion of dissociated isocohumulone contributed to harsher bitterness more than other alpha acid analogues. The theory influenced hop breeding for decades. Low cohumulone ratio cultivars were preferred. Advances in separation methods and analytical instrumentation at the end of the last century have enabled the separation and analyses of individual alpha acid analogues for experimental brews. Detailed studies indeed revealed differences in physical, chemical, and sensorial properties among alpha acid analogues. But no negative effect of cohumulone on the character of beer bitterness was found. The fact that the yield of cohumulone and *iso*–cohumulone are higher during the brewing process was confirmed. The only negative effect of *iso*–cohumulones is the lower beer foam stability. The whole cohumulone history is described in detail below.

Bitterness Intensity

Alpha acids are formed by seven cohumulone analogues that are currently known (cohumulone, humulone, adhumulone, pre-, post-humulone and two others that have not been named yet). The heterogeneous character of alpha acids was discovered by Rigby and Bethune in 1952 (Rigby, Bethune, 1952) by means of the countercurrent distribution. Individual analogues differ by the side acyl chain R at the second carbon of the aroma ring (see Fig. 1).

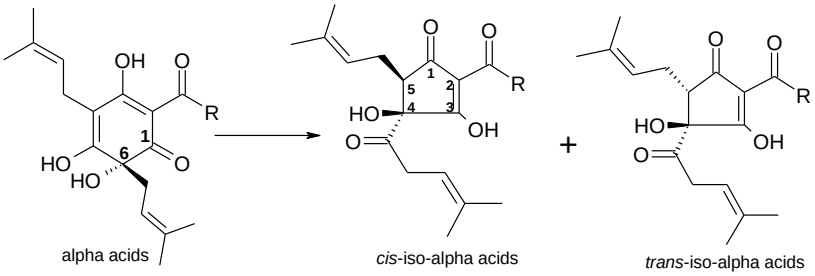


Fig. 1: Structure of alpha acids and their isomerization to *iso*–alpha acids

Cohumulone, humulone, and adhumulone form more than 99% of natural alpha acids. Other analogues are present in negligible amounts. Cohumulone ratio, easily determined by liquid chromatography (Fig. 2), is a chemotaxonomic parameter of hop varieties. Adhumulone ratio ranges between 15–20% rel. in most cultivars. Cohumulone ratio is found in a broader range of 15–45% rel. The analogues proportion changes during hop maturation and ripening. Cohumulone content gradually increases to a level typical for a specific variety. The cohumulone ratio of Czech and German hop varieties shown in Fig. 3 indicates that the ranges of many cultivars overlap. Therefore, variety identification based on the cohumulone content solely is not possible, only the group selection is feasible. Alpha acids are weak non–carboxylic acids (pK_a=4.7–5.7). Pure substances have no taste or smell. The structure and selected properties of alpha acids are summarized in Table 1.

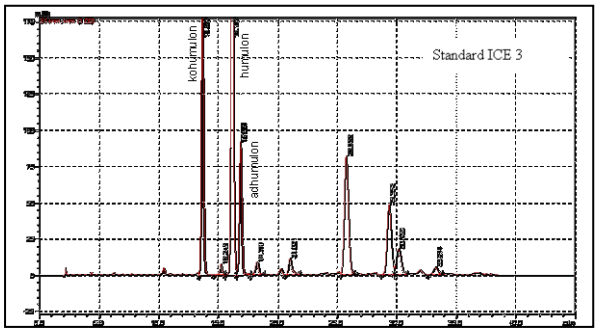


Fig 2: HPLC chromatogram of alpha and beta acids

Table 1: Structure and some properties of alpha acids analogues (Hough, 1991)

Acyl side chain (R)		Name	Melting point (°C)	pK_a
$-CO.CH.(CH_3)_2$	isobutyryl	cohumulone	oil	4.7
$-CO.CH_2.CH.(CH_3)_2$	isovaleryl	humulone	64.5	5.5
$-CO.CH.(CH_3).CH_2.CH_3$	2-methyl butyryl	adhumulone	oil	5.7
$-CO.CH_2.CH_3$	propionyl	posthumulone	-	-
$-CO.CH_2.CH_2.CH.(CH_3)_2$	4-methylpentanoyl	prehumulone	-	-

Table 2: Isomerization yield of the main analogues of hop alpha acids during wort boiling

Isomerization yield (%)	Duration of boiling (min.) at 100 °C					
	10	20	30	45	60	90
cohumulone	5.3	9.2	15.4	24.9	32.0	41.0
humulone	3.9	6.9	11.5	19.6	25.7	33.3
adhumulone	4.3	7.6	13.7	21.4	28.0	36.0

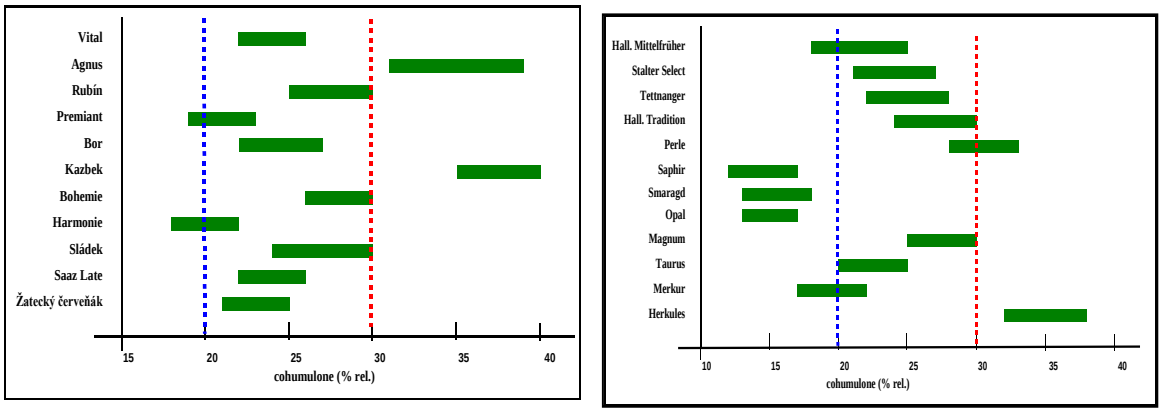


Fig. 3: The ratio of cohumulone in Czech (left) and German hop varieties

Isomerization is the most important reaction of alpha acids. The contraction of the six-member ring to a five-member one occurs (Fig. 1). *Iso*–alpha acids are the main products of the reaction (> 98%). Because cohumulone has a higher dissociation constant (lower pK_a, Table 1), it is more soluble and reactive in wort and beer than other analogues. Its conversion to *iso*–cohumulone during wort boiling is higher than the one of humulone and adhumulone. The results of the detailed kinetic study of the alpha acids isomerization according to Jaskula (Jaskula, 2008) are presented in Table 2. The *iso*–cohumulone ratio in beer is higher than the cohumuone ratio in hops. The difference is 5–7%. The high cohumulone hops have a broader utilization in the brewing process. The acidity of boiling wort (pH ≈ 5.5) is not optimal for conversion of alpha acids to *iso*–alpha acids. If the reaction runs in an alkaline environment at pH=8.0–10.0 under the catalytic effect of magnesium (Mg^{2+}) or calcium ions (Ca^{2+}), the conversion reaches more than 95%. Each analogue is present in the form of two *cis*- and *trans*–stereoisomers. According to a recent study (Urban, 2012), the chiral center is located at C5 of the cyclopentene ring, not at C4 as has been reported up to now (Verzele, 1991). Stereoiosmers have different physical and sensorial properties. Significant differences were found in bitterness of individual *iso*–alpha acid stereoisomers. Cis, trans *iso*-cohumulones and cis, trans *iso*-humulones were separated with the help of preparative HPLC by Hughes and Simpson (1996). The highest level of bitterness was established at *cis*–isohumulone, 1.82 x higher than a *trans*–isohumulone. The lowest bitterness, 74% of *trans*–isohumulone, was found at a *trans*–iso–cohumulone. The bitterness comparison of the most important *iso*-alpha acids is lined up below:

cis-iso-humulone (1.82 x) > *trans*-iso-humulone ≈ *cis*-iso-cohumulone > *trans*-iso-cohumulone (0.74 x)

Under wort boiling conditions, the *cis*-*trans*-stereoisomers and iso–alpha acids are formed at the ratio of 68:32. If pre–isomerized hop products are used for hopping then the *cis*-*trans*–stereoisomers ratio is 80:20.

Character of Bitterness

Character of bitterness is an integral part of the sensorial assessment of beer. It is most frequently described as fine, harmonic, balanced, harsh, unpleasant, clinging etc. Beer acidity (pH) is an important parameter which effects the sensorial impression of the bitterness. Brenner (1956) showed that beers with a higher pH have unpleasant bitterness. As weak acids, isohumulones are found in beer in the form of anions and not dissociated molecules. Rigby (1972) assumes that among the dissociation constants of *iso*–alpha acids there are appr. the same differences as among alpha acids. Anion forms of *iso*-alpha acids disturb the taste cells much more than the undissociated molecules. It means that in beer, the *iso*–cohumulones are more dissociated than the *iso*–humulones. Rigby deduces that hops with a high cohumulone ratio provide beers with harsher and less pleasant bitterness. This conclusion has been presented more or less as a theory not based on a thorough experimental verification. It is surprising that this theory influenced the opinion on cohumulone among hop breeders and brewers for such a long period of time. For many years, varieties with the cohumulone ratio below 30% rel. were preferred in hop breeding. Narzciss (1992) recommends the maximum of 28% of cohumulone for aroma hops, 20–25% being the optimal level. The preference of new hops with a low cohumulone ratio became evident in breeding programs; the target value was set up to 25% rel. (Haunold, 1988). German varieties Smaragd, Opal, and Saphir registered in the period of 2000–2010 have a very low cohumulone content. It took 20 years until several independent studies had shown that Rigby's theory was erroneous.

Warkerbauer (1992) found no negative effect of cohumulone on beer bitterness. He carried out brewing tests with pure preparations of cohumulone, humulone, and adhumulone. Lower beer foam stability was the only negative effect of *iso*–cohumulones. The work of Shellhammer (2004) did not confirm any negative effect of cohumulone on the character of beer bitterness either. Experiments involving two varieties with extremely different cohumulone proportions were conducted (Topaz, 52% rel.; Horizon, 20% rel.). Results of the sensorial tests were assessed by means of the state–of–the–art statistical methods. A recent study comes from Germany and falls to the period when the Herkules variety (35% rel. of cohumulone) was introduced on the market. Full scale brewing tests were carried out using Merkur hops (20% rel. cohumulone) with T90 pellets. Beers were tested for sensorial quality as fresh and after 3 and 6 months of maturation. The results were presented on the 31st EBC Congress in Venice (Kusche, 2007). No statistically significant differences were found during assessment of beers in the sensorial tests.

Table 3: Characteristics of hops and beers – brewing test in pilot scale, HRI Žatec, CZ

Variety	hops			beer		
	alpha acids (% w/w.)	beta acids (% w/w.)	cohumulone (% rel.)	bitterness of beer (IBU)	ranking assessment	final order
Herkules	55.8	18.3	35.0	33.9	162	1.-2.
Magnum	47.4	22.6	26.6	31.9	147	4.
Vital	47.0	27.9	24.6	30.1	162	1.-2.
Agnus	43.3	30.6	33.8	32.6	149	3.

In the course of the last decade, several series of pilot-scale beers have been prepared in the Hop Research Institute in Žatec (CZ) to test cultivars with varied cohumulone ratios. To avoid possible effects of hop polyphenols on beer bitterness, beers were hopped exclusively by CO_2-extracts. The input parameters of hops and results of the sensorial assessment of beers are summarized in Table 3. The cohumulone ratio in the tested hops (Herkules,

Magnum, Vital, Agnus) ranged in the interval of 24–25% rel. Beers were tested for sensorial quality by a ranking test carried out at the brewing seminars that regularly take place in the institute. The tasting panel consisted of 60 to 80 non-professional members as well as professional brewers. No negative effect of high cohumulone levels was found in this test and neither in all other tests.

Conclusion

Individual analogues of alpha acids differ in structure and physical properties (state, solubility in water, optical rotation). These differences cause a specific behavior during the brewing process in respect to the kinetics of isomerization, intensity of bitterness or stability of beer foam. The opinion of the role of cohumulone in brewing underwent a complicated process. For many years, harsh bitterness of beer was blamed on the cohumulone levels although a considerable number of brewing tests did not confirm this hypothesis. Thanks to the higher solubility in wort, cohumulone provides a higher yield and better utilization of alpha acids in the brewing process. The whole history concerning cohumulone is important in regard to breeding of new hop varieties and has been best expressed by Ch. Schönberger (2009) who said that „*Cohumulone is much better than its reputation*".

Acknowledgements

This work was financially supported by Czech Ministry of Industry and Trade within the project FR-TI1/012.

References

Brenner, M.W. et al. (1956): A Study of Hop Bitters (Isohumulones) in Beer. Proceedings of Am. Soc. Brew. Chem. 48-61.

Haunold, A. (1988): Brew. Digest, 63 (10), s. 40.

Hough, J.S. et al. (1991): Malting and Brewing Science, Chapman&Hall, London. ISBN 0412165902.

Hughes, P., Simpson, W.J. (1996): Bitterness of Congeners and Stereoisomers of Hop-Derived Bitter Acids Found in Beer. J. Am. Soc. Brew. Chem. 54(4), 234-237.

Hughes, P., Marinova, G. (1997): Variations in iso-alpha acids distribution and behavior of different hop products. Brewer´s Guardian, 26-30.

Jaskula, B. et al. (2008): A Kinetic Study on the Isomerization of Hop Alpha Acids. J. Agric. Food Chem. 56, 6408-6415.

Krofta, K., Patzak, J. (2011): Zjišťování autenticity českých odrůd chmele pomocí hemických a genetických analýz. Kvasný průmysl 57, 296-304.

Kusche et. al. (2007). Influence of the new high alpha hop variety Herkules on beer quality. Proc. 31th EBC Congress, Venice, Italy.

Narziss, L. (1992): The new Hop Varieties: Hopfen-Rundschau International, 18-25.

Rigby, F.L., Bethune, J.L. (1952): Countercurrent Distribution of hop Constituents. Proceedings of Am. Soc. Brew. Chem. 98-105.

Shellhammer, T. et al. (2004): A Comparison of the Bitter Quality of Beer Produced with High and Low co-humulone Hop Varieties. Oral presentation. World Brewing Congress, San Diego.

Schönberger, Ch. (2009): Why cohumulone is better than its reputation. Brauwelt International III, 159-160.

Urban J. et al. (2012): Beer ´s bitter structural chirality (solved). Oral presentation. III. ISHS Humulus Symposium, Žatec, Czech Republic, September 9 -14.

Verzele, M., De Keukeleire, D. (1991): Chemistry and Analysis of Hop and Beer Bitter Acids. Developments in Food Science 27. Elsevier. ISBN 0-444-88165-4.

Wackerbauer, K., Balzer, H. (1993): Hop bitter compounds in beer, Part II: The influence of cohumulone on beer quality. Brauwelt International, 11, 116-118.

CHANGES OF HOP PRENYLFLAVONOIDS CONTENT DURING MATURATION, HARVESTING, AND PROCESSING

Krofta, K., Pokorný, J., Křivánek, J., Ježek, J.

Hop Research institute Co., Ltd, Žatec, Czech Republic
E-mail: krofta@chizatec.cz

Abstract

The xanthohumol and desmethylxanthohumol (DMX) prenylflavonoids are important secondary metabolites in hops. Numerous studies have proven a considerable number of beneficial physiological and medical effects of xanthohumol. DMX is a potential precursor of 8-prenylnaringenine, which is the most potent known phytoestrogen. Changes to the content of the above mentioned prenylflavonoids were tested in the Czech variety Vital during three vegetation seasons 2010–2012. The Vital variety has an above the average content of xanthohumol (0.70–0.90% w.) and a high content of DMX (0.20–0.40% w.) in dried cones immediately after harvesting. These characteristic properties may be utilized in other industry branches like the pharmacy or the food supplements production. While xanthohumol is a very stable compound, DMX is prone to decomposing under heat exposure and open air conditions. Monitoring of DMX in green cones during ripening and maturation showed that the content of DMX in green cones reached the level of 0.50-0.70% w/w in dry matter. A dramatic decrease in the DMX content by more than 50% rel. occurred during the full scale drying in belt and chamber dryers, too. It is necessary to store the harvested hops immediately after packaging in an air-conditioned warehouse. The CO_2-extraction seems to be the most convenient processing method with respect to the prospective utilizations of spent hops which contain the highest amount of prenylflavonoids.

The content of desmethylxanthohumol showed a permanently decreasing trend depending on the processing and storage conditions. The total loss of DMX in the chain of mature green cones–dry cones–CO_2-extraction–spent hops reached up to 70% rel. In spite of this, the residual content of DMX in spent hops was mostly found in the range of 0.15-20% w/w. On the contrary, the losses of xanthohumol were very minor. The experiments resulted in a proposition of several measures in order to preserve the prenylflavonoids contents in hops at the highest possible level.

Keywords: hops, Vital variety, hops processing, xanthohumol, DMX

Acknowledgements

This work was financially supported by Czech Ministry of Industry and Trade within the project FR-TI1/012.

INCREASING THE POLYPHENOL AND FLAVONOID CONTENTS IN HOP CELL CULTURES THROUGH ELICITATION

Pšenáková, I., Michalková, I., Faragó, J.

Department of Biotechnology, Faculty of Natural Sciences, University of SS. Cyril and Methodius in Trnava, Slovak Republic,
E-mail: juraj.farago@ucm.sk

Hop (*Humulus lupulus* L.) is a well known crop important for the brewing industry. Recently, it has been recognized also as a plant having very interesting chemical composition, that renders it potentially usable for food industry and medical applications. Especially, hop polyphenols and flavonoids are standing in the centre of research interest. Plant tissue cultures, notably callus and cell suspension cultures, provide a convenient model system to study the biosynthesis of biologically active hop compounds, as well as a potentially reliable tool for their production.

The aim of our study was to study the possibility of increasing the accumulation of polyphenols and flavonoids in hop cell suspension cultures using the elicitation strategy. Jasmonic acid (JA), a plant growth regulator, playing a key role in responses of plant cells to exogenous signals from the surrounding environment, was chosen as the elicitor of secondary metabolism. Cell suspension cultures were established in two hop genotypes, K-72/6/13 and PRM/3, derived from the cultivars Saazer (clone Osvald's clone 72) and Premiant using a meristem culture technique to eradicate viruses. Callus cultures were initiated by plating leaf segments (LS) and internodal segments (IS), excised from *in vitro* maintained shoot cultures, onto two culture media B2D2 (MS salts + WS vitamins + 2 mg/l 6-benzylaminopurine [BAP] + 2 mg/l 2,4-dichlorophenoxyacetic acid [2,4-D]) and B2N2 (MS salts + WS vitamins + 2 mg/l BAP + 2 mg/l α-naphthylacetic acid [NAA]), respectively. Callus proliferation was induced in conditions of photoperiod (PP, 16 h light/8 h dark) or in continuous dark (D). After 4 weeks of cultivation, calli were subcultured onto media B1D1 and B1N1, differing from the previous ones by halving the concentrations of plant growth regultators. Cell suspension cultures (CSCs) were established by placing 8-weeks-old calli into 40 ml of B1D1 or B1N1 liquid media in 100ml Erlenmeyer flasks. Seven days after the CSCs establishment, the elicitor – jasmonic acid – was added to the liquid cultures in final concentrations of 0 (control), 20, 40, and 100 mg/l. The CSCs were agitated on a rotary shaker (rpm = 120), and incubated in PP or D conditions at 23°C. Total polyphenol (TPC) and flavonoid (TFC) contents in CSCs were determined spectrophotometrically using the methods of Singleton & Rossi (1965), and Rakotoarison et al. (1997). TPC and TFC in callus cultures differed greatly, depending on the genotype, explant type, culture medium composision, and culture conditions. In calli, the TPC ranged between 49.6-261.1 µg/g FW. The content of flavonoids varied between 17.2-58.5 µg/g FW. In cell suspension cultures, JA in the whole concentration range stimulated the accumulation of polyphenols and flavonoids. In the case of polyphenol production, 17-31% increase was achieved in media containing JA at higher concentrations (40-100 mg/l). The JA-induced increase in flavonoid accumulation was lower (9-23%) and shifted to the lower concentrations (20-40 mg/l) of elicitor in media. In conclusion, supplementation of culture media with jasmonic acid proved to be a useful strategy to increase the polyphenol and flavonoid production in hop cell suspension cultures.

References

Rakotoarison, D., Gressier, B., Trotin, F., Brunet, C., Dine, T., Luyckx, M., Vasseur, J., Cazin, M., Cazin, J.C., Pinkas, M. 1997. Antioxidant activites of polyphenolic extracts from flowers, *in vitro* callus and cell suspension cultures of *Crataegus monogyna*. Pharmazia, 52: 60−63.

Singelton, V.L., Rossi, J.A. 1965. Colorimetry of total phenolics with phosphomolybdic-phosphotungstic acid reagents. Am. J. Enol. Viticult., 16: 144–158.

IV. Session: Management of Hop Diseases and Pests

RESIDUE ANALYSIS OF HOPS – STRATEGY, SUITABLE METHOD, RESULTS

Biendl, M., Kittsteiner-Eberle, R., Jaeger, G., Schmidt, C.

Hopsteiner HHV GmbH, Auhofstr. 16, 84048 Mainburg, Germany
E-mail: mbiendl@hopsteiner.de

Keywords: Multi-residue method, gas chromatography tandem mass spectrometry, high pressure liquid chromatography tandem mass spectrometry

Hop growers carefully balance between combating pests or pathogens and producing a crop that can pass the import tolerances or maximum residue limits (MRLs) established by the various countries to which hops are exported. To control MRLs the official standard methods for analysing different crops cannot be applied to hops due to the more complex matrix of this plant (high content of resins and polyphenols).

The classical multi-method “DFG S-19”, published in 1985 (now officially called “ASU §64 LFGB L00-0034”), includes several steps of sample preparation like extraction of solid plant material, liquid-liquid extraction (LLE), gel permeation chromatography (GPC) and finally solid phase extraction (SPE). Therefore it is very time consuming. For analysing hops chemists found that even additional steps of GPC and SPE are necessary in order to achieve sufficient purification before the injection of a sample into the measurement system (e.g. gas chromatography with electron capture detector). Such a modified procedure suitable for hops was described by Fuchsbichler & Tkaczyk in 1989.

Nowadays residue analysis using gas chromatography (GC) and high pressure liquid chromatography (HPLC) each time coupled with tandem mass spectrometry (MS/MS) are state-of-the-art. Since these selective and sensitive analytical detection systems are available, efforts have been made to cut down the sample preparation. For less complex plant matrices much simpler procedures like the “Klein & Alder” or “Quechers” methods are widely established today (officially called “ASU §64 LFGB L00-00113” or “ASU §64 LFGB L00-00115”). Both methods are straightforward (without GPC, the most time consuming step) but not applicable to hops.

The goal was to develop a shortened sample preparation procedure for hops based on the very reliable first extraction steps of the official multi-method “DFG S-19” but considering the possibility of selective MS/MS detection. Actually this could be achieved by leaving out the GPC step and tailor-made further separate purification steps for GC or HPLC analysis respectively.

Extraction of 5 g ground hops (or pellets) with acetone/water (2/1 v/v) and following LLE with dichloromethane are carried out according to the established original method. Then the resulting organic phase is split. One part is evaporated to dryness, re-dissolved with ethyl acetate, passed through a SPE column filled with “PSA” (primary/secondary amine) which is then eluted with acetonitrile. After evaporation to dryness and re-dissolution in 1 ml methanol/buffered water (50/50 v/v) the sample is ready for analysis by HPLC-MS/MS. The second part of the organic phase is also evaporated to dryness, then re-dissolved with cyclohexane/dichloromethane (85/15 v/v), passed through a column filled with silica gel on “Florisil” which is finally eluted with toluene/acetone (95/5 v/v). For analysis by GC-MS/MS the eluate is evaporated and concentrated to a volume of 1 ml.

When analysing hop extracts (sample weight: 0.5 g) sample preparation starts with direct dissolution either in ethyl acetate or cyclohexane/dichloromethane (85/15 v/v) followed by the same procedures as described above.

Up to now this multi-residue approach was validated for 37 active ingredients (see table) showing recovery rates in the range of 70-120 % when spiked to organic grown hop pellets without any pesticide residues. For hops and pellets a limit of quantification (LOQ) at 0.1 mg/kg (ppm) or lower could be achieved for all the different active ingredients. For hop extracts the LOQs are a factor of 2-5 higher.

This multi-method was applied both for random control of raw hops after crop 2012 and then for full control of all hop products prepared during the following processing season. More than 800 samples were analysed. Any residues detected were consistently below the MRLs. Thus all hop products could be confirmed as tradable.

Active ingredient	Analysis according to GC-procedure	Analysis according to HPLC-procedure	LOQ (mg/kg) in hops/pellets
Abamectin		x	0.1
Acetamiprid		x	0.1
Alpha/Cypermethrin	x		0.1
Azoxystrobin		x	0.1
Boscalid		x	0.1
Captan	x		0.05
Cinidon-ethyl		x	0.1
Clothianidin		x	0.02
Cymoxanil		x	0.06
Dimethomorph		x	0.1
Fenpropimorph		x	0.1
Fenpyroximat		x	0.1
Flonicamid		x	0.1
Fluazifop-p-butyl		x	0.05
Fluopicolid		x	0.02
Folpet	x		0.1
Hexythiazox		x	0.1
Imidacloprid		x	0.1
Lambda-Cyhalothrin	x		0.1
Linuron		x	0.1
Mandipropamid		x	0.1
Metalaxyl		x	0.04
Myclobutanil		x	0.1
Propamocarb		x	0.04
Propargit		x	0.1
Pymetrozin		x	0.1
Pyraclostrobin		x	0.1
Pyraflufen-ethyl		x	0.06
Quinoxyfen	x		0.1
Spirodiclofen	x		0.1
Tebuconazol		x	0.1
Terbuthylazin		x	0.1
Thiamethoxam		x	0.1
Tolylfluanid	x		0.1
Triadimenol		x	0.1
Trifloxystrobin	x		0.1
Zoxamid		x	0.04

References

Amtliche Sammlung von Untersuchungsverfahren nach § 64 LFGB 00.0034: Untersuchung von Lebensmitteln – Modulare Multimethode zur Bestimmung von Pflanzenschutzmittelrückständen in Lebensmitteln (Erweiterte Neufassung der DFG-Methode S 19), September 2010: 1-73.

Fuchsbichler, G. & Tkaczyk, M. 1989. Bestimmung von Endosulfan in Hopfen und Bier (Quantification of endosulfan in hops and beer). Landwirtsch. Forsch. 42: 1-5.

EU-COMMODITY EXPERT GROUP MINOR USES HOP – A COOPERATION TO CLOSE GAPS AND TO HARMONIZE PLANT PROTECTION AT EU-LEVEL

Sichelstiel, W., Weihrauch, F., Schwarz, J.

Plant Protection Hop, Bavarian State Research Center for Agriculture,
Hüll 5 1/3, 85283 Wolnzach, Germany;
E-mail: wolfgang.sichelstiel@lfl.bayern.de

Abstract

With the Regulation (EC) 1107/2009 of the European Parliament and of the Council on June 14, 2011 coming into force, new framework conditions for the authorization of plant protection products and therefore also for minor uses are permitted in the EU. Future work in the field of minor uses is planned and executed in close co-operation of the concerned EU Member States in order to share costs and capacities.

Newly established technically-oriented commodity expert groups minor uses (CEG) are the most important elements of the EU minor uses activities. They take care of the specific plant protection problems of minor crops and minor uses. The commodity expert group hops was founded in Hüll in September 2012. Members are representatives of plant protection working groups of the hop institutes of Germany, Slovenia, the Czech Republic and Poland, plant protection experts of the hop growers associations from France, Belgium, United Kingdom, Austria and Germany and representatives of the hop industry. The aim of the group is to close plant protection gaps in hops in Europe sharing labor and costs. The basics for zonal applications by article 51 of Regulation (EC) 1107/2009 for new agents and active ingredients shall be developed together.

The main tasks of CEGs are appointment of pest management problems in small cultures and joint search for solutions. Joint projects, the data development and data exchange are the basics to create zonal authorization requests for minor use in EC.

On the first meeting of the CEG Hop in February 2012, joint projects regarding efficacy trials with new active ingredients have been agreed on the basis of the available data. On the other hand, the procedure of mutual recognition according to article 40 of Regulation (EC) 1107/2009 shall be plumbed in order to transfer existing approvals of plant protection products from one member state to another.

The data compilations of CEG hops and of the other CEGs can be seen in the EUMUDA, the European Union Minor Uses Database (www.eumuda.eu). This database contains information on minor uses and minor crops in the EC Member States, work and project lists of the EU commodity expert groups minor uses and lists of members of the CEGs, and a list of contact persons of the plant protection companies.

Keywords: EU Commodity Expert Group Minor Uses, EUMUDA, harmonization

DOWNY MILDEW CONTROL IN ORGANIC HOP BY THE MINIMAL USE OF COPPER FUNGICIDES – HOW LOW CAN WE GO?

Weihrauch, F., Schwarz, J.

Bavarian State Research Center for Agriculture
Institute for Crop Science and Plant Breeding, Hop Research Center
Hüll 5 1/3, 85283 Wolnzach, Germany
E-mail: Florian.Weihrauch@LfL.bayern.de

Abstract

A range of new copper products and formulations with reduced amounts of copper (2 and 3 kg Cu/ha per year) were tested during 2012, as the third consecutive year, to control hop downy mildew *Pseudoperonospora humuli* in an organic hop garden in the Hallertau, 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 pesticides was assessed. Compared to the untreated control plot (92.8 % cone infection at harvest on 03 September), all treatments provided distinct, highly significant better control (0.3 – 7.1 % cone infection). The controlling effect in the plots with 3 kg Cu/ha was always better than that in according 2 kg Cu/ha plots. The addition of plant strengtheners always led to an even better control, with 'Frutogard' providing always the best results. However, it has to be taken into account that 'Frutogard', a product that contains phosphonate, has currently been taken from the market and is generally discussed highly controversial in organic farming. The results from the first three project years substantiate that, with the recently registered copper hydroxides, the control of downy mildew in organic hops with 3 kg Cu/ha will be possible in future. Furthermore, the results emphasize the possibility that with novel copper formulations of the next generation, the amount of copper used can probably be reduced by 50 % in future, at least in cultivars tolerant to hop downy mildew.

Keywords: Organic hops, hop downy mildew, *Pseudoperonospora humuli*, control, copper fungicides, plant strengtheners

Introduction

Fungal diseases and their control are 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 synthetic pesticides are prohibited for organic growers, they are completely dependent on compounds that do also occur naturally. Hence, currently copper compounds - e.g., copper sulphate 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, 2013). 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 report for the second time (cf. Weihrauch et al., 2011) on a four years' research project concerning the reduction of the use of copper application rates in organic hops, and focus on the results of the third project year 2012.

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 another, adjacent organic hop field field, a Burkard spore trap was set up

to monitor the actual local disease pressure (numbers of sporangia) from 08 May until late August, briefly before 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 ('Funguran'; application rate of 4 kg Cu/ha and year) as the organic standard when the project started; two new, recently registered copper hydroxides ('Funguran progress', powdery formulation, 2 and 3 kg Cu/ha) and 'Cuprozin progress', (liquid formulation, 3 kg Cu/ha); the tribasic copper sulphate 'Cuproxat' at 3 kg Cu/ha; the encapsuled copper sulphate 'CuCaps' at 3 kg Cu/ha; and three different plant strengtheners ('Herbagreen', 'Biplantol' and 'Frutogard'), each applied together with 'Funguran progress' at 2 and 3 kg copper/ha, respectively. To each spraying a customary farm mixture of sulphur and fine mineral powders was added.

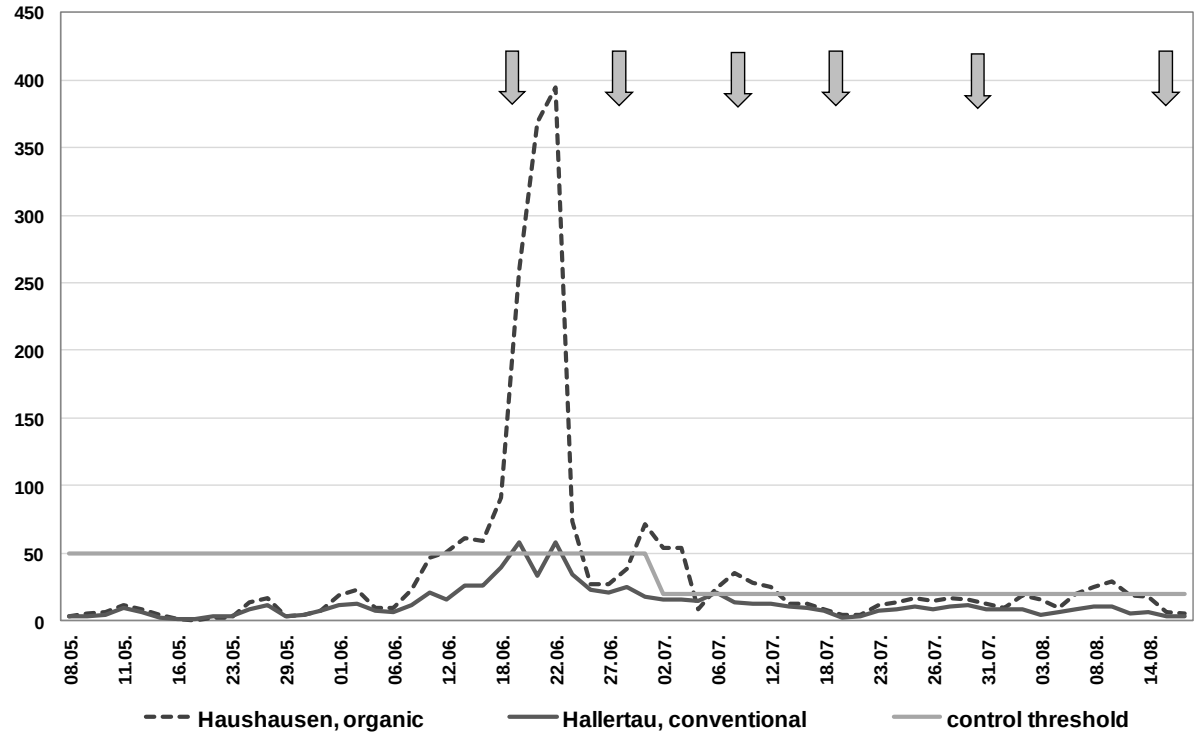


Figure 1: Downy mildew sporangia numbers caught in four days, respectively, in a Burkard spore trap in an organic hop garden in Haushausen, compared to the average number from four other traps in conventional gardens in the Hallertau, Germany, during 2012. The arrows indicate spraying dates in Haushausen.

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 had been released by the regional disease forecasting system. Altogether six sprayings were applied in all plots (18 June, 28 June, 09 July, 19 July, 31 July, and 14 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 several controls of primary infection at random and five monitorings of secondary infection (21 June, 10 July, 27 July, 13 August, and 23 August), split into infection of leaves, flowers, and cones, where appropriate. Finally, during harvest (03 September) an experimental harvest was performed to determine yield and alpha acid content of most plots, and cone samples of all plots were taken and the level of infestation was assessed on dried cones.

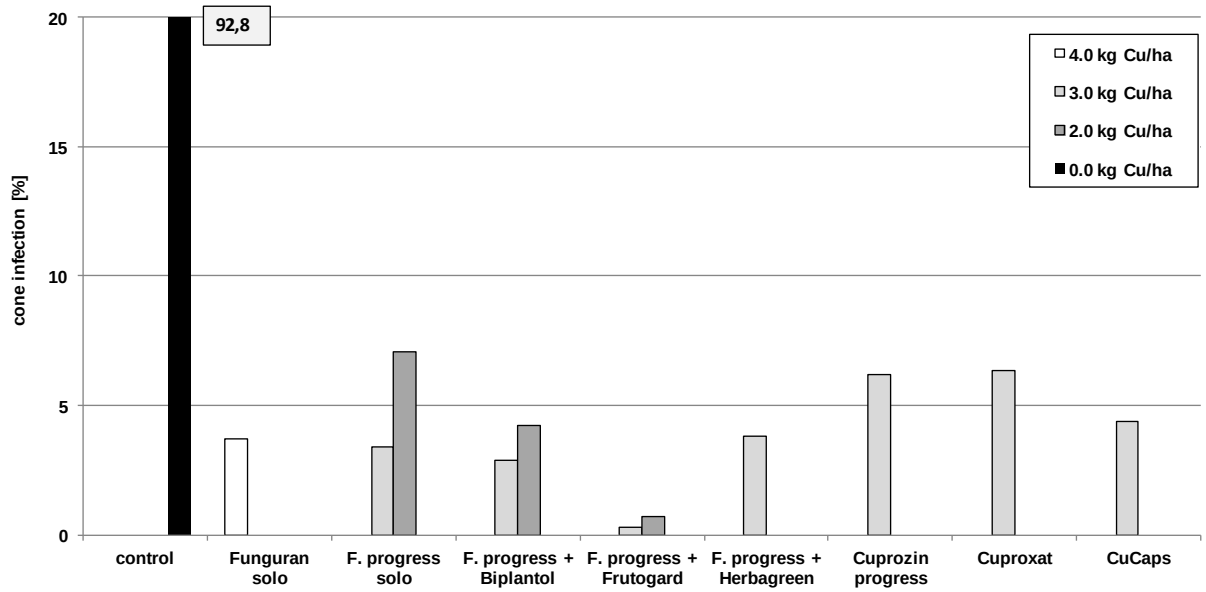


Figure 2: Downy mildew cone infection [%] in an organic hop garden in Haushausen, Hallertau, Germany, at harvest on 03 September 2012. Means of four replications, respectively. Comparison of the different treatments under consideration of the amount of copper applied.

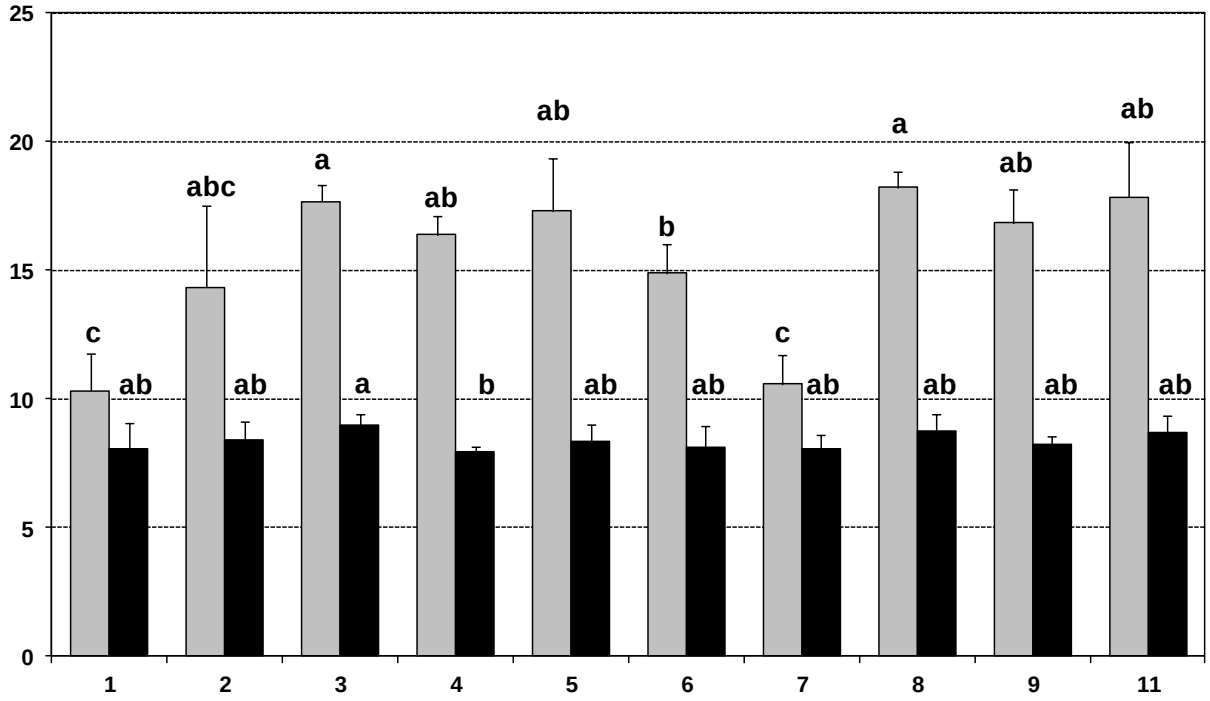


Figure 3: Yield [dt/ha; grey bars] and alpha acid content [%, black bars] determination in an experimental harvest in an organic hop garden in Haushausen, Hallertau, on 03 September 2012. Means ± s.e. of four replications, respectively. Bars with the same letter are not significantly different (ANOVA, $p<0.05$). Experimental plots: 1 control; 2 Funguran (4 kg Cu/ha); 3 Funguran progress (3 kg Cu/ha); 4 Funguran progress (2 kg Cu/ha); 5 Cuprozin progress (3 kg Cu/ha); 6 Cuproxat (3 kg Cu/ha); 7 CuCaps (3 kg Cu/ha); 8 Funguran progress (3 kg Cu/ha) + Herbagreen; 9 Funguran progress (3 kg Cu/ha) + Biplantol; 11 Funguran progress (3 kg Cu/ha) + Frutogard.

Results

Unlike the previous two project years, 2012 was a year with a moderate disease pressure by downy mildew, and the numbers of sporangia caught in the spore trap in the experimental organic field distinctly exceeded the average values in conventionally managed fields only once aruond 20 June (Fig. 1). All the same, this moderate disease pressure led to 87.5 % infected cones in the untreated control plot on 23 August and 92.8 % 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 and the better control effect of 3 kg versus 2 kg Cu/ha clearly visible (Fig. 2). The plot with 4 kg Cu/ha, applied as copper oxychloride, did not achieve significantly better results that the 3 kg variants, applied in from of novel copper hydroxides or copper sulphates.

The experimental harvest revealed practically no significant differences in yield between single copper treatments, which were all significantly higher that the untreated control plot. The significant yield loss in the 'CuCaps' plot as the only exception in this regard was caused by a phytotoxic effect that was due to a defective application of this product in 2012 (Fig. 3).

Discussion

The results of the first three project years are extremely promising. Obviously the recently registered copper hydroxides will be able to reduce the amount of copper used for the control of fungal diseases by 25 % without too much risk for the growers, at least in tolerant cultivars. Their higher efficacy as compared to copper oxychloride is based on the finer distribution of the copper molecules in these formulations, accounting for a better coverage of copper ions – as the effective active ingredient – on the leaf surface. This mode of action is supposed to be honed by the experimental formulation of 'CuCaps', where copper sulphate is encapsuled in fatty acid, to slowly release the $Cu^{2\pm}$-ions when the capsule becomes moist.

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, has ever been discussed highly controversial in organic farming and is currently not available on the market. However, the results from meanwhile three project years emphasize the possibility that with novel formulations the use of copper for the control of fungal diseases can probably be reduced by 50 % in future: In all three project years, the 2 kg Cu/ha variants never exceeded 10 % cone infection at harvest, and in combination with plant strengtheners cone infection was always below 5 %. Therefore, we regard the option to control hop downy mildew in organic hops with 2 kg Cu/ha, at least in cultivars tolerant to this disease, as possible and as the next hurdle that has to be cleared by specific research. In this context, it would be extremely helpful for growers and their acceptance of this reduction if they would be furnished with a 'copper account' to balance an according copper input over, e.g., three years with a maximum use of 6 kg Cu/ha during that time span. Thus, growers could react in years with heavy disease pressure with more copper and save a portion of copper to be used during years with low disease pressure.

On the other hand, the results again substantiate the fact that for the control of fungal diseases in organic hops a complete substitution of copper is still not in sight and that growers have to rely on the future availability of this compound.

References

Bioland (2013): Bioland-Richtlinien. Fassung vom 18. März 2013. Bioland e.V., Verband für organisch-biologischen Landbau, Mainz. URL: <http://www.bioland.de/fileadmin/bioland/file/bioland/qualitaet_richtlinien/Bioland_Richtlinien_18_M%C3%A4rz_2013.pdf> (accessed 30-iv-2013).

Weihrauch F., Schwarz J., Sterler A. (2011): Downy mildew control in organic hops: How much copper is actually needed? International Hop Growers' Convention I.H.G.C., Proceedings of the Scientific Commission, Lublin, Poland, 19-23 June 2011: 76-79.

RESEARCH ACTIVITIES AGAINST HOP WILT IN GERMAN HOP GROWING REGIONS

Seefelder, S.[1], Maurer, K.[1], Niedermeier, E.[1], Berg, G.[2], Javornik, B.[3], Radišek, S.[4]

[1]Bavarian State Research Centre for Agriculture,
Institute for Crop Science and Plant Breeding, Hop Research,
Hüll 5 1/3, 85283 Wolnzach, Germany,
[2]Graz University of Technology,
Environmental Biotechnology, Petersgasse 12, A-8010 Graz, Austria
[3]Centre for Biotechnology and Plant Breeding, University of Ljubljana,
Biotechnical Faculty, Jamnikarjeva 101, Ljubljana, Slovenia;
[4]Slovenian Institute of Hop Research and Brewing,
Cesta Žalskega tabora 2, Žalec, Slovenia;
E-mail: stefan.seefelder@lfl.bayern.de

Abstract

The control of Verticillium is a crucial problem in all affected host plants worldwide. There are no chemical fungicides for the efficient control of this disease. Moreover, there are negative impacts of other strategies such as e.g. fumigants, especially in hop as perennial plant where the control of this soil-borne pathogen is more difficult than in crops with fast crop rotation. Knowledge about the virulence of the Verticillium strains, a fast and reliable detection system, agronomical advisory, the testing of biological control agents and increasing the effort in resistance/tolerance breeding are the aims pursued since the occurrence of lethal Verticillium strains in Hallertau.

Keywords: *Verticillium*, bioantagonist, molecular diagnostics, disease management

Introduction

Verticillium wilt caused by soil-borne fungi *Verticillium albo-atrum* and *V. dahliae* is an important vascular disease of hop and other dicotyledone plants. In Germany the first appearance of this disease was in 1952 (Zattler 1959), but in the mild form which can be tolerated by Huell hop cultivars. Since 2005 the more aggressive lethal form, caused by *V. albo-atrum,* has been threatening some regions of the Hallertau with drastic yield losses. Since 2008 research has been conducted only at a limited level, to assess intensity of the infections and to identify most affected areas. (Seefelder et al. 2009).

Over the last 5 years an increase of infected hop gardens has been observed and there is no doubt that official measures are necessary to prevent a wieder spread of this lethal type in the Hallertau. In other countries frequent monitoring of hop gardens, continous sampling of hops, the use of, certified planting material, laboratory analyses and expert support showed as effective strategies to control (Radišek 2009). For the high numbers of samples which have to be analysed from the Hallertau a DNA based method was established to detect the Verticillium fungus directly from the hop bine (Maurer et al. 2013). Because of the size and intensity of German hop production the implementation of disease control like is monitoring of hop gardens should be adapted to the German situation.

Research activities

- Identification of causal agent of lethal outbreaks by AFLP molecular analysis and pathogenicity testing
- Real time PCR molecular testing of seedlings from the hop research center and the nursery for wilt infection
- Establishing artificial infection tests on hop plants and soil testing techniques
- Testing bioantagonists for protection of hop plants (laboratory and field trials)

- Long term field studies for investigations of wilt influencing parameters
- Screening of breeding lines/wild hops by natural infection in field and PCR testing.

Conclusion

An effective Verticillium diagnostic system, the generation of wilt tolerant/resistant varieties and the willing of the farmers to follow the advisory means will be the only way in the future to get out of this serious problem. More research activities in different strategic ways will be necessary in the next decades and synergies to Verticillium activities in other perennial crops have to be forced.

Acknowledgement

We would like to thank the Erzeugergemeinschaft Hopfen HVG e.G. and the Wissenschaftsförderung der Deutschen Brauwirtschaft e.V. for their financial support in the beginning of the new German Verticillium hop research.

References

Berg, G. 2009: Plant-microbe interactions promoting plant growth and health: perspectives for controlled use of microorganisms in agriculture. Appl. Microbiol. Biot. doi: 10. 1007/s00253-009-2092-7.

Jernej, J., Cernak, A., Radišek, S., Satovic, Z., Javornik, B. 2013: Identification of quantitative trait loci for resistance to Verrticillium wilt and yield parameters in hop (*Humulus lupulus* L.)

Maurer, K., A., Radišek, S., Zachow, C., Berg, G., Seefelder, S. 2013: Molecular in planta test for the detection of Verticillium species in hops and initial steps towards biological control. 11th International Verticillium Symposium, Göttingen, Germany, pp 143.

Maurer, K. A., Radišek, S., Berg, G., Seefelder, S.: Real-time PCR assay to detect *Verticillium albo-atrum* and *V. dahliae* in hops: development and comparison with a standard PCR method. J. Plant Dis. Protect. *in press*.

Radišek, S., Kolenc, U., Persolja, J., Čerenak, A., Knapic, V., Pavlic-Nikolic, E., Javornik, B. 2009: Verticillium wilt on hops in Slovenia: Phytosanitary control and disease management. Proceedings of 10th International Verticillium Symposium, Corfu, Greek pp. 106.

Radišek, S., Kosir, I., Ceh, B., Javornik, B. 2013: The suppression of Verticillium wilt of hop by biofumigation. Proceedings of 11th International Verticillium Symposium, Göttingen, Germany, pp. 146.

Seefelder S, Seigner E, Niedermeier E, Radišek S, Javornik, B. 2009.:Genotyping of Verticillium pathotypes in the Hallertau: Basic findings to assess the risk of Verticillium infections. In: Seigner E. (ed) CICH—IHB—IHGC International Hop Growers‘ Convention. Leon, Spain, pp 67–69

GENOMIC ANALYSIS OF FUNGAL PATHOGEN *VERTICILLIUM ALBO-ATRUM*

Jakše, J.[1], Rot, G.[2], Jelen, V.[1], Radišek, S.[3], Mandelc, S.[1], Majer, A.[1,] Zupan, B.[2], Javornik, B.[1]

[1]University of Ljubljana, Biotechnical Faculty, Jamnikarjeva 101, SI-1000 Ljubljana, Slovenia;
[2]University of Ljubljana, Faculty of Computer and Information Sciences, Tržaška cesta 25 SI-1000 Ljubljana, Slovenia
[3]Slovenian Institute for Hop Research and Brewing, Cesta Žalskega tabora 2, SI-3310 Žalec, Slovenia;
E-mail: Branka.Javornik@bf.uni-lj.si

Abstract

Our research group has been studying the phytopathogenic fungus *Verticillium albo-atrum*, which causes lethal damage to hop (*Humulus lupulus* L.). By the use of molecular analysis and pathogenicity tests, we first identified two types of isolates in Slovene hop gardens: isolates (PG1) that induce a mild form of hop wilt and isolates (PG2) that kill the plants (Radišek et al., 2003). Comparison of these two pathotypes with hop *V. albo-atrum* isolates from other European hop growing regions and isolates of *V. albo-atrum* from other hosts have shown genetic differentiation between all lethal and mild isolates, as well as isolates from other hosts, and a close genetic relationship between Slovene PG2 isolates and lethal hop isolates from England (Radišek et al., 2006). Comparative analysis of mycelium proteomes of the two forms additionally show similarities between lethal pathotypes from Slovenia and England and mild pathotypes from the two countries, as well as differentially expressed proteins that might explain the increased virulence of the lethal pathotype (Mandelc et al., 2009). Preliminary studies of the genome size of lethal and mild isolates suggest that lethal isolates have larger genomes than mild types and pulse field electrophoresis has revealed different karyotypes for them. We extended our research by the employment of new generation sequencing technologies. We sequenced six genomes of *Verticillium albo-atrum* mild and lethal hop strains from three different geographical regions. By comparative analysis of the sequenced genomes, we anticipate that we can find enough genomic differences between the strains to explain the higher virulence of the lethal pathotype, detect virulence-associated factors and elucidate pathogenicity in *Verticillium* spp. Illumina technology was used for sequencing four insert libraries (370 bp, 500-600 bp, 1000 bp and 5000 bp) for a reference strain, producing 95 M reads. Another five strains were sequenced to a depth of 4.8 up to 11.5 M reads. *De-novo* assembly of the reference genome and reference mapping of the other five genomes was performed using CLC software. The final assembly resulted in 439 contigs with a total length of 35.6 Mb, of which 0.5 Mb of DNA was present only in lethal strains. Contigs were mapped on an optical map of the reference strain consisting of ten chromosomes; 2.3% of the sequences remain unplaced. For annotation of the transcribed part of the genome, 38.3 M RNA-seq reads were produced from one mild and one lethal transcriptome in three biological replicates. Gene prediction tools, supported by Exonerate protein alignments and RNA-seq analysis, resulted in 9858 gene models. In the lethal-specific region, 91 gene models were predicted, with additional evidence of several regions being expressed but not predicted by the software tools. The obtained genome data provide a good genomic resource for studying *V. albo-atrum* strains.

References

Mandelc S, Radišek S, Jamnik P, Javornik B. 2009. Comparison of mycelial proteomes of two Verticillium albo-atrum pathotypes from hop. Eur J Plant Pathol, 125: 159–171

Radišek S, Jakše J, Simončič A, Javornik B. 2003. Characterization of *Verticillium albo-atrum* field isolates using pathogenicity data and AFLP analysis. Plant Disease, 87: 633638

Radišek S, Jakše J, Javornik B. 2006. Genetic variability and virulence among *Verticillium albo-atrum* isolates from hop. European Journal of Plant Pathology, 116: 301–314

RESEARCH ACTIVITIES FOCUSED ON HOP VIROID DISEASES IN SLOVENIA

Radišek, S.[1], Oset, M.[2], Čerenak, A.[3], Jakše, J.[4], Knapič, V.[5], Matoušek, J.[6], Javornik, B.[7]

[1,2,3]Slovenian Institute for Hop Research and Brewing, Cesta Žalskega tabora 2, SI-3310 Žalec, Slovenia; E-mail: sebastjan.radisek@ihps.si

[4.7]University of Ljubljana, Biotechnical Faculty, Jamnikarjeva 101, SI-1000 Ljubljana, Slovenia;

[5]Administration of the Republic of Slovenia for Food Safety, Veterinary sector and Plant Protection, Dunajska 22, SI-1000 Ljubljana, Slovenia;

[6]Biological Centre v.v.i. Academy of Sciences of CR, Institute of Plant Molecular Biology, Branišovská 31, 370 05 České Budějovice, Czech Republic

Abstract

Viroids are single stranded, circular and self-replicating non-coding RNA molecules, with sizes ranging from 246-475 nucleotides (nt). They are the smallest known plant pathogens and cause severe to mild diseases in many economically important crop species (Flores et al., 1997). To date, more than 30 different viroid species have been identified, which are grouped into two families: *Pospiviroidae* and *Avsunviroidae*. Three viroids belonging to *Pospiviroidae* are currently known to infect hop: hop latent viroid (HLVd), hop stunt viroid (HSVd) and apple fruit crinkle viroid (AFCVd). HLVd is distributed throughout the world and is symptomless in most cultivars. HLVd infected plants have reduced alpha-acid content (up to 30%) in the cones and have significantly reduced rooting and establishment of softwood cuttings. HSVd and AFCVd have been detected only in some hop growing countries and causing severe hop stunt diseases. The first hop viroid research activities in Slovenia started in 1996 and were focused on the development of HLVd identification techniques based on R-PAGE, dot blot hybridisation and RT-PCR analysis. The second important milestone was an HLVd elimination program, which ran from 2006-2009, when the primary nuclear stock of all Slovene varieties was subjected to mericloning, and a new generation of viroid/virus-free mother plants was established. Two-year field trials of the new generation plants confirmed the economic value of the HLVd elimination program and a positive effect was also observed on plants after HLVd reinfection. Until 2007, only HLVd was present in Slovenia but in that year, a severe outbreak of an unknown disease was found in a hop field (cv. Celeia) in the central part of the Savinja valley. Affected plants developed symptoms of severe stunting, including leaf curling, small cone formation and dry root rot. In the following years, 2008-2012, the disease was found in 25 hop fields located in the vicinity of the first outbreak, and in 2 hop fields located in the Koroška region, 60 km away. The majority of the affected hop fileds were planted with the variety Celeia, and the rest with the varieties Bobek, Savinjski golding and Aurora. The disease incidence varied among hop fields from 1-30% and increased rapidly (up to 10%) each subsequent year, predominantly along plant rows. Diagnostic analysis of symptomatic plants lately revealed a mixed infection with hop stunt viroid (HSVd) and citrus bark cracking viroid (CBCVd). In order to prevent further spread and eradicate the disease, official emergency measures have been implemented since 2011. The Slovenian Institute of Hop Research and Brewing (SIHB) has carried out a systematic survey of hop fields, which included visual inspections of hop plants, sampling, laboratory analysis and instructions on sanitation measures. In addition, strict phytosanitary measures with roughing out the infected hop fields and compensation to growers have been taken by phytosanitary service in last three years to eradicate HSVd/CBCVd infections.

Keywords: viroids, hop, *Humulus lupulus*, hop stunt disease

References:

Flores R, Di Serio F, and Hernández C, 1997. Viroids: the non coding genomes. Seminars in Virology, 8, 65-73.

Radišek et al. First Report of *Hop stunt viroid* Infecting Hop in Slovenia. Plant Disease, 96 (4): 592, 2012.

USING SMALL RNA TECHNOLOGY TO IDENTIFY VIROIDS ON HOP

Pokorn, T.[1], Radišek, S.[2], Javornik, B.[1], Jakše, J.[1]

[1]Centre for Biotechnology and Plant Breeding, University of Ljubljana, Biotechnical Faculty, Jamnikarjeva 101, Ljubljana, Slovenia;
[2]Plant Protection Department, Slovenian Institute of Hop Research and Brewing, Cesta Žalskega tabora 2, Žalec, Slovenia;
E-mail: tine.pokorn@bf.uni-lj.si

Fast and efficient detection of a pathogen is important to prevent further spread of infection. The majority of current methods for pathogen detection are based on either serological or PCR techniques. When a new pathogen occurs, or one that has never before been reported in the investigated crop plant, a new, more investigational approach has to be used. Hop growers in the Savinja valley reported noticeable symptoms on plants, such as reduced plant vigor, shorter internodes, smaller leafs with epinasty and cones with fewer lupulin glands. Samples from infected and healthy plants were collected and RNA was isolated. Bulk RNA was made from different plant samples and subjected to high-throughput parallel sequencing of small RNAs. Small RNA (sRNA), which are 21, 22 and 24 bp long sequences, can be used in *de novo* assembly to construct a virus or viroid genomes. Such an approach was confirmed to be suitable by Kreuze et al. (2009) to find pathogens in sweet potato. Small interfering RNAs (siRNA) are part of sRNA and are produced in plants as a defense mechanism against viroid or virus infection. sRNA were sequenced using Illumina technology and yielded 33 M small RNA reads. A reference mapping approach and PFOR *de novo* assembly algorithm was used to discover the presence of pathogenic sequences. Both approaches confirmed the presence of two viroids in infected samples – hop latent viroid (HLVd) and citrus bark cracking viroid (CBCd/CVd-IV). Further RT-PCR tests confirmed the presence of CBCd/CVd-IV in most of the diseased plants. In our further work we will investigate the role of viroid sRNAs in the silencing mechanism of hop genes.

MONITORING OF HOP STUNT VIROID AND DANGEROUS VIRUSES IN GERMAN HOP GARDENS

Seigner, L.[1], Lutz, A.[2], Seigner, E.[2]

[1]Bavarian State Research Center for Agriculture, Institute for Plant Protection, Lange Point 10, 85354 Freising, Germany
[2]Bavarian State Research Center for Agriculture, Institute for Crop Science and Plant Breeding, Hop Research Center Huell, 85283 Wolnzach, Germany
E-mail: Luitgardis.Seigner@LfL.bayern.de

Introduction

Viroids, especially the Hop stunt viroid (HSVd), and viruses are important pathogens in hop production worldwide causing significant economic losses. They are spread efficiently by mechanical means during cultivation activities. Viroids and viruses cannot be controlled by plant protection measures. The majority of viruses are transmitted by aphids; due to the non persistent way of transmission spraying is ineffective in controlling virus infections. For plant breeding no resistance genes are available. For all these reasons, preventive measures are of utmost importance. In this context a broad scale monitoring of important viruses and HSVd in German has been started in 2011 to detect and eradicate primary sources of potential HSVd infections and to elucidate the current virus situation in Germany's hop production.

Methods

Leaf samples were taken in all German hop growing regions; plants showing symptoms of abnormal growth were preferred. Samples were analysed for HpMV, ApMV and Arabis mosais virus (ArMV) by ELISA by the virus laboratory of the Bavarian State Research Center for Agriculture (LfL). Moreover samples were analysed by RT-PCR for HSVd and Hop latent carlavirus (HpLV); part of the samples were tested for American hop latent carlavirus (AHpLV). RNA extraction was performed as described by Seigner et al. (2007). Primers and thermal cycling conditions were kindly provided by Dr. Ken Eastwell, Washington State University, Prosser, USA. An internal nad5 mRNA based RT-PCR control was included.

Results

From 2008 to 2012 a total of approximately 1,170 samples was analysed. The dangerous HSVd was only detected in 9 plants which were eradicated effectively in 2009. This result also emphasizes the value of our monitoring program. Furthermore our studies show that in contrast to HSVd, viruses are wide spread in Germany's hop production although the real virus situation might be more or less overestimated due to the preferred testing of symptom showing plants. HpMV, HpLV and ApMV were the dominating viruses in both years. Mixed infections with up to 4 viruses occurred in a considerable percentage of samples. ArMV was rarely detected.

Keywords: *hops, AHpLV, ArMV, ApMV, HpLV, HpMV, HSVd, monitoring, virus, viroid*

Acknowledgement

Special thanks goes to the Wissenschaftliche Station für Brauerei in München e.V. for the financial support. We also thank Vanessa Auzinger, Susanne Kaiser, Franziska Nachtmann, Linda Keckel, Christine Huber, Margerete Kistler, Dorothea Köhler and J. Kneidl for their skilled assistance. Thanks are also due to all our partners providing the hop samples.

Kremheller, H. T., Rossbauer, G., and Ehrmaier, H. 1989 a: Reinfection of virus-free planted hop gardens with Prunus necrotic ringspot and hop mosaic virus. Effects of the virus infection upon the yield, alpha acids, and the disease symptoms of the various hop varieties. 133-136 in: Proc. Int. Workshop Hop Virus Dis. Giessen.

Seigner, L., Kappen, M., Huber, C., Kistler, M., Köhler, D. 2008: First trials for transmission of Potato spindle tuber viroid from ornamental Solanaceae to tomato using RT-PCR and an mRNA based internal positive control for detection. Journal of Plant Diseases and Protection, 115 (3), 97–101.

ASSESSMENT OF ApMV AND HMV PRESENCE IN RECOVERED SAAZER

Svoboda, P., Malirova, I.

Hop Research Institute Co., Ltd, Kadanska 2525, 430 46 Zatec, Czech Republic
svoboda@chizatec.cz

Hop (*Humulus lupulus* L.) as a vegetatively propagated perennial crop is frequently attacked by pathogens. All the original Saazer plants were fully infested by viruses and viroids. Recovery process was therefore necessary to get virus-free planting material with higher biological value. Long-range observation of healthy status in hop gardens planted with recovered hop plants is an inseparable part of our breeding program.

Hop gardens planted with Saazer (Osvald clones no. 31, 72, 114) in Žatec (Saaz) and Úštěk (Auscha) regions in the spell since 1991 till 2007 were submitted to ELISA tests in 1992-2012. It was found that re-infection of these viruses is very low and hop plants are able to keep a good health status practically for the whole lifetime. It is very important knowledge for hop growers, propagators and breeders.

Keywords: hop, Humulus lupulus L., viruses, ApMV, HMV, ELISA test, Saazer, health status, recovery process, re-infection

Acknowledgement

Ministry of Agriculture of CR institutional support for the development of research organization RO1486434704.

THE CONTROL OF TWO-SPOTTED SPIDER MITE (*TETRANYCHUS URTICAE* KOCH) WITH THE HELP OF RELEASED PREDATORY MITES *TYPHLODROMUS PYRI* SCHEUTEN AND NATIVE ACAROPHAGOUS PREDATORS WITHIN IPM AND ORGANIC HOP GROWING SYSTEMS IN CR.

Vostřel, J.

Hop Research Institute, Co., Ltd., Žatec (Saaz), Czech Republic
E-mail: vostrel@chizatec.cz

Abstract

After three years of the necessary temporary period certified organic fine aroma Saazer has been at disposal since August 2012. Whereas damson-hop aphid (*Phorodon humuli* Schrank) is controlled with the help of extract of the tropical plant *Quassia amara* as well as by native predators, mainly aphidophagous coccinellids, predatory mite *Typhlodromus pyri* Scheuten is released to support native populations of acarophagous predators to control two-spotted spider mite (*Tetranychus urticae* Koch). Nevertheless, optimal release rate is still questionable. It depends on a locality and actual endangering by spider mites. The fact that this species is able to hibernate in hop gardens and control *T. urticae* in the following years is of great importance within the project of organic hop growing as well as IPM systems in Czech Republic where selective aphicides pymetrozine and flonicamide are commonly used to control damson-hop aphid. Neither these selective zoocides nor fungicides, applied to control downy mildew (*Pseudoperonospora humuli*), have negative effect on predatory mites and other species of acarophagous predators so as they are able to keep spider mites under the economic threshold.

Keywords: acarophagous predators, organic hops, IPM, two-spotted spider mite (*Tetranychus urticae* Koch), predatory mites, *Typhlodromus pyri* Scheuten, release, over-wintering, control, damson-hop aphid (*Phorodon humuli* Schrank), selective aphicides.

Introduction

The beginning of organic hop growing in Czech Republic dates back to 2009. Nevertheless, it has its origin in the early eighties of the 20th century within a research project in the cooperation between Hop Research Institute and Entomological Institute of Czechoslovak Academy of Sciences (Růžička et al., 1988). Unfortunately, there used to be no demand for organic hops from breweries at that time and therefore we did not continue in this research even though some field trials with predatory mites *Typhlodromus pyri, Amblyseius californicus, Amblyseius cucumeris* or *Phytoseiulus persimilis* were carried out in the nineties and at the beginning of the new millennium (Vostřel, 2001; 2003).

The situation has changed considerably nowadays and there are emerging breweries, which demand organic hops so as to be able to produce organic beer. It was also the reason why Hop Research Institute persuaded some hop growers in CR to enter the transition period. The acreage of organic hop gardens in CR amounts to 10.5 ha at present. The first officially certified organic Czech hops have been available from the 2012 crop.

Efficient control of downy mildew and major pests: damson-hop aphid and two-spotted spider mite is the most important task to manage to produce good quality conventional as well as organic hops. In the case of T. urticae creation of natural balance between spider mites and their enemies is very important. Predatory mites T. pyri are released to *help indigenous natural enemies to prevent damage caused by spider mites (Vostřel, 2012).*

Field trials with predatory mites were carried out as early as the beginning of the 1970s in hop yards in Yakima valley (WA). *Typhlodromus occidentalis* appeared to be a better candidate than *P. persimilis* (Pruszynski & Cone, 1972). In hop yards of Willamette valley (OR) inoculative release of Phytoseiid mites and the important question of spatial

aggregation and refugia of *T. urticae* and predatory mite *Neoseiulus fallacis* were studied by Strong & Croft (1995; 1997). The most effective control was reached by *N. fallacis* and *T. occidentalis* or by mixture of the both species. In English Kent Campbell & Lilley (1999) studied the effect of timing and rates of *P. persimilis* release. On all treatments the numbers of *T. urticae* decreased when the prey and predator ratio reached the value of 10:1. In Hallertau two-spotted spider mite appeared to be more manageable pest for biological control than damson-hop aphid (Benker, 1997).

In the trials carried out in Czech Republic *P. persimilis* was able to control two-spotted spider mite on leaves of young hop plants in a nursery. *T pyri* and *A. californicus* succeeded in keeping *T. urticae* under its economic injury level on a small experimental hop garden under weather conditions suitable for the development of this pest (Vostřel, 2001). At the beginning of the 1990s, however, there seemed to be little hope of using *T. pyri* or other predators to control spider mites within IPM as aphids had to be controlled by chemicals, which were not sufficiently selective to avoid destroying the predators (Neve, 1991). Nevertheless, the situation has changed with the registration of a selective insecticide pymetrozine. The impact of pymetrozine application on Phytoseiids and a guild of spider mite/aphid predators in commercial hop yards in Washington were evaluated by James (2002). Pymetrozine was non-toxic to all predators at all rates. Phytoseiid *Gallendromus occidentalis* Nesbitt and *Neoseiulus fallacies* Garman populations in hop yards appeared unaffected by the full rate of this aphicide showing thus a great potential as a selective pesticide. This insecticide was also used to control aphids in an experimental hop garden together with *T. pyri* and *A. californicus* and in this way the both major pests became manageable (Vostřel, 2003). Recently, the spectrum of selective pesticides has been enriched by another efficient aphicide, flonicamid (Vostřel & Filkuka, 2008), which is a good promise for an efficient IPM system in hop cultivation.

Material and Methods

The trial no. 1 (organic hop growing) was conducted in a hop garden of 1,5 ha in Listany (Rocov cooperative farm), which is situated in Saaz hop region. The cultivated variety is Saazer. Since 2009 the hop garden has been in the transition period from commercial to organic hop growing system. This period has been finished by the harvest in 2011 and therefore the crop of 2012 has already had the status of fully certified organic hops. As the successful control of spider mites with the help of predatory mites *T. pyri* was presented at the Third International Humulus Symposium, which was held in September 2012 in Žatec (Vostřel, 2012), the focus will be aimed at utilization of this species within IPM system (trial no. 2). This trial was established in a hop garden of 0.9 ha in a village of Rybňany, which is a part of a hop research farm in Stekník belonging to Hop Research Institute in Žatec, where a breeding plot with perspective genomes is found.

Fifty leaves were sampled in the intervals of 1-2 weeks. Predatory mites *T. pyri* were bought from Biola Chelčice, which is an official distributor of bio-agents including *T. pyri* in CR.

Predatory mites were released (3 strips of cloth containing 5 gravid females per a pole = 1.5 mites/plant) on May 26, 2009. Samples were taken to the laboratory in plastic bags where numbers of eggs and mobile stages of spider mites and predatory mites as well as other species of acarophagous predators were either directly checked or put into a refrigerator and checked the following day. Mites were counted with the help of a binocular microscope. No predatory mites were released in 2010-2012 there.

Totally 11 ha of commercial hop gardens are held under IPM system including spider mites control with the help of Phytoseiid *T. pyri* in our research farm. Only selective insecticides (flonicamide and pymetrozine) are used there to control aphids and to enable predatory mites to survive and control spider mites next year. Common fungicides are applied (fosetyl-Al, azoxystrobin, metalaxyl, cymoxanil and cooper fungicides) against downy mildew.

Results and Discussion

As obvious from Tables 1-3 over wintering populations of Phytoseiids together with other species of acarophagous predators were able to control two-spotted spider mites within the experimental hop garden for three years following the release of *T. pyri.* Number of spider mites never exceeded the threshold level (5 mobile stages per leaf) during this period. Anthocorid bugs of the genus *Orius,* acarophagous tiny lady birds *Stethorus* spp., predatory gall midges *Feltiella acarisuga*, acarophagous thrips and rove beetles *Oligota* spp. were common indigenous predators of *T. urticae* during that period. Low population density of *T. urticae* corresponded with low numbers of predatory mites. Most numerous predatory mites were in the middle of August 2011, when all the developmental stages were observed on the leaves sampled from the experimental hop garden (Table 2).

Our experience hitherto with predatory mite *T. pyri* can be summarized in the following way.

1. Phytoseiid *T. pyri* is a suitable bio-agent for the control of two-spotted spider mites within IPM as well as in organic hops. Nevertheless, optimal release rate is still questionable. It is not impossible to determine it in a uniform way as it depends on a location of a hop garden as well as on the population density of *T. urticae.*

2. This species is able to over-winter in Bohemian hop gardens. The over-wintering generation can significantly reduce population density of *T. urticae* in the following season. Nevertheless, other research should be aimed at the improvement of over-wintering conditions.

3. If *T. pyri* is used within IPM systems applications of selective insecticides (flonicamide, pymetrozine) are necessary to save not only Phytoseiids but all the complex of acarophagous and aphidophagous predators as well.

References

Benker, U., 1997: Population dynamics of pests (damson-hop aphid *Phorodon humuli* Schrank) and two-spotted spider mite *Tetranychus urticae* Koch) and beneficial organisms in hops. In Proc. Scient. Comm. I.H.G.C., Žatec, Czech Republic: 21-27.

Campbell, C.A.M., Lilley, R., 1999: The effect of timing and rates of release of *Phytoseiulus persimilis* against two-spotted spider mite *Tetranychus urticae* on dwarf hops. Biological Science and Technology 9: 453-465.

James, D.G., 2002: Selectivity of the miticide bifenazate, and aphicide, pymetrozine, to spider mite predators in Washington hops, Internat. J. Acarol., 28: 175-179.

Neve, R.A., 1991: Hops. Chapman and Hall, London: 266 pp.

Pruszynski, S., Cone, W.W., 1972: Relationship between *Phytoseiulus persimilis* and other enemies of the two-spotted spider mite on hops. Environmental Entomology 1: 431-433.

Růžička, Z., Vostřel, J., Zelený, J., 1988: Interaction between *Phorodon humuli* and indigenous predators in a pesticide untreated hop garden. In IOBC WPRS Bull. XI/5, East Malling, England: 64-72.

Strong, W.B., Croft, B.A., 1995: Inoculative release of Phytoseiid mites (Acarina: Phytoseiidae) onto the rapidly expanding canopy of hops for control of *Tetranychus urticae* (Acarina: Tetranychidae). Entomological Society of America 24: 446-453.

Strong, W.B., Croft, B.A., 1997: Spatial aggregation and refugia of the mites *Tetranychus urticae* and *Neoseiulus fallacis* (Acari: Tetranychidae, Phytoseiidae) on hop. Environmental Entomology 26: 323-339.

Vostřel, J., 2001: The control of *Tetranychus urticae* by predatory mites *Phytosiulus persimilis* Athias Henriot, *Typhlodromus pyri* Scheuten and *Amblyseius californicus* Mc. Gregor (Acari: Tetranychidae, Phytoseiidae) on hops. In Proc. Scient. Comm. I.H.G.C., Canterbury, Kent, England: 66-70.

Vostřel, J., 2003: Biological control of *Tetranychus urticae* with the help of predatory mites on hops. In Proc. Scient. Comm. I.H.G.C., Dobrna, Czech Republic: 46-49.

Vostřel, J., Filkuka, I., 2008: Hop Protection against Pests and Diseases in Central Europe in 2020. In Proc. International Hop Symposium. 5/6 May 2008. Wolnzach, Germany: 63-67.

Vostřel, J., 2012: Protection of organic Saaz hops against two-spotted spider mite (*Tetranychus urticae* Koch) with the help of released predatory mites *Typhlodromus pyri* Scheuten. In. Proc. of Third International Humulus Symposium. Acta Horticulturae. ISHS, Žatec, Czech Republic. In Press.

Acknowledgements

The work was supported by Czech Ministry of Industry and Trade within the Research Project No. FR-TI3/376: "Czech BIO-BEER" and by Ministry of Agriculture within the Research Project MZe RO 0411 "Research of quality and production of Czech hops."

Table 1: Population dynamics of two-spotted spider mites and acarophagous predators, experimental IPM hop garden – Rybňany (research farm, Stekník 2010)

Date	Number of spider mites per leaf (mobile stages/eggs)	Acarophagous predators (number per 10 leaves))
09.07.	1.6/4.1	4x *Stethorus* larvae, 1x thrips, 2x *Feltiella acarisuga* larvae
20.07.	1.9/2.0	1x *Orius* larvae, 1x thrips
26.07.	3.2/2.6	2x *Stethorus* larvae, 1x *Stethorus* pupae, 1x *Orius* larvae, 1x, *F. acarisuga* larvae, 1x *Oligota* larvae
02.08.	3.7/3.7	1x Thrips, 1x *Orius* larvae, 1x *Stethorus* egg, 3x *Stethorus* larvae, 1x *T. pyri* egg, 1x *Oligota* imago
10.08.	3.7/4.2	1x *Stethorus* egg, 1x *Stethorus* larvae, 1x *Stethorus* pupae, 1x *T. pyri* egg, 1x *T. pyri* larvae, 1x *F. acarisuga* larvae, 4x Thrips, 1x *Oligota* larvae
16.08.	2.6/4.3	1x *Stethorus* egg, 2x *Stethorus* larvae, 1x *Stethorus* pupae, 2x *Orius* egg, 1x *F. acarisuga* larvae, 2x thrips, 1x *T. pyri* imago
26.08.	2.8/5.7	1x thrips, 1x *F. acarisuga* larvae, 1x *T. pyri* larvae, 1x *T. pyri* imago, 3x *Stethorus* larvae
08.09.	4.6/5.3	1x *Stethorus* egg, 5x *Stethorus* pupae, 1x *F. acarisuga* larvae, 1x *T. pyri* imago

Table 2: Population dynamics of two-spotted spider mites and acarophagous predators, experimental IPM hop garden – Rybňany (research farm, Stekník 2011)

Date	Number of spider mites per leaf (mobile stages/eggs)	Acarophagous predators (number per 10 leaves))
27.06.	0.2/0.1	
12.07.	0.1/0.4	1x *Stethorus* egg
19.07.	0.2/0.2	1x *T. pyri* egg
26.07.	0.4/1.4	1x *Orius* larvae, 1x *Stethorus* egg, 1x *Stethorus* larvae, 1x *T. pyri* egg
03.08.	0.9/3.0	1x *Stethorus* larvae, 2x *T. pyri* egg, 1x *T. pyri* imago
12.08.	4.9/13.9	2x *Orius* egg, 4x *F. acarisuga* larvae, 2x trips, 3x *T. pyri* egg, 2x *T. pyri* imago
16.08.	1.1/6.5	1x thrips, 3x *F. acarisuga* larvae, 3x *T. pyri* egg, 1x*T. pyri* larvae, 3x *T. pyri* imago, 3x *Stethorus* egg
22.08.	1.9/3.1	5x *F. acarisuga* larvae, 1x *T. pyri* egg, 1x *T. pyri* imago
01.09.	2.1/4.5	1x thrips, 9x *F. acarisuga* larvae, 1x *Stethorus* egg, 2x *Stethorus* larvae, 1x *T. pyri* imago
30.09.	1.3/0.3	1x *F. acarisuga* larvae

Table 3: Population dynamics of two-spotted spider mites and acarophagous predators, experimental IPM hop garden – Rybňany (research farm, Stekník 2012)

Date	Number of spider mites per leaf (mobile stages/eggs)	Acarophagous predators (number per 10 leaves)
30.04.	0.4/0.7	
11.05.	0	1x thrips, 1x *Stethorus* egg
29.05.	0.2/0.3	1x thrips, 1x *F. acarisuga* larvae
14.06.	0.2/1.5	2x thrips, 2x *Stethorus* egg, 1x *Orius* larvae, 1x *T.pyri* egg, 1x *T. pyri* larvae
28.06.	0.7/0.8	3x thrips, 2x *Stethorus* egg, 2x *T. pyri* egg
10.07.	1.7/2.4	12x thrips, 1x *Stethorus* larvae, 1x *T. pyri* imago
24.07.	1.9/2.4	1x *T. pyri* egg 1x *T. pyri* imago, 2x *F. acarisuga* larvae, 1x *Orius* larvae
08.08.	2.7/1.4	1x *T. pyri* imago

MONITORING OF FLONICAMID (TEPPEKI) BIOLOGICAL EFFICIENCY ON CZECH AND MORAVIAN FIELD STRAINS OF DAMSON-HOP APHID (*PHORODON HUMULI* SCHRANK) IN BIOASSAYS.

Vostřel, J.

Hop Research Institute, Co., Ltd., Žatec (Saaz), Czech Republic
E-mail: vostrel@chizatec.cz

Abstract

Hop protection against damson-hop aphid *(Phorodon humuli* Schrank*)* in Czech Republic is based on the application of synthetic insecticides. Whereas imidacloprid and pymetrozine we frequently used in the last nearly two decades, nowadays spirotetramat (Movento) and flonicamide (Teppeki) have become the most important insecticides for the control of resistant damson hop aphid.The data show the mortality of aphids attacking hop plants on the territory of Czech Republic after flonicamide applications on field strains sampled within Czech and Moravian hop growing regions in the beginning of June 2012. No surviving aphids were observed after application of Teppeki in 0.01, 0.005 and 0.0025% concentrations. If applied in lower tested rates the mortality dropped to approximately 98-99% (0.00125%), with the exception of Tršice strains (100%), to 94.0 – 97.6% (0.00062%) and to 87.3 – 93.2% (0.00031%), resp. (Table 1).

If we take into consideration the fact that Teppeki is registered and commonly recommended in the rate of 180 g/ha in 2000 lt of water (0.009% conc.) as well as its high selectivity to beneficial insects we can see great potential of this efficient aphicide within IPM system in hops for the years to come.

Keywords: damson-hop aphid, flonicamide, Teppeki, field strains, bioassays, Potter tower, biological efficiency, IPM in hops, beneficial insects.

Materials and methods

Samples of damson-hop aphids were taken from the selected hop-yards within the hop regions in Czech Republic in 2011 (Žatec region: 5 samples from Louny district and 3 samples from Rakovník district; 3 samples from Úštěk and 2 samples from Tršice regions). Aphids were collected in the first decade of June before insecticide treatments

Field samples of *P. humuli* populations were transferred into breedings. Their offspring was used in laboratory tests. Aphids were placed in an air-conditioned room at a temperature of 20-22°C and 16-hours photoperiod. Relative humidity was kept at 60-70%. As a host plant hop seedlings were used. These plants were grown in a glasshouse all over the year. Hop leaves with petioles were taken from untreated or residue-free hop plants.

Decapitated leaves were placed with their back side up on the bottom of a sedimentation tower (30 cm in diameter and 96 cm high) and sprayed with 1 ml of the solution of Teppeki with the help of Potter's nozzle under the pressure of 0,2 MPa. After the sedimentation time (10 minutes) treated leaves were removed from the tower. The method (Hrdy, Kuldová, 1981), requires glass cylinders (22 mm in diameter and 15 mm high) stuck on the inside of hop leaves with the help of paraffin and bee-wax mixture that was melted to 50 °C before they were used. Glass cylinders were coated with fluon to prevent escaping aphids. Then they were placed into panels with openings for vials containing water, into which leaf stalks of the treated leaves were inserted.

Two to three hours after spraying thirty-three aphids were transferred into each cylinder by a fine, slightly moist little brush in the following sequence: non-treated (control) leaves and treated leaves in order from the lowest to the highest tested concentration. Mortality of aphids was counted 48 hours after each treatment. The knocked down aphids and the ones, which were unable to move, were considered dead. The mortality of non-treated (control)

leaves must not have been higher than 20% (if so, the experiment had to be repeated). Each test was carried out three times. That means 100 aphids were tested under each concentration in a geometric row.

Acknowledgements

The work was supported by Czech Ministry of Agriculture within the Research Project No. MZe RO 0411 “Research of quality and production of Czech hops.”

References

Hrdy, I., Kuldova, J., 1981: A standardized spray-residue method for measuring, and dip-test for monitoring resistance in aphids. In IOBC WPRS Bull. IV/3, Liblice, Czechoslovakia: 21-28.

Table 1: Biological efficiency of flonicamide (Teppeki) in laboratory bioassays on resistant field strains of damson-hop aphid (*Phorodon humuli* Schrank) sampled within Czech and Moravian hop growing regions in 2012.

Field strain	**Concentration tested (%)**					
Žatec (Saaz) hop growing region, district Louny	**0.01%**	**0.005%**	**0.0025 %**	**0.00125%**	**0.00062%**	**0.00031%**
Dubčany	100	100	100	100	100	95
Markvarec	100	100	100	100	100	97
Orasice	100	100	100	99	95	89
Ročov	100	100	100	100	97	95
Stekník	100	100	100	99	96	90
Average mortality	***100%***	***100%***	***100%***	***99.6%***	***97.6%***	***93.2%***
Žatec (Saaz) hop growing region, district Rakovník	**0.008 %**	**0.004 %**	**0.002 %**	**0.001 %**	**0.0005 %**	**0.00025 %**
Kounov	100	100	100	98	94	85
Mutějovice	100	100	100	99	95	90
Nesuchyně	100	100	100	98	93	87
Average mortality	***100%***	***100%***	***100%***	***98.3%***	***94.0%***	***87.3%***
Úštěk hop growing region	**0.008 %**	**0.004 %**	**0.002 %**	**0.001 %**	**0.0005 %**	**0.00025 %**
Liběšice	100	100	100	97	92	85
Polepy	100	100	100	99	95	89
Vědomice	100	100	100	100	99	94
Average mortality	***100%***	***100%***	***100%***	***98.7%***	***95.3%***	***89.3%***
Tršice hop growing region	**0.008 %**	**0.004 %**	**0.002 %**	**0.001 %**	**0.0005 %**	**0.00025 %**
Doloplazy	100	100	100	100	98	93
Tršice	100	100	100	100	97	92
Average mortality	***100%***	***100%***	***100%***	***100%***	***97.5%***	***91.5%***

THE METHODS OF MONITORING AND MANAGEMENT THE EUROPEAN CORN BORER (OSTRINIA NUBILALIS) IN SLOVENIAN HOP GARDEN

Rak Cizej M., Persolja J.

Plant Protection Department, Slovenian Institute of Hop Research and Brewing, Cesta Žalskega tabora 2, 3210 Žalec, Slovenia
E-mail: magda.rak-cizej@ihps.si

Abstract

The European corn borer (ECB; *Ostrinia nubilalis* Hübner) is polyphagous. In Slovenia, it causes the most damage to corn (*Zea mays* L.) and hops (*Humulus lupulus* L.) but it is also often found on vegetables (tomato, pepper) and ornamentals. The European corn borer is a serious pest of hop in Slovenian hop fields (most part of Savinja valley) for a long time, which larvae cause yield losses and quality of hop. In the last 10 years, the presence of ECB increased significantly in Slovenia and we also observed increasing economic damage on host crops. Reasons for increasing are: farmers have a lot of corn fields, reduced use of insecticides, climatic changes, no execute of phytosanitary hygienic measures. In the central part of Slovenia, the E strain of ECB is present on hops and corn. The Slovenian Institute of Hop Research and Brewing has been trapping ECB on hops in Žalec with a light trap for over 35 years, which has proven to be a very effective way of monitoring the population of ECB. In 2010, we started using pheromone-baited traps to monitor ECB moths. At first, we used delta sticky traps, but in comparison with light traps on pheromone baits, no moths were caught. In 2011, we started monitoring ECB with wire mesh cone traps on which we captured ECB male moths; however, we were not successful in 2012. In 2011 and 2012, in addition to the E strain pheromones, we also used phenylacetaldehyde as an attractant to capture ECB female moths. Monitoring ECB with pheromone-baited traps cannot yet be used in practice to forecast ECB. The European corn borer has a two-generation per year on hop, but usually second generation causes economic damage. Control of European corn borer with contact insecticide with a.i. lambda-cyhalothrin don't have satisfactory efficacy. In the future we will include for larvae control new biological methods.

Keywords: European corn borer, *Ostrinia nubilalis*, monitoring, pheromones, delta sticky traps, wire mesh cone traps, control

Introduction

The European corn borer (ECB; *Ostrinia nubilalis*) has been known as a corn and hop pest in the Savinja Valley for a long time and lately has caused significant economic damage. In Slovenia, the last record shows that ECB also caused damage to vegetables and ornamental crops. Ten years ago, the damage of ECB on corn and hop fields was insignificant, but nowadays ECB is present in large numbers throughout the Savinja region (Rak Cizej et al., 2009, 2012a). In Slovenia, in the Savinja Valley, ECB has two generations per year on hop and corn. The larvae of the first ECB generation cause the most damage by boring into the hop stems, while the second generation larvae also bore into leaf and hop cones. The ECB larvae are responsible for stunted growth and the development of plants, which results in a substantial decrease in yield quality and quantity. Hop plants with a lot of ECB larvae are completely destroyed in dry and hot seasons (Rak Cizej et al., 2009, 2012a). ECB larvae controlled with contact insecticide with a.i. lambda-cyhalothrin which is not enough efficacy.

The light trap is one of the most reliable methods of monitoring the moths, and we have used it for more than 35 years to monitor ECB on hops in Slovenia (Rak Cizej et al., 2009). The downside of a light trap is that it cannot be used for monitoring ECB at all locations, and furthermore, it is an expensive method. This is the reason why we started monitoring ECB by using pheromone traps in 2010 (Rak Cizej et al., 2012b). In the central part of Slovenia, we have the E strain of ECB on hops and corn (Rak Cizej et al., 2010).

Materials and methods

Monitoring of ECB by using light traps

From 2010 to 2012, we monitored ECB moths by using light traps (Figure 1A) on hops from April until the end of September on location in Žalec (location 1). The examination of ECB was performed in the laboratory, where we also determined the sex of ECB.

Monitoring of ECB by using pheromone traps

In 2010, we began to monitor ECB moths by using pheromone bait from the end of May until the third week of September. At first we used sticky delta traps (Figure 1B) with E strain pheromones (producer Isagro, Italy). With delta sticky traps we had no success, so in the years of 2011 and 2012 we monitored ECB moths using wire mesh cone traps (Figure 1C). In addition to E strain pheromones, we also used phenylacetaldehyde as an attractant for catching females (producer Isagro, Italy). The ECB were monitored using pheromone traps on two locations in hops: one location was 100 metres away from the light trap in Žalec (location 1); the second trap was placed in hops at Roje near Žalec (location 2), where each year we recorded large populations of ECB. Pheromone traps were located on the edge of the hops, at a height of 2,1m. E strain pheromones were changed every 14 days and attractants every 30 days. Once a week, we checked pheromone traps for any captured ECB moths.

Figure 1. A - Light trap for monitoring ECB, B - delta sticky trap, C - wire mesh cone trap.

Visual assessment of damage on hops and corn caused by ECB

From 2010 to 2012, near the pheromone bait trap at location 2 (Roje near Žalec), we assessed the damage on corn and hops caused by ECB larvae. In August, we checked 100 randomly selected hop plants. On each plant, we counted the number of holes and determined the percentage of damaged plants. In the same manner, we assessed the damage on corn at the beginning of September.

Results and Discussion

The populations of ECB were monitored for three years at the Žalec location. In 2010, the populations of ECB were very large. On the light trap we caught a total of 831 moths. In the following two years, the ECB populations were lower (Table 1). In 2010, the light trap caught 571 male moths, in 2011, 277 male moths were caught, and in 2012, 294 were caught (Table 1). In parallel with light traps ECB populations were monitored with pheromone traps as well, in which we used E-strain pheromones. In 2010, we attempted to catch ECB with delta sticky traps, which were unsuccessful. This is the reason why we began to catch ECB moths with the wire mesh cone trap in 2011 and 2012. Field studies showed that the wire mesh cone traps capture approximately six times more ECB males than delta traps (Pélozuelo & Frérot, 2006). We had the first successful attempts with pheromone traps in 2011, where we used wire mesh cone traps at both locations (locations 1 and 2). The pheromone trap at location 1 (location Žalec), which was 100 metres away from the light trap, captured 28 ECB male moths, which is 10 times less than on the light trap. In 2011, at location 2 (location Roje near

Žalec), throughout the season, we caught 130 ECB male moths with the pheromone trap (Table 1).

Table 1. Comparison of ECB moths captured with light traps and pheromone traps (E-strain pheromone and attractant phenylacetaldehyde) from the end of May until the end of September in the years 2010 to 2012.

Year	Location	Type of trap	No. of males	No. of females	Total catch of ECB
2010	Location 1	Light trap	565	266	831
	Location 1	Pheromone trap (delta sticky trap)	0	/	0
	Location 2	Pheromone trap (delta sticky trap)	0	/	0
2011	Location 1	Light trap	272	89	361
	Location 1	Pheromone trap (wire mesh cone trap)	28	1	29
	Location 2	Pheromone trap (wire mesh cone trap)	130	10	140
2012	Location 1	Light trap	292	96	388
	Location 1	Pheromone trap (wire mesh cone trap)	0	0	0
	Location 2	Pheromone trap (wire mesh cone trap)	0	0	0

The potential of ECB moths at location 2 (Roje near Žalec) is shown in Figure 2. In all three years (2010 - 2012), the potential of ECB near the pheromone trap was enormous, which shows on the damage to corn (49 - 55%) and damage to hops (90 - 100%).

Figure 2. Damage caused by ECB larvae on hops and corn at location 2, Roje near Žalec.

In 2012, we did not catch any ECB moths with pheromone traps, in spite of a large population of ECB moths on the light trap at location 1 and a high percentage of ECB damage on hops and corn at location 2. At this moment, we do not know what the reasons are for the lack of success in monitoring ECB with pheromone traps in 2012. Perhaps new ECB strains appeared or the pheromone component was not attractive enough. In 2011 and 2012, we attempted to catch female ECB moths by using the attractant phenylacetaldehyde. Despite the fact that the light traps caught large female ECB moths, we have been less successful when using the attractant. The monitoring of ECB with pheromone-baited traps

cannot be used for the forecasting of ECB yet in Slovenia, because in order to manage ECB eggs and larvae, exact data is required.

Conclusions

In previous ten years the ECB population has increased substantially in Slovenia hop gardens. The reasons for increased ECB can be attributed to different factors which primarily include: untimely remove leftovers of host plants after the harvest, application of contact insecticides in agriculture have been restricted, phytosanitary and hygienic measures are not implemented rigorously enough, the climate has changed, etc.

In Slovenia for control of ECB larvae in hop gardens using contact insecticide with a.i. lambda-cyhalothrin has proven insufficiently effective. In the future, new biological methods will be introduced in order to control these pests, while regular implementation of phytosanitary and hygienic measures will remain on the top of the first. In Slovenia, it would be necessary to reinstate the Decision on the control of the ECB, which was valid until 2001.

References

Rak Cizej, M., Leskošek, G. and Radišek, S. 2009. European corn borer in Slovenian hop fields. *Zbornik seminarja*. Slovenian Institute of Hop Research and Brewing: 107-113.

Rak Cizej, M., Kárpáti, Z., Leskošek, G. and Radišek, S. 2010. Determination of the strain of European corn borer (*Ostrinia nubilalis* Hübner) in Savinja valley; preliminary monitoring of male European corn borer with pheromone bait. Hop Bulletin, 17: 65-73.

Rak Cizej, M., Radišek, S. and Leskošek, G. 2012a. European corn borer, a notorious pest in hop. *Hmeljar* (*Žalec*), 74: 23-25.

Rak Cizej, M., Cilenšek, A. and Persolja J. 2012b. Beginning of monitoring European corn borer (*Ostrinia nubilalis* Hübner) on hop (*Humulus lupulus* L.) with pheromone traps in Slovenia. *Semio-chemicals: the essence of green pest control:* IOBC/WPRS Conference, Turkey, Bursa; October 2012, p. 130.

Pélozuelo L. and Frérot B. 2006. Behavior of male European corn borer, *Ostrinia nubilalis* Hübner (Lep.:, Crambidae) towards pheromone-baited delta traps, bucket traps and wire mesh cone traps. J. Appl. Entomol. 130 (4): 230-237.

V. Session:
Molecular Investigations on Hops

REGULATION OF LUPULIN BIOSYNTHESIS BY HOP TRANSCRIPTION REGULATION FACTORS AND SOME STRATEGIES TO ENTER THE REGULATION NETWORK.

Matoušek, J.[1], Patzak, J.[2], Duraisamy,S. G.[3], Kocábek, T.[4] , Krofta, K. [5], Piernikarczyk, R.J.J.[6], Radišek S.[7], Jakše, J.[8], Steger, G.[9]

*[1,3,4]Biology Centre of the ASCR, v.v.i., Branišovská 31, 370 05 České Budějovice, Czech Republic (*jmat@umbr.cas.cz*); [2,5]Hop Research Institute Co., Ltd., Kadaňská 2525, 438 01 Žatec, Czech Rep.; [6,9]Institute of Physical Biology, Heinrich-Heine-Universität Düsseldorf, 40204 Düsseldorf, Germany; [7]Slovenian Institute for Hop Research and Brewing, Cesta Žalskega tabora 2, 3310 Žalec, Slovenia; [8]University of Ljubljana, Biotechnical Faculty, Jamnikarjeva 101, 1000 Ljubljana, Slovenia.*

Abstract

Intensive work on various models like *A. thaliana* discovered in the last years network regulation of many processes including metabolome regulation and their influencing by stress conditions or pathogenic processes. The same is expected for genetic background co-determining levels and composition of hop metabolome in the lupulin glands, and in particular, for regulation of Xanthohumol (X). The last steps of the X pathway are controlled by the chalcone synthase chs_H1, prenyltransferase and O-methyltransferase. Our recent results showed the involvement of several key transcription factors from Myb (M), bHLH (B) and WDR (W) families in the regulation of *chs*_H1 and *omt*1 gene by WRKY, W1 and silencing suppressors. We have identified lupulin specific ternary *Hl*s-M3B2W1, M2B2W1, binary *Hl*s-M3B2; M2B2; B2W; M1W1; WRKY1W1 complexes, promoter activity modulators like *Hl*bZip1 and 2 TFs and inhibitors like *Hl*Myb7 that are quite selective for different promoters. Recently, to enter the network regulation of X, we initiated analysis of key TF promoters to identify secondary TFs and possible TFs autoregulation. To analyze the regulatory networks, we include viroid-induced mRNA-imbalancing pathogenesis, where target mRNAs were predicted by the bioinformatic methods.

Keywords: transcription factors; TF complexes; viroid pathogenesis; lupulin biosynthesis; silencing targets; *Humulus lupulus* L.;

Introduction

The recent analyses of hop and in particular metabolic pathways are facilitated by the new generation of sequencing, availability of promoter sequences and by fast accumulating *in silico* analyses of transcription factors (TFs) and their regulation involved in specific networks especially in model plants like *Arabidopsis thaliana* where most of TFs were identified (Riechmann and Ratcliffe, 2000). The polyphenol group produced in hop cones, including prenylchalcones, such as xanthohumol (X) and desmethylxanthohumol (DMX) represent important hop metabolites, which were found to have applications in medicine as anticancerogenic and phytoestrogenic compounds (Van Cleemput et al., 2009). However, the concentration of these compounds in cones of world hop cultivars is rather low, ranging from 0.2 to 1 % of DW and up to 0.2% of DW for X and DMX, respectively (De Keukeleire et al., 2003). Therefore, there is a common interest to increase the levels of these metabolites. According to our recent knowledge about the biosynthetic pathway leading to X and DMX in lupulin glands, the synthesis of these medicinally important compounds is controlled by the chalcone synthase chs_H1 (Matoušek et al., 2006), prenyltransferase PT-1 (Tsurumaru et al. 2010) and O-methyltransferase OMT1 (Nagel et al., 2008). The available data (Nesvadba et al., 2011) suggest a complexity of regulators involved in X production, and an involvement of specific networking in this regulation. Several important hop regulatory factors form specific complexes (Matoušek et al., 2012a) obviously involved in the networking as they are transcribed independently. Hop metabolome regulation is further influenced by pathogenesis reactions induced by various pathogens and in particular, by viroids from *Pospiviroidae* family that can change the metabolome accumulation (Patzak et al., 2001) and imbalance of

expression of important biosynthetic and regulatory genes (Füssy et al., 2013). In the present work we show some strategies to enter the lupulin regulatory networks.

Material and Methods

The Saaz Osvald's hop clone 72 served as a starting material in all experiments. The genes were cloned from the cDNA libraries which were established from glandular tissue-enriched cones (Matoušek et al., 2007; 2010) or from isolated lupulin glands (Matoušek et al., 2012a). The expression of the hop genes was confirmed by quantitative real-time PCR (q-RT PCR) as described in Matoušek et al. (2007; 2010; 2012a). Promoter binding sites are predicted as previously (e.g. Matoušek et al., 2012a). cDNAs of hop genes were cloned into binary vectors as described earlier in Matoušek et al. (2007). These vectors were introduced into *Agrobacterium tumefaciens* strain LBA 4404. To determine promoter regions of the genes of interest, a phage library screen (Lodish et al., 2000) or inverse or TAIL PCR approaches (Ochman et al., 1988; Liu et al., 1995) were applied. To identify potential target sites of viroid-specific RNAs (vsRNAs) within the the hop cDNA library, the circular viroid sequences were processed into all sequences of length 21 nt. The Venn Diagram (Fig.1b) was drawn using the VennDiagram package (Chen and Boutros, 2011).

Results and Discussion

To analyze regulatory network involved in the lupulin biosynthesis, we selected several subsequent steps based on our initial regulatory model (Fig.1a) assuming an involvement of several key transcription factors with significant specificity for lupulin. Some of studied TFs formed ternary and binary complexes (Fig.1a) involved in the regulation of *chs*_H1 (Matoušek et al., 2012a) and *omt*1 genes (unpublished). Our model depicted in Fig.1a includes independent action of some TFs and their ability to either activate or modulate (s-*Hl*MYB3, *Hl*bHLH2, *Hl*MYB1, *Hl*bZIP1) or to inhibit (*Hl*MYB7) of *chs*_H1 or *omt*1. The first step to approach the network includes the extraction of TFs promoter elements that is performing either by screening of phage library of hop or using PCR-based methods (Table 1, Fig 1c) unless hop genomic sequence is available. The second step involves bioinformatic analysis of promoter regions and prediction of TFs binding sites. For instance, according to our recent analyses for *Hl*Myb1 we identified two Myb, five bHLH (Myc), three bZIP, eight WRKY and ten DOF binding boxes, suggesting simultaneously these regulators as TFs of interest. For *Hl*Myb3 promoter two Myb and two DOF binding sites were found so far and for promoter of *Hl*WRKY1 three units of WRKY-binding sites were discovered, suggesting possible autoregulation. As the next step we aim to check for mutual promoter activation within group of so-far isolated TFs using *in vitro* systems (Matoušek et al. 2007; 2010 and 2012a) and for possible TFs auto activation. Moreover, new lupulin specific TFs are analyzed based on recent hop transcriptome databases. To date, additional seven Myb, eight WRKY, four bHLH and three bZIP TFs were identified from trichome-specific database, cloned and are recently analyzed for the degree of lupulin specificity. Finally, the system involves ectopic overexpression of TFs, as performed recently by Gatica-Arias et al. (2013), or ectopic silencing to analyze their influence on X biosynthesis and on the levels of hop TFs within possible "overexpressed network(s)". Significant changes in TFs network(s) can be observed in viroid-infected hops showing specific or strong symptoms (Radišek et al., 2012, Füssy et al. 2013). Viroid pathogenesis is presumably induced by vsRNAs within the mechanism of RNA silencing (Matoušek et al., 2012b) and therefore, there is a possibility to predict viroid mRNA targets that can lead to changes in specific TFs network(s). For instance, according to our results three hop viroid pathogens HpLVd (Patzak et al., 2001), HSVd g and c variants (Füssy et al., 2013) and CVd IV (unpublished) could target and silence 164, 90, 36 and 80 hop genes as unique targets, respectively (Fig. 1b). Their combinations could allow modifying of pathogenesis and TFs network(s).

Acknowledgements
The project was supported by the Czech Science Foundation (GACR 13-03037S) and with institutional support RVO:60077344. We acknowledge the use of research infrastructure that was built with the aid of MODBIOLIN project (FP7-REGPOT-2012-2013-1316304).

Table 1. Selection of promoters by Lambda phage screening

TF	AC	lambda phages detected	DNA with positive fragments isolated	sequenced promoter region	iPCR/TAIL primers designed	PCR products obtained	sequenced promoter region
*Hl*Myb1●	AJ876882	-	-	-	yes	550bp	250bp
*Hl*Myb2*	AB292245	yes	no	-	no	-	-
*Hl*Myb3*	AM501509	yes	yes	in progress	yes	500bp	146bp
*Hl*Myb7	FR873650	yes	in progress	-	no	-	-
*Hl*bZip1	FN395065	no	-	-	yes	no	-
*Hl*bZip2	AM998490	no	-	-	yes	no	-
*Hl*bHLH1	FN646080	no	-	-	no	-	-
*Hl*bHLH2*	FR751553	yes	yes	in progress	no	-	-
*Hl*WDR1*	FN689721	yes	yes	no	no	-	-
*Hl*WRKY1*	FR751557	yes	yes	1051 bp	yes	300bp	152bp

● Promoter sequence was finally determined from contig1099b by bioinformatics approach
*TFs forming regulatory complexes.

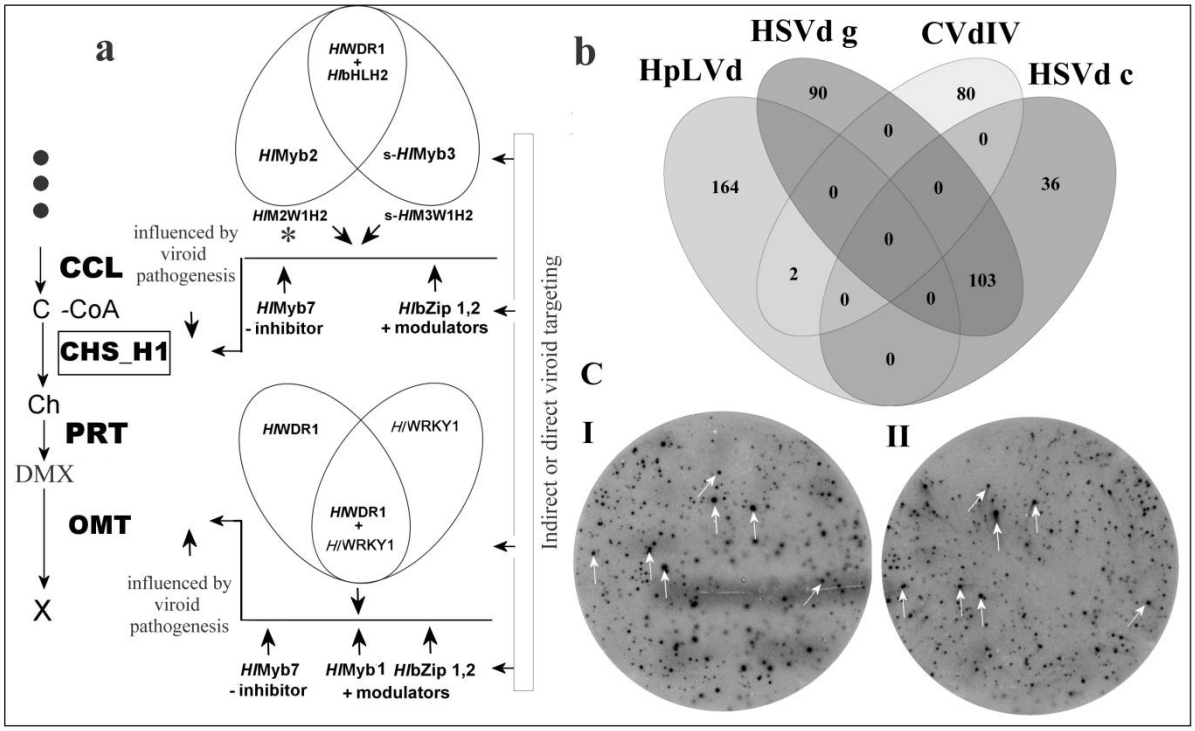


Fig.1. **(a)** The model scheme of xanthohumol (X) regulation on levels of *chs*_H1 and *omt*1 genes.and their influence of viroid pathogenesis. *- TF complex is influenced by HSVd according to Füssy et al. (2013). **(b)** Venn diagram of predicted vsRNA-targeted and cleaved mRNAs. The diagram illustrates vsRNA-targeted host transcripts shared by the hop latent viroid (HpLVd); cucumber isolate of hop stunt viroid (HSVd c); grapevine isolate of HSVd (HSVd g) and citrus viroid IV (CVd IV). **(c)** An example of the primary screening of hop lambda phage library for the *Hl*Myb7 sequence. I – original membrane with plaques giving positive signal for the *Hl*Myb7 probe; II – duplicate membrane to distinguish specific from spurious signals. The arrows indicate some of the best positive plaques selected for the secondary screening. Abbreviations: C-CoA, 4-coumarate CoA; Ch, chalcone; DMX, desmethylxanthohumol; CCL- ligase; CHS_H1 – chalcone synthase; PRT – prenyltransferase; OMT- O-methyltransferase.

References

Chen, H. and Boutros, P.C. 2011. VennDiagram: a package for the generation of highly-customizable Venn and Euler diagrams in R. BMC Bioinformatics. 12: 35.

De Keukeleire, J., Ooms, G., Heyerick, A., Roldán-Ruiz, I., Van Bockstaele, E. and De Keukeleire, D. 2003. Formation and accumulation of α-acids, β-acids, desmethylxanthohumol and xanthohumol during flowering of hops (Humulus lupulus L.). J. Agric. Food Chem. 51: 4436-4441.

Füssy, Z., Patzak J., Stehlík J. and Matoušek J. 2013. Imbalance in expression of hop (*Humulus lupulus*) chalcone synthase H1 and its regulators during hop stunt viroid pathogenesis. J. Plant Physiol. 170: 688-695.

Gatica-Arias, A., Stanke, M., Häntzschel, K.R., Matoušek, J. and Weber, G. 2013. Over-expression of the transcription factor HlMYB3 in transgenic hop (*Humulus lupulus* L. cv. Tettnanger) modulates the expression of genes involved in the biosynthesis of flavonoids and phloroglucinols. Plant Cell Tiss. Organ. Cult. 113: 279–289.

Liu, Y.G., Mitsukawa, N., Oosumi, T.and Whittier, R.F. 1995. Efficient isolation and mapping of Arabidopsis thaliana T-DNA insert junctions by thermal asymmetric interlaced PCR. Plant J 8: 457–463.

Lodish, H., Berk, A. and Zipursky, S.L. 2000. Constructing DNA libraries with λ phage and other cloning vectors. In: Molecular Cell Biology (Lodish et al. eds.). 4th edition. Freeman & Co., New York.

Matoušek, J., Vrba, L., Škopek, J., Orctová, L., Pešina, K., Heyerick, A., Baulcombe, D. and De Keukeleire, D. 2006. Sequence analysis of a „true“ chalcone synthase (chs_H1) oligofamily from hop (Humulus lupulus L.) and PAP1 activation of chs_H1 in heterologous systems. J. Agric. Food Chem. 54: 7606-7615.

Matoušek, J., Kocábek, T., Patzak, J., Škopek, J., Maloukh, L., Heyerick, A., Füssy, Z., Roldán-Ruiz, I. and De-Keukeleire, D. 2007. HlMyb3, a putative regulatory factor in hop (Humulus lupulus L.), shows diverse biological effects in heterologous transgenotes. J. Agric. Food Chem 55: 7767-7776.

Matoušek, J., Kocábek, T., Patzak, J.,Stehlík, J., Füssy, Z., Krofta, K. Heyerick, A. Roldán-Ruiz, I., Maloukh, L. and De Keukeleire, D. 2010. Cloning and molecular analysis of *HlbZip1* and *HlbZip2* transcription factors putatively involved in the regulation of the lupulin metabolome in hop (*Humulus lupulus* L.). J. Agric. Food Chem. 58: 902-912.

Matoušek, J., Kocábek, T., Patzak, J., Füssy, Z., Procházková, J. and Heyerick, A. 2012a. Combinatorial analysis of lupulin gland transcription factors from R2R3Myb, bHLH and WDR families indicates a complex regulation of *chs*_H1 genes essential for prenylflavonoid biosynthesis in hop (*Humulus lupulus* L.). BMC Plant Biol. 12: 27.

Matoušek, J., Riesner, D. and Steger, G. 2012b. Viroids: the smallest known infectious agents cause accumulation of viroid-specific small RNAs. In From Nucleic Acids Sequences to Molecular Medicine (Erdmann, V.A. & Barciszewski, J., eds), RNA Technologies. Springer-Verlag: New York, Berlin, pp. 629–644.

Nagel, J., Culley, L.K,. Lu, Y., Liu, E., Matthews, P.D., Stevens, J.F. and Page, J.S. 2008. EST analysis of hop glandular trichomes identifies an O-methyltransferase that catalyzes the biosynthesis of xanthohumol. Plant Cell 20: 186-200.

Nesvadba, V., Krofta, K. and Polončíková, Z. New knowledge in czech hop breeding. In: Seigner, E. (ed), Proceedings of the Scientific Commission, International Hop Growers Convention, s. 15-18, Lublin, Poland, 19-23 June, 2011.

Ochman, H., Gerber, A.S. and Hartl, D.L. 1988. Genetic applications of an inverse polymerase chain reaction. Genetics. 120: 621-623.

Patzak, J., Matoušek ,J.,Krofta, K. and Svoboda, P. 2001. Hop latent viroid (HLVd)-caused pathogenesis: effects of HLVd infection on lupulin composition of meristem culture-derived hop (Humulus lupulus L.). Biol. Plant. 44: 579-585.

Radišek, S., Majer, A., Jakše J., Javornik, B. and Matoušek, J. 2012. First report of Hop stunt viroid infecting hop in Slovenia. Plant Disease 96: 592.

Riechmann, J.L. and Ratcliffe, O.J. 2000. A genomic perspective on plant transcription factors. Curr. Opin. Plant Biol. 3: 423-434.

Tsurumaru, Y., Sasaki, K., Miyawaki, T., Momma, T., Umemoto, N. and Yazaki, K. 2010. An aromatic prenyltransferase-like gene HlPT-1 preferentially expressed in lupulin glands of hop. Plant Biotechnol. 27: 199-204.

Van Cleemput, M., Cattoor, K., De Bosscher, K., Haegeman, G., De Keukeleire, D. and Heyerick, A. 2009. Hop (Humulus lupulus)-derived bitter acids as multipotent bioactive compounds. J. .Natur. Prod. 72: 1220-1230.

CHANGES IN ENDOGENOUS PHYTOHORMONE LEVELS AND INVOLVED GENES EXPRESSIONS IN DWARF HOP PLANTS

Patzak, J.[1], Dobrev, P.[2], Motyka, V.[2]

[1] Hop Research Institute, Co. Ltd.,
Kadanska 2525, 43846 Zatec, Czech Republic;
[2] Laboratory of Hormonal Regulations in Plants,
Institute of Experimental Botany AS CR, Rozvojova 263, 16502 Prague 6, Czech Republic;
E-mail: patzak@chizatec.cz

Abstract

The cultivated hop (*Humulus lupulus* L.) is normally grown in 5-8 m tall wirework to produce commercial yields of inflorescences known as cones. Relatively recently, dwarf hops have been developed in the United Kingdom, which have shorter internode lengths and are cultivated in low trellis systems. Phytohormones are associated with plant growth and developmental processes including vegetative growth, internode elongation, flower induction, shoot formation, leaf senescence, chlorophyll production, etc. Growth hormones (auxins, gibberellins, cytokinins) and stress hormones (abscisic acid, jasmonic acid, salicylic acid) were measured by LC-electrospray tandem-mass spectrometry in female plants of normal and dwarf cultivars. Various tested plant tissues (apex buds, young leaves, old leaves, inflorescences) markedly differed in the phytohormone levels, independently on hop genotypes. Enhanced levels of auxins and gibberellins (GA_{19}, GA_{20}, GA_{29}) were detected in inflorescences suggesting these classes of hormones might be involved in flowering, maturating and generative growth of plants. In cytokinins, the statistically significant difference between dwarf and normal hop plants was revealed for *cis*-zeatin-9-riboside-*O*-glucoside exhibiting enhanced levels in all tested tissues of dwarf cultivars. Slightly higher levels of abscisic acid were detected in apex buds of normal cultivars. Enhanced levels of salicylic acid occurred in apex buds and young leaves of dwarf cultivars in some years. Our results indicate an involvement of both growth and stress hormones in the dwarfism as well as their relationships during vegetative and reproductive development of hop plants. In molecular expression analysis by qRT-PCR we also found the overexpression of abscisic acid synthesis genes in normal plant tissues compared to dwarf hop tissues.

Keywords: abscisic acid, auxin, cytokinin, gibberellin, jasmonic acid, salicylic acid

Introduction

The cultivated hop (*Humulus lupulus* L.) is normally grown in 5-8 m tall wirework to produce commercial yields of inflorescences known as cones. Several years ago, the development simplified a growing system known as “low-trellis” (2.5-3 m), which allows much greater mechanisation and reduction of labour requirements. The first breeding selections were identified for establishment in farm trials by 1992 and registered as First Gold, Herald and Pioneer, the world’s first dwarf hop varieties, in 1996 in the United Kingdom (Darby, 2004).

The low stature, short internode length and low flowering node are characteristic for dwarf hop cultivars. Their altered phenotypes clearly indicate changes in phytohormone homeostasis compared to normal varieties. Phytohormones are defined as a group of naturally occurring organic substances which influence physiological processes at low concentrations (Davies 2004); they are generally known to regulate a great number of plant growth and developmental processes including vegetative growth, internode elongation, flower induction, shoot formation, leaf senescence, chlorophyll production, etc. Phytohormones are grouped into several classes based on their chemical structure similarities and on their effects on plant physiology. The main groups are auxins, cytokinins (CKs) and gibberellins (GAs) (Osbourne and McManus, 2005). Another group of phytohormones is represented by stress hormones. Abscisic acid (ABA) is known to function in many plant developmental processes, including bud dormancy. ABA-mediated signalling

plays a very important part in plant responses to environmental stress and plant pathogens. Salicylic acid (SA) and jasmonic acid (JA) are also involved in plant defense responses, especially to pathogen attacks. Ethylene is a gas phytohormone that affects numerous physiological processes in plants including shoot and root growth and differentiation, leaf and fruit abscission and fruit ripening. It is synthesized by most tissues in response to stress, and regulates other hormones including ABA and stress hormones (Wang et al., 2007).

Data concerning phytohormone profiles in hop plants are rather scarce. Villacorta et al. (2008) characterized endogenous growth hormone (auxin, GAs and CKs) levels and changes associated with both vegetative and reproductive development in hop. They collected the samples approximately every 15 days during plant development from initial shoot sprout time (V1), during vegetative development (V2 and V3), and after development of the flowers (F1 and F2). The increasing information about hop DNA gene sequences (258472 ESTs in GeneBank EST database, http://www.ncbi.nlm.nih.gov/blast/blast.cgi) provides for us possibility to look for specific genes for biosynthesis and regulation of phytohormones. Taking advantage of advanced analytical methodology (LC-electrospray tandem-mass spectrometry, RT-qPCR), the main objective of our study was to reveal possible differences in growth and stress hormones and expressions of some their biosynthetic genes between normal and dwarf hop genotypes during development in field conditions.

Material and methods

Plant material

Samples collected from female plants of five normal (Saazer, Sladek, Admiral, Phoenix, Magnum) and five dwarf (First Gold, Herald, Crusader, 5021, 5060) cultivars grown in experimental hopgardens of Hop Research Institute, Zatec, CR, were used for analyses. Apex buds, first expanded young leaves, old green leaves and flowers were collected in the morning of 20th May and 9th July 2009 (V2 and F2 phases according to Villacorta et al., 2008), 7th June and 2nd July 2011 (V3 and F2 phases) and of 21st June 2012 (F2 phase). Tissue samples were immediately deeply-frozen by liquid nitrogen and stored at -80°C until analyses.

Phytohormone analyses

Samples were purified and analyzed as described in Dobrev and Kamínek (2002) and Dobrev and Vankova (2012). Stable isotope labeled internal standards were added for the majority of determined compounds immediately after homogenization. Two fractions were separated using reverse phase and ion exchange chromatography: (1) fraction A – containing acidic and neutral character hormones (auxins, ABA, SA, JA and GAs), and (2) fraction B – containing basic character hormones (CK derivatives). Hormones were quantified using HPLC (Ultimate 3000, Dionex, Sunnyvale, CA, USA) coupled to hybrid triple quadrupole/linear ion trap mass spectrometer (3200 Q TRAP, Applied Biosystems, Framingham, MA, USA) set in selected reaction monitoring mode. Quantification of hormones was done using isotope dilution method with multilevel calibration curves (r^2>0.99). Data processing was carried out with Analyst 1.5 software (Applied Biosystems, Framingham, MA, USA). Statistical analyses of data were performed using STATISTICA 8.0 CZ (StatSoft, Tulsa, OK, USA).

Molecular gene expression analyses

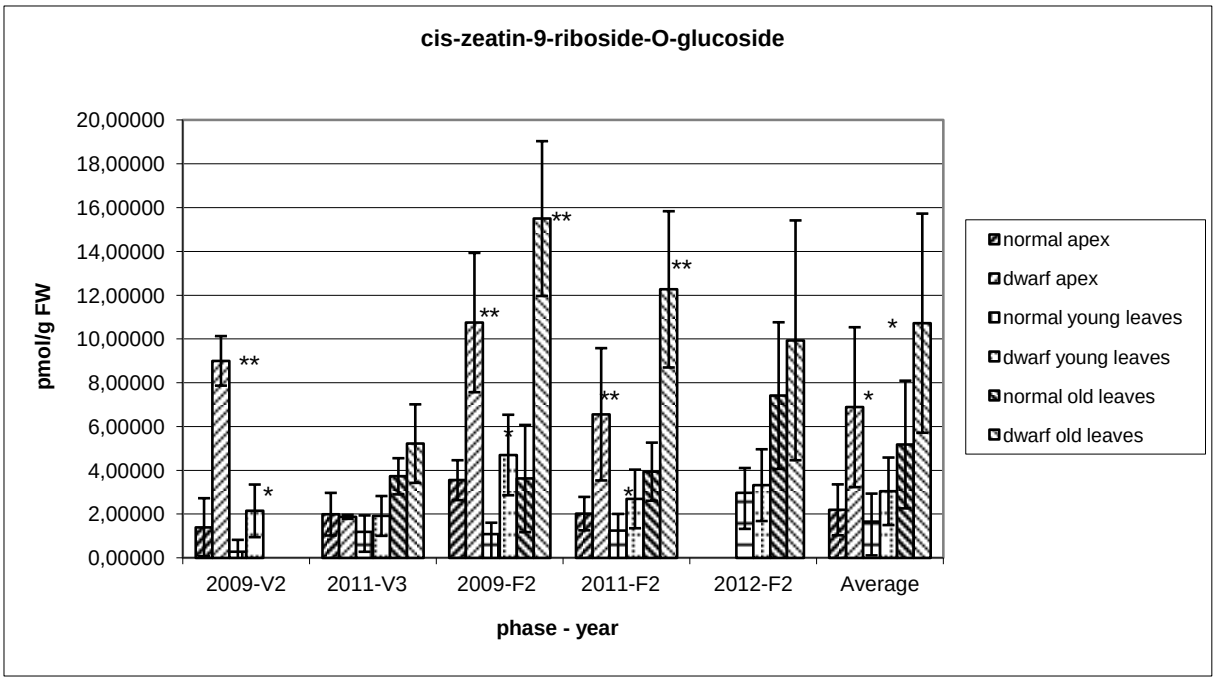
For real-time quantitative PCR (RT qPCR), total RNA was isolated from 100 mg of samples (FW) using Concert™Plant RNA Reagent (Invitrogen, Carlsbad, CA, USA) following RNA purification and DNA cleavage (DNAseI, Qiagen, Hilden, Germany) on column (RNeasy Plant Total RNA kit, Qiagen, Hilden, Germany). Four micrograms of total RNA were reverse transcribed using oligo $(dT)_{18}$ primer and First Strand cDNA Synthesis Kit (Roche Diagnostics, Mannheim, Germany) at 50°C for 60 min. A total of 2 µl of 50 × diluted cDNA was used for a 20 µl PCR reaction with FastStart universal SYBR Green Master (Roche Diagnostics, Mannheim, Germany) and specific primers 300 nM each. All amplifications were carried out on a Lightcycler 2.0 (Roche Diagnostics, Mannheim, Germany) for 60 cycles

(94°C for 10 s, 58-60°C for 30 s, 72°C for 1 s) following an initial denaturation/Taq activation step (94°C for 10 min) in the Institute of Chemical Technology, Prague. All specific primers were derived on sequences of hormone biosynthetic pathway genes from EST hop trichome library (http://planttrichome.org/trichomedb) by Universal Probe Library Assay Design Center (Roche Diagnostics, Mannheim, Germany). The abundance of a reference transcripts, glyceraldehyde-3-phosphate dehydrogenase (GAPDH) and DEAD-box RNA helicase 1 (DRH1) were estimated in parallel in each sample (Maloukh et al. 2009; Matoušek et al., 2012). The relative values were standardized to reference genes with the “Delta-delta method" and normalized to the sample expression by REST 2009 (Relative Expression Software Tools v.2.0.13, Pfaffl et al., 2002).

Results and Discussion

In the first part of our study, we measured endogenous phytohormone contents in different tissues of normal and dwarf hop genotypes during development in field conditions. The most of endogenous phytohormone contents were varied in plant tissues independently on hop genotypes. Higher levels of auxins (IAA) and gibberellins (especially precursors of bioactive forms GA_{19} and GA_{20} and a deactivation product GA_{29}) were detected in inflorescences suggesting these phytohormones might be involved in flowering, maturating and generative growth of plants. The occurrence of IAA and GAs was previously reported by Villacorta et al. (2008) in the F2 growing phase of hop plants in the apex buds and young leaves, however, neither old leaves nor inflorescences were used for phytohormone analyses in their study.

Figure 1: Endogenous contents of *cis*-zeatin-9-riboside-O-glucoside in fresh tissues of normal and dwarf hop plants. * - significant at 0.05 probability level, ** - significant at 0.01 probability level.



As for CKs, *trans*-zeatin (Z) and dihydrozeatin (DHZ) types were found to prevail in all tissues followed by *cis*-zeatin (*cis*Z) and N^6-(Δ^2-isopentenyl)adenine (iP) types (Table 1). This is inconsistent with a prevalence of *cis*Z over Z (ca. 3 : 1) reported for shoots of hop plants (Gajdošová et al. 2011). Our analyses showed that *cis*Z types were present in considerably higher amounts in dwarf genotypes compared to normal ones. The statistically significant difference was revealed in *cis*Z-9-riboside-O-glucoside content exhibiting enhanced levels in all tested tissues of dwarf cultivars (Figure 1). The contents of *cis*-zeatin and total O-glucosides were not influenced and did not differ between dwarf and normal hop plants. In general, a vast majority of all CKs occured as bioactive forms and storage *O*-glucosides in

young organs (young leaves, apex buds, inflorescences) while inactive or weakly active *N*-glucosides predominated in old leaves.

Higher levels of ABA were detected in apex buds and flowers of normal compared to dwarf cultivars. Generally, derivatives such as phaseic acid, dihydrophaseic acid, neophaseic acid and ABA-glucose ester were similar to ABA for dwarf and normal genotypes, although a few exceptions to this generalization were found. ABA is involved in abscision, seed dormancy and stomatal closure and ABA-mediated signaling and also plays an important part in plant responses to environmental stress and plant pathogens (Seo and Koshiba, 2002). Plant growth and development are complex, strictly regulated processes and ABA shows close interactions with other phytohormone pathways (Divi et al., 2010). One of them is an antagonistic interaction with SA (Yasuda et al., 2008).
We found considerably higher levels of SA in apex buds and young leaves of dwarf cultivars compared to normal plants, only in 2011 F2 phase. SA is primarily involved in Systemic Acquired Resistance (SAR) to pathogens (Yasuda et al., 2008), but recent studies showed that it is also involved in growth, germination, photosynthesis, flowering and senescence. (Rivas-San Vicente and Plasencia, 2011). Differences in SA contents between normal and dwarf cultivars (Fig. 3) could be related mainly to the dwarf growth, which is in accordance with recently published data demonstrating high levels of SA in *Arabidopsis* dwarf mutants or growth inhibition of chamomile plants by exogenous SA application (Rivas-San Vicente and Plasencia, 2011). These differences do not seem to be associated with SAR, because concentration of JA, another compound involved in plant response to pathogens (Yasuda et al., 2008), was not apparently influenced.

In the second part of our study, we performed out the molecular expression analyses of known biosynthetic pathway phytohormone and signal transduction genes in the same samples from 2011 F2 phase. Even though, some key biosynthetic genes were not possible to find in EST hop trichome library, we found some correlations between gene expression and phytohormone levels in dwarf and normal plant tissues. Relative expression of abscisic-aldehyde oxidase and xanthoxin dehydrogenase showed overexpression (10-50 times) in normal plant tissues (apex and young leaves) compared to dwarf hop tissues. By contrast, relative expression of zeatin O-glucosyltransferases showed overexpression (6-10 times) in dwarf young leaves compared to normal hop tissues.
Our results clearly indicate an involvement of both growth and stress hormones in the dwarfism as well as their relationships during vegetative and reproductive development of hop plants. Delicate mechanisms and regulation of their action, either individually and/or in complex hormonal crosstalk, however, need to be studied in detail and further elucidated.

Acknowledgements

This work was supported by the Ministry of Education, Youth and Sports of CR in EUREKA project LF11008 and the Grant Agency of the Czech Republic in projects 13-03037S and P506/11/0774. The authors are grateful to Alena Henychová and Marie Korecká for excellent technical support.

References

Darby, P. 2004. Hop growing in England in the twenty first century. J. Royal Agricult. Soc. England 165: epub. http://www.rase.org.uk/what-we-do/publications/journal/2004/08-67228849.pdf

Davies, P.J. 2004. The Plant Hormones: Their Nature, Occurrence, and Functions. In: *Plant Hormones. Biosynthesis, Signal Transduction, Action!*, ed. P. J. Davies, 1-35, Kluwer Academic Publishers.

Divi, U.K., Rahman, T. and Krishna, P. 2010. Brassinosteroid-mediated stress tolerance in *Arabidopsis* shows interactions with abscisic acid, ethylene and salicylic acid pathways. BMC Plant Biol. 10: 151.

Dobrev, P. and Kamínek, M. 2002. Fast and efficient separation of cytokinins from auxin and abscisic acid and their purification using mixed-mode solid-phase extraction. J. Chrom. 950: 21-29.

Dobrev, P.I. and Vankova, R. 2012. Quantification of abscisic acid, cytokinin, and auxin content in salt-stressed plant tissues. in *Plant Salt Tolerance: Methods and Protocols,* Methods in Molecular Biology. vol. 913, Springer Science + Business Media, eds. Shabala S., and Ann Cuin T., ch. 17, 251-261

Gajdošová, S., Spíchal, L., Kamínek, M., Hoyerová, K., Novák, O., Dobrev, P.I., Galuszka, P., Klíma1, P., Gaudinová A., Žižkova, E., Hanuš, J., Dančák, M., Trávníček, B., Pešek. B., Krupička, M., Vaňková, R., Strnad, M. and Motyka, V. 2011. Distribution, biological activities, metabolism, and the conceivable function of cis-zeatin-type cytokinins in plants. J. Exp. Bot. 62: 2827-2840.

Kamínek, M., Březinová, A., Gaudinová, A., Motyka, V., Vaňková, R. and Zažímalová, E. 2000. Purine cytokinins: a proposal of abbreviations. Plant Growth Regul. 32: 253-256.

Matoušek, J., Kocábek, T., Patzak, J., Füssy, Z., Procházková, J. and Heyerick, A. 2012. Combinatorial analysis of lupulin gland transcription factors from R2R3Myb, bHLH and WDR families indicates a complex regulation of chs_H1 genes essential for prenylflavonoid biosynthesis in hop (*Humulus lupulus* L.). BMC Plant Biol. 12: 27.

Maloukh, L., Matoušek, J., Van Bockstaele, E. and Roldán-Ruiz, I. 2009. Housekeeping gene selection for Real Time-PCR normalization in female hop (Humulus lupulus L) tissues. J. Plant Biochem. Biotech. 18: 53-58.

Osborne, D.J. and McManus, M.T. 2005. Hormones, signals and target cells in plant development. Cambridge University Press. p. 158

Pfaffl, M.W., Horgan, G.W. and Dempfle, L. 2002. Relative expression software tool (REST©) for group-wise comparison and statistical analysis of relative expression results in real-time PCR. Nucleic Acids Res. 30(9): e36

Rivas-San Vicente, M. and Plasencia, J. 2011. Salicylic acid beyond defence: its role in plant growth and development. J. Exp. Bot. 62(10): 3321-3338.

Seo, M. and Koshiba, T. 2002. Complex regulation of ABA biosynthesis in plants. Trends Plant Sci. 7(1): 41-48.

Villacorta, N.F., Fernandez, H., Prinsen, E., Bernad, P.L. and Revilla, M.A. 2008. Endogenous hormonal profiles in hop development. J. Plant Growth Regul. 27: 93-98.

Wang, Y., Liu, C., Li, K., Sun, F., Hu, H., Li, X., Zhao, Y., Han, C., Zhang, W., Duan, Y., Liu, M. and Li, X. 2007. *Arabidopsis* EIN2 modulates stress response through abscisic acid response pathway. Plant Mol. Biol. 64(6): 633-644.

Yasuda, M., Ishikawa, A., Jikumaru, Y., Seki, M., Umezawa, T., Asami, T., Maruyama-Nakashita, A., Kudo, T., Shinozaki, K., Yoshida, S. and Nakashita, H. 2008. Antagonistic interaction between systemic acquired resistance and the abscisic acid–mediated abiotic stress response in *Arabidopsis* http://www.plantcell.org/content/20/6/ - fn-4. Plant Cell 20(6): 1678-1692

VI. Session:
Physiology of Hop

CHARACTERIZATION OF CHANGES IN PHOTOSYNTHETIC RATE AND TRANSPIRATION OF HOP INFECTED BY DOWNY MILDEW (*PSEUDOPERENOSPORA HUMULI*) AND HOP-FLEA-BEETLE (*PSYLLIODES ATTENUATUS*)

Pokorný J.[1], Křivánek J.[1], Ježek J.[1], Pulkrábek J.[2]

[1]Hop Research Institute Co., Ltd., Kadaňská 2525, Žatec, Czech Republic
[2] Department of Crop Production, Czech University of Life Sciences, Faculty of Agrobiology, Food and Natural Resources, Kamýcka 129, Praha 6, Czech Republic
E-mail: pokorny@chizatec.cz

Abstract

In the article, we are evaluating the influence of leaf damage (Agnus variety) by the downy mildew (*Pseudoperenospora humuli*) and the hop-flea-beetle (*Psylliodes attentuatus*) to the rate of photosynthesis and transpiration of hop. The rate of photosynthesis and transpiration was measured by the LC pro+ machine which is a mobile infra-red analysator. The measurement took place in the Czech Republic in Steknik, at the hop garden called Zimmermann VI. planted with the Agnus variety in the Saaz area during different periods of the year 2012.

Keywords: hop, physiology, photosynthesis, transpiration

Introduction

Yield formation of hops can be characterized as the result of a complex of interacting factors, which take place during the growing season and outside the growing season in plants. Field crops use from 1.0 to 2.5% of solar radiation throughout the growing season. Photosynthesis is a fundamental physiological process of plant growth and one of the important factors in the yield of field crops (Wullschleger, Oosterhuis, 1989, Yu et al., 2001). All above-ground organs of hop are ready for the intensive course of photosynthesis outside the fruit. Vine and lateral branches leaves are most important. Lateral branches leaves show a higher photosynthetic activity than vine leaves (Rybacek, 1980). The relatively high rate of photosynthesis takes place in the old and yellowed leaves. Rate of photosynthesis in these leaves is equal to intact leaves (Hnilicka, 2000). Leaf damage is a major limiting factor of photosynthesis. Transpiration is output, in the form of water vapor, from the surface of the plant into the atmosphere. Transpiration is subject to physical laws determining evaporation (irradiance, temperature, humidity, air flow) and anatomical-morphological structure and physiological state of plants (openness, size and density of stomata) (Prochazka et al., 1998). The leaf damage is a significant factor, which affecting transpiration. The course of photosynthesis can be observed by various methods, but the study of photosynthetic production of higher plants requires gazometric methods and the reduction of CO_2 (Sestak, Catsky, 1966).
Downy mildew is a very dangerous disease of hop, which is prevalent in all the hop-growing areas of Europe and overseas. The way to protect against this disease of hop is very well developed. In some cases, this disease is not kept below the threshold of economic harmfulness (especially in wet years). Hop-flea-beetles are the most common pest of hops. Hop-flea-beetle is not a major pest in the moment, but typical perforation have to changing physiological processes in lateral branches leaves in July and August.
The aim of the measurements was to determine how the leaf damage, affected by downy mildew and hop-flea-beetle, change the rate of photosynthesis and transpiration of lateral branches leaves against healthy lateral branches leaves.

Methods

Rate of photosynthesis and transpiration were measured on attacked lateral branches leaves (variety Agnus) by hop flea beetle (*Psylliodes attenuatus*) and downy mildew (*Pseudoperenospora humuli*). Selected lateral branches leaves were marked and measured during the whole experiment. The attack of lateral branches leaves is described in Table 1 (expressed in percentages). Healthy leaves were the control variant.
We measured in terms 10.7., 19.7., 24.7., 2.8. 2012 in the morning between 5:00 to 10:00 h. Individual measurements were about 25 min. We measured in three repeats + control variant the above mentioned terms.
We used to measure for selected physiological parameters LC pro+ device. LC pro+ (leaf ifrared analyzer – ADC, BioScientific Ltd., UK), to measure the basic physiological processes in the leaf without its separation from the plant. LC pro+ follows the physiology of leaf inside the measuring chamber, which is controlled by temperature and lighting. It is a method of gazometric analysis. The principle of measurement is the detection of changes in the concentration of CO_2 and water vapor in the air passing around a leaf, which is hermetically closed in the measuring chamber. The rate of photosynthesis and transpiration inside the measuring chamber are calculated every 20 seconds from differences in the concentration levels of gas and air flow. CO_2 measurement is carried out by an infrared gas analyzer (IRGA). H_2O measurement is carried out two high-quality humidity sensor. The measured values are automatically stored on a PCMCIA memory card. We measured at a constant temperature of 23 °C and 600 nm irradiation. Rate of photosynthesis (A) and transpiration (E) is expressed in units $\mu molCO_2.m^{-2}(of\ leaf).s^{-1}$ respectively $mmolH_2O.m^{-2}(of\ leaf).s^{-1}$.

Table 1. *The attack of lateral branches leaves (variety Agnus) by hop-flea-beetle and downy mildew in selected periods in 2012 (expressed in percentages)*

	the attack by hop-flea-beetle (**%**)	the attack by downy mildew (**%**)
10.7.	30	15
19.7.	30	20
24.7.	30	20
2.8.	30	25

Results

During the monitored period, the photosynthesis rate of lateral branches leaves affected by hop-flea-beetle (*Psylliodes attentuatus*) moved from 3.38 to 6.12 $\mu molCO_2.m^{-2}.s^{-1}$. The photosynthesis rate of those leaves affected by downy mildew (*Pseudoperenospora humuli*) moved from 1.43 to 3.83 $\mu molCO_2.m^{-2}.s^{-1}$. The photosynthesis rate of healthy lateral branches leaves moved between 3.96 – 5.51 $\mu molCO_2.m^{-2}.s^{-1}$. The transpiration rate of leaves affected by hop-flea-beetle moved from 0.58 to 0.91 $mmolH_2O.m^{-2}.s^{-1}$. The transpiration rate of leaves affected by downy mildew moved from 0.20 to 0.75 $mmolH_2O.m^{-2}.s^{-1}$. The transpiration rate of healthy leaves moved from 0.59 to 0.79 $mmolH_2O.m^{-2}.s^{-1}$.
The overall comparison of average values from individual measurements of photosynthesis and transpiration rates of affected hop lateral branches leaves for different terms of 2012 are shown in charts no. 1 and 2.
The results show that average values of measurement of the photosynthesis rate of lateral branches leaves affected by hop-flea-beetle are not significantly different from average values measured to healthy leaves (the chart no.1). On average, the healthy lateral branches leaves have shown 108% of photosynthesis rate of those leaves affected by hop-flea-beetle. In case of the measurement of transpiration rate, the healthy leaves have shown 96% of transpiration rate of leaves affected by hop-flea-beetle. We suppose that the typical perforation increases a plant transpiration, i.e. supply of water, by affected parts of a leaf (see the chart no. 2).
In case of lateral branches leaves affected by downy mildew, different results were found out.

The healthy lateral branches leaves have shown, on average, 196% of the photosynthesis rate of leaves affected by downy mildew. The rate of photosynthesis has decreased simultaneously with increasing leaf damage. It is caused by lower quantity of photosyntheticly active pigments and by progressive drying of leaves. However, we have to state that even so damaged leaves (table 1) are photosyntheticly active. The differences in the transpiration rate between the healthy lateral branches leaves and those damaged by downy mildew are similar to the results of 2011. On average, the healthy leaves have shown 133% of transpiration rate of lateral branches leaves affected by downy mildew. We suppose that the transpiration in the parts of leaves affected by downy mildew is not possible owing to progressive drying. In case of healthy leaves, the active transpiration has been observed in all measured part.

Conclusion

- values of measurement of the photosynthesis rate of lateral branches leaves (Agnus variety) affected by typical perforation of hop flea beetle (*Psylliodes attentuatus*) are not significantly different from average values measured to healthy leaves.
- on average, the healthy leaves have shown 108% of photosynthesis rate of leaves affected by hop-flea-beetle.
- values of measurement of the photosynthesis rate of lateral branches leaves (Agnus variety) affected by downy mildew (*Pseudoperenospora humuli*) are significantly different from values measured to healthy leaves.
- the healthy leaves have shown, on average, 196% of the photosynthesis rate of leaves affected by downy mildew.
- even lateral branches leaves significantly damaged by downy mildew are photosynthetically active.
- the transpiration rate of healthy leaves was lower than the leaves affected by hop-flea-beetle.
- the healthy leaves have shown 96% of transpiration rate of leaves affected by hop-flea-beetle.
- on average, the healthy leaves have shown 133% of transpiration rate of leaves affected by downy mildew.

Chart 1. *The comparison of mean values and the total average values of photosynthesis rate ($\mu molCO_2.m^{-2}.s^{-1}$) of healthy lateral branches leaves with lateral branches leaves damaged by hop flea beetle and downy mildew in selected periods in 2012*

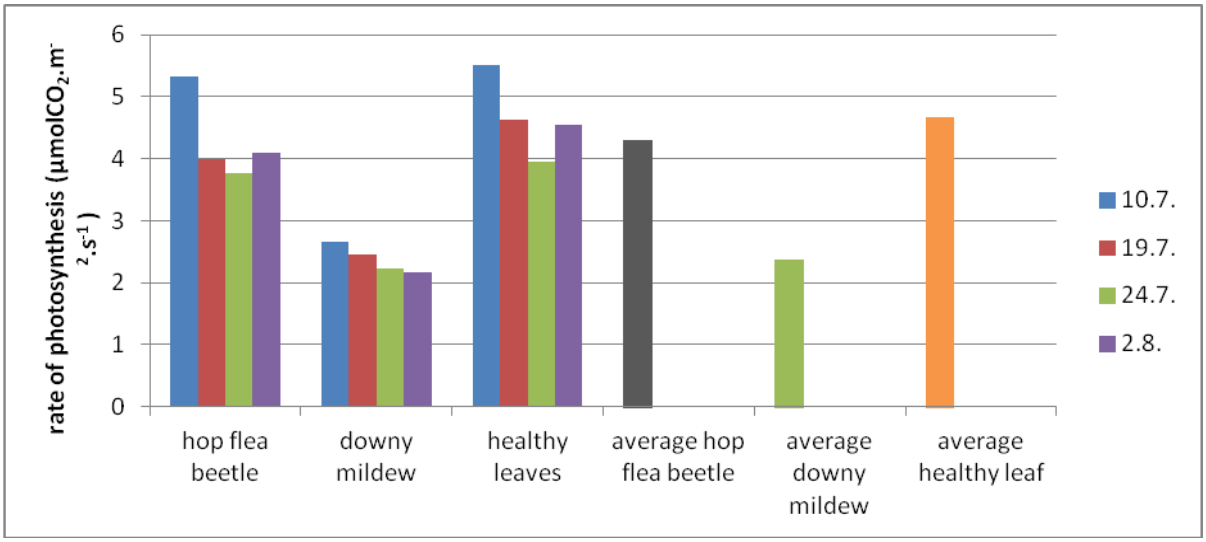
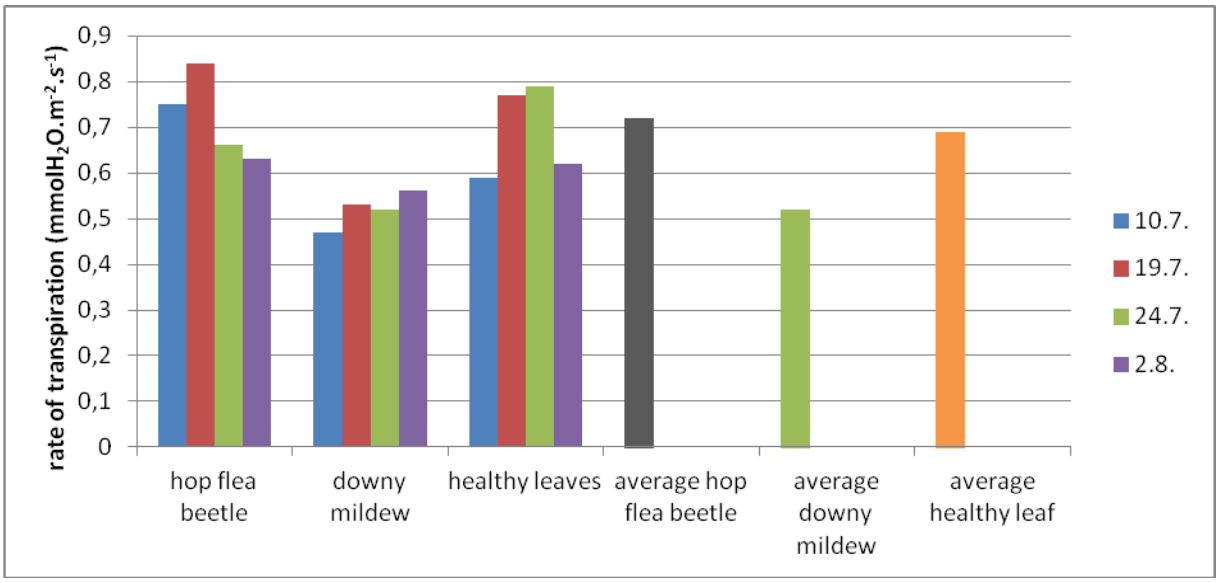


Chart 2. *The comparison of mean values and the total average values of transpiration rate (mmol$H_2O.m^{-2}.s^{-1}$) of healthy lateral branches leaves with lateral branches leaves damaged by hop flea beetle and downy mildew in selected periods in 2012*



References

Hnilickova, H.; Hejnak, V.; Hnilicka, F. 2000. Studium fotosyntetického procesu u chmele. Chmelařství (4): 69-70

Prochazka, S.; Gloser, J.; Havel, L.; Nátr, L.; Macháčková, I.; Krekule, J.; Prášil, I.; Procházka, S.; Sladký, Z.; Šantrůček, J.; Šebánek J.; Tesařová, M.; Vyskot, B. 1998. Fyziologie rostlin. Praha: Academia Praha, ISBN 80-200-0586-2: 72, 130

Rybacek, V. 1980. Hop production. Praha: Státní zemědělské nakladatelství: 61-62

Sestak, Z.; Catsky, J.; Avratovščuková, N.; Bartoš, J.; Janáč, J.; Kubín, Š.; Květ, J.; Nečas, J.; Slavík, B.; Smetánková, M.; Šetlík, I.; Vozněsenskij, V. L. 1966. Metody studia fotosynthetické produkce rostlin. SZN Praha: 58-60

Wullschleger, S. D.; Oosterhius, D. M. 1990. Photosynthesis of individual field-grown cotton leaves during ontogeny. Photosynth. Res., vol. 23: 163 – 170

Yu, G. R.; Zhuang, J.; Yu, Z. L. 2001. An attempt to establish a synthetic model of photosynthesis-transpiration based on stomatal behavior for maize and soybean plants grown in field. Journal Plant Phisiology, vol. 158: 861 – 874

Acknowledgement

This paper is a result of our working activities within research project NAZV QH81049: *"Integrated system of hop growing"* (2008-2012) with the financial support from Czech Ministry of Agriculture.

VII. Session:
Hop Production

REDUCTION OF PESTICIDES BY USING SENSOR TECHNOLOGY IN ROW TREATMENTS

Portner, J.

Bavarian State Research Center for Agriculture, Institute for Crop Science and Plant Breeding, Hop Research Center Huell, Kellerstr. 1, 85283 Wolnzach Germany; E-mail: Johann.Portner@LfL.bayern.de

Introduction

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 approx. 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 of the spraying fan between the hop plants the amount of spraying liquid could be reduced significantly at the same efficacy while protecting the environment.

Methods

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 wire 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) and two years (2011 and 2012) the amount of pesticides, which could be reduced by switching off the spaying fan, was found out in comparison with the full-length row treatment.

Fig. 1 + 2: State of the art in practice for continuous row treatment


Fig. 3 + 4: Sensor-controlled application technique for the first Application date (19/04/2011) to 40 cm plant height



Fig 5 - 7: Sensor-controlled application technique for the second time of application (02/05/2011) to 1.5 m height

Results and Discussion

For the first trial (BBCH 15:19/04/2011 and 02/05/2012) hop plants with a shoot length of 5-40 cm pre-treated with a circular cultivator were sprayed in a band treatment with 2 flat fan nozzles per side. Depending on the width of the band switched-off between the plants sensor technology brought compared to continuous treatment a saving of 61-70 % of spray mixture and thus pesticides.

At the second application date (BBCH 32: 02/05/2011 and 15/05/2012) after pruning and training the plant height has been approximately 1.5 m. Therefore, 3 flat fan nozzles were arranged in a vertical linkage and between the training wires switched off by sensor. The savings in spray and pesticides amounted to 55-60%.

There was no visual difference in between the leaf wetting strip processing and sensor-driven application technique. Efficacy trials were not performed.

Advantages in the use of sensor technology for early crop protection applications in the hops are mainly seen in the improved user protection and the exact placement and dosage of the plant protection product. Of economic importance is that the device that was originally designed for the watering treatment, by a simple conversion can be used for two other applications and thus reduce fixed costs. The saving of more than half of pesticides and the required support staff for application also contributes to reduce costs and make the device interesting for smaller farms. From an ecological point the device serves the political goals of reducing plant protection and thus contributes to the integrated pest management.

DEVELOPMENT AND OPTIMISATION OF A MACHINE FOR AUTOMATIC PICKING OF HOPS

Portner, J[1]., Gobor, Z.[2], Fröhlich, G.[2], Kammerloher, T.[2]

Bavarian State Research Center for Agriculture,
[1]Institute for Crop Science and Plant Breeding, Kellerstr. 1, 85283 Wolnzach, Germany;
[2]Institute for Agricultural Engineering and Animal Husbandry,
Vöttinger Str. 36, 85354 Freising, Germany
E-mail: Johann.Portner@LfL.bayern.de

Introduction

Although Germany produces more than a third of the world's hop, the technical progress, particularly in picking machine has barely developed since their introduction 50 years ago. Still the hop vines are hooked into the picking machine by hand. These strenuous (7 days a week, 12 hours per day), ergonomically stressful (work related) and accident-prone work (hand injury) is mainly carried out by foreign seasonal workers. Due to the improved employment opportunities in countries of origin and increasing labor costs, it is increasingly difficult to attract suitable persons for these activities. Therefore, the considerations are to automate the manual mount of hop vines into the picking machine. Then the entire hop harvest from tearing off the vines on the field up to pressing the dried hop into the bales would be automatable.

Objectives

In the framework of a research project, funded by the Federal Ministry of Food, Agriculture and Consumer Protection (BMELV) in cooperation with the Federal Agency for Agriculture and Food (BLE) under the program to encourage innovation, manual mount of hop vines shall be replaced by the procedure that vine stacks of 6-7 m long hop vines dropped by the transport vehicles are loaded dosed into the new picking machine which pre-cuts and pre-picks the stacks in about 1 m long pieces before reaching the main picking unit.

The aims are:

- Elimination of manual mount of the hop vines
- Increase the picking rate to 1000 vines per hour at savings of up to 4 workers
- Improved safety according due to the EC machinery directive 2006/42/EC
- Minimizing losses and maintaining quality at least on the current state

Results

Based on a digital prototype created in 3D model construction, the functions of new modules were tested virtually and kinematic analysis was carried out. In this way, potential construction faults were minimized before the assembling and the time of development has been significantly reduced. Based on the optimized digital prototype a delivery ramp supplying the vines stack, the pre-cut system and the pre-pick unit were built and tested in practical use in 2012. The operation can be described as follows: The vine stacks deposited by the transport vehicle are supplied via an inclined ramp with a scraper floor into a pre-cutting unit which cuts approximately 1 m long pieces by bottom-up cutting blades. A pre-picking belt which is arranged adjacent to the cutting device and moves upwards with the cutting tool, picks a portion of cones during the cutting operation and conveys the vine portions separated on a conveyor belt. In this section the vine pieces are supplied to a yet to be constructed main and side picking unit. On this way, a separation of the already picked hop cones and leaves of the vine pieces is performed by a sorting belt. The first results of pre-cutting and pre-picking were promising. In first tests the precision of picking were analyzed and compared to conventional hop picking machine results.

FERTILIZATION OF ORGANIC HOPS

Solarska, E., Marzec, M., Potocka, E.

University of Life Sciences in Lublin, Poland
20-704 Lublin, Skromna 8, E-mail: ewa.solarska@up.lublin.pl

Abstract

The use of fertilizers, including chemical fertilizers and manures, to enhance soil fertility and crop productivity has often negatively affected the environment. Organic nutrient management system is needed to maintain agricultural productivity and protect the environment. Probiotic microorganisms are promising components of such management systems. The aim of this study was to develop methods of organic hop fertilization using composted manure with probiotic microorganisms and basalt meal.

Highest yields of cones were harvested from the hop-garden fertilized with composted manure in mixture with basalt meal and probiotic microorganisms. In both, composted horse manure with basalt meal and probiotic microorganisms as well as in cattle slurry with basalt meal and probiotic microrganisms higher content of nitrogen and phosphorus was found in comparison with manure and slurry without additions. The highest content of humus was found in parts of hop-gardens fertilized with composted horse manure with basalt meal and probiotic microorganisms.

Keywords: hops, organic farming, fertilization, manure, basalt meal, probiotic microorganisms

Introduction

The continued use of chemical fertilizers and manures for enhanced soil fertility and crop productivity often results in unexpected harmful environmental effects, including leaching of nitrate into ground water, surface run-off of phosphorus and nitrogen run-off, and eutrophication of aquatic ecosystems. Increases in emissions of CO_2, CH_4, and nitric oxide (N_2O), the three most important greenhouse gases, have been linked to fertilizer applications. Increasing atmospheric N_2O is considered an important factor in ozone layer depletion. Gases such as N_2O and ammonia emissions from livestock and fertilizers contribute to acid rain and the acidification of soils and freshwater ecosystems. In organic farming application only organic fertilizers such as green manure, organic manure, compost, compost extract is allowed to enhance crop production and/or control of plant pathogens. However, it is important to emphasize that agro-environmental problems are not limited to the use of chemical fertilizers but also occur with animal waste (Adesemoye, Kloepper 2009). Organic nutrient management system is needed to maintain agricultural productivity and protect the environment. Probiotic microorganisms are promising components of such management systems.

The aim of this study was to develop methods of organic hop fertilization using composted manure with probiotic microorganisms and basalt meal.

Material and methods

The experiment was conducted on two hop-gardens away from each other by about 3 km:
- in Jastków a hop-garden with cultivar Magnum was area of 1.56 ha,
- in Natalin a hop-garden with cultivar Marynka was area of 2.36 ha.

Fertilization was applied on two dates: 9.IV - composted horse manure after spraying with probiotic microorganisms in amount of 1 liter per 1 m3 of manure, applied to the entire surface of each hop-garden in the amount of 15 t / ha
• 4.V - horse manure (35 t / ha) and basalt meal were used in such a way that the hop-gardens in Jastków and Natalin were divided into three parts: - in the first part basalt meal in dose of 1,2 t per ha was used. - in the second part composted horse manure with the

addition of basalt meal in dose 2 tons per 1 ha after spraying with probiotic microorganisms in amount of 1 liter per 1 m3 of manure were used

- in third part composted horse manure after spraying with probiotic microorganisms in amount of 1 liter per 1 m3 of manure were used. Each of the three parts was divided into three successive parts, in which 7.V following plants were sown in interrows of hops: a - triticale (130 kg / ha), b - oats (180 kg / ha), c - no undersown crop.

Additionally 23.VII cattle slurry was applied at a dose of 80 l / ha with the addition of probiotic microorganisms in dose 40 l / ha. Cattle slurry and compost obtained after the decomposition of horse manure were evaluated for nutrient parameters such as Nitrogen (N), Phosphorous (P), Potassium (K). Content of humus in soil of experiment was also defined.

Results

The estimation of yield showed that it was higher than in the previous year, despite that during cold winter from 16% to 39% of the hop plants were damaged by frost. Therefore, for an objective assessment of cones yield on hop-gardens number of plants converted to full plant density and referred to the 1 ha. The highest yields of cones in Jastków were found on those parts of the hop-garden, on which basalt meal was used and it amounted 1285 kg / ha and where manure composted with basalt meal and probiotic microorganisms was used and it amounted 1266 kg / ha (tab. 1). But definitely the highest yields of cones were harvested in Natalin from the hop-garden fertilized with composted manure in mixture with basalt meal and probiotic microorganisms (1465 kg / ha) and fertilized with the composted manure with probiotic microorganisms and they amounted 1495 kg / ha. It was found a clear influence of field formation on quantity of crop. Both the Jastków, as well as part of the hop-garden Natalin were on the field for quite a considerable slope, and yields of cones from these parts of hop-gardens were the lowest: 1189 kg per hectare in Jastków and 1128 kg per hectare in Natalin (tab. 1). Applied triticale and oats undersown clearly influenced the increase in yield (tab. 1). In both composted horse manure with basalt meal and probiotic microorganisms as well as in cattle slurry with basalt meal and probiotic microrganisms higher content of nitrogen and phosphorus was found in comparison with manure and slurry without additions (tab. 2, 3). The highest content of humus was found in parts of hop-gardens fertilized with composted horse manure with basalt meal and probiotic microorganisms (tab. 4).

Table 1. Yields of hops in kg/ha on parts of hop garden with different types of fertilization in Jastków and Natalin in 2012

Object (cultivar, locality)	Type of fertilization and undersown								
	Basalt meal (1,2 t/ha)			Manure composted with basalt meal and probiotic microrganisms (35 t/ha)			Manure composted with probiotic microrganisms (35 t/ha)		
	undersown of trticale	undersown of oat	without undersown	undersown of trticale	undersown of oat	without undersown	undersown of trticale	undersown of oat	without undersown
Magnum, Jastków	1298	1326	1231	1331	1245	1222	1192	1232	1142
	mean = 1285			mean = 1266			mean = 1189		
Marynka, Natalin,	1043	1127	1215	1484	1460	1452	1388	1567	1529
	mean = 1128			mean = 1465			mean = 1495		

Table 2. The content of macronutrients in composted horse manure with microorganisms and basalt meal

Type of manure	dry weight in %	The content of macronutrients in% (in fresh weight of the sample)				
		total nitrogen (N)	phosphorus (P)	potassium (K)	calcium (Ca)	magnesium (Mg)
Manure composted with basalt meal and probiotic micro-organisms	33,2	0,73	0,12	0,49	0,45	0,15
		The content of macronutrients in% (dry weight of the sample)				
		2,20	0,35	1,47	1,37	0,45

Type of manure	dry weight in %	The content of macronutrients in% (in fresh weight of the sample)				
		total nitrogen (N)	phosphorus (P)	potassium (K)	calcium (Ca)	magnesium (Mg)
control manure	77,4	1,37	0,22	1,02	1,10	0,23
		The content of macronutrients in% (dry weight of the sample)				
		1,77	0,28	1,32	1,42	0,30

Table 3. The content of macronutrients in cattle slurry with basalt meal, probiotic microorganisms

Type of animal waste	Dry weight in %	The content of macronutrients in% (in fresh weight of the sample)				
		total nitrogen (N)	phosphorus (P)	potassium (K)	calcium (Ca)	magnesium (Mg)
slurry with basalt meal, prrobiotic microorganisms	2,80	0,38	0,01	0,62	0,038	0,029
		The content of macronutrients in% (dry weight of the sample)				
		13,57	0,36	22,14	1,36	1,04

Type of animal waste	**Dry weight in %**	**The content of macronutrients in% (in fresh weight of the sample)**				
		total nitrogen (N)	**phosphorus (P)**	**potassium (K)**	**calcium (Ca)**	**magnesium (Mg)**
Control slurry	1,20	0,27	0,0025	0,29	0,008	0,014
		The content of macronutrients in% (dry weight of the sample)				
		22,50	0,21	24,17	0,67	1,17

Table 4. The content of humus (in %) in soil on parts of hop-garden with different types of fertilization in Jastków and Natalin in 2012

Object (cultivar, locality)	Type of fertilization and undersown								
	Basalt meal (1,2 t/ha)			Manure composted with basalt meal and probiotic microrganisms (35 t/ha)			Manure composted with probiotic microrganisms (35 t/ha)		
	undersown of trticale	undersown of oat	without undersown	undersown of trticale	undersown of oat	without undersown	undersown of trticale	undersown of oat	without undersown
Magnum, Jastków	1,60	1,60	1,65	1,50	1,66	1,86	1,72	1,78	1,34
	mean = 1,62			mean = 1,67			mean= 1,61		
Marynka, Natalin	1,77	1,64	1,24	2,01	2,30	2,11	2,02	1,88	1,96
	mean = 1,55			mean= 2,14			mean = 1,95		

Discussion

Results obtained in this study showed that cones yield increased when hop plants were fertilized with composted horse manure with addition of probiotic microorganisms and basalt meal. The nitrogen and phosphorus levels in the composted manure increased; these results could account for the higher crop yields that are often reported when organic manures are inoculated with probiotic microorganisms (Bosco et al. 2007). Hops have a great demand especially for micronutrients such as boron and zinc (Rossbauer et al. 1991). Basalt meal contains the mentioned trace elements as well as iron, magnesium and other. Therefore this fertilizer greatly increase plant growth and survival (Arango et al. 2011). Studies with microbial inoculants and organic fertilizers have demonstrated that some inoculants can improve plant uptake of nutrients and thereby increase the use efficiency of applied fertilizers and manures. These proofs of concept studies will serve as the basis for vigorous future research into nutrient management in organic farming.

References

Adesemoye A.O., Kloepper J. W.: Plant–microbes interactions in enhanced fertilizer-use Efficiency, Appl Microbiol Biotechnol (2009) 85:1–12

Arango C. A., Despaigne-Matchett G., Despaigne-Ceballos G., Goreau T. J., Goreau M., Lufkin F., Solis R., Campe J.: Basalt Powder Restores Soil Fertility and Greatly Accelerates Tree Growth on Impoverished Tropical Soils in Panama. Proceedings of the symposium Symposium on Soil Ecology and Restoration, Merida, Mexico, 2011

Bosco M., Giovannetti G., Picard C., Baruffa E., Brondolo A., Sabbioni F.: Commercial plant-probiotic microorganisms for sustainable organic tomato production systems. In Niggli U., Leifert C., Alföldi T., Lück L., Willer H. (eds): Improving Sustainability in Organic and Low-Input Food Production Systems. Proceedings of the 3rd QLIF Congress. Stuttgart, March 20-23, 2007. FiBL, Frick, p. 268-271.

Rossbauer, G. et al.: Hopfen: Anbau, Duengung, Pflanzenschutz - Hinweise fuer das Jahr 1991. Hopfen Rundschau No.3/91, Germany (1991)

NITROGEN IN THE HOP FIELD SOIL IN A DRY YEAR

Čeh, B. and Čremožnik, B.

Slovenian Institute of Hop Research and Brewing,
Cesta Žalskega tabora 2, Žalec, Slovenia
E-mail: barbara.ceh@ihps.si

Abstract

In the year of 2012 with lack of precipitation soil Nmin was monitored in the field experiment with hop cultivar Aurora, which was conducted and treated the same way from 2010. Nmin (NH_4^+- N and NO_3^--N) sampling (0-30 cm, 30-60 cm) was done seven times in the season at four different treatments (in three replications), which differ in the form of N fertilizer for the second and the third hop side-dressing (KAN or cattle slurry in the amount 26 m^3/ha). In the spring (28th April 2012) there was no significant difference in Nmin in soil (0-60 cm) among treatments; the quantity was between 78 and 92 kg/ha. Before the second hop side-dressing there was still no significant difference in soil Nmin; there was 165 to 187 kg/ha Nmin (0-60 cm). Before the third hop side-dressing there was significant difference in Nmin in soil among treatments; significantly higher Nmin in soil was at treatments where KAN (70 kg/ha N) was used for the second hop side-dressing (approximately 400 kg/ha Nmin 0-60 cm) compared to the treatments where cattle slurry was applied (265 kg/ha Nmin). The lack of rainfall probably did not allow KAN to be dissolved and so N was not available to be absorbed by plants; it was only accumulating in soil. On the other hand less N was applied with cattle slurry at the second side-dressing compared to KAN (35 kg/ha plant available form applied with cattle slurry) and it was more available to the plants because cattle slurry contains also water, so plants were able to absorb some more N compared to the KAN treatment. High amounts of Nmin in soil were recorded also in mid summer (6th August) and after harvest, the differences among treatments in soil Nmin continued to be present. After harvest there was as much as 264 kg/ha Nmin in the soil (0-60 cm); significantly lower at treatment where cattle slurry was used for the second and the third hop side-dressing (142 kg/ha), and from 224 to 264 kg/ha (0-60 cm) at the other investigated treatments. Unfortunately the Nmin did not stay in the soil until the next hop season but it was leached away from the upper 60 cm layer of the soil; after very rainy autumn (209 mm only in September) we detected on 29th November less than 50 kg/ha Nmin in soil (0-60 cm) at all treatments. Nmin sampling is urgent in such extreme years to get familiar with the quantity of plant available N already present in soil before deciding about the next hop side-dressing when there is still time to act and avoid such situations, which lead to environmental burdening and reflect also money wasted on fertilizers and bad management.

Keywords: hop, *Humulus lupulus* L., fertilization, side-dressing, nitrogen in soil, Nmin

Acknowledgements

The research was funded by the Slovenian Ministry of agriculture and the environment, within professional task Technology of hop production and processing.

TEMPORARY PERMANENT GRASSING IN HIGH AND LOW TRELLIS

Křivánek, J., Ježek, J., Pokorný, J., Krofta, K.

Hop Research Institute Co., Ltd., Kadaňská 2525, Žatec, Czech Republic
E-mail: krivanek@chizatec.cz

Abstract

Integrated production of agricultural crops is aimed at sustainable procedures of soil cultivation or at utilization of ecological infrastructures inside and outside production sites. Such procedures are green manuring and grassing of the land between crops in hop gardens. For high and low trellis in Czech growing conditions, authors have introduced the term temporary grassing of hop gardens (the duration of approximately 2-3 years) and have proposed sowing rates of various crops. The mixture of selected crops for low and high trellis includes Red Fescue, Meadow-fescue, Perennial Ryegrass, Annual Ryegrass, White Clover, Kentucky Bluegrass, Crown Vetch, Black Medic, and Trefoil. The percentages of each crop representation in the sown seeds are specified as well.

Keywords: hop, integrated production, low trellis, grassing

Introduction

Integrated plant production is an agricultural system that represents a modern method of soil management. It prefers sustainable methods of plants cultivation and protection, and minimizes inputs of agrochemicals that have potential side effects. Ecologically and economically acceptable measurements are introduced into the production process aiming at the highest quality of products while minimizing the contents of extraneous compounds. Integrated agricultural systems are an important intermediate between conventional and organic production. These systems promote reduction of nutrients unbalance risk and also a more rational utilization of nutrients. Thanks to their well-worked out management methods, efficient cultivation procedures, crop protection and weed control, the integrated agriculture and production significantly decrease the consumption of agrochemicals. In the EU, integrated production is considered the first feasible step to the solution of current problems of intensive agriculture. In the Czech Republic, the system has been used for cultivation of fruits, grapes, and vegetables for many years. Legislative rules are currently being prepared for hop production as follows from the *Directive 2009/128/EC of the European Parliament and of the Council establishing a framework for Community action to achieve the sustainable use of pesticides.*

The integrated crop protection and non chemical methods are defined in article 3. ***"Integrated pest management"*** means careful consideration of all available plant protection methods and subsequent integration of appropriate measures that discourage the development of populations of harmful organisms and keep the use of plant protection products and other forms of intervention to levels that are economically and ecologically justified and reduce or minimize risks to human health and the environment. "Integrated pest management" emphasizes the growth of a healthy crop with the least possible disruption to agro-ecosystems and encourages natural pest control mechanisms.

"Non-chemical methods" means alternative methods to chemical pesticides for plant protection and pest management, based on **agronomic techniques** such as those referred to in point 1 of Annex III, or physical, mechanical or biological pest control methods.

Annex III is called the General principles of integrated pest management. The following is stated in point 1: The prevention and/or suppression of harmful organisms should be achieved or supported among other options especially by:

- crop rotation,
- use of adequate cultivation techniques (e.g. stale seedbed technique, sowing dates and densities, under-sowing, **conservation tillage,** pruning and direct sowing),
- use, where appropriate, of resistant/tolerant cultivars and standard/certified seed and planting material,
- use of balanced fertilization, liming and irrigation/drainage practices,
- preventing the spreading of harmful organisms by hygiene measures (e.g. by regular cleansing of machinery and equipment),
- protection and enhancement of important beneficial organisms, e.g. by adequate plant protection measures or **the utilization of ecological infrastructures** inside and outside production sites.

Utilization of **green manuring and permanent grassing** in hop gardens represents saving procedures of soil cultivation. In our experiments, we focused more on permanent grassing that is called temporary. Under conditions available in the Czech Republic, the permanent temporary grassing means grassing of a hop garden for the duration of 2–3 years, not fully permanent for the hop garden lifetime.

Since 2008, the Czech hop industry has been going through the renaissance of hop cultivation on low trellis system. First experiments were carried out by the Hop Research Institute in Žatec at the beginning of 1990s. At that time, the performance of Saaz, Premiant and Sladek cultivars was investigated. After several seasons it was concluded that the Saaz variety was not suitable for the low trellis cultivation system and that profitability was achievable when using the Sladek variety (yield exceeding 1.1 tons per hectare). Due to the poor results, this technology was not found promising at that time and further cultivation tests were abandoned.

In 2006-2007, the Czech hop industry faced the problem of critical shortage of labor force for spring seasonal operations (attaching training wires, training of hop shoots). Therefore, a few first hop growers decided to build low trellis again and started to cultivate the Premiant, Sladek, and Agnus varieties having found inspiration in England. In 2012, the total low trellis hop garden area was approximately 47 ha.

Season 2011 was extremely wet and rainy with torrential rainfalls which made crop harvest very difficult. Permanent grassing in low trellis showed to be an acceptable solution in situations when soft soil surface did not enable the mobile harvester to operate in the hop garden. The aim of our tests was to choose crops suitable for cultivation in inter-rows of hop gardens.

Methods

Sowing of permanent grassing in low trellis is possible during the whole vegetation season, providing soil conditions are favorable. The best time for grassing is up to the end of June in low trellis and turn of May-June in high trellis. In low trellis, no shading of seeded crops occurs. The clover-grass mixtures (Tables 1 and 2) are able to create firm unbroken turfs very quickly. It is necessary to mow and mulch the turf several times during the season in order to improve its structure. The clover-grass mixtures have generally a slower start into creating an uninterrupted growth due to different proportions of individual components. Weed infestation usually occurs during the first vegetation season. Therefore, mulching or cutting is important for weed suppression. Moreover, it is effective to choose grass mixtures that include fast growing species that will be successful at competing with weeds. To some extent weeds help protect emerging grass against sunshine (covering effect), however, later on, weeds become competitors. Therefore, weed-off cutting should be performed to prevent weed plants from shedding seeds. During the first vegetation season a single weed-off cutting is usually adequate for grass to create a continuous carpet.

Table 1: Perennial grass mixture for permanent grassing in low trellis

Crop	Latin term	Proportion (% w/w)
(Creeping) Red Fescue	*Festuca rubra L.*	20
Meadow-fescue	*Festuca pratensis L.*	15
Perennial (English) Ryegrass	*Lolium perenne L.*	15
White Clover (Dutch Clover)	*Trifolium repens L.*	15
Annual (Italian) Ryegrass	*Lolium multiflorum Lam.*	10
Kentucky Bluegrass (Smooth or Common Meadow-grass)	*Poa pratensis L.*	10
(Purple) Crown Vetch	*Securigera varia L.*	5
Black Medic(k)	*Medicago lupulina L.*	5
Clustered birdsfoot-trefoil	*Lotus ornithopodioides L.*	5

Table 2: Perennial grass mixture for permanent grassing in high trellis

Crop	Latin term	Proportion (% w/w)
Perennial (English) Ryegrass	*Lolium perenne L.*	25
Annual (Italian) Ryegrass	*Lolium multiflorum Lam.*	20
Kentucky Bluegrass (Smooth or Common Meadow-grass)	*Poa pratensis L.*	15
White Clover (Dutch Clover)	*Trifolium repens L.*	15
Meadow-fescue	*Festuca pratensis L.*	15
(Creeping) Red Fescue	*Festuca rubra L.*	10

Results

In both high and low trellis cultivation systems, the sowing rate of 25-30 kg/ha proved to be effective for temporary permanent grassing in hop gardens. The composition of grass mixtures suitable for temporary permanent grassing is displayed in Tables 1 and 2. In high trellis, the best time for permanent grass seeding is by the end of May at the latest due to grass shading during advanced season time. In low trellis, the best time for seeding is up to the end of June. It is recommended that the lawn is mowed or mulched at least once a year in order to improve the quality of the turf structure (Fig. 2).

Conclusion

From the viewpoint of hop cultivation, permanent grassing of the inter-rows in hop gardens has the following effects:

- maintaining the herbaceous cover in the inter-rows area is considerably less energy-demanding than the cultivation of the whole area of the soil surface,
- also, moving of tractors and other machinery on grass surface in the inter-rows area in hop gardens is less energy-demanding in comparison to movement on soil surface,
- herbaceous vegetation that is rich in species and flowers for most of the vegetation period is necessary for occurrence of many beneficial insect species,
- furthermore, the possibility to enter the hop garden with the cultivation machinery at any time (even shortly after rainfall) and treat hop plants in critical moments is very important,
- grassing is a suitable anti-erosion measurement on slope plots.

In low trellis, the permanent grassing is useful not only for movement of the cultivation machinery but also for smooth and regular passing through of the mobile harvester that

makes straddle movements and its undercarriage moves in the middle of rows where the soil is usually very loose and soft and causes sinking of the heavy harvester making it very difficult for the machine to move about in the terrain. Clover-grass mixtures create firm turf suitable for regular cutting or mulching. Summer cultivation of hop gardens is not necessary.

After 2-3 years of utilization and regular mowing of the permanent grassing, it is beneficial to till the lawn into the soil by the hop plough in autumn, and to establish new temporary permanent grassing of the inter-rows again in the upcoming year.

Acknowledgement

This paper is a result of our activities within the research project NAZV QH81049: *"Integrated system of hop growing"* (2008-2012) with the financial support from Czech Ministry of Agriculture.

Fig. 1: Permanent grassing in low trellis helps the smooth operation of mobile harvester HUN-30
(photo by J. Ježek)

Fig. 2: A mulcher is operating in a hop garden.
(photo by J. Ježek)

MULCHER TO HOP GARDEN

Křivánek, J.[1], Ježek, J.[1], Pokorný, J.[1], Krofta, K.[1], Ostratický, R.[2], Ostratický, J.[2]

[1]Hop Research Institute Co. Ltd., Kadaňská 2525, Žatec, Czech Republic
[2]Ostratický Co., Ltd., Hrušecká 388, Týnec u Břeclavi, Czech Republic
E-mail: krivanek@chizatec.cz

Abstract

Innovation of horizontal mulch machine for hop gardens is based on the possibility to adjust working swath and throw of plant mass into the sides of the rows with the help of specially bent knives. The machine is suitable for the surface mowing and mulching of grass, weeds, natural seeding of shrubs and trees till the diameter of 1 cm not only in hop gardens but also in vineyards including crush of bines.

Keywords: hop, mulcher, mulch machine, green manure, grassing, integrated production

Description

Machinery for side throw of mulch interim crop in the space between rows with variable working width consists of three sections: central, right and left. Supporting frame with the main gearbox, which shifts rotating moment from shaft outlet of the tractor to the right and left sections, forms the central section. Two diagonal brackets are placed at the central section. They support right and left section. The both sections are secured with a socket and fixing screws for adjustment of the working width. Terminal gearboxes are put on the adjustable right and left sections. They shift the motion from the main gearbox to the rotors.

Rectifiers have been installed in the front, central, right and left sections to throw mulch mass. With their help plant material is put aside into the outlets placed on the right and left sections.

Working width of this machine (2050 mm – 2200 mm – 2300 mm) can be set up and in this way we are able to regulate the swath of mulching according to the width of rows in the hop garden. This operation is carried out with the help of fix screws so as to shift right and left sections on the demanded width. Fix screws also serve to fix right and left sections back to the diagonal supports. Better grinding and throwing of mulch mass through the outlets are provided with the help of bent knives.

Technical certification of the machine is following: working machine supported, horizontal mulch machine, trademark: OSTRATICKÝ, type HM4/HM5. Technically is the machine identical with the type authorized by Ministry of Transport (no. S-0243-03-02). Weight of the machine is 590 kg; maximal transport speed of all the machine alternatives is 20 kilometers per hour.

Three-point tow is placed in the front part of the carrying part as a pendulum. Chains connect it with the rear carrying profile. Pendular suspension of the third point is necessary for optimal terrain tracing.

Acknowledgement

This machine is a result of our working activities within research project NAZV QH81049: *"Integrated system of hop growing"* (2008-2012) with the financial support from Czech Ministry of Agriculture.

VIII. Session:
Alternative Applications of Hops

THE EFFECT OF HOPS (*HUMULUS LUPULUS* L.) ON IN VITRO RUMEN MICROBIAL ACTIVITY

Lavrenčič, A.[1], Levart, A.[1], Košir, I.J.[2], and Čerenak, A.[2]

[1]University of Ljubljana, Biotechnical Faculty, Department of Animal Science, Domžale, Slovenia
[2]Slovenian Institute of Hop Research and Brewing, Žalec, Slovenia
E-mail: andreja.cerenak@ihps.si

Besides of its bittering and aromatic compounds it is well known that hops have also antimicrobial and/or microbe modulating properties. Part of our complex research was to determine the effect of two hop varieties differing mainly in the ratio between alpha and beta acids content on *in vitro* gas production.

Total mixed ration (TMR) was used as substrate, calculated for a dairy cow producing 30 kg of milk per day. Hop varieties Dana (alpha/beta acid ratio 3.37) and Aurora (alpha/beta acid ratio 2.37) were added into the inoculum in concentrations equal to those fed to 650 kg dairy cow in amounts of 50, 100 and 200 g hops per day. The individual gas production measurements over 96 hours of incubation were fitted with Gompertz model.

The gas production parameters such as total potential gas production (parameter "B"), gas production till 24 h of incubation (Gas24), maximum fermentation rate (MFR) and time of maximum fermentation rate (TMFR) were significantly affected by the hop concentration, while hop variety affected parameter "B", Gas24 and TMFR with the highest concentration having generally the greatest effects. The parameter "B" decreased from 314 ml/g dry matter (DM) to 204 and 170 ml/g DM and TMFR decreased from 6.0 to 3.9 hours when Dana hop variety was added. However, the greatest decrease in MFR was observed if 100 g of Dana variety will be fed to dairy cows (from 18.8 with TMR to 14.3 ml/h with 100 g of Dana). The amount of gas produced in 24 hours of incubation (Gas24) varied from 169 ml/g DM when the highest concentration of Dana was incubated with TMR to 297 ml/g DM when only TMR was incubated. The volatile fatty acid (VFA) contents determined after 24 hours of incubation partially confirmed above results. The Dana hop variety had always the greatest effects on individual VFA contents. It significantly decreased acetic and butyric acid contents from 3.28 to 2.07 mmol/g DM and from 0.78 to 0.21 mmol/g DM, respectively, and slightly ($P > 0.05$) increased the amount of propionic acid from 1.14 to 1.40 mmol/g DM.

From these results we can conclude that the supplementation of diets with hops changed the fermentation pattern *in vitro.* However, these data are not sufficiently conclusive to give a definitive recommendation about the variety and amount of hops fed to dairy cows or other ruminants, so further trials and analysis will be done.

Reference:

Lavrenčič, A., Levart, A., Košir, I.J. and Čerenak, A. Influence of two hop (*Humulus lupulus* L.) varieties on in vitro dry matter and crude protein degradability and digestibility in ruminants. Journal of the Science of Food and Agriculture – submitted