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Foreword

Almost a quarter of a century has passed since the Scientific-Technical Commission (STC) of the International Hop Growers' Convention (IHGC) has met for the last time in France's most important growing region, the Alsace – in fact, that was in July 1995 in Strasbourg, under the label of 'Scientific Commission' then. I as one was rather new in hop research at that time and wasn't yet mature enough to attend the 1995 meeting, but Bernhard Engelhard already presented results of my trials in his first talk at a Scientific Commission meeting. My very first professional visit to Alsace dates back to the year 2003, when I first met the two 'Girls from Alsace', Bernadette Laugel and Michèle Dauger, on site to count spider mites together under Strisselspalt hops… Both Bernadette and Michèle have been the assets of hop research in Alsace then already and still are today, two decades later. And without forgetting all other French IHGC fellows – Bernard Ingwiller, Francis Heitz, Jean-Marc Meyer or Freddy Merkling, to name but a few – who definitely all did a wonderful job in arranging the current meeting as hosts, I suppose it is chiefly the merit of Bernadette and Michèle that the STC is facing such an attractive and well-organised event at a perfect site in beautiful Alsace. Merci infiniment!

Due to the great organising of the 2019 STC conference by our French fellows, the international community of hop scientists is facing another highlight of a meeting under the auspices of the International Hop Growers' Convention and more than 60 participants from 11 hop-growing nations have registered. We are grateful to all scientists who were ready to enliven our conference by presenting their current work and have submitted an oral presentation or a poster. Altogether, 35 talks have been submitted and will be presented during nine scientific sessions – hop breeding, phytopathology, entomology, hop chemistry, hop cultivation and management, molecular investigations, hops and brewing, hops and health and 'hops under the Southern Cross'; finally, a poster session presenting 15 posters will make our meeting perfect. In addition to the scientific agenda, an excursion to the Alsace hop fields will give us the opportunity to get to know, or learn much more, about this traditional European hop growing region.

We are also expressing our gratitude to the sponsors of our conference; the generous financial backing by Barth-Haas Group, Comptoir Agricole, Hopfenverwertungsgenossenschaft HVG and Hopsteiner supports the mission of the STC and facilitates the participation of many scientists.

In closing, I wish all participants a fruitful and pleasant meeting with many interesting discussions and encounters that will strengthen international cooperation and networking within the small world of hop science, jointly together with the hop and brewing industry. Welcome to Alsace!

Dr Florian Weihrauch
Chairman, Scientific-Technical Commission of the I.H.G.C.

I: Hop breeding

Cost effective genotyping for 21st century hop breeding

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Abstract

Hops are a high value horticultural crop with historical and cultural importance in the UK. For the last century English hop breeders have relied on classical breeding approaches, where characterisation of desirable traits is conducted exclusively through phenotyping and molecular breeding techniques are not used. However, genome-assisted breeding approaches could add enormous value to hop breeding programmes. In order for genomic-assisted hop breeding to be economically viable, the cost of genotyping must be low.

We are currently evaluating repeat Amplification Sequencing (rAmpSeq), a new PCR and sequencing based genotyping method that uses transposon sequence derived markers for robust genotyping. rAmpSeq technology uses a small number of primers to amplify hundreds to thousands of amplicons that are variable within and between individuals.

Preliminary rAmpSeq PCR primers have been identified from the “Cascade” reference genome and will be further optimised on whole genome sequences generated for key parental lines selected from the UK hop breeding programme. Preliminary in silico tests with one rAmpSeq primer pair revealed a high abundance of genetic polymorphisms present across the genome. With the further optimisation of this technology, a cost effective large-scale discovery of markers represented by unique combinations of single nucleotide polymorphisms (SNPs) could be achieved. A single rAmpSeq PCR primer pair could amplify large numbers of unique genetic variants that may be used to identify QTLs associated with desirable crop traits.

Key words. Cost-effective genotyping, hops, marker-assisted breeding, rAmpSeq

Introduction

The European hop *Humulus lupulus* var. lupulus has a large genome; flow cytometry experiments suggest a diploid genome size of greater than 5.4 Gb (KINGAN et al. 2018). Current assemblies of the hop genome are highly fragmented (NATSUME et al. 2015; HILL et al. 2017; KINGAN et al. 2018) reflecting the difficulty in assembling a genome comprised of approximately 60 % repetitive regions (PISUPATI et al. 2018). There is no current SNP chip available for hop and the development of a hop SNP array is currently expensive. Restriction-enzyme based genotyping by sequencing (GBS) strategies have been previously conducted on hop (HENNING et al. 2016) but with limited success due to the large genome size. Restriction enzyme GBS also has the disadvantage of PCR competition between the various amplicons. By focussing on the repetitive fractions of the genome, where the sequences are nearly identical in length, this competition could be reduced (BUCKLER et al. 2016). The high repeat content, and variability of repeats within plant genomes has been exploited for variant discovery and trait mapping in Maize (BUCKLER et al. 2016). This technique, repeat Amplification Sequencing (rAmpSeq), could provide a simple and cost effective way to map and identify genetic polymorphisms which may prove informative to hop breeding programmes.

In this study we aim to develop genomic resources for hop breeding programmes and evaluate molecular marker assisted breeding techniques. This study is broken down into four objectives: 1) Preliminary rAmpSeq primer design from the “Cascade” reference genome, 2) Genome sequencing of eight UK parental lines and further in silico and in vitro optimisation of rAmpSeq primers, 3) Amplicon sequencing and QTL analysis on individuals from biparental mapping populations, 4) Implementation of findings in genomic selection breeding techniques.

In this paper we report the progress in “Study objective 1” and outline our future study plans.

Material and methods

The “Cascade” genome assembly was downloaded from hopbase.org (HILL et al. 2017). Preliminary rAmpSeq primer design has been carried out using a modified pipeline based on the approach described in BUCKLER et al. (2016), originally developed for maize. The “Cascade” genome was fragmented into 20 bp sequences (Kmers) and Kmer occurrence was counted using KAT (MAPLESON et al. 2017). Kmers in single copy represent non-duplicated regions of the genome. In contrast, Kmers in high abundance represent highly duplicated regions of the genome and putative transposable DNA sequences. 20 bp Kmers that were present in the genome in more than a thousand copies were selected as potential primer candidates for rAmpSeq. These primer candidates were filtered for 35-65% GC content and presence of a GC-clamp within 3 bp of the 3’ end. This filtered set of primers was used to identify all potential 125-200 bp amplicons in the “Cascade” genome assembly. Identified primer pairs were ranked by their abundance and selected primer pairs were further evaluated. Sequence alignments were performed and further visualised using Geneious R10.03 (KEARSE et al. 2012).

Preliminary results

Preliminary candidate rAmpSeq primer pairs have been designed from high copy Kmers in the “Cascade” genome. The most abundant primer pair was found throughout the genome in 4433 contigs out of 8836 contigs. This primer pair was present 12393 times in the genome. A high level of sequence variation was observed between these amplicons, with 9852 unique haplotypes. For example, in contig 000000F of the “Cascade” genome, 31 variants were identified of which 29 were unique sequences (Fig. 1). Primer pairs from various abundance ranges in “Cascade” showed that amplicon uniqueness is present at different amplicon frequencies (Table 1).

Table 1. Primer pairs present in various copy numbers in the “Cascade” genome and their predicted amplicon numbers; total and unique.

Primer pair	GC content (primer1/primer2)	Predicted amplicons	Predicted unique sequences	Predicted unique sequences (%)
A	50/45	12393	9852	79.5
B	55/45	12037	9512	79.0
C	50/50	11706	9186	78.5
D	60/50	10019	7713	77.0
E	35/45	10008	7471	74.7
F	40/50	9952	5622	56.5
G	50/60	5001	3854	77.1
H	35/40	5000	3494	69.9
I	55/50	4998	3873	77.5
J	50/65	1000	783	78.3
K	50/40	1000	991	99.1
L	35/40	1000	742	74.2

Amplicon count and sequence uniqueness in the “Cascade” genome using various rAmpSeq primer pairs show promising results. However the distribution of these amplified sequences has not yet been investigated. Understanding the underlying nature of transposon distribution within and between different hop genomes is essential for the further optimisation of rAmpSeq. These preliminary results demonstrate the first step of optimising rAmpSeq primer pair design for cost effective genotyping of hop.

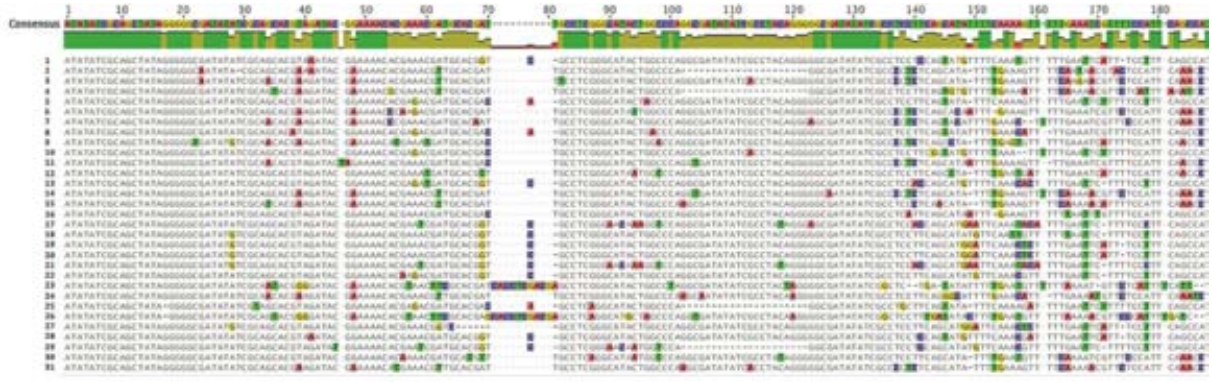

Figure 1. Alignment of 31 putative amplicons in “Cascade” contig 000000F from a rAmpSeq primer pair showing 30 unique sequences.

Future work

Genomes of the main UK hop progenitors will be sequenced and rAmpSeq primers will be further assessed to ensure variability of amplicons between parents and presence and absence of variants. Even distribution through the parental genomes will also be assessed. Primer pairs identified from the parental genomes that are evenly distributed across the genome will be selected for further *in vitro* PCR optimisation. Amplicon sequencing (genotyping) of individuals from a biparental mapping population will be combined with phenotyping and QTL analysis to identify genetic markers for breeding.

Discussion

Transposons constitute a large fraction of the DNA in many species of plants including hop (FEDOROFF 2002). Transposable elements have previously been referred to as 'junk DNA', however it is now apparent that they play important roles in shaping genome evolution (DUBIN et al. 2018). Furthermore, the use of transposable DNA regions as genetic markers in genomic breeding techniques has been investigated (BUCKLER et al. 2016).

rAmpSeq genotyping has the potential to be deployed on thousands of hop plants during early stages of breeding to identify the genetic basis of agronomically important traits such as resistance to major pest and diseases, dwarfing and sex. This technology could provide a robust background for the potential future implementation of marker assisted selection (MAS) and genomic selection (GS) techniques. It is believed that both MAS and GS offer advantages compared to conventional breeding strategies by increasing selection accuracy due to removing the influence of environmental variation on the estimated breeding values. Additionally, both may reduce cost and manpower requirements of phenotyping traits that are expensive or otherwise difficult to measure (COLLARD & MACKILL 2008). GS also has the potential to reduce the duration of a breeding cycle, as it allows selection to be made at juvenile stages.

The implementation of rAmpSeq could lead to more effective pre-screening of hop breeding material and the use of genomic prediction to determine breeding values for both male and female lines. In recent years global hop breeding has been shifting from classical breeding to molecular breeding approaches. Environmental factors and market competition drive the hop breeder to breed new varieties quicker, cheaper and more efficiently. If appropriate primer sets can be identified, then rAmpSeq technology has great potential to become a versatile tool in the armoury of the 21st century's hop breeder.

Acknowledgement

This study was supported by the British Hop Association. We also acknowledge Mike Baldock at Wye Hops Ltd by providing hop material for future DNA extractions and genome sequencing.

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An attempt to elucidate the mechanism forming a hop lupulin gland for the breeding of a new hop variety in Japan

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Abstract

Hop Breeding has ever carried out very long time using conventional method, for example in my case hop variety made from cross breeding by female plant cross male plant for long time. We know a variety of hop is different from where they are grown. In Japan we have bred many varieties in the northern part of the country. Now I would breed on a new variety of hop. I tried to elucidate the mechanism forming hop lupulin glands. Then using this information I am trying to breed a new variety of hop. Present study gives information on 1) Aromatic hop characters in the biosynthesis of terpenes, 2) recovery of Hop stunt viroid-free plants from HSVd-infected hop and hop transformation, 3) a new development possibility of lupulin glands on the bottom surface of leaves, 4) elucidated genesis of male and female hops and 5) a vision of hop breeding by my idea. This time I would like to present on some of them.

Key words. Hop breeding, lupulin glands, Aroma, terpenoid synthesis pathway, hop stunt viroid

Introduction

Hop (*Humulus lupulus*) is a perennial, dioecious plant belonging to the Cannabaceae family. Humans have long used wild and cultivated hops for their medicinal properties and as a key ingredient in brewing beer (HORNSEY 2003).

The female inflorescences (cones) of hop are rich in terpenoid essential oils and terpenophenolic resins. In addition to the terpenophenolic acylphloroglucinols (e.g., humulone) that give beer its characteristic bitter flavor, hop cones also contain 1 % of xanthohumol, a prenylchalcone with potent cancer preventive properties (STEVENS & PAGE 2004). Xanthohumol and other hop terpenophenolics accumulate primarily in peltate glandular trichomes, termed lupulin glands, which are visible as yellow structures at the base of bracteoles in hop cones. A trichome-specific CHS gene, chs_h1, has been cloned from hop (NOVAK et al. 2003). A key and unique feature of glandular trichomes is their ability to synthesize and secrete large amounts, relative to their size, of a limited number of specialized metabolites: mainly terpenoids (GERSHENZON et al. 1992; GERSHENZON & DUDAREVA 2007) but also phenylpropanoids (GANG 2001; DESCHAMPS et al. 2006; XIE et al. 2008), flavonoids (VOIRIN et al. 1993; TATTINI et al. 2000), methylketones (FRIDMAN et al. 2005), and acyl sugars (KROUMOVA & WAGNER 2003; SCHILMILLER et al. 2010; WEINHOLD & BALDWIN 2011). Over the long term, the ability to modulate the density and productivity of such secreting structures in plants would be of great biotechnological interest. This requires the identification and characterization of the genes initiating, regulating, and driving the development of such glandular structures. Awareness of, and detailed knowledge on, the biology of plant glandular trichome development and metabolism will generate new leads to turn trichomes into biochemical factories using metabolic engineering approaches (TISSIER 2012), tap their largely unexploited potential in plant resistance to pests, and lead to the improved production of important specialized metabolites (LANGE & TURNER 2013). In the future, awareness of, and detailed knowledge on, the biology of plant glandular trichome development and metabolism will generate new leads to tap the largely unexploited potential of glandular trichomes in plant resistance to pests and lead to the improved production of specialized metabolites with high industrial or pharmacological value.

Recently I have been breeding hop using some varieties with the objective to make a new variety of hop. During the past few years I achieved some candidates from this approach and now I am studying their quality and yield. Objective of this study is to inform hop breeders of the world of my ideas, to discuss them and to receive feedback from colleagues.

Materials and methods

1. Recovery of hop stunt viroid-free plants from hop stunt disease hop & hop transformation

We studied the relation with pathogen and host plants using viroid-free hops and viroid-infected hops. The original Morioka isolate of HSVd was purified as previously described (TAKAHASHI 1981; YOSHIKAWA & TAKAHASHI 1982). The purified HSVd (5 µg/ml) was used to inoculate hop plants (cv. Shinshuwase) by razor-slashing the young shoots in early spring. One year later, the inoculated plants were stunted, and their upper, young leaves showed epinasty and other characteristic symptoms of HSV infection (TAKAHASHI & TAKUSARI 1979a; TAKAHASHI 1981). The presence of HSV in the inoculated hop plants was ascertained by cucumber assay (TAKAHASHI & TAKUSARI 1979b). Tissue samples were taken at developing stages from the fourth to the eighth leaves (exhibiting typical epinasy) below the terminal but of hop plants as well as from comparable leaves of the un-inoculated, HSVd-free controls. Parallel studies were made with HSVd-infected cucumber plants (Cucumis sativas cv. Suuyou); inoculation was carried out by mechanical rubbing of the first true leaves of Suuyou cucumber plants (TAKAHASHI & TAKUSARI 1979b), and 2-3 weeks later, tissue materials from the apical leaves showing the typical rugosity (Fig. 11) were sampled from microscopic observations.

2. A new development in lupulin glands

Stem tips ranging in size from 1 to 2cm long were cut off from terminal or lateral shoots of inoculated, field-grown hops, and were surface-sterilized in sodium hypochloride (0.5% w/v, available Cl) containing 0.1 % Tween 20 for 5-10 minutes. They were then rinsed five times in sterile distilled water and were aseptically dissected. The excised apical meristem (0.2-5.0 mm long) were grown on bridges of filter paper (paper wick method, Baker & Phillips 1962) which dipped into 10 ml of Adams' medium (ADAMS 1975), supplement with 0.2 ppm of 6-benzuladenine (BA). Then the young shoots (2-10 mm long) grown from apical meristems were transferred to a second medium solidified with 0.6 % agar medium containing no BA when they were grown up to 5 cm long. Cultured tubes were kept in growth chamber (at 25±1°C) with continuous illumination (1,000 to 3,000 lux) by fluorescent lamps. Thereafter, rooted cultures were potted in polyethylene pot (5x5x5 cm) containing a mixture of vermiculite, perlite and peat moss (1:1:1) and were watered with solution of major element of MURASHIGE & SKOOG (1962). After two weeks, the plantlets thus obtained were transferred to clay pot (10 cm in diameter) and were raised.

3. A vision of hop breeding

We are now working on making a new variety of hop. Most advanced varieties are in 7th generation and are now cultivated in my private garden. We are trying on the identification of those varieties. In the near future we could get some varieties in those.

Results and discussion

1. Recovery of hop stunt viroid-free plants from hop stunt disease hop & hop transformation

In parallel with the electron microscopic observations, we tried to produce HSVd-free hop plants by means of meristem tip culture; fifty-five out of 129 shoot tips obtained from infected hop plants survived and became established plants (Table 1), and three out of 55 plants were found to be HSVd-free. As can be seen in Table 1, the percentage of plants free of HSVd was higher when the size of excised shoot tips was smaller. The shoot apical meristems, comprising the apical dome plus the first two primordial of ca 0.2 to 0.3 mm length, were again found to be free of HSVd.

Table 1. Survival of shoot apical meristem grouped according to size and elimination of HSVd (taken from MOMMA & TAKAHASHI 1983)

Size of shoot tip	Number of shoot tips		
[mm]	Cut	Survived to plants	HSVd-free
0.2	8	6	2
0.3	33	8	1
0.4	10	1	0
0.5-1.0	27	4	0
1.1-2.0	11	7	0
2.1-3.0	28	20	0
3.1-4.0	5	3	0
4.1-5.0	7	6	0
Total	**129**	**55**	**3**

The surface structure of the leaves of HSVd-free and HSVd-infected hop plants was observed under a scanning electron microscope.

In shoot apical meristems of the infected hop plants, the commonly noted cytopathic effects were induction of cell wall abnormalities; undulation of cell walls and irregularities in thickness of cell walls, which indicate that these changes are characteristic for HSVd-infected hop cells. Therefore, we tried to examine the corresponding cells of the shoot apical meristem of HSVd-free hop plant obtained by meristem tip culture. Cell wall development was normal in all cells of the comparable primordial examined, as in the non-inoculated hop plants (MOMMA & TAKAHASHI 1982).

Ultrastructural studies of the symptom-bearing have HSVd-infection indicated that walls in the infected hop leaf tissue appear to be extremely distorted with irregular profiles and thylakoid membranes of chloroplast are loosely arranged, so that poor stacking of the grana occurs (MOMMA & TAKAHASHI 1982). In the present observation of ultrastructure of shoot apical meristem of hop plant infected systemically with HSVd, no measureable cytopathic changes were found in shoots up to 0.2 mm long and composed of the apical meristem bearing two pairs of the primordial.

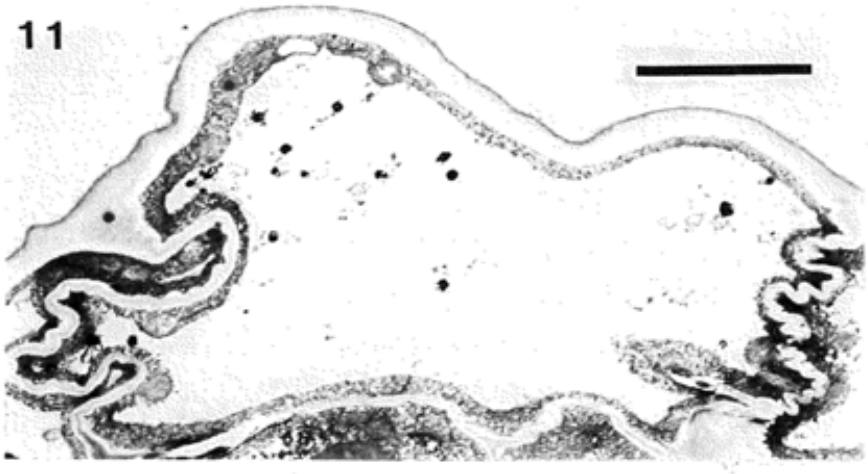

Figure 1. Transmission electron micrograph of upper epidermis in HSVd-infected hop leaf tissue. The cell walls in epidermal cell appear to be undulated and of narrow thickness, resulting in an irregular shape of the cell. In the cuticular layer of the epidermis, the fold-like structures could not be observed. Bar represents 5 µm (taken from MOMMA & TAKAHASHI 1983).

2. A new development in lupulin glands

We established a meristem culture of hop. Then we got three viroid-free plants from HSVd-infected plants. After that we observed various phenomena in both HSVd-infected and non-infected hop plants (MOMMA & TAKAHASHI 1983). This time I got the new information that there are lupulin glands reserving a resin, not only in cones but also on the reverse side of leaf. Lupulin glands are formed on the back of a leaf. We observed the situation two different varieties (Shinshuwase and Toyomidori).

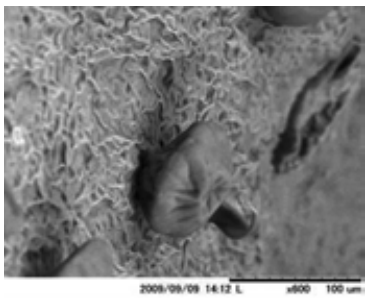

Figure 2. SEM photograph of reverse side of whole hop leaf grown in vitro

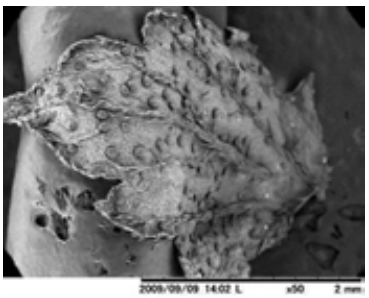

Figure 3. SEM photograph of reverse side of hop leaf grown in vitro showing lupulin glands (600x)

3. A vision of hop breeding

We have bred on some varieties of hop so far. We have now a candidate of hop variety, for example eJONNY and wSHION. They are bred on Hallertauer Mfr. ♀ × Shinshuwase ♂.

In a few years we could get a new variety of hop and spread these varieties in any location. We are now checking the alpha-acids, beta-acids and other components in these plants.

Conclusion

In the viroid-infected cells, no helpful means have been established for detecting the infected cells because viroid molecules could not be confirmed in the cell in situ. They could not be detected by using serological methods why we regarded the cells bearing wall abnormalities as the infected cells. This target was found to be a useful criterion for HSVd-infected hop leaf cells.

Nevertheless, for practical purposes the bioassay is still the most reliable way to test for the presence or absence of HSVd in hop plants. Then we have had a technique to make HSVd-free plants from HSVd-infected plants. Now we tried to get a new variety of hop and are trying to breed on several hops. In the near future we could get a new variety of hop.

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Breeding of aroma hops in the Czech Republic

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Abstract

Karel Osvald began breeding aroma hops in the Czech Republic 100 years ago. His clones 31, 72, and 114 occupy 85 % of today's area in the Czech Republic. The first hop crossing in the Czech Republic, for new aroma varieties of hops, was begun in 1954 by Lubomir Vent. In the 1960s František Bránek was the main breeder. He is the creator of the first hybrid aroma varieties, Bor, Sládek and Premiant, which were registered at the end of the 20th century. Since 2000, the other aroma varieties Harmonie, Kazbek, Bohemie and Saaz Late have been registered. In 1997, a new breeding program was launched for the creation of a new generation of aromatic varieties, which will have the origin of Saaz Saazer and will have similar brewing characteristics. In 2019, the new four aromatic varieties Saaz Brilliant, Saaz Comfort, Saaz Shine and Mimosa will be registered.

Key words. hop (*Humulus lupulus* L.), hop breeding, aroma hops, perspective genotypes

Introduction

Czech Republic is well-known for the production of fine aroma hops with excellent brewing quality, which ensues from historical tradition of growing Osvald clones of the Saazer landrace. Nevertheless, since the 1990s new hop varieties (Sladek, Premiant, Harmonie, Saaz Late, etc.) have been grown here as well. Hop breeding aimed at aroma varieties still continues with the objective to register new cultivars with stable qualitative and quantitative parameters and excellent brewing characteristics. Breeding of a new generation of aroma cultivars was begun in 1999. Each year, nearly 20 crossings were carried out and the genotypes were at first tested for susceptibility to Downy and Powdery mildew. The best genotypes were selected within tolerant and resistant progenies and tested within our breeding program. Five perspective aroma genotypes were transferred into registration trials in 2015 and four others in 2016. Hop breeding aimed at new genotypes suitable for growing in low trellis in Czech Republic has been studied since 2008. Within the 'Eureka' program we worked on the development of new cultivars of dwarf hops in cooperation with 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. Breweries are still more and more interested in hops with specific aroma in the recent years. Especially, small breweries produce special beers, which are different from the common Czech lager beers.

Material and methods

In the framework of the long-term breeding concept, in 2017 the selection of existing and new breeding material continued with the preference for genotyping with resistance to climatic changes. In total 7,340 genotypes were evaluated from the collection of breeding material. In order to get new perspective genotypes that will show resistance. Testing, reverse, inhalation, convergence and combination crosses were realized. From the offspring of Sm16, 25 female genotypes were selected, of which four genotypes with specific scents, two genotypes of bitter type, and remaining aromatic type. In 2017, selections were focused on the creation of aroma genotypes. On the basis of pre-harvest descriptions from unfinished breeding material, 343 genotypes were selected and evaluated. These are genotypes of both aroma and bitter types, and with specific odour. The alpha bitter acid content ranges from 2.97 % (lit. 5600) to 11.19 % (lit. 5086). All genotypes show good yield levels. Many genotypes have yields above 3 tons per ha.

In 2017, the evaluation of UKZUZ registration exams for promising new breeding lines (4849, 4914, 4915, 4932, 4964) was completed, of which two new bitter-type Gaia (4849) and Boomerang (4914) were selected and registered. Currently, hop breeding is focused on ending the registration experiments of aroma hops.

Chemical analyses for the determination of the contents and compositions of hop resins in hop cones were made by HPLC (EBC 7.7) method. Hop essential oils were specified with the help of gas chromatography (EBC 7.12).

Results and discussion

Average values from chemical analyses of hop resins obtained in 2018 are shown in Table 1. All genotypes have a low ratio of cohumulone, which is a very important brewing characteristic. The best results show genotypes 4801, 4979, 5045 and 5227. As genotypes 5045 and 5227 had the highest yield they were planted in another hop garden so as to be subject to other research. In 2017 we managed to harvest a sufficient number of hops for chemical analyses and trial brews in our pilot brewery and other breweries in Czech Republic. Susceptibility to agrotechnical operations needed for successful growing of these hops is determined within field trials in practical conditions of hop gardens. In 2018, a sufficient amount of hops was harvested for chemical analysis and, above all, for brewing tests, both in the experimental brewery of the Hop Institute, s.r.o. Žatec, as well as for verification batches in Czech breweries.

Origin and characteristics of the new genotypes

Saaz Brilliant has its origin in the selection from hybrid progeny after mother Saazer x male Saazer (inzucht crossing). Good yield.

Saaz Comfort has its origin in the selection from hybrid progeny after mother Serebrianka (Russia) x male Saazer. Good tolerance to downy mildew.

Saaz Shine has its origin in the selection from hybrid progeny after mother Sladek x male Saazer. Hop resins and oils similar to Saazer. Good yield. Fine hoppy aroma. All these varieties are suitable for the 2nd and 3rd hopping. Excellent for lager beers with hoppy aroma.

All of these genotypes have been tested in brewing tests in the pilot brewery of the Hop Research institute in Žatec and in Research Institute for Brewing and Malting in Prague. Saaz Brilliant and Saaz Comfort have been tested in Holba brewery in Hanusovice. Saaz Shine has been tested in the Japanese brewery Suntory.

Mimosa has its origin in the selection from hybrid progeny after mother from Czech breeding material x male from South African breeding material. Aroma is light citrusy and floral. Suitable for dry hopping and used as flavour hop.

Table 1. Average yield, composition of hop resins and ration of farnesene of new Czech aroma hop genotypes in the period from 2013 till 2018.

Genotype	Yield [t ha^{-1}]	Alpha-acids [% w/w]	Beta-acids [% w/w]	Cohumulone [% rel.]	Farnesene [% rel.]
4799	2.0	4.8	4.1	23	11
4801 **Saaz Brilliant**	2.7	4.1	4.0	26	12
4975 **Saaz Comfort**	2.8	6.4	6.5	18	15
4979	2.4	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	2.7	8.2	7.4	25	2
5045 **Saaz Shine**	2.8	3.7	3.8	24	12
5227 **Mimosa**	2.8	2.4	7.6	31	1

Conclusion

Registration of new aroma varieties is supposed in 2019. We expect interest of brewers not only from Czech but all over the world as well. New aroma varieties of hops have good agronomical and brewing properties. They are tested in breeding practice and in breweries. At present, the demand for these new aromatic varieties is hops.

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The new Hüll aroma hop cultivars – ready for the future in providing enhanced resilience to climatic stress and versatility in brewing

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Abstract

Since 2012 the Hop Research Center Hüll has released five new hop cultivars in close cooperation with the hop and the brewing industry. These aroma varieties with unique flavor profiles paved the way to the craft beer market. But – special flavor potential is not the only benefit, these new Hüll cultivars provide much more: In addition to enhanced yield potential, broad disease resistance and improved nitrogen efficiency, these hop varieties have demonstrated increased resilience to drought and heat in the extraordinary summer of 2018 in showing significantly smaller reductions in yield and quality than older varieties and landraces. Furthermore, due to the limited supply of the traditional aroma varieties after the harvest 2018 the new Hüll aroma cultivars - so far almost exclusively used for dry hopping - convinced brewers and beer tasters as well by revealing harmonious classical hoppy flavor profiles when added at the start of boiling and in the wort boiling process in beers.

Keywords. climatic change, stress tolerance, stable yields and quality, brewing potential

Introduction

In recent years initiated by the US craft beer movement five new aroma cultivars with unique aroma and flavor potential have been released paving the way of German hops into this highly interesting beer market. But Mandarina Bavaria (MBA), Hallertau Blanc (HBC), Huell Melon (HMN), Callista (CAL), and Ariana (ANA) have proven much more benefits for German hop growers and the brewing industry.

Methods

Selection procedure and growing trials

Crosses combining European and North American germplasm were conducted in 2006 at the Hop Research Center Hüll focusing to develop aroma varieties which impart unique fruity as well as hoppy flavor compositions into beers. All seedlings from these crosses were preselected for resistance or tolerance towards powdery and downy mildew after artificial inoculation in the greenhouse and were assessed in a growth hall the first year. Those female seedlings showing vigor, twining ability, female flowers and subsequently good cone set were transplanted to the Hüll breeding yard and assessed as single plants for three years. The best selections were propagated and grown in replications at two different sites for four years before they were tested in on-farm growing trials. For Callista and Ariana also large scale growing trials on a hectare basis were performed by hop growers funded by GfH members to collect data on their agronomic performance and disease as well as pest resistance/tolerance.

Brewing trials

In addition, in 2015 already before their release standardized brewing trial commissioned by the Society of Hop Research were conducted with Callista and Ariana by the trial brewery of the Bitburger Braugruppe providing information on their brewing quality when used at the start of boiling, in the whirlpool and in the dry hopping procedure (HANKE et al, 2015, 2016).

Only recently also based on a standardized protocol Callista, Ariana and Mandarina Bavaria were tested in trial brews funded by the Society of Hop Research to produce single hopped Lager beer.

Results

Progress in breeding

The new Hüll aroma cultivars Mandarina Bavaria, Hallertau Blanc, Huell Melon, Callista, and Ariana were developed by the Hop Research Center Hüll based on crosses conducted in 2006, 2009 and 2010, respectively. They were released by the Society of Hop Research in 2012 and 2016, respectively aiming to provide unique fruity and floral flavor compositions for the craft beer scene. Today they are grown on an acreage of 770 ha.

When used in the dry hopping procedure they impart intriguing fruity aroma and flavor of mandarine, grapefruit, apricot, passion fruit or black currant, but also provide a hoppy and herbal basic note.

However, special flavor potential is not the only benefit of these new Hüll breeds. Table 1 shows their agronomic performance and resistance to major diseases and pests.

Table 1. Overview of the new Hüll aroma cultivars

		Quality			Resistance towards				
	Yield kg/ha	Hoppy Flavor plus	Oil Content (ml/100g)	α-Acids (%)	Vert. wilt (mild)	Downy Mildew	Powdery Mildew	Red Spider Mite	Hop Aphid
Mandarina Bavaria	2,300	mandarin, grapefruit	1.5 - 2.5	7- 10	+/-	+	+ +	+/-	+
Huell Melon	2,000	honeydew melon, apricot	1.5 - 2.4	5 - 8	+	+ +	+ +	+/-	+
Hallertau Blanc	2,300	mango, white wine	1.3 - 2.1	8 - 11	+/-	+ +	+ + +	+/-	+
Callista	2,300	apricot, passion fruit	1.4 - 2.0	2 - 4	+ +	+ +	+ +	+/-	+
Ariana	2,300	black currant, blackberry	1.5 - 2.3	9 - 11	+ + +	+ +	+ +	+	+/-

With a yield potential of 2,300 kg per ha on average these varieties (MBA, HBC, CAL, ANA) clearly exceed the yield of older Hüll cultivars such as Perle and Hallertauer Tradition with around 2,000 kg / ha, while Huell Melon remains at this prior level.

Based on LfL-own growing trials as well as on feedback and data from commercial hop production the new Hüll aroma cultivars proved enhanced broad resistance and tolerance respectively. Resistance to powdery mildew is based on one or two fully effective major resistance/s so far unbroken in practice. Also – especially in the season 2016 with highly conducive conditions for DM infections the new Hüll cultivars showed a higher level of tolerance to downy mildew. A significant progress has been achieved in their tolerance towards *Verticillium* wilt in its mild form with Callista and in particular Ariana displaying less or no wilting symptoms and no dying off.

Already for decades the focus in our breeding work has been to develop hop varieties which get along with lower amounts of nitrogen fertilizer while still producing high and stable yields. Based on strict reduction in the supply of nitrogen during the whole selection process in our breeding yards all Hüll breeds show optimized nitrogen efficiency which is an essential contribution to protect ground water and surface waters in German hop growing regions. Furthermore, less nitrogen supply can help to mitigate the *Verticillium* wilting problem in particular with regard to the mild form of this wilting disease.

In particular, in the growing season of 2018 with very high temperatures (around 4 ^{0}C above long-term mean values) and drastically reduced precipitation in the main vegetation period (April – August) of more than 100 mm in comparison to long-term mean values the new Hüll cultivars could demonstrate their increased resilience to stress caused by extreme drought and heat in Germany.

Hallertau Mittelfrüher and other landraces along with older Hüll cultivars such as Perle and Hallertauer Magnum showed drastically reduced yields and alpha acid contents. When comparing the yield expressed in kg alpha per hectare of 2018 to that of 2017 the Hallertau growers had to tolerate losses of minus 33 % with Hallertauer Mittelfrüher, minus 23 % with Perle and minus 38 % with Hallertauer Magnum. While the new Hüll cultivars having been selected since the 1990s under already manifesting climatic changes proved a much higher tolerance to drought and heat stress. This is most important since only 20 % of the total acreage can be irrigated. Notably Mandarina Bavaria and Hallertau Blanc defied the stress conditions of 2018 and delivered in comparison to 2017 the same alpha acid yields. Similar stress tolerance applies to Herkules and Polaris with losses of only 6 or 8 % in yield from 2018 to 2017. While Huell Melon with a yield reduction of 35 % in alpha acids per ha represents only average in climatic tolerance.

Even when comparing the data in kg alpha acids per ha from the year 2018 to 2016 with its higher than average yields the new Hüll cultivars clearly demonstrate their improved stability towards climatic stress. The yields in kg alpha per hectare with Hallertauer Magnum, Perle and Hallertauer Mittelfrüher were 40 to 60 % below the 2016 level while Mandarina Bavaria and Hallertau Blanc revealed only a deficit of below 20 %. Since Callista and Ariana were released in 2016 sound data on yield and alpha acids are still missing, but preliminary results suggest a higher level of stress tolerance.

Versatility in brewing

As consequence of the limited availability of traditional aroma varieties in 2018 due to these extreme weather conditions brewers added the new Hüll cultivars Mandarina Bavaria, Callista and Ariana which so far were almost exclusively used in the dry hopping process at the start of boiling and in the wort boiling process with convincing results in classical beer types such as Lager, Helles, Pilsner, and Märzen. In addition, in standardized brewing trials - conducted by the research brewery of the Technical University Munich-Weihenstephan, and funded by the Society of Hop Research - the excellent brewing quality of the new Hüll aroma cultivars MBA, CAL and ANA in the wort boiling process was confirmed. This could be proven by respective analytical data and evaluations by an experienced tasting panel. The single hopped Lager beers with MBA, CAL and ANA as hop additions at the start of boiling and in the wort boiling process revealed their classical hoppy basic flavour and a pleasant, mild bitterness. These Lager beers were also tasted and evaluated by professional visitors and experts from the brewing and hop industry at the Brau Beviale in Nuremberg 2018. Here also all tasters confirmed the highly balanced flavor and aroma with its pronounced hoppy note and its high drinkability.

Moreover, feedback from numerous brewers worldwide after using the new Hüll aroma cultivars in their brewing recipes were valuable information about these cultivars on their brewing quality when used in the hot and cold phase of brewing.

Conclusion

In the first place, the new Hüll aroma cultivars were developed for the German hop growers to get access to the booming craft beer market, but beside unique flavor profiles they show apparent progress in breeding. With their higher yields, enhanced spectrum of disease resistance or tolerance, improved nitrogen efficiency and in particular by revealing increased tolerance toward heat and drought in the extremely hot and dry summer of 2018 the new Hüll breeds fully met the demands of the hop and brewing industry. Moreover, in trial brews and in commercial beers they have proven versatility in the brewing process. When added in the

wort boiling process they imparted their elegant, harmonious hoppy flavor. When used in the dry hopping process the Hüll aroma cultivars displayed their unique, intriguing fruity flavor profiles. Thus, it is quite clear Mandarina Bavaria, Hallertau Blanc, Huell Melon, Callista, and Ariana already paved the way to tackle with future demands and challenges of the hop and the brewing industry.

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We would like to give special thanks to our hop breeding team for their valuable work in the greenhouses and hop breeding yards. Many thanks go to the team of the hop analytics for providing the chemical data of the Hüll breeds. We highly appreciate the support of our growers for their testing of the Hüll hop varieties in their fields and of all hop growers involved in the large scale growing trials. Special thanks go to Walter König, managing director of the Society of Hop Research (GfH), for his great support, especially in planning and organizing the various trial brews. We say thanks a lot to all brewers involved in brewing trials, in particular to Christoph Neugrodda, Research Brewery, Brewing Faculty, Technical University München-Weihenstephan, to Dr. Stefan Hanke, Bitburger Braugruppe, and to Andreas Gahr, Hopfenveredlung St. Johann.

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Reflections on the changing nature of Wye hop breeding

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Abstract

This paper is a personal review of hop breeding at Wye Hops over the last four decades; a period when there have been great changes in the objectives for hop breeding reflecting changes in the brewing industry as well as recognising wider concerns about the environment. The main aims in the early 1980s were to increase the alpha-acid content whilst reducing production costs to address the commodity hop market for the large brewing companies. In contrast today, these quantifiable traits have been replaced by breeding for aroma and flavour to create a premium product for smaller brewers, notably the craft sector. This challenges the breeder with subjective and ill-defined objectives. Whilst breeding for resistance to the main hop diseases has remained a constant factor throughout this period, the discovery of resistance to aphid pests has introduced a completely new trait. As the arsenal of plant protection products for hops declines and the effects of climate change become apparent, it is clear that the identification of multiple pest and disease resistances using new molecular selection methods will become a higher priority in the future.

Key words. Hops, breeding, objectives

Introduction

The objectives for hop breeding are determined by many factors; the requirements of brewers, the constraints on production for growers, public concerns over the impact of hop husbandry on the environment, and the scientific techniques and resources available. This paper reviews how these diverse parameters have changed over the last four decades and shaped the hop breeding activities at Wye College, and its successor Wye Hops Ltd.

Increased alpha-acid content

In 1981, the principal aim of hop breeding was to increase the alpha-acid content. Building on the pioneering work of Prof. Salmon, 'Wye Target' had been released in 1972. It was the first variety in commercial production to exceed 10 % alpha-acid and it had become the most widely-grown UK hop variety. Its release accelerated the trading of hops on the basis of the cost of a kilo of alpha-acid. Alpha-acid had come to be regarded as a commodity and this benefitted the larger, international breweries and processors for whom the economics of their operations was paramount. Consequently, brewing and processing industries demanded higher alpha-acid content and growers looked to maximise the production of alpha-acid per hectare. For the hop breeder, it was simple – to breed for increased alpha-acid content and the goal at that time was a "super-alpha" 15% content. Fortunately, alpha-acid could be measured easily in large progenies by titration (LCV). With high heritability and polygenic inheritance, improvement by selection was both incremental and successful. However, a few obstacles slowed breeding progress in the Wye programme. The trait was sex-limited, necessitating progeny testing of male parents. Furthermore, there was an adverse genetic interaction with resistance to wilt, an essential trait for any new variety in the UK. R2 resistance to powdery mildew gave field immunity at that time but there was also a strong suggestion that incorporating this resistance was restricting progress. Although exceeded in breeding lines, the commercial 15 % goal was eventually realised in the development of 'Admiral', from a cross made in 1984.

As a commodity, the varietal origin of the alpha-acid was unimportant. Indeed, brewing trials aimed to see if the test variety could substitute, undetected, for an existing alpha

source, usually ‘Target’. For the hop processing industry, the stability of the alpha-acids during storage became an important associated breeding aim.

Measurement of alpha-acid by LCV was replaced at Wye in 1988 by HPLC and the ability to detect different alpha-acids, notably cohumulone, heralded the end of selection for generic alpha. Varieties with a lower cohumulone content were preferred by the more traditional regional brewers. Progeny show a continuous range of cohumulone values and the characteristic appears highly heritable. Thus, progress through selection was rapid, reducing the minimum cohumulone levels in Wye breeding lines from 19 % in 1988 to <11 % by 1998.

Because of smaller acreage and higher production costs, the UK could not compete with the USA in producing alpha-acid for the international brewers. Breeding emphasis shifted towards dual-purpose varieties supplying the UK regional breweries; hops with an alpha-acid content considerably higher than a traditional aroma variety, but with a desirable flavour for dry hopping. Despite a current shortage of alpha-acid supply, it is unlikely that Wye will seek to develop further “super-alpha” varieties.

Dwarfness

To address the economics of alpha-acid production, growers looked to reduce the costs of production. A simplification of the growing system was seen as the key with low-trellis wirework supporting short stature plants. Although Prof. Salmon recorded dwarfness in hop seedlings at Wye in 1911, the main breeding programme originated from the discovery in 1977 of a mature, productive plant of short stature (~4 m tall). Its progeny, and that of most subsequent families, had segregated for the short height characteristic with a 5:3 ratio of conventional height to short plants, consistent with the action of a single dominant gene, modified by a dominant epistatic gene. Hand-harvesting in 1981 of short-stature selections indicated that the yield potential of some genotypes was comparable with that of conventional production. Detailed analysis in 1984 of the growth habits of families segregating for dwarfness revealed that it consisted of several independent traits, including short stature, short internode length and low flowering node. With knowledge of the inheritance and expression of dwarfness, selection of parents and progeny was more efficient. From crosses made in 1985, the programme developed ‘First Gold’, registered in 1996 as the world’s first dwarf variety. Followed by ‘Pioneer’, ‘Sovereign’ and ‘Endeavour’, low trellis production has been on a commercial scale since then with approximately 23 % of the 2019 UK hop area growing dwarf varieties. Breeding for low-trellis production, which started with just a single plant in 1977, has expanded to become a main priority for the current programme.

Although initially developed to address the economics of hop production, hops grown on a low-trellis wirework system provide a smaller, more accessible spray target, requiring lower volumes of pesticides applied in lower volumes of water. The continuous contact between plants on such a system also facilitates the action of beneficial predatory insects. Dwarf hops were seen as a means to reduce environmental impact from hop growing and this attracted public funding, particularly during the 1990s, consolidating this objective within the programme.

Aroma and flavour

The demand for aroma hops in the UK in 1981 was largely met by the production of the traditional ‘Fuggle’ and ‘Golding’ varieties. Both were highly susceptible to wilt disease but their production was protected by regulations defining eradication areas where only susceptible varieties could be grown. There was no pressure to develop a new, wilt-resistant aroma variety as such a variety would compete directly with the stable, established market for these traditional aroma varieties. Several developments have changed that concept.

The continued spread of wilt disease during the 1980s led to pressure to reduce the eradication zones, permitting producers to grow resistant varieties in these areas. The Progressive Verticillium Wilt Order of 1965 was replaced in 1987 by a new Plant Health

Order and the area maintained free of wilt disease declined. British growers now looked to breeding to provide a wilt-resistant “traditional” aroma-type variety. This was achieved in ‘Sovereign’ from a cross made in 1995 and released to farms in 2006.

But the most important development was the craft brewing movement which had begun in the USA in the late 1970s. These small breweries championed hops for their flavour as a premium product, irrespective of the content, quantity or cost of the alpha-acid. Craft brewing gained momentum steadily in the USA and accelerated during the early part of the twenty-first century following the rediscovery of ‘Cascade’ and the release of ‘Citra’ in 2007. It concentrated on producing beers with an assertive hop character. Demographic changes in beer consumers, active on social media, further consolidated the demand for craft beer and it has become the main driving force in brewing worldwide over the last decade influencing larger regional breweries and many of the international companies. Elimination of the distinction between bitter and aroma hops by craft brewers changed the aims of Wye hop breeding drastically.

Breeding to meet this new market began at Wye in 2002 and has become its principal objective since 2011. The aim is to develop hop varieties which provide a powerful and distinct flavour. In contrast to breeding for alpha-acid content, this new objective is highly subjective and no single measure defines it. In general, suitable varieties are high in oil content with a relatively high proportion of monoterpenes, and often high in resin content with higher cohumulone than traditional aroma varieties. But these parameters are ill-defined and incremental breeding progress cannot be measured by routine analyses. The challenge has been addressed by working closely with taste panels and merchants who supply this particular market, initially selecting on dry cone aroma and following quickly with pilot brews. Pedigree has been an important consideration and at Wye, as with many other programmes worldwide, ‘Cascade’ has proved to be a useful parent giving progeny providing markedly different flavours. ‘Endeavour’, the first of a series of seedlings of ‘Cascade’, is currently completing farm trials. Similarly, wild USA material has proved a useful germplasm resource for strong flavour and ‘Ernest’, a seedling of *neomexicanus*, is also on farm trials.

Pest and disease resistance

In contrast to the major changes in breeding objectives from alpha to flavour, generic commodity economics to a premium product, and measurable to subjective criteria, breeding for disease resistance has remained very constant in importance and techniques over the last four decades. Benefitting all sectors of the industry, less reliance on pesticides reduces environmental impact, potential residues and production costs. Using well described procedures, the screening of seedlings continues for downy mildew and powdery mildew resistance which was initiated at Wye in 1968. Similarly, routine testing of selections for wilt resistance began in 1956 and continues today using similar techniques.

However, a survey of the germplasm collection held at Wye between 1981 and 1983 revealed several accessions on which aphid infestation appeared to be less than on commercial cultivars. Analysis of aphid populations on putatively resistant genotypes indicated that several expressed their resistance as a true antibiotic effect, rather than simple non-preference. The aphids on these genotypes reproduced more slowly, produced fewer and markedly smaller offspring than on reference varieties. The most resistant accession was INT101, a wild male plant collected from a mountainous region in Japan. The resistance derived from INT101 has been shown to be controlled by the action of two dominant major genes and, in low-trellis in particular, can be augmented by the action of natural predators sufficient to control damson-hop aphid throughout the season without pesticide applications. Selection for field resistance to aphids has been a regular part of the Wye programme since 1988 with seedlings being left unsprayed with aphicides throughout the growing season. ‘Boadicea’, released from this programme in 2005 (registered EU PVR 2008), was the first commercial variety with such aphid-resistance. Further selections, including ‘Merlin’, are currently on farm trials.

The future

Undoubtedly, climate change will be a major factor shaping the hop breeding at Wye Hops during the next decades. Some effects can be predicted such as warmer spring temperatures inducing dormancy, and hotter and drier summers inducing stress, abnormal flowering and premature ripening. It is certain also that the spectrum of pests and diseases will be affected. With an ever-diminishing arsenal of plant protection products, the brewing and hop-growing industries will look to hop breeders to embrace new molecular selection techniques to develop rapidly multiple pest and disease resistant varieties, resilient against climatic stress.

But many other developments cannot yet be predicted. The lesson of the last four decades is that anything and everything can change!

Development of the new flavor hop variety “Furano Magical”

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Abstract

A new flavor hop variety, “Furano K906901060 Go” (named “Furano Magical”), was bred by Sapporo Breweries Ltd., Japan. This variety has the world’s highest level of 4-methyl-4-sulfunylpentan-2-one (4MSP), one of the most odor active compounds in hops, which imparts a black currant-like fruity aroma to beer. This variety was derived from the crossing of our own male to a female breeding line performed in 1989 as part of the breeding program of Sapporo Breweries Ltd. It had been kept as a mid-mother plant from 1997, until in 2014 it was evaluated by sensory evaluation of hop water extract by boiling (HWEB) and found to have an extremely strong fruity flavor. Furano Magical has 80–129 µg/kg of 4MSP in its dried cones, which is higher than the world’s current leading flavor hop varieties, such as Citra (37–114 µg/kg) or Simcoe (51–112 µg/kg). Beer that was late-hopped with Furano Magical had a characteristic tropical, mango-like flavor, with an aroma intensity that was stronger than that of beer hopped using Nelson Sauvin.

Key words. 4-methyl-4-sulfunylpentan-2-one, flavor hop, sensory evaluation, breeding, beer

Introduction

The development of new flavor hop varieties has been actively pursued for the last two decades due to the strong demand for this type of hops from craft breweries. These craft breweries, whose sales have been growing (especially in the USA), prefer flavor hops. They also use ten-times more hops to brew the same quantity of beer compared with the average of the brewing industry.

Though the requirement for new flavor hops had been apparent, their development presented a new challenge for hop breeders. We, the hop breeders of Sapporo Breweries Ltd., also faced this difficulty, despite the fact that we have much experience of hop breeding. We are one of the few breweries to have our own breeding program for barley and hops. This is rooted in our history, since we tried to establish the domestic agricultural production of raw materials of beer, together with the development of the brewing industry in Japan, during the late 19th century. One key improvement, the construction of a sensory evaluation system for hop water extract by boiling (HWEB), allowed us to tackle the development of flavor hops. The method, simulating the late-hopping process in beer brewing and resulting in a similar volatile compounds profile to late-hopped wort[1)] enables the evaluation of fruity flavor characteristics of hops.

In this report we describe how we applied the sensory evaluation system to the screening of the breeding lines, and how in doing so we were able to develop a novel flavor hop variety we named “Furano Magical”.

Materials and methods

Sixteen varieties and clones were selected in the preliminary selection using the sensory evaluation of the HWEB (KOIE et al. 2016) from about 200 varieties and clones harvested in 2013 in Hokkaido, Japan. Cone samples of 51 breeding lines in the Sapporo Breweries’ breeding program in 2013 were harvested in Hokkaido, and 25 commercial varieties from around the world were also used in this study.

Ground hop cones of Furano Magical harvested in 2014 and in 2015 in Hokkaido were used for the brewing trials and the analyses of polyfunctional thiols. Type 90 pellets of Nelson Sauvin hops imported from New Zealand were used as the reference variety for the brewing trial.

Plants of Furano Magical were evaluated for their agricultural characteristics together with Little Star (bred by Sapporo Breweries Ltd., JP registration number 13532), the major variety in Hokkaido, Japan, as a reference. Maturity, yield, and resistance to diseases were observed.

Aroma characteristics of hops were evaluated by sensory evaluation of HWEB. Hop powder containing 0.1g of □-acids was mixed with 200 mL water in a glass flask capped with aluminum foil. The mixture was heat-treated using an autoclave at 105°C for 5 min, then cooled and filtered, and the filtrates were used for the sensory evaluation. Nine sensory descriptors were sued: “Hay,” “Tea,” “Woody,” “Citrus,” “Grape/Currant,” “Fruits/Flower,” “Green,” “Spicy/Herbal,” and “Bouillon”. The panel consisted of five persons, who were required to record the magnitude of the sensation of each attribute using a scale from 0 (no sensation) to 4 (very strong). Principal component analysis (PCA) was carried out based on the sensory scores after HWEB using the “princomp” function in the open-source platform R for Windows.

The volatile aroma compounds in 23 varieties of hop cones after being selected by the sensory evaluation of HWEB, and in the hop samples of Furano Magical and Nelson Sauvin used for the brewing trial, were analyzed. Linalool and geraniol contents in hop cones were analyzed using GC-MS following their extraction by n-hexane. Linalool, geraniol and □-citronellol in beer were analyzed using GC-MS after the solid-phase microextraction. Polyfunctional thiols in hop cones were analyzed using GC-MS/MS after their extraction according to a previously published method using solid phase extraction with an ASPEC GX-274 (Gilson, Middleton, WI, USA). Polyfunctional thiols in beer were also analyzed using a previously described method (TAKAZUMI et al. 2017).

Single hop beers using Furano Magical and Nelson Sauvin were made. All malt wort was used to make a mash at an original gravity of 12 %. The base bittering was performed such that the beer made using each variety had a similar level of bitterness, while late-hopping was done at the whirlpool at a hop dosage rate of 1.11 g/L. After the mash was cooled, fermentation with lager yeast was carried out for about 1 week less than 13°C, and then preserved for 3 weeks, before being filtered and bottled. A panel of 42 people conducted the sensory evaluation of the trial beers. Seven criteria were used: “Flowery”, “Fruity”, “Citrus”, “Tropical”, “Green”, “Sulfur-like”, and “Impact”. Panelists’ comments about the flavor of the beers were also recorded. A four-point scoring system (0-3) was applied.

Results

Furano Magical received the highest score for “Grape/Currant” in the sensory evaluation of HWEB among the varieties tested, which included Nelson Sauvin and Citra, the leading flavor hop varieties in the world (Table 1). The results of the PCA using the sensory scores of HWEB showed that the first principal component (PC1) was positively related with the scores of “Tea” and “Bouillon”, and negatively related with the scores of “Citrus”, “Grape/Currant” and “Fruits/Flower”. The second principal component (PC2) was positively related with the scores of “Grape/Currant” and negatively related with the scores of “Fruits/Flower”. Among all the varieties, Furano Magical had the lowest score for PC1 and the highest score for PC2 (Fig. 1).

Table 1. The contents of the volatile aroma compounds and the scores of the sensory evaluation of the hop water extracts by boiling (HWEB) of the hop varieties.

3SH, 3-sulfunylhexan-1-ol; 4MSP, 4-methyl-4-sulfunylpentan-2-one;
3S4MP, 3-sulfunyl-4-methylpentan-1-ol; 3S4MPA, 3-sulfunyl-4-methylpentyl-1-acetate

Variety / Clone No.	Country	Aroma compounds in hop cone						Sensory scores of HWEB		
		3SH	4MSP	3S4MP	3S4MPA	linalool	geraniol	Citrus	Grape/Currant	Fruits/Flower
		□g/kg	□g/kg	□g/kg	□g/kg	mg/kg	mg/kg			
Cascade	USA	5	17	18	n.d.	176	57	0.0	0.0	0.0
Citra	USA	18	67	52	2	47	36	1.4	0.9	1.1
Columbus	USA	n.d.	8	10	6	45	52	0.5	1.0	2.0
Ekuanot	USA	4	11	175	4	79	93	0.5	0.8	0.5
HBC431	USA	n.d.	34	8	n.d.	45	52	0.3	1.3	1.3
Amarillo	USA	10	9	98	57	115	56	0.5	0.7	1.2
Sorachi Ace	USA	n.d.	n.d.	n.d.	n.d.	102	67	0.2	0.0	1.3
Nelson Sauvin	New Zealand	9	31	492	11	80	55	0.5	1.8	1.2
Saazer	Czech Republic	n.d.	n.d.	n.d.	n.d.	20	12	0.0	0.0	0.8
Hallertau Tradition	Germany	n.d.	n.d.	n.d.	n.d.	32	32	0.3	0.0	0.7
Polaris	Germany	4	3	3	n.d.	65	67	0.3	0.2	0.3
Mandarina Bavaria	Germany	8	n.d.	53	9	22	37	0.0	0.0	0.7
Hallertau Blanc	Germany	18	2	432	89	29	33	0.7	1.0	0.5
Huell Melon	Germany	n.d.	n.d.	n.d.	n.d.	20	28	0.7	0.5	2.0
Furano Beauty	Japan	3	6	42	14	162	107	1.0	1.5	0.2
Furano Blanc	Japan	2	12	90	3	65	37	0.5	0.3	0.9
Furano Flora	Japan	n.d.	6	10	n.d.	86	86	0.6	1.0	1.3
Furano Rosa	Japan	n.d.	n.d.	14	n.d.	94	229	0.2	0.3	0.5
Hokuto Ace	Japan	n.d.	n.d.	n.d.	n.d.	158	37	0.0	0.2	1.0
0710D	Japan	2	4	49	5	143	24	0.6	0.8	1.5
0705A	Japan	n.d.	n.d.	n.d.	n.d.	132	70	0.2	0.3	1.1
Furano Magical (2013)	Japan	4	85	111	18	230	86	1.0	2.5	1.0
Furano Magical (2014)	Japan	10	80	68	n.d.	-	-	-	-	-
Furano Magical (2015)	Japan	2	129	50	n.d.	-	-	-	-	-
69KBH66	Japan	4	5	80	5	167	152	0.2	1.2	1.5

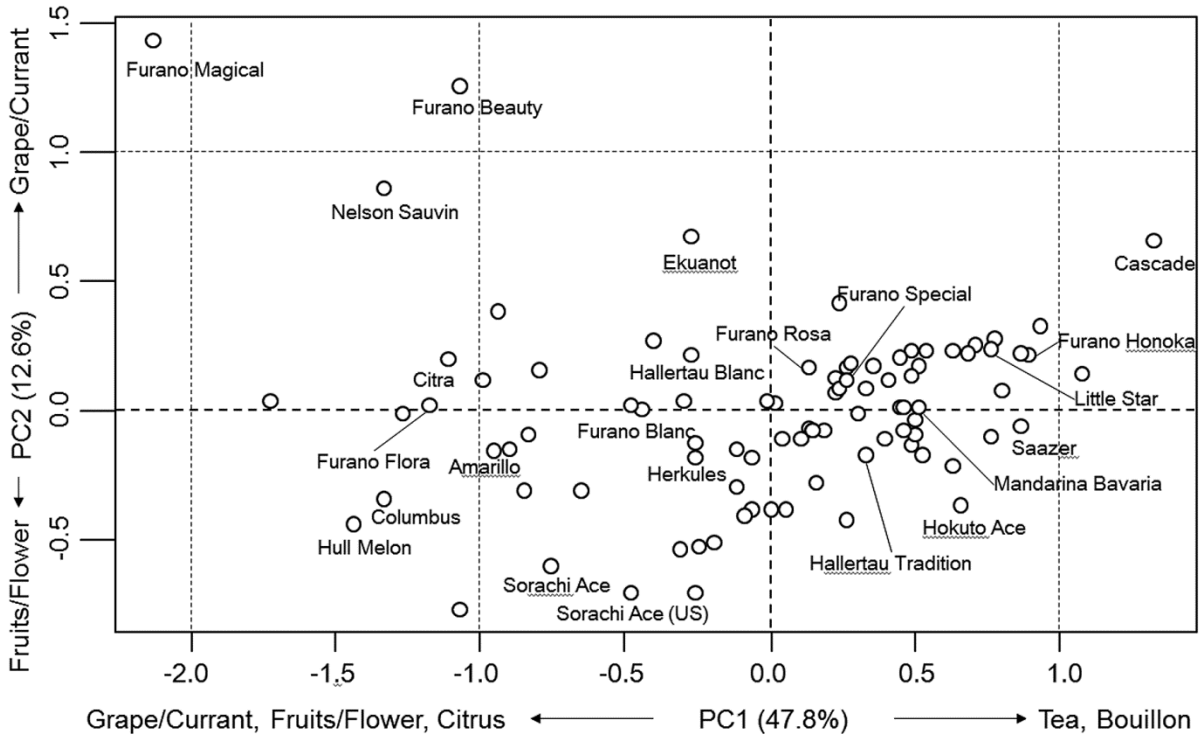

Figure 1. Scatter-plot of the hop varieties using the principal components 1 (PC1) and 2 (PC2) of the principal component analysis using the sensory evaluation scores of the hop water extract by boiling (HWEB).

The volatile aroma compounds present in the test varieties are shown in Table 1. Citra and Hallertau Blanc contained the highest amount of 3-sulfunylhexan-I-ol compared with the other varieties. The 4MSP content of Furano Magical was the highest among all varieties tested. The amount of 4MSP in Furano Magical cones was 85, 80 and 129 µg/kg in the crop years 2013, 2014 and 2015, respectively. The highest previously recorded levels of 4MSP in hop cones were reported to be 51–112 µg/kg for Simcoe (KISHIMOTO et al. 2008; REGLITZ & STEINHAUS 2017) and 37–114 µg/kg for Citra (REGLITZ & STEINHAUS 2017; TAKAZUMI et al. 2017), so Furano Magical is revealed to have the worlds' highest level of 4MSP content in its hop cones at present. Furano Magical was found to have the highest linalool content, while Furano Rosa contained the highest amount of geraniol.

The scores of "Tropical" and "Impact" of the beer using Furano Magical were significantly higher than those of the beer made using Nelson Sauvin ($p < 0.05$) (Fig. 2). There were more comments related to "Tropical" and "Mango" notes for the beer made using Furano Magical than there were for the beer made using Nelson Sauvin (Table 2).

Table 2. Summary of the comments about the trial beers single-hopped with Furano Magical or Nelson Sauvin. The number of panelists who made similar comments is shown in parentheses (total number of panelists = 42).

Variety	Comments
Furano Magical	Tropical (8), Mango (3), Pineapple, Grape (2), White wine, Citrus (4), Green (5), Peach (2)
Nelson Sauvin	Tropical (3), Pineapple, Grape (2), White wine, Flint, Citrus (5), Green (2), Berries

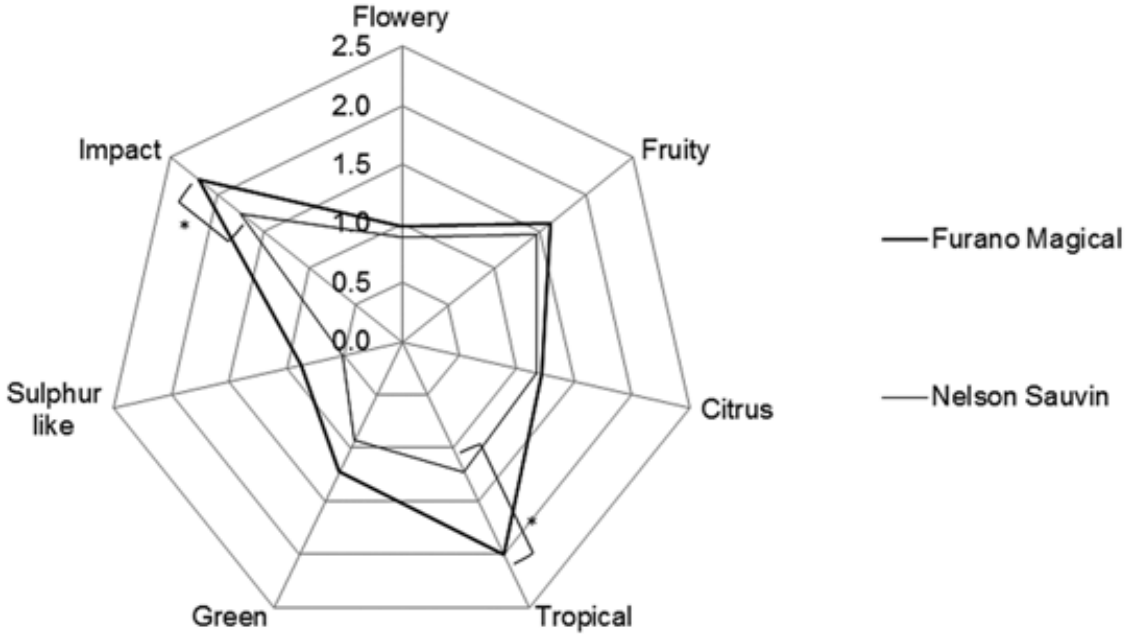

Figure 2. The scores of the sensory evaluation of the trial beers single-hopped with Furano Magical and Nelson Sauvin. *Denotes significant differences ($P < 0.05$)

The 4MSP and linalool content of the beer made using Furano Magical was higher than that of the beer made using Nelson Sauvin (Table 3). The level of 4MSP, 3S4MP and linalool in both test beers was more than their flavor thresholds of 1.5 ng/L (KISHIMOTO et al. 2006), 70 ng/L (TAKOI et al. 2009) and 1.0 □g/L (KISHIMOTO et al. 2006), respectively. 4MSP and linalool were considered to contribute to the tropical or mango-like flavor of the test beer made using Furano Magical because they are known to have a synergistic effect and to impart tropical flavor when they coexist (TAKOI et al. 2014).

Agricultural data showed that Furano Magical has comparable resistance to disease to that of Little Star; the cone yield of Furano Magical, however, is less than that of Little Star (Table 4).

Table 3. Volatile aroma compound content in the trial beers single-hopped with Furano Magical or Nelson Sauvin
3SH, 3-sulfunylhexan-1-ol; 4MSP, 4-methyl-4-sulfunylpentan-2-one;
3S4MP, 3-sulfunyl-4-methylpentan-1-ol; 3S4MPA, 3-sulfunyl-4-methylpentyl-1-acetate

		Furano Magical	Nelson Sauvin
3SH	ng/L	87	67
4MSP	ng/L	32	9
3S4MP	ng/L	164	335
3S4MPA	ng/L	12	8
linalool	□g/L	87	21
□-citronellol	□g/L	9	10
geraniol	□g/L	6	4

Table 4. Maturity, yiled, and resisitance to disease of Furano Magical and Little Star grown in Hokkaido, Japan.

	Maturity	Yield (t/ha)	Resistance to disease	
			Powdery mildew	Downey mildew
Furano Magical	8.21	1.22	not resistant	slightly resistant
Little Star	8.23	1.99	not resistant - weak	slightly resistant

Discussion

Furano Magical was not initially developed as a flavor hop when the variety was first under development. The seedlings from the crossing in1989 (Fig. 3) aimed to produce a classical aroma variety, and growing began in 1990. After six years of observation, the breeding line was judged not suitable for to proceed to the next selection level and was kept as a mid-mother plant. About 20 years later the breeding line was re-discovered by the screening program as described above and found to have special flavor notes.

Applications to have Furano Magical recognized as a new hop variety, “Furano K906901060 Go”, were made in Japan in 2016 (Application number, 31508) and in the European Union (EU) in 2017 (Application number, A201701994), and it has been protected as a new hop variety in the USA (United States Patent PP30067). We began commercial scale production of the variety in Japan from 2018 onwards to increase the level of supply.

The genetics of the trait that confers the content of polyfunctional thiols in hop cones is one of the next interesting subjects to explore. A biosynthesis pathway for 4MSP has been proposed (VERMEULEN et al. 2006), and this could be helpful in searching for genetic factors related to the contents of compounds present in hop cones. A greater understanding of the biosynthesis of these compounds could lead to the development of new hop varieties with even higher levels of these compounds in their cones than those of the existing varieties.

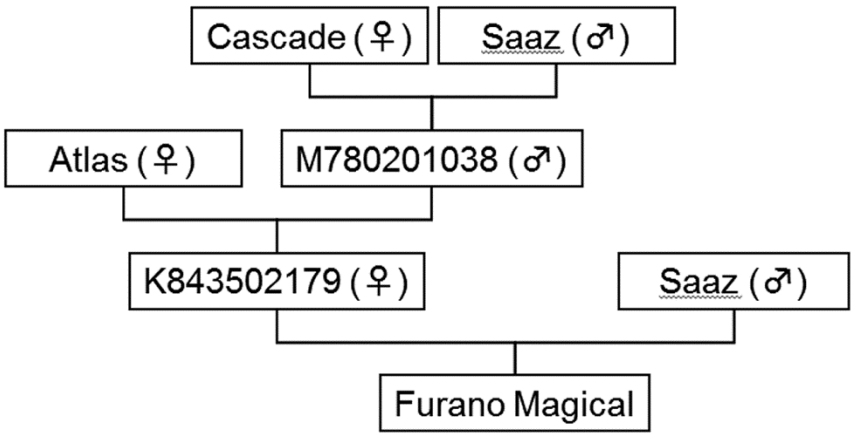

Figure 3. The pedigree of Furano Magical. The male parent “Saaz” is not Czech Saazer, but a Sapporo male parent line considered related to Czech Saazer.

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Slovenian hop breeding program – recent advances and challenges caused by citrus bark cracking viroid (CBCVd)

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Abstract

Slovenian hop fields are planted to 98 % with Slovenian hop varieties, bred at the Slovenian Institute of Hop Research and Brewing. In the last year two flavour varieties were registered, Styrian Dragon and Styrian Fox, in addition to the already established varieties Styrian Wolf, Styrian Cardinal, Styrian Eagle, and Styrian Kolibri. The farmers recognized them as an alternative addition to classical varieties and therefore their acreage increases continuously. Nevertheless, Slovenian hop industry is still mainly using and known for its traditional aroma varieties, with Celeia and Aurora as the leading ones. This successful collaboration between research institute and hop industry has advanced hop research together with the University of Ljubljana revealing in developed new selection methods.

Annually, selections on main diseases (Downy mildew, Powdery mildew) are performed at the stage of seedlings and correlations from data obtained in last 10 years will be presented.

Since the infection of hop plants with CBCVd is threatening research program at the Institute strict eradication management measures are performed also at hop breeding program. We present challenges of preservation and re-establishing of planting material to ensure the continuation of the Slovenian hop breeding program.

Key words. varieties, selection to diseases, citrus bark cracking viroid (CBCVd), *Humulus lupulus*

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The application of metabolomics and genomics in hop breeding

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Abstract

The major goal of hop breeding is to develop new competitive varieties for an efficient and resource-saving hop industry. Resistance to diseases, pests and changing climatic conditions as well as consistency in yield and quality are specific breeding targets. An important tool to master these requirements is the implementation of molecular genetics and metabolomics. Studying the relationship between metabolite content levels and trait expression enables the identification of molecular processes involved in resistance against abiotic and biotic stress.

For example, Downy mildew of hop, also called Peronospora, is caused by *Pseudoperonospora humuli* and generates significant losses in cone quality and yield as well as rootstock death. To identify the molecular processes conferring natural Downy mildew resistance (DMR) and to determine genetic and metabolic markers for breeding, a metabolome-genome-wide association study was carried out.

An F1 hop population consisting of 192 individuals from parents contrasting in DMR was germinated and grown under ex situ conditions. Inoculation of the population of full-siblings with the fungus P. humuli led to both variation in specialized metabolites and downy mildew resistance phenotypes. ANOVA between infected and control plants showed that metabolites of almost all phytochemical classes were induced 48 hours after induction, providing evidence for a general and massive allocation of carbon into pathways with function in pathogen defense. But this approach did not lead to the identification of metabolites with direct activity against the pathogen. Using Pearson correlation analysis, a small number of metabolites with potential protective function against downy mildew were identified and mapped to the phenylpropanoid biosynthetic pathway. These metabolites were even correlated to DMR in mock-infected plant set, suggesting that DMR is established prior contact with the pathogen. Genome-wide association study and genetic mapping detected a co-localization of the major downy mildew resistance locus and the phenylpropanoid pathway metabolite markers, indicating that the major contribution to resistance is mediated by these metabolites, in a heritable way.

In an independent validation experiment, a mix of three putative prophylactic phenylpropanoids was co-inoculated alongside with *P. humuli* on Downy mildew susceptible genotypes. This external application led to a reduced leaf infection, thus confirming the phenylpropanoid`s protective activity either directly or as precursors of active compounds.

These novel metabolic and genetic markers provide a better basis for the precise selection of crossing partners and progeny in hop breeding strategies in the future and may facilitate the development of bio-based fungicides for secured and sustainable cultivation of hop and other plant species affected by Downy mildew.

Key words. Downy mildew resistance, untargeted metabolomics, genome-wide association study

II: Hop chemistry

Recent development of the content of undesirable and foreign substances in Czech hops

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Abstract

In addition to a number of important brewing substances (resins, essential oils, polyphenols), hops also contain some undesirable and extraneous substances coming from the environment. One example are residues from chemical plant protection, which is still necessary to maintain plant health. Undesirable substances include, in particular, nitrates, which are natural components of plants and which in some organs may be present in large quantities. Such organs include hop cones in which nitrates accumulate naturally and where their content is usually in the range of 5,000 to 15,000 mg kg^{-1}. Unlike other substances, nitrate content in hops does not change significantly between years. Due to their very good water solubility, nitrates quickly get into the wort during hop boiling and significantly affect the final nitrate content of beer. Many breweries therefore require nitrate analysis as part of their quality systems.

Heavy metals are serious environmental contaminants. In the context of hops, copper, which in various formulations is part of a wide range of copper fungicides, has been investigated in recent years. On the basis of implementing regulation EU No. 2015/232 and EFSA recommendations, an application of max 4.0 kg of pure copper per hectare and year is allowed in the Czech Republic since 2017. Keeping this dose, the copper content of the hops does not exceed 100 mg kg^{-1}. This measure was very quickly reflected in the significant reduction of elementary copper in Czech hops. In 2017 and 2018, the average copper content decreased to 85 mg kg^{-1} (2017) and 80 mg kg^{-1} (2018), respectively. The highest levels were found in the range of 250 to 350 mg kg^{-1}, which is considerably less than the time of unlimited application of copper fungicides, when copper contents were often in the range of 500 to 1,000 mg kg^{-1}.

Unlike nitrates, the residual content of active substances of pesticides applied in hop gardens is highly variable in terms of both the product range and the quantity. The most commonly used pesticides in recent years include ametoctradin, azoxystrobin, bifenazate, boscalid, dimetomorph, mandipropamid and spirotetramat. To a lesser extent, fenpyroximate, cymoxanil, metalaxyl and hexythiazox were also applied. Very sensitive instruments that are capable to detect trace amounts are used for analysis. As a result, the presence of substances that were not directly used in the hop gardens but were applied to adjacent fields with another crop was recorded. Positive findings of pesticides in wild hops, which grow in the hop regions but also beyond, show that a pesticide can be transported by wind and air currents even over many kilometers away from the application site. Large amounts of pesticides remain on the leaves and bines, and as a biological admixture, they can contaminate the harvested hops, although hop cones themselves are free of residues. Dynamics of pesticide decomposition also are greatly affected by the weather conditions after application. The fact that no unauthorized pesticides are found in Czech hops is evidence of the good technological discipline of hop growers.

III: Phytopathology

Transmission pathway studies of citrus bark cracking viroid (CBCVd) on hop

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Abstract

Since 2007, Slovenian hop production has been faced with the appearance of a dangerous disease called »severe hop stunt disease«, caused by citrus bark cracking viroid (CBCVd). CBCVd has a highly negative impact to hop production, since infected plants develop severe stunting in the first year after infection and die in 3-5 years. Like other viroids CBCVd spreads mainly mechanically; longer distance spreading is only possible by infected planting material and plant remains. It is assumed that the appearance of CBCVd in Slovenia is a consequence of a rare transmission event during which hop plants had been exposed to remains of infected citrus fruits in the primary outbreak location. The spread of CBCVd in hop gardens is extremely rapid, due to the specific agro-technical practices in hop production, which creates ideal conditions for mechanical transmission, and due to the physiological characteristics of hop as a green herbaceous plant. On this way CBCVd infections can reach more than 40 % of plants in hop gardens in three successive years. Since the CBCVd infected plants are incurable, basic management depends mainly on eradicative phytosanitary measures; however, experiences from farms show different levels of success. In the presentation transmission pathway studies and sensitivity testing of hop cultivars are considered important factors in future disease management strategy.

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

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

Response of the hop transcriptome after infection with HLVd, CBCVd and their co-infection and estimation of disease severity

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Abstract

Viroids are the smallest know plant pathogens in the form of single-stranded, covalently-closed circular noncoding RNA up to ~400 nucleotides long. They solely rely on host factors for their replication and can cause severe diseases and crop loses in several economically important crop plants including hops. The mechanisms of viroid pathogenesis are under investigation and include either direct interaction of the viroid RNA with cellular machinery, or post-transcriptional and transcriptional gene silencing mediated by viroid-derived small RNAs (vd-sRNAs) generated by the host RNA interference pathway. Currently, four different viroid species are infecting hop plants: HLVd, HSVd, AFCVd and recently discovered CBCVd. Different range of symptoms are recorded on plants infected with these viroids from presumably symptomless plants in case of HLVd to complete dieback of plants in case of CBCVd infection. There is also no information available on viroid co-infections in hop. HLVd and CBCVd are attractive model system to study viroid-hop plant interactions, due to the symptomless infection of the HLVd and severe symptoms induced by the CBCVd. Powerful tool to study the gene expression response on a global scale is possible through high-throughput sequencing of RNA or RNA-seq. RNA-seq experiment was performed to address the question of hop response by single and mixed HLVd and CBCVd infections. Viroid free hop plants of Slovenian cultivar 'Celeia' were inoculated using HLVd and CBCVd viroid constructs and phenotypically evaluated. Isolated total RNA was enriched for mRNAs by mRNA Direct Kit and strand specific NGS Sequencing was performed on Ion Proton system at depth of approx. 20 M sequences per biological replicate. Sequences were aligned to available hop draft genome sequence and the analysis of differential gene expression was performed by EdgeR Bioconductor package implemented in CLC Genomics Workbench tool. Analysis showed the dynamic changes in activity of genes of CBCVd infection as compared to single HLVd infection or their co-infection including genes involved in defense, phytohormone signaling, photosynthesis, RNA regulation, processing and binding, protein metabolism and modification and others. However, in mixed infection genes involved in proteolysis mechanism are more active suggesting co-infection probably result in interference with more host factors inducting more severe symptoms, which was also confirmed by phenotypical evaluations of plants.

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Molecular-genetic analysis of downy mildew (*Pseudoperonospora humuli*) population in Czech hop growing regions

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Abstract

Hop downy mildew *Pseudoperonospora humuli* is an obligate biotrophic oomycete pathogen, causing severe yield and quality losses of hops in all hop growing regions. Obligate nature of the pathogen causes that there are only few molecular genetic data available for genomic and population analyses. *Pseudoperonospora humuli* populations from the Czech Republic were analyzed for genetic variation using certain gene loci and screening of microsatellites (SSRs). Isolates of *P. humuli* were collected during the spring season 2017-2018 from commercial fields. Basal spikes were collected from Žatec region in 2017, and from all hop growing regions in 2018. Spikes were collected mainly from sensitive cultivars. DNA was extracted directly from mycelium using DNeasy Plant Mini Kit (Qiagen) and EZNA Fungal DNA Mini Kit (Omega). The internal transcribed spacers of nuclear ribosomal DNA (ITS) region, β-tubulin gene (β-tub), and the cytochrome c oxidase (cox) cluster (cox2, cox2-cox1 spacer and cox1) were amplified with the specific primers to evaluate the specification of primers. We also used markers such as SSRs and tested 18 of them by electrophoresis in PAGE. The genetic structure of the Czech populations differed a little and there was no detectable nucleotide variation in selected loci. It is suggested that *P. humuli* populations have clonal character in the Czech Republic. It is important to analyze more isolates from other regions and examined different genes and SSRs.

Effect of lethal hop wilt strains (*Verticillium nonalfalfae*) and different nitrogen fertilizer levels on the indicator plant eggplant (*Solanum melongena* L.)

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Abstract

The experiment consists of a 2-factorial test, to set a nitrogen fertilizer level on the development of symptoms of various Verticillium strains on the eggplant. The plants are artificially infected by lethal Verticillium strain. Furthermore, three different nitrogen fertilizer levels are used. During the experiment, evaluations of the plant status are carried out every week. The plant size is measured, and yellowing, necrosis or curling of the leaves are documented. Furthermore, the most recent fully developed leaf is measured by SPAD-Meter (Soil Plant Analysis Development). The nitrogen- and nitrate-amount are measured on the most recent fully developed leave. From the results should be derived, how the differences in the nitrogen nutritional status of the plants affect the development of symptoms related to the fungus.

Key words. *Verticillium* wilt, *V. nonalfalfae*, *V. albo-atrum*, indicator plant, eggplant

Introduction

If wilting symptoms appear on hops (*Humulus lupulus*), they may have been caused by a variety of fungal pathogens. In addition, the rare diseases *Alternaria*, *Phoma* and *Fusarium* spp. have also been described as the cause of wilting (LFL 2019). However, *Verticillium* wilt is a pathological disease that can lead to considerable losses in cone yield and is induced in hops chiefly by the pathogen *Verticillium nonalfalfae*, which was described only in 2011 (INDERBITZIN et al. 2011). Formerly, this disease had been ascribed to *Verticillium albo-atrum* Reinke & Berthold, but *V. nonalfalfae* is in fact a distinct species causing the vast majority of *Verticillium* outbreaks in hop; more rarely the disease is caused by *Verticillium dahliae* Klebahn (RADIŠEK 2009)

Verticillium can be found in living and rotten leaves, bines and roots. The fungus invades the root and colonizes the whole hop plant via the xylem elements. *Verticillium* also decomposes the cell walls of the host plant through the excretion of certain metabolic products (EPPO 2007; RADIŠEK 2009). The symptoms are manifested at first by yellowing of the leaves, which becomes necrotic. Furthermore, there is a thickening of the bines and a brownish discoloration of the xylem elements (EFSA 2014). In particular, there are no chemicals available to combat this disease. The disease can also be influenced by the nitrogen nutrient level. High nitrogen levels often cause a higher vulnerability to *Verticillium* (RADIŠEK 2009).

Since hops do not show symptoms until months after infection and no soil test is available, a indicator plant is a proper possibility to show the infection earlier. This could be found with the eggplant (*Solanum melongena* L.), which can also be infected by *Verticillium* wilt (ELMER & FERRANDINO 1994; ANGELOPOULOU et al. 2014).

Material and methods

The experiment based on a two-factorial test, to set a nitrogen fertilizer level on the aggressiveness of various *Verticillium* strains on the eggplant cv. *Violetta di Firenze*. Six weeks after seeding, the plants are artificially infected by a lethal *Verticillium* strain via the dip method (experiment 1). The roots of the plants are cut back and kept in a spore suspension (FLAJSMAN et al. 2017). Four weeks later a half of the control plants were poured by inoculum (experiment 2) to compare the two different artificially infection methods. Furthermore, for the second trial factor three different nitrogen fertilizer levels are set. These can be divided into a nitrogen deficiency variant, a nitrogen abundance variant and an adapted nitrogen fertilization variant. To ensure the same amount of nitrogen is applied to all plants within a variant group, liquid fertilizers are used. During the experiment, evaluations of the plant status are carried out every two weeks. The plant size is measured, and yellowing, necrosis or curling of the leaves is documented. Furthermore, the most recent fully developed leaf is measured by SPAD, which shows the chlorophyll content of the leaves and thus reflects the N-nutritional status of the plant (CAMPBELL et al. 1990).

At the end of the experiment, the fresh and dry mass of each plant is documented. The nitrogen- and nitrate-amounts are measured and for half of the plants which show *Verticillium* symptoms, qPCR will be carried out on the petiole of a visibly infected leaf (MAURER et al. 2013). All plants were successfully infected and had *Verticillium*-mycelium in the channels of the stems.

Results and Discussion

The experiment also shows that the methodological procedure for an artificial infection with *Verticillium* needs to be adjusted even better to make the differences between the experimental variants clear. Initially a too high inoculum concentration was chosen and the eggplants started wilting after only three weeks. Due to the rapid death of the plants no observations regarding the effect of the two fertilizers used were made (ELMER & FERRANDINO 1994). Neither the nitrate nor the ammonium variant performed better in terms of wilting disease. Also, the plants showed no differences in regard to the three fertilizer levels. All plants were equally affected by *Verticillium* wilt. From these results, a pot system for the greenhouse is to be developed to test the effect of different plant health management systems.

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Molecular detection of *Verticillium nonalfalfae* in planting material and in the field

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Abstract

Verticillium dahliae and especially *Verticillium nonalfalfae* are important fungal vascular pathogens of hop. In the case of *V. nonalfalfae*, a mild and a lethal form exist, the latter of which has recently been recorded from an increasing number of European countries. As no real curative options exist, two main strategies for dealing with this pathogen are avoidance of introduction via pathogen-free planting material and planting hop in *Verticillium*-free soil. To help facilitating this, a first objective of our study was to improve methods for the detection of these Verticillium species in plants and in soil.

For the sensitive detection of *Verticillium* in plant material, we optimized tissue maceration, DNA extraction and qPCR methods. Sensitive detection of the pathogen is possible in rhizomes from infected plants, providing opportunities for detection in bulked rhizome material that is used for making new planting material. Bulked petioles can also be used to screen mother plants during the growing season. However, the presence of the pathogen in such material should preferentially be tested late in the season, from the lower part of the plant and using at least two opposite petioles from each bine, as the pathogen was distributed irregularly in the plant and only started to move upwards in July. Detection in soil of *V. dahliae* was facilitated via the extraction of microsclerotia from 100 g soil samples using a zonal centrifuge. This was followed by DNA extraction and qPCR, resulting in a detection limit of less than 0.1 microsclerotia per gramme of soil.

A second objective of our study was to determine which *Verticillium* species and forms are present in Belgian hop fields. We conducted a survey in 10 hop fields in which symptoms of the disease were present. Verticillium nonalfalfae was the main species detected. We tested Genotyping by Sequencing (GBS) as a method for the characterization of the resulting Verticillium isolates and compared it to alternative methods. Using an international reference collection we could show that even though the genetic diversity within *V. nonalfalfae* is low, GBS was suited to identify molecular markers linked to specific geographic and/or pathogenic groups of isolates. This included a few markers that were able to discriminate the lethal from the mild form of *V. nonalfalfae*. The results with these new markers were congruent with the results of the discriminative test in the EPPO diagnostic protocol. We are now characterizing our own *V. nonalfalfae* population using the GBS technique.

Population structure and genetic diversity of *Podosphaera macularis*

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Abstract

Powdery mildew is one of the most problematic diseases of hop. The disease was first reported in western U.S. growing areas in the mid-1990s. More recently, the disease has reemerged in newly planted hop yards of the eastern U.S., as hop production expanded to meet demands of local craft brewers. The spread of strains adapted to extant sources of host resistance, available fungicides, and the MAT1-2 mating type to the western U.S. all threaten the sustainability of hop production. We sequenced the transcriptome of 104 isolates of *Podosphaera macularis* collected throughout the western US, eastern US, and Europe to quantify genetic diversity of pathogen populations and elucidate the origins of pathogen populations in the western U.S. Discriminant analysis of principle components grouped isolates into 3 to 5 geographic populations, dependent on stringency of the grouping criteria. Isolates from the western U.S. were categorized into one of three pathogenic races based on phenotype on differential cultivars. Western U.S. populations were clonal, irrespective of pathogenic race. Isolates originating from wild hop plants in the eastern US were genetically differentiated from all other populations, whereas isolates from cultivated hop plants in the eastern U.S. mostly grouped with isolates originating from the west. Mating types of isolates originating from cultivated western and eastern U.S. hop plants were entirely MAT1-1. In contrast, a 1:1 ratio of MAT1-1 and MAT1-2 was observed with isolates sampled from wild plants or Europe. Within the western U.S. a set of highly differentiated loci were identified in *P. macularis* isolates associated with virulence to the R-gene R6. The foregoing genetic and phenotypic patterns suggest a European origin of the *P. macularis* populations in the western U.S., followed by spread of the pathogen from the western U.S. to re-emergent production regions in the eastern U.S. Furthermore, R6-compatibility appears to have been selected from an extant isolate within the western U.S.

Key words. Disease ecology, epidemiology, genetic diversity, mating type, Powdery mildew.

Introduction

Hop powdery mildew (caused by *Podospheara macularis*) is one of the oldest fungal diseases known on cultivated hop (*Humulus lupulus*), dating back to reports in England from the 1700s (ROYLE 1978). *P. macularis* can now be found in most hop growing regions in the northern hemisphere. In the United States, nearly all of the hop industry is centered in the Pacific Northwestern states. Although powdery mildew has been present and recorded in eastern North America since the early 19th century (BLOGETT 1913) and can still be found on wild and feral plants (WOLFENBARGER et al. 2015), the fungus only became established in the Pacific Northwest in the mid-1990s (OCAMB et al. 1999). The fungus is now endemic in the western U.S. and powdery mildew becomes epidemic annually.

About 2 % of the U.S. hop crop is grown in states outside of the Pacific Northwest, primarily in the Midwest and the northeast. Outside of the western U.S., powdery mildew can be found on wild and feral hop plants (e.g., CLAASSEN et al. 2017; WOLFENBARGER et al. 2015), but at present is observed only occasionally on cultivated hop plants (GENT et al. 2015).

The source of *P. macularis* that introduced the pathogen into the Pacific Northwestern U.S. is speculative, but the population is believed to be the result of a single introduction due to absence of the ascigerious stage of the fungus. *P. macularis* is heterothallic but only the *MAT1-1* idiomorph of *P. macularis* is known to occur in this region, forcing the fungus to reproduce solely by asexual means. In contrast, both mating types are broadly distributed in Europe and eastern North America, and the ascigerious stage frequently occurs in these populations (BLOGETT 1913; ROYLE 1978; WOLFENBARGER et al. 2015).

Important questions about the population genetic diversity and structure are unresolved in *P*. *macularis*. It is unknown if the population in the Pacific Northwestern U.S. is structured by state or pathogenic race, if virulent races were selected from extant isolates or introduced, how the population in the U.S. is related to other populations in the world, and the origin of *P. macularis* that is now endemic in the Pacific Northwest. These questions motivated two overarching objectives of this study. We sought to determine if the population of the fungus is structured among different geographic regions and pathogen races. Secondly, we sought to characterize the relatedness of the populations in the Pacific Northwest to those in eastern North America and Europe to infer the possible origin of *P. macularis* in the primary hop production regions of the U.S.

Material and methods

Isolates of *P. macularis* were maintained on detached hop leaves and transferred every two to three weeks. A total of 104 isolates of *P. macularis* were obtained from a variety of populations. These isolates were derived from the Pacific Northwestern U.S. (49 isolates), east of the Pacific Northwestern U.S. (25 isolates), England (13 isolates), and continental Europe (17 isolates). Efforts were made to collect isolates representing each of the three dominant races found in the western U.S. PCR assays were used as described by Wolfenbarger et al. (2014) to determine whether isolates were *MAT1-1* or *MAT1-2*.

The first node of completely unfurled leaves of a powdery-mildew-susceptible cultivar was detached, inoculated, and incubated for 14 to 17 days. RNA was extracted from the fungal mycelia. RNA sequencing was performed using paired-end 150 bp Illumina HiSeq 3000 technology at the Oregon State University Center for Genome Research and Biocomputing. A *de novo* transcriptome assembly was assembled using SPAdes using RNA sequence data from an isolate obtained from Oregon after mapping to a hop reference genome to reduce hop-related transcripts. Genetic variants were called using the GATK's haplotype caller and quality filtered on depth.

In order to explore global population structure of *P. macularis* we used the discriminant analysis of principal components (DAPC) method to identify clusters of genetically related isolates. The isolates were assigned to a range of group numbers (2 – 5 groups) and the Bayesian information criterion (BIC) was used to determine support for these groupings.

Results

The assembly resulted in 5,269 transcripts containing 8.49 million base pairs with an N50 of 1,626. These transcripts were slightly enriched for A/T with most transcripts being about 60% A/T. Variant calling resulted in 166,601 variants; 243 variants were retained (due to low coverage and missingness) after filtering.

K-means clustering demonstrated little if any improvement in BIC when samples were divided into more than three groups (Fig. 1A). A scatter plot of the discriminant functions indicated that even if assigning isolates to five groups, three of these groups cluster together resulting in only three well distinguished clusters (Fig. 1B). Barplots of posterior probabilities of group membership indicated that the eastern U.S. population typically included the highest number of groups (Fig. 1C). And at *K* = 5 the eastern U.S. had a low frequency group that was not observed elsewhere. Isolates from the Pacific Northwest belonged to one group independent of *K*, indicating clonality and that this group was shared among populations.

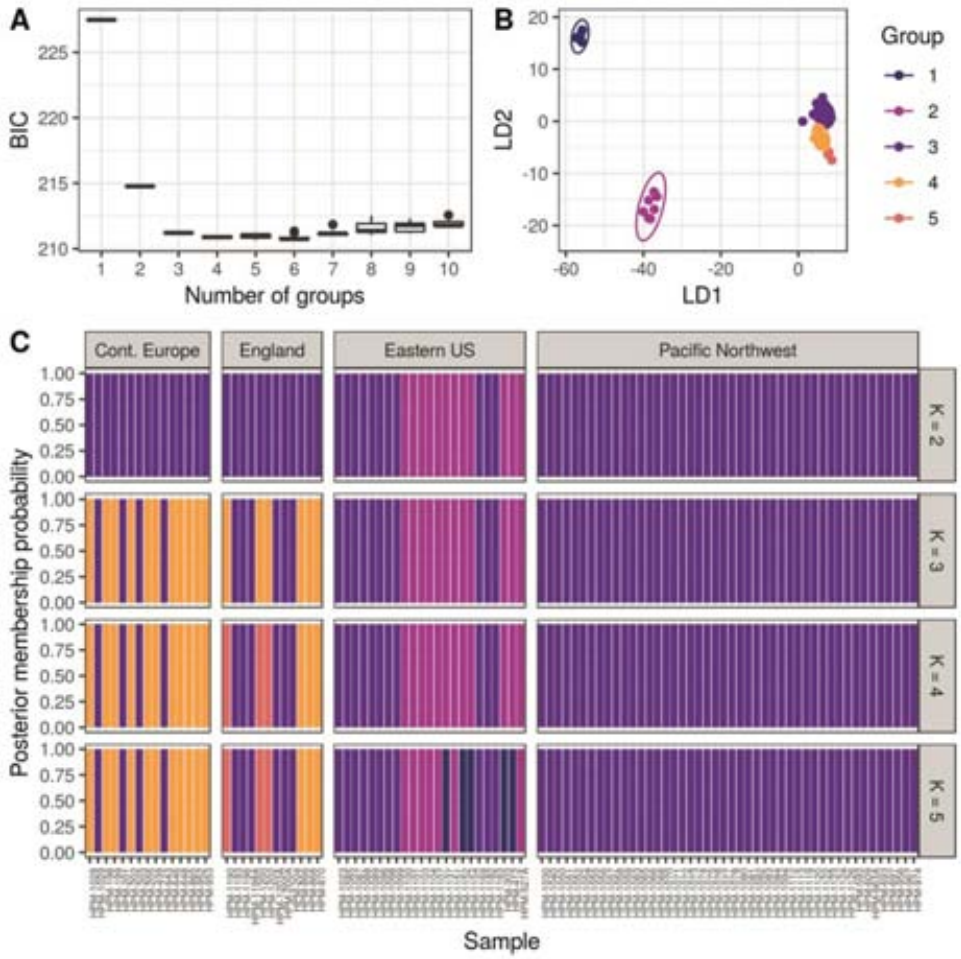

Figure 1. Discriminant analysis of principal components. **A,** *K*-means clustering (10 replicates for each value of *K*). **B,** Scatterplot of discriminant functions based on *K*=5. **C,** Barplots of the posterior probability of group membership for each individual based on *K* = 2 to 5.

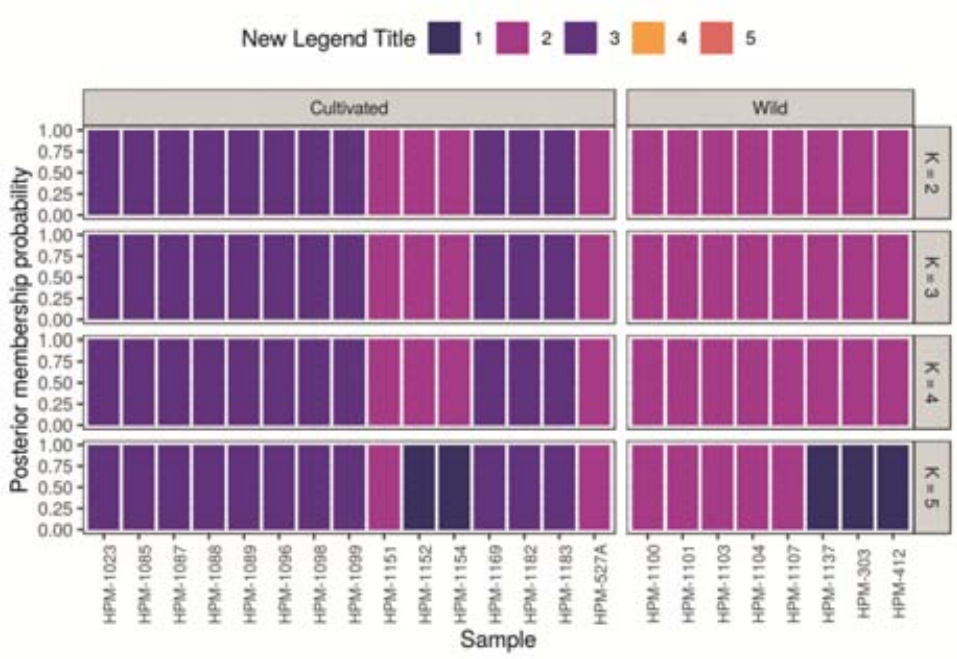

Figure 2. Posterior probability of group membership with varying number of groups (*K*) for isolates of *Podosphaera macularis* originating from cultivated hop yards or wild plants in the eastern U.S.

In the eastern U.S., membership of the isolates into genetic groups varied depending on the number of groups defined and also whether isolates originated from wild or cultivated hop plants (Fig. 2). The population originating from wild plants was unique from all other populations, independent of the number of groups defined. In contrast, on cultivated plants (at all values of *K*) 10 of the 14 isolates (71.4%) belonged to the genetic group that contained all isolates from the Pacific Northwestern U.S. Four isolates were atypical on cultivated plants, belonging to the genetic groups found only on wild plants.

Mating type assays indicted further similarity between isolates originating from the Pacific Northwest and those found on cultivated plants in the eastern U.S. All isolates from the western U.S. were mating type *MAT1-1*, as expected. However, all isolates from cultivated plants in the eastern U.S. were also identified as *MAT1-1*. In contrast, both mating type idiomorphs were detected at similar frequencies in isolates derived from wild plants. The ratio of mating types in populations from Europe also was approximately 1:1.

Discussion

This research indicates that the population of *P. macularis* in the western U.S. is genetically clonal and highly similar to populations of the pathogen found in England and continental Europe. However, the population is differentiated from that found on wild and feral hop plants in eastern North America. Given the clonal structure of the pathogen population it is not possible to assign probabilistic functions to the likelihood that *P. macularis* in the western U.S. originated in Europe. Nonetheless, the genetic structure of the populations are consistent with a European origin of the western U.S. population. Dissemination of *P. macularis* from Europe to the western U.S. in association with plant material seems plausible given the geographic isolation of the production regions.

We did not find evidence of pronounced genetic differentiation among the three pathogenic races of *P. macularis* that occur in the western U.S. This supports the hypothesis that these races were selected from an extant isolate in the western U.S., coincident with shifts in cultivars with different sources of resistance to powdery mildew.

The genetic and phenotypic relatedness of the population in the western U.S. and the population found on cultivated plants in eastern North America also indicates that *P. macularis* spread from the western U.S. to re-emerging production regions in the eastern U.S. With few exceptions, isolates from both populations belonged to the same genetic group and were invariably the *MAT1-1* idiomorph. *P. macularis* can be found growing on wild and feral hop plants in the Midwestern U.S. and eastern North America (BLODGETT 1913; WOLFENBARGER et al. 2015). However, the genetic groups found on these plants usually were distinct from the populations found on cultivated plants. Wild or feral plants also harbored both mating types in approximately a 1:1 ratio. Therefore, the dominant population that occurs on wild or feral plants appears distinct from the population on cultivated plants. Again, the most plausible explanation for this intracontentinenal spread of *P. macularis* is in association with infected planting material as new yards have been established in the central and eastern portions of the U.S. Sanitation measures during propagation and quarantine policies should be considered to limit further spread of novel genotypes of the pathogen.

Acknowledgement

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IV: Hop cultivation and management

Influence of weather conditions, irrigation and plant age on yield and alpha-acids content of Czech hop (*Humulus lupulus* L.) cultivars

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Hop (*Humulus lupulus* L.) is an essential crop for the brewing industry. With more than one thousand years hop growing history, the Czech Republic is on the third position worldwide in the acreage and production after the USA and Germany. As the climate changes, weather conditions during growth of hop plants strongly influence the production of hops in both quantity and quality. In this study, we quantified effects of weather conditions, irrigation and plant age on yield and alpha-acids content of Czech hop cultivars Saaz, Sládek, Premiant and Agnus since 1993.

Rainfall was the most significant factor that positively influenced the yield of Saaz hops with correlations r = 0.5904 and 0.6061 for total seasonal rainfall, 0.6498 and 0.6027 for days with rainfall over 3 mm, 0.3866 and 0.5771 for rainfall in May and 0.4953 and 0.3225 in July in Saaz region and Stekník farm, respectively. The yield of other cultivars Sládek, Premiant and Agnus was not influenced by rainfall, but there was a positive effect of irrigation level on increasing yield with correlations of 0.5831, 0.5471 and 0.4868. High air temperatures during the summer, expressed as average monthly temperatures, number of tropical days and tropical nights, were the most significant factors that negatively influenced the alpha-acids content with high correlations ranging from -0.5577 to -0.8292. Saaz and Premiant cultivars were more sensitive than Sládek, while Agnus showed a stable weather-independent alpha acid content. Yield and alpha-acids content were also influenced by plant age since younger plants were more productive and after 15 to 20 years it was time for replanting.

Hop irrigation in Slovenia – overview and research

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Abstract

In the time of climate changes water is becoming more and more precious resource and the future improvements in irrigation efficiency will play a very important role. In Slovenia we had to cope with with many droughts during the last 17 years: 2000, 2001, 2003, 2006, 2007, 2009, 2012, 2013 and 2017. Therefore, irrigation is an important element in the field of hop production. We are presenting a short review of scientific contributions (e.g., NAGLIČ 2014; NAGLIČ et al. 2014, 2016) and research projects in the field of hop irrigation in Slovenia in last 50 years. Contributions relate primarily to the water needs of hop plants and the impact of irrigation on quantity and quality of hop yield, taking into consideration different irrigation technologies, the irrigation impact on the leaching of nutrients (nitrate) into groundwater and decision support about sprinkler irrigation. We are also presenting the current understanding of the area and highlighting the loopholes and future possibilities in the area of hop irrigation management.

Key words. hop, irrigation, irrigation scheduling, modelling

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Effects of irrigation on hop (*Humulus lupulus* L.) cv. Nugget in Galicia: yield and quality aspects

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Abstract

A hopyard, established in Mabegondo (Galicia, NW Spain), was monitored during the 2018 growing season. Two treatments were established: 1) irrigation strategy common in the region (100 %), and 2) a deficit irrigation (80 %). Both treatments were applied through a drip irrigation system, amounting 2310 m^3 ha^{-1} and 1848 m^3 ha^{-1}, respectively for 100 and 80 %. The following indicators were determined: air temperature, rainfall and sunlight availability, soil organic matter and pH, soil water content, phenology, yield and quality of hop cones (3 plants per treatment).

Applied water amounts influenced significantly the soil water content, with 100 % irrigation treatment presenting the highest values. However, irrigation treatments did not cause significant differences on yield parameters, such as the ratio dry matter/fresh weight (80 %=0.228; 100 %=0.226) and total hop cone fresh weight per plant (80 %=3.47 kg; 100 %=3.56 kg); and quality traits such as alpha-acids (80 %=13.5 %; 100 %=13.4 %) and beta-acids (80 %=4.30 %; 100 %=4.37 %). These findings suggest that plants did not suffer from water stress, indicating that the irrigation applied by the producer on 2018 exceeded hop water needs and reducing irrigation by 20 %, did not compromise hop yield and quality. This information is useful for optimizing irrigation, saving water and money, and supporting a better planning of irrigation in the future.

Key words. Deficit irrigation, Spain, hop cone fresh weight, alpha- beta- acids

Introduction

Hop production demand increased in the last years because of the requirements of the brewing industry. This situation requires the development of agricultural techniques that maximize yield while reduce the consumption of water resources (CANCELA et al. 2017). Moreover, there is a trend for hop varieties adapted to local conditions, or at least being produced within the region of the brewery and/or under ecological cultivation. Therefore, assessing the feasibility of producing the same hop variety under different water stress situations is a challenge (BARRY et al. 2018). Several authors studied the effect of irrigation management on hop production, as reviewed by CANCELA et al. (2017); however, few works determined the water amount to apply and its effects on hop yield and quality (KROFTA et al. 2013; FANDIÑO et al. 2015; NAKAWUKA et al. 2017). These studies reported that irrigation increased yield and did not negatively affect the alpha-acid content, although impacts of water and temperature stress related with the phenological stages when occurred.

In this context, the main objective of the current study was to assess the impact of irrigation depth on hop (*Humulus lupulus L.* cv. 'Nugget') yield and quality, during the 2018 season, under the climate conditions of Northwestern Spain.

Material and methods

This research was conducted in a hop yard (cv. 'Nugget') of 'L.U.T.E.G.A, S.C.L', located in Abegondo (A Coruña, Galicia, NW Spain), during 2018. The experimental site, 'Borreiros', covers approximately 2 ha (43°13'27.8"N 8°16'29.7"E; elevation 94 m a.s.l.). Plant spacings were 1.5 x 3.0 m (2,222 plants ha^{-1}) and the plot was equipped with surface drip irrigation. The lateral pipes were equipped with in-line non-compensating emitters (2 L h^{-1}) spaced 100 cm along the crop row, thus 1.5 emitters per plant.

The soil at this site is loamy (33.7 % sand, 46.7 % silt, 19.6 % clay, pH 6.1, 6.7 % organic matter and water storage capacity of 200 mm m^{-1}).

Two treatments were implemented: 1) irrigation strategy common in the region (100 %), and 2) a deficit irrigation (80 %). Water was applied between July 1 and September 8 amounting 70 irrigation events of 5 and 4 h for the 100 % and 80 % treatments, respectively. The 100 % treatment corresponded to the irrigation strategy applied by hop growers in the region and represented the crop evapotranspiration (ET_c), as defined by ALLEN et al (1998), on the basis of the use of crop coefficients (K_c) for the different crop phenological stages (Table 1).

Table 1. Crop coefficients (K_c) of hop in different phenological BBCH stages, adapted from ALLEN et al (1998).

K_c	Value	Phenological BBCH stage
$K_{c\ initial}$	0.15	00 – 20
$K_{c\ middle}$	1.00	60 – 79
$K_{c\ final}$	0.80	80 – 89

Several variables were determined over the crop growing cycle (soil water content, weather variables, phenological stages). Soil water content was determined with a TDR100 (Campbell Scientific) at 0.40 cm depth in six plants per treatment (FANDIÑO et al. 2015). Weather data were collected from the Mabegondo station (Xunta de Galicia) accounting for reference evapotranspiration (ET_o) (ALLEN et al. 1998), rainfall, sun hours and temperatures over the growing cycle. Phenological stages were determined over the growing season using BBCH scale.

In addition, yield was assessed in four plants per treatment in order to evaluate the effect of the irrigation strategy on hop production and quality components, including dry matter/fresh weight ratio, total hop cone fresh weight per plant; and alpha and beta-acids concentrations. Data were analyzed by means of ANOVA using the R software (https://cran.r-project.org/). Differences between treatments were considered significant at $p < 0.05$.

Results

During the growing season of 2018, mean temperature was 15.2ºC, similar to that for the period of 2001 to 2017 (Table 2). Sun hours in 2018 were slightly lower, about 5 %, than in the period 2001 to 2017. In contrast, rainfall was greater in 2018 than in the period from 2001 to 2017, approximately 58 % higher (Table 2).

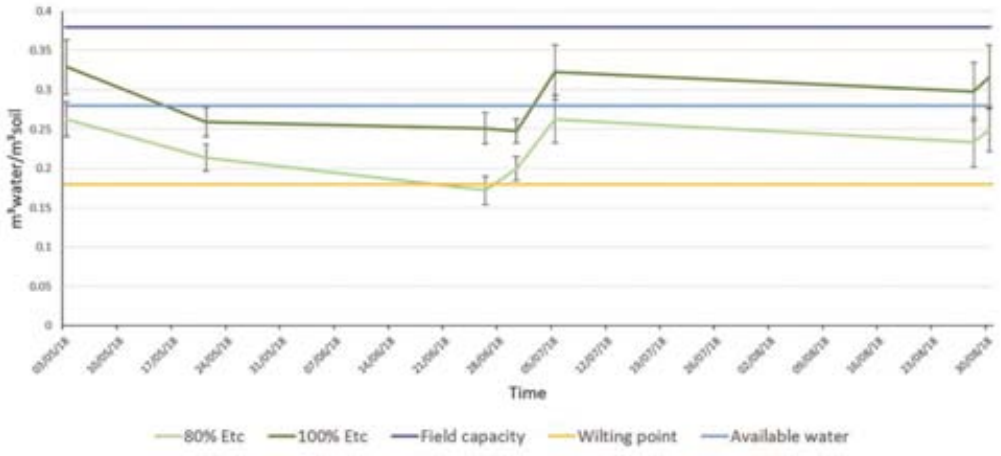

Figure 1. Soil water content over the growing season for the 100 % and 80 % irrigation treatments (data are averages ± standard deviations).

Figure 1 shows the dynamics of soil water content over the growing season for the two irrigation treatments, as well as the values of field capacity and wilting point (FANDIÑO et al. 2015; CANCELA et al. 2017), which are 0.38 % and 0.18 %, respectively. Between May 3 and June 28, soil water content resulted from rainfall, whereas, from July 1 onwards, irrigation was triggered (Figure 1). Soil water content was significantly higher in the 100 % treatment than in the 80 % treatment all over the growing season (Figure 1).

Irrigation treatment did not affect yield components (Table 3). The dry matter/fresh matter ratio was similar between treatments, whereas yield decreased by less than 2.5% in fresh weight under the 80% irrigation treatment (Table 3). When considering yield at 10% humidity, the 80% irrigation treatment yield only decreased by 1.9% (Table 3).

Hop cone composition was similar for the two irrigation treatments (Table 4). The contents of alpha and beta acids, as well as those of cohumulones and colupulones are within the intervals required by the brewing industry. No significant differences were detected for any of the compositional traits determined in the current study (Table 4).

Table 2. Monthly mean temperature, sunlight and rainfall during 2018 and for the 2001-2017 period.

Month	2018			2001 – 2017		
	Mean temperature (ºC)	Sunlight (hours)	Rainfall (mm)	Mean temperature (ºC)	Sunlight (hours)	Rainfall (mm)
March	8.9	120.0	325.1	10.6	167.3	109.3
April	11.7	178.4	105.8	12.0	198.5	85.4
May	14.0	221.3	44.4	14.2	205.7	76.3
June	17.2	182.3	82.9	17.2	224.1	45.9
July	19.5	199.3	29.8	18.7	236.8	34.2
August	19.7	296.0	27.0	18.8	233.5	34.4
Average/Total	*15.2*	*1197.3*	*611.0*	*15.3*	*1265.8*	*385.5*

Table 3. Hop yield components as a function of the irrigation treatment.

Irrigation treatment	Dry/fresh matter ratio	Hop yield (fresh weight) per plant [kg]	Hop yield (fresh weight) per hectare [kg]	Hop yield (10 % humidity) per hectare [kg]
80 %	0.228	3.473	7717.75	1930.42
100 %	0.226	3.563	7917.73	1967.50

Table 4. Hop yield components as a function of the irrigation treatment.

Irrigation treatment	Alpha acids [%]	Beta acids [%]	Cohumulone [%]	Colupulone [%]	Alpha/Beta ratio
80 %	13.50	4.30	21.17	47.07	3.17
100 %	13.40	4.37	22.33	48.53	3.07

Discussion

Using data from the hop yield per plant in fresh weight, the dry/fresh matter ratio, plant density and commercialization humidity (10 %), it is possible to forecast the actual yield per hectare under the two irrigation strategies employed in the current study. Yield for the 80 % and 100 % irrigation treatments was 1,930.42 kg ha^{-1} and 1,967.50 kg ha^{-1}, respectively (Table 3). These values are lower than those expected for this region and hop variety according to MAGADÁN et al. (2011), namely, they are lower than 2,200 kg ha^{-1}, although they are within the interval between 1,900 kg ha^{-1} and 2,500 kg ha^{-1} expected by the a private hop enterprise in the USA (HOPSTEINER 2018).

Overall, considering the significant difference between the two irrigation strategies studied in water applied and soil water content, and the fact that this was not reflected on hop yield, requires further explanations. Since a control of the irrigation with soil sensors (NAKAWUKA et al. 2017) or by modelling approaches (FANDIÑO et al. 2015) as in other regions of the world, as well as the need for an update in the calibration of the irrigation system, our results might indicate that the irrigation at 100 % was not completely optimized. In fact, it was verified that irrigation to the 80 % of that recommended was able to fully satisfy hop water requirements. It must also be noted that this study was carried out for the year 2018 only, and the rainfall recorded this year was 35 %, approximately, higher than in a standard year. Moreover, rainfall distribution over the year favored hop growing, with a high rainfall amount in June (82.9 mm in 2018 against 45.9 mm in a standard year) that guaranteed a sufficient soil water storage for hop development and that was not considered when planning irrigation.

In this study, irrigation to 80 % (deficit) and 100 % (common practice) corresponded to 1,848 and 2,310 m^3 ha^{-1}, within the theoretical interval considered adequate 1,200 to 2,500 m^3 ha^{-1} for drip irrigation in hopyards. Consequently, despite the significant effect between irrigation treatments on the water applied, plants did not show negative responses in terms of phenological development, yield or hop cone quality. This information is useful for hop growers since it allows for optimizing irrigation, saving water and money, and supporting a better planning of irrigation in the future.

Acknowledgements

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Satellital multispectral images for the management of hop plantations

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Abstract

Through the use of the satellite images of Landsat-7, Landsat-8 and Sentinel 2, it is intended to study if the characterization and management of hop cultivation is possible in two areas in Spain (Carrizo de la Ribera in the province of León and Abegondo in the province of A Coruña). In order to create a model for the proper management of the crop in the coming years as it has been done in other crops.

Key words. Remote sensing, satellite images, Vegetation Index, hop

Introduction

Hops are a crop that, after an important setback in recent decades, is experiencing a new and incipient development in Spain linked to the beer sector and, in part, to new trends in the development and consumption of craft beers. All this leads to an increase in the cultivated area of hops to take advantage of the growing demand (CANCELA et al. 2017).

This requires, among others, an efficient use of production factors and an adequate management of them. Satellite images and Remote sensing have a lot to offer in this regard (CASTERAD et al. 2010).

The images acquired at different stages of growth of a crop provide clues about the optimum not always possible during the season. The performance patterns and management zones identified from hyperspectral and multispectral images can be very useful for management before, during and after the growing season (LOSADA 2019; LOSADA et al. 2019).

Material and methods

The study was carried out in hop plantations (cv. Nugget) located in Carrizo de la Ribera (León, Spain) and in Abegondo (A Coruña, Spain).

Data acquisition

For the present study, multispectral satellite images corresponding to Landsat-7, Landsat-8 and Sentinel 2 were used. A total of 211 images were downloaded, 115 for Carrizo de la Ribera and 96 for Abegondo, although only 87 and 69 have been used respectively. The seasons studied were from May to September 2008 to 2018 in Carrizo de la Ribera and from 2012 to 2018 in Abegondo.

Data evaluation

Vegetation index (VI): Using the different wavelengths of each of the images, the following indexes were calculated NDVI, NDRE, SAVI, PCDI that are related to the vigor of the plant, SIPI with the sanitary status of the plant and NDWI and MSI with the content in water and water stress of the plant (PANDA et al. 2010).

Statistical analyses

The results were analyzed by Pearson correlation using VI and the agro-climatic parameters of the hop in SPSS to test for significant correlation ($p < 0.05$).

Results

Multitemporal analysis of vegetation index

The first analysis of the obtained data was simply qualitative from the representation of the polynomial lines of the calculated indexes. The results show that the polynomial lines describe an upward and then slightly downward curve coinciding with the growth stages of the crop. The smallest values are found during the period of development and growth of the main stems (May) while the largest ones, during the peak period of the plant (August). During spring, fodder turnip is sown in Abegondo, explaining the increase in the value of the indexes at those dates.

If we compare the three satellites with each other, it can be seen that the values of Sentinel 2 are higher than those obtained with Landsat-8 and Landsat-7.

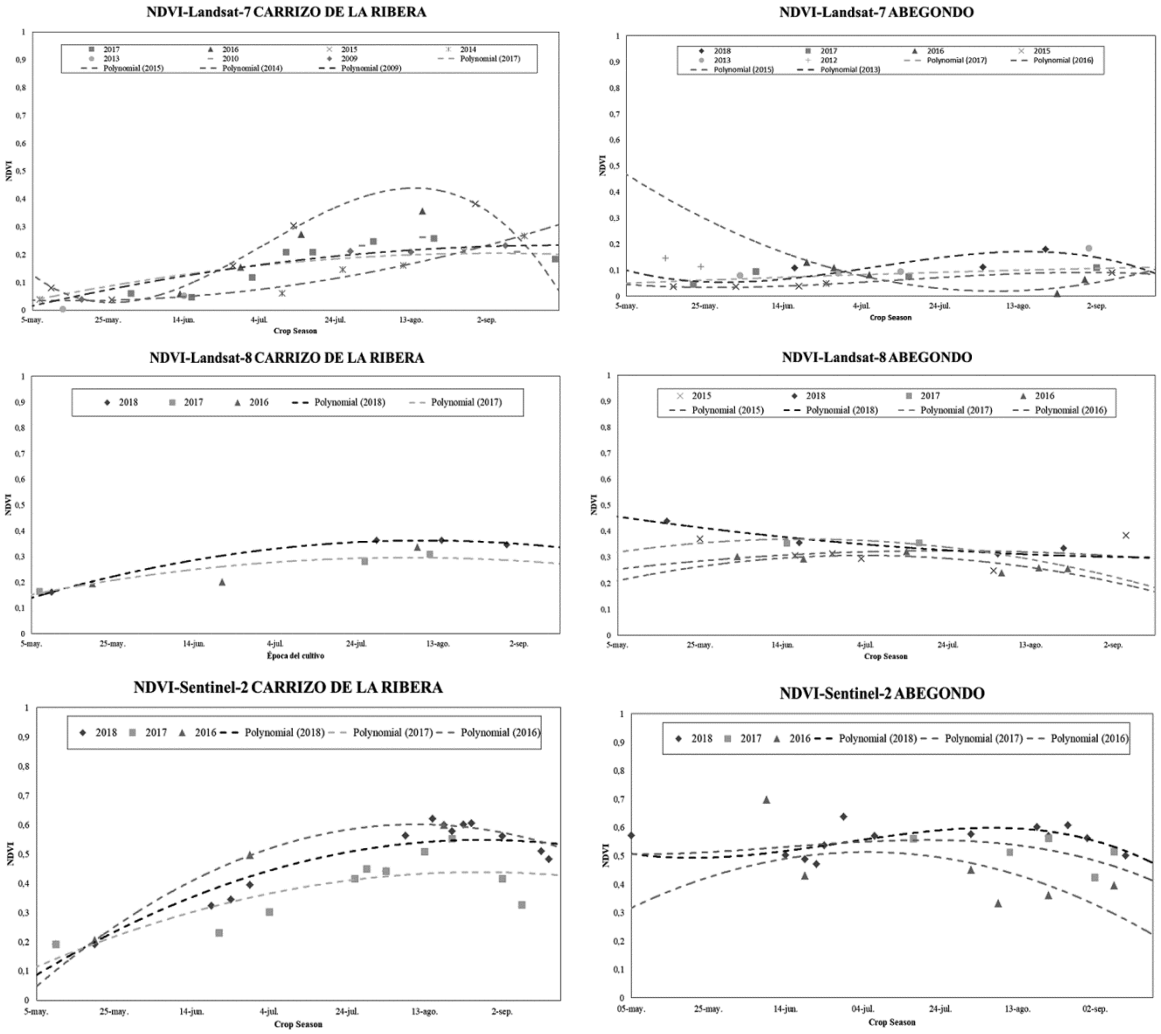

Figure 1. Concentrations temporal evolution of the NDVI Index for each Landsat-7, Landsat-8 and Sentinel-2 satellite for each study area. Polynomial lines are represented only in the years in which there were a greater number of 3 images to be more representative.

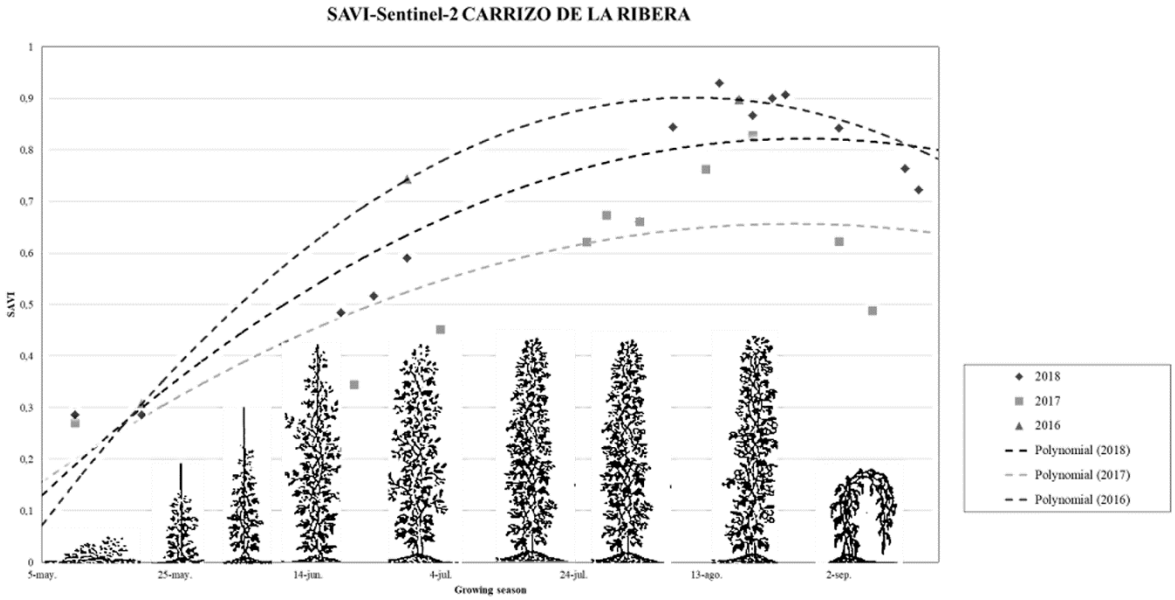

Figure 2. Relationship between spectral data and agronomic variables

If we compare the production with the SAVI of any of the satellites, it follows that the SAVI is related to production. If we observe any polynomial line it is affirmed that the polynomial line of the SAVI with greater vigor is above the rest and therefore has higher production. Coincidentally, the same happens for the opposite case, the year with the lowest production has a polynomial line below the rest, registering less vigorous.

Statistical analysis

Satelite	Pearson Correlation´s	
Sen	NDRE	SIPI (-0,875*)
L8	MSI Max	NDVI (-0,891*), SAVI (-0,894*), PCDI (-0,916*)
L8	Hours sun	SIPI (0,709*) y SIPI Max (0,730*)
L7	Production	NDVI Max (0,738*), SAVI Max (0,742*) Y PCDI Max (0,726*)
L7	Monthly precip. period cultivation	NDWI7 (-0,681*), NDWI7 Max (-0,686*), NDWI6 (-0,739*) NDWI6 Max (-0,701*) MSI (-0,726*) y MSI Max (-0,692*)
Significant correlation * 0,05		

As a result of the statistical analysis, it is determined that the NDVI, SAVI and PCDI are related to each other for Sentinel 2 as well as the NDVI, SAVI and PCDI of Landsat-8. These in turn have an inverse correlation with the Landsat-8 MSI. This means that the lower the vigor, the greater the stress. There is also an inverse correlation of the SIPI index with the NDWI (6) and NDWI (7) of Landsat-8. These data suggest that if the plant is stressed its cellular structure is affected.

And the SIPI index max. with the sun hours of the crop period and inversely proportional to the NDVI, SAVI and PCDI. In the same line it is observed that both indexes, SIPI average and max., are related to the frosts of February and the frosts of May.

However, the indexes derived from the Landsat-7 data: NDWI (6) has a negative relationship with the monthly precipitation of the period, and with the January freezes. It is also found that there is a positive relationship between SIPI and the hours of sunlight of the crop.

Discussion

The results obtained from the research carried out corroborate to a certain extent that the spectral data, transformed into vegetation indexes, have a great potential to be used in prediction models of Hop plantations.

The Vegetation Indexes analyzed NDVI, SAVI and PCDI have a correlation among each other, in the three satellites. In addition these allow estimating the vigor of the hops so they are useful to predict production. The MSI and NDWI indexes can be useful for detecting water content and water stress and, therefore, of great help for irrigation management in the crop.

Of the three satellites studied, Sentinel-2 is the one that presents the highest values of vigor, this could be due to the size of the pixel, being able to better characterize the plot.

Acknowledgement

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V: Entomology

Establishment of predatory mites on undersown crops in hop cultivation

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Abstract

The two-spotted spider mite *Tetranychus urticae* is one of the two major pests in hop cultivation. In organic hops there is up to date no effective way of controlling spider mites. In vineyards or orchards established populations of predatory mites solve this problem. However, in a hop garden the entire plant biomass is removed from the field at harvest, and no habitat remains for predatory mites to overwinter in the field. Therefore, we test three different undersown crops in the driving lanes as hibernation quarters for beneficials: Festuca arundinacea as well as a grassland mixture of legumes and grasses provide not only habitat but also grass pollen as food for predatory mites. The third variant was strawberries as ligneous plants in the lanes. The focus of the project is the native predatory mite Typhlodromus pyri, a well-established species in vineyards, which can be transferred to hop gardens by grape wine cuttings. We also tested purchasable predatory mites: In the first year of the project we used a mix of Phytoseiulus persimilis and Neoseiulus californicus as well as Amblyseius andersoni. For those allochthonous predatory mites we also tested different ways of dispersal in the hop garden: On bean leaves, on vermiculite dispersed with mini air bug or strewn by hand, and in sachets at different stages of development.

Key words. two-spotted spider mite, predatory mite, hops, undersown crops

Introduction

The Two-spotted spider mite *Tetranychus urticae* is one of the two major pests in hop cultivation. Conventional growers use acaricides to control spider mites, often in a preventive manner, whereas there is up to date no effective way of controlling spider mites in organic hops. In vineyards or orchards established populations of predatory mites, in Central Europe most notably the autochthonous predatory mite, *Typhlodromus pyri*, solve this problem. Successful control of *T. urticae* in hops by *T. pyri* has already been reported by VOSTŘEL (2003) and ENGELHARD & WEIHRAUCH (2005). Main object of this project is to establish this native species in hop gardens for effective natural control of spider mites.

However, other than in vineyards or orchards, in a hop garden the entire plant biomass is removed from the field at harvest, and no habitat remains for predatory mites to overwinter in the field. Therefore, we tested three different undersown crops in the driving lanes as hibernation quarters for beneficials: Tall fescue *Festuca arundinacea* already showed promising results in a previous project and provides not only habitat but also grass pollen as food for predatory mites in spring. Second, a grassland mixture was sown as food source for the mites and to create more attractive habitat for beneficials. ENGEL (1991) showed that Poaceae like *Alopecurus pratensis* or *Poa pratensis*, which are part of the grassland mixture, can allow high reproduction rates and stable populations for *T. pyri*. Legumes are popular with organic farmers due to the biological nitrogen fixation. The third variant were strawberries as ligneous plants in the lanes, providing comparable hibernation quarters to vineyards or orchards without hampering the farmers regular works in the hop garden.

In addition we test the application of purchasable allochthone predatory mites two control the two-spotted spider mite in hops. A mixture of *Phytoseiulus persimilis* und *Neoseiulus californicus* showed promising results in first test in the field in 2007 (WEIHRAUCH 2008) as well as in the previous predatory mite project by JEREB & WEIHRAUCH (2016, 2017). Now the objective is finding the best way and time to release those predatory mites in the hop yard.

Material and methods

This project runs on five hop yards, two conventionally und three ecologically farmed. The undersown crops *Festuca arundinacea* and a grassland mixture of six legumes and eight grasses (e.g., *Alopecurus pratensis, Poa pratensis, Festuca pratensis*) are sawn in the driving lanes, in addition in one of the hop gardens strawberries are planted in the driving lanes of one variant.

The focus of the project is the native predatory mite *T. pyri*, a well-established species in vineyards. Therefor we got grapevine cuttings in May during pruning of vineyards with high numbers of *T. pyri* from the Bavarian State Institute for Viticulture and Horticulture. These grapevine branches were cut into small pieces and dispersed in experimental hop gardens.

We also tested purchasable predatory mites. In the first year of the project we used a mix of *Phytoseiulus persimilis* and *Neoseiulus californicus* as well as *Amblyseius andersoni*. For those allochthonous predatory mites we also tested different ways of dispersal in the hop garden: On bean leaves, on vermiculite dispersed with mini air bug or by hand, and in sachets at different stages of development.

During the growing season we do ratings in each experimental hop yard every two weeks to monitor numbers of *T. urticae* and predatory mites as well as their eggs. We also do experimental harvest.

Figure 1. Grapevine cuttings transferred to the experimental hop garden

Figure 2. Different variants of dispersal of allochthonous predatory mites; on bean leaves, in sachets and on vermiculite dispersed with mini air bug

Results

In 2018 we achieved only results regarding the different predatory mite species. We were not able to interpret the three undersown crops in the lanes as summer was unusually dry and hot, why the different grasses didn't grow well. Therefore, there was nearly no habitat for native predatory mites to overwinter, why *T. pyri* has to be released again in spring 2019.

In two of the experimental hop gardens the number of spider mites increased only at the end of growing season, so we were not able to see differences between the variants. In the other hop gardens we could see at least a tendency or even significant differences between the predatory mite variants or between the predatory mites and the untreated control. So far, the predatory mites mix on bean leaves yielded best results and seemingly was most user-friendly for the growers. *T. pyri* also performed quite well in one experimental field until heat and drought affected the predatory mites. However, we realized increasing numbers of predatory mites over the growing season. Due to the high mobility numbers of predatory mites are only hints but at least the number of eggs shows the possibility to establish populations of predatory mites in hop gardens.

Table 1. Sum of predatory mites/ predatory mite eggs over all allotments during rating date 1 to 6 in each experimental hop garden

	1	2	3	4	5	6
Benzendorf	2/5	4/2	6/9	0/16	6/27	2/33
Laipersdorf	2/1	4/17	4/11	3/16	6/31	1/60
Oberulrain	66/66	166/149	278/366	798/299	74/19	9/6
Starzhausen	29/26	37/48	91/178	97/93	382/156	745/60
Ursbach	0/24	1/20	0/55	1/39	0/46	16/124

Discussion

After the first year of the project we can exclude on variant of dispersal of commercial predatory mites as the results in the mini air bug variant were significant worse than same predatory mite mix on bean leaves in the same hop yard.

Overall the hot and dry summer led to high infestation by *T. urticae* in the Hallertau in 2019 (EURINGER 2019). For this efficacy of the use of predatory mites was satisfactory in the first year of this field experiment. Especially in the end of the growing season numbers of spider mites got to high and predatory mites were hardly able to control the pest under these climatic conditions. Partial very high infestation in the hop yards is not an evidence for insufficient efficacy of predatory mites but is probably based on differences in ground and for this different micro climate.

Outlook

In 2019 we compare grapevine cuttings from pruning in May with branches of winter pruning. Undersown crops shall be renewed or completed.

Acknowledgement

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Trapview AURA – a light trap with an automated system for the monitoring of European corn borer *Ostrinia nubilalis* in hop gardens

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Abstract

In Slovenia, European corn borer (ECB) *Ostrinia nubilalis* has long been known as a corn and hop pest and has recently also caused significant economic damage to vegetable crops and ornamental plants (RAK CIZEJ et al. 2009). It is a serious pest of hop in most parts of the Savinja valley, causing loss of yield and affecting the quality of the hop. In the last 10 years, the presence of *O. nubilalis* increased significantly and an increase of economic damage to host crops, especially hop and corn, was observed likewise (RAK CIZEJ & TREMATERRA 2017). Reasons for the increases are farmers having a lot of corn fields, the reduced use of contact insecticides, climatic conditions (temperature, rainfall, relative humidity, wind) and the absence of phytosanitary measures (RAK CIZEJ et al. 2012).

Adults of *O. nubilalis* are active at night and a light trap is reliable equipment for monitoring both females and males in order to gather necessary data for ECB management under an IPM strategy. The use of a light trap is limited in most hop fields by the supply of electricity to run the trap. For that reason we were looking for many years for an opportunity to exchange classical light traps with a 160 W Osram-Hwl mercury bulb and chemical chloroform to another system. Spectral characteristics of light needed are around 350 and 400 nm, with most moths being caught at 362 nm (RAK CIZEJ et al. 2014).

In this regard, during the present preliminary study we analysed the suitability of a classical light trap compared to an automated Trapview AURA light trap equipped with LED lights for the monitoring of *O. nubilalis* moth flights in Slovenian hop gardens in year 2018. Trapview AURA is an experiment focused on the implementation of an automated insect pest monitoring system using UV polarized light to attract ECB. Trapview AURA combines automated devices/sensors in the field for insect collecting. The automated traps are equipped with a solar panel and battery, thus giving it complete energy independence, while several high resolution cameras take pictures of catches in the traps and transfer the images, GPS data and other data collected by advanced electronics to the Trapview AURA cloud via mobile network. In the cloud Artificial Intelligence algorithms will be developed for detecting ECB and processing ECB related data.

Another objective of the Trapview AURA experiment is reducing the need for in-field maintenance and travel. Therefore, trap prototypes will be equipped with a self-cleaning mechanism, meaning that the users will be able to clean the traps remotely by using the Trapview application. The self-cleaning mechanism is controlled remotely via the main electronics board of the automated trap.

In 2018, we monitored the flight of the second generation of *O. nubilalis* with a classical mercury lamp light trap and compared that to an automated Trapview AURA light trap equipped with LED lights. ECB was monitored on the wing from end of July to middle of September at the edges of two hop gardens (Žalec and Roje, near Žalec). The dynamics of the ECB flight recorded by the classic light trap was the same at both sites. A difference was however recorded in the abundance of the population of ECB, which was larger at the Roje site. The peak of the second generation of ECB was recorded in the second decade of August. The dynamic of the flight of ECB was similar on the classical light trap compared to Trapview AURA, but only 5-10 % of the population of *O. nubilalis* was caught by the latter trap. During the next years we will continue working to optimise use Trapview AURA in practice.

Key words. European corn borer, *Ostrinia nubilalis*, monitoring, hop gardens, light trap, automated trap

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Hop-flea beetle revisited: In search for attractants

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Abstract

In the course of a three-year research project concerning the control of Hop flea beetle *Psylliodes attenuatus* in organic hops (WEIHRAUCH et al. 2017; MUMM et al. 2017) it soon became clear that meaningful flea beetle control can only be achieved during two limited points in time. The first option is in early spring, when hibernating adults attack the young shoots of hops in late March or April and therefore concentrate on the small hop plants of early growth stage. The second point is during the soil passage, when *P. attenuatus* adults have vanished in May-June after egg-laying and only the larvae are present underground, concentrated chiefly in the hop rows. With photoeclectors set up for several weeks in an organic field in Hersbruck during the summers of 2017 and 2018 we recorded emergence of the new generation of beetles from the hilled rows during seven weeks (weekly counts, early July – late August). Beetles emerged in huge numbers that reached 2,500 to 4,500 individuals per square meter altogether. A conservative projection of catches from 12 eclectors reaches a 'production' of 6 million beetles per hectare, or 3,000 beetles per hop plant.

Under consideration of these facts, we conclude that meaningful control measures should either be executed in early spring against adult beetles or in June against *P. attenuatus* larvae. Control of adults was investigated by various attractants, of which only linalool had a certain, moderate effect as a lure. Control of larvae was investigated by the use of an entomopathogenic fungus, *Metarhizium brunneum*, applied in various formulations. The tests regarding larval control were hitherto not successful, but we found that larvae were obviously attracted by CO_2 released from the *M. brunneum* formulations. However, further studies are needed to verify our preliminary results.

Key words. *Psylliodes attenuatus*, Alticinae, pest control, CO2

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Online identification tool for harmful organisms in hop growing: http://pflanzenschutz.oekolandbau.de

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Abstract

For organic farmers, especially on newly converted or diverse farms, assessing information concerning pest and disease identification and organic regulation options requires large efforts. A concept for a user-friendly diagnostic aid for relevant pest organisms in hop growing and other crops (storage pests, pests diseases and weeds in arable and fruit crops) in organic farming is presented, which is based on a flexible filter structure. Recent scientific results and market availability of regulation options in organic farming are being reviewed and included.

Key words. hop, plant protection, organic farming, knowledge transfer, diagnosis

Introduction

The almost 20,000 organic farms in Germany, in particular the 400 to 800 farms that are under conversion annually, have a high and special need for information on organic plant protection. Existing online tools for determining pests and diseases are either linked to advertising for plant protection products or only cover a limited spectrum of pest and diseases. An ongoing project is developing an identification aid for organic farming and compiling organic regulation options on the basis of current research results and available plant protection products. The current project has so far been published on the portal http://pflanzenschutz.oekolandbau.de for the topics of storage protection, arable farming, weed regulation, fruit and wine growing. The hop growing section is being developed, followed by vegetable and medicinal and spice plant cultivation.

Material and methods

Current research results on the regulation possibilities of the respective harmful organisms have been researched in international research databases (e.g., JKI 2015; CABI 2018; Organic eprints 2018) and among the research projects funded within the framework of the German Ministry of Agriculture. It was supplemented with information on beneficial organisms, plant strengthening products, basic substances and plant protection products. Harmful organisms were taken from insect breeding at the Julius Kühn-Institute as well as trapped on farms and depicted in high-quality macro photographs. In cooperation with the Hop Research Center Hüll (Germany), the harmful organisms in the hop gardens were photographed between 2018 and 2019. Pictures of different development stages and symptoms were combined into identification plates. Historical drawings and pictures were also viewed and checked for usability. The description of alternative regulatory measures specifically for organic farming was also carried out in cooperation with the Hop Research Center Hüll.

Results and discussion

The determination aid is designed as a filterable, image-based complete list of harmful organisms that allows any combination of selection options. Predefined decision paths and microscopic features are avoided.

In addition to characteristics of the organisms, it is also possible to filter for larval characteristics, infested products (storage protection), site conditions (weeds), or infested plant parts and harmful symptoms and then determine them with high-quality images.

For crops with a low organic cultivation volume such as rape, sugar beet, barley and maize, eco-specific information on plant protection is necessary in order to overcome existing cultivation obstacles. On the other hand, the inclusion of rarer crops, such as sunflowers, oil flax, oats and hemp, which are particularly cultivated in organic farming, poses a challenge. For hops, more than 10 harmful organisms and their alternative control measures are described; for examples see Figures 1-4. For organic fruit growing, the areas of pome and berry fruit were expanded, and the special significance of quince, walnut, elderberry, sea buckthorn and aronia berry would also have to be taken into account. Beneficial insects available on the market are also described in linked profiles. Current research enables more recommendations with regard to control options, e.g., with thermal processes, inert gases and beneficial insects.

Figure 1. Two-spotted spider mite *Tetranychus urticae*

Figure 2. Downy mildew *Pseudoperonospora humuli*

Figure 3. Hop flea beetle *Psylliodes attenuatus*

Figure 4. Rosy rustic moth *Hydraecia micacea*

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VI: Molecular investigations on hops

Some metabolome changes in hop glandular trichomes due to transgenes *Hl*WRKY/*Hl*WDR1, MBW, gene tissue-specific expression and light

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Abstract

In our previous work (MATOUŠEK et al. 2012, 2016; MISHRA et al. 2018) we characterized two principal type of complexes of lupulin-specific transcription factors *Hl*WRKY/*Hl*WDR (WW) (1) and *Hl*Myb/*Hl*bHLH/*Hl*WDR (MBW) (2) that are able to activate metabolome genes identified and cloned previously from hop glandular trichome library. In the present work we prepared single- or double- hop transformants with "oligo cDNA-bearing" plant vectors and analyzed them for gene and metabolome expression in leaf and lupulin tissues. It was found that WW complexes show wide effects on gene expression influencing hop physiology and metabolism (3) and modify phenolics as well as some volatile substances in lupulin. Fine analyses showed that various WW lines modified expression of alpha and beta acids, colupulone, humulone and posthumulone compared to the wildtype. Moreover, WW and expecially MBW complexes modify phenolics expression in hop leaves differently under various blue- and red light regimes. The analysis of lupulin and leaf glandular trichome morphogenesis and metabolome expression, where recently cloned *Hl*Glabra2_1 and *Hl*Mixta1 genes could be involved within specific regulatory network(s) is in progress.

Acknowledgement

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Implication of the CRISPR/Cas9 technique in Czech hop genome editing

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Abstract

The clustered regularly interspaced short palindromic repeats-associated protein 9 (CRISPR/Cas9) system is a powerful tool for editing plant genomes. In this current study, we designed two guide RNAs for targeted mutagenesis of the *Hl*Myb7 transcription factor gene, the repressor of the flavonoid biosynthetic pathway in lupulin glands (MATOUŠEK et al. 2012), and obtained more than 20 transgenic plants via *Agrobacterium*-mediated transformation regenerated from nodal segments of the Saaz hop Osvald's clone 72. We confirmed successful transformation events as well as Cas9 nuclease expression driven by the constitutive ubiquitin4-2 promoter of *Petroselium cripsum*. The heteroduplex analysis indicated heterogenity of the *Hl*Myb7 target region(s) and the preliminar target sequencing confirmed *Hl*Myb7 frameshift in some transgenic plants. More detailed analysis of the target sites is still in progress. After confirmation of the mutation of the target gene, the tissues will be subjected to detailed metabolome analysis. We conclude that the CRISPR/Cas9 system represents a useful tool for gene functional analysis and hop molecular breeding. Nowadays, we developed a strategy to knock out small R3 Myb inhibitors of trichome development to achieve higher production of prenylflavonoids and bitter acids through increasing the density of lupulin glands in hop cones.

Acknowledgement

The work was supported by the Czech Science Foundation project 19-19629S and by the institutional support RVO: 60077344.

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Comparative transcriptomic-based gene expression analysis associated with floral leaf transition to lupulin gland development in hop (*Humulus lupulus* L.)

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Abstract

The female plants of hop (*Humulus lupulus* L.) produce cone-like inflorescences, commonly referred to as “cones” or “hops” that contain a large number of highly metabolically active glandular trichomes (lupulin glands) on the inner side of bracts and bracteoles. During their phased maturation, the lupulin glands synthesize and/or secrete specific secondary metabolites such as essential oils, bitter acids and prenylated flavonoids, which are used in brewing as well as pharmaceutical industry. To gain insight into the transcriptome dynamics and gene regulatory mechanism in the context of secondary metabolite production and key genes and pathways that coordinate the lupulin gland morphogenesis and density, we performed a comprehensive global transcriptome analysis of leaves, lupulin glands and cones devoid of lupulin glands. Analysis of differentially expressed tissue specific genes, combined with gene ontology and Kyoto Encyclopedia of Genes and Genomes pathway analyses, showed that many biological processes were highly diminished in lupulin glands. These included catabolic processes and signalling pathways of hormones and photosynthesis pathways, whereas secondary metabolite biosynthesis including phenylpropanoids, flavonoids and terpenes were highly enriched. Furthermore, our systematic analysis provided comprehensive transcriptomic information regarding trichome development, repressors and activators involved in morphological differentiation and development of lupulin glands.

Acknowledgement

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Strategy for gene-targeted marker development in hop

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Abstract

The global hop market is constantly changing; thus hop breeders do not only have to react on climate change and resistance against pests and pathogens, but also on new market trends. Molecular markers have been successfully used to enhance the selection process in breeding programs of major crops. However, despite some reports on marker development for hop, marker-assisted selection is not widely used in most hop breeding programs. This is likely due to the low number of available markers of high quality to accurately predict specific traits.

The marker development strategy presented here is based on the assumption that genetic variance within the regulatory regions of a given gene is highly correlated with gene function and consequently directly linked to the expression of an associated trait. The starting point of the process is to select a gene-of-interest and to predict the according gene structure; however, only limited hop sequences of varying quality are online available and thus are not all equally useful for gene structure prediction. Moreover, the potential regulatory regions of each gene need to be identified based on the discrimination of repetitive elements and based on the presence of potential transcription factor binding sites. In this review, different targeted sequencing techniques will be discussed in order to give a recommendation for the use in a gene-targeted marker development approach.

VII: Hops and brewing

Barbe Rouge: impact of hopping method on sensorial and analytical properties of beers

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Abstract

Beers were hopped with Barbe Rouge, a hop variety with remarkable aromatic potential, following three hopping methods: late hopping, whirlpool and dry hopping. To study the impact of hopping method, volatile compounds of beers were evaluated using both GC-MS and olfactometry (GC-O), after Stir Bar Sorptive Extraction (SBSE). The aromagrams of the 3 beers were built using Nasal Impact Frequency (NIF) on the GC-O data with 5 to 6 panelists. GC-MS analyses show that Barbe Rouge brings a great diversity of esters to the beers (more than 65 esters have been identified) and that the aromatic profile in late hopping is similar to the one in whirlpool, while dry hopping is distinguished by higher concentrations of volatile compounds. The aromagrams confirm that the red fruit flavors detected in the beers produced with Barbe Rouge are related to the presence of ethyl isobutanoate and ethyl butanoate. These smells of strawberry and red fruits are more frequent with whirlpool and dry hopping. This study highlights the unique aromatic qualities of Barbe Rouge and shows that the combination of hop varieties and hopping methods can be crucial in maximizing the aromatic impact of hops on beer.

Key words. Hops, Volatile compounds, SBSE, GC-MS, GC-O.

Introduction

With yeast, hop provide some of the major key-compounds involved in the beer typicity. This is the reason why, the choice of hop variety has a major influence on beer aromatic profile (KISHIMOTO et al. 2006). Hop derived compounds like sesquiterpenes, monoterpenes or branched-chain fatty acids are known to be odor-active in beer but the influence of the hopping methods on their final concentration are not known. Previous results showed that Barbe Rouge beer contained high concentration of α-humulene (balsamic, wood), β-caryophyllene (clove, black pepper) and linalool (floral) (BRIGNIER et al. 2018). Moreover, Barbe Rouge is characterized by its typical esters composition bringing notes of red fruits such as strawberry and exotic fruits to the beer[2]. In this context, the aim of the present study was to identify the most efficient hopping method to give red fruit aroma to beer 1) by studying the impact of three hopping methods (late hopping, whirlpool and dry hopping) on the volatile compounds of beers using Stir Bar Sorptive Extraction-Thermal Desorption-Gas Chromatography-Mass Spectrometry (SBSE-TD-GC-MS), 2) by building the aromagrams of the 3 beers using Nasal Impact Frequency (NIF) on the GC-O data with 5 to 6 panelists, 3) and by identifying the most odor-active compounds with a focus on red fruit aroma compounds which is the specifity of Barbe Rouge hop variety.

Material and methods

Beers were provided by Cophoudal/Comptoir Agricole (Hochfelden, France). These beers were hopped with Barbe Rouge, following three hopping methods: late hopping, whirlpool and dry hopping.

Extraction of volatile compounds of beers was performed, in triplicate, by Stir Bar Sorptive Extraction and Thermal Desorption (SBSE-TD) (Gerstel, Mülheim an der Ruhr, Germany).

Volatile analyses were performed by GC-MS (Agilent, USA). Volatile compounds were identified by comparing their mass spectra with those from the MS library database (NIST14) after automated peak extraction and the automated deconvolution of mass spectra.

Reconfirmation was performed by comparison of the mass spectrum (MS), retention index (RI) and odor description with personal database (TWIST). The data were subjected to statistical treatment using one-way ANOVA analysis (Minitab16 Statistical Software), with the level of significance set at $p < 0.05$. Clustering analysis was done by Principal Component Analysis (PCA) performed with the package FactominerR and R-software.

The SBSE-TD-GC–Olfactometry (GC-O) analyses were performed by five or six panelists. The aromagrams of the three beers were built using Nasal Impact Frequency (NIF) according the manufacturer software (ODP-Chemstation).

Results and discussion

A total of 208 volatile compounds were identified in the Late Hopping (LH), Whirlpool (W) and Dry Hopping (DH) beers. Among these compounds, 179 compounds were significantly different between the three beers. For this study, the total data set was summarized using the sum of the compounds by chemical family.

A Principal Component Analysis (PCA) was performed on the chemical family of the volatile compounds to compare volatile compound profiles between the three beers (Figure 1). The first principal components (PC1) accounted for 67.8 % of the variance in the data set and showed a high positive correlation to esters, terpenes, ketones, alcohols, furans and phenols in the DH. PC2 accounted for 32.2 % of the variance in the data set and explained differences between LH beer (thiols and aldehydes) and W beer (acids). Statistical analyses confirmed that LH beer and W beer were more similar (only 82/179 are significantly different between these two beers) than LH and DH beers (166/179) and W and DH beers (156/179).

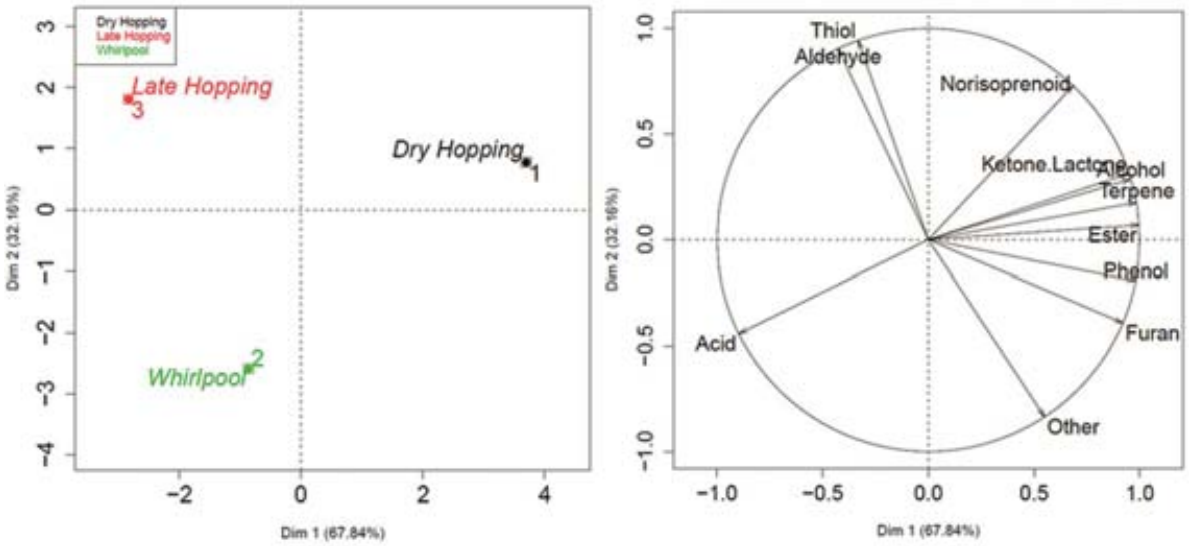

Figure 1. PCA of volatile compounds families of Late Hopping, Whirlpool and Dry Hopping beers.

Olfactometry analyses were investigated to study the impact of hopping method on odor-active compounds and to identify compounds responsible of red fruits aroma of Barbe Rouge. Figure 2 depicts the aromagrams of the three beers, More than 70 odorants were detected but only odours detected by at least 50 % of the panelists are shown (NIF >50 %). A total of 19, 27 and 38 odorants respectively in LH, W and DH beers were detected and identified thanks to MS.

Table 1. Odorant compounds identified in Late Hopping, Whirlpool and Dry Hopping beers

RT (min)	Odor description	NIF [%]			Identification (based on odor, MS and RI)
		LH (n=5)	W (n=5)	DH (n=6)	
7,48	**Red fruit, strawberry**		61,4	55,0	Ethyl isobutanoate (fruit, sweet, strawberry)
8,19	Chemical, solvent			50,0	Isobutyl acetate (fruit, floral, banana)
8,62	**Red fruit, strawberry**		60,0	54,4	Ethyl butanoate (fruit)
8,91	**Strawberry**		75,8	53,8	Ethyl 2-methyl butanoate (fruit, apple)
9,16	Fruit		60,0	50,0	Ethyl isopentanoate ,butyl acetate (fruit, floral)
9,77	Burnt, plastic		60,0		Unknown
9,93	Brewery, vegetal*	60,0	60,0	56,5	geranic oxide* (herbal, rosemary), Hexanal* (green, grassy)
10,12	Banana*	60,0	71,4	51,2	Isoamyl acetate* (banana, fruit, sweet)
10,27	Old hop, underwood			50,0	Unknown
10,51	Green, grass, hay*	60,0	60,0	50,0	b-myrcene* (spicy)
11,37	**Fruit, strawberry**	63,3		55,6	3-Methylbutyl-2-methylpropanoate (sweet, fruit)
11,54	Unpleasant, old hop	66,7		60,8	Isoamyl alcool (alcoholic, malty, fusel)
12,16	Fruit	66,7	60,0		Ethyl hexanoate (fruit)
13,57	Almond, roasted*	66,7	66,0	50,7	Acetol* (nutty), 3-methyl-2-butenol* (herbaceous)
13,82	Unpleasant, old hop			50,0	Unknown
14,84	Green, grass, vegetal*	66,7	67,9	57,0	trans-3-Hexenol* (green, moss)
15,18	Floral, rose, mushroom	66,7		54,2	Ethyl octanoate (fruit, floral)
15,79	Earthy, forest, hop		68,6	59,9	Unknown
16,20	Mushroom, mosse		60,0	52,0	Furfural (alkane, sweet, floral)
17,01	Unpleasant, plastic		60,0	50,0	Propanoic acid (pungent, rancid, soy)
17,29	Floral, roasted	63,3		51,4	Linalool (floral, citrus, fruit)
17,49	Unpleasant, white flower	60,0			1-Octanol (green, floral, rose)
18,26	Soap, rancid		60,0	50,0	Butanoic acid (rancid, cheesy)
18,98	Old hop, cheese*	60,0	66,1	55,6	3-methyl butanoic acid* (rancid, cheesy)
19,48	Hot milk, vanilla			50,0	Unknown
20,31	Floral, soap, wood		60,0		Citronellol (rose, green)
20,90	Tabacco, soap, floral, lemon, wax			50,0	isogeraniol (rose), Nerol (floral), Ethyl-2-phenylacetate (honey, fruit)
21,31	old garage, soap, floral, lemon*	60,0	64,6	50,0	Hexanoic acid* (cheesy, rancid), 2-phenylethylacetate* (floral, rose), Geraniol* (rose, floral) (coelution ?)
21,49	Fruit, applesauce, rose*	60,0	74,7	50,0	b-Damascenone* (floral, tobacco)
21,74	Floral, roasted	60,0		50,0	Benzyl alcohol (floral, fruit), guaiacol (burnt, smoky)
22,13	Burnt, floral			60,3	Ethyl dihydrocinnamate (floral, fruit, sweet)
22,32	Floral, rose, white flower		60,0	50,0	2-Phenylethanol (rose, floral, honey)
22,73	Rose, fruity			50,0	a-calacorene (wood)
23,23	Roasted, smoky		60,0		Phenol (medicinal, smoky)
23,58	Roasted, burnt, candy, red fruit*	60,0		53,3	4-Ethylguaiacol* (spicy, clove, smoky), furaneol*(caramel, burnt)
23,71	Dust, powdery, burnt		67,1	55,4	Ethyl tetradecanoate (wax)
24,00	Floral, coconut		60,0	57,9	g-Nonalactone (coconut, peach)
24,14	urine		60,9	50,0	p-Cresol (phenolic, cattle, medicinal)
24,29	vanilla, honey, caramel		71,3	54,5	1,10-di-epi- Cubenol
24,44	Caramel, leather			50,0	Unknown
24,74	Rubber, burnt		60,0		Nonanoic acid (fatty, rancid)
25,14	Floral, fruit	60,0			Ethyl cinnamate (honey, floral)
25,44	**Fruit, red fruit, marshmallow**	60,0		50,0	2-Phenethyl hexanoate (fruit)
25,78	Roasted, spicy			51,7	4-vinyl guaiacol (spicy, clove, smoky)
26,42	Unpleasant, motor oil*	60,0	65,7	64,9	Decanoic acid* (rancid, soapy)

* NIF>50 % in the three beers

GC-O analyses allowed to describe several floral, soap-like aromas related to terpenes like linalool and citronellol. Fruit aromas, in particular red fruits and strawberry-like aromas, related to esters, were also detected and tentatively identified as Ethyl isobutanoate (7.45 min), Ethyl butanoate (8.61 min), Ethyl 2-methylbutanoate (8.90 min), 3-Methylbutyl-2-methylpropanoate (11.37 min) and 2-Phenethyl hexanoate (25.44 min) (Table 1 and Figure 2). The first three compounds have been detected in W and DH beers and the last two compounds in LH and DH beers (NIF > 50 %). As already reported, ethyl butanoate and ethyl 2-methylbutanoate are key-aroma of strawberry (DU et al. 2011; SCHWIETERMAN et al. 2014), highlighting that these compounds are involved in the typicity of Barbe Rouge.

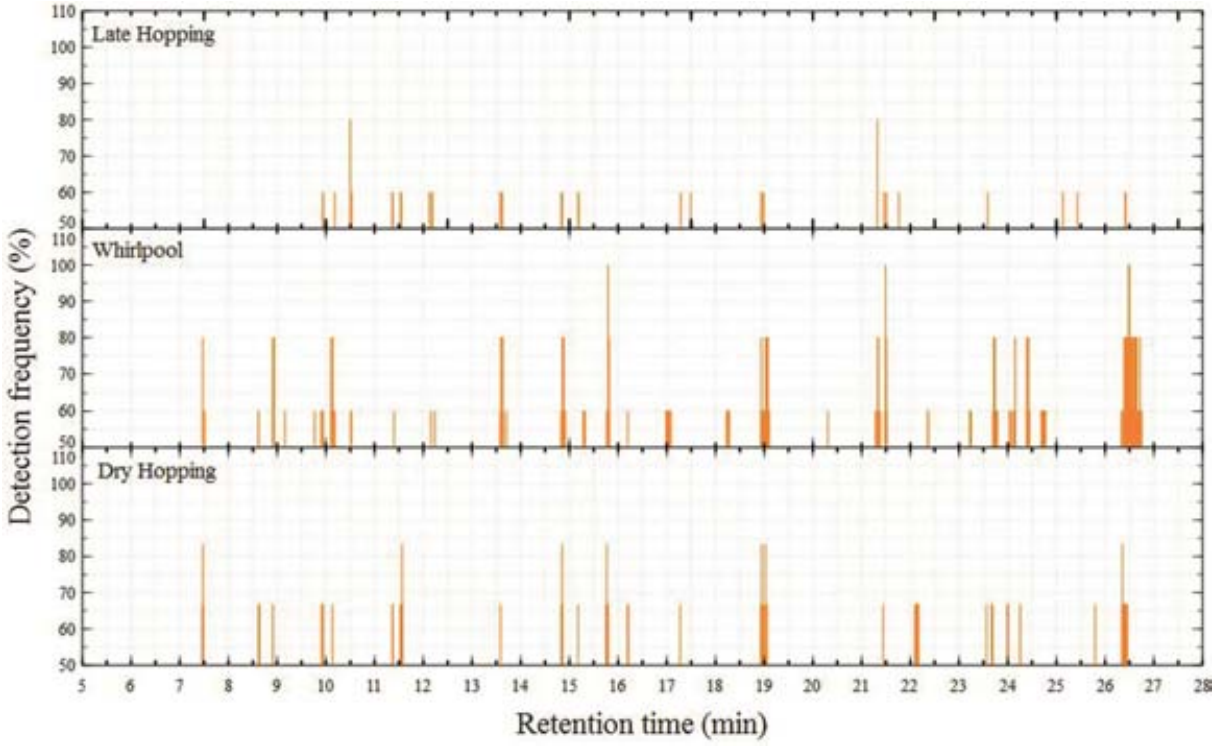

Figure 2. Aromagrams of Late Hopping, Whirlpool and Dry Hopping beers.

Conclusion

In this study, the volatile compounds and odor-active compounds of LH, W and DH beers hopped with Barbe Rouge were compared by GC-MS and GC-O. The three hopping methods allowed to obtain three different beers with typical aromas of Barbe Rouge. GC-MS analyses showed quite similar volatile profiles between LH and W beers. However olfactometry highlighted W and DH beers contains more odor-active compounds than LH. Among these components, five compounds linked to red fruits, strawberry-like aromas were identified as Ethyl isobutanoate, Ethyl butanoate, Ethyl 2-methylbutanoate, 3-Methylbutyl-2-methylpropanoate and 2-Phenethyl hexanoate. In our conditions DH and W are the most efficient hopping methods to improve red fruit aroma in beer using Barbe Rouge variety in comparison to LH.

Acknowledgement

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Impact of Climate change on hops – exemplified by the Hallertau growing region

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Climate change is no longer denied. In European hop production areas we are also experiencing hotter and dryer summers and more extreme weather conditions in form of storms and heavy rainfall. In the Hallertau the average temperature in the most relevant months for hop growth (June-August) increased in the last 10 years in comparison to the period 1961 to 1990 (generally accepted as period before obvious climate change) by 2.0 °C. In order to understand the influence of the weather conditions on hop growing and the impact on different hop varieties we propose two options.

Method 1 − Comparison of two crops with very different weather: This was the case in crop 2015 (dry and hot) and 2016 (moderate). The comparison of precipitation, average temperature and hot days (>30°C) in June to August 2015 *vs* 2016 is 184 *vs* 334 mm, 19.5 *vs* 17.7 °C and 36 *vs* 7 hot days. We calculated furthermore a ratio of the yield of alpha-acids in kg per hectare of each variety as following:

$$Factor = \frac{\text{yield alpha kg/ha in 2015}}{\text{yield alpha kg/ha in 2016}}$$

The following tables show values for traditional aroma varieties, bitter hops and recent new aroma hops, also called flavor hops.

Table 1. Ratio of alpha-acid yield between harvest years 2015 and 2016 in the Hallertau for traditional aroma varieties, bitter hops and recent new aroma (flavour) hops

Aroma	Spalter	28%
	Perle	34%
	Nothern Brewer	36%
	Spalter Select	39%
	Hallertauer mfr.	40%
	Saphir	42%
	Hall. Tradition	45%
	Opal*	45%
	Tettnang Tettnanger	46%
	Hersbrucker	56%
	Smaragd*	66%
	Average	43%

Bitter	Taurus	52%
	Nugget	56%
	Herkules*	64%
	Magnum	71%
	Polaris*	77%
	Average	64%

Flavor	Cascade	52%
	Hall. Blanc*	56%
	Hüll Melon*	65%
	Mandarina Bavaria*	71%
	Average	61%

It is obvious that all varieties, but especially the traditional varieties, react sensitively to weather conditions. This sensitivity or tolerance fluctuates in a wide range between 28 % for Spalter (very climate sensitive) to 77 % for Polaris (more climate tolerant). The advantage of this method besides getting a feeling for climate tolerance of all varieties is the possibility to describe also new varieties. The problem and disadvantage is the lack of a long-term observation. This may include the risk to draw wrong interpretations.

Background for both methods is the calculation of the yield of alpha-acids per hectare. The publicly available figures are the total acreage, the harvest yield in tons and the average of alpha-acids in % (w/w). What has to be considered regarding the calculation of the alpha yield per hectare is the fact that hop planted on new acreage produces lower yield in the first year. There are existing empiric factors in form of the ratio new vs. old acreage for each variety. They fluctuate for instance between 0 (older varieties), 0.25 (Hüll Melon, Smaragd, Opal), 0.3 (Mandarina Bavaria, Hallertau Blanc) and 0.45 (Herkules). Total acreage is calculated: Production acreage + new acreage x factor.

Method 2 − long term calculation: Basis is on the one side the same description of a hop crop in form of kg alpha-acids per hectare. Furthermore we developed a climate factor with the precipitation and the average temperature from June to July, as following:

$$\textbf{Climate Factor} = \frac{\sum \textbf{Rainfall June to August [mm]}}{\text{Average } \textbf{Temperature} \text{ June to August [°C]}}$$

We calculated the climate factors from 2002 to 2018 and the alpha-acid yields. In order to compare different varieties the relative yield was calculated:

$$\textbf{alpha−yield } [\%\text{−rel}] = \frac{\text{kg alpha / ha } \textbf{in a specific crop}}{\text{average kg alpha / ha } \textbf{from 2002−2018}}$$

The relative yields are plotted versus the climate factor. Figure 1 shows this correlation for Hallertauer Magnum and Perle.

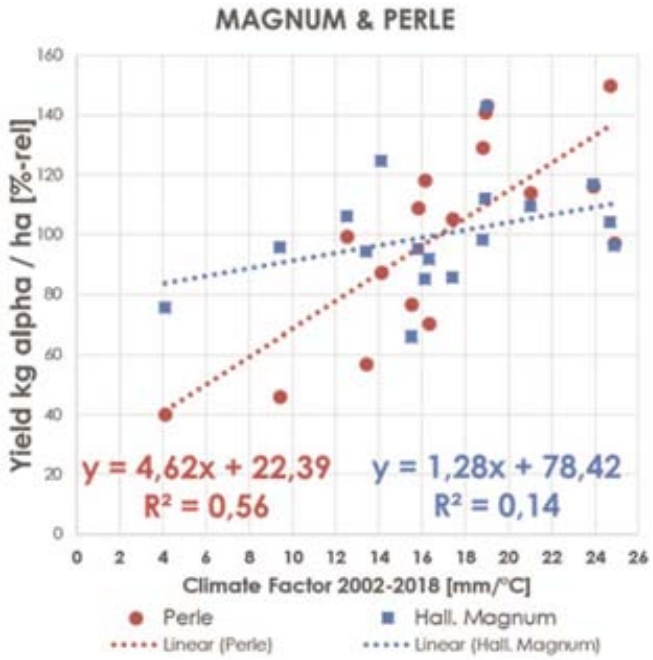

Figure 1. Correlation of relative yield and Climate Factor

Table 2. Slopes of the regression lines of relative yields plotted versus the climate factor (for calculation see text), serving as a good indicator how sensitive a variety reacts on weather. Asterisks (*) indicate new varieties with less data points.

Hall. Magnum	1,3
Herkules*	2,3
Taurus	2,5
Nugget	2,7
Hall. Tradition	3,1
Hersbrucker	3,9
Spalter Select	4,1
Smaragd*	4,2
Hallertauer Mfr.	4,2
Opal*	4,3
Perle	4,6
Saphir*	4,8
Northern Brewer	4,9

While Magnum has a weak correlation with a slope of 1.28 and a not significant correlation factor of $R^2 = 0.14$, Perle has a clear slope of 4.62 with a ** significant R^2 of 0.56. The two varieties behave differently, Magnum is more climate-tolerant and Perle very sensitive. The slope of the regression line serves as a good indicator how sensitive a variety reacts on weather. Table 2 shows the slopes of all investigated varieties.

The climate dependency varies between 1.3 (more tolerant) to 4.9 (very sensitive). The advantage of this method is the long-term observation with more reliable information as in method 1. However, new varieties are not included.

Finally, we correlated the two methods (method 1 plotted *vs* method 2), as shown in Figure 2. The results on climate dependency of German hop varieties in a two-point analysis (2015/2016) and the associated long-term consideration are well together. Considering only two climatically different years is acceptable for a first and early assessment of the climate stability of a new variety.

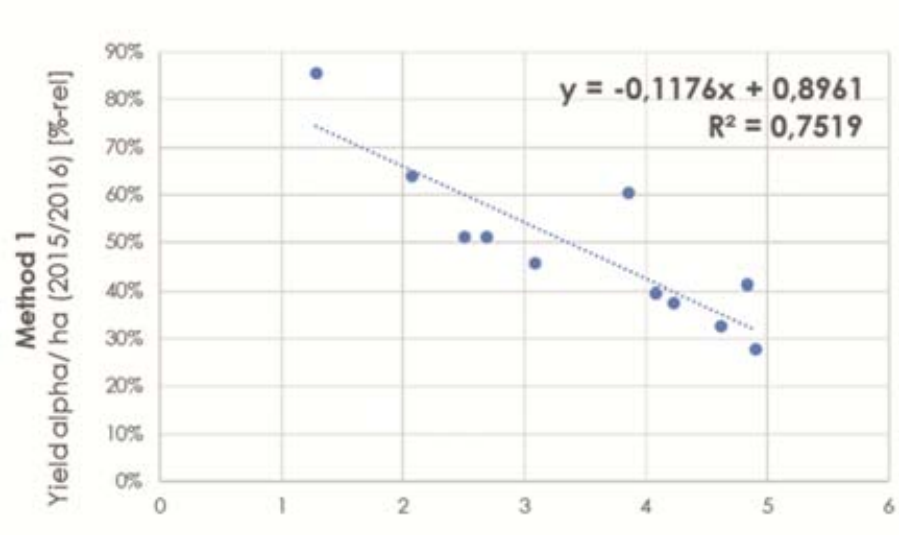

Figure 2. Correlation of method 1 plotted *vs* method 2

Conclusion

Traditional aroma hops are affected stronger regarding their alpha yield by (extreme) weather conditions than bitter and new aroma varieties (flavor hops). A substitution of such varieties is recommended. It is open when, to what extent and with which “better” variety or varieties. Irrigation helps but is only part of the solution. Breeders have to do a big job and may have to simulate different climate conditions in greenhouses. Climate change is a global issue and affects all growing areas.

Comparison of Saaz hops grown in two regions in climatically different years

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Hops have been grown in the Czech Republic and especially in the Žatec (Saaz) area for centuries. Almost 80 % of the Czech hops with their main variety *Saaz* are produced there. Besides among a few ignoramuses global warming is no longer denied. But when it comes to the effects of the climate change the consequences are realised locally. In Central Europe we are experiencing warmer and dryer summers and more frequent extreme weather events (storm, heavy rainfall). Aim of this study was to find out which impact the altered weather conditions can have on hop growing in the Saaz area and whether it is an alternative to grow the variety *Saaz* in another growing area.

The climate change in the area south and north of the Ore Mountains (Erzgebirge) has been subject of a recent study (LFULG 2015). The authors come to the conclusion that there is already an increase in temperature and that there is no change in the amount of precipitation yet. So the available amount of water for the agriculture is declining. Hop is a huge climbing plant and needs much water to grow and to produce its valuable secondary metabolites, particularly alpha-acids and essential oils. If water supply is not sufficient, there is a threat of lower yields (amount of harvested hops per hectare) and lower alpha-acid content in the cones. When looking to the average alpha acid content of *Saaz* hops in the years 1994-2018 there is a significant decline of almost 1 % alpha acid within two decades (MIKYŠKA & JURKOVÁ 2019).

SCHÜLL & FORSTER (2019) examined climate effects using the Hallertau as an example. They developed a so-called “climate factor” (CF), which includes summerly precipitation and the average daily temperatures in the months from June to August according to the following equation:

$$\mathbf{CF} = \frac{\mathbf{Sum\ of\ Precipitation\ (June-August)\ [mm]}}{\mathbf{Av.\ daily\ Temprature\ (June-August)\ [°C]}}$$

The CF was calculated for the years 2002-2018 and showed a good correlation with the relative hop yield value, which is the quotient of the average yield [kg □/ha] in the respective year and the average yield of all years in the observation period. The slope of the linear regression line served as an indicator of how sensitive a variety reacts on weather conditions. Calculating CFs for other German growing areas showed that there were different weather conditions in the same year in different growing regions.

It was of great interest if this model could be transferred to other growing areas e.g. Saaz. Therefore, weather data from the Czech Hydrometeorological Institute (http://portal.chmi.cz) and hop harvest data from the Barth Reports 2011-2018 (https://www.barthhaasgroup.com/en/media-library/downloads) and Bohemia Hop Reports (http://www.bohemiahop.cz/reports) were retrieved and the CF and relative hop yield for the variety Saaz were calculated. Unfortunately weather data were available only for the years since 2011 but the linear regression showed even for this short period a significant relation between the CF and the relative hop yield in the years 2011-2018 (Fig. 1).

The yield of other Czech varieties, namely *Sladek* and *Premiant*, are also highly correlated with the CF. *Agnus* yield shows low weather impact (Fig. 1). The slope as a measure for the climate tolerance of a variety cannot directly be compared with those from Hallertau (Schüll &

Forster 2019) because it has been calculated for a different period, but it clearly demonstrates the variety dependence.

Comparing the relatively good crop 2016 with the problematic crop 2018, almost 38 % less *Saaz* hops were harvested per hectare. *Sladek* (-35 %) and *Premiant* (-42 %) followed this trend. When looking at the alpha acid yield [kg α/ha], the difference to 2016 was even higher. Only *Agnus* (-9 %) nearly brought the same yield in 2018.

A few questions are arising from the fact that climate change occurs and hops are reacting to the changing conditions. Can varieties, which are showing clear climate dependence, be grown in future? And can measures like more irrigation or growing a variety in another area solve this problem? Czech authorities already began to support an irrigation program and the variety *Saaz* is already cultivated in Germany, in the Elbe-Saale area on more than 100 ha, and growing of the variety in the Hallertau area has started, too. For brewers one of the most interesting questions is whether these hops are as good from a quality point of view as the original and whether they are showing the same properties in beer.

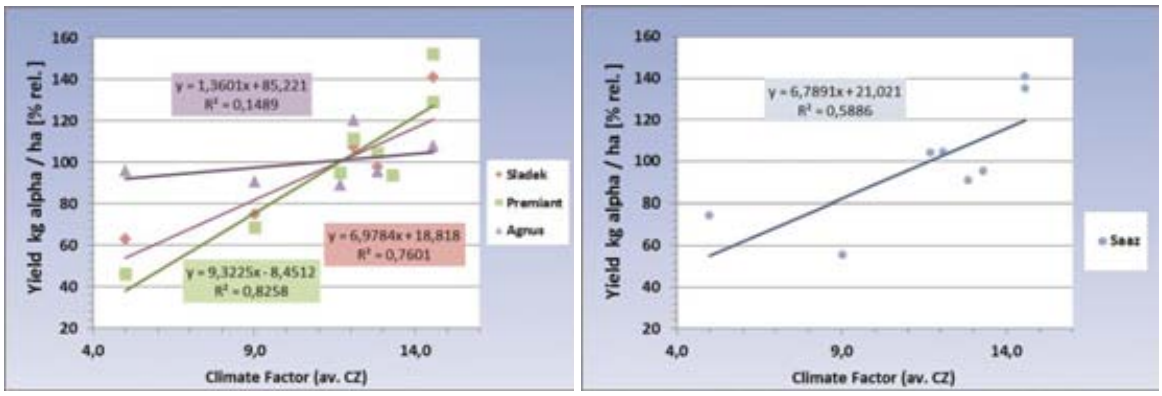

Figure 1. Climate Factor *vs* relative hop yield of cultivars *Saaz*, *Sladek*, *Premiant* and *Agnus* in Czech Republic in the years 2011-2018

Saaz variety hops from Elbe-Saale/Germany (ESAZ) and Žatec/Czech Republic (CZ-SAZ) from four commercial pellet productions between 2 and 33 tons cropped in 2016 and 2018 were collected. The hops were stored and processed in a similar way.

The hop analysis revealed only minor differences. Alpha acid content was comparable low and in terms of α-acid ESAZ seems to be a bit more susceptible to hot and dry weather conditions. The HSI of all four pellets was comparable. Hop essential oil content varied slightly within the variance of the analysis (R=0.15 ml/100g). Total polyphenols were not influenced by the weather conditions; ESAZ had a bit lower content than CZ-SAZ.

With these four hop pellets brewing trials were done in a research brewery on a 2-hl scale. The beers were brewed with 100 % Pilsener malt and an infusion mashing program as single variety beers. Hops were added to the following ratios:

- begin of boil: 4g α/hl
- end of boil: 150g/hl
- whirlpool: 150g/hl

The beers were bottom fermented with a W34/70 yeast one week at 9°C, matured *ca* one week at 15°C and stored for another three weeks at 0°C before they were kieselguhr filtered and bottled on a semi-automatic hand filling device at low oxygen levels. 2018 beers were brewed with different malt as 2016.

The values of the general beer analyses (OG, alcohol, ADF, pH …) were comparable for each pair of beers. Also bitterness and especially isohumulones were on the same level. Linalool and Polyphenols may seem different, but when having a closer look to dosage, transfer rates and analytical variation have to be regarded as similar (Table 1).

Table 1. Values of general analyses of beers hopped with cv. Saaz (2016 and 2018 harvest) from Elbe-Saale/Germany (ESAZ) and Žatec/Czech Republic (CZ-SAZ)

		CZ-SAZ 16	ESAZ 16	CZ-SAZ 18	ESAZ 18
Bitterness	IBU	**19**	**17**	**17**	**16**
Isohumulones	mg/l	**13,8**	**13,2**	**11,8**	**11,7**
R-Linalool	µg/l	56	37	**45**	**41**
Transfer Rate Linalool	%	63	41	**58**	**67**
Total Polyphenols	mg/l	299	277	**270**	**293**
Transfer Rate Hop PP	%	58	59	**48**	**53**

The beers were tasted by 11-15 panellists. The beers were rated similarly in their beer quality (enhanced DLG tasting; Fig. 2). Slight differences in overall hop aroma quality and intensity were detected in different crop years and growing areas. Intensity of the single aroma descriptors was similar in different crop years and growing areas.

Intensity and quality of the bitterness showed no differences when comparing crop years or growing areas. So the whole tasting results did not allow to state a significant quality or intensity difference between the beers brewed from CZ or German Saaz hops.

Saaz hops react sensitively to the changes of the weather conditions in CZ. The transfer of the cultivation to Elbe-Saale area had brought hops of similar quality and yield. But the climate in the Elbe-Saale area does not differ substantially from that of the Saaz area. So is this the solution for the climate problem? Certainly not.

The obvious climate change is more decisive to the survival of distinct hop varieties than to the growing area. All partners in the hop supply chain (breeders, growers, merchants and brewers) have to work together on a strategy to overcome the upcoming problems in the field of tension between sustainable hop growing and security of supply.

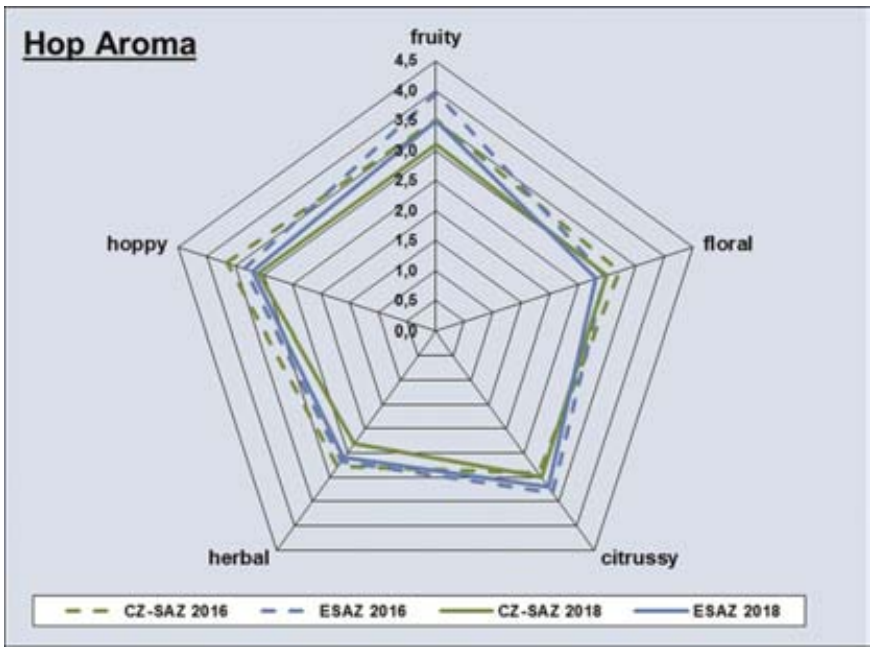

Figure 2. Results of tastings comparing beers hopped with cv. Saaz (2016 and 2018 harvest) from Elbe-Saale/Germany (ESAZ) and Žatec/Czech Republic (CZ-SAZ)

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Hops – aspects from variety to technology and beer quality

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Today, in Germany 22 “own” hop varieties are available. Of those, 10 are classical aroma varieties; five are new aroma (flavor) varieties and seven bitter (high-alpha) varieties.

The German varieties are discussed with respect to their level and composition of their bitter substances and aroma components. They can be distinguished by the ratio of beta- to alpha-acids, which is for bitter (high-alpha) hops 0.3-0.4 and for aroma hops 0.7-2.0, thereby affecting the composition of the bitter substances in wort and beer, i.e., the ratio EBC-BU to iso-alpha-acids. The higher this factor, the better the bitter taste and its harmony will be.

Thus, the hop variety determines the character of the bitter taste. But also a splitting of the hop dosage to 3 or 4 different times during boiling influences the ratio EBC-BU to iso-alpha-acids and, to a certain extent, the bitter quality of the resulting beer. But nevertheless a difference between bitter- and aroma hops will remain.

In this context the last addition to the whirlpool is of interest, if beer with a pointed hop aroma should be produced. This step should be improved to avoid differences from brew to brew. Either hop pellets should be added with hot water (60-80°C) or a “hop back” used between whirlpool and wort cooler for a defined impact of the wort with the hops. All these measures can help to an even better differentiation of the beers.

VIII: Hops and health

Specialized metabolites of hop: a great potential for health, in particular to fight bacterial resistance

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Abstract

Interest for hop has never been so strong as pharmacological research keeps demonstrating the great potential of its specialized metabolites in human health and in particular to fight bacterial resistance. This is of particular importance as the World Health Organization declared that antimicrobial resistance is an increasingly serious threat to global public health, requiring action across all government sectors and society (WHO 2018).

Up to now, hop has been studied for its many pharmacological activities, including sedative, estrogenic, antioxidant, anti-inflammatory and antimicrobial activities (BOCQUET et al. 2018a). Moreover, if the antibacterial properties on Gram-positive strains have already been demonstrated for some prenylated compounds, it is still underexploited to fight against bacteria resistant to antibiotics (BOCQUET et al. 2018b).

This presentation will be an opportunity to highlight the great potential of the specialized metabolites of hop in human health, and in particular the double antibacterial and anti-biofilm action of some prenylated compounds against some clinical isolated of Methicillin-resistant *Staphylococcus aureus* strains (MRSA) (BOCQUET et al. 2019)

Acknowledgment

We thank the family Beck (Bailleul, France) for providing us hops every year; the platforms CUMA (University Lille 2, Pr. J.F. Goossens) and LARMN (University Lille 2, Pr. N. Azaroual) for the access to equipment of mass spectrometry and NMR; as well as the team of the laboratory of bacteriology of the Faculty of pharmacy of Lille (INSERM U995) and in particular Dr C. Neut for this collaboration. We also thank the region Hauts de France and the University of Lille 2 for financing the PhD thesis of Laetitia Bocquet. This work is also carried out within the Charles Viollette Institute as part of the Alibiotech project funded by the European Union, the French State and the region Hauts-de-France.

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IX: Hops under the Southern Cross

Overview on Mapuche, Traful and Nahuel, local hop varieties cultivated in Patagonia, Argentina

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Abstract

The hop industry in Argentina was born at the end of the 1940s. In the 1980s, Quilmes Brewing and Malting Company started its own hop breeding program with the aim of developing new varieties adapted to the local agro-climatic environment and obtaining better yields. As a result, in the 1990s, Mapuche and Traful varieties were registered, and another cultivar was registered under the name of Nahuel in 2018. Due to the greater demand from craft breweries interested in hops with different aroma profiles, in the last 10 years these varieties are having an increasing growing area, with better-known yields. Nahuel stands out for its high yield, while Traful has the lowest. Mapuche achieves average yields around 1,800 kg/ha and shows a higher total oil content compared to the other two cultivars. The three varieties provide an acceptable bitterness quality in beer despite the high cohumulone ratio relative to total humulone. Regarding sensory evaluations, Mapuche is studied best, having a citrusy and slightly floral aroma profile. None of the three varieties could be considered a flavour hop, and even though they do not have an exciting fruity profile, they have unique characteristics supporting their growing demand. Hop production in Argentina should probably rely upon local varieties, properly adapted to the particular environment and to the relatively low latitude. Hop breeding efforts are necessary in the country to promote the development of not only higher yielding varieties but also diverse flavour profiles in order to respond to local craft market requirements.

Key words. Hop varieties, breeding program, flavour profile, Patagonia, Argentina.

Introduction

The hop industry in Argentina was born at the end of the 1940s as a consequence of the experience suffered by the world conflicts in terms of the insecurity of a normal supply of this essential raw material for brewing. Initial trials were made with the Spalter variety, which acclimatized and predominated over others that were tested (Semsch and Tettnanger). Subsequently, searching for greater yields, the varieties Atlas, Neoplanta, Late Cluster, Brewers Gold, Bullion and Cascade were tested. Cascade arrived in Patagonia at the end of the 1970s and was undoubtedly the one that best acclimatized, expressing high quality and good performance similar to its place of origin. In the 1980s, Quilmes Brewing and Malting Company started its own hop breeding program. The relatively low latitude of hop growing areas in Patagonia (more similar to those in Australia) suggested that it was necessary to develop varieties adapted to the local agro-climatic environment. The main goal of Quilmes breeding program was to develop new varieties to allow a sustainable hop industry, able to increase efficiency in brewing their lager styles, looking for more alpha but without too much interest in particular flavour profiles. In the 1990s, the first varieties were registered and since then they have been cultivated, although without much prominence compared to the production of Cascade hops.

The 1990s were not very encouraging for the hop industry, and in Argentina the area under crop was reduced to almost 50 %; also as a consequence of high production costs due to local macroeconomic environment. In recent years, craft breweries are still more and more

interested in hops with specific aroma. Most of them want to brew special beers, which are different from the traditional industrial lager beers.

The trend in consumption in Argentina points to American ale styles, with a marked leadership of the American IPAs and APAs over the rest of the beers. Although the great number of newly released cultivars of flavour hops worldwide, which the local craft brewers immediately demanded (assuming high import costs in significant volumes of hop pellets), no breeding program was started in Argentina. In this context, local varieties were revalued in this decade, because of the adoption by many craft breweries. Slowly, Mapuche, Traful and Nahuel are having an increasing growing area. As observed in the local statistical registers about 60 % of the volume of hops produced in Argentina corresponds to Cascade, with a slight downward trend in the last 10 years.

Beyond the laboratory evaluations that were conducted in the 1990s, for many years there were no exhaustive analytical studies or brewing tests in Argentina on these varieties. Specific knowledge about their behavior from a brewing point of view began in the last decade, largely as a result of the growth boom in the craft industry. Nowadays, some premium beers from South America and Europe use these varieties (especially Mapuche), and the beer flavor profile is better described.

The hops and beer production represent a strength for the Patagonia region, which generates scientific and technical needs. Since 2016 research institute IPATEC (Instituto Andino Patagónico en Tecnologías Biológicas y Geoambientales) in San Carlos de Bariloche, provides important support to both industries. They have a reference laboratory for hop growers as well as local brewers and thanks to the close link that exists between the public and private sectors, important knowledge is generated adding value to these young industries. In particular, the institute highlights the brewing yeast bank, which includes a collection of native strains.

Currently, there are positive results in favor of local hops, both in agronomic and analytical and sensory aspects. Without being varieties with great differentiation in terms of exciting flavour profiles, they are appreciated by the market, are well adapted to the local soil and weather conditions, and could possibly serve as parents for future breeding programs.

Material and methods

Plant breeding

The crosses were made in 1984, in Quilmes experimental field in Fernandez Oro (latitude 38°95'S), Río Negro province, northern Patagonia, Argentina. Cascade variety was used as mother and Quilmes own lines as father plants (Q82M2 and Q82M21). All the important data regarding the resistance to diseases (especially Downy mildew), and yields were collected since 1985. Mapuche variety was registered in 1992 and later Traful variety in 1994. For more than 20 years the line CQ86227 was kept under observation and then cultivated on a larger scale, and in 2018 it was registered under the name of Nahuel. There are still some lines that remain under evaluation, and new variety registrations may arise in the short term.

Resin and oil analyses

Hops were analyzed as pellets, type 90, stored at -20ºC, from different batches of the 2015 harvest until the 2019 harvest. Quantification of α- and β-acids were done by UV-spectrophotometry (HOPS-6) (Shimadzu UV-1800) and by HPLC according to protocol Hops-14 (ASBC). ICE-3 (ASBC) was used as standard, in a C18 column (Phenomenex Luna 5 µm C18, 25 cm x 4.6 mm). Isocratic runs included a mobile phase of 90 % methanol and 10 % acidified water (17:0.25, water: phosphoric acid) with a constant flux of 0.8 ml/min. Detector was set at 314 nm (HPLC Waters Delta 600, detector Waters PDA 2898). For identification and quantification, chromatographic peak areas of standard and samples were compared (Software Empower 2). The four identified and resolved peaks included i) cohumulone, ii) adhumulone + n-humulone, iii) colupulone, and iv) adlupulone + n-lupulone. Oil content

was analyzed following ASBC Hops 12 protocol and EBC 7.12. Samples were analyzed by Quilmes Brewing and Malting Company and some from harvest 2018 in collaboration with Hopsteiner (Mainburg, Germany).

Results

There are several years of agronomic records about yields and certain characteristics of each variety, which determine field management practices. The harvest maturity date is one of the main differences between Traful and Mapuche; while the first is usually early (beginning of March), Mapuche is always the last variety to be harvested, without altering too much its chemical parameters of bitter acids. It has great vegetative growth which usually causes some harvest complications. The average yields are around 1,800 kg/ha, and in the best years it reaches 2,300 kg/ha. When cultivated in the 42nd parallel the harvest can be delayed until the beginning of April and the risk of strong frosts can be a big problem to the point of losing whole plots in the higher fields (440 meters above sea level). Traful yielding is a little poorer, with values that average 1,300 kg/ha. The low weight of their cones is evident. The recently registered Nahuel variety expresses the best yields among the three local lines. In average tends to overcome 2,000 kg/ha, and in the best years it reaches 3,000 kg/ha. It has a great growth rate, which sometimes complicates the training in slightly windy conditions due to the long internodes with few turns of the guide on the string.

In the last five years the chemical determination of bitter acids and gas chromatography-mass spectrometry for determination of aroma compounds in local hops were performed repeatedly. An overview on the major components of both varieties is shown as average of the last five years in Table 1.

Table 1. Resin and oil content of Mapuche, Traful and Nahuel hop pellets, type 90.

Method		Units	Mapuche	Traful	Nahuel
Spectrophotometry Hops 6A (ASBC 2014)	alpha-acids	%-w/w	6.4	7.2	5.9
	beta-acids	%-w/w	4.8	5.8	4.7
	ratio beta:alpha		0.8	0.8	0.8
HPLC Hops 14 (ASBC 2008)	alpha-acids	%-w/w	5.8	6.7	5.5
	Cohumulone	%-w/w*	46 %	48 %	50 %
	beta-acids	%-w/w	4.1	4.6	4.0
	ratio beta:alpha		0.7	0.7	0.7
Hops 13 (ASBC 2008)	Total Oil	ml/100 g	1.11	0.84	0.86

∗Expressed as % of total humulone

All varieties have relatively low alpha acids content and also relative to its Cascade parental. The high beta to alpha acids ratio is indicating a good presence of auxiliary bitter compounds and despite the high cohumulone ratio relative to total humulone, the quality of the bitterness in beer is acceptable, as it happens with Ales single hopped with Cascade. No great differences are apparent between varieties, beyond some slight superiority in the total oil content in Mapuche compared to the other two. Table 2 shows the results of major aroma components of the local varieties in mg/100g (EBC 7.12). To have greater support, further analyses of future crops will be needed since only two years samples were examined.

Table 2. Major aroma components of Mapuche, Traful and Nahuel hop pellet, type 90.

	Units	Mapuche	Traful	Nahuel
Myrcene	%-rel	33.0%	29.0%	29.5%
Linalool	%-rel	0.5%	0.7%	0.6%
beta-Caryophyllene	%-rel	11.3%	8.9%	9.8%
Farnesene	%-rel	10.4%	9.5%	8.7%
alpha-Humulene	%-rel	19.1%	15.7%	15.7%

The relative amount [%] for mono- and sesquiterpenes are higher in Mapuche, while Traful and Nahuel alternate similar values. Traful showed the greatest linalool relative amount, so if this aroma compound gives a good correlation between perceived flowery-fruity hop aroma in beer, its low total oil content (as shown in Table 1), is compensated and in late additions it has an aroma potential that could surpass Mapuche.

Regarding sensory profile of each variety, the most studied is Mapuche as the one that is produced in greater volume, which is also present in several beers. Its specific aroma has been described as citrussy and slightly floral (orange blossom). Occasionally, it expresses sweet notes (chocolate), green tea and some pine. In Traful a spicy profile predominates, with notes of black pepper, some fennel and anise. In pilsner beers, a fruity aroma (dehydrated fig) may appear slightly woody. Nahuel has not been described yet, but expresses a floral/spicy profile, with notes of green tea and vegetables. None of the three varieties could be considered as a flavour hop, and even though they do not have an exciting fruity aroma, they have their particularities. Craft brewers need to differentiate, and generally try to vary the citrus profile of Cascade hops, which is achieved well with Mapuche. Local varieties are also found in Belgian ales, with very auspicious results.

Discussion and conclusions

As it is being done in Australia, hop production in Argentina should probably rely upon locally bred varieties. Hop growing regions of those countries have one aspect in common: latitude. Both Victoria and Tasmania in Australia (or Nelson in New Zealand), as well as the hop growing valleys in Patagonia, are areas below 43rd parallel. That latitude is quite distant compared to the main hop growing regions of the world. Hallertau and Yakima are located in the 48th and 46th parallels, respectively. It is a very significant feature when looking for attributes to be able to determine that a hop growing area constitutes a particular "terroir". Day length determines hop plant phenology, which is surely related to biomass yields and the synthesis of specific compounds.

Breeding efforts in Argentina are necessary to promote the development of not only higher yielding varieties but also diverse flavour profiles in order to respond to local craft market requirements. Since the hop industry in Argentina is such a small sector, it is necessary to target a specific business oriented to special varieties for exclusive demands, as is done by other countries that are also under the Southern Cross.

Acknowledgement

Our thanks are due to all hop growers in Patagonia for providing valuable information on the agronomic performance of local hops. Many thanks to Johann Pichlmaier and Florian Schüll (HVG); John Paul Maye, Willi Mitter, Frank Peifer, Richard Shaye and José Antonio Magadán (Hopsteiner) for collaborating and clearing technical doubts. Thanks to Cervecería y Maltería Quilmes (AB InBev) and many other brewers for conducting individual brewing trials with our local hops. Special thanks to Diego Libkind (IPATEC, CONICET-UNComahue) and his team for many of the chemical analyses of our hop varieties.

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20+ years of hop research in Australia – a personal retrospective

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Abstract

For more than 20 years, I have had the privilege and pleasure to undertake a collaborative research partnership with Hop Products Australia. This research has incorporated modern technologies into the Australian hop breeding program and has involved a multidisciplinary approach, including, cell biology, plant biology, genetics, molecular biology, plant breeding, analytical chemistry and biotechnology. This successful university-industry collaboration could not have occurred without staff and students from the University of Tasmania as well as colleagues from Hop Products Australia and research institutions from around the world. This collaborative effort has advanced hop research and breeding efforts in Australia and has assisted the Australian hop industry to not only remain internationally competitive, but to also grow and thrive.

X: Posters

New hop varieties in the Czech Republic

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Abstract

From 2017 till 2019 ten new hop varieties were released in the Czech Republic. Gaia and Boomerang belong to bitter varieties; both are suitable for basic hopping of beers. Boomerang, thanks to its spicy aroma, is suitable for top fermentation as well as for dry hopping. Country, Jazz and Blues are the first Czech dwarf varieties of the aroma type. Jazz and Blues can also be recommended for top fermentation and dry hopping. Aroma varieties Saaz Brilliant, Saaz Comfort, Saaz Shine and Mimosa have their origin in Saazer. Mimosa is very good for dry hopping of APA and IPA beers. Uran belongs among flavour hops and is typical for its strong spicy aroma, which is very suitable for IPA, Double IPA, Belgium Imperial, etc. At present all the mentioned hop varieties are tested in Czech and foreign breweries.

Elimination of hop viroids from pollen

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Abstract

Several viroids − single-stranded, non-coding, circular RNA parasites of plants − are able to infect hop. They represent a significant danger due to their fast spreading and difficult eradication from plant tissues. Some hop viroids like hop stunt viroid are transmissible through pollen to seeds and new generation, while others like hop latent viroid are not. We analyze the molecular background of elimination of two hop viroids, apple fruit crinkle viroid (AFCVd) and citrus bark cracking viroid (CBCVd) from male gametophyte cells. As both viroids are able to replicate in pollen model, *N. tabacum*, the mechanism of eradication is in parallel analyzed by NGS, complex RNA and protein expression profiling using infected and transformed tobacco anther tissues at different developmental stages. Our results show that in pollen the viroid replication pathway involving DNA-dependent polymerase II complex is depressed in comparison to leaves and simultaneously specific and unspecific degradation proceeds in pollen and in growing pollen tubes. The analysis is in progress to identify specific roles of argonauts, dicers, tudor binding factors and nucleases in the elimination mechanism. According to our results, enhanced propagation of both these hop viroids in pollen mediated by plant transformation leads to some physiological disorders of tobacco pollen development and germination.

Acknowledgement

The work was supported by the Czech Science Foundation project 18-10515J to JM and DH, by the institutional support RVO: 60077344 to JM and by the DFG to GS.

Some changes in RNA expression caused by hop viroids in pollen

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Abstract

Our recent research shows the elimination of hop viroids in pollen. In this study, we aimed at providing deeper insights into the molecular mechanisms of viroid elimination and pathogenesis using gene expression profiling of AFCVd-infected tobacco (*Nicotiana tabacum*) covering three stages early bicellular pollen, late bicellular pollen and germinating pollen pollen (GP). Our analysis revealed the dynamic changes in activity of genes in three stages, which could be correlated with effective elimination of AFCVd from pollen stages as well as with induction of viroid pathogenesis on the level of pollen, as we found that the presence of AFCVd leads to depression of GP and pollen biomass accumulation. Genes involved in pollen nucleolytic activities, defense-related genes, receptor-like kinases, Argonaute proteins and transcription factors were found to be strongly activated, whereas activity of certain classes of RNA polymerase was significantly declined. Functional classifications of genes with enriched expression in AFCVd-infected pollen cells showed that DNA repair, ubiquitin-mediated proteolysis, and cell cycle progression are overrepresented Gene Ontology categories. We believe that our results will contribute to decipher the mechanism of viroid elimination and pathogenesis in pollen. A major output of our study is two different high-quality databases representing global changes in gene expression associated with pollen germination and viroid induced pathogenesis coupled with developmental changes in gene expression.

Funding

This research was funded by bilateral Czech Science foundation project (18-10515J/DFG STE 465/10-1).

The first detection of hop stunt viroid (HSVd) on hop 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.

Viroids are the smallest known pathogens that consist of non-capsidated, single-stranded non-coding RNA replicons and exploit host factors for their replication and propagation.

Viroids that can infect hop (*Humulus lupulus* L.) include Hop latent viroid (HLVd), Hop stunt viroid (HSVd), Citrus bark cracking viroid (CBCVd) and Apple fruit crinkle viroid (AFCVd).

HSVd infection of hop plants was previously found in Japan, Korea and USA and Slovenia. Symptoms are stunting, leaf curling, chlorosis, and poor plant vigor. Plants infected by HSVd produce less and smaller cones, yield is 50 % decreased, alpha and beta acids content is decreased up to 50 to 70 % compare to viroid-free plants (SANO 2013). The HSVd has been found in grapevine, citrus trees, plum, peach and several other fruit plants (HATAYA et al. 2017).

The qRT-PCR method was successfully used for detection of HSVd in hop plants (PATZAK et al. 2017; GUČEK et al. 2017, 2019).

We have used this method to control the health of hop plants in the World Collection of hop varieties from around the world in 2018. We checked 18 varieties of hops from the Czech Republic, USA, England, Ukraine, Lithuania and Slovenia. HSVd was found in four plants of the Horizon variety.

This work was supported by the Ministry of Agriculture of Czech Republic in conceptual project MZE–RO1319.

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Eggplant *Solanum melongena* as indicator plant for *Verticillium*

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Abstract

Verticillium nonalfalfae, the pathogen that is the main cause for *Verticillium* wilt on hops, persists for up to five years in the soil, thus representing a constant source of infection for hop plants. Soils infected with *Verticillium* have to be disinfected. In order to check the efficacy of various soil disinfection methods, a search was made for a practical fast detection method. Usually, today a qPCR-based diagnosis is performed to detect if there is *Verticillium* in the hop plant. However, there is no practicable way to detect and quantify *Verticillium* in the soil. A growth test of soil does not fit practical needs for detecting *Verticillium* in fields. A hop plant is difficult to infect artificially and wilting symptoms often appear only with delay. Therefore, hop itself is no suitable indicator plant even for testing soil in pots. One possible indicator plant is eggplant. Therefore, soil from naturally infected hop fields was collected. In addition, infected root stocks were cut into small pieces and mixed with the soil. Eggplants were cultivated in planting substrate for eight weeks. After potting them into the naturally infected soil with infected rootstocks cuttings, symptoms developed after five to six weeks post inoculation (Fig. 1). Wilt symptoms were noted at 50 % of plants potted into this infected soil. *Verticillium nonanfalfae* in the eggplant was confirmed then by qPCR.

Key words. *Verticillium* wilt, *V. nonalfalfae*, *V. albo-atrum*, indicator plant, eggplant

Figure 1. Eggplant showing *Verticillium* symptoms six weeks after inoculation with the pathogen

Cooperation

Dr. S. Radišek, Slovenian Institute of Hop Research and Brewing

Acknowledgement

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Identification and differential expression analysis of microRNAs in hop plants (*Humulus lupulus* L.) in response to *Verticillium nonalfalfae* infection

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Abstract

MicroRNAs (miRNAs) are involved in gene silencing processes at the post-transcriptional level, thereby regulating a wide variety of biological processes and ensure a fine-tuned regulation of genes involved in defense mechanisms during the stress response and defense against pathogens. Limited knowledge is available on the miRNA population of hop (*Humulus lupulus* L.) which is highly susceptible to *Verticillium* wilt, a disease that threatens European hop production. The unique pathosystem of hop plants and fungus *Verticillium nonalfalfae* is an attractive model to study the profile of phenolic compounds and miRNA response during infection.

The aim of our study was 1) to monitor the colonization of *V. nonalfalfae* during the pathogenesis of verticillium wilt, 2) to determine the phenolic response, and 3) to identify differentially expressed miRNAs as a response to *V. nonalfalfae* infection in susceptible Celeia (SGC) and tolerant Wye Target (TRG) variety.

Artificial inoculation of two hop varieties was conducted with lethal strain T2 of *V. nonalfalfae*. A sampling of 3 control and 15 treated plants per variety was performed at 1, 3, 6, 12, 15, 18 and 30 days post-inoculation (DPI). The colonization pattern of *V. nonalfalfae* during infection was obtained by quantitative PCR (qPCR) using *V. nonalfalfae* specific primers. Furthermore, we used HPLC-MS method to quantify phenolic compounds responsive to colonization with *V. nonalfalfae*. Fungal colonization and phenolic compound profiles help us to select the most appropriate sampling points for miRNA-Seq experiment and to characterize and identify the responsive differentially expressed miRNAs during verticillium wilt pathogenesis.

Fungal colonization and phenolic compound profiles show an increasing tendency in stems of the susceptible variety Celeia (SGC), while in the resistant variety such trend is not noticeable. On average, a higher total phenolic content was found in infected and control samples of susceptible variety SGC compared to the resistant variety Target (TRG). In a miRNA-Seq analysis of infected and control TRG and SGC root samples from 1 DPI, we identified 941 miRNA candidates supported by draft hop genome. Of the identified, 128 miRNA candidates mapped to 30 known miRNA gene families from miRBase. Using DESeq2 we identified 14 and 12 differentially expressed miRNAs in infected samples of SGC and TRG cultivars, respectively. In SGC, nine miRNAs belong to three known families (miR167, miR828, miR159) and five miRNAs are novel, whereas in TRG 11 miRNAs belong to five known families (miR156, miR319, miR160, miR408, miR159) and one miRNA is novel. Identified responsive hop miRNAs will be further validated using RT-qPCR. We will perform identification and computational functional analysis of hop genes/transcripts, which are targeted by selected identified hop miRNAs. Finally, genome-wide analysis of hop targets, which are cut by the action of miRNA silencing, will be performed.

Genome-wide identification and expression analysis of RNA interference core components in *Verticillium nonalfalfae*, a vascular wilt pathogenic plant fungus of hops

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Abstract

The RNA interference mechanism (RNAi) has become a focus of intense scientific investigation in the fungal kingdom in recent years. Novel studies have proposed that RNAi is a major contributor to the virulence of fungal pathogens as a result of so-called trans-kingdom RNA silencing. In the present study, we identified the three RNAi core proteins in the pathogenic plant fungus *Verticillium nonalfalfae*, a soil-borne plant pathogen that causes severe wilting disease in hops (*Humulus lupulus* L.). Our phylogeny results confirm the existing taxonomy in the Ascomycete fungal phylum. The expression analysis revealed a potential role of RNAi in the pathogenicity of the fungus since all the RNAi genes were highly upregulated in the highly virulent isolate T2 and were also differentially expressed in the *V. nonalfalfae*-susceptible 'Celeia' and resistant 'Wye Target' cultivars.

Key words. *Verticillium nonalfalfae*, RNAi, Hops.

Introduction

Verticillium sensu stricto is an anamorphic genus within the pathogenic plant fungi of Ascomycetes that causes *Verticillium* wilt, a vascular disease affecting many important crops worldwide (INDERBITZIN et.al. 2011). One of the most susceptible hosts of the *Verticillium nonalfalfae* species is hop (*Humulus lupulus* L.), primarily cultivated as an essential product for beer production. The most severe outbreaks in hops are caused by highly virulent *V. nonalfalfae* pathotypes that produce infections inducing rapid plant dieback and lethal *Verticillium* wilt symptoms.

Recently, it was shown that pathogenic plant fungi, such as *Verticillium* species, also use RNA interference mechanisms and the small RNAs as virulence factors by exporting them to the host to mediate host defence in the host-pathogen interactions (WEIBERG et. al. 2013). Therefore, in the present study, 1) the core components of RNA silencing in *V. nonalfalfae* were investigated and 2) expression analysis was performed to examine expression patterns in the highly virulent and less virulent fungal pathotypes and to predict the potential effects of fungal RNAi *in vivo* during *V. nonalfalfae* infection in hop host plants.

Material and methods

The homologues of AGO, DCL, and RdRP in the *V. nonalfalfae* genome were identified using BLAST analysis with available fungal protein sequences for AGO, DCL, and RdRP from the UniProtKB database and the genome sequence of *V. nonalfalfae* available in our laboratory. All identified genes were examined using the Conserved Domain Database, Pfam 31.0, and the Simple Modular Architecture Research Tool to predict the conserved domains.

For the phylogenetic analysis, the protein sequences were aligned using the MUSCLE algorithm, and a maximum likelihood neighbour-joining tree was constructed for the AGO, DCL, and RdRP groups of proteins.

RNA was extracted using Spectrum™ Plant Total RNA Kit (Sigma-Aldrich) form mycelia and conidia of the highly virulent T2 and less virulent Rec fungal isolates and from roots and stems of susceptible 'Celeia' and resistant 'Wye Target' cultivars. Quantitative RT-PCR was performed using the 7500 Real-Time PCR System (Applied Biosystems).

The relative expression levels of the target genes were calculated based on the mathematical model proposed by PFAFFL (2001).

Results

A total of two AGO, two DCL, and two RdRP genes were identified in the *V. nonalfalfae* genome. All the proteins contained the conserved domains typical for RNAi proteins. All *V. nonalfalfae* RNAi genes were expressed in both of the investigated fungal tissues, conidia and mycelia grown in xylem-simulating media (XSM). The highest expression levels of the AGO, DCL, and RdRP genes were encountered in the XSM mycelia of the highly virulent pathotype T2. In hop plants infected with the highly virulent isolate T2, different expression levels were observed for the fungal RNAi genes in the roots and stems of the susceptible Celeia and resistant Wye Target cultivars (Fig. 1). In the stems of the susceptible Celeia cultivar, all fungal RNAi genes were abundantly expressed, with fold increases ranging from 3.4 to 30.1.

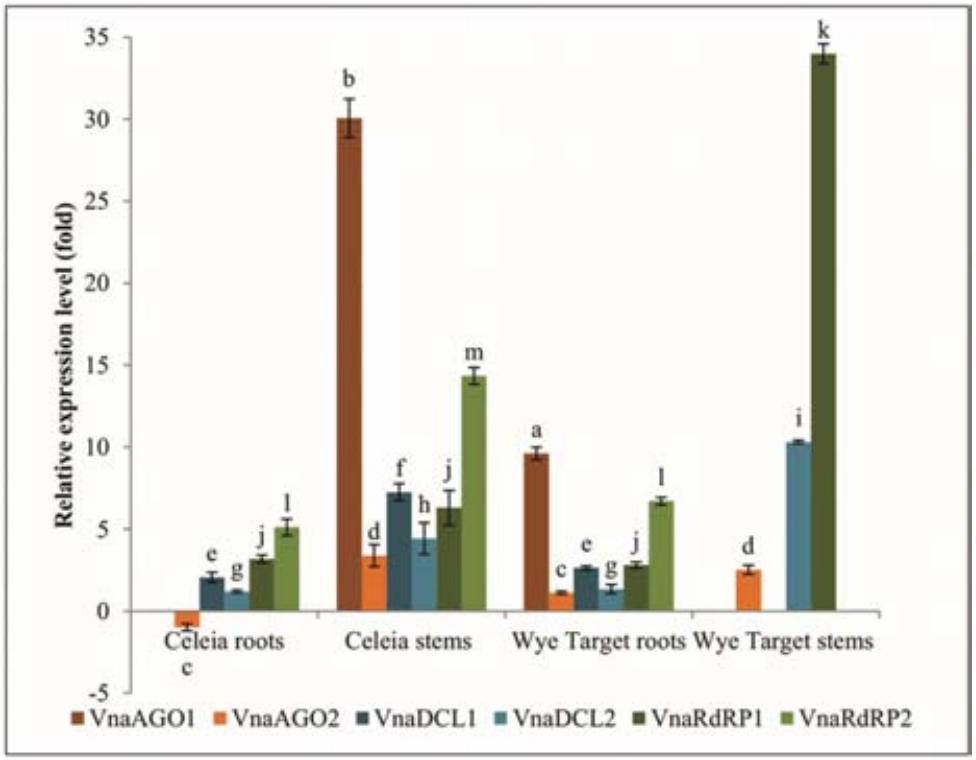

Figure 1. Expression levels of the *V. nonalfalfae* RNAi genes in the T2-infected hop roots and stems of the susceptible Celeia and resistant Wye Target cultivars; the results are shown as fold-changes relative to the expression in the mycelia grown in CD from the isolate T2.

Discussion

In *V. nonalfalfae*, a pathogenic fungus of hops, all three groups of RNAi core genes were identified and all the conserved domains characterized. The highest expression levels of all six *V. nonalfalfae* RNAi genes were observed in the highly virulent pathotype T2, in which all the genes were upregulated in the mycelia grown in media simulating the conditions in plant xylem tissue in which fungi actively spread in the host. The data gathered during the present study suggest a plausible role of RNAi in the pathogenesis of *V. nonalfalfae*.

Acknowledgement

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Detached leaf assay to evaluate downy mildew tolerance of hops

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Key words. breeding for resistance, selection, leaf test system

Downy mildew (DM) caused by the fungus *Pseudoperonospora humuli* is a destructive disease in hop production. Breeding of hops with enhanced tolerance towards this fungus is a crucial contribution to solve this problem. Assessment of tolerance is quite complex due to the occurrence of systemic infections starting from "inside" as well as leaf or cone infections as non-systematic form of DM disease symptoms (2, 3). A seedling test system with artificial inoculation in the growth hall had been in use for decades. In recent years a detached leaf assay in the laboratory based on standardized inoculation and incubation conditions was elaborated to assess the tolerance of advanced hop selections towards this fungus.

Methods

Leaves of vigorously growing test plants were taken from the third node (using at least three replicates) and the abaxial side of each leaf was inoculated with a suspension of *P. humuli* at 2 – 5 x 10^4 sporangia / ml. Leaves were visually evaluated and finally assessed 14 days after inoculation (dpi). Ratings for sporulation, chlorosis and necrosis were on a scale of 0 to 5: 0 = no symptoms, 1 = 1-10 % of leaf area infected; 2 = 11-30 %; 3 = 31-60 %; 4 = 61-80 %; 5 = 81-100 %. Finally, the index of disease severity according to TOWNSEND & HEUBERGER (1943) of each cultivar or experimental line was calculated, based on 6-19 observations obtained from 2016 to 2018, and statistically evaluated.

Results

A detached leaf assay to evaluate DM tolerance has been optimized (JAWAD-FLEISCHER 2014) based on knowledge from UK, USA and Germany (ROYLE & KREMHELLER 1981; DARBY 2005; MITCHELL 2010). A temperature cycle of 22 ^{0}C during light phase and 13 ^{0}C during the 12-hour-darkness induced vigorous sporulation of the fungus on leaves of DM susceptible plants within the first days after inoculation. Later the necrotizing of host cells followed. A clear differentiation of both disease reactions (sporulation of the fungus and/or necrosis of host cells) could be achieved. Sporulation ratings were most suitable to assess non-systematic tolerance towards DM (DARBY 2005). The DM tolerance of several hop cultivars has been assessed using this leaf assay. Preliminary results on the tolerance or susceptibility of Hüll cultivars and experimental lines to DM over two to three years of testing showed that there are three levels of DM reactions: highly tolerant individuals with Hallertau Tradition as the most tolerant one, highly susceptible with Polaris and Saphir as examples and the majority of cultivars and experimental lines with medium level of tolerance and susceptibility respectively. In most cases the reactions in the leaf assay correspond with their reaction based on field records.

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Realtime PCR based diagnostics and meristem culture – essential tools for healthy hops

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Abstract

With the occurrence of lethal forms of the *Verticillium* wilt in German hop production and the detection of two highly deleterious viroids – *Hop stunt viroid* (HpSVd) alone or in combination with *Citrus bark cracking viroid* (CBCVd) in hop production abroad - the focus in our research was to establish and optimize powerful diagnostic tools to detect these pathogens in hop plants. Since there are no chemicals available to fight these pathogens a crucial part in the control of these diseases is the identification and eradication of infected plants as well as the production of healthy planting material for the sake of the hop and brewing industry.

Thus, an *in planta* multiplex TaqMan® realtime PCR was worked out as highly sensitive, specific and reliable method to identify *Verticillium nonalfalfae* and simultaneously discern mild and lethal strains of this fungus in hop bines. For the detection of HpSVd and CBCVd in hops a TaqMan® realtime RT (reverse transcriptase)- PCR was developed proving high sensitivity, specificity and robustness in the validation process. In all these diagnostic methods an internal control confirms a fully functional PCR run. In addition, ELISA and RT-PCR as well-established techniques in our laboratory were employed for virus testing.

With the aim to cure infected hop plants the meristem culture in combination with cold or heat treatment was optimized so that healthy plants could be regenerated from the meristematic shoot tips of *Verticillium* and virus infected hops. Efforts to eliminate viroids were only in rare cases successful – since HpSVd or CBCVd infected hops were not available – plants with HpLVd were used in all investigations.

Key words. hop viroids, Verticillium, realtime PCR, meristem culture

Acknowledgement

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Control of two-spotted spider mite *Tetranychus urticae* by predators with the help of released *Typhlodromus pyri* within IPM and organic hop growing in Saaz hop growing region (eleven years' experience)

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Abstract

Two-spotted spider mite *Tetranychus urticae* is the most dangerous pest of hops, especially in Saaz hop growing region, typical for low precipitations. Contemporary hop protection against this pest, based entirely on application of miticides, has become difficult as their future using is uncertain either because of their decreasing efficiency or their toxicity on non-target organisms. One possible way of controlling resistant populations of *T. urticae* is to increase the efficiency of the natural control by native acarophagous predators supported by release of the predatory mite *Typhlodromus pyri*.

In 2008 *T. pyri* was released in nine hop yards (10.5 ha) under IPM management and in four hop yards under organic hop growing management (6.5 ha) at the research farm of Hop Research Institute (IPM, 2x organic) and Líšťany (2x organic). Spider mites and their predators have been checked in regular intervals during growing seasons. Whereas in some of the hop yards one release was enough, in most of them predatory mites have had to be released repeatedly (2-3x) since 2008. Thanks to predatory mites the density of acarophagous predators has established at the level able to control *T. urticae* without the necessity of miticide applications. The ability of predators to keep spider mites under the economic threshold showed also under the extraordinary hot and dry weather conditions of the year 2018, when numerous miticide sprays had to be carried out to control this dangerous pest in other conventional hop yards.

Pesticide residue analysis of hops (crop 2016 – 2018)

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Abstract

Current agriculture is focused on high yields and quality of the harvested products.

Hopwever, due to deteriorating climatic conditions it is sometimes difficult to find suitable agrochemicals for the protection of hop gardens that meet the health requirements of the final product.

The quality of hops is evaluated from several points of view. One is the brewing value of hops and the other, which is still gaining importance, is the food safety of the processed hops (content of heavy metals, residues of pesticides, nitrates etc.).

Our laboratory uses the internal QuEChRS methods as a primary reference for pesticide analysis in hops. Analysis is conducted employing GC-MS/MS and LC-MS analyses. Profiles are designed to meet maximum residue levels (MRLs) established for both domestic and foreign MRL requirements whenever possible.

Acquired results from hop fields (dissipation curves of spirotetramat, mandipropamid, ametoctradin and dimethomorph) and hop analyses on pesticide residue content is presented for the period 2016 to 2018.

Key words. Hop, pesticide residues, dissipation curves, MRL

Non-destructive assessment of plant nitrogen status in hops using leaf chlorophyll measurements

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Abstract

In many crops non-destructive methods have been used successfully to determine the current nitrogen nutrition status of plants and afterwards have been utilized to optimize nitrogen fertilization.

Since the nitrogen nutrition status is correlated with the content of chlorophyll, one option is to determine the relative amount of chlorophyll by measuring the light absorbance of the leaf at certain wavelengths. Chlorophyll has absorbance peaks in the blue and red regions, with no absorbance in the near-infrared. The SPAD-502Plus (SPAD = Soil Plant Analysis Development) uses this characteristic of chlorophyll and calculates a numerical value which is proportional to the amount of chlorophyll present in the leaf.

Within nitrogen fertilization trials, located in the Hallertau growing region, a SPAD-502Plus has been used in order to monitor the chlorophyll content of hop plants and evaluate the correlation of chlorophyll content and nitrogen nutrition status. First results show high correlation (R^2 = 0.87–0.95) with a statistical significance (p <0.001). Therefore the SPAD-502Plus can be used to determine the nitrogen nutrition status of a hop plant. Trial options with different amounts of nitrogen applied can well be distinguished through SPAD-Meter readings. It was also observed that there is no further increase of yield and quality of hops if SPAD-Meter readings exceed a certain value depending on the plant's development stage. A final evaluation of this non-destructive method is not yet possible.

Key words. Nitrogen, plant nutrition, chlorophyll, SPAD

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Hop harvest residues: Options for effective usage in terms of nitrogen efficiency

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Abstract

In the Hallertau almost 900 growers cultivate hops on an area of more than 16,000 ha. Every year about 230,000 tons of hop harvest residues accrue at the stationary harvest centers. More than 75 % of these residues are applied as an organic fertilizer again in hop yards after harvest. Due to the fact that hop harvest residues contain a significant content of nitrogen, it is very important to use them as effectively as possible to achieve a good fertilizing effect. In terms of environmental protection it is also important to apply the nitrogen contained in the residues properly.

The objective of the study is firstly to develop and evaluate different sustainable and practicable recycling options for hop harvest residues, in order to reduce nitrogen casualties throughout the winter months. Secondly, the generated composts and substrates from hop harvest residues in the first step are used in field trials to investigate the nitrogen fertilizing effect, the potential of nitrogen casualties and the feasibility of the respective method.

The experimental setup of this project is divided in four parts: 1) Experimental basis is a small-scale composting experiment, in which the conditions for a proper aerobic composting are examined. Therefore several variants of aerobic composts with hop harvest residues have been started, only differing in the interval of turning. In all compost piles of this study continuous temperature and gas, carbon, nitrogen and rotting degree measurements were made. In order to sanitize the material from diseases like *Verticillium* wilt the temperature is decisive. 2) At the same time four different methods to recycle harvest residues are analyzed: a) the usual deposition and application shortly after the harvest, b) the aerobic composting, c) an alternative composting method by Witte (microbial carbonization) and d) the ensiling of the material. This composting trial under practical conditions has several objectives. The knowledge gained from the small-scale trials should be tested under practical conditions. Also the composts and substrates are generated in this part of the study that are used for following field trials. 3) In the third part of the study the gained materials are tested in plot trials with rye and 4) field trials in a hop yard. In both trials the N-fertilization effect and the N-mineralization are analyzed via soil samples, determination of grown biomass and N-contents and yield evaluation.

Key words. Hop harvest residues, Nitrogen

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Fertigation as an intensification tool of hop production

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Abstract

The fertigation (water + water soluble fertilizers) distributed in drip irrigation (fixed at the ceiling of hop construction) can reasonably increase hop yield.

Key words. Hop, fertigation, drip irrigation

Introduction

The usual way of mineral nutrition of Czech hops consists of application of industrial mineral fertilizers in autumn or early spring around the time of pruning. It is followed by application of nitrate forms of nitrogen-based mineral fertilizers right before first hill-building ploughing. There is optional third fertilization (usually nitrate nitrogen only) before second hilling, but it is based on leaf analysis and is not practiced every year. Foliar application of micronutrients (also based on leaf analysis) together with plant protection products also occurs during vegetation. This way of fertilization is used on farms without drip irrigation.

The drip irrigation provides, besides watering, distribution of water-soluble fertilizers. We changed the approach to hop nutrition in 2017 and 2018. The basic dose of fertilizers was applied during sprouting, followed by quintuple fertigation and foliar application during vegetation period. The hop gardens were irrigated as required.

Material and methods

Locality – hop growing area Saaz, variety Saaz, planted in 2004, V-shape training, spacing 300 cm x 100 cm (distance between rows x plants).

Standard treatment – ammonium sulfate (26 % N; 13 % S) 300 kg/ha, ammonium phosphate (11 % N; 52 % P_2O_5) 300 kg/ha, potassium chloride (60 % K_2O) 300 kg/ha [March, mounted spreader]; ammonium nitrate with limestone (27 % N) 250 kg/ha [May, mounted spreader]; Zinc (700 g/l) 0,5 l/ha and magnesium sulfate 5 kg/ha [May, June]; leaf fertilizer 'Vegaflor' 10 l/ha (6 % N; 5,7 % P_2O_5; 6 % K2O) [June, July 2x].

Trial – YaraMila NPK (20 % N; 7 % P_2O_5; 10 % K_2O; 4 % S; 2 % MgO) 640 kg/ha [April, row spreading]; leaf fertilizers 'YaraVita Zeatrel' (29,5 % P_2O_5; 5 % K_2O; 4,5 % MgO; 3,1 % Zn) 3 l/ha and 'Bortrac' (10,95 % B; 150 g/l) 1 l/ha [both June and June]; YaraVita Zintrac 700 (40 % Zn; 700 g/l) 0,5 l/ha [June]; YaraVita Thiotrac 300 (15,2 % N; 22,8 % S; 300 g S/l) 5 l/ha [July]; quintuple fertigation: YaraTera Kristalon Super 42,5 kg/ha (12 % N; 12 % P_2O_5, 36 % K_2O; + micro) [June–July].

Results

This experiment with fertigation led to yield increase by 27 % in 2017 and by 33 % in 2018, compared to control. Alpha-acids content remained unaffected in 2017, decreasing by 3 % (statistically not significant). On the other hand, alpha-acids increased by 14 % in 2018 (statistically significant).

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