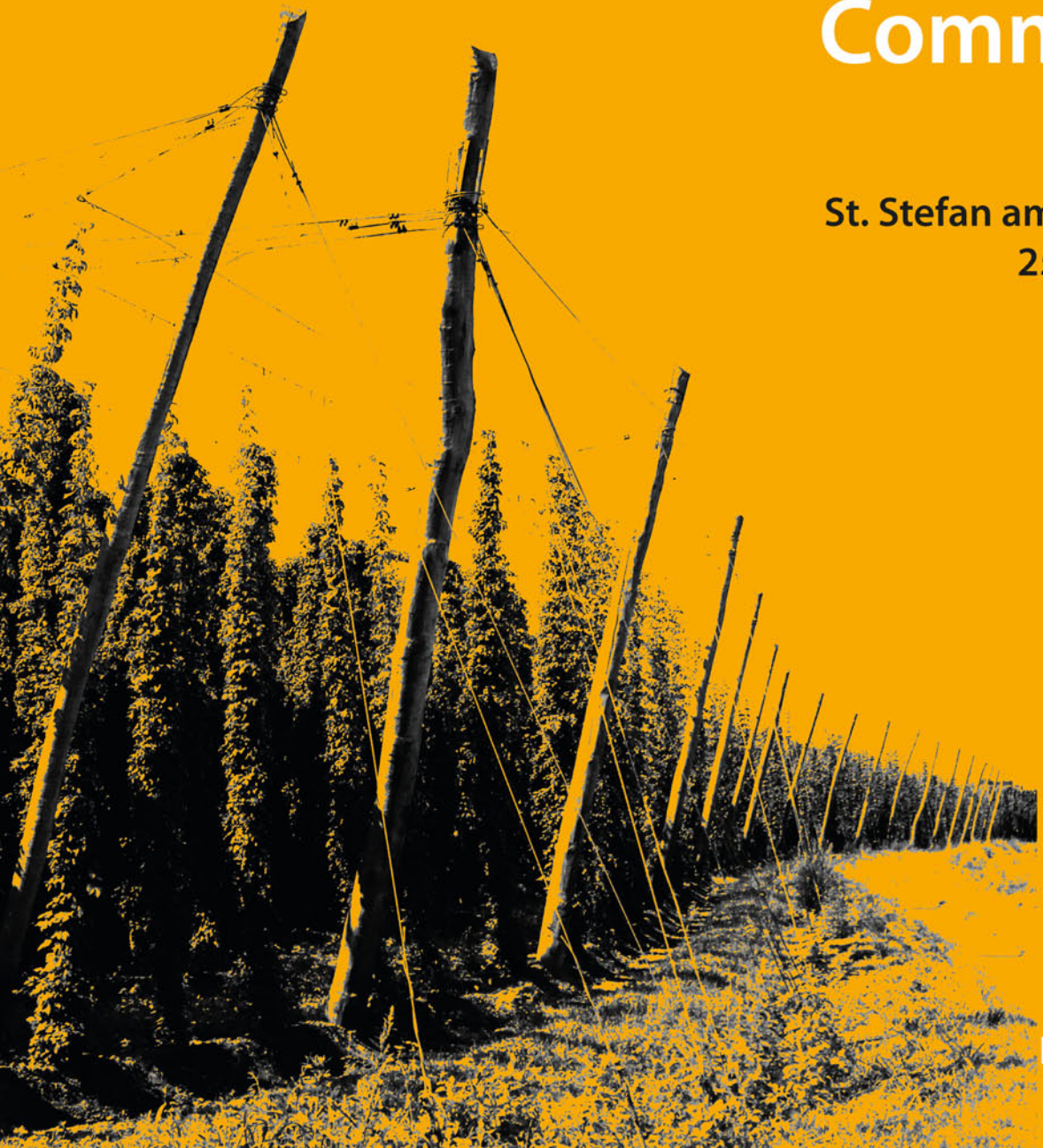
International Hop Growers' Convention I.H.G.C.

INTERNATIONAL HOP GROWERS` CONVENTION
IHGC
Austria • Mühlviertel • 2017

Proceedings of the Scientific-Technical Commission

St. Stefan am Walde, Austria
25–29 June 2017



ISSN 1814-2206

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Imprint

Scientific-Technical Commission of the
International Hop Growers' Convention, I.H.G.C.
c/o Hop Research Center, Hüll 5 1/3, 85283 Wolnzach, Germany
www.lfl.bayern.de/ipz/hopfen/024299/index.php

Edited by Florian Weihrauch
Editorial assistance by Alexandra Hertwig
Editorial deadline 31 May 2017

Cover design by Birgit Rödder Mediendesign, Bad Münstereifel, Germany, www.scidesign.de;
cover photograph by F. Weihrauch

ISSN 1814-2206

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Foreword

As chairperson and secretary of the Scientific-Technical Commission (STC) of the International Hop Growers` Convention (I.H.G.C.) it is my pleasure to welcome all of you in St. Stefan am Walde, Austria. The hop community of the STC gladly accepted the invitation of the Hop Growers' Cooperative of the Mühlviertel, represented by its CEO Hermann Bayer, to hold our meeting in this nice hop growing region for the very first time. With its 157 ha hop acreage out of a total of 261 ha in Austria this is only a small hop producing area but nevertheless for Austrian breweries a crucial hop provider of top quality of regional origin.

We say thank you to Hermann Bayer, vice-chairman of the STC and host, for his excellent work in organizing and preparing this meeting and also for his time he spent in all this work. We are also grateful to Hermann for conducting the pick-up service from the Linz airport and train station for several participants.

Also just at the beginning I would like to give my special thanks to my colleague Dr Florian Weihrauch who took over the majority of tasks to organize this meeting of the STC. Many thanks to him for his excellent work already performed at the run up of this meeting. In addition, I highly appreciate Florian´s pick-up service from the Munich airport.

I am very happy that after this break of four years for the STC – due to the introduction of this alternating cycle of the ISHS Humulus Symposium and the I.H.G.C. Scientific-Technical Commission meeting – more than 60 attendees have come to our meeting to share their knowledge on hops with the STC community.

In these days the focus of interest is on research activities in the various fields of hop and beer. Scientists and experts from 14 nations present their exciting work in papers and posters comprising the following topics: hop breeding, molecular investigations and biotechnological approaches, phytopathology, bacteriology and entomology including chemoecology, hop physiology, hop chemistry, hop cultivation and management. Moreover, this time in a special session you will be informed on hop and its impact on beer flavor which extends the range of topics covered by the STC.

We are grateful to all participants for their valuable contributions and for providing the manuscripts of their papers or posters for the Proceedings, which finally will also be available on the internet and in the ISSN library.

In addition to the scientific program, an excursion arranged by Hermann Bayer will give you the opportunity to learn more about the Mühlviertler hop growing region with a lot of beautiful sites.

Our thanks are due to our sponsors – the Barth-Haas Group, the Hopfenverwertungsgenossenschaft HVG and Hopsteiner; with their financial backing they are supporting the mission of the STC.

In closing, I would like to wish you a pleasant and fruitful conference with 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-Technical Commission

I: Hop breeding

Recent advances in hop research in Australia

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Abstract

The Australian hop industry has a rich history of continued commitment to R&D dating back to the 1950s. To remain internationally competitive, hop production in Australia relies upon varieties that have been bred in the Australian environment. Since the mid-1990s, a research partnership between the University of Tasmania and Hop Products Australia has focused on incorporating modern technologies into the Australian hop breeding program. This includes the use of flow cytometry for the development of triploid hop varieties, discovering hop genetic information using molecular markers and understanding the complex chemical composition of hop using analytical chemistry. This successful university-industry collaboration has advanced hop research and breeding efforts in Australia and promoted the development of higher yielding, seedless varieties with diverse and exciting flavour profiles and provides a flexible framework to respond to future research requirements.

Hop Breeding in the Czech Republic

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Abstract

Hop breeding in Czech Republic has been aimed at aroma and bitter hops for a long time. Recently, we have managed to get some new genotypes with a specific flavour. In 2017 the cultivars Gaia and Boomerang have been released. New genotypes 5193 and 5164 have been submitted to registration process. A new generation of flavour hops is characterised by the wide variability of the various types of hop aroma.

Key words: *Humulus lupulus* L., flavour hops, perspective genotypes.

Introduction

In recent years, breweries are still more and more interested in hops with specific aroma. Especially small breweries produce special beers, which are different from the common Czech lager beers. It concerns particularly beers produced by top fermentation (IPA, APA, Ale, IBA, etc.), which are wanted not only by American breweries but also by breweries all over the world. It is the reason why many hop breeders, including Czech ones, have aimed at getting new varieties of flavour hops, which is obvious from the great number of newly released cultivars of this type. The first Czech variety of this type is Kazbek, with an increasing growing area in Czech Republic because of interest from Czech as well as foreign breweries. At present, there is a number of perspective flavour genotypes in our trials including some dwarf varieties (NESVADBA 2016). Wild hops are used within this breeding process as well (FARAGO et al. 2013).

Material and methods

The process of hop breeding aimed at aroma and bitter hops still continues in CR. Nevertheless, in 2012 and 2013 fifteen crossing were made focused on specific aroma (NESVADBA et al. 2013). Varieties Kazbek, Columbus, wild hops and developing new genetic material served as parental components. We planted 2,430 hop plants totally. After the assessment of the flavour of fresh cones we chose 84 perspective genotypes for other breeding work. After the first evaluation of hop cones after drying the set of 27 genotypes was selected. We preferred the ones with interesting flavour even after drying. The samples of these hops were assessed by 48 experts. On the base of this evaluation we chose twelve most interesting genotypes for cold hopping, which were divided into four groups. The names of the planets of our solar system were chosen to replace numbers of the individual genotypes for better clearness. We decided for planets because the flavours are not common hoppy ones, as if they come from another planet.

The first group consisted of genotypes with the best evaluation of citrusy flavour. From this point of view the “Eris” genotype seems to be very interesting as it shows entirely this type of flavour. The genotype “Earth” was the best within the second group, where floral flavour was evaluated. High intensity of floral as well as citrusy and spicy flavours are typical for the “Pluto” genotype. Fruity flavour represented the third group, where the highest intensity of this flavour was evaluated. It was found out that “Venus” is characteristic not only by fruity but also by spicy flavours. Nearly the same ratio was found out in the “Saturn” and “Neptune” genotypes, with lower intensity in “Neptune”. The fourth group is represented by two flavour genotypes, “Ceres” and “Haumea” with the highest intensity of spicy aroma and the “Sun” genotype with the most distinctive woody aroma.

Results and discussion

Selected samples were used for cold hopping when an Ale type of beer was brewed. Agnus and Kazbek were used for the first hopping. Ground cones of the individual tested genotypes in the dose of 3.0 g/L, added ten days before bottling, were used for cold hopping.

Twenty-three brew masters evaluated the beers. Kazbek was used as a reference sample within citrusy flavours. It is obvious from Figure 1 that the genotypes “Eris” and “Jupiter” were the most favoured ones within citrusy aromas. Floral flavours were not as distinctive as citrusy ones. Therefore, the tasters preferred the “Earth” genotype because of its intensive floral aroma. Among fruity types, ”Saturn” was the most preferred one, even though it is not unambiguous. The “Sun” genotype was the best within the last group. All tasters agreed that is very suitable for IBA production. We can say that each genotype is interesting for its specificity. We can demonstrate it in the group of fruity flavours. Even though the “Neptune” genotype showed just mild peach aroma, one of the taster preferred it for its fine aroma.

Two perspective genotypes with aroma different from Kazbek are in registration trials at present (Table 1). Other crossings were carried out and the obtained genotypes are typical by their interesting specific aromas. Within the program aimed at dwarf hops, the genotypes N2 and N8 seem to be the most perspective ones.

Table 1. Perspective genotypes, currently in registration trials, with specific aroma

Genotype	Alpha acids [% w/w]	Beta acids [% w/w]	Cohumulone [% rel.]	Aroma
5193	10.4	5.5	32	Medium, hoppy, fruity and citrusy
5164	12.1	6.3	28	Strong spicy

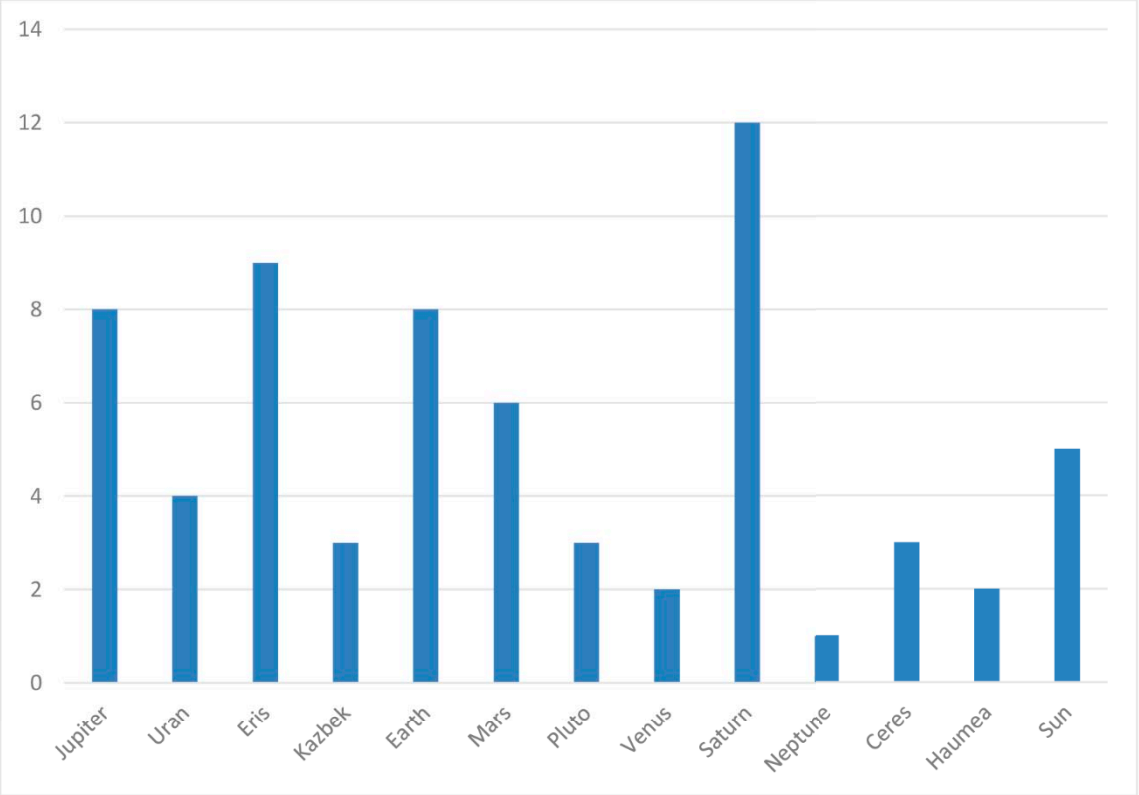

Figure 1. Preference of brew masters (n=23) for genotypes used for cold hopping

In 2017 two new hop varieties, Gaia and Boomerang, have been registered. Contents and compositions of hop resins and essential oils are shown in Tables 2 and 3. Gaia originates from Agnus and it was named after the Greek goddess of Earth because of its vigour and very good productivity. Gaia is characteristic for its hoppy and spicy aroma. As it ensues from pilot tests Gaia is suitable not only for the first but for the second hopping as well. Boomerang is also after Agnus. As it is typical by its specific aroma it can be used as flavour hops too. If added in the form of cold hopping the flavour comes back as a boomerang into a fine aroma of beer. Aroma is intensively spicy and citrusy. Boomerang is suitable for both the first and the second hopping of top fermented ALE, IPA and IBA beers. It can be used in the form of 100% hopping as well as for cold hopping in these types of beer.

Table 2. Content and composition of hop resins in cvs Gaia and Boomerang

Variety	Alpha acids [% w/w]	Beta acids [% w/w]	Alpha/beta ratio	Cohumulone [% rel.]
Gaia	12 - 15	5 - 10	1.3 – 2.7	20 - 29
Boomerang	10 - 14	5 - 10	1.5 – 2.3	27 - 32

Table 3. Content and composition of hop essential oils in cvs Gaia and Boomerang

Variety	Content [% w/w]	Myrcene [% rel.]	Caryophyllene [% rel.]	Farnesene [% rel.]	Humulene [% rel.]	Selinene [% rel.]
Gaia	1.5 – 2.5	23 - 37	9 - 12	5 - 7	2 - 4	25 - 27
Boomerang	1.5 – 3.0	30 - 53	7 - 11	0.4 -1.0	17 - 24	1 - 2

Conclusion

Hop breeding aimed at specific flavours is very difficult because of changing aroma during the assessment. Many genotypes lose their aroma during the drying process and many of them do not transmit it into beer. On the contrary, some genotypes influence also the taste of beer in an unwanted manner.

However, the above-mentioned genotypes with specific flavour have been tested in numerous small breweries. In 2016, we started to propagate these perspective genotypes and in 2018 more plants of these genotypes will be planted. At present, we know that the new genotypes have higher intensity and variability of flavours than Kazbek or genotypes 5193 and 5164, which are in registration trials. The results show that we have managed to get new very good flavour genotypes with very interesting aromas. The originality of the flavours ensues from wild hops used within the crossings. Thanks to experimental batches in pilot and small conventional breweries, the new genotypes have been brought to attention of brew masters. This is the reason why the demand is higher than the supply. Nevertheless, we believe that we will be able to satisfy this increasing demand for high quality flavour hops and there will be a sustainable supply for breweries.

Acknowledgement

This work was supported by Czech Ministry of Education within the Research Project EUREKA no. LF 15020 and by Ministry of Agriculture of CR within Institutional Support for the Development of Research Organization RO1486434704.

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Development of a new Huell hop cultivar: innovative strategies together with the hop and the brewing industry

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Abstract

In the context of the new Special Flavor hop breeding program the selection process has been optimized at the Hop Research Center Huell. In close collaboration with the hop and the brewing industry innovations have been implemented which increase the efficiency of the selection procedure and fulfill the demand from all relevant economic circles for more transparency before the release of a new hop cultivar: a panel of hop experts assists the evaluation process of experimental lines. In addition, large-scale farm trials with highly promising breeding lines and their testing in standardized brewing trials are complementing the selection process. The first candidates which passed this process were the new Special Flavor cultivars Callista and Ariana released in April 2016. At current, the breeding line 89/02/25 and 96/01/24 with classical aroma profiles are being tested. Thus, all new Huell hop cultivars are released with crucial information for growers, hop traders and brewers concerning their agronomic performance, resistance properties and brewing characteristics already before a new cultivar appears on the market which is unique.

Keywords. hop breeding, innovations, hop expert group, large-scale growing trial, trial brews

Introduction

Hop breeding research at Huell is a proud tradition starting in1926 as reaction of German brewers towards downy mildew which threatened and almost stopped hop production in Germany in those years. For decades focus has been put exclusively on the development of classical aroma varieties until the 1970s. Then a high alpha breeding program has been started. In both programs the development of varieties with enhanced disease resistance and increased agronomic performance were fixed goals. Only recently, in 2006 initiated by the US craft brewers and by creative brewers worldwide a Special Flavor breeding program has been started. Along with this new breeding trend in Huell in 2014 several innovations were introduced in the selection process of hop varieties by the Society of Hop Research, the German Hop Growers` Association and the German Hop Trading Association together with the LfL.

Results and discussion

During the first phase of the developing of new varieties (Fig. 1) comprising crosses, seedling selection and advanced growing trials with promising experimental lines the LfL is taking full control of all steps. Then in the second phase in which promising breeding lines are tested in farm trials the hop and the brewing industry got strongly involved in the whole selection process. By establishing the hop expert group as well as by introducing large-scale growing trials and standardized brewing trials the efficiency and transparency of the selection procedure could be optimized. Moreover, this involvement of all relevant economic groups guarantees that new hop cultivars will be released which fully meet the requirements and wishes of the hop and the brewing industry.

A four-stage-process (Fig. 2) taking effect during the second phase of the development of new hop cultivars has been implemented:

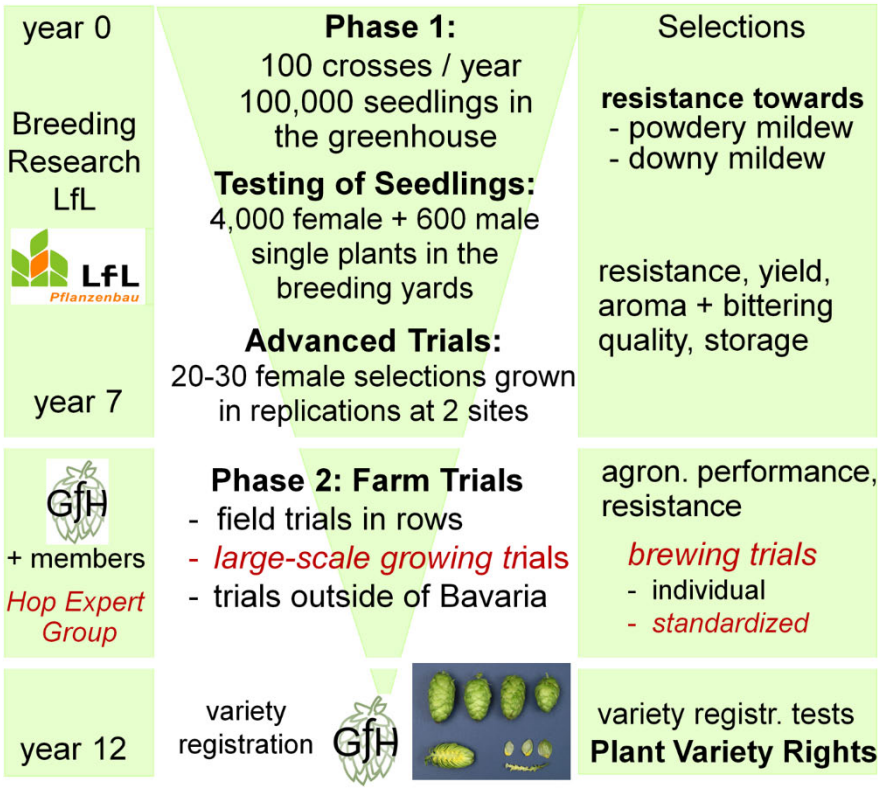

Figure 1. Scheme on the development of new Huell hop cultivars including the innovations (in italics)

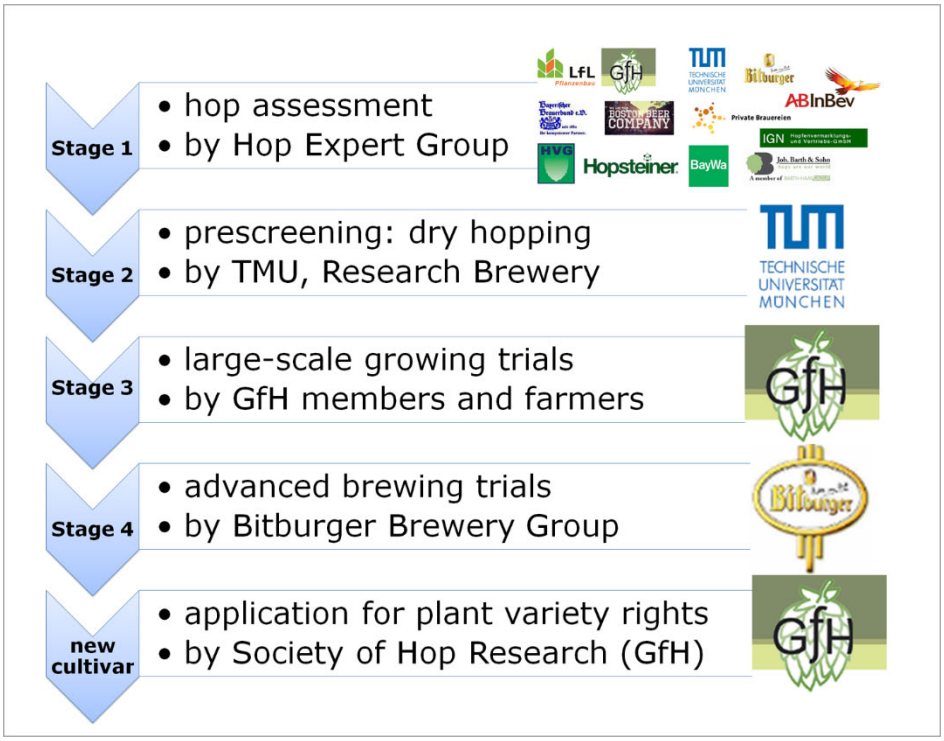

Figure 2. Four-stage-process during the development of new varieties involving the hop and the brewing industry

Hop expert group – hop assessment

In stage 1 a group of 17 hop experts representing the entire value added chain of the hop and the brewing industry with Anton Lutz, breeder and head of this panel, is supporting the selection process. This group characterizes the aroma of promising experimental lines and evaluates their aroma and bitter potential as well as their resistance and agronomics from the perspective of the industry.

Pre-screening: standardized dry hopping brewing trials

Based on this rating the most promising breeding lines are tested for their dry hopping aroma in stage 2. Based on a concept drawn up by the hop expert group these experimental lines are tested in bottom- and top-fermented beers with various contact times and different amounts of dosed hop oil. These dry hopping trials are conducted at the Weihenstephan Research Brewery, Technical University Munich, Brewing Faculty, and financed by the Society of Hop Research (GfH).

Large-scale growing trials

Hop varieties revealing exciting dry hopping aroma characteristics are released by the board of the GfH as candidates for large-scale growing trials. Members of the GfH, in general hop trading firms take charge of these field trials on a hectare basis with farmers. Comprehensive information on the agronomic performance and resistance of these breeding lines at different locations can be gained during this phase. Moreover, thereby enough hops is produced for individual and standardized brewing trials conducted in stage 4 and finally after the release of a fully convincing breeding line as new cultivar sufficient planting material will be available for propagation purposes.

Last but not least the large-scale field trials also allow conducting studies concerning the processing of experimental lines. The pelleting of the cones of Polaris with its exceptionally high alpha acid content of up to 24 % was tested at the St. Johann pelleting factory long before its release.

Advanced standardized brewing trials

Breeding lines with confirmed good agronomic features and enhanced resistance in large-scale and LfL-own growing trials are tested in advanced brewing trials in stage 4. The protocol for these standardized brewing trials was drawn up by the hop expert group. Here the bittering potential as well the whirlpool and dry hopping aroma of the breeding lines under test are being evaluated. So far the Bitburger Brewery Group's experimental brewery conducted all these brews. Based on a catalogue of descriptors worked out by the hop expert group the bottom- and top-fermented beers of highly promising breeding lines are characterized and evaluated.

Individual brewing trials with obligatory reporting

Individual brews have been conducted by interested brewers worldwide for decades. But now obligatory reporting on these brews according to the expert group's reporting sheets and beer-tasting guidelines brought significantly more information on the bittering and aroma potential of experimental lines under test. In the case of breeding line 89/02/25 and 96/01/24 various medium-sized breweries and major brewing corporations as well as trial breweries conducted very successful brews with these classical aroma lines. Finally they provided comprehensive data and evaluation of their brewing potentials.

Application for cultivar registration

Finally based all this information collected in all stages of this selection process the board of the GfH can decide whether this new breeding line should be registered as new cultivar.

All the above described innovations have already been employed during the selection of Callista and Ariana, the two Special Flavor cultivars released in 2016. At current two experimental lines representing the classical aroma sector are being tested following this 4-stage-process.

Conclusion

All innovations introduced in the selection process of new Huell hop varieties have already proven their effectiveness so that all partners involved are highly satisfied with this way of proceeding. Thus, in collaboration with hop farmers, hop traders, brewers, and brewing associations hop cultivars are developed by the Hop Research Center Huell and have already proven during their selection process that they meet the demands of all relevant economic circles which is unique worldwide.

Acknowledgement

We would like to give our thanks to all members of the Hop Expert Group of the GfH for their support during the selection process of Huell hop varieties. Many thanks are due to Dr Stefan Hanke, Experimental Brewery of the Bitburger Brewery Group, and to Dr Johann Tippmann and Dr Florian Schüll (till April 2015), Research Brewery of the Technical University Munich-Freising for conducting the standardized brewing trials. Our thanks go to AB InBev, the Trial Brewery St. Johann and many other brewers worldwide for conducting comprehensive individual brewing trials with our experimental lines. Our thanks are due to all hop growers involved in growing trials for providing valuable information on the agronomic performance of Huell experimental hops. Special thanks to Dr Klaus Kammhuber and his team for conducting the chemical analyses of our hop varieties.

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Evolution of hop breeding: Crossbreeding using male flowers induced on female hop plants

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Abstract

We developed a method for controlling the sexuality of hop plants by applying a chemical to a female hop plant one or more times to enable a fertile male flower to produce pollen epiphytically on the female plant. Specifically, the chemical induces a reaction with the endogenous ethylene in the female plant. By using this method, an embryo or seed, hop plant, and cone can be obtained. Therefore, our method can be used for screening hop plants and producing a new hop plant variety.

Keywords: *Humulus lupulus*, dioecious, male-female crossing, seed

Introduction

A hop cone, the female flower of the dioecious hop plant (*Humulus lupulus*), is an important raw material used for brewing beer. The cone of hop plant contains bitter acids, terpenoids, and polyphenols, and is therefore closely associated with the flavor of beer. Only the cone is used as the raw material why only the female plant is used for hop production. Unpollinated cones are good quality raw materials, whereas using male plants, which are not needed for cone production, result in poor quality product and, hence, are not cultivated intentionally.

The male hop plant is used for breeding, i.e., the practice of cultivating, propagating, and evaluating individual plants derived from seeds obtained by crossbreeding the pollen of a male flower epiphyte on a male plant with a flower on a female plant. This is followed by screening for suitable individuals. Male plants cannot produce a female flower and are of no use for brewing, but they are presumed to possess genetic characteristics that can be used to evaluate the cones of individual sister plants or offspring produced by crossbreeding with other female plants. Hop plants propagate vegetatively and have high genetic heterozygosis, which makes it difficult to presume that male plants possess such genetic characteristics. Thus, an efficient breeding strategy for the cone and its evaluation are needed.

To overcome these problems and develop efficient breeding practices, a strategy has been conceived where the male plant is not used, but rather a male flower is intentionally induced to develop epiphytically on female plants, which are subsequently allowed to crossbreed. Presently, a male flower can grow epiphytically on a female hop plant by spraying 500 ppm α-(2-chlorophenylthio)-propionic acid sodium salt, which is similar to the plant hormone auxin (WESTON 1960). However, this study merely reported the epiphytic development of a male flower and it is not clear whether pollen formation, fertility, and other related activities were achieved. To our knowledge, the only case of the induction of an epiphytic male flower on a hop female plant requiring the use of an auxin-related substance was documentted half a century ago, and since then, α-(2-chlorophenylthio)propionic acid has not been used for breeding hop plants any more.

Material and methods

Crossbreeding and seed production

Since the hop female flower does not have any stamen, plant emasculation or stamen removal is not required and, thus, crossbreeding can be achieved by adhesion of the pollen of an epiphytic male flower to the stigma of a female flower of a self- or cross-female plant. However, since hop is an anemophilous plant, the possibility of pollination by unintended pollen should be eliminated as far as possible to obtain seeds by targeted crossbreeding. An effective strategy for this is blocking the unintended pollination using a process such as bagging after artificial pollination has been performed. Where an unintended pollination may occur, bagging before the pollination is also effective. Two to three months after pollination, fully grown cones are harvested to obtain the seeds. Furthermore, the present invention also provides a method for obtaining seeds from the cone obtained by producing a female plant with epiphytic male flowers by controlling the hop sexuality as described above. This is followed by crossbreeding of the pollen of the female plant with the female flower of the female plant or a female flower of another female plant.

Breeding

The breeding procedure of the proposed method is similar to that of conventional methods, except that the treatment for inducing a male flower epiphyte is carried out. Specifically, the cultivation, crossbreeding, seed production, progeny breeding, propagation or multiplication, evaluation, and selection procedures are not different from those used in conventional methods, and therefore, they may be practiced by those currently breeding hop plants.

Breeding using the present method can crossbreed female plants with each other and it is possible to obtain progenies by directly using parents with already evaluated cone traits. The crossbred progeny obtained using the present method is further bred by conventional methods. The presently developed method enables breeding using parents with confirmed cone traits and productivity, thereby making it easy to accumulate the desired cone traits and productivity. The present method is effective for but not limited to breeding a variety. For example, we could obtain a variety with a combination of traits from both parents plant that each have a characteristic flavor component; a variety with a high probability of producing high α-acid content based on the parent plants; and a variety with a high α-acid content inherited from one parent plant and a characteristic flavor component from the other.

This invented method further contributes to enhancing the efficient breeding of varieties with many useful traits without limiting the products to the cone traits. Unlike the male plants, in the varieties used for cone production in practice, various traits such as cultivation properties, resistance to diseases, and environmental adaptability, in addition to the cone traits and cone productivity, are grasped in cone producing districts. When these varieties are used as parents, a variety with a combination of useful traits or those that complement the unnecessary traits of the parents can be bred more directly.

Cone

The present method further provides a cone of a novel plant produced from the embryo or seed obtained. The novel plant obtained by crossbreeding has new cone qualities differing from those of the parents. A fertile male flower capable of forming pollen is made epiphytic on a female plant using the method mentioned above and, subsequently, the female plants are crossbred with each other to obtain novel plants and cones.

Screening hop plants

The present method further provides a strategy for screening for hop plants. This involves breeding hop plants from different embryos or seeds, determining a DNA polymorphism linked to a trait characteristic as a genetic marker of each hop plant, creating a genetic linkage map between the genetic marker and the trait, and screening for the hop plant of interest from the hop plants using the genetic linkage map.

Using the present method enables a progeny to be obtained between female plants and following female plant self-fertilization. The traits of these progenies were examined and genetic analysis was carried out for the segregated traits. The genetic analysis involves the use of routine techniques, a hereditary pattern is presumed, and the genetic linkages among the traits are calculated. The traits used in the process are DNA polymorphisms. A specific DNA polymorphism linked to a specific trait can be used as a genetic marker.

The genetic linkage map between traits is constructed from the calculated genetic linkage distances. Further, for quantitative traits, a genetic marker can be identified using the QTL analysis using the genetic linkage map of DNA polymorphisms.

The progenies between female plants are theoretically all female plants, and the traits and productivity of the cones can be evaluated. Since conventional crossbreeding between female and male plants produce more than a few male plants, all the progenies could not be evaluated for cone traits and productivity. The present invention would allow the cone traits and productivity of all the progenies to be evaluated and, so, the precision of the genetic analysis of the cone traits and productivity is improved.

Specifying a chromosomal region containing a hop gene

The present method provides a strategy for determining a chromosomal region bearing a hop gene using the genetic linkage map constructed using the above method. When the genetic linkage map constructed using the present invention is used, the chromosomal region with the target gene can be identified. More specifically, when the genetic markers linking both sides of the target trait are detected on a chromosome, the intended gene is revealed to be present in the region sandwiched between these genetic markers. When a clone is identified in the cloned hop DNA using an indicator genetic marker linked to the target trait, it becomes possible to specify the clone with the DNA that lies near the intended gene. The stronger the linkage between the genetic marker and the intended trait, the nearer the clones are to the target gene and, therefore they can be identified.

Results

In dioecious plants whose sexuality is genetically determined, the XY type is the most common, but the ZW type is also known. The sex determination of these plants is roughly divided into species where the male sex determination factor is presumably present in the Y chromosome even in the XY type and those where the sex is determined by the ratio of the X chromosome to the autosome (MATSUNAGA 2000). This suggests that a diverse genetic background exists in the sex determination. It has been reported that the sexuality of the hop plant is determined by the ratio of the X chromosome to the autosome (SHEPHARD et al. 2000). Morphic sex chromosomes have been reported in asparagus and hop and, thus, the sex is determined by the presence of sex genes on the sex chromosomes (ATWELL et al. 1999). Auxin and ethylene promote feminization of cucumber, pineapple, papaya, and date plants; gibberellin promotes the masculinization of Cucurbitaceae such as mulberry, which belongs to the Moraceae family, and oil palm; and cytokinin induces hermaphrodite flowers in the male grape.

Plant hormones are frequently involved in the sex determination and sexual differentiation of plants, which is documented with substances such as auxin, gibberellin, cytokinin, and ethylene, and probably no plant hormone is common in higher plants (MATSUNAGA 2000). It has been suggested that corn, which is a dioecious flower, exhibits pistil degenerated induced by jasmonic acid, a plant hormone, and subsequently transforms into a male flower (ACOSTA et al. 2009). Thus, in sex determination of plants, mechanisms differ because of the various plant hormones and each plant is considered to have independently evolved. Sex conversion of plant species associated with ethylene has been investigated using the Cucurbitaceae. These are dioecious plants and their female and male flowers are rendered epiphytic on a genetically identical single plant by physiological factors.

The formation of epiphytic female flowers by ethylene treatment has been reported in Cucurbitaceae, and the stamen development in cucumber has been suggested to be inhibited by ethylene with the subsequent formation of an epiphytic female flower (WANG et al. 2010). An epiphytic hermaphrodite flower was formed when a female *Momordica dioica* (Cucurbitaceae) was treated with silver nitrate, which is an ethylene inhibitor (ALI et al. 1991). Applying silver nitrate or a silver thiosulfate (STS) anionic complex, an ethylene inhibitor, to a growing point of the dioecious *Cannabis sativa* plant turns it black (MOHAN RAM & SETT 1982). Furthermore, an intersex flower, an incomplete male flower with few stamens or a complete male flower, and a female flower were made epiphytic on the main branch and lateral branches that grew after the treatment (MOHAN RAM & SETT 1982).

Discussion

Bitter acids, terpenoids, and polyphenols are constituents of the cone and are critically responsible for imparting flavor to beer. We crossbred the pollen of a male flower implanted into a male strain with a female flower, and new varieties of hop plants were developed by choosing an individual plant derived from a provided seed. Because a cone was not implanted, the male strain must be used, does not enable the evaluation of quality and productivity. Furthermore, it was a large male flower, and the efficiency of developing new plant was inferior to that of other plants. We discovered a strategy for inducing the development of a male flower on a female plant in this study. This method enabled mating in females with clear characteristics, and the use of this technique could lead to innovative progress in developing new varieties of hop plants. We focused our attention on the action of ethylene and $AgNO_3$ (ethylene action repressor), STS, chrysal K-20C (STS-related agent), and aminoethoxyvinyl glycine (AVG, ethylene composition repressor) and examined their effects on a male flower.

The process involved a method we used on a leaf. We used six varieties: Shinshuwase (Kirin No.2), Kaikogane, Kitamidori, Toyomidori, Hallertauer Mfr., and Hersbrucker Spät. A male flower was formed on all six varieties after the use of $AgNO_3$, STS, Chrysal K-20C, and AVG. The condition of the male flower varied according to the variety used, agent application density, and number of times the agents were applied. We confirmed the fertility of the pollen based on the extension of the pollen tube by using stainable acetocarmine and nutrient agar medium. In addition, we obtained seeds after crossbreeding the pollen obtained from the male flower that developed on Shinshuwase with the female flowers of five different varieties. Of these seeds, seminal germination was confirmed for Shinshuwase and Hallertauer Mfr. DNA analysis of the mated plant revealed inheritance of the DNA polymorphism of the male stock. A variety interval (female) and mating in the same variety that had known evolution was already decided that I was not able to accomplish so far in this way were enabled. By using this newly developed method, various heredity analyses became easy, in addition to efficiently developing new varieties. Thus, we believe our method can be used to develop unique traits in plant breeding and contributes to hop breeding in a manner that previous techniques do not.

Acknowledgements

We thank Dr. Teruo Sano (Hirosaki University) and Dr. Rym Fekih for their helpful discussion as well as Masako Otsuka and Noboru Ohnishi (Kirin Co.) for their technical support.

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Verticillium wilt on hops: Real-time PCR and meristem culture – essential tools to produce healthy planting material

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Abstract

Verticillium wilt in its lethal form caused by specific strains of the fungus *Verticillium nonalfalfae* Inderbitzin has become a new challenge in German hop production. In particular, since there are no chemicals available to combat this disease a crucial part in the management and control of this disease is the use of healthy planting material. Basic problem at the hop breeding yard in Huell is that *Verticillium* in its mild form has been tolerated for decades in order to be able to select hop seedlings with enhanced tolerance towards this vascular disease. But hops from the Huell breeding yard being used in advanced growing trials in replications at other locations have to be free of this dangerous fungus. Therefore, all seedlings before being propagated and transplanted have to be tested for *Verticillium*. A highly sensitive and reliable in planta real-time PCR procedure has been developed and employed as diagnostic method to identify *Verticillium* contaminations of hop plants. In addition, meristem culture so far only used to eliminate viruses provided the option to regenerate healthy plants from the meristematic shoot tips of *Verticillium* infected hops.

Keywords. Hop, *Verticillium*, real-time PCR, meristem culture, planting material

Introduction

Verticillium nonalfalfae (formerly *V. albo-atrum*), and in rare cases *V. dahlia*, are the soil-borne pathogens causing wilting in hops. Their conidia, mycelium and resting mycelium (or microsclerotia for *V. dahlia*) which can be found in living and rotten leaves, bines, cones, roots and even in soil particles are the spreading and infecting structures. *Verticillium* invades the root and via the xylem elements colonizes the whole hop plant.

In 1924 *Verticillium* wilt in its mild (fluctuating) form was observed in hops in the UK for the very first time, followed by severe outbreaks in Germany starting in 1956 and in Slovenia in 1974. A much more aggressive, so-called progressive (lethal) form of this pathogen evolved in the UK in 1933 and in Slovenia in 1997. Finally also in Germany around 2005 severe wilting symptoms occurred on Perle, Northern Brewer, Hallertau Tradition, and other cultivars which so far had been described as being tolerant towards *Verticillium*. Pathogenicity and molecular tests (SEEFELDER et al. 2011) confirmed the occurrence of lethal strains of *Verticillium* in German hop growing regions and subsequently also in the Huell breeding yard.

In the Huell hop breeding yard *Verticillium* in its mild form had been tolerated. Moreover, until the 1980s soil had been enriched by artificial *Verticillium* inoculation in order to improve selection conditions for tolerance of seedlings towards this fungal disease. But after the confirmation of lethal (progressive) *Verticillium* strains in the Huell breeding yard this attitude has completely changed. Chemicals are not available to control or at best to eradicate this pathogen and even in resistant hops such as Wye Target *Verticillium* could be detected in its roots and also in its bines although to a lower extent (CREGEEN et al. 2015). All seedlings showing *Verticillium* wilting symptoms during their 3-year-testing period in the *Verticillium*-contaminated Huell breeding yard were discarded. But promising seedlings without characteristic wilting symptoms which should be propagated and transplanted into other fields for advanced growing trials had to be tested for this fungus. Thus, a reliable detection method of *Verticillium* was urgently needed. MAURER et al. (2013) developed the highly sensitive *in planta* multiplex real-time PCR method to detect *V. albo-atrum* (= *nonalfalfae*) and *V. dahliae* simultaneously; the aim here was not the quantification of the fungal DNA. In addition, conventional PCR using *Verticillium* specific primers for mild and lethal strains (RADIŠEK et al. 2004; DOWN et al. 2007) was employed to differentiate both Verticillium forms.

Both molecular tests were key tools to identify *Verticillium*-free and infected planting material. Especially in the case of valuable but contaminated seedlings which should be maintained the elimination of this pathogen would be vital for our breeding work. Furthermore, for the contract nursery *Verticillium*-free mother plants of newly registered cultivars are crucial to be able to provide certified virus- and *Verticillium*-free planting material for the growers.

Meristem culture (ADAMS et al. 1983; KREMHELLER et al. 1989) has been a well-established technique in our biotechnological laboratory for decades, and in mint and peppermint the elimination of *V. dahlia* could be achieved via shoot tip culture (WANG & REED 2003). Based on the assumptions that shoot apical meristems without functioning junction to the vascular system should be free of *Verticillium* and that ultimately heat treatment of the meristematic cells for several days should inactivate already invaded infectious structures of *Verticillium* meristem culture appeared to be the most suitable method to produce *Verticillium*-free hop plantlets. Additionally, virus contaminations would be eliminated by this procedure, too. Another objective was to accelerate the regeneration of meristems into plantlets by improving various culture conditions.

Material and methods

Tests for viruses

Leaves of young hop plants derived from root cuttings grown in pots in the greenhouse were tested for viruses using the DAS-ELISA (Double Antibody Sandwich Enzyme Linked Immunosorbent Assay) and RT-PCR (Reverse Transcriptase Polymerase Chain Reaction) techniques (SEIGNER et al. 2014). From meristem-derived plantlets at least five *in vitro* cloned plants were investigated for virus infections. Field material (leaves) in general is only tested for apple mosaic virus and hop mosaic virus using DAS-ELISA.

Tests for Verticillium

Core pieces from the base of hop bines were used to test for *Verticillium*. From each plant at least three base-near bines were tested using the real-time multiplex PCR assay following the protocol of MAURER et al. (2013) which allowed to detect and differentiate *V. albo-atrum* (i.e., *V. nonalfalfae*) and *V. dahliae* simultaneously. Each run included two positive controls: one with pure *Verticillium nonalfalfae* DNA from a reference strain and the other with DNA from a *Verticillium*-infected hop plant. In addition, a non-template control using nuclease-free water was run in each testing. DNA-samples were tested separately with primer pairs for polyubiquitin in a conventional PCR reaction according to MAURER et al. (2013) to exclude false negative results due to failed DNA extractions or PCR inhibitors. From 2016 onwards, as internal control of amplification primer pairs for the genes CAC (clathrin adaptor complexes medium subun.) or DRH1 (DEAD box RNA helicase) (GUČEK et al. 2015) were included in the multiplex real-time PCR run. Moreover, starting in 2016 real-time results were checked once more with multiplex real-time PCR using the primer pair 9-1gs recently developed by GUČEK et al. (2015) which identified mild and lethal strains of *V. albo-atrum* (i.e., *V. nonalfalfae*) as well as *V. dahliae*. Moreover, mild and lethal strains of *V. nonalfalfae* were discerned using the conventional PCR according to the EPPO guidelines (RADIŠEK et al. 2004; DOWN et al. 2007) and in this way also real-time based PCR results could be verified although with lower sensitivity.

Meristem culture

Shoot tips of infected hop plants grown in the greenhouse in pots were cut and meristems (0.3–0.5 mm) were excised from shoot tips of hop plants pretreated with heat for seven days. Regeneration of meristematic tissue started on semi-solid Murashige-Skoog (MS) medium for 3 weeks (KREMHELLER et al. 1989). Regeneration continued on a slightly different semi-solid MS medium with BAP concentrations between 0.5 and 1 mg/l. For the liquid culture phase in RITA^{TM} vessels MS based regeneration medium was used without agar. Meristem-derived plantlets were cloned using the node culture technique. Two to three cloned plantlets were tested for *Verticillium* in order to prove the elimination of this pathogen before being transplanted into earth and used as starting material.

Results and discussion

Hop bines were tested for *Verticillium* infections using the real-time PCR providing a higher level of sensitivity to detect also traces of fungal contaminations in breeding and planting material. In addition, these plants were tested for virus contaminations. From plants identified as being infected shoot tips were cut and pre-treated *in vitro* with heat for seven days. From these shoot tips meristems were excised and cultured on semi-solid MS based medium for three weeks. Thereafter, the small leaf-like structures emerging from the meristems were transferred to plastic jars with semi-solid medium following the procedure described by KREMHELLER et al. (1989). On the other side based on the experience gained by SCHWEKENDIEK et al. (2009), GATICA-ARIAS & WEBER (2013) and PENZKOFER (2010) the RITA^{TM} temporary immersion system was tested to increase the regeneration efficiency of meristem tissue. For this purpose the meristem-derived structures were placed into the RITA vessels which were flushed every 4 hours for 1 minute with liquid medium.

The following steps and parameters were investigated in order to optimize the whole regeneration process:

- testing of various systems to fix plant tissue during the flooding phase in RITA^{TM} vessels which turned out to be not necessary or even impedimental for undisturbed root development
- influence of age and size of used starting material: 3-week-old meristem-derived leaf structures were most suitable for this transfer into the RITA vessels
- optimizing the flushing intervals: in the first weeks of regeneration the vessels were flushed every 4 hours for 1 minute; at more advanced stages of regeneration the flushing interval was prolonged to every 6 hours
- effect of phytohormones: the best regeneration could be achieved with the same phytohormone regime as in the semi-solid medium
- interval of medium change: every 4 weeks
- transfer of plantlets from RITA vessels on semi-solid medium was necessary when light and gas provision appeared as limiting growth factors due to vigorous, dense growth of the regenerating plantlets

When comparing the growth and regeneration of meristems into plantlets on agar solidified medium in plastic jars and in the RITA liquid system the benefits of the liquid culture system became quite obvious. Due to the better provision of nutrients the meristem-derived plantlets were more vigorous and the time necessary to regenerate plants including their cloning could be reduced from 8-10 months to 4-7 months. At the same time the genotypic effect on the regeneration process could be minimized. On semi-solid medium on average only 45 % (0-59 % range of variation) of all meristems could be regenerated to fully grown plants, while some recalcitrant genotypes did not produce a single plant. Using the RITA system with 16 different genotypes tested so far on average 90 % (range of variation: 43-100 %) of all meristems regenerated into vigorous plants.

ELISA and RT-PCR were used to confirm the virus-free state of the meristem-derived plants and with real-time multiplex PCR the absence of *Verticillium* was checked. In many cases conventional PCR was also performed to confirm real-time PCR results, although at a lower level of sensitivity. Since 2013 *Verticillium* and virus contaminations have been successfully eliminated from sixteen genotypes. Over the years the question was addressed whether remaining *Verticillium* or virus infections in the heat-treated meristem tissue could be the source of cross-contaminations when several meristems were floating together in one RITA vessel over weeks. So far there are no proofs for such a spread of infection during the liquid culture phase in the RITA vessels but this has to be controlled constantly.

By optimizing the various steps of the meristem procedure the level of remaining *Verticillium* and virus infections in the meristem-derived plants could be reduced from approximately 40 % to less than 6 %. Still contaminated plants were discarded so that finally healthy planting stock was available for various growing trials and for the contract nursery.

Conclusion

The real-time PCR was used as sensitive, quick and after years of optimizing as reliable diagnostic technique to identify *Verticillium*-infected hop plants from our hop breeding yard. Meristem culture in combination with the RITA^{TM} liquid culture system is the essential tool to eliminate *Verticillium* and viruses from promising breeding lines. Due to the non-homogenous distribution of *Verticillium* and viruses thorough testing and re-testing of experimental lines coming from the Huell breeding yard is necessary before they can be propagated and transplanted to another so far *Verticillium*-free hop breeding yard for further growing trials. Finally, after years of field testing of highly promising experimental lines their *Verticillium*- and virus-status has to be re-checked before propagation starts for farm trials and for the contract nursery.

Acknowledgement

Thanks go to the HVG Hop Producer Association, the Scientific Station of Munich Breweries, the Scientific Fund of the German Brewing Industry for funding the various parts of this research. Many thanks are due to Dr Stefan Seefelder for all his work on *Verticillium* as project leader from 2008 till 2015. We say thank you to Dr Bianca Büttner for her support in molecular issues. We highly appreciate the contribution of Simon Euringer during his internship semester and the support of Michael Penzkofer in establishing the RITA system. Special thanks are due to the virus diagnostic group from the Institute for Plant Protection, LfL, for conducting all virus tests. Many thanks go to Dr Sebastjan Radišek, Slovenian Hop Research Institute, Slovenia, for his valuable continued support in our work on *Verticillium*.

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II: Hop microbiology

Microbial community of hop (*Humulus lupulus* L.) phyllosphere – preliminary results

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Abstract

The above-ground parts of plants are normally colonized by a variety of bacteria, yeasts, and fungi. The aerial habitat colonized by these microbes is called the phyllosphere and the inhabitants are epiphytes. Some are beneficial to the plant, others function as plant pathogens. However, the majority of microbial colonists on any given plant have no detectable effect on plant growth or function. Research into the characteristics of microbial life in the phyllosphere is of great commercial importance to the agricultural industry for two reasons. Firstly, understanding the survival of plant disease-causing bacteria and fungi is vital for developing new ways to control their spread. Secondly, there has been a recent rise in the number of food poisoning cases associated with fruit and vegetables contaminated with bacteria. Most of the research on phyllosphere microbiology has focused on leaves, which constitute a very large microbial habitat. Bacteria are by far the most abundant inhabitants of the phyllosphere. Epiphytic bacteria populations differ greatly in size among and within plants of the same species, as well as in close proximity and over short time scale over the growing season. During the two growing seasons 2015-2016, the composition of the microbial community on the surface of leaves and cones of hops cultivated in the Saaz hop growing area (Czech Republic) was studied. During the vegetation, three leaf samplings were carried out in the course of June-September. Sampling of cones was done immediately prior to harvesting. After the harvest, the survey was extended to dried hops and hop pellets. On the above-ground parts of the hop plant, there are aerobic bacteria, facultative anaerobic bacteria, and spores of anaerobes that are not able to germinate in the air.

The microorganisms were washed away from the surface of leaves or cones by physiological saline solution. The extract was applied in several dilutions of a medium specific to bacterial, fungi and yeast culture. Pure microorganism cultures were obtained by sequential sprouting on agar plates. All species of bacteria were identified using the microbiological ID system Biolog (Hayward, CA). The database currently contains 2,700 kinds of bacteria. The fungi were identified on the generic level using microscopic observations and the morphological appearance of the colonies on the dishes. Their identification is only indicative. In the bacterial community, Bacillus (megatherium, idriensis, indicus, endophyticus, licheniformis) and Pantotea species (aglomerans, dispersa) were most frequently identified in both years. There is much greater variability in regard to fungi probably due to diametrically different weather conditions in years 2015 and 2016. Only two fungi of the genus Penicillium and Rhizopus from the total number of eight were identified in both years. The occurrence of yeasts was only rarely found on hop leaves (2015, Candida parapsilosis). The presence of bacteria was detected in dried hops and hop pellets. This demonstrates that post-harvest hops processing, kiln drying and pelletizing are not sterilization treatments.

Acknowledgment

This work was financially supported by the Czech Ministry of Agriculture within the project NAZV QJ1610202.

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III: Hop physiology

Effects of drought stress on hop (*Humulus lupulus* L.): physiological and proteomic view

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Abstract

Drought stress has a big impact on crop plants, including hop, because it reduces crop yield and its quality. In spite of new published studies in the last years, hop response to drought stress is not well understood yet. Detailed understanding of hop stress mechanisms to drought would contribute important knowledge in the breeding process to develop cultivars tolerant to water deficit. This motivated us for the research in which a combination of methods (proteome analysis and physiology methods) was used with the purpose to obtain better insight into hop response to water deficit.

High importance was given to optimal plot experiment design in order to have the most possible equated hop plants and to have the best possible control over the escalation of drought. Plants exposed to drought stress were gradually sampled six times in different stages of daily increasing water deficit, whereas the plants in control conditions were optimally watered. The results of physiological measurements revealed that hop has a very stable water balance, because the relative water content values in leaves dropped only when drought was very severe. Similar results were obtained with water potential measurements. Extremely small changes in water potential lead us to classify hop to isohydric plants. The better water use efficiency in hop under drought stress was obtained because of a decrease in stomatal conduction with the effect on transpiration. A decrease in photosynthesis was measured because of the decrease in stomatal conductance, which indicated stomatal inhibition of photosynthesis. We were not able to find any differences between included hop cultivars in response to drought stress among all measured parameters.

Leaf proteins were analyzed with 2D-DIGE method and one-way ANOVA revealed 132 statistically significant changes in protein spots for cultivar SG and 143 for AU. According to the statistical significance and reproducibility, the 44 protein spots were analyzed with tandem mass spectrometry (MALDI-TOF/TOF), out of which 28 were successfully identified. Among all the identified protein spots, 9 of them were classified into sugar metabolism, 11 into photosynthesis process, 4 into nitrogen metabolism, 1 into ROS related pathway and 3 protein spots, which were classified into different processes in cell. Proteomic analysis also showed the carbon metabolism to be most affected. There was a strong decrease in photosynthetic proteins and proteins of the energetic metabolism.

With our best knowledge we were able to connect proteome analysis results with physiological measurements for photosynthesis. Physiological measurements revealed a decreasing trend of photosynthesis with progressive drought. On the other hand, the results of proteome analysis showed a decrease of proteins, important for photosynthesis, with more severe drought stress. These results are a useful and important starting point for further studies to understand hop mechanisms during water deficit. Otherwise, it makes sense to continue research with targeted search of hop mechanisms answering drought stress.

This abstract is based on the following original article:

KOLENC Z., VODNIK D., MANDELC S., JAVORNIK B., KASTELEC D & ČERENAK A. 2016. Hop (*Humulus lupulus* L.) response mechanisms in drought stress. Plant Physiology and Biochemistry 105: 67-78.

IV: Hop cultivation and management

Test of an alternative training system for hop bines in high trellis

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Abstract

In a field trial with hop cvs Perle and Herkules, the traditional training system (V-type) was compared with an alternative training system (self-training, wall of foliage). The self-training did not work because the distance between soil and the supporting material was too large (48 cm). After manual training, the yield in the alternative system was significantly lower.

Key words. Hops training, self-training of hops

Introduction

In collaboration with the Weihenstephan-Triesdorf University we examined whether the time requirement for the most time-consuming jobs in hop growing, namely training and stripping the hop shoots, could probably be reduced or even eliminated by devising an alternative to the current system. The idea was to profit from the merits of a low-trellis system, the principal advantage of which is self-training, by transposing them to a high-trellis system. To do this, designated strips with an alternative system (a wall of foliage) were integrated into a typical 7 m-trellis system, and then compared to a traditional training system (V-shaped training wire). This took place in randomized blocks at an experimental site where the varieties Perle and Herkules were grown, i.e., the two most important hop varieties in terms of acreage in the Hallertau. The objective was not only to test whether self-training could be successful, but also to find out what impact this system would have on yield performance, plant growth, and quality.

Figure 1. The alternative training system in cv. Perle at the beginning of August.

Material and methods

The experimental field in the Hallertau hop growing region near Volkenschwand was planted with the cvs Perle (2 ha) and Herkules (2.14 ha). In each of these two cultivars, four trial strips using the traditional V-shaped training system, and four strips using the alternative system were laid out (4 replications, respectively). The individual plots consisted of three rows each, but only the hops from the central hill were harvested. Each strip was equivalent in length to the distance between two poles and contained 14 hop plants.

In the alternative system the plants had to climb up a vertical string arranged in the form of a web. The training string was affixed near the plant to a barbed wire running about 45-50 cm above the cutting level. From there, the string, which was endless, was taken upwards to the barbed wire, threaded into a metal hook there and then passed down again where it changed direction once more to create a narrow V-pattern of training string along the row.

Figure 2. and Figure 3. Attaching the strings to the trellis in the alternative training system

In the conventionally managed plots, standard training of three shoots to each wire was conducted on 6 May in Perle and on 12 May in Herkules. In the plots with the alternative system, no training was done initially because the shoots were expected to grow towards the climbing supports autonomously, without help. However, it soon became clear that the distance between the ground and the lowest part of the training string (Ø 48 cm) was too high and self-training was not possible in either hop type. Thus, both cultivars were trained by hand on 17 May in order to close gaps in the crop and to prevent major yield loss.

Results were collected by measuring the height of the developing crop every week, and also via various assessments and exact harvesting. The cone volume was also recorded by measuring exactly 500 dry cones per plot.

Results

The time recorded for the job of setting up the training elements showed that attaching the training string did not take longer than installing and affixing the wire in the traditional system. If the alternative system could be perfected to an effectively working self-training, this would save growers lots of time in spring. However, even if self-training was successful, an implementation of the system in practice will only happen if yield and the quality of the hops are the same as in the conventional system.

In the case of cv. Herkules, there was hardly any discernible divergence in plant development between the two systems. On the other hand, in cv. Perle plant growth in the strips with alternative training lagged behind that in the conventional strips until the end of July, probably because training was carried out 11 days later than in the conventional strips. The question is: did the divergence in plant growth later affect yield?

The results for Perle using the alternative training system were indeed significantly lower with respect to both yield (kg/ha) and alpha acids levels (kg/ha), than those from the V-type system. In contrast, in the high alpha cv. Herkules, the differences in cone yield and bitter compounds yield were far less.

With respect to alpha acids content, the values for both varieties tended to be lower with the V-type training method than with the alternative version.

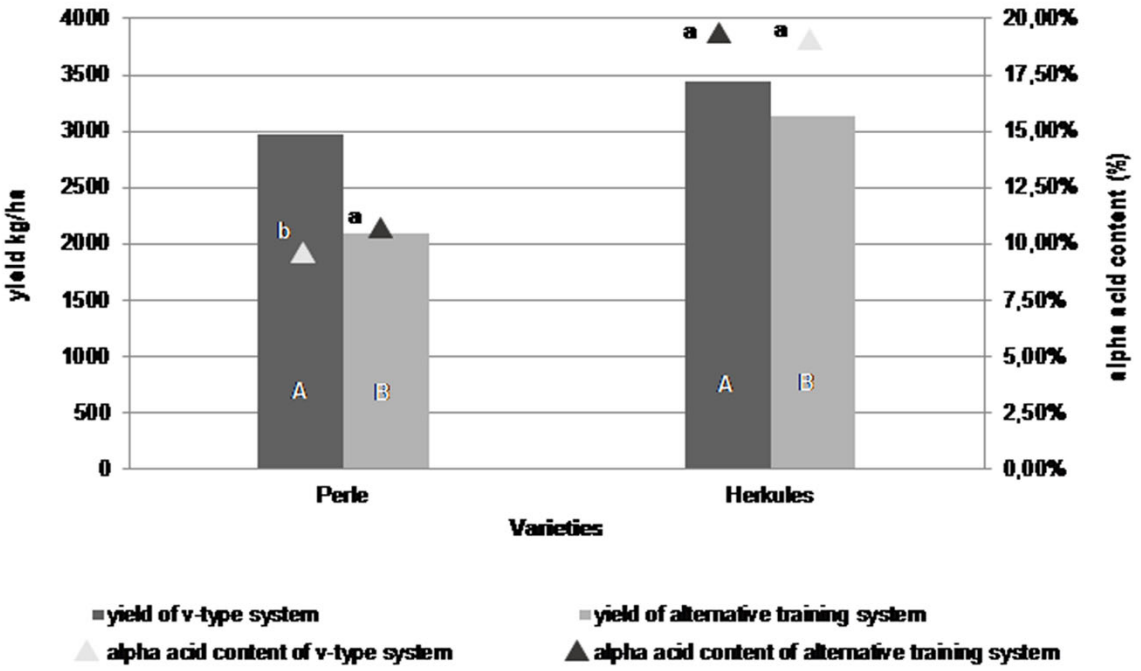

Figure 4. Yields and alpha acids in cvs Perle and Herkules, according to training system

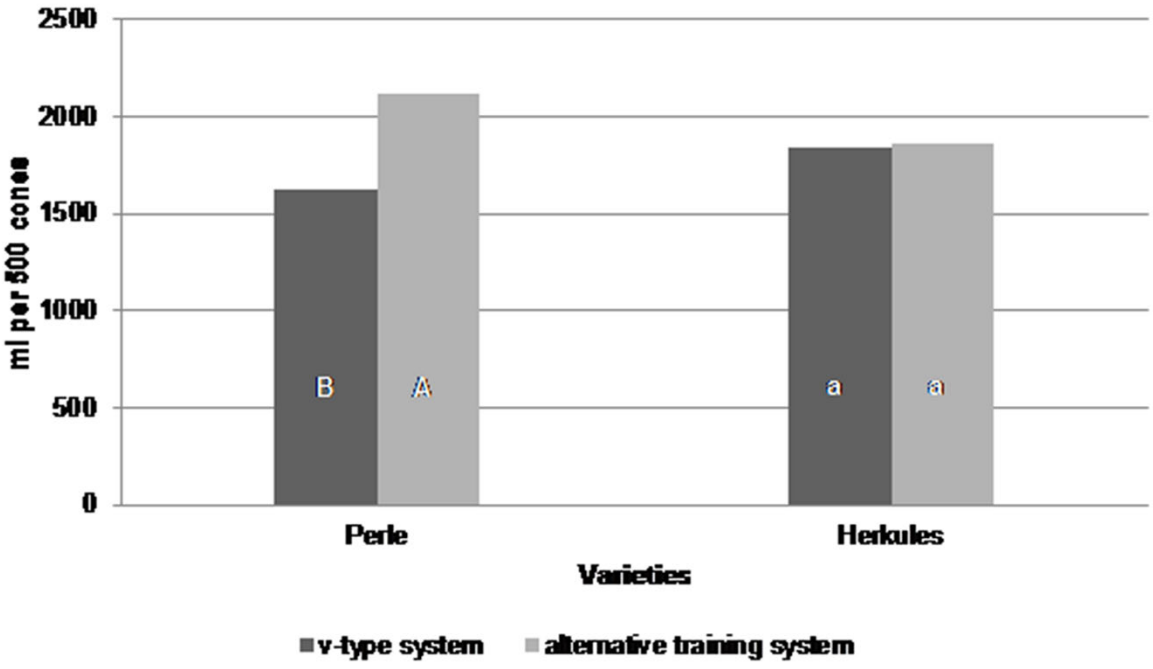

Figure 5. Cone volume of cvs Perle and Herkules, according to training system

The poor yield for the alternative system with the aroma cv. Perle was mirrored in the cone volume, which was much greater in the alternative system. In the case of Herkules, however, no difference between the two systems in cone volume was detected.

Discussion

In the trial the linear growth of the self-training plots was delayed as compared to the conventional system. As a consequence, the hop plant spent a great deal of energy on maintaining a large number of shoots. In addition, there was no help with climbing available for quite some time – something that would have had a positive impact on growth. With Herkules, the difference between the training dates was about half that number of days and, here, the divergence was minimal and only hardly noticeable.

The yield difference in Perle was higher than in Herkules. Thus, the significant difference in yield from the trial in Perle can be attributed to the delayed training in the alternative system.

In the conventional system a lower alpha acid content was determined. The reason for this is unclear. It is possible that a higher yield has some kind of diluting effect.

The cone volume in the V-type system in Perle was significantly less. In theory, if the plant produces fewer cones (i.e., yield), these cones are naturally always bigger. Therefore, the smaller number of cone clusters in the aroma hop led in the alternative system to a 30 % greater cone volume than in the conventional system.

Outlook

Training systems offering an alternative to the traditional high-trellis V-type system can only be regarded as promising if they can either offer a reduction of costs or if they are beneficial to the growers' labour management economics. It will be a precondition that any advantage of an alternative training system are not undermined by reduced yields or lower market profits and that technical challenges of the self-training system have been solved − especially concerning plant protection and harvesting.

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PORTNER J. & OBSTER R. 2017. Jahresbericht 2016 Sonderkultur Hopfen, Bayerische Landesanstalt für Landwirtschaft und Gesellschaft für Hopfenforschung, 67-73

Mobile hop picking and a new cleaning line for low trellis hops in the Czech Republic

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Abstract

Since 2007 the resumption of low trellis hop has been investigated in the Czech Republic. A breeding program with Czech dwarf hop varieties is going on. The harvest mechanization was designed or innovated, produced (prototypes) and verified.

Key words. Low trellis, mobile hop picking machine HUN-30, special trailer, cleaning line.

Introduction

In 2007, the company Chmelařství, cooperative Žatec, organized an excursion to England. Some hop growers became enthusiastic about low trellis hops. At that time, before the world crisis arose, Czech hop industry had been struggling with provision of seasonal workers as the interest in spring work of Slovak and Ukrainian workers was decreasing. Therefore, the Czech leadership revived the idea of growing hops on low trellis. The harvest was partially solved, since the company Chmelařství has already sold several mobile hop picking machines HUN-30 to China and the USA since the 1990s. The questions of a special trailer for transporting hop biomass and of an appropriate cleaning line had to be solved however.

Results

Special trailer for transporting hop biomass

The invented trailer for a hop biomass is of all-welded structure. It consists of a two-axle chassis formed by a backbone-type frame terminated in its front section by a towing pole for attaching to a tractor. A hydraulic distributor is located in the rear section of the chassis.

The chassis carries a car body formed by two lateral sidewalls followed up with faces, wherein the rear face is tiltable, and a sliding bottom, which consists of two shafts fitted with chain wheels with chains. Those chains are fitted with transverse supports for lamellas and together are forming an endless conveyor belt. A scraper for scraping residual adhered hop product is mounted in the rear bottom section of the trailer's body. The conveyor belt as well as the rear tiltable face are arranged for driving with a hydraulic control motor disposed on a driving shaft.

Mobile hop picking machine HUN-30

The HUN-30 hop picker is a mobile machine pulled by a tractor and designed to harvest hops in 3 m high vertical trellis system. The trellis system remains intact during harvest and individual hop bines are not cut. The harvested biomass is collected in a trailer, which moves along with the hop picker. The flexible chassis of the hop picker allows to adjust the height and side angle of the picking system. Individual picking banks are movable and placed in the frame to easily navigate around trellises to get at the hops. After harvesting a row the picking banks are drawn back into the machine by a hydraulic system. The harvested hops are cleaned by a stationary cleaning system.

Tractor requirements: 52 kW (70 HP), auxiliary shaft revolutions, 1,000 rev/min, switch to super-crawl speed up to 0.7 km/hr at maximum engine speed. Technical parameters: machine length: 5,000 mm, machine width: 3,000 mm, machine height: 3,900 mm, height regulation: 300 mm, maximum permissible load on the trailer drawbar per coupling: 1,200 kg, maximum permissible axle load: 3,500 kg.

Low trellis PT-2000 cleaning line

The cleaning line is a machine designed to sort the hop cones from the mixture of hop stems, leaves, and other impurities. The cleaning line is a stationary machine and thus located in a hall. The hop mixture is transported to it by special trailer for low-trellis hop fields. The mixture is obtained by picking low-trellis hop fields using mobile hop picking machine HUN-30.

The machine is placed along one of the long sides of the hall. The exhaust fans for the first and second vacuum cleaning are mounted on the walls of the hall. The air ducts for these cleaners and the flake separator are located on the outside wall of the hall. The waste conveyor (leaves and stems) also runs through this wall.

The cleaning line in comprised of the following assemblies: loading conveyor, 1st separator, secondary picker, 2nd separator, product conveyor, waste conveyor, 1st air purifier, roller track – separator, small pocket conveyor, 2nd air purifier, large pocket conveyor, inclined conveyor, set of waste conveyors and bagger.

The cleaning line has a width of 2,000 mm. A throughout of 2,500 kg of fresh hops per hour (= 2,231 lb/acre per hour) was achieved.

Dual harvest (low & high trellis)

Based on the new experience and the use of the existing cleaning line, a stationary picking line was designed, including a duplex PT-30 picking wall, a duplex hanging tract for two hanging sits, a 19-chain secondary picker, a drum cutter and split separation of hop cones.

Discussion

The sources about low trellis harvest mechanizations are scarce. The history of development of hop picking machines was published by DARBY (2004).

Acknowledgement

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This paper is a result of our activities within the research project TA03021046 'Research and Development of Hop Technology and Equipment for Cultivation of Hop Under Low Trellis' (2013–2016) with the financial support from Technology Agency of the Czech Republic.

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Figure 1. Special trailer for hop mass and mobile hop picking machine HUN-30

Figure 2. Mobile hop picking machine HUN-30 in a low trellis system in the Saaz hop growing region

Figure 3. Loading conveyor ('feeding table') of a new PT-2000 cleaning line for low trellis

Figure 4. The new design of a PT-2000 cleaning line for low trellis

Hop water requirements: a review and future goals

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Abstract

Irrigation management in hop cultivation requires studies of soil water balance and we present a small review of previous studies. The use of modeling tools such as SIMDualKc facilitates the management of irrigation using soil and climatic data, once it has been calibrated for local conditions. Soil water modeling results are showed for cv. Nugget in Galicia (Spain), for the years 2012-2014. The modeling adjusts the measurements of the soil water content, made with TDR, for both irrigated and rain-fed plots. Together with the installation of a wireless sensor network at key sampling points in a plot as well as with the use of hyperspectral and thermal images, the use of this model is presented as key tool for the adequate management of water resources, the application of nutrients and phytosanitary treatments, just as it is being applied in other crops.

Key words. Irrigation scheduling, soil water content, modelling, vegetation index, wireless sensor network.

Introduction

Water supply is one of the key factors in agriculture and therefore the achievement of a sustainable water management where water productivity is a good index to water management is crucial. Currently, a successful crop water management, especially in high water demand crops such as hop, is of increasing significance. In this regard, we have performed a small review on main studies related to irrigation in *Humulus lupulus*, with an additional case study in Galicia (Spain) and pointing at future goals.

Several authors have shown the relevance of precipitation and temperature for an adequate growth of hop and for yield and quality, e.g., SREČEC et al. (2004, 2008) in Croatia; KOŘEN (2007) and MOZNY et al. (2009) in Czech Republic; ENGELHARD (2004) in Germany; BAVEK et al. (2003) and PAVLOVIČ et al. (2012, 2013) in Slovenia; BENÍTEZ et al. (1998) in Spain, and WAMPLE & FARRAR (1983) in the USA. MOZNY et al. (2009) reported on climate change effects concluding that hop may be a particularly vulnerable crop. In addition, various studies tackled the effects of irrigation on production at the farm scale (e.g., WAMPLE & FARRAR 1983; SVOBODA et al. 2008; DELAHUNTY et al. 2011; NAKAWUKA et al. 2017; FANDIÑO et al. 2015) or at plant scale (DE KEUKELEIRE et al. 2007; HNILIČKOVÁ et al. 2009; GLOSER et al. 2013). These studies reported that irrigation increases yield and do not negatively affect the alpha-acid content although impacts of water and temperature stress relate with the phenological stages when occurring. However, few studies have focused on in-depth determination of hop water requirements (BÁREK et al. 2009; KROFTA et al. 2013) and there are limited studies providing for an in-depth analysis of evapotranspiration, soil water balance and irrigation scheduling (FANDIÑO et al. 2015).

There are several methods and techniques that may be applied to measure and estimate crop evapotranspiration, e.g., lysimeters, heat pulse, heat balance, Bowen ratio energy balance (BREB), surface renewal energy balance, eddy covariance and the soil water balance (ALLEN et al. 2011). URBAN et al. (2012) applied the sap flow technique and BREB with cv. 'Agnus' in Czech Republic, KROFTA et al. (2013) measured the sap flow with cv 'Premiant', and FANDIÑO et al. (2015) soil water balance with cv. 'Nugget'. Generally, those studies estimated hop transpiration but did not quantify soil evaporation, groundwater contribution or the runoff components of the water balance, except FANDIÑO et al. (2015). Hence, considering the importance of knowing the dynamics of the various soil water balance components throughout the hop season, it is required to apply a model that allows related computation with a daily time step.

Soil water balance models are applicable to a large number of crops, including when allowing the partition of ET_c and/or are used to support irrigation scheduling advising (ALLEN et al. 2011). Models adopting the referred K_c - ET_o approach have shown to be appropriate for using either the single or the dual K_c as recently reviewed by PEREIRA ET AL. (2015). The SIMDualKc model (ROSA et al. 2012) has proved to appropriately perform the soil water balance and partitioning ET_c in Hop (FANDIÑO et al. 2015), as well as to estimate the groundwater contribution with small errors of estimates.

Main objective of our study was to determine the soil water balance in a cv. 'Nugget' hop field during three seasons (2012-2014), allowing to determine an irrigation scheduling with soil water content measures. Moreover, a brief description about future goals concerning water requirements in hops in order to manage irrigation in a reasonable way is included.

Material and methods

The research was conducted over a period of three seasons (2012-2014) in a hop field (*Humulus lupulus* L.) cv. 'Nugget' of 'Centro de Investigaciones Agrarias de Mabegondo (CIAM) - Xunta de Galicia', located in Abegondo (A Coruña, Galicia, NW Spain). The experimental site, 'Govia', presented plants aged 8 years at the beginning of the study, arranged in 6 m trellis with 19 rows and 63 plants per row (3 x 2 m), with a density of 1,667 plants ha^{-1}, and equipped with surface drip irrigation. The lateral pipes were equipped with in-line non-compensating emitters spaced by 50 cm along the rows, thus resulting in four emitters per plant with a flow rate of 2 L h^{-1}, respectively. Irrigation depths and number of irrigations are defined in Table 1. Soil water content measures were determined with a TDR100 (Campbell, Sci.) every ten days.

SimDualKc model was used to determine soil water content daily (ROSA et al. 2012). More details about experimental design, calibration data and methods can be found in FANDIÑO et al. (2015).

Table 1. Treatments and irrigation depths

Year	Treatment	Number of irrigation	Irrigation depth [mm]
2012	Rainfed	0	0
	Drip Irrigation	14	128
2013	Drip Irrigation	15	65
2014	Drip Irrigation	15	144

Results

SimDualKc calibration and validation corresponded well to actual soil water content measures, with a coefficient of determination of 0.88 to all data. In Figure 1 it is possible to determine the effect of no irrigation (Fig. 1a), where a lower value of θ_p was achieved from first days of July to harvest; this result was linked to a lower yield per hectare (FANDIÑO et al. 2015). In addition, an excess of irrigation is observed in Figure 1d in 2014, which suggests an energetic cost and poor use of the water resources. In general, the measurement of soil water content will allow us to know the actual situation of the crop, in relation to water stress, and to plan the irrigations. However, the use of the SimDualKc model based on climatic parameters, once calibrated for each site, eliminates the need to determine the soil water content periodically, thus allowing the irrigation schedules to be performed in advance.

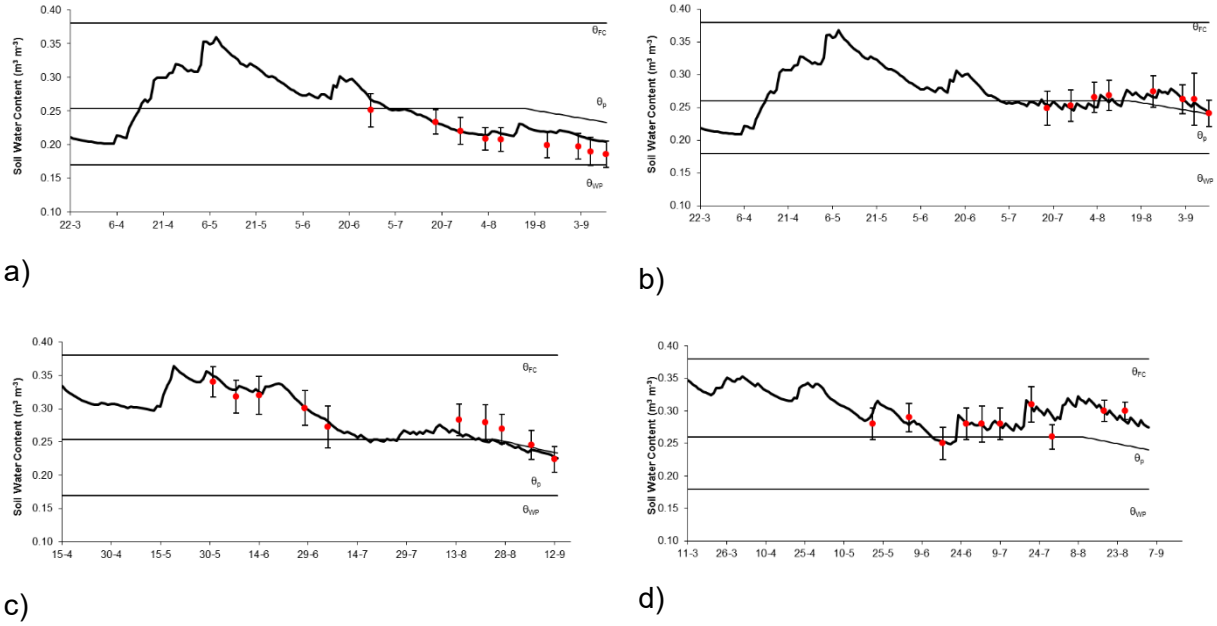

Figure 1. Simulated soil water content curve (bold line) and observed values (●); (a) rain-fed, 2012, (b) irrigated, 2012, (c) irrigated, 2013, and (d) irrigated, 2014. Curves θ_{FC}, θ_{WP} and θ_p represent soil moisture at field capacity, wilting point, and when depletion equals the fraction p, respectively. Error bars represent standard deviation (n= 6).

Discussion

The modeling tools of soil water balance allow understanding the situation of the crop during the vegetative cycle. When the water content in the soil is below the soil water depletion for no stress line (θ_p), irrigation should be started to maintain a correct water status of the plant. At present, in intensive (horticultural), or extensive crops such as maize and woody species (olive groves, vineyards, etc.), real-time control methods have been developed that allow to know the soil water content and to manage the irrigation. The methods used are related to soil sensors, which are linked to a wireless network, and can be consulted online via the web. Likewise, the sensors configure alerts when the soil water content reaches a certain value with which irrigation should begin. Irrigation management based on soil water content should be related to studies of the effects on the plant, in terms of physiological parameters such as photosynthesis or stomatal conductance. In addition, they should be connected to the nutritional needs of the plant during the different stages of vegetative cycle, for which the irrigation system can be used as a fertigation system. Moreover, the use of multispectral and thermal images could help to determine different areas in a plot, in order to manage water and nutrients in a precision agriculture system correctly. Unmanned aerial vehicles (UAV) or satellites are key to achieve images with different resolution and time frame, allowing determination of vegetation index, determining homogeneous areas to use of variable rate technology (VRT) to apply irrigation, sprayers, etc.

Acknowledgement

To the enterprise ‘Hijos de Rivera Inversiones Corporativas, S.L.’ (Estrella de Galicia beer) and ‘Centro de Investigacións Agrarias de Mabegondo’ (INGACAL-CIAM), Xunta de Galicia, Spain.

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Norwegian hops and small-scale cultivation systems

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Abstract

Hop is an old cultivated plant in Scandinavia and in Norway the oldest written evidence of hop cultivation derives from the 12th century. However, for the last 100 years hop cultivation has been almost forgotten and revitalization of plant varieties as well as agronomic knowledge is needed. Due to long days, low temperatures and high rainfall, the selection of appropriate plant material is important. The tested hops from the Norwegian germplasm collection have all been described as possible aroma hops with an α-acid content below 5-6 %. The production in Norway is aimed at niche production and alternative cultivation systems have been tested to improve yield and economy. We found that cultivation of hops in plastic tunnels increased the yield two to three-fold and that variation in yield between clones of collected Norwegian wild hops was high.

Introduction

Hops have been cultivated in the Nordic region since mediaeval times and are mentioned already in the old laws from the 12th century, Frostatingsloven (HØEGH 1975). Under the Danish and Norwegian Union, from the 15th century until the middle of the 18th century, farmers were obliged to grow hops on the farm from the far South all the way to middle-northern Norway, North-Troendelag. However, we know that hops were cultivated even further to the North, e.g., in the Lofoten Islands (68°N, 14°E). Whether hops were introduced to the country or whether the plant is part of the natural flora is uncertain. There are indications that some hop plants may have come from the East in times of the Finno-Ugric tribes or with Slavic people. Due to a long joint history, an import from countries south of Norway such as Denmark would also be likely. To reveal any similarities, DNA fingerprint studies were performed on plants in the Danish and in the Norwegian germplasm collection. Together with observations on morphological traits and chemical content, these results allowed to separate the individual clones and also the individual regions (SOLBERG et al. 2013).

As a consequence of centralisation of the breweries and cheaper import from Germany, Danish and Norwegian hop production declined markedly in the 19th century. In Norway the last data we have from hop experiments are from 1926, where a number of German varieties were tested in the south-western part of the country and were reported to give good yield and quality. Figures from Denmark show that the hop production fell from 1103 ha in 1881 to 200 ha only 20 years later, before almost disappearing in the 20th century (SOLBERG et al. 2013). Hop production in Scandinavia is now very much related to historical practices, but new trends may lead to changes in this regard. In Scandinavia the trend to local and Nordic food raised a dramatic interest in recent years. This includes a distinct interest in brewing. In Norway today 100 farm- and microbreweries (members of the Brewers association, BROD) produce approximately 9-10 million litres of beer annually, which is 4 % of the total beer production in Norway. Following this tremendous interest concerning brewing of good Norwegian beer there is also a high demand for local Norwegian ingredients.

The knowledge about growing of hops in Norway has mostly disappeared and we need to build up experience again around growing techniques as well as finding and selecting the appropriate genetic lines or cultivars for growing in the different climate zones in the country. For a new start of successful cultivation of hops in Norway, with long days and a large variation in summer temperatures and precipitation incidences, we first need to test and select the proper genetic plant material but may also find new innovative ways for production.

In Norway a germplasm collection of hops were established at the Norwegian Institute for Bioeconomy Research in 2000 consisting of plants from furthest South to furthest North in the country. For the experimental work, we selected clones from this collection.

Material and methods

The hop clones used in the experiments were selected both in order to represent a South to North gradient and also from clones that had been observed to develop ripe cones and amongst those the least susceptible to diseases (Table 1).

Table 1. Origin of clones of Norwegian hops selected for this study.

Hop clone	Locality	Elevation [m a.s.l.]	Latitude
6	Luster	10	61°29'N 07°36'E
7	Ringsaker	140	60°55'N 10°42'E
27	Nome	100	59°13N 09°14'E
37	Vevelstad	10	65°43'N 12°3'5E
40	Kvinesdal	60	58°20'N 06°57'E

Plants were either grown in open field (all five clones), or in growth plastic tunnel (clones 7, 37 and 40).

Open field system
Poles extended to 5 m above ground with 9.5 m between the poles, respectively, and 4 m row distance. Irrigation was given by trickle irrigation and fertilizer as chicken manure in pellets calculated to 100 kg of N per ha. The irrigation water was enriched with 3 % Resistim (Taminco, Ghent, Belgium), a plant nutrient supplement containing potassium and phosphorus. The soil in the rows was covered by woven polypropylene 'Mypex' groundcover (Don & Low, Forfar, UK).

Tunnel system
Height of the training strings was approximately 4 m, limited by the roof height, and row distance was 4 m as in open field. Irrigation and ground cover were the same as in open field. The tunnels, 8 x 40 m each, were open at the lower 1.5 m at the sides and completely open in both ends. In the tunnel roof water irrigators were sprinkling the plants at irregular intervals as a prevention of powdery mildew infestation. The irrigation was deactivated daily early in the afternoon for the plants to dry before nightfall to reduce the risk of *Botrytis* infestation.

Temperature and humidity were recorded throughout the experimental period. We found that the plastic tunnel increased sum day degrees from 1328 in open field to 1383 in the plastic tunnel.

At harvest the plants were cut down completely and cones removed by hand. In 2014 all plants in each plot were harvested, while in 2015 three plants per plot were harvested and in 2016 one plant per plot was harvested. The cones were dried in a dryer cabinet at 50°C until dry weight remained stable. The plants and cones were measured for morphological characteristics and chemical analyses were performed at NATEC$_2$ in Wolnzach, Germany.

Statistical analyses
The results were analysed by means of the GLM-procedures in MINITAB (Minitab, 2011) to test for significant treatment effects. Effects were considered to be significant with P <0.05. Experiments were laid out in completely randomised block designs with three replicates. The All Pairwise Comparison Procedure of the Tukey Test was used for multiple comparisons.

Results

During the three growing seasons, we have seen that there are major differences between the clones we selected as well as between the cultivation systems open field and plastic tunnel. Yield is approximately 2-3 times higher in tunnels than in open field (Table 2). In average over the three years, the three clones grown in both tunnel and in open field yielded respectively 616 g and 226 g of dry cones per plant. Clone number 40 gave highest yield in both cultivation systems.

The hops have been tested in laboratory; and for aroma in dry cones (Table 3) as well as in a number of different brews. There are individual differences and preferences but overall the brewers have found that the Norwegian hops can create a good and interesting aroma in the brew.

Table 2. Cone yield in open field and in growth tunnel as average over three years.

	Open field		Growth tunnel	
Clone	**Fresh weight [g/plant]**	**Dry weight [g/plant]**	**Fresh weight [g/plant]**	**Dry weight [g/plant]**
6	916	216		
7	665	173	1354	362
27	1268	285		
37	759	203	2556	579
40	1318	303	3761	907

Table 3. Aroma in dry cones evaluated in common trials by non-trained persons.

Dry hops	**Clone**				
Aroma, % of participants	**6**	**7**	**27**	**37**	**40**
Citrusy	50	44	63	30	0
Flowery	38	70	85	80	44
Fruity	38	40	50	60	33
Spicy	75	64	63	60	67
Woody	75	60	63	60	56
Bitter	38	40	25	30	44

Conclusion

For improved cultivation success, selection of clonal material is important. In small-scale cultivation systems use of plastic growth tunnels provides a more stable and higher yield of hops. In Norway the revitalization of hops and hop production has just begun, but we hope for this to become an interesting niche crop on many farms.

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The influence of various hop cultivation conditions on hop quality, beer quality and yield

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Abstract

We investigated the correlation between the cultivation factors such as pruning, blooming and harvest time and hop aroma characteristics, chemical components, appearance, yield, and beer quality in four locations and in three years. Among the cultivation conditions investigated in this study, harvest time had a significant impact on the amount of essential oils, especially mono-terpenes. Among them, linalool, geraniol and myrcene those are contributing beer hoppy aroma increased with delay in harvest time. These impact caused by harvest time were confirmed in all 4 locations and in all 3 years.

Key words. *Humulus lupulus*, harvest, pruning, hop aroma.

Introduction

Hop (*Humulus lupulus* L.) provides bitterness, fullness, and flavor to beer, all derived from the humulones, polyphenols, terpenes, etc. present within the hop plant (INUI et al. 2013). In order to control beer taste and flavor, different cultivars (KOVAČEVIC & KAČ 2002; STEENACKERS et al. 2015) and different processed hop products, such as pellets or hop extracts (each containing different components) are generally used.

To some extent, it is possible to achieve the desired beer quality simply by selecting specific cultivars, adding hop products, and brewing it properly. However, hop is an agricultural crop and its quality is itself unstable, varying according to climate (KROFTA & KUČERA 2010), root properties (e.g., age, viral infections), and cultivation conditions such as fertilizing, pruning, and harvest (SHARP et al. 2014). Therefore, it is important to control cultivation conditions in order to obtain high quality hop.

Cultivation factors can be classified into three types: 1) 'of natural origin' − e.g., the soil type and hop plant age; 2) 'culture-controlled' − e.g., fertilizer application, pruning, and harvest times; 3) 'of climatic origin' − e.g., temperature, rainfall, and the amount of sunlight. Among these three types, only the 'culture controlled' factors can be regulated. In this study, we investigated the culture-controlled factors, pruning, blooming, and harvest times, and analyzed the relationship between these three variables and hop aroma quality.

Material and methods

Test conditions

Hop gardens in four villages − Rybňany, Lipenec, Blšany, and Kroučova − of the Saaz region (Bohemia, Czech Republic) were selected and used as test fields for experimental cultivation. These four gardens were located in the north-western, northern, south-western, and south-eastern part of the Saaz region, respectively (Fig. 1), at a distance of about 10 km from each other. The kind of soil and plant age differed between the test gardens (Fig. 1). In 2012, 15 combinations of pruning and harvest times were tested within each garden: three pruning (April 5, 15, and 25) conditions were combined with five harvest times (August 15 and 25; September 3, 10, and 20); eight bines were harvested for each combination of pruning and harvest conditions. The influence of harvest time was also examined in 2010 and 2011, in Rybňany.

Hop blooming and cone formation

Reproductive growth characteristics, i.e., flowering and cone formation were recorded every week from the end of June to the middle of August. Flowering and cone formation seasons were defined by the 50 % flowering/ cone formation ratio, calculated from the average of that observed in the eight vines collected in each experiment.

Hop color measurement

A dried hop cone was pulverized using a coffee mill (Melitta Japan Ltd.). Milling was adjusted to get identical particle sizes. The color of pulverized hop was evaluated using colorimetric values in a CIE L*a*b system measured with a CM-2022 spectrophotometer (Minolta Co., Ltd, Tokyo, Japan), and the data were calculated under a 10º observer and D65 illuminants. Color data were analyzed using SpectraMagic^{TM} NX color-control software (Minolta). The closest RHSCC number to hop's cone color, based on its CIE L*a*b value, was obtained by visual discrimination and measurement with the device mentioned above. RHSCC number is a standard reference for plant color identification in the horticultural industry selected by the Color Classification System version 2.1.1 (The Japan Research Institute Co., Ltd.) and can be used for objective selection of the closest RHSCC number by color data.

Brewing trials

Trials were conducted in a pilot scale brewery (100 L) with a malt ratio of 100 %. Hops were added twice: at the beginning (kettle hopping) and at the end (late hopping) of wort boiling. In order to compare differences in hop aroma characteristics, an identical amount of each hop sample was added during the late hopping stage, and only at this stage. The amount of hop extracts added at kettle hopping was decided to be the same, in order to provide identical bitterness to beer samples. Worts were fermented at 10ºC using lager yeasts.

Results

Influence of the pruning date on the blooming date

We analyzed the relationship between pruning and blooming (cone formation) dates at the four locations, in 2012. It can be seen that pruning date does not influence blooming date as the slope of the line corresponding to their correlation is very small. There was no correlation between the date of pruning and the date of cone formation. The cumulative temperature calculated from the average temperature in each day was 290 ºC in April (pruning period) and 1,942 ºC from April to July (blooming period). Hence, the ratio of cumulative temperature in April is only 15 %, suggesting that the 20 days difference in pruning dates in April did not affect the blooming dates.

Influence of the harvest date on hop quality and yield

The influence of different harvest times was examined at the four locations in 2012 and at Rybňany in three consecutive years. Significant effects at the 99.9 % level were detected in the period from blooming to harvest for essential oils, linalool, and sensory score. Blooming times were almost identical for all gardens (within 1 week). The amount of essential oil and linalool increased at all locations until the middle of September; these increases only continued until the end of September in Blšany and Kroučova. It is difficult to understand why these two patterns exist. In association with the increase in essential oils and linalool, hop aroma intensity also increased; α-acid and yield were almost stable during the harvest period. Six mono-terpenes (linalool, geraniol, myrcene, ocimene, β-pinene, D-limonene) and four sesqui-terpenes (β-farnesene, bergamotene, α-humulene, β-caryophyllene) in hop were analyzed. In the four locations, sesqui-terpenes were generated early in the harvest period and mono-terpenes were generated later. These results suggest that harvest timing affects both intensity and balance of the hoppy aroma in beer.

We also investigated the influence of the harvest date through three consecutive years (2010, 2011, and 2012) at Rybňany. Significant effects at the 99.9 % level were found in the period from blooming to harvest for essential oils and linalool.

According to the climatic data, differences in temperature and rainfall between the three years in one location are larger than those observed between the four locations in one year. Essential oils and linalool content in hop increased during the harvest period in all years; while sesqui-terpenes were mainly generated in the beginning of the harvest period, mono-terpenes were generated towards the end of the harvest period. This behavior is therefore similar to that found for the four locations in 2012.

Influence of the harvest date on beer hoppy aroma

We brewed beer using the hops harvested in Rybňany in 2011 and 2012. Results confirmed that the intensity of hoppy aroma was stronger in beer brewed with later harvested hop compared to that in beer brewed using the hop harvested in an early period. Beer linalool content showed the same tendency as aroma intensity (data not shown). The balance of hoppy aromas is different between harvest times and these aromatic characters also differ between years. This tendency is generally consistent with that of other varieties (SHARP et al., 2014). Therefore, although hoppy aroma intensity may be an indicator for suitable harvest timing, it may be difficult to predict beer aroma character based on hop aroma intensity alone because of its yearly fluctuation.

Change in hop color during the harvest period (*Rybňany in 2011*)

Historically, many brewers evaluating hop color might prefer bright green color to the brown color from the overall view point. And there are some post-harvest methods to maintain its bright green color such as sulfur treatment at kilning process. In this study, hop color changed during the harvest period and the results of hop color measurements are shown. Early harvested hops showed a bright green color, which gradually changed to yellow-brown towards the end of the period. The a-value (indicating color change from green to red) tended to increase during the harvest period whereas the hue angle 'h' (indicating the direction of the rectangular coordinates - the horizontal axis is from green to red, the vertical axis is from blue to yellow) decreased during the harvest period. Although the data are only from Rybňany in 2011, the same color changes were recognized in the other samples (three years in Rybňany and four locations in 2012). These results suggest hop color could be a good indicator to estimate hop aroma quality. The brown colored hop harvested later in September was analyzed for infections by downy mildew using qRT-PCR, but downy mildew infection was not confirmed (data not shown). It can therefore be assumed that the change in hop color is due to the oxidation of polyphenols or to the decrease of chlorophyll in hop bract during the harvest period.

Acknowledgement

The authors would like to thank all the members of the Hop Research Institute Co., Ltd. in Saaz, Czech Republic for their contribution to hop analyses, and all the members of Žatec Hop Company Ltd. for their kind support to this study. The authors would also like to thank all farmers who allowed them to observe their hop gardens and provided hop samples.

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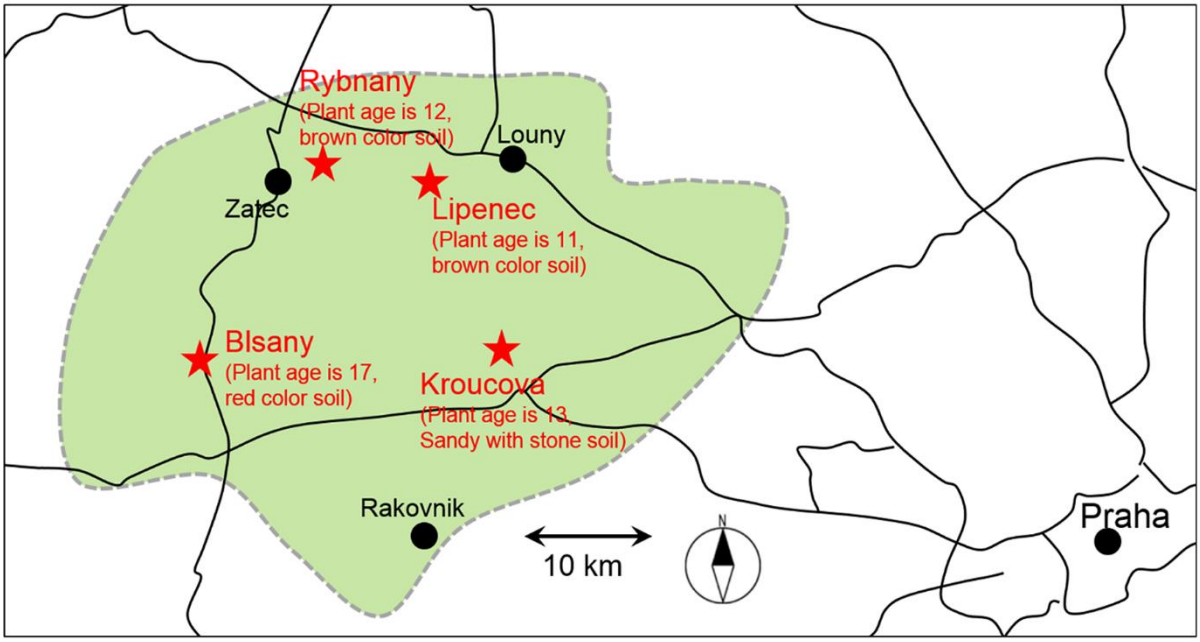

Figure 1. Geographic situation of the four test localities in Bohemia, Czech Republic. The Saaz hop growing region is the area surrounded by a dotted line.

This manuscript is an edited version based on the following original article:

MATSUI H., INUI T., OKA K. & FUKUI N. 2016. The influence of pruning and harvest timing on hop aroma, cone appearance, and yield. Food Chemistry 202: 15-22. DOI:10.1016/j.foodchem.2016.01.058

V: Phytopathology

Management of citrus bark cracking viroid (CBCVd) on hop in Slovenia

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Abstract

Viroids are the smallest known plant pathogens and cause diseases in many economically important crop species. They are single stranded, circular and self-replicating non-coding RNA molecules, with sizes ranging from 246-401 nucleotides (nt). Four viroids are currently known to infect hop: hop latent viroid (HLVd), hop stunt viroid (HSVd), apple fruit crinkle viroid (AFCVd) and in Slovenia recently discovered citrus bark cracking viroid (CBCVd). All four viroids have negative impact on hop but CBCVd causes the most aggressive infections resulting in severe plant stunting, disturbed cones formation, and plant dieback 3-5 years after infection. Before finding on hop, CBCVd had been described as a pathogen of citrus plants that did not cause any economic damage. Hop is therefore a new and highly susceptible host for CBCVd. The new hop disease named »severe hop stunt disease« has been currently discovered in Slovenia, which signifies the appearance of CBCVd in a new area outside citrus production regions. The spread of CBCVd in hop gardens is extremely fast, due to the specific agro-technical practices in hop production creating ideal conditions for mechanical transmission. Since the disease is incurable, basic management depends mainly on eradicative phytosanitary measures such as destroying infected plants and hop fields. In this process, infected plants can be visually detected only one year after infection, while asymptomatic infected plants in the stage of incubation cannot be observed. These infected asymptomatic plants represent hidden infection potential, which can only be eliminated by destroying the entire infected hop field, and by utilising adapted agro-techniques on infected areas. Based on epidemiology studies of CBCVd on hop, critical spreading points connected to hop cultivation practices were defined. As a response to that, new procedures in hop technology were developed, which prevent infection and the survival of CBCVd.

Key words: viroids, citrus bark cracking viroid (CBCVd), *Humulus lupulus*

Acknowledgement

The authors acknowledge the financial support of the Slovenian Research Agency (V4-1405, L4-6809) and the Administration of the Republic of Slovenia for Food Safety, Veterinary and Plant Protection.

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Hop protection against Downy mildew (*Pseudoperonospora humuli*) in Czech Republic

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Abstract

Hop downy mildew *Pseudoperonospora humuli* (DM) is the most dangerous harmful organism of hops in all hop growing areas of the Czech Republic. It is necessary to carry out many treatments every year to prevent damage caused by this pathogen. The strategy of hop protection against this disease is based on an early belt spray of fosetyl Al. The treatment is recommended in the time when young hop plants reach the height of 10-15 cm. Just after the first training in the second half of May another spray with fosetyl Al is usually carried out to eliminate the occurrence of primary infection in form of spikes later during the season. Since the beginning of June till the harvest usually four to seven treatments are made to control secondary infection of the pathogen. These sprays are recommended according to short time prognosis, which is based on the number of days with precipitations. Despite quite a lot of fungicides registered, most of them are copper-based, which can be used just in a limited scale. Problems for Czech hop growers, who have been accustomed to use copper fungicides for many decades, have arisen recently. Hop protection against DM is getting more difficult for them especially in the second half of the vegetation period, when copper fungicides were the most frequently used ones, particularly because of their short time pre-harvest interval and good efficacy on DM in the time of hop cone formation.

Restriction of copper fungicides brings about the necessity in searching for other efficient fungicides to replace them gradually. One of the ways is the replacement of this group of fungicides by new, efficient ones. Mandipropamid was in CR registered several years ago as Pergado F but as a matter of fact it was practically not used because of problems with drift of captan from neighbouring orchards, which is very similar to folpet, the compound that together with mandipropamid was active ingredient of Pergado F. Another efficient fungicide is Orvego (ametoctradin + dimethomorph). These fungicides are not only efficient to control DM but have a short time pre-harvest interval, which enables them to become a good replacement for copper fungicides in CR. Copper fungicides with lower contents of copper in the form of copper hydroxides (Cuprozin progress, Defender, Funguran progress and others) showed good efficacy as well and under a medium pressure of DM they will be able to help to control this disease in the second half of the vegetation period as well. Nevertheless, difficulty may arise under heavy pressure of the pathogen when lower contents of copper may be insufficient to control this dangerous disease.

In the connection with the restriction of copper fungicides we can ask the following questions: is the process of copper limitation reasonable? Are new synthetic fungicides, which replace them, more environmentally sound? Are copper fungicides more harmful than synthetic ones? If so, why have they been allowed in organic agriculture unlike synthetic ones? Are there differences in toxicity to earthworms between copper hydroxide, oxychloride and sulphate? All these questions should have been answered before the decision of severe restriction of copper fungicides began.

Acknowledgement

The work was supported by Ministry of Agriculture of CR within Institutional Support for the Development of Research Organization RO1486434704.

An overview on forecasting systems for Downy and Powdery mildew in Hallertau hops

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Abstract

Downy (*Pseudoperonospora humuli*) and Powdery mildew (*Podosphaera macularis* ssp. *humuli*) are the main fungal diseases of hop and both have a high potential of reducing the hop yield significantly in quality and quantity. Financial losses are also caused by the regular use of pesticides for their control. Therefore, the establishment of forecasting systems to manage the two diseases and to reduce the amount of fungicides for both is on focus.

For Downy mildew secondary infection a pre-warn system is established since the 1980s (KREMHELLER 1979) and shows adequate results in disease management. From April until September the zoosporangia of the oomycete are caught by five specific traps distributed over the Hallertau and are counted daily. If the determined threshold of spores is exceeded, the farmers get an advice to treat their plants. A prerequisite for a good working pest management with the warn system is the control of primary infection in the hop garden and no wild hops in the vicinity of the garden. A number of contact as well as systemic active ingredients are available to control Downy mildew infection effectively during the whole season.

For Powdery mildew a running forecasting system is still lacking. In the last 20 years a lot of efforts have been made to create an effective warning system. The simple method of counting spores could not be applied to this biotrophic fungus. Hence, a system based on meteorological data was developed by Engelhard in the 2000s, followed by a more sophisticated version by SCHLAGENHAUFER (2010). The calculation is based on micro-climatic conditions favouring fungal growth, viz. temperature (~ 18°C), preferably with no differences between day and night, global radiation (<4000 Wh/m²) and precipitation and resulting relative humidity (>70 %). The audit of this warning system is planned for summer 2017 in cooperation with the website ISIP (www.isip.de), which allows the exact calculation of weather data for a hop garden based on GPS and interpolation of data from a meteorological station.

The results of ISIP are currently screened at five different sites in the Hallertau. The comparison of the farmer's own common practice to a point of application based on weather data should help to connect the biology of the fungus, the potential of sporulation in the environment and the exogenous factors during the field season.

Forecasting systems should support the farmer in practicing resistance management against pests and diseases. This requires a wider range of active ingredients with different mode of actions and the optimal time for application. Thus, to achieve effective disease control and management, the biology of the fungus and its adaptive potential in the specific environment hop has to be better understood.

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Survival, persistence, and landscape-level development of Powdery mildew

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Abstract

Podosphaera macularis survives by means of infection of crown buds where the ascigerous stage is absent. Experiments were conducted to describe the overwintering process associated with bud infection and persistence of *P. macularis*. Buds of varying sizes remained susceptible to powdery mildew until late September to early October, with susceptibility decreasing substantially thereafter. Emergence of shoots with powdery mildew (flag shoots) was asynchronous in spring, although flag shoots were most numerous when plants were inoculated with *P. macularis* in early summer. Historical flag shoot data collected from Oregon and Washington were analyzed to identify factors associated with their occurrence. The occurrence and incidence of flag shoots was correlated with prior occurrence of flag shoots, grower pruning method, and winter temperature. Extensive sampling in Oregon during 2014 to 2017 found flag shoots in 24 of 490 yards evaluated. Of yards where flag shoots were found, 21 yards (87.5 %) were chemically pruned or not pruned; 2 (8.3 %) were mechanically pruned in mid-April after the initial emergence of flag shoots; and 1 (4.2 %) was mechanically pruned during March. Thus, the fungus appears to persist in a subset of chronically affected hop yards, primarily those not mechanically pruned. Primary inoculum dose of *P. macularis* also appears linked to epidemic severity of powdery mildew.

Key words. epidemiology, hop, *Humulus lupulus*, *Podosphaera macularis*, powdery mildew

Introduction

Perennation of Powdery mildew fungi may be by either the ascigerious stage (chasmothecia) or as mycelium in association with living host tissue. The latter may be due to the fungus surviving vegetatively on host tissue that does not become fully dormant, long distance dispersal, survival on an alternate host, or via invasion of dormant buds. Bud infection may lead to development of the so-called flag shoots, which have been described on various hosts, including hop. Bud perennation is particularly important on hop in the Pacific Northwest. *P. macularis* is heterothallic and chasmothecia occur in regions where both mating types of the fungus are present (WOLFENBARGER et al. 2015). However, only the MAT1-1 mating type is known to occur in the Pacific Northwest (WOLFENBARGER et al. 2015). This situation is particularly unique from a disease management perspective as practices that reduce bud perennation may be especially efficacious in delaying disease.

ROYLE (1978) noted that outbreaks of powdery mildew occurred earlier in the season when mechanical pruning of plants in spring was replaced with chemical desiccation of shoots, owing to development of flag shoots. LIYANAGE & ROYLE (1976) also reported that flag shoot development was associated with infection of buds later in the season (i.e., August). Field observations and experiments using potted hop plants kept in varying environments also indicated the number of flag shoots was related to temperature in winter (LIYANAGE & ROYLE 1976), similar to bud perennation of powdery mildew pathogens on other hosts.

Flag shoots occur at a low frequency on hop in the Pacific Northwest, being found on approximately 0.69 % of susceptible plants in Washington and only 0.02 % of susceptible plants in Oregon (GENT et al. 2008). Given the extraordinarily poor overwintering survival of the fungus, relatively few flag shoots actually provide the inoculum that incites regional disease epidemics. Furthermore, elimination of flag shoots potentially could have multiyear effects because in some perennial hosts the flag shoot development is associated with chronic infection of the same plants (YPEMA & GUBLER 2000).

In this research we sought to characterize when crown bud infection may occur and the association of infection timing with flag shoot development in the ensuing season. We also sought to identify risk factors for flag shoot development and quantify the subsequent spread of powdery mildew at the landscape level.

Material and methods

Susceptibility of crown buds to powdery mildew. Plants were produced in 4 liter pots and were deployed to an experimental farm in May of each of 2012, 2013, and 2014. Beginning in early July, the number and diameter of surface crown buds was determined regularly on a set of 20 plants. Bud assessments continued until mid-October. To determine the relative susceptibility of crown buds of various sizes over time, on each of three dates 25 pots were retrieved from the field site. On each of 5 plants, buds of varying size were identified and inoculated with *P. macularis*. After inoculation, plants were placed in a growth chamber maintained at 18°C; 10 to 12 days later the percent bud area colonized was quantified.

Flag shoot development. Over four years, studies were conducted using potted plants to determine when crown bud infection by *P. macularis* is most likely to lead to successful overwintering of the pathogen. The general procedure was to produce potted plants free of powdery mildew, deploy the plants to an isolated field site to simulate environmental conditions in a natural setting, sequentially inoculate batches of plants, overwinter the plants outdoors, and observe plants in the following season to quantify flag shoot development.

Beginning in February to early March of the following year, the number of shoots on the plants were counted every one to two weeks and inspected for the presence of powdery mildew. When the number of newly emerged flag shoots ceased to increase or became indistinguishable from secondary spread the foliage was desiccated with carfentrazone. Assessments continued for up to five more weeks as new shoots emerged.

Persistence of mycelia in and on overwintering buds. During July and October of 2015 and 2016, 30 to 40 additional plants were inoculated and overwintered outdoors. In the following November and January, 15 to 20 pots from each inoculation time (July and October) were selected arbitrarily. Surface crown buds on each pot were removed, enumerated, and examined to determine the number of infected bud scales.

Long-term data on flag shoots. A database was constructed that contained relevant historical data on flag shoot occurrence in commercial hop yards and production practices. The data base included 169 hop yards or plots in Oregon and 244 hop yards in Washington evaluated during 2000 to 2017. Historical weather data was obtained from the nearest regional weather station. Associations between flag shoot occurrence and incidence were assessed by correlation, the nonparametric K-S test, and mixed model analyses.

Intensive assessment of flag shoots in Oregon. To further understand where the powdery mildew fungus persists overwintering, during 2014 to 2017 intensive evaluations of flag shoots and powdery mildew levels were made in all hop yards in the eastern hop production regions of Marion County in Oregon. In each year, every commercial yard on all farms was assessed for flag shoots. This was a total of 108 yards in 2014, 122 in 2015, 124 in 2016, and 136 in 2017. Sampling was conducted during late March to mid-April. A similar sampling approach was followed in the same yards in May, June, and July to quantify disease progression at the landscape level.

Results

Susceptibility of crown buds to powdery mildew. Small surface crown buds were present by mid-July and both the number and size of the buds increased linearly over time in all years. Crown buds in all size classes were susceptible at varying levels to powdery mildew during inoculations in August and September, although their susceptibility decreased significantly when inoculated in October.

Flag shoot development. Flag shoots emerged over an extended period of time, in sync with plant growth in late winter and spring. In repeated measures analyses conducted by year, the number of flag shoots produced depended on the rating date ($P \leq 0.020$ in all years), although the effect of timing of inoculation during the previous year varied in a year-dependent manner. In general, the most flag shoots were produced when plants were inoculated earlier.

Persistence of mycelia in and on overwintering buds. In both years of the study, in November the incidence of buds with powdery mildew was similar in plants inoculated in July or October. However, by January the incidence of infected buds and bud scales was signifycantly greater in plants inoculated in July as compared to plants inoculated in October.

Long-term data on flag shoots. Several variables related to temperature in late autumn and early winter were significantly related to the presence of flag shoots in Washington, but not in Oregon. In Washington, temperature during October to February was associated with flag shoot occurrence (K-S test $P \leq 0.05$) although the strength of the association was greatest in December. There was weak evidence that December temperature was associated with flag shoot occurrence in Oregon.

Among disease related variables, in Washington there was a significant correlation between the incidence of plants with flag shoots and disease levels in the previous season (Spearman rank correlation $S = 0.25$; $P = 0.027$) and plants with flag shoots in the next season ($S = 0.16$; $P = 0.080$). In Oregon, the incidence of plants with flag shoots was correlated with disease levels in the previous year ($S = 0.29$; $P = 0.028$), disease levels on leaves in the current season ($S = 0.31$; $P = 0.002$), and also flag shoots in the next season (S=0.37; P=0.005). Therefore, in both Oregon and Washington there was some evidence for high disease levels in the previous year and prior occurrence of flag shoots to influence future flag shoot occurrence.

A mixed model analysis further indicated that prior season disease levels were positively associated with the occurrence of flag shoots, and also the likelihood of flag shoots occurring in the ensuing season. In Oregon, but not Washington, there was a general association between grower fungicide use patterns and the presence or absence of flag shoots. Growers made on average 1.8 more fungicide applications per season in yards where flag shoots were present versus yards where flag shoots were absent. This was associated with a 13 day delay in the timing of the first fungicide application.

The severity of powdery mildew on leaves was significantly associated with the thoroughness of pruning in Washington ($P = 0.149$), but less so in Oregon ($P = 0.363$). In both states, there was a trend for the incidence of plants with flag shoots in the following season to be influenced by the thoroughness of pruning in spring.

Intensive assessment of flag shoots in Oregon. Flag shoots were identified in seven yards in 2014, three in 2015, six yards in 2016, and seven yards in 2017. In all but three instances, flag shoots were detected in yards that were either not pruned or pruned by chemical desiccation. From these initial foci powdery mildew increased regionally, later being found in 35 to 68% of yards at the landscape level.

Over the four years of the assessments, where flag shoots occurred previously was associated with the likelihood of subsequent flag shoot occurrence (Table 1). The odds of a flag shoot occurring in a given yard was significantly associated with prior occurrence of a flag shoot on the same farm, in a yard adjacent to where a flag shoot occurred previously, or, most strongly, prior occurrence of a flag shoot in that yard.

Discussion

Successful bud perennation by *P. macularis* requires the coincident occurrence of susceptible crown buds, presence of the pathogen, and environmental conditions permissive for infection and persistence of the fungus. The intersection of these factors occurs during early summer to early autumn, with buds remaining most susceptibile during approximately 10 weeks after bloom. Therefore, the overall tendency was for earlier inoculation to lead to more flag shoots; however, even the latest inoculation timing did not preclude flag shoot development. *P. macularis* declined more rapidly and survived at lower levels in buds infected in October of the preceding year as compared to July. Successful bud perennation appears to require infection that penetrates multiple bud scales before bud susceptibility declines in early October with the onset of dormancy.

Flag shoot occurrence is associated with severe powdery mildew in the previous season, which itself is associated with the occurrence and density of flag shoots in that season. Chronic occurrence of flag shoots also is evidenced in both Oregon and Washington. This cycle seems to be interrupted, at some level, by elimination of flag shoots by thorough pruning, particularly mechanical pruning. However, the overall efficacy of this strategy seems to vary by state, potentially reflecting differences in inoculum density. In Oregon, powdery mildew flag shoots occur at lower incidence than in Washington and some yards escape the disease entirely because inoculum density appears to be limiting to epidemic development. Because of greater overwintering of the fungus in Washington, and thus greater potential for dispersal within and among yards, the overall impact of sanitation measures on flag shoot development and persistence appears to be less. Nonetheless, targeted management of flag shoots in a subset of hop yards could delay disease development on foliage. The benefit of sanitation measures could be magnified at the landscape level due to the ability of the pathogen to disperse over long distances.

Table 1. Association between previous occurrence of a powdery mildew flag shoot and risk of flag shoot development in the subsequent year in Oregon

Risk factor	Increased odds of a flag shoot in a given yard	AUROC curve[a]
Flag shoot present on same farm last year	11.1	0.76
Flag shoot present in same or adjacent yard last year	7.2	0.69
Flag shoot in same yard last year	29.2	0.73

[a] Data is from evaluation of 490 hop yards in Oregon during 2014 to 2017.

[b] Area under the receiver operating characteristic curve. This statistic ranges from 0 to 1 and provides a measure of overall predictive accuracy, with 1 being perfect prediction.

Acknowledgement

Financial support was provided by USDA-ARS CRIS 5358-21000-040-00D, the Oregon Hop Commission, Washington State University, the Hop Research Council, Washington State Commission on Pesticide Registrations, Washington State Department of Agriculture Specialty Crops Research Block Grant program, and the Western IPM Center.

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VI: Entomology and chemoecology

Two-spotted spider mite, *Tetranychus urticae* Koch, has developed resistances to chemical acaricides in Slovenian hop gardens

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Abstract

Two-spotted spider mite, *Tetranychus urticae* Koch, is a polyphagous pest that attacks more than 200 different plant species. Among these, the hop (*Humulus lupulus* L.) is an important host plant and subject to severe economic damage caused by spider mites in all hop-growing regions of the world. Depending on weather conditions, a complete developmental cycle of *T. urticae* may be completed in 7-20 days. The various generations overlap and all stages (eggs, larvae, protonymph, deutonymph, and adults) can be found on hop leaves from late spring to late summer (i.e., from May to September). Economic damage is chiefly caused spider mites feeding on cones, resulting in dry, brittle, red cones with both reduced quality and quantity of hop yield. Hot and dry weather conditions favour the development of severe infestations on hop. Spider mites are able to develop more than 10 generations during the vegetation period on hops in Slovenia. These rapidly developing generations are the main reason for the development of resistances to chemical acaricides in Two-spotted spider mites.

Spider mite control in Slovenian hop gardens is exclusively based on foliar applications of acaricides. The availability of registered acaricides is varying from year to year and in average two acaricide sprays per growing season are applied in Slovenian hop gardens. For more than 20 years, plant protection against *T. urticae* in Slovenian hop gardens was solely based on two compounds only, a.i. abamectin (Vertimec 1.8 EC) and a.i. hexythiazox (Nissorun 10 WP).

However, during the last two growing seasons a decrease in the efficacy of both active ingredients to control spider mites was observed in Slovenian hop gardens, especially concerning abamectin. In preliminary laboratory tests we detected the same low efficacy of abamectin. The efficacy of various other active ingredients will be tested in future in laboratory experiments on spider mite populations collected from different Slovenian hop growing regions.

Currently Slovenia does not have enough effective and registered active substances of acaricides for the control of spider mites in hop production. New active ingredients for a successful control of spider mites, with different mode of action by IRAC classification, are urgently needed in future in the framework of an efficient Integrated Pest Management.

Flea-beetle control in organic hops: Are there options?

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Abstract

In earlier days of hop cultivation, hop flea-beetles were considered one of the two major pests of this crop (e.g., HEIKERTINGER 1913; NEWTON 1929). Subsequently, excessive use of non-selective insecticides during several decades of the 20th century had repressed hop-flea beetles to an almost negligent level. However, the recent ban of most according compounds has led to a renaissance of this pest in hops, at least if they are managed in an organic regime. Therefore an environmentally friendly control method for hop flea-beetles is badly needed not only in organic hops, but under consideration of the constant loss of registered insecticides also as a 'Plan B' for conventional growers.

In the Palaearctic, the leaf-beetle called "hop flea-beetle" means the species *Psylliodes attenuatus* (Koch, 1803) (Chrysomelidae, Alticinae). It should be however taken into account that a small percentage (in the Hallertau approx. 5 %) of flea-beetles attacking hops are *Chaetocnema concinna* (Marsham, 1802). As soon as the first hop shoots appear above ground in spring, the beetles begin feeding on young vines and leaves. Typically, this injury gives the leaves the appearance of being covered with fine shot holes. In bad cases, many leaves are skeletonised, photosynthesis may be reduced and growth retarded. However, the infestation is usually confined to the lower 1 to 2 m of the bines and the rapid growth of hop plants in spring normally allows them to outgrow the damage relatively quickly. Later in the season, from July onwards, the newly emerged generation of adult beetles can feed on the bracteoles of young cones up to a scaffold height of 5-6 m and can cause a significant loss of yield. It has been reported that in some cases this damage caused the complete destruction of a crop (e.g., CHITTENDEN 1909), but this degree of damage however is extremely rare.

First results from 2016 indicate that the number of flea-beetles produced in the investigated organic hop garden was 1.2 million individuals per hectare or 600 individuals per plant. Therefore, all hitherto tested mechanical measures for reduction of imaginal beetles can be classified as 'actionism' and did not lead to a significant reduction of flea-beetles. The most elegant control method would be yellow Moericke's traps combined with a species-specific attractant for *P. attenuatus*, which has not been identified yet. However, in some instances with linalool as a lure more beetles were caught in the traps in the field as compared to other tested volatiles.

Key words. Psylliodes attenuatus, Alticinae, pest control.

Acknowledgement

This study was supported by Bavarian State Ministry for Food, Agriculture and Forests in the framework of the aid programme 'BioRegio Bayern 2020'.

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Elucidation of the role of volatile compounds in the chemical communication of the hop flea beetle *Psylliodes attenuatus*

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Abstract

The hop flea beetle *Psylliodes attenuatus* is an emerging pest in hop. In spring the overwintering adults can damage the leaves while in summer the next adult generation can cause severe damage on the cones resulting in a loss of yield. Currently, no biological management of this pest is available causing challenges particularly in organic hops. In this study we aimed to elucidate volatile organic compounds produced specifically by the beetles or by the host plants and may be exploited for trapping beetles in the field. Analyses of the headspace of infested hop resulted in a number of candidate volatile compounds that might be produced by the beetles. Some of these candidate compounds are biochemically related to male specific aggregation pheromones of other flea beetle species.

Key words. Pheromone, kairomone, headspace analysis, gas chromatography mass spectrometry

Introduction

The hop flea beetle, *Psylliodes attenuatus* (Koch) (Chrysomelidae), is an emerging pest in hop culture. The main damage is caused by the 2-3 mm big adults and happens mainly in two phases. First, in the early spring the overwintering adults emerge and feed on the young hop shoots. Severe damage can result in a retarded growth of the plants. Even more problematic is the damage caused by the following adult generation emerging in the summer, which prevalently feeds on the hop cones (WEIHRAUCH 2009). This damage can result in significant loss of yield. Currently, pest management strategies are based on applying insecticides as no tools are currently available that would enable a proper biological control of the flea beetles. The latter would, however, be essential for a sustainable production of organic hops without synthetic pesticide use.

In this study we aimed to identify volatile cues involved in the chemical communication of *P. attenuatus* that may be exploited for trapping beetles in the field either for monitoring or mass trapping purposes. Many phytophagous insects use volatile organic compounds (VOCs) emitted by their host plants (so called kairomones) to locate suitable feeding spots (SCHOONHOVEN et al. 2005). In addition, insects often produce so called pheromones in order to find mating partners. These mainly VOCs are either produced specifically by either males or females to attract the other sex or can also be produced by both sexes. Recently, an aggregation pheromone that is specifically produced by males of the closely related flea beetle *Phyllotreta striolata* has been described that also interacts with kairomones from the host plant (BERAN et al. 2011, 2016).

We used a combined chemical and behavioural approach to investigate whether certain volatile compounds are produced by males and/or females of *P. attenuatus* that are attractive to the same or the other gender. We developed a non-invasive method to sex flea beetles. VOCs from both females and males and a combination of both were collected by dynamic headspace trapping and extracts were analysed by coupled gas chromatography mass spectrometry. In addition, we also analysed total extracts from both female and male beetles.

Material and methods

Plants and beetles. Hop plants used for experiments were obtained by the Bavarian State Research Center for Agriculture. Beetles were collected from an organic hop field near Wolnzach, Bavaria. Beetles were kept in aerated petri dishes at 10°C and provided with hop leaves until used for experiments. To distinguish the gender, beetles were anesthetized with CO_2 and were then sexed based on the different shape of the abdomen tip under a binocular.

Volatile collection. Volatiles emitted by hop plants and beetles were collected by dynamic headspace trapping using a setup described by BAARS et al. (2011). In a first experiment plants were either uninfested or infested with either 60 beetles (males and females mixed). Infested and control plants kept in glass jars in a climate cabinet at 20°C and a 16:8h LD regime for 5 days. Every day volatiles from both 4 infested and 2 uninfested plants were collected for 8 hrs by sucking air out of the jars during the photoperiod and for 16 hrs during the scotoperiod on cartridges filled with 200mg of Tenax TA as adsorbent. Experiments were replicated 4 times. In a second experiment VOCs of uninfested hop leaves or leaves infested with either 30 male or 30 female *P. attenuatus*, a mix of both or were collected. VOC trapping was done in 10mL glass vials under conditions as described above. Volatiles were trapped as aforementioned for 6 hrs during the photoperiod.

GC-MS and data analysis. Headspace samples were analyzed GC-MS by thermally desorbing the Tenax cartridges similarly as described by HUIGENS et al. (2011). Raw GC-MS data were processed using an untargeted metabolomics approach that was developed at WUR (MUMM et al. 2016). Processed data were analyzed by Principal component analysis (PCA) using the software SIMCA-P (14.0).

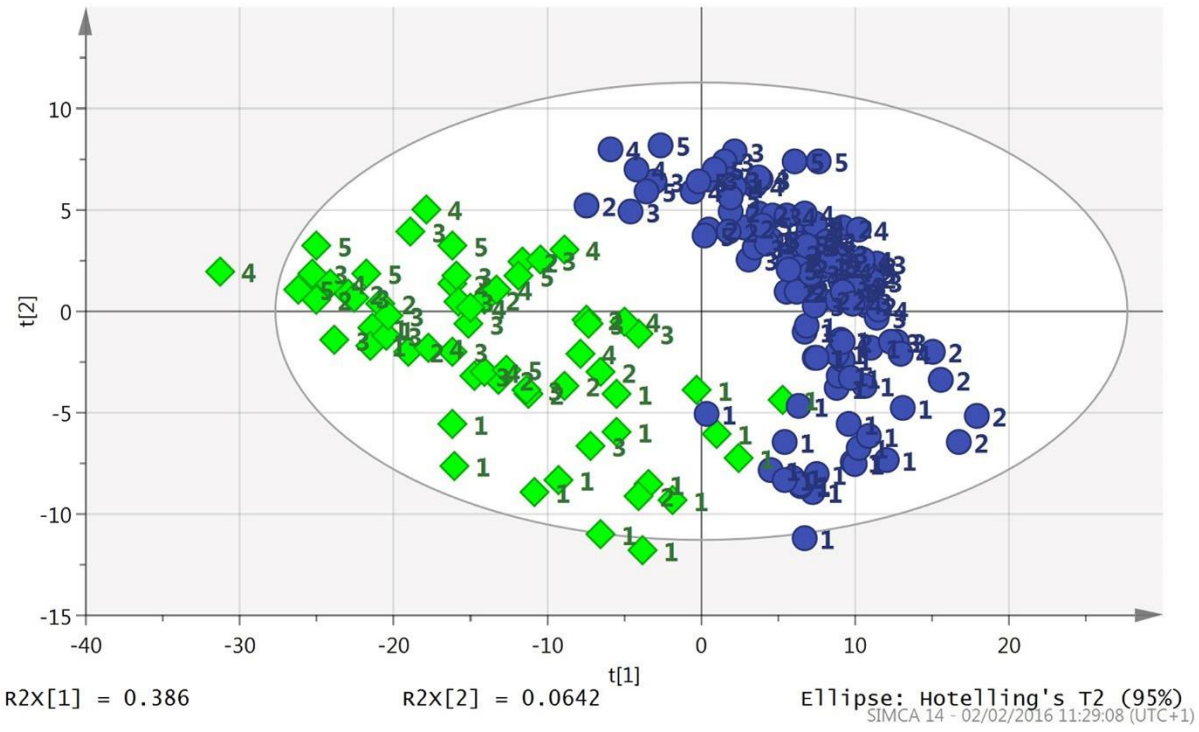

Figure 1. PCA score plot of VOCs blends of uninfested (diamonds) and hop plants infested with *P. attenuatus* (circles) during the photoperiod. PC1 vs PC2 is given explaining 45 % of the total variation. Numbers indicate the day after infestation. Ellipse indicates the 95 % Hotellings-T^2 confidence interval.

Results and Discussion

Multivariate analysis of 250 VOCs detected in a total of 186 samples of uninfested and infested hop plants showed that the presence of beetles induced clear changes in the VOC blends already from day one onwards (Fig. 1). These changes can most likely be attributed to the feeding activity of the beetles because the detected VOCs were mainly of plant origin emitted due to the wounding of the plant tissue. There was no significant difference in the pattern of the VOC blends collected during the scotoperiod (not shown). Because the differences between infested and uninfested plants were so pronounced and dominated by VOCs from the plants it was difficult to detect a possible pheromonal signal that was produced by the beetles. Therefore, we performed another experiment where we reduced the plant biomass to a minimum in order to detect potential pheromone compound more easily.

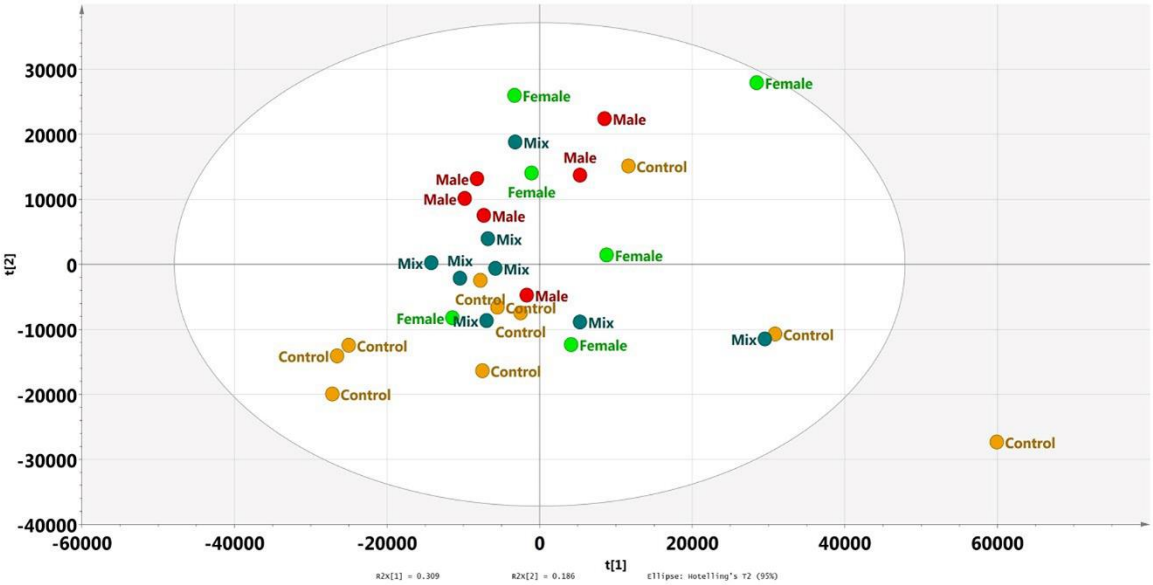

Figure 2. PCA score plot of VOCs blends of uninfested (control) hop or infested with either male, female *P. attenuatus* or a combination of both. PC1 vs PC2 is given explaining 49.6% of the total variation.

A total of 291 VOCs were detected 30 in samples. The majority of VOCs likely are emitted by the plants. PCA did not indicate a clear separation of the VOC profiles of leaves infested with males, females or a combination of both compared to the control plants (Fig. 2). This may not be surprising as one or few pheromones emitted by the beetles will not have a strong influence on a PCA being dominated by plant VOCs. The data were subsequently scrutinized by visual inspection and subjected to classification techniques to find candidate VOCs. Five VOCs having an interesting differential pattern between control samples and those with beetles are given in Figure 3. Four out of five compounds have a terpenoid signature of which some of them could be tentatively identified based on their mass spectra and retention index. One seems to be a long chained alcohol. This compound is exclusively detected when males are present although it was not found in all samples. Three of the four terpenoid compounds were not detected in the non-infested control samples. Although it is not clear whether these terpenoids are produced by the beetles or are induced in the plant by feeding these compounds have been described in related species. For example, male specific aggregation pheromones of several flea beetle species of genus *Phyllotreta* have been shown to be sesquiterpenes (BARTELT et al. 2011; BERAN et al. 2011, 2016; TÓTH et al. 2012) similar as VOC shown in Figure 3a-c from this study. Interestingly, the attractiveness of some of them is synergistically increased when offered with certain cues originating from the host plant.

Future studies will need to focus on a more detailed elucidation of the candidate VOC found in the headspace of hop plants being infested with males or females of *P. attenuatus*. Their

potential bioactivity should be tested in bioassays incorporating the possibility of a synergism of a pheromone and a kairomone coming from the host plant.

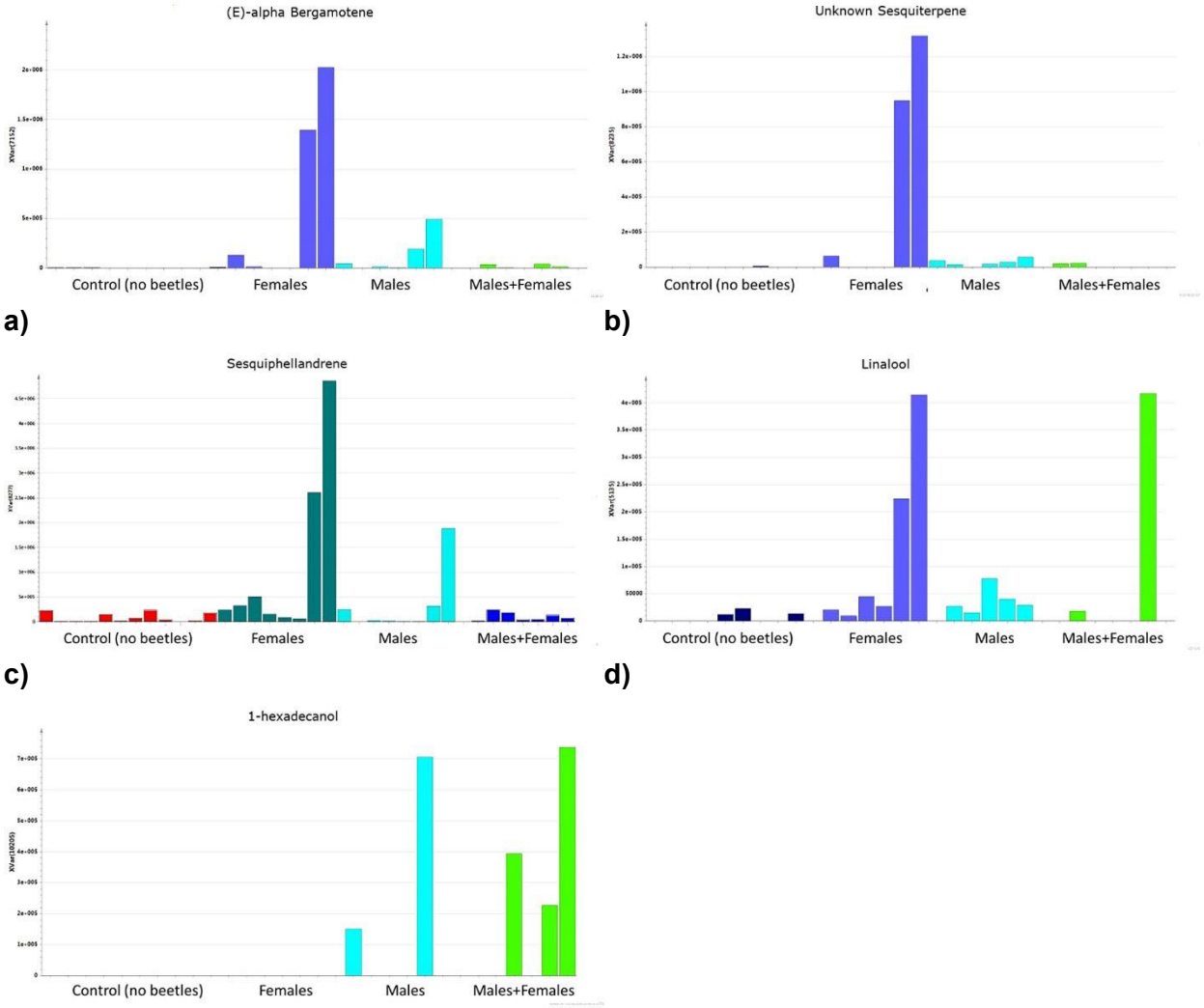

Figure 3a-e: Profiles of different VOCs showing interesting emission pattern across the tested samples.

Acknowledgement

The authors thank Antje de Bruin, Gerrie Wiegers, Leo Poleij and Jacques Davies for their support with the experiments, Anna Baumgartner, Daniel Eisenbraun and Silvana Wolf for the collecting of flea-beetles and Jutta Kneidl for constant supply with hop plants. This study was supported by Bavarian State Ministry for Food, Agriculture and Forests in the framework of the aid programme 'BioRegio Bayern 2020'.

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The use of metabolomics to elucidate resistance markers against Damson-hop aphid

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Abstract

Hop is very rich in secondary metabolites such as bitter acids (alpha-acids), prenylflavonoids and essential oils, which contribute to hop flavor and aroma. The vast majority of hop secondary metabolites are found in the cones, why phytochemical analyses of hop have focused on cone material. Importantly, the winged females (*alatae*) of the Damson hop-aphid *Phorodon humuli* colonise hop as their summer host long before the cones have been formed. Therefore, the leaf metabolites are responsible for host selection behavior during the aphid's spring migration.

In this study we profiled apolar secondary metabolites with GC-MS in leaves of a range of hop genotypes, representing a range of aphid susceptibility. Our objective was to correlate the leaf metabolome and aphid behavior. To validate this approached the metabolic changes occurring during aphids infestation was compared in 20 genotypes gown in the field and green house at two time points. Significant metabolic changes induced by aphid infestation were only detected in early summer. After statistical analysis we estblished a good correlation between specific terpenoids and aphids resistance in hop.

Key words. Aphids, resistance, metabolomics, terpenoids.

Acknowledgement

This work was supported by Erzeugergemeinschaft HVG e.G., Wolnzach, Germany.

The full manuscript to this abstract has been submitted to a peer-reviewed journal.

VII: Hops and brewing

Characterization of odor-active compounds in Huell Melon and Polaris hops

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Abstract

The volatiles isolated from samples of the German flavor hop varieties Huell Melon and Polaris were isolated by solvent extraction and solvent assisted flavor evaporation and screened for odor-active compounds by application of an aroma extract dilution analyses (AEDA). Results revealed myrcene, linalool, 2- and 3-methylbutanoic acid, and geraniol as most potent odorants in both hop varieties. Differences in the FD factors between the two flavor hops suggested that the intense fruity, cantaloupe-like aroma of Huell Melon is due to the presence of high concentrations of the fruity smelling esters ethyl 2-methylpropanoate, ethyl 2-methylbutanoate, and propyl 2-methylbutanoate, whereas fruity, banana-like smelling 3-methylbutyl acetate and eucalyptus-like smelling 1,8-cineole account for the characteristic aroma of Polaris hops. Quantitation assays using stable isotopically substituted analogues of the target compounds as internal standards confirmed the AEDA data.

Key words. Hop aroma, Huell Melon, Polaris, AEDA, SIDA, 1,8-cineole.

Introduction

Driven by the demand of the US craft brewers community for hops with strong and extraordinary aroma characteristics, so-called flavor hops were first developed and marketed in the United States. Inspired by the success of American flavor hops, the team at the Hop Research Center in Hüll, Germany started breeding trials targeted at German flavor hop varieties in 2006. Traditional German aroma hops were crossed with North American hop varieties. Five years later, eight promising "Hüller Special Flavor Hops" were presented to the public. Four of them were registered and released for cultivation. Among them were the variety Huell Melon, characterized by intense fruity, cantaloupe-like aroma notes, and the variety Polaris, described as fruity with a pronounced minty note (LUTZ et al. 2012; SEIGNER et al. 2012).

The aim of the present study was 1) to gently isolate the volatiles from samples of the two German flavor hop varieties Huell Melon and Polaris using a mild, artifact-avoiding workup procedure based on solvent extraction and solvent assisted flavor evaporation (SAFE) (ENGEL et al., 1999), 2) to screen the volatile fraction for odor-active compounds by application of an aroma extract dilution analysis (AEDA) (SCHIEBERLE & GROSCH 1987a), 3) to structurally identify the most potent odor-active compounds, i.e. the compounds exhibiting the highest flavor dilution (FD) factors and 4) to objectify FD factor differences found by quantitation using stable isotope dilution assays (SIDA) (SCHIEBERLE & GROSCH 1987b).

Material and methods

Hops were obtained as pellets, type 90, from HHV (Mainburg, Germany) and stored at -20°C. Harvest year was 2014 for Huell Melon and 2012 for Polaris.

For the screening experiments, the pellets were cryomilled at the temperature of liquid nitrogen. The resulting hop powder was extracted with dichloromethane and nonvolatiles were removed by SAFE. The distillate was concentrated (1 mL) and subjected to AEDA. FD factors were determined as the dilution factor of the highest dilution in which the respective odorant was detected by GC-O.

The structures of the odor-active compounds were assigned by comparison of their retention indices on two columns of different polarity, their odor properties as perceived at the sniffing port and their mass spectra in EI and CI modes with data of authentic reference compounds recorded under the same conditions.

The quantitation assays started with the addition of isotopically substituted standards and solvent to hop powder, followed by anhydrous sodium sulfate. Solvent was dichloromethane except for the quantitation of esters, where diethyl ether was employed. After stirring (≥3 h), the mixture was filtered, nonvolatile material was removed by SAFE, and the distillates were concentrated. Except for the quantitation of linalool, volatile hydrocarbons were removed by silica gel chromatography (STEINHAUS 2015) before GC-MS.

GC-MS measurements were accomplished either by comprehensive two-dimensional gas chromatography-mass spectrometry using a GC×GC-TOFMS system (1,8-cineole) or by two-dimensional heart-cut gas chromatography-mass spectrometry using a GC-GC-ITDMS system (other compounds) (REGLITZ & STEINHAUS 2017).

Results

Application of the AEDA revealed a total of 40 odor-active compounds in the hop samples of the varieties Huell Melon and Polaris. FD factors ranged from 16 to 2048. High FD factors in both varieties were found for geranium leaf-like smelling myrcene, citrusy, bergamot-like smelling linalool, cheesy smelling 2- and 3-methylbutanoic acids, geranium leaf-like smelling (5*Z*)-octa-1,5-dien-3-one, flowery, rose-like smelling geraniol, and black currant-like smelling 4-mercapto-4-methylpentan-2-one. Myrcene, linalool, and 2- and 3-methylbutanoic acids are well established odor-active hop constituents (STEINHAUS & SCHIEBERLE 2000; STEINHAUS et al. 2007). 4-Mercapto-4-methylpentan-2-one was first identified in Cascade hops and shown to be responsible for the typical black currant-like aroma note characterizing this variety (STEINHAUS & SCHIEBERLE 2007).

In the Huell Melon sample, outstanding high FD factors were additionally found for the fruity smelling compounds ethyl 2-methylpropanoate, ethyl 2-methylbutanoate, and propyl 2-methylbutanoate. These esters were also detected in Polaris, but FD factors were clearly lower. These results suggested that ethyl 2-methylpropanoate, ethyl 2-methylbutanoate, and propyl 2-methylbutanoate vitally contributed to the strong fruity, cantaloupe-like aroma of the Huell Melon hops. The fruity, minty aroma of Polaris, on the other hand, corresponded to the presence of odor-active amounts of banana-like smelling 3-methylbutyl acetate and eucalyptus-like smelling 1,8-cineole. Both compounds were not detected in the Huell Melon extract.

To objectify the AEDA results, selected odor-active compounds were quantitated in the samples of both varieties by using SIDAs. Concentrations of linalool and geraniol ranged between 10 and 90 mg/kg. In Polaris, concentrations of both compounds were clearly higher than in Huell Melon (Fig. 1). Quantitation of the odor-active esters confirmed the results of the AEDA experiments. In the Huell Melon sample, extraordinary high concentrations of ethyl 2-methylpropanoate, ethyl 2-methylbutanoate, and propyl 2-methylbutanoate were found. Concentrations were up to 100 times higher than the respective concentrations in the Polaris sample. Data thus confirmed the role of these three esters for the pronounced fruity note in the sensory profile of Huell Melon. By contrast, 3-methylbutyl acetate, but also methyl 2-methylbutanoate concentrations were clearly higher in Polaris. The same was found for 1,8-cineole, which was present at a level of 63 µg/kg in Polaris, whereas the concentration in the Huell Melon sample amounted to only 1 µg/kg.

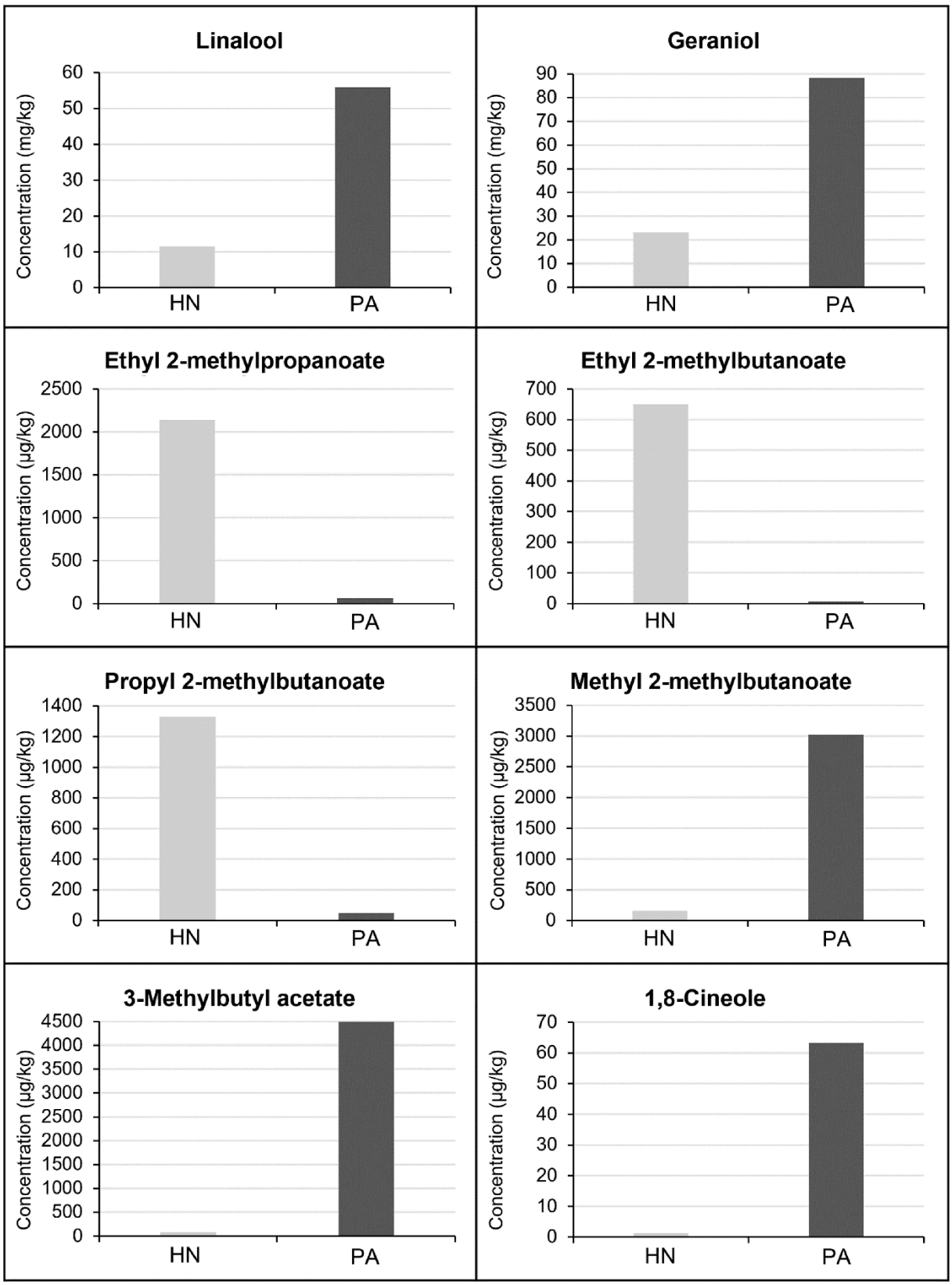

Figure 1. Concentrations of linalool, geraniol, ethyl 2-methylpropanoate, ethyl 2-methylbutanoate, propyl 2-methylbutanoate, methyl 2-methylbutanoate, 3-methylbutyl acetate, and 1,8-cineole in samples of the hop varieties Huell Melon (HN) and Polaris (PA)

Discussion

The SIDA quantitations clearly confirmed the results of the screening experiments. In summary, the intense fruity, cantaloupe-like aroma of Huell Melon could be ascribed to high concentrations of the fruity smelling esters ethyl 2-methylpropanoate, ethyl 2-methylbutanoate, and propyl 2-methylbutanoate, whereas high concentrations of fruity, banana-like smelling 3-methylbutyl acetate and eucalyptus-like smelling 1,8-cineole were shown to be responsible for the characteristic aroma of Polaris hops.

Acknowledgement

This IGF Project of the FEI was supported via AiF within the programme for promoting the Industrial Collective Research (IGF) of the German Ministry of Economic Affairs and Energy (BMWi), based on a resolution of the German Parliament. Project-Nr.: AiF 18069 N.

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Procedure for variety substitution taking the example of Herkules and Polaris

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Abstract

Polaris was registered in 2012 as a special flavor hop variety. However, due to its high α-acid content (approx. 18–20 %) it is also interesting as a high alpha variety. Furthermore, in the Elbe-Saale growing region Polaris thrives better than Herkules. It was therefore interesting to compare Herkules, the currently leading bitter variety in Europe, with Polaris. Regarding the basic analysis Polaris differs from Herkules particularly in α-contents (18.6 vs 16.7 %), cohumulone level (26 vs 35 % rel.) and hop oil (1.7 vs 3.2 ml/100 g). In the composition of the aroma substances Polaris has a greater aroma potential.

In trial brews both varieties were added at begin of boil. The following differences could be determined: 1) The iso-cohumulone ratio of the Polaris beers was with an average of 34.6 % significantly lower than that of Herkules beers with 44.3 %. 2)The content of some hop esters was decidedly more prominent in the Polaris beer than in the Herkules beer. Sensorily the Polaris beers did slightly better than the Herkules beers: 1) In the comparison of the Hallertau hops Polaris was preferred significantly more and was assessed as having a more intensive aroma. 2) The bitterness of the Polaris beers had a tendency to be assessed as better. Therefore, what regards quality, Polaris is a viable alternative to Herkules as a bitter hop.

Key words. Hop varieties, bitter hops, differentiation of hop varieties

Introduction

Herkules (HS) is a German high α-breed, which currently has the greatest acreage in Germany with approx. 5,860 ha in 2017. In only few years it has significantly surpassed Magnum and Taurus, the formerly leading high α-varieties in the Hallertau growing region. Furthermore, Herkules has now become an established bitter variety on the world market and is convincing not only in its agronomic properties of yield and bitterness but also in its quality. Herkules is currently the benchmark for European high α-hops.

In 2012, due to its very specific aroma, Polaris (PA) received official approval as a “Special Flavor Hop”. Its growing area in the Hallertau and the Elbe-Saale region covered approx. 140 ha in 2017. However, the PA α-acid content of almost 20 % is so high that one could consider using it as a bitter hop for an early addition.

Data about growing areas and yields are available in the annual reports of the Society of Hop Research (GfH; online at http://www.lfl.bayern.de/publikationen/). Information about agrotechnical data and chemical components of both varieties are listed by BIENDL et al. (2014) and GERMAN HOP GROWERS ASSOCIATION (2016).

Task

The present work deals with the differences between both varieties in the major substance groups with a differentiation between the Hallertau and Elbe-Saale growing regions. A description of the brewing properties of both varieties is based on six trial brews. Two comparisons were made with pellets of both growing regions and one with CO_2 extract.

Components of both varieties

An overview on the major components of both varieties is shown in Table 1.

Table 1.		HS	PA
α-acids (EBC 7.4)	%-w/w	16.6	18.6
β- : α-acids (EBC 7.7)		0.3	0.3
Cohumulone ratio (EBC 7.7)	%-rel.	35	26
Polyphenols (EBC 7.14)	%-w/w	4.1	4.0
Xanthohumol (EBC 7.7)	%-w/w	0.62	0.79
Total Oil (EBC 7.10)	ml/100 g	1.7	3.2

Two differences are noticeable: 1) The cohumulone ratio relative to the total humulone is significantly lower in Polaris with 26 % than in Herkules with 35 %; 2) Polaris contains about two times as much hop oil as Herkules. No great differences are however apparent neither in the total polyphenols nor in the xanthohumol content. Table 2 shows the results of major aroma components of both varieties from both growing regions (H=Hallertau; E=Elbe-Saale) in mg/100g.

Table 2.	HHS	EHS	HPA	EPA
Myrcene	850	728	1938	1659
β-Caryophyllene	86	101	236	179
α-Humulene	250	307	592	417
Hydrocarbon fraction	**1486**	**1317**	**3004**	**2453**
Isobutylisobutyrate	18	13	26	21
Isoamylpropanoate	12	8	24	17
Isoamyl-3-methylpropanoate	8	5	17	14
Isoamyl-2-methylpropanoate	66	41	74	56
Methylheptanoate	7	5	14	10
Methyloctanoate	4	4	61	55
Methyl-4,8-decadienoate	12	10	29	22
Octylisobutanoate	4	2	50	41
Sum of 8 esters	**131**	**88**	**295**	**236**
Linalool	6	6	8	7
Geraniol	5	8	8	5
Geranylacetate	1	0.2	17	11
Oxygen fraction	**258**	**206**	**462**	**368**

A significantly higher dosage of mono- and sesquiterpenes that are volatile in steam, like myrcene, β-caryophyllene and humulene at the beginning of the boil has no effect on the beer aroma. The oxygen fraction which dissolves better could help towards a somewhat fruitier beer aroma with a plus of 80 % with Polaris as compared to Herkules. The values for the Elbe-Saale region are slightly lower. Since only single samples were examined, the data gathered is without substantial backup.

Methyl octanoate, octyl isobutanoate, geranyl acetate and geranyl isobutyrate content can be taken as examples for particularly striking variety-specific 15 to 23 times higher contents in Polaris than in Herkules.

Trial brews

Six brews with the following characteristics were brewed in the 2 hl St. Johann Research Brewery:

- Bottom-fermented lager beer with an original extract of 11.5–12.0 %
- Targeted bitterness of 20 IBU in the pellet beers and 15 IBU in the extract beers
- Hop addition at the beginning of the boil

The Herkules and Polaris beers differ only in their iso- cohumulone ratio. With an average of 34.6 %, Polaris beers were significantly lower than the Herkules beers with 44.3 %.

There are no differences between the linalool content of the varieties, which however can also be explained by the minimal differences in the hops. Remarkable is the four times higher content of four selected hop esters in the Polaris beer as compared to the Herkules beer (Hallertau growing region).

All the beers were tasted in pairs by 29 panelists each. The pellet beers with the Hallertau samples could be clearly differentiated. The Polaris beer performed better than the Herkules beer regarding aroma and preference. There was a slight tendency to prefer the bitterness of the Polaris beers. The tastings of the Elbe-Saale beers showed a slight preference for the Polaris beer, which was due to the more intensive aroma and a more pleasant bitterness. Thus, sensorily the Polaris beers had the slight edge over the Herkules beers, when pellets were dosed. No significant differences occurred with extract beers.

Conclusion

Polaris was officially released in 2012 and initially introduced as a “Special Flavor Hop”. Due to its high α acid values of 18- 20 % it has now also gained interest as a bitter hop. At some growing sites PA proved to be more suitable than HS, in particular in the Elbe-Saale region. There are differences between PA and HS in the spectrum of components:

- The cohumulone ratio is lower in PA (26 % rel. vs 35 % in HS).
- PA contains not only significantly greater quantities of aroma components, but is also different in the spectrum. Its unmistakable aroma was and continues to be the reason to use it also for dry hopping. There is a chance – depending on the brewery – that even with early hop additions a trace of fruity aroma survives the brewing process.
- Polaris beers show a lower iso-cohumulone ratio than Herkules beers, which can be considered to be a rather positive characteristic. In comparisons, sensorily the Polaris beers were on one occasion significantly better (Hallertau) and on another slightly better (Elbe-Saale). The extract beers showed no difference.

From a brewing viewpoint, Polaris is therefore on a par with Herkules. Some aspects make it even more favorable. Even with additions at the beginning of the boil, sometimes the more intensive aroma of Polaris is noticeable as a slightly fruity scent in the final beer.

The presented results have been published previously by FORSTER & GAHR (2016, 2017).

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Description and classification of two “new” Hüll aroma breeding varieties

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Abstract

In the past 13 years no new aroma varieties have been approved in Germany for traditional hopping during the wort boil. Of the 20 breeds last approved worldwide 18 are "special flavor hops" in particular for dry hopping. Interest was lost for the "normal" aroma hops despite the fact that promising varieties are in the pipeline.

We consider two of these breeding lines (89/25 and 96/24) to be interesting, especially the line 89/25. Both have as mother hops of the Saaz group of varieties (89, Spalt; 96, Tettnang). In a brewing series both breeds were compared with their mothers and Saphir. The dosage was made at end of boil according to hop oil content (6 ml/hl).

The breeding lines differ essentially through lower polyphenol content and a greater aroma potential. In particular the 89/25 is significantly richer in well soluble hop esters and linalool. These characteristics can also be found in the beers. The ester content is spread in a range from 18-55 µg/l with Tettnanger, Spalter and the 96 line via 180 µg/l with Saphir to 220 µg/l with the 89 line. The linalool values range from 134 µg/l (Tettnanger) via approx. 150 µg/l (96 line, Spalter and Saphir) to 215 µg/l with the 89 line.

The beers had sensory differences. The 89 beer was preferred significantly. Especially 89/25 is a promising candidate for registration as a new aroma variety for late hopping purposes.

Key words: Hop varieties, aroma hops, hop aroma substances, hop aroma in beer

Introduction

At present new hop varieties with unique aroma and flavour are popping up like flowers at springtime. But many of those are emerging from a rush job in order not to miss the “Flavour Hop Train”. However, it must not be forgotten that there might be a need for classical aroma varieties as well in the future, no matter if quality, efficiency (aroma yield), yield (kg/ha) or disease and climate tolerance will be the driving factors.

Although a lot of new flavour varieties were released by the Hop Research Center in Huell, Bavaria, the last few years (Polaris, Mandarina Bavaria, Huell Melon, Hallertau Blanc, Ariana and Callista) there are still promising “normal” aroma breeding lines in the experimental gardens that have a number only. On two of those we want to draw your attention to: 89/002/025 and 96/001/024.

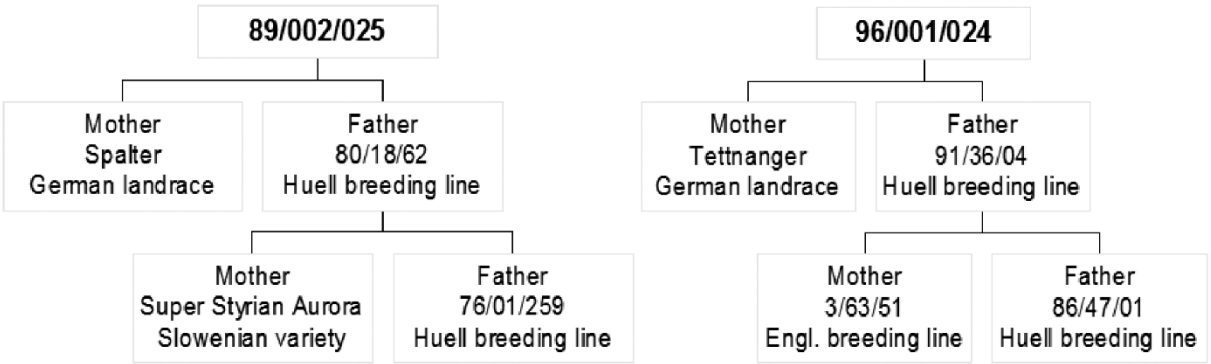

Figure 1. Pedigree of 89/002/025 and 96/001/024 (source: LfL, Huell).

Hop analyses

Table 1 shows analytical data including the total polyphenols and the sum of low molecular polyphenols (HPLC). The breeding lines are lower in total and esp. in the low molecular polyphenols.

Table 1. Hop Analyses of the two experimental varieties compared to the two mother varieties Tettnanger (TTE) and Spalter (SSP) and Saphir (HSR); LM:TP ratio = indicator for low molecular character of the polyphenols

		89/25	96/24	SSP	TTE	HSR
α-acids	% w/w	5.3	4.1	3.7	3.3	2.8
β : α		0.89	1.00	1.86	1.55	2.11
Cohumulone ratio	% rel.	22	23	26	25	14
Hop oil	ml/100g	1.70	1.75	0.70	0.60	0.95
Xathohumol	% w/w	0.42	0.44	0.46	0.32	0.38
Sum of low molecular-weight polyphenols (LM)	mg/100g	1181	1007	1777	1756	1837
Total polyphenols (TP)	% w/w	5.7	5.7	6.4	6.5	6.7
LM : TP	%	21%	18%	28%	27%	27%

Some of the hop aroma substances and key figures are listed in table 2. Both breeding lines, esp. 89/25 are rich in beer soluble components like esters, linalool and consequently in the oxygen fraction of the hop oil.

Table 2. Hop aroma substance analyses

		89/25	96/24	SSP	TTE	HSR
Sum of Monoterpenes (MT)	mg/100g	733	659	340	248	342
Sum of Sesquiterpenes (ST)	mg/100g	353	478	195	191	204
Hydrocarbon fraction (HCF)	mg/100g	1086	1137	535	438	546
Farnesen (%) of ST	mg/100g	26	3	29	29	7
Sum of 6 Esters	mg/100g	54	18	12	5	37
Linalool	mg/100g	17	12	6	5	7
Geraniol	mg/100g	2	2	6	4	2
Sum of 4 Epoxides	mg/100g	10	9	19	14	21
Oxygen fraction (OF)	mg/100g	129	95	71	54	132

The major differences in hop analyses are, that 89/25 and 96/24 are lower in total and low molecular polyphenols, but have a high aroma capacity (total oil, oxygen fraction, linalool).

Brewing trials

The main parameters of the brewing trials in the 2-hl Research Brewery in St. Johann are:

- 100% malt, infusion, 12 % original extract
- Bitter hop addition begin of boil: Herkules pellets (to 20 IBU)
- Late hop addition in form of pellets type 90 according to oil content (6 ml/hl)
- Bottom fermentation with yeast strain W34/70
- Fermentation 8°C, maturation 14°C, cold conditioning 0-1°C
- Kieselgur filtration, low-oxygen bottling

Table 3. Dosed amounts of other criteria and ratio max/min when dosing 6 ml hop oil/hl

		89/25	96/24	SSP	TTE	HSR	Max : Min
Amount	**g/hl**	**353**	**343**	**857**	**1000**	**632**	**2.9**
α-acids	**mg/l**	**187**	**141**	**317**	**330**	**177**	**2.3**
Oxygen fraction	**mg/hl**	**455**	**328**	**609**	**540**	**771**	**2.4**
Linalool	**mg/hl**	**60**	**41**	**51**	**50**	**44**	**1.5**

Beer analyses results

Original extract, alcohol, app. extract, degree of fermentation and pH show good reproducibility with variation coefficients of 0.8 to 1.9 %. Iso-a-acids vary moderately from 13 to 15 mg/l and the bitterness from 18 to 22 IBU.

89/25 and 96/24 are significantly lower in hop derived low molecular (LM) polyphenols (Figure 2) mostly as a result of lower dose of hop amount (Table 3)

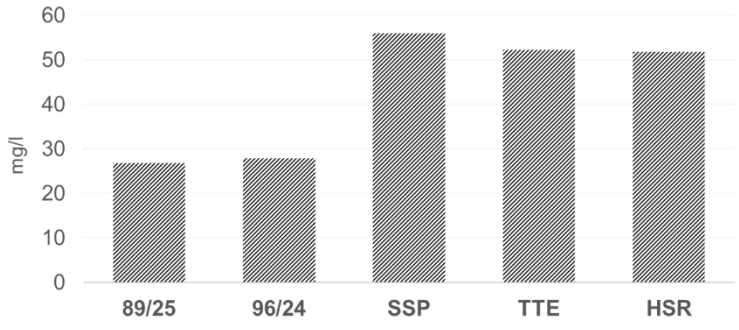

Figure 2. Hop derived low molecular polyphenols

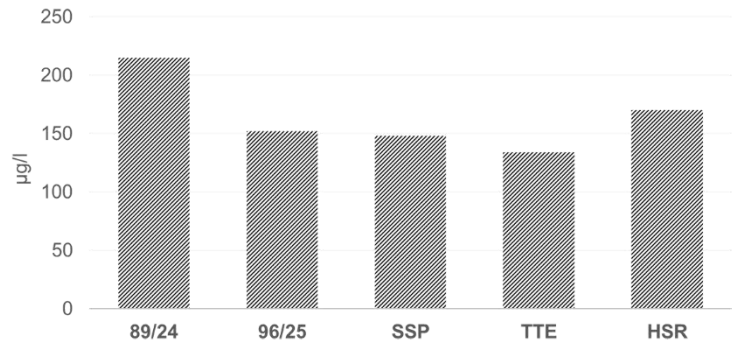

Figure 3. Total Linalool

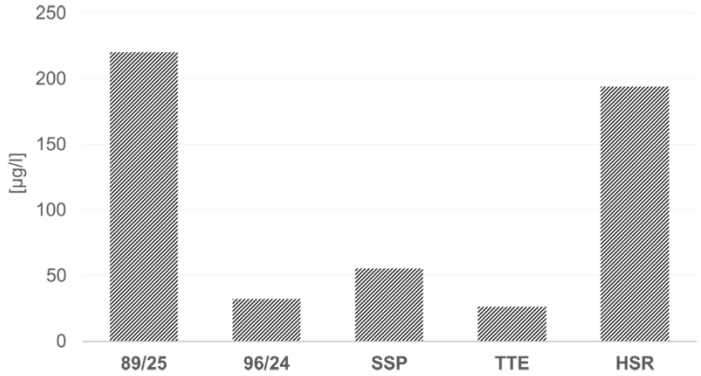

Figure 4. Sum of 6 esters

89/25 shows significant higher linalool content in beer (Figure 3); the beer values follow the dose rates. Figure 4 exhibits the ester contents in beer. 89/25 and Saphir show the highest values by far. Some single components can exceed their threshold levels.

Sensory analysis results

Beers were tasted by the St. Johann taste panel (Table 4). All beers became good average marks, 89/25 was significantly preferred

	89/25	96/24	SSP	TTE	HSR
DLG (average grade)	4.47	4.27	4.48	4.41	4.21
Preference	1*	3	2	3	5

Table 4. Tasting Results (enhanced DLG form, St. Johann panel, n=13)

In a small consumer panel 89/25 has been preferred by tendency 6:1 to TTE and 5:2 to SSP.

Summary

Two Huell aroma breeding varieties, 89/002/025 and 96/001/024 are compared to their mothers Spalter and Tettnanger as well as to Saphir, the aroma variety with the highest aroma capacity in Germany yet. Both breeds have reduced low molecular polyphenolic character compared to mother varieties. 89/25 shows a high farnesene content, an indicator for its affiliation to the Saaz hop family. Although the mother of 96/24 is a member of the Saaz hop family the properties of those haven´t been passed on evidently.

Especially 89/25 is very rich in aroma substances of good solubility like linalool and esters. A dosage according to hop oil content resulted in a reduced throw of the breeding varieties: 35 % of TTE, 41 % of SSP and 55 % of HSR. The 89/25 beer showed the highest values of flavour-active compounds (linalool and esters). Particularly 89/25 has been rated well in the tastings and the beer has been preferred significantly.

In order to achieve a comparable hop aroma in beer the breeding lines can be dosed at significantly lower quantities.

Due to the results HVG has decided to grow both varieties on an amplified acreage this year: 89/25 on 3 ha and 96/24 on 1 ha.

Especially 89/002/025 is a very promising candidate for a registration as a new aroma variety for late hopping purposes.

Beer bitterness is much more than only iso-alpha-acids!

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Abstract

The term auxiliary bitter compounds in hops refers to all bitter compounds in the hop resin which are transferred to the beer and are not iso-α-acids. This includes all components found in fresh hops and excludes those formed through oxidative aging reactions. There are numerous auxiliary bitter compounds found in hops that are either present in a directly soluble form in hops or are formed from α-acids and β-acids during the wort boiling process.

The majority of these substances are considered desirable from a sensory perspective. They mask a harshness and lingering character of the bitterness and make a positive contribution to the quality and harmony of the bitterness in beer. The ratio of the non-specific EBC bittering units (spectrophotometric method) to the specific iso-α-acids (HPLC method) serves as an indicator for the amount of auxiliary bitter compounds in beer. This ratio is equal to 1 in beers brewed with only one hop addition of high-alpha hops at the beginning of the boil, in which case the bittering units are equivalent to the iso-α-acid content (Fig. 1).

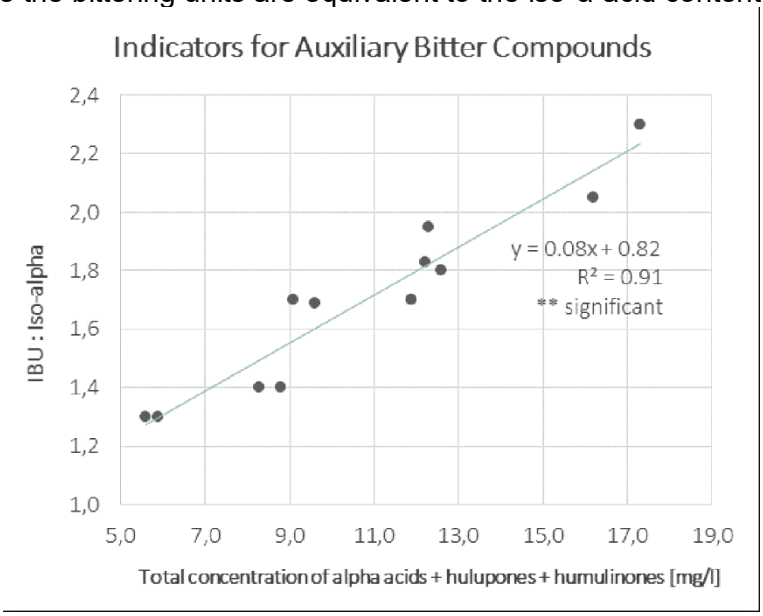

Figure 1. IBU to iso-α-acids as a function of the concentration of α-acids, humulinones and hulupones in 12 beers

By contrast, beers brewed with a complex hopping regime, e.g., with several additions of aroma hops, exhibit significantly higher levels of bittering units than iso-α-acids. Here, the values for IBU to iso-α-acids have been measured as high as 2. In this situation, the bitter units derived from non-iso-α-acids equal the iso-alpha-acid bitter units. Thus, auxiliary bitter compounds make up a sizable portion of the bittering units (Fig. 2).

The results from the brewing trials described previously show that the bitterness of beers with higher ratios of IBU to iso-α-acids is less harsh and lingering, while the overall impression is more balanced and pleasant (Figs 3 and 4).

Adding larger amounts of aroma hops over several additions does not only influence the aroma but also serves to round out the bitterness of the beer.

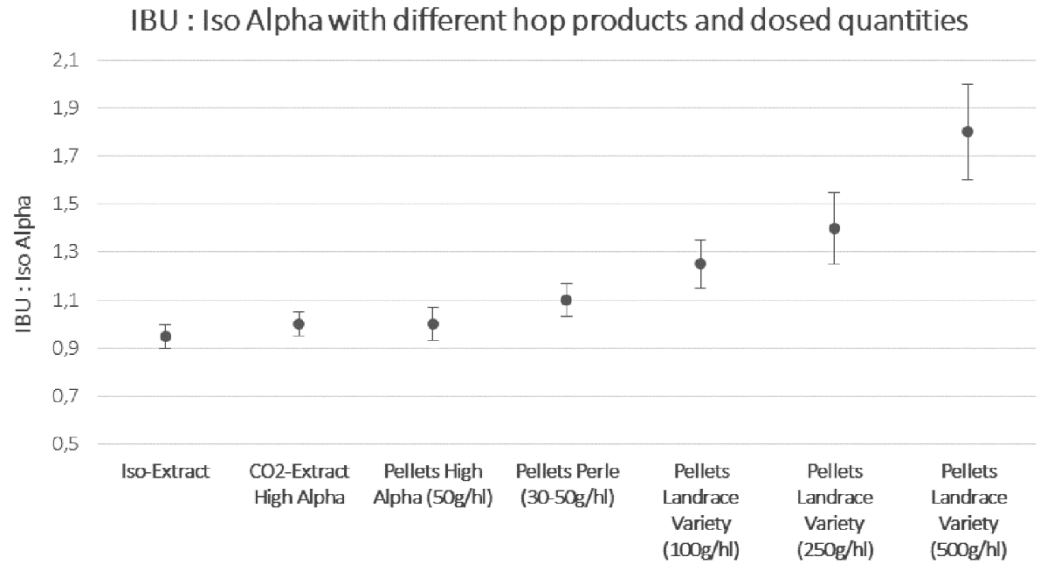

Figure 2. Ranges of IBU to iso-α-acid ratios for different hop products and dosed quantities

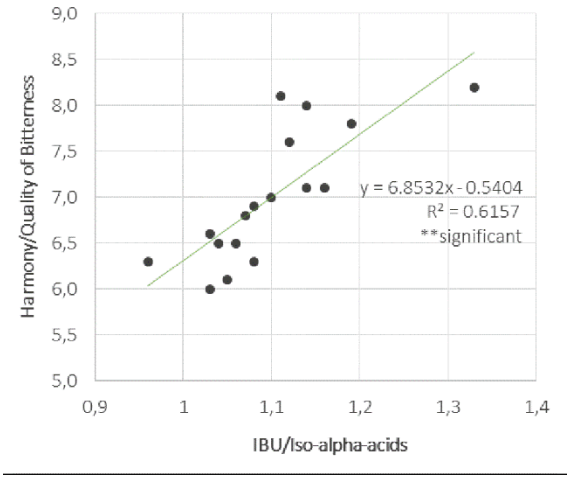

Figure 3. Quality of the bitterness in 16 single-hop beers plotted against the ratio of IBU to iso-α-acids

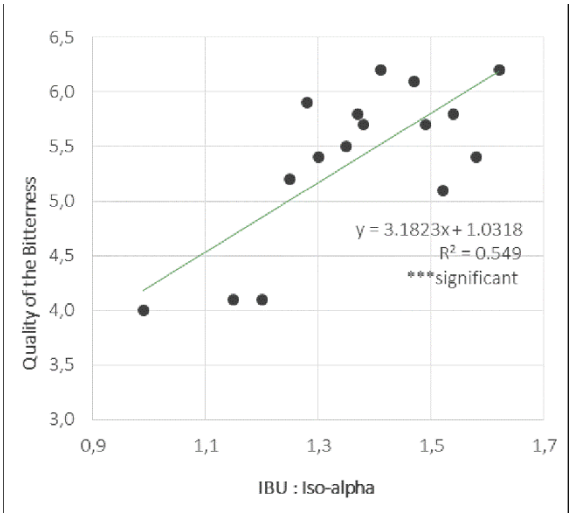

Figure 4. Quality of the bitterness in 17 beers plotted against the ratio of IBU to iso-α-acids

The full publication to this abstract, entitled “What are auxiliary bitter compounds in hops and how do they affect the quality of bitterness in beer?”, has been submitted to *BrewingScience* and is in the review process.

Examination of brewing value in Slovenian hop breeding program

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Abstract

The main goal of Slovenian breeding program is to develop new varieties which enable sustainable hop industry and are desirable on the global market. Since the majority of hops is used in the brewing industry, the examination of brewing value is one of the most important procedures in the breeding process. The chemical determination of bitter acids and gas chromatography-mass spectrometry for determination of aroma compounds in selected experimental lines are performed annually. When all other important data regarding the resistance to diseases and quantity of the yield have been collected, pilot brews of single hopped beers are prepared in our own pilot brewery, recently in kettle and kettle + dry-hopped variants using a standard variety (usually Aurora) for comparison. Additionally, Slovenian craft brewers as well as the biggest breweries are involved for obtaining more adequate data on bigger scale brewing trials. Since more than 95 % of Slovenian hops are exported, the Slovenian Institute of Hop Research and Brewing collaborates with breweries outside Slovenia, in Europe and USA, especially with the Research Brewery St. Johann, Germany and with the Research Institute of Brewing and Malting, Czech Republic. During the presentation the overview and results obtained from different brewing trials will be given and sensory behavior of hop varieties in beer will be exposed.

Key words: hops, brewing value, breeding, collaboration

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VIII: Molecular investigations on hops

The introduction to gene network influencing lupulin and some approaches to molecular study of hop metabolome regulation.

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Abstract

In our previous work we characterized several transcription factors (TFs) and TFs complexes that activate or suppress hop (*Humulus lupulus* L.) lupulin-specific genes involved in the regulation of prenylflavonoids. Among them there are regulatory genes from Myb, bHLH, WRKY, WD40 and bZIP families. The purpose of our work during several past years was to analyze some network connections especially within regulation of the final steps of prenylflavonoid biosynthesis involving genes encoding enzymes like chalcone synthase CHS_H1, prenyltransferase (*Prt*) and O-methyltransferase 1 (*omt*1). Several specific TF genes like *Hl*Myb3 (e.g., MATOUŠEK et al. 2007; GATICA-ARIAS et al. 2013), *Hl*Myb7 (MATOUŠEK et al. 2012), *Hl*Myb8 (unpub.) and TF complexes like *Hl*Myb3/*Hl*bHLH2/*Hl*WDR1, *Hl*Myb2/*Hl*bHLH2/*Hl*WDR1 (MATOUŠEK et al. 2012) and *Hl*WRKY1/*Hl*WDR1 (WW) (MATOUŠEK et al. 2016) are of special interest and they were mapped in the initial regulatory network. In the case of WW complexes we described wide dependency of *Hl*WRKY1 on phosphorylation, autoactivation and RNA silencing (MATOUŠEK et al. 2016). According to our recent analyses hop gene modulation by tripartite complexes is, in addition, strongly dependent on experimental light conditions enriched either by blue, red or UV-A spectra. Thus, all factors analysed so far suggest high complexity of lupulin regulatory network.

This complexity was now confirmed by new results obtained from transcriptome profiling of recently prepared hop transgenotes using high-throughput RNA-Seq technology (NGS). According to these profiles WW overexpression in Osvald's 72 hop led to modulation of 522 unigenes with predominant gene activation, *Hl*Myb7 overexpression included modulation of 788 unigenes with predominant suppression as expected according to the suppressor role of *Hl*Myb7 (MATOUŠEK et al. 2012) and 2145 unigenes were modulated in *Hl*Myb8-overexpressing transgenotes − see related abstracts in this issue by MISHRA et al. (2017) and KOCÁBEK et al. (2017). Bioinformatic processing of NGS transcription profiles from leaves and lupulin glands of controls and transgenic lines together with the analysis of promoter elements of particular hop genes (DURAISAMY et al. 2016) and their responses in transient expression systems (e.g., MATOUŠEK et al. 2016) will help to discover new regulatory network connections important for lupulin biogenesis, as well as for morphogenesis of glandular trichomes. These analyses are in progress.

Acknowledgement

The work was supported by GACR 13-03037S and by the institutional support RVO:60077344.

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Functional analysis of R2R3 transcription factor HlMyb8 from hop (*Humulus lupulus* L.) and its role in lupulin-specific flavonoid biosynthesis

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Abstract

Hop lupulin glands consist of several biologically active components, including prenylflavonoids, with beneficial effects for human health. Biosynthesis of the flavonoids is controlled by the transcription factors (TFs) belonging to the different families such as WD40 repeat (W), bHLH (B), MYB (M), WRKY or bZIP as well as by exogenous factors. A combinatorial action of lupulin gland specific transcription factors in the regulation of the *HlChs_H1* gene has already been demonstrated (MATOUŠEK et al. 2012). The early steps of flavonoid biosynthesis are driven independently by Myb TFs without the involvement of MBW complex. In order to gain in-depth knowledge of regulatory role of other unidentified TFs in flavonoid biosynthesis pathway in hop lupulin glands, we isolated and identified a new putative TF based on its significant similarity to MYB12 (At2G47460) of *Arabidopsis thaliana* (MEHRTENS et al. 2005).

We amplified and cloned cDNA of *HlMyb8* from a lupulin-specific c-DNA library. Phylogenetic analysis based on amino-acid sequence showed that HlMYB8 grouped with other representatives of subgroup 7 flavonol clade. qRT-PCR analysis indicated that *HlMyb8* expression is highly lupulin-specific. Transient expression analysis in the *Nicotiana benthamiana* leaves using promoters of the genes encoding the enzymes of the terminal steps of the prenylflavonoid or bitter acid pathways showed that HlMYB8 activates predominantly chalcone synthase gene (HlChs_H1) whereas it has negligible effect on the activation of other genes, like *Hl*Omt1, *Hl*Pt1L and *Hl*Vps. We successfully performed *Agrobacterium*-mediated transformation of hop using its nodal segments with 35S::*HlMyb8* construct. Three independent transformants were selected for further analyses. qRT-PCR from RNA isolated from transgenic leaf tissue confirmed the enhanced expression of CHS_H1 gene. Based on the previously reported results of functional role of *At*Myb12 (MEHRTENS et al., 2005) we have also investigated the expression profile of other potential targets of *Hl*Myb8 (FLS, F3H, F3'H and CHI) using qRT-PCR. The expression profile of these genes suggests the involvement of *Hl*Myb8 TF in flavonol-specific branch. The Illumina-sequencing and differential gene expression profiling (DEG) using leaf tissue of HlMyb8 overexpressing lines showed that 318 unigenes were up-regulated, whereas 1827 unigenes were down-regulated. The DEG profiling confirmed our previous findings of the regulatory role of HlMyb8 in flavonol biosynthesis pathway. In addition, some new genes related to flavonoid metabolism as well as other biological processes such as signaling and biotic/abiotic stresses were observed to be differentially regulated.

Acknowledgement: The work was supported by the Czech Science Foundation project (GAČR 13-03037S) and by the institutional support RVO:60077344.

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Comparative transcriptome analysis of *Humulus lupulus* wild-type and transformant co-expressing *Hl*WRKY1 and *Hl*WDR1 transcriptional factors

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Abstract

Hop (*Humulus lupulus* L., Cannabaceae) is a dioecious, twining perennial flowering plant, native to Europe, western Asia and North America. The lupulin glands, which are glandular trichomes present on hop cones (high density) and leaves (low density) are composed of biosynthetic cells. They secret a specific but complex metabolome consisting mainly of terpenophenolics (hop bitter acids and prenylflavonoids) and terpenoids (essential oil components), which serve as essential raw ingredient in beer, contributing the distinctive bitterness, flavour and aroma, as well as preservative activity (MATOUŠEK et al. 2002; NAGEL et al. 2008). The terminal step of biosynthesis of prenylated flavonoids in hop cones is mediated by chalcone synthase CHS_H1, prenyltransferase (*Prt*) and O-methyltransferase 1 (*omt*1) enzymes (MATOUŠEK et al. 2013). Recently, we have shown that lupulin gland-specific transcription factor *Hl*WRKY1, close homologue of *At*WRKY75 in the binary combination of WD40 repeat protein1 (*Hl*WDR1) activate the promoters of genes (*HlOmt*1, *HlChs_H1, HlPrt1(2)* and *HlVps*) involved in the final steps of prenylflavonoid (PF) and bitter acid (BA) biosynthesis pathway (MATOUŠEK et al. 2016).

In the present study, transgenic hop lines (WW) constitutively expressing *Hl*WDR1 and *Hl*WRKY1 transcription factors have been developed and using high-throughput RNA-Seq technology, leaf transcriptome was analysed. Differentially expressed gene analysis showed the modulation of 522 unigenes (385 up-regulated and 137 down-regulated) in WW-transformant lines. In addition, several genes involved in various other biological process such as signalling, biosynthesis, metabolism, biotic/abiotic stresses were observed to be differentially regulated. Another intriguing observation of transcriptome analysis was found to be modulation of newly identified TFs (MYB, bHLH, WRKY), their involvement in the regulation of PF and BA biosynthesis pathway remains to be elucidated utilizing CRISPR/Cas9 gene editing technique which compromise our future area of work. Currently we are closely monitoring the growth and morphological patters of WW transformant lines and once cone will be developed, they could be used further for metabolite profiling. Our research would open the new avenue to engineer TFs as sophisticated and reliable strategy for further enhancement of lupulin metabolome content to meet the increasing demand for hop production.

Acknowledgement

The work was supported by the Czech Science Foundation project (GACR 13-03037S), MSMT Kontakt II LH14255 and by the institutional support RVO:60077344.

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The full paper to this abstract is under review by the Journal of Experimental Botany and publication is expected still in 2017.

Utilization of an enlarged set of molecular markers for control of authenticity and an evaluation of genetic variability within actual hop cultivars

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Abstract

Traditional hop cultivars have been used in brewing industry for a long time. About ten new breeding lines were realized for the world-wide market in every decade from the 1970s onwards. In the last decade, it was six times more, why it is necessary to control their genotype and origin. A utilization of DNA molecular genetic methods is the best solution for this purpose. We developed the efficient marker system for authenticity control of hop genotypes based on EST-SSR. The set of 11 loci effectively differentiated all used cultivars, breeding lines and wild hops, except for originally same genotypes of Saaz- and Fuggle-derived cultivars. We evaluated molecular genetic variability within 238 hop genotypes by 333 amplified molecular markers. Molecular genetic variability analyses corresponded with genealogical and geographical origin of individual cultivars, incorporated breeding events and influences of key cultivars.

Key words. expressed sequence tag-simple sequence repeat (EST-SSR); authenticity control; cluster analysis; principal coordinate analysis

Introduction

Nowadays, an increasing demand for production of hops (*Humulus lupulus* L.), raw material for brewing industry, was predominantly caused by craft breweries. They still require new hop cultivars for the beer market and therefore breeders enlarge a list of cultivars every year in all hop countries. BARTH-HAAS GROUP (2016) listed a record of 146 hop cultivars in common use worldwide, compared to 125 cultivars in 2016 and 108 cultivars in 2009. This rapid development brings no easy orientation within cultivar's portfolio when their origin is sometimes secret or briefly described. Every cultivar is exactly characterized by contents of bitter acids, essential oils and polyphenols in hop cones, but there are overlapping and influenced by season, growing and environmental conditions (KROFTA & PATZAK 2011). Nowadays, a utilization of DNA molecular genetic methods is the best tool for the evaluation of individual cultivars and genotypes. Microsatellite SSR (Simple Sequence Repeat) markers became a standard DNA identification method for species and cultivars within different organisms. A recent technical advance in next generation sequencers (NGS) opened a way to obtain huge transcriptome (NAGEL et al. 2008; CLARK et al. 2013; XU et al. 2013) and whole genome sequence information (NATSUME *et al.* 2015), which provided us by possibility to look for new gene specific molecular markers. From these information, new type molecular markers were derived as Expressed Sequence Tag-Simple Sequence Repeat (EST-SSR) (PATZAK & MATOUŠEK 2011; JAKŠE et al. 2011; KOELLING et al. 2012; SINGH et al. 2012) and Single Nucleotide Polymorphism (SNP) markers (MATTHEWS et al. 2013; YAMAUCHI et al. 2014; HENNING et al. 2015). Recently, we reported the efficient marker system for genotyping and authenticity control of Czech hop cultivars based on EST-SSR, which was implemented into the identification of hop genotypes and control of cultivar rootstocks purity (PATZAK & MATOUŠEK 2013a; b). This marker system is not only successful and efficient for cultivar determination but also for evaluation of molecular genetic variability with enlargement set of highly polymorphic molecular markers (PATZAK et al. 2007; PATZAK & MATOUŠEK 2011; PATZAK & HENYCHOVA 2017a, b).

Material and methods

In this study we analysed 175 traditional and new world hop cultivars, 36 breeding lines and 27 wild hops by molecular analyses.

DNA was isolated from the young leaves of samples from the hop garden of the world hop collection of Hop Research Institute Co.Ltd., Žatec and from dried cones or pellets from samples obtained from hop merchants (Yakima Chief – Hopunion, LLC., Belgium; Simon H. Steiner, Hopfen, GmbH, Germany; John Barth & Sohn GmbH, Germany; Charles Faram & Co.Ltd., United Kingdom; Comptoir agricole, France; South African Breweries, South Africa; Slovenian Institute of Hop Research and Brewing, Slovenia) by CTAB method according to PATZAK (2001). Six SSR (JAKŠE et al. 2002; ŠTAJNER et al. 2005), five STS (Sequence-Tagged Sites) (PATZAK et al. 2007) and thirty-seven EST-SSR (PATZAK & MATOUŠEK 2011; PATZAK & HENYCHOVA 2017a, b) loci were used in PCR reactions (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). Amplification products were resolved via 5% denaturing (8M urea) polyacrylamide gel vertical electrophoresis and visualized by silver-staining (PATZAK 2001). Eleven EST-SSR fluorescence labelled markers were resolved via capillary electrophoresis on Applied Biosystems 3130 genetic analyzer (ThermoFisher Scientific, USA).

Hierarchical cluster analyses and principal coordinates analysis (PCoA) were used for evaluation of molecular genetic variability within hop genotypes. They were based on Jaccard's similarity coefficient and Neighbor-Joining (NJ) clustering by Unweighted Pair Group Method with Arithmetic means (UPGMA) in DARwin v. 5.0.155 (Dissimilarity Analysis and Representation for Windows, http://darwin.cirad.fr/darwin) and NTSYS-pc v. 2.11V for WINDOWS (Exeter Software, New York, NY, USA). The dendrograms were visualised by Geneious Pro 4.8.2 (Biomatters Ltd., Auckland, New Zealand).

Results and Discussion

Information in DNA sequence is useful for genotyping hop cultivars by molecular genetic methods. Recently, we reported the efficient marker system for genotyping and authenticity control of Czech hop cultivars based on EST-SSR, which was implemented into the identification of hop genotypes and control of cultivar rootstocks purity (PATZAK & MATOUŠEK 2013a, b). This system in PCR amplifies alleles of these genes: WRKY transcription factor 1 (WRKY1), 2-C-methyl-D-erythritol 2,4-cyclodiphosphate syntase (CMPS), leucoanthocyanidin reductase 1 (LAR1) and calcium-binding EF hand family protein (CaEFh). Meanwhile we enlarged the set of EST-SSR markers by MinimalMarker analyses (FUJII et al. 2013) of highly polymorphic molecular markers (OLŠOVSKÁ *et al.* 2016; PATZAK & HENYCHOVA 2017a, b). From these sets, we selected next seven EST-SSR loci with high polymorphism within hop genotypes. There were loci in genes: chalcone synthase 1 (CHS1), flavanone 3-hydroxylase (F3H), MYB transcription factor 8 (HlMYB8), nucleotide DNA-binding protein (NDBP), cellulase 1 (CEL1), small auxin upregulated RNA protein 1 (SAUR1) and gibberellic acid 2 oxidase 2 (GA2oxy2). Markers amplification profiles enable to combine them in three fluorescence capillary electrophoresis runs (Table 1).

Table 1. Amplification and polymorphism characteristics of EST-SSR markers

Marker	PCR size range (bp)	Number of common alleles	Number of unique alleles	Number of genotypes with same profile	Fluorescence label	Run
WRKY1	198-244	10	4		6-FAM	1
LAR1	165-179	4	3		6-FAM	1
CMPS	225-251	5	2		6-FAM	2
CaEFh	185-210	5	2		6-FAM	2
CHS1	236-267	4	1		HEX	1
F3H	176-192	3	6		6-FAM	3
HlMYB8	160-184	4	1		HEX	2
NDBP	210-248	7	3		HEX	3
CEL1	206-251	7	10		HEX	2
SAUR1	226-248	4	5		NED/Cy3	1
GA2oxy2	220-233	7	4		6-FAM	3

In total, this set of 11 loci amplified 101 molecular markers, which effectively differentiated all used cultivars, breeding lines and wild hops, with exception of varieties derived in history from Saaz (Saaz, Spalt, Tettnang, Nadwislawsky and Strisselspalt) and Fuggle (Fuggle, Hersbruck, Savinjski Golding, Golding, Izabela).

The results were useful also for evaluation of molecular genetic variability within hop genotypes in comparison to total 333 amplified polymorphic molecular markers in all reactions. It was based on Jaccard's similarity coefficient and NJ clustering by UPGMA (DARwin v. 5.0.155). When we compared both dissimilarity matrixes by Mantel's Z statistic cophenetic correlation analysis (NTSYS-pc v. 2.11V), we found high correlation (r=0.779) and the selected set was useful for evaluation of genetic variability. The hierarchical cluster analysis of all molecular markers (Fig. 1) corresponded with combination of genealogical, geobotanical and analytical characteristics of individual cultivars. Hop cultivar germplasm is divided into European and North American genotypes by different molecular method analyses (SEEFELDER et al. 2000; ŠTAJNER et al. 2008; HOWARD et al. 2011; HENNING et al. 2015). A lot of genotypes are influenced by their breeding history when cultivars progressively originated from European landrace germplasm by introduction of North American wild germplasm (BASSIL et al. 2008; ŠTAJNER et al. 2008; PATZAK et al. 2010; HOWARD et al. 2011). This was also imprinted in cluster analysis when clusters were divided into European and North American germplasm. European germplasm was divided into continental European landraces including genotypes originating from it, and cultivars originating from Fuggle, Golding and Northern Brewer (UK), spaced by the European wild hops group (Fig. 1). In opposite, North American wild hops were the genetically most distant types. North American germplasm was joined with South African and New Zealand clusters.

The principal coordinate analysis (PCoA) was also used for estimation of genetic diversity structure (not shown). The first principal coordinate (PCo) represented 11.87 % of variation and the second PCo represented 4.85 % of variation. PCoA corresponded with the dendrogram (Fig. 1) when it divided cultivars to four quadrants: 1) continental European landrace origin and wild hops, including South African hops, close to axis, 2) European cultivars of Fuggle and Northern Brewer origin, 3) North American and Australian germplasm cultivars originating from Brewers Gold, including mixed germplasm hops, close to axis, 4) New Zealand cultivars originating from Late Cluster, cultivars bred from Cascade, and distant North American wild hops in the corner.

Acknowledgement

This work was supported by Grant Agency of CR in project 13-03037S and by Technological Agency of CR in Centre of competence TE02000177. We thank Larisa Havlínová (Yakima Chief – Hopunion, LLC., Belgium), Alexander Feiner (Simon H. Steiner, Hopfen, GmbH, Germany), Christina Schönberger and Mark Zunkel (Joh. Barth & Sohn GmbH, Germany), Andreja Čeranak (Slovenian Institute of Hop Research and Brewing, Slovenia), Francis Heitz (Comptoir agricole, France), Beverley-Anne Joseph (South African Breweries, South Africa) and Will Rogers (Charles Faram & Co.Ltd., United Kingdom) for providing us by actual hop samples from Germany, Slovenia, France, South Africa, United Kingdom, Australia, New Zealand and USA.

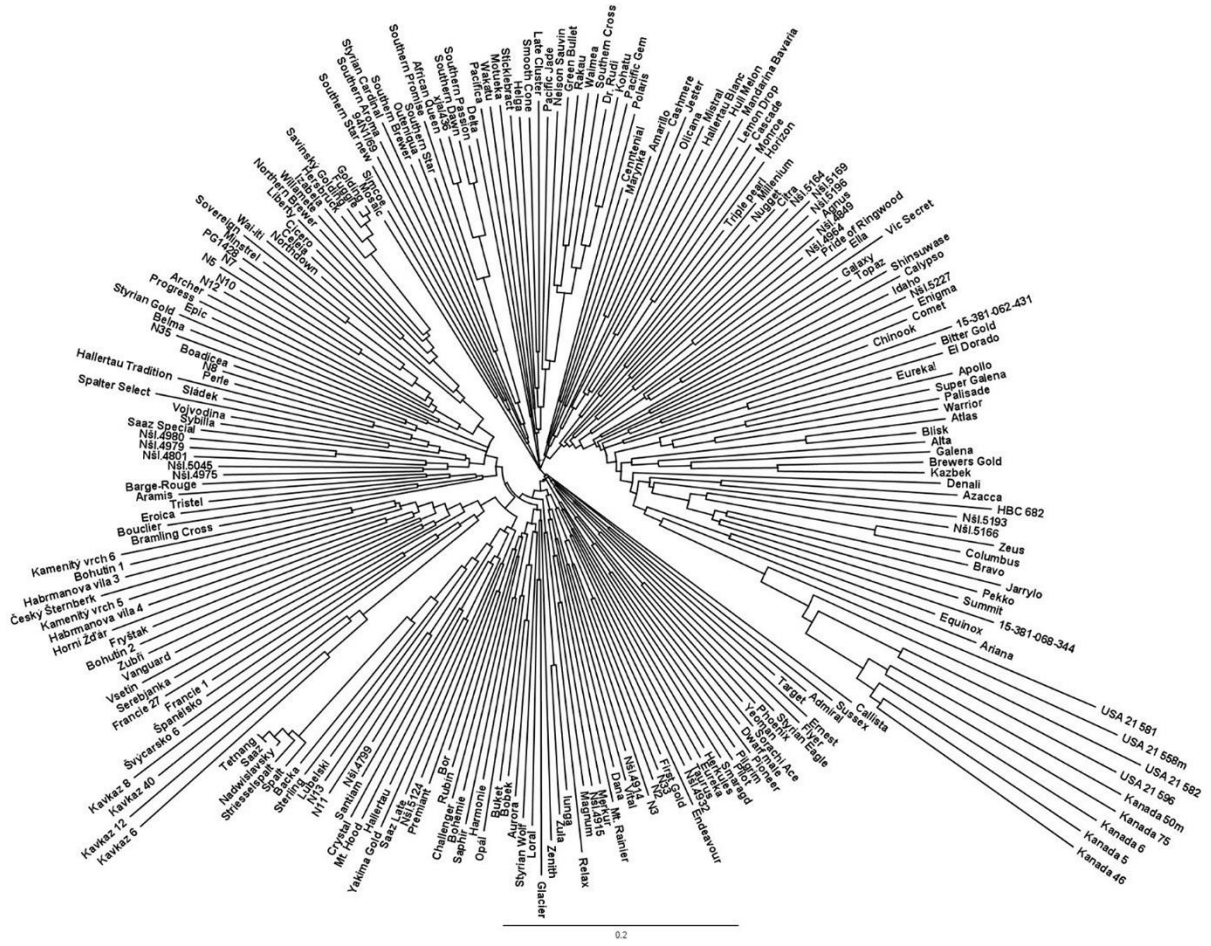

Figure 1. Dendrogram of genetic distances of 238 world hop cultivars and breeding materials revealed by Unweighted Pair Group Method with Arithmetic means (UPGMA) and Neighbor-Joining (NJ) clustering based on Jaccard's similarity coefficient determined by 333 polymorphic molecular markers.

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Detailed hop transcriptome for *in-silico* prediction and validation of potential gene targets for viroid derived small RNAs*

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Abstract

Viroids can cause considerable damage and economic losses in plant production systems. Hop production has recently been threatened by Citrus bark cracking viroid (CBCVd), which causes the most aggressive symptoms among all viroid species infecting hops. Plants show the first symptoms 4 months after infection, which manifest as severe stunting and other phenotypic abnormalities after first dormancy and complete dieback in the following years. Hop plants are currently hosts to four known viroids, of which hop latent viroid (HLVd) and the recently confirmed CBCVd are very interesting models for studying plant-viroid interactions. HLVd is present in all hop plants and is considered symptomless in terms of visible symptoms, while CBCVd causes severe stunting disease, manifested in drastic phenotype changes. The model of viroid pathogenicity is still under research and is puzzling researchers. One of the models, with recent experimental evidence, is attenuation of transcripts by transcriptional- (TGS) or posttranscriptional (PTGS) gene silencing through viroid-derived small RNAs (vd-sRNAs), which are present in hop cells in high concentrations, as evident from NGS sequencing experiments. To study the proposed PTGS model in HLVd and CBCVd infected plants, we performed the following experiment. We first performed *in-silico* prediction of target transcripts for HLVd- and CBCVd-derived sRNAs, using two bioinformatic tools. For this purpose, we assembled a very deep NGS hop transcriptome from 35 Gb of raw data. Assembly resulted in over 74 Mb of scaffolds, representing hop transcripts from various tissues and developmental stages. Prediction models revealed that 1062 and 1387 hop transcripts share nucleotide homologies with HLVd- and CBCVd-derived small RNAs, respectively. According to the silencing model, these transcripts may be targets of the RNA interference process. Seventeen hop transcripts were selected and their expression was monitored by RT-qPCR in leaves, flowers and cones of viroid-free, HLVd- and CBCVd-infected plants. The selected transcripts were chosen based on 1) their involvement in the pathways of plant hormone metabolism, 2) their involvement in small RNA biogenesis, 3) showing the highest homology with viroid derived small RNAs and 4) being targeted by CBCVd-derived small RNAs, which have highest concentration in hop cells as reveled from NGS mapping data. Additional expression profiles of five pathogenesis related (PR) genes were also monitored. The majority of transcripts showed expression fluctuations in leaf, flower and cone tissues compared to viroid free plants, confirming drastic transcriptional changes in HLVd and CBCVd infected plants. Possible evidence of silencing was observed for two genes: GATA transcription factor targeted by CBCVd-derived small RNA and linoleate 13S-lipoxygenase gene targeted by HLVd-derived small RNA. Interestingly, expression of PR genes revealed high expression levels of four pathogenesis related genes in the leaves of both group of infected hop plants, indicating pathogenicity also of the symptomless HLVd. These efforts aim to achieve better understanding of the viroid's disease molecular mechanisms. They could initiate novel strategies to fight these diseases and help in the search for possible genetic resistance in hop against viroid infections.

Acknowledgement

The authors acknowledge the financial support of the Slovenian Research Agency (J4-4153, P4-0077, T.P. supported by 1000-11-310098) and the Administration of the Republic of Slovenia for Food Safety, Veterinary and Plant Protection (2321-12-210006).

The full manuscript to this abstract is currently under review in PLOS ONE.

Metabolome-genome-wide association study of downy mildew resistance in hops (*Humulus lupulus* L.) reveals metabolite interaction

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Abstract

The plant metabolome is typically known as the readout of the physiological state leading to variation in complex traits, such as growth, stress response or pathogen-induced secondary metabolite accumulation. Studying the relationship between statistical modeling of metabolite content levels and trait expression identifies molecular processes involved in disease resistance. Advances in high-throughput profiling and genotyping technologies coupled to metabolome-genome-wide association studies provide a powerful and alternative tool to dissect biochemical genetics of the plant metabolism. We identified both canonical and novel metabolites profile changes correlated to downy mildew disease resistance status caused by *Pseudopersonospora humuli* treatments in hops using large untargeted metabolomics data generated by liquid chromatography high resolution mass spectrometry

Specifically, an F1 hop population consisting of 192 individuals showing phenotypic variance in downy mildew symptoms was cloned and then spore-inoculated and mock-challenged under controlled, environmental chamber conditions. After an inoculation with the fungus the family revealed preadapted and specialized, induced metabolites. Using linear regression, content variation of a small number of metabolites with potential protective function against downy mildew were identified and mapped to the phenylpropanoid pathway.

Key words. downy mildew resistance, phenylpropanoid biosynthesis, metabolome-genome-wide association study

The full manuscript to this abstract is currently prepared for peer-reviewed publication and will soon be submitted.

IX: Posters

Development of new hop aroma varieties for Czech beer

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Abstract

Czech Republic is famous for production of fine aroma hops with excellent brewing quality, which ensues from historical tradition of growing Osvald clones (Saazer). Nevertheless, since the 1990s newly bred Czech hop varieties (Sladek, Premiant, Harmonie, Saaz Late, etc.) have been grown here as well. Hop breeding aimed at getting new aroma varieties still continues with the objective to register new aroma varieties with stable qualitative and quantitative parameters and excellent brewing characteristics. Breeding of new generation aroma cultivars began in 1999. Each year nearly twenty crossings were made and the genotypes were at first tested regarding their susceptibility to Downy and Powdery mildew. The best genotypes were selected within tolerant and resistant progenies and tested within our breeding program. Five promising aroma genotypes were transferred into registration trials in 2015 and four others in 2016.

Average values from chemical analyses of hop resins obtained in 2016 are shown in Table 1. All genotypes have a low ratio of cohumulone, which is a very important brewing characteristic. The best results showed genotypes 4801, 4979, 5045 and 5227. As genotypes 5045 and 5227 had the highest yield they were planted in another hop garden to be subject for further research. In 2016, we managed to harvest a sufficient amount of hops for chemical analyses and trial brews in our pilot brewery and other Czech breweries. Susceptibility to agrotechnical operations needed for successful growing of these hops is found out within field trials in practical conditions of hop gardens.

Registration of new aroma varieties is expected in 2018. We expect interest of brewers not only from Czech but from brew masters all over the world as well.

Table 1. Characteristics of nine new, promising Czech hop genotypes in the period from 2013 to 2016

Genotype	Yield [t ha^{-1}]	Alpha acids [% w/w]	Beta acids [% w/w]	Cohumulone [% rel.]	Farnesene [% rel.]
4799	2.0	4.8	4.1	22	11
4801	2.4	4.1	4.0	26	12
4975	2.4	6.4	6.5	18	15
4979	2.3	2.9	4.4	24	14
4980	2.2	5.2	4.2	26	18
5030	2.3	5.3	5.3	21	10
5044	2.4	8.2	7.4	25	2
5045	2.8	3.7	3.8	24	12
5227	2.5	2.4	7.6	31	1

Acknowledgement

This work was supported by Czech Ministry of Education within the Research Project TE02000177 „Centre for Innovative Use and Strengthening of Competitiveness of Czech Brewery Raw Materials and Products“.

‘Magnat’ and ‘Puławski’ – the new Polish hop cultivars

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Abstract

In 2014 two new hop cultivars, ‘Puławski’ and ‘Magnat‘, have been released in Poland.

‘Magnat’ belongs to the high-alpha hops. It originates from hybrid progeny of cv. ‘Hallertauer Magnum’. Typical morphological characteristics of ‘Magnat’ include: a huge habit with very long laterals exceeding 1 m and numerous, egg-shaped cones with a thick density and mean size of 34 mm. ‘Magnat’ is a late cultivar. In Polish climate conditions it reaches harvest maturity in the second half of September. This cultivar is characterized by high yield in the range of 2.5-3.0 t/ha and high alpha acids content from 13 to 16 %. Average content of essential oils is 1.2 mg/100g. Their composition is characterized by a relatively high amount of α-humulene (16.6 % rel.) and β-farnesene (5.1 % rel.). On account of high alpha acids content, ‘Magnat’ is particularly useful for production of hop extracts. Its high yield combined with high alpha acids content contributes to the increase of effectiveness of hop production.

‘Puławski’ is aroma cultivar originating from hybrid progeny with predominance of the Polish cv. ‘Marynka’. ‘Puławski’ is a medium late cultivar and is usually harvested in the middle of September. The plant has cylindrical shape with laterals of 80-90 cm length. Cones are oval in shape with characteristic flatten tips. Mean size of the cone is 29 mm. Compared to the other aromatic hops, ‘Puławski’ is characterized by a high content of alpha acids from 8 to 10 %. Average content of essential oils is on the level of 1.6 mg/100g and their composition is characterized by high content of α-humulene (21 – 23 % rel.) and caryophyllene (7 % rel.). In brewing trials both cultivars have shown a good influence on the taste and aroma of beer.

The introduction of ‘Puławski’ and ‘Magnat’ to practical cultivation was preceded by elimination of viruses and *Hop latent viroid* from the plants and the production of healthy planting material for growers. This contributes to the utilization of the full biological potential of these cultivars and reduces the spread of pathogens in the agricultural environment. So far, 90,000 healthy plants of ‘Puławski’ and ‘Magnat’ have been transferred to farmers.

Key words: Hop, cultivars, breeding

The first Czech dwarf hops

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Abstract

Hop breeding aimed at new genotypes suitable for growing on low trellis in the Czech Republic has been solved since 2008. Within Eureka program we studied dwarf hops in the cooperation with our English colleagues from 2011 to 2014. During this project we managed to carry out 24 crossings, which resulted in 75,502 seeds. Saazer was used as the standard of the best aroma quality as a female plant whereas English dwarf male hops were used as father plants. They have been the donors of genetic dwarfishness as well as tolerance to fungal diseases. Since 2011 we have evaluated 22,327 plants in breeding trials placed on 6 ha of low trellis. We have selected 91 perspective genotypes, which represent only 0.41 % of the initially tested array.

In the period from 2014 to 2017, the twelve best genotypes entered registration trials. Genotypes N33 and N5 were the most productive with an average yield of 3.16 resp. 2.86 kg of fresh hops/plant, which corresponds to 2.0 tons of dry hops/ha. Genotypes N2, N3, N11 and PG1428 show good yield of 2.0 kg of fresh hops/plant. This corresponds to 1.5 tons of dry hops per hectare. Commercial assessments, carried out in all these genotypes, were aimed at aroma, overall appearance and damage caused by Downy and Powdery mildew. None of them showed damage caused by these fungal diseases. According to their aroma they can be divided into hoppy (N7, N12, N13 and PG1428), spicy (N3, N5, N8 and N35) and specific genotypes (N2, N10, N11 and N33). In samples harvested in 2016, the alpha acid content does not exceed 9.0 % w/w. The highest content of alpha acids was found out in genotypes N11, N12, N3 (7.7 %; 7.5 %; resp. 7.0 % w/w). Genotypes N5, N7 and N10 had lower content of alpha acids (ca 3.0 % w/w). Content of beta acids in all these hybrids is lower than alpha acids, with a ratio in the range from 1.5 to 2.3. Cohumulone ratio moves in relatively wide interval, from 15.0 % rel. (N10) to 32.4 % rel. (N13). In most of the genotypes the cohumulone ratio is in the range of 20-25 % rel.

Genotype N2 is the richest in essential oils (1.0–1.5 g/100 g). Several other have essential oil contents up to 0.6 g/100 g (N5, N7, N10, N12, N13 and N35). The composition of essential oils in the dwarf hops is highly varied. Typical for them is a relatively low content of myrcene, mostly in the interval 15-30 % rel. Extremely high content of beta-selinenes (up to 30-35 % rel.) is specific for genotypes N2, N3, N5, M7, N10 and N33. Beta-farnesene, the terpenic hydrocarbon typical for Saazer, was found in higher amount (>5 % rel.) only in few genotypes (N5, N10, N11, M12 and N13). The richest in it is genotype N11 (20-25 % rel.).

Genotypes with the highest variability in the content and composition of hop resins and essential oils (N5, N7, N8, N10, N33 and PG 1428) were chosen for brewing tests because of sensoric variability in beers. Genotype PG1428 was not distinctive in the beer but its bitterness was pleasant. Therefore, we can recommend it for the third hopping. Genotypes N5, N7 and N10 show higher sensoric bitterness with pleasant dying away. Therefore, they are suitable for the second and third hopping. Very interesting are genotypes N8 and N33, which are characteristic of their specific aroma. They bring spicy and fruity flavours into beer. The highest intensity was found out in N8 genotype. Therefore, these two genotypes can be used for the production of special beers.

Acknowledgement

This work was supported by Czech Ministry of Education within the Research Project EUREKA no. LF 15020. 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.

Collection of hop genetic resources in the Czech Republic

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Abstract

The Czech Hop Research Institute has two rich collections of genetic resources. The first holds hop varieties grown all over the world in the frame of "National Program of Conservation and Utilization of Genetic Resources in Plants and Biodiversity" issued by Czech Ministry of Agriculture. There are 395 items, representing old and new hop cultivars, which serve as donors of important characteristics within the breeding process. The second collection holds wild hops, which have been regularly collected since 1997. There are 275 items from Europe (108), North America (73) Caucasus (76) and eastern Russia (18). Wild hops show genetic, chemical and phenotypic variability. It is necessary to bring wild hops from habitats into field conditions to find out if demanded features are based genetically and not just influenced by environment. Assessment is carried out at least for five years and each genotype is planted there in three replicates, which are evaluated individually. All the characteristics are transferred into the information system with the help of our classifier.

Since July 2015, we have been operating the National documentation system on PGR GRIN Czech (currently in English only). This new system, adapted to the Czech Republic from a globally recognized system of documentation of genetic resources GRIN Global, was provided to CRI by the workplace USDA Agricultural Research Service (National Germplasm Resources Laboratory, Database Management Unit, Beltsville). GRIN Global was developed from the original documentation system GRIN in cooperation with USDA Agricultural Research Service, Biodiversity International and the Global Crop Diversity Trust.

Documentation of PGR, which leads in accordance with § 17 of Decree No. 458/2003 Coll. person in charge of the National Programme, consists of:

a) Passport data – the general characteristics of plant genetic resources which are common to all PGR. Currently, the applicable standard is the document Multi-Crop Passport Descriptors (MCPD).

b) Characterized and evaluation data – assessment of morphological, biological, and biochemical characteristics in the form of descriptors, which are genus or species-specific and are evaluated according to the specific descriptors list (classifier) indicating the method of evaluation of expression of each character.

c) Storage data –basic storage information is provided for all samples of genetic resources (number of items, date of harvest, date of start of preservation, date of recovery, conservation method and others). Data are also recorded about provided samples to users.

The first collection is utilized within our breeding process as well as for research and study works and each item is available for every Czech and foreign workplace in all the forms (plants, dry cones, leaves, DNA). Ministry of Agriculture supports genetic resources in the form of grant called "National Program of Conservation and Utilization of Genetic Resources in Plants and Biodiversity". The second collection is utilized just by Hop Research Institute for breeding purposes because the Institute has invested into the expeditions from its own sources and therefore these items are not at disposal. In 2015 we enriched the collection by 22 wild hops, typical by their wide diversity.

Acknowledgement

This work was supported by Czech Ministry of Education within the Research Project EUREKA no. LF 15020. 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.

Genotyping-by-Sequencing (GBS) utilization for construction of high density linkage map in hop (*Humulus lupulus* L.)

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Abstract

The main aim of the study was to promote genomic-assisted plant breeding and high-throughput analysis of genetic variability in hops with a genotyping by sequencing (GBS) approach. GBS is a simple, fast and affordable approach for sequencing specific plant genes and genomic regions. The study focused on identification of marker-trait associations of hops for yield parameters, chemical content and disease tolerance, the most important parameters of hops for its use in brewing and the pharmaceutical industry. The main objectives were as follows: 1) selection of a subset of custom targets and creation of a customized gene panel; 2) primer design for ultrahigh-multiplex PCR amplification using custom assay design pipelines; and 3) analyses of data and comparison of commercial workflows and pipelines available in the public domain.

Based on publicly available datasets of transcriptomic data of five different hop varieties, we discovered 13,000 polymorphisms, of which 6,669 polymorphisms (SNP mostly) occurred simultaneously in all screened genotypes and are therefore useful for large-scale genotyping studies on hops. Based on the additional criterion of being heterozygous in the parental genotype, they are expected to segregate in a mapping population and are therefore useful for mapping studies. A F_1 mapping population segregating for disease resistance, chemical and yield components was derived from a cross between the VW (*Verticillium* wilt) highly resistant English variety Wye Target and the susceptible Slovene male breeding line BL2/1 and consists of 144 F_1 full-sib genotypes.

Using a GBS approach, the identified sequence polymorphisms covered a much higher proportion of the genome than previous marker technologies and the higher number of molecular markers is significant for saturation of the current genetic map of hops based on the F_1 mapping population.

The study is of significant relevance to the hop-related work program since it allows analysis of hundreds of genes simultaneously; these are regions of interest in hops implicated in biochemical pathways contributing bitterness, flavour and aroma to beer and regions responsible for disease resistance, particularly the most severe vascular fungi disease, Verticillium wilt.

Key words. GBS, genotyping by sequencing, Verticillium wilt, mapping, molecular markers

Improved selection system to test for Downy mildew tolerance of hops

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Abstract

Downy mildew caused by the fungus *Pseudoperonospora humuli* has been a serious threat to hop production in the Hallertau region in recent years. A crucial contribution to solve this problem with downy mildew (DM) is the breeding of hops with enhanced tolerance towards this fungus. Since decades, each year thousands of seedlings have been tested for tolerance towards DM in the growth hall. Here, a detached leaf assay in the laboratory was elaborated to provide additional information based on standardized inoculation and incubation conditions to predict and specify the tolerance of advanced hop selections towards this fungus.

Leaves randomly taken from the third node of vigorously growing test plants (at least two replicates) were harvested and the abaxial side of each leaf was inoculated with a suspension of *P. humuli* (2-5 x 10^4 sporangia/ml). Five to seven days after inoculation (dpi) leaves were visually evaluated and finally assessed 14 dpi. Ratings for sporulation, chlorosis and necrosis were on a scale of 0 to 5: 0 (highly tolerant), no sporulation; 1 (tolerant), 1-10 %; 2 (medium), 11-30 %; 3 (susceptible), 31-60 %; 4 (highly susceptible), 61-80 %; 5 (extremely susceptible), 81-100 % of leaf area infected. Results are based on at least 4-5 replicated examinations per year in 2015 and 2016.

A detached leaf assay for testing DM tolerance has been investigated (JAWAD-FLEISCHER 2014) based on knowledge from UK (ROYLE & KREMHELLER 1981; DARBY 2005), USA (PARKER 2007; MITCHELL 2010) and Germany (KREMHELLER 1979). The age of leaves was identified as being crucial. Vitality of downy mildew spores during the inoculation procedure was improved (JONES 2001) resulting in a better reproducibility of disease symptoms on leaves. Moreover, the temperature regime was optimized. Following a temperature cycle of 13^0C during the 12-hour-darkness and 22^0C during light phase vigorous sporulation of the fungus occurred on leaves of DM susceptible plants within the first days after inoculation followed by the necrotizing of host cells in a later phase of infection (large necrotic spots). A clear differentiation of both disease reactions (sporulation of the fungus; necrosis of host cells) could be achieved. Sporulation ratings were most suitable to assess non-systematic tolerance towards DM. The DM tolerance of several hop cultivars has been assessed using this leaf assay and compared to their tolerance in the field over several years.

Keywords: Hop, downy mildew, detached leaf assay, selection, disease tolerance

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Detection of hop viruses and viroids by qRT-PCR in the Czech Republic

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Abstract

The hop plant, *Humulus lupulus* L., is a dioecious perennial species, and only female cones are used for beer brewing. Hop as a perennial and vegetative-propagated crop is endangered by viruses and viroids. The most important viruses are: Apple mosaic virus (ApMV) from the genus *Ilarvirus*, Hop mosaic virus (HMV) and Hop latent virus (HLV) from the genus *Carlavirus*. Hop latent viroid (HLVd) from the group *Pospiviroid* is the most frequent viroid found out in hops. DAS-ELISA, Dot-blot molecular hybridization and RT-PCR have been used for control of infection hop plant status (PATZAK et al. 2001; SEIGNER et al. 2014; ZIEGLER et al. 2014). Nowadays, the use of real time PCR systems have enabled to precise quantification of viruses and viroids (ZIEGLER et al. 2014; WINKOWSKÁ et al. 2016; PAPAYIANNIS 2014).

In our study, we developed real-time quantitative RT-PCR (RT qRT-PCR) specific primers for detection of ApMV, HMV, HLV and HLVd RNA molecules in hop plants. The results of virus infection plant status were comparable with DAS-ELISA results. We confirmed that HMV antibodies could cross-react with HLV coat protein. Reference genes, (GAPDH, DRH1) were used for relative virus quantification (MATOUŠEK *et al.* 2012). Viroid quantification was absolute to known concentration levels in tissues obtained by Dot-blot molecular hybridization. We proved that this one-step RT qRT-PCR has been the valuable tool for molecular detection of virus and viroid pathogens within hop plants.

Key words. Apple mosaic virus (ApMV); Hop mosaic virus (HMV); Hop latent virus (HLV); Hop latent viroid (HLVd); quantitative real time reverse transcription PCR (RT qRT-PCR); DAS-ELISA

Acknowledgement

This work was supported by the Ministry of Agriculture of CR in conceptual project RO1486434704.

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Computational prediction of novel microRNAs in the genome of soil-borne hop pathogen *Verticillium nonalfalfae*

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Abstract

Micro RNAs (miRNAs) are coded by genes and processed into small non-coding RNAs containing about 21-24 nt; they are considered a small RNA (sRNA) species. They pair with mRNAs and direct post-transcriptional repression by mRNA degradation. They are therefore known to be negative regulators of gene expression. Several classes of sRNAs exist, including miRNA-like sRNAs (mil-RNAs). They have been recently shown to exist in filamentous fungi. Moreover, some plant pathogens use them during the infection process as protein effector molecules to suppress the components of the plant's innate immunity. However, no miRNAs or mil-RNAs have to date been reported in the phyto-pathogenic fungus, *Verticillium nonalfalfae*, a soil-borne plant pathogen causing vascular wilt in many important crops worldwide, including hops. Two pathotypes of *V. nonalfalfae*, mild strain and lethal strain, have been isolated from Slovenian hop fields, with the lethal strain causing severe symptoms in hop plants, resulting in rapid and intense withering and complete dieback of the plant. Previous genomics and proteomics studies have confirmed differences between these two pathotypes. With the available genome sequence and detailed transcriptome of the two strains, we aimed to identify and characterize miRNAs or mil-RNAs in the two pathotypes of *V. nonalfalfae* and elucidate their possible involvement in the pathogenesis process. In this study, two Slovenian isolates were analyzed, one causing mild and the other lethal symptoms. Small and total RNA fractions were isolated from four different tissue types: spores, mycelia, mycelia grown on simulated xylem fluid medium (SXM) and resting mycelia. Isolated small RNAs were used for small RNA library construction and sequenced using the Ion Proton NGS sequencing platform. Raw sequences were subjected to quality control analysis. Using the RFAM 12.0 database, the quantity of rRNA, tRNA, snRNA and snoRNA species was determined. Fungal mil-RNA precursors were predicted using MIReNA software and the results were further inspected with the aid of the CLC Genomic Workbench package. Validation and selection of predicted precursors was performed manually, applying the criteria for plant miRNA candidates proposed by Mishra et al. (2015). Additionally, some of the predicted candidate mil-RNA precursors were selected and were validated with stem-loop RT-PCR using mil-RNA-specific primers. This NGS and bioinformatic based approach confirmed the existence of mil-RNA structures of *V. non-alfalfae*. Future studies will be performed to determine their endogenous targets and to investigate their role in the infection process.

Key words. Verticillium wilt, *Verticillium nonalfalfae*, miRNA, phytopathogenic fungi

A comparison of weather conditions in four hop-growing countries in Europe

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Abstract

We compared data of precipitation and temperatures in four hop-growing countries (Slovenia, Germany, Austria and Czech Republic) in the years 2008 to 2010. Precipitation and temperatures differed a lot between locations.

Key words. *Humulus lupulus* L., weather conditions, growing conditions, precipitation, temperatures

Introduction

Weather conditions influence a lot on hop growing and yield. In the investigation, we compared data on precipitation and temperatures in four hop-growing countries (Slovenia, Germany, Austria and Czech Republic) in the years 2008 to 2010.

Results and discussion

The average day decade temperatures were almost in all years and decades the highest at the most southerly locality in Slovenia, followed by Austria, while the lower and the most comparable between localities were temperatures in Germany and Czech Republic. An example is presented for the year 2010 in Figure 1. The average day decade's temperature in the growth season (April – September) was above 17°C in Slovenia each year, in Austria it was between 15.5°C (in 2010) and 16.6°C (in 2009). In the Czech Republic, it was between 14.6°C (in 2010) and 15.9°C (in 2009), while in Germany it was between 14.1°C (in 2010) and 15.5 C (in 2009). The lowest precipitation amount was each year in Czech Republic (from 304 mm in 2009 to 529 mm in 2010) and the second lowest in Germany (from 509 mm in 2009 to 680 mm in 2010). The highest amount of precipitation was in Austria during two years (1338 mm in 2009 and 886 mm in 2010) and in 2008 in Slovenia (733 mm). Amount of precipitation did not show the same pattern with regard to the locality.

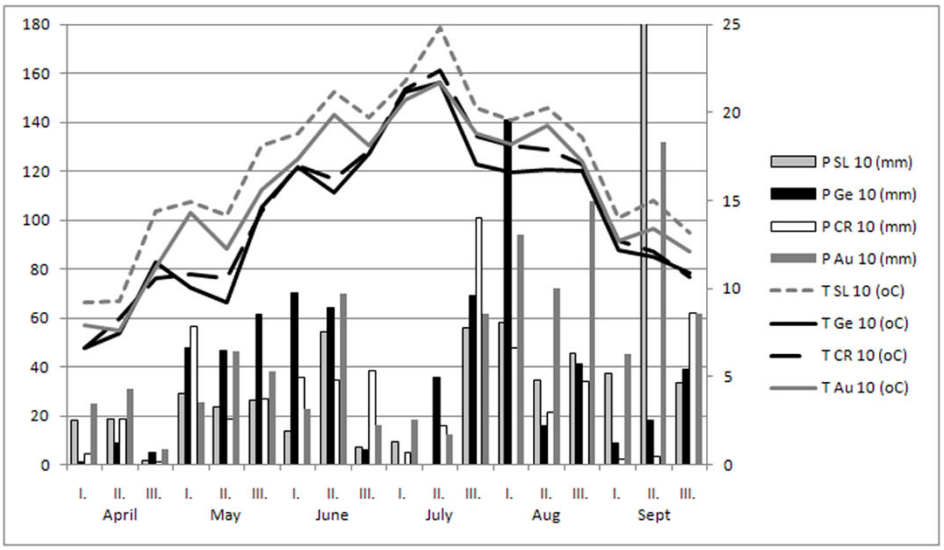

Figure 1. Average day decade temperatures T [°C] and amount of precipitation P [mm] in the hop growth season in year 2010 (10) for all investigated locations (Slovenia - SL, Austria - Au, Germany - Ge, Czech Republic - CR)

Acknowledgement

We would like to thank colleagues from the Hop Research Institute, Žatec, in the Czech Republic, the Bavarian State Research Center for Agriculture, Hüll-Wolnzach, in Germany, and Mr Gerald Pronegg, hop producer from Leutschach, Austria, for weather data.

Production of polyploids during prolonged tissue cultures of *Humulus lupulus* L.

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Abstract

Polyploidization of plant genomes is one of the methods of creating biological progress in agriculture. It is also used in hop breeding in order to obtain high yielding cultivars as well as relatively seedless cones (ROY et al. 2001). We report research on successful induction of tetraploids from long-term callus cultures of *Humulus lupulus*. Stem fragments and petiole explants of popular and highly valued by the brewing industry Polish cultivar Marynka were cultured on callus induction medium for three months. Then callus clumps were transferred to regeneration medium MS supplemented with 0.5 mg/l IAA in combination with two cytokinins: BA (1 and 2 mg/l) and TDZ (0.25 and 0.5 mg/l). TDZ was dissolved in 96 % ethanol to carry out the studies. The type of regenerated callus was assessed. The number of explants producing shoots and the number of shoots per explant were scored after 12 weeks of culture. Callus-derived shoots were also verified for ploidy level.

Most of explants formed cream-colored lumpy and fragile callus about 9-12 days after culture initiation. Shoot regeneration from stem fragments was higher than that obtained from petioles. The percentage of explants producing shoots and the number of shoots per explants were influenced by concentration of cytokinins. The highest number of shoots per explant occurred on stem-derived callus cultured on medium with 2 mg/l BA, while the lowest from petiole fragments on medium containing 0.25 mg/l TDZ. The regenerated shoots longer than 2 cm were cut off from callus, rooted and evaluated for DNA content. Flow cytometry analysis revealed the presence of diploids and tetraploids. There was a variation in the number of tetraploids depending on the initial explant and plant growth regulator. The stem-derived explants had a higher potential of tetraploid induction compared to petiole fragments. Application of TDZ to the regeneration medium did not affect the ploidy of the regenerated plants.

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Parthenogenesis and its role in increasing the capacity of hop (*Humulus lupulus* L.)

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Abstract

Pollination of hop by xenogamous pollen can induce active parenchyma cell proliferation of the ovary wall and initiate growth processes of adjacent tissues. This results in seedless fruits that are formed with normally developed pericarp. If the ovule is under development or the embryo sac degraded, perianth tissues preserve the parenchymal isomorphous structure longer. Polyphenols accumulate in an epidermis of perianth and bract. Induced parthenocarpy leads to active development of peltate trichomes with bitter and aromatic compounds. This effect has practical value and allows increasing the productivity of a valuable crop.

Key words. Parthenocarpic fruit, grandular tissue, ovule, pollen, integument.

Introduction

Wide interest in hops (*Humulus lupulus* L.) is due to unique biochemical components that accumulate in glandular trichomes. The secretory and reproductive systems of the pistillate plant are of significant applied importance for the biology of hop. Yet up to present, anthecological factors have not been taken into account in the agricultural techniques for cultivation of this technical crop. The issues related to the pollination of hop pistillate flowers by xenogamic pollen, parthenocarpy induction and the impact of parthenocarpic fruit on the biosynthesis of bitter and aromatic substances remain understudied (SEELEY & WAIN 1955; THOMAS & NEVE 1976; SCHILMILLER et al. 2008). The aim of our research was the study of hop parthenocarpy and its influence of glandular trichomes formation.

Material and methods

Nearly 100 flowers and cones of variety “National” have been selected at different stages of development for embryological and anatomical analysis. Research of reproductive organs tissues was carried out on permanent preparations. Staining of perianth and fruit tissues was used by aceto-basic fuchsin. Study of peltate trichomes was carried out on native preparations. Conventional methods of histochemical analysis were used for identification of polyphenols, cellulose, lignin, proteins and lipids.

Results

In the mesocarp parenchyma the symplastic growth of separate cells transforms into apical intrusive growth. The cells lengthen significantly and inculcate into the intercellular space of the parenchyma. During this process, the previously compact homogeneous tissue is transformed into a heterogeneous one, in the composition of which there are obvious prolonged cells rich in cytoplasm structurally looking like a conductive tissue of the stylodium. In the endocarp close-fitting to a zone of active cell division, sclereids increase in size and cellular walls become thicker (Fig. 1). The initiation of periclinal and anticlinal cell division is observed, resulting in the formation of two-three (more seldom six-nine) row endocarp. The basal part of the stylodium does not fall down for a long time after dying-off. The inner space of the fruit is filled with quickly spreading tissue of inner and outer integuments, while nucellus cells gradually degrade. Morphologically it is difficult to distinguish between a parthenocarpic fruit and a normally formed fruit. Its most important peculiarity is that, in the period of abnormal spreading of ovary walls and integument tissue, active differentiation and

development of peltate trichomes where bitter and aromatic substances are synthesized is observed. The grandular tissue is formed as a result of intensive spreading of the apical part of the integument and is histochemically essentially different from the main tissue (Fig. 1e).

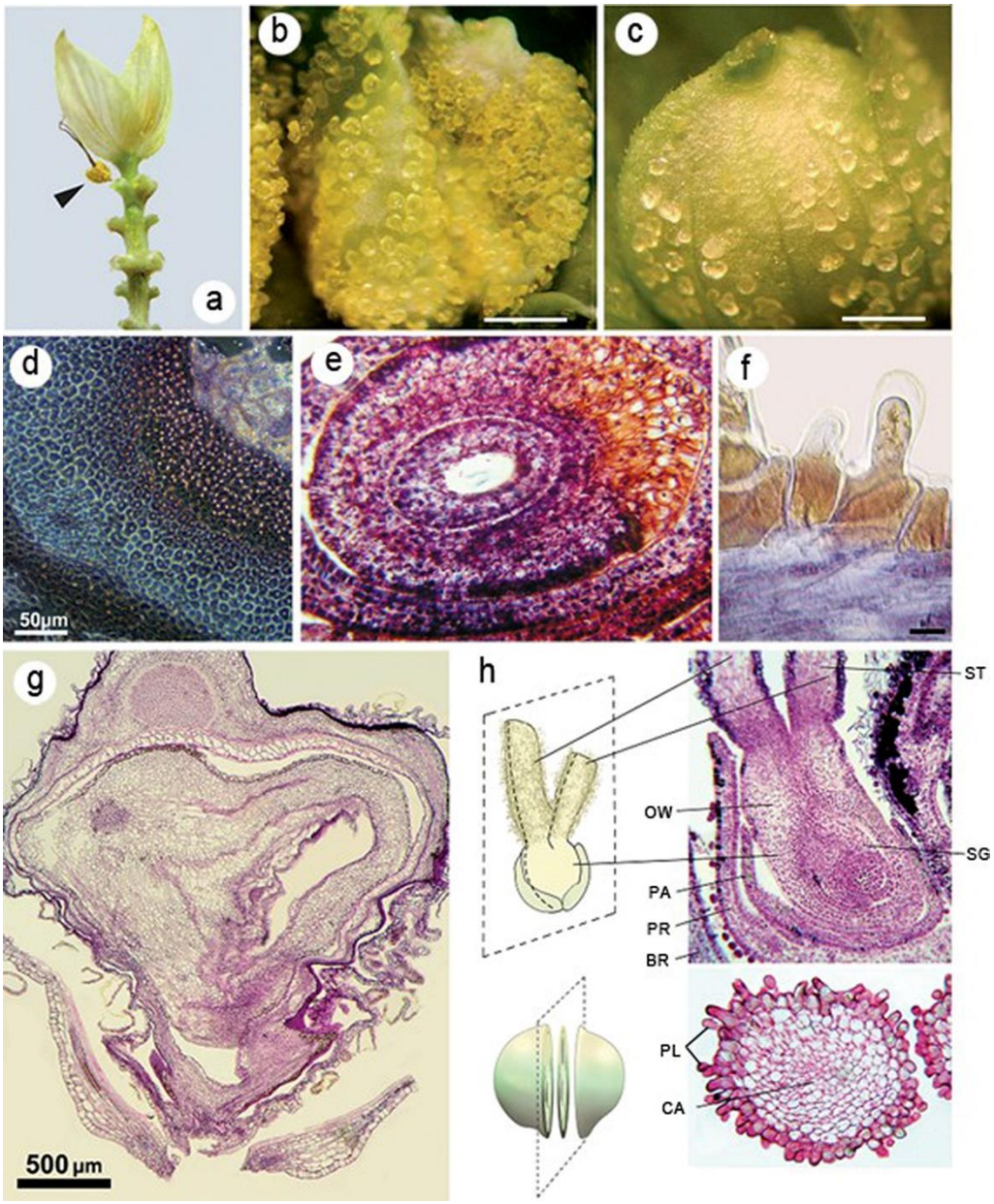

Figure 1. The structure of the pistil and parthenocarpic fruit. a − parthenocarpic fruit, the rest elements of the cone is removed; b − seedless (parthenocarpic) fruit, on the surface of the perianth, which formed a significant number of lupulin; c − normally shaped nut (line 500 µm); d − endocarp cell configuration (view in the plane); e – ovule: cells with predominantly oxyphilic (alkaline) components that are painted in colors of blue, basophilic (acid) components in red. Outer integument secretory tissue is stained with red; f − localization of proteins in stylodium cells: histochemical reaction of proteins on bromophenol blue in the presence of mercuric chloride (fibers stained in shades of blue); g − longitudinal section of the fruit, formed with massive integument tissue with abnormal growth (staining with acetofucsin); h − longitudinal section of the pistil: OW, ovary wall; PA, perianth; BR, bracts; PR, prophyllum; SG, bitegmic campylotropous crassinucellate ovule; ST, stylodium; cross section of stylodium: PL, papillas; CA, conducting area

Discussion

Our multiyear studies show from the moment of ovule formation and ovule ability to be fertilized, the hop ovary represents a complex system consisting of at least three subsystems: ovary walls, ovule integumentary tissues and the nucellus, and the embryo sac. Each subsystem is clearly arranged spatially and hierarchically. The subordination levels 'from inferior to higher' are distributed centripetally — from the perianth external epidermis to the ovule. The functioning of each subsystem structures is mainly conditioned by its spatial position and physiological activity of its tissues synthesizing biologically active substances, hormones and phenol compounds.

It is known that there is an auxin gradient in the tissues of a developing fruit the concentration of which increases from the pericarp external tissues to the embryo. Such model of the fruit development is a norm; yet, as we mentioned earlier, the agricultural techniques of hop growing is ecologically arranged in such a way that the proper formation of plant generative organs is minimal. As a result of the artificial sexual isolation in the atmospheric air surrounding the pistillate plants, there is hardly any hop pollen or its quantity is not enough for normal fertilization. At that, the xenogamic pollen of other anemophilous species (*Zea mays* L., *Urtica dioica* L. and others) from the surrounding environment gets on hop flowers. The pollen grain triggers the most complicated mechanisms of molecular identification and biochemical exchange on the pistil stylodium. As a rule, the growing processes of the xenogamic pollen cease quickly, but over a short period, hormones and other biologically active compounds manage to diffuse from the pollen tube cytoplasm in the stylodium. Moving basipetally, the exogenous auxin reaches the ovary parenchyma, which transforms in the mesocarp. The hormone initiates the proliferation of cells. The pericarp tissue grows thickly and forms the centre of endogenic hormone synthesis. Being a powerful attractant, the anomalously increased parenchyma draws out nourishing substances and stimulates the growing processes of close-fitting tissues.

According to our findings, during the formation of the parthenocarpic fruit the number of peltate trichomes filled with bitter and aromatic compounds of the "National" hop variety increases by 1.2-1.5 times. It is also supposed that the phenomenon of parthenocarpy is promoted by special grandular structures we found in the distal integument of the bitegmal ovule of some hop varieties. This feature is not well developed in wild species and that is probably why it has not been noticed by other researchers so far.

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Conservation of the Czech hop germplasm by cryopreservation

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Abstract

Hop (*Humulus lupulus* L.) belongs to the most important crops in the Czech Republic. This crop is propagated and maintained vegetatively. Hop as a perennial and vegetative-propagated crop is endangered by viruses and viroids. Conservation of hop germplasm in the field collections increases the risk of accidental loss of valuable genotypes. This risk can be reduced using the method of cryopreservation, which allows safe storage of the plant samples at ultra-low temperatures. This method contributes to the conservation of genetic stability and prevent from ageing. It is used for conservation of genetic resources of cultural and wild plants in such virus free material, which is endangered by biotic and abiotic stresses if multiplied in field conditions. Development of cryocollection is the best way to eliminate the risk of an accession loss (REED et al. 2005).

In vitro cultures were derived from extracted meristems tips according to procedure described by (SVOBODA 1992) and maintained by (SVOBODA 1991; FALTUS et al. 2007).

Simple cryopreservation method was used for cryopreservation of selected genotypes of the Czech hop germplasm collection. Nodal cutting were acclimated by low temperature and sucrose treatment. Isolated shoot tips were loaded with 0.7M sucrose for overnight and simultaneously dehydrated above silica gel for approximately 100 minutes on aluminium plates. Shoot tips were plunged directly into liquid nitrogen. Control explants were thawed at 40°C water bath and regenerated on medium for eight weeks. Altogether 45 hop genotypes have been cryopreserved with average recovery rate of 40 %. A plant recovery higher than 30 % was observed in 79 % of all accessions. The minimal number of plants to recover for each cultivar was calculated as a sum of minimal numbers of viable plants in particular cryopreservation procedures according to a probability tool developed by DUSSERT et al. (2003). The methods used and results are presented.

Key words: *Humulus lupulus* L., *in vitro*, cryopreservation, liquid nitrogen

Acknowledgement

This study was supported by the research project QJ1630301 of the Ministry of Agriculture of the Czech Republic.

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Spatial layout of the root system of hop in Czech Republic

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Abstract

For a full understanding of the plant's response to habitat conditions, among other things the knowledge of the development, morphology and functionality of the root system is fundamental. In terms of its anatomy, spatial layout, physiology and chemical composition the root system provides basic information about interactions between the plant and its environment. Soil conditions and characteristics of the soil profile influence the formation of the root system of hops. Between 2015 and 2017 several excavations of the root system of three Czech hop varieties ranging in age from 3 to 14 years were carried out in the Saaz hop growing area (locality Steknik near Žatec, elevation 197-203 m a.s.l.). The soil profile at the evaluated localities was characterized by quaternary sediments from the holocene and pleistocene, consisting of topsoil and brown to red clays. Under the surface layer, the sediment deposits were represented by black-gray clay and sandy clays. Large amount of soil was removed by means of heavy machinery. Preparation of the root system was completed by manual removing of the soil from the root zone. During the preparation of the roots, their spatial layout was recorded in the soil by means of markers placed on the spine roots. The position of the roots was determined based on axial coordinates x /y /z from the center of the crown. After the roots were removed from the ground, their spatial reconstruction and subsequent graphical analysis were performed using infrared images.

Depending on the age of the hops, the depth of the roots ranged between 80 cm (Saaz, 3 years old plant) and 130-140 cm (Agnus, 14 years) to 150 cm (Harmony, 4 years). The lateral penetration, perpendicular to the line, was limited, mostly to the distance of 50-60 cm, measured from the center of the crown, to the depth of about 50 cm. Capillary roots growing from the stronger ones have been found throughout the root system profile. The limited formation of horizontal roots at the top of the soil profile (perpendicular to the line) is caused by the repeated cultivation of hop gardens to the depth of 10-15 during vegetation. The predominant feature of the 14-year-old root system of the Agnus variety was the mutual proliferation of the roots of adjacent plants in a horizontal direction along the line axis. To disclose the entire root system of two plants it was necessary to uncover the roots of another two adjacent plants in the upper layer to the depth of about 30 cm. In the depths of 40-80 cm, dead roots of about 10-15 mm were found. At the transition of the dead and the living plant parts, new weaker and thinner roots were growing out.

The growing technology, based on regular cultivation of the interlayer, fundamentally influences the formation of the root system of hop. The spatial distribution of the root system in the soil is the basic requirement for efficient agrotechnical procedures of hop growing. In particular, it enables a targeted application of nutrients in the soil to the rooting zone and creation of conditions for water infiltration (rain and irrigation) to the root system.

Key words. Hop, *Humulus lupulus* L., root system, soil

Acknowledgment

This work was financially supported by Czech Ministry of Agriculture within the project NAZV QJ530181 and Research concept VZ-CHI16-001.

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Structural changes in the guild of aphidophagous coccinellids in Bohemian hop gardens

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Abstract

The spectrum of aphidophagous coccinelids recently has changed considerably in Bohemian hop gardens. It was caused by the invasion of Asian harlequin ladybird *Harmonia axyridis* (Pallas). Whereas, in the period before its invasion, the native species *Coccinella septempunctata* L., *Adalia bipunctata* L. and *Propylea quatuordecimpunctata* L. used to be the dominant predators of damson hop aphid *Phorodon humuli* (Schrank), *H. axyridis* is the most frequent ladybird species in the Žatec (Saaz) hop growing region nowadays. Six years ago, the harlequin ladybird represented just 10 % of the total Coccinellidae, but since 2015 it has become the most abundant ladybird species. This may have been caused by the aggressiveness of *H. axyridis*, whose larvae were observed to feed on eggs and young larvae of the native species. To prevent attacks from larvae of *H. axyridis*, females of *C. septempunctata* appeared on hop plants and began to lay eggs earlier, as illustrated by the presence of the fourth instar larvae in times when larvae of *H. axyridis* reached smaller size and were not competitive enough. This phenomenon was observed in our experimental garden in Stekník again in 2016. Whereas *H. axyridis* represented 77.4 % of all coccinellid adults, native species, especially *C. septempunctata* and *A. bipuctata*, were very scarce. Therefore, the arrival of this aggressive invasive species does undoubtedly have a negative effect on native guild of aphidophagous coccinellids because of its aggressiveness and so its presence is questionable.

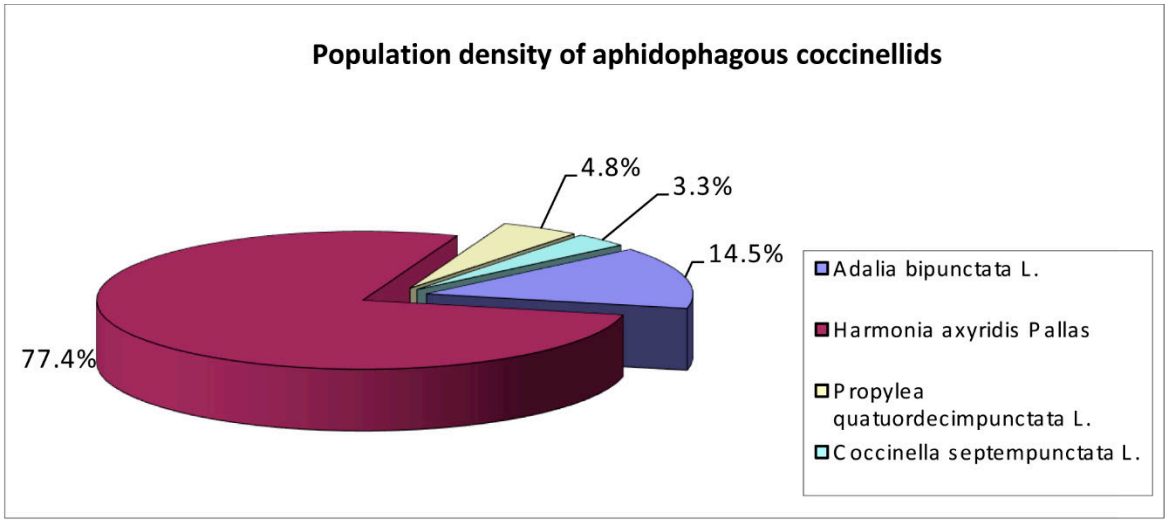

Figure 1. Population density of aphidophagous coccinellids in the experimental hop garden Stekník near Žatec, June 2016

Acknowledgement

The work was supported by Ministry of Agriculture of CR within Institutional Support for the Development of Research Organization RO1486434704.

Reducing European roe deer browse damage in hop gardens in the Czech Republic

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Abstract

Czech hop growers deal with browse damage of young hop shoots. Some deterrent methods are tested, but a temporary polytape electric fence around a hop garden is highly effective.

Key words. Hop, damage, European roe deer, browsing, electric fence.

Introduction

The Czech hop growers have been recently dealing with a problem how to protect young shoots against browse damage caused by a wild game. Signs of animal presence (droppings, footprints) and abundance or observation have denoted roe deer, *Capreolus capreolus*, which belongs naturally to the Czech fauna.

Growth stages from sprouting after cutting (BBCH 09) until climbing up to approximately 2 m (BBCH 32-33) are crucial. A high priority against browse is to protect the apex of the hop bine. In the Czech Republic, human hair, contact and area repellents have been hung on anchor cables to discourage deer. The contact repellent called 'Trico' (sheep fat substance) has been added to the methodology recently. However, a temporary polytape electric fence is probably most effective.

KOPP (2007) summarized the methods of deterrents against browse damage.

Material and methods

Human hair from hairdressers in perforated bag is hung on anchor cables.

Area foam repellents, e. g. Hagopur, Antifer, Pacholek, are applied on hop poles or hung on anchor cables. Formerly, a liquid cartridge was sprayed on stripes of textiles.

Aluminium strips are hung on anchor cables. They include a part for spraying area repellents containing predator or human odors. The effect is optical and noisy (they reflect sun rays and they jingle).

Contact repellent 'Trico' (sheep fat), approved in a methodology, is applied with a spraying machine. A side effect of supplementary soil substance 'Eutrofit' might discourage browse damage as well.

Electric polytape fence is installed around a hop garden.

Results

Czech hop growers combine various methods of deterrents against browse damage. If roe deer density is high, electric fencing may be the best option. It is recommended for localities with high abundances of roe deer where a hop garden adjoins a forest or a grove.

Acknowledgement

The work was supported by Czech Ministry of Agriculture within the Research Project No. RO1486434704.

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Pesticides in hops and beer

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First presentation at 3rd German Hop Convention, Bad Gögging, Germany, 2016

Abstract

Hops have to be treated with plant protection agents. Otherwise the supply of the brewing industry with hops whether in quality nor in quantity cannot be secured.

The German Federal Institute for Risk Assessment (BfR) inspects and approves in an extensive procedure the registration of agrochemicals. Maximum residue levels (MRLs) in hops and processing factors (PF) from hops to beer are specified. The task of the BfR is thereby not the avoidance of any pesticide residues but the exclusion of risks during application and for the consumer.

In the first part of the poster all measures that guarantee the compliance with the MRL legislation are displayed. This implies a comprehensive monitoring from hop grower to hop processor.

Furthermore in addition to the EBC poster (A method to enable systematic studies on the transfer of pesticides from hops into beer), which is also exhibited at this meeting, the residue situation in beer is discussed.

The detection limits of pesticide residues in beer are lowered constantly with progressing analytical techniques. Therefore hop pesticides can be detected especially in strongly hopped beers. This is the logical result of the pesticide residues in hops and the solubility of their active ingredients in wort and beer.

The publication of processing factors by the BfR confirms this context. The BfR does not see a threat to the consumer as long as the MRLs are not exceeded.

All members of the supply chain from hops to beer have to be aware of the fact that, contrary to somewhat unrealistic consumer expectations, hop pesticides can be detected in beer.

A method to enable systematic studies on the transfer of pesticides from hops into beer

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First presentation at EBC Congress, Porto 2015

Abstract

The legal situation regarding pesticides in hops and beer is focusing on hops only. There are clear rules for registration including brewing trials and fixing maximum residue levels (MRLs). In beer, there is no need to establish MRLs for pesticides from hops as long as the MRL in hops is not exceeded. The transfer of pesticides from hops into beer can be elucidated with the help of brewing trials only. To date rare literature with limited information on few pesticides and not on the actual spectrum is available. Furthermore, the way of hopping beer changed within the last 20 years. Wort hopping up to 500 g/hl and additionally dry hopping up to 500 g/hl is not unusual in craft beers. Also the detection limit of pesticide analyses in hops and beer has been constantly lowered. It is therefore reasonable to undertake systematic studies on the transfer of pesticides from hops to beer.

Pesticides show a wide range of solubility in beer. The transfer of pesticide residues from hops into beer is not fully understood. The main reason is a lack of samples that are contaminated with more than a few pesticides at a suitable level. Spiking of pellets with all relevant pesticides is the only method to get valuable information on the behavior of these substances in the brewing process.

The transfer of 16 selected pesticides from hops to beer is determined in a 2 hl pilot brewery with following hopping regimes:

- Dry hopping in a tank and a keg (500 g/hl)
- Late hopping in the brew kettle (200 to 450 g/hl)
- With each 500 g/hl late in the brew kettle and for dry hopping

By means of dry hopping in kegs, pesticides can be classified due to their solubility as follows:

unsoluble	**Fluopicolide, pyraclostrobin, quinoxyfen**
poor	**Hexythiazox, spirodiclofen, trifloxystrobin**
medium	**Azoxystrobin, boscalid, dimethomorph, mandipropamid, myclobutanil, triadimenol, pymetrozine**
good	**Flonicamid, imidacloprid, metalaxyl**

Regarding the transfer of pesticides, it has been confirmed that there are no significant differences between the use of originally contaminated and spiked hop pellets.

The dosage of hops in the brewhouse results in higher transfer rates than with dry hopping. In all 10 medium to good soluble pesticides, no significant losses due to fermentation, storage, centrifugation or filtration are detectable.

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