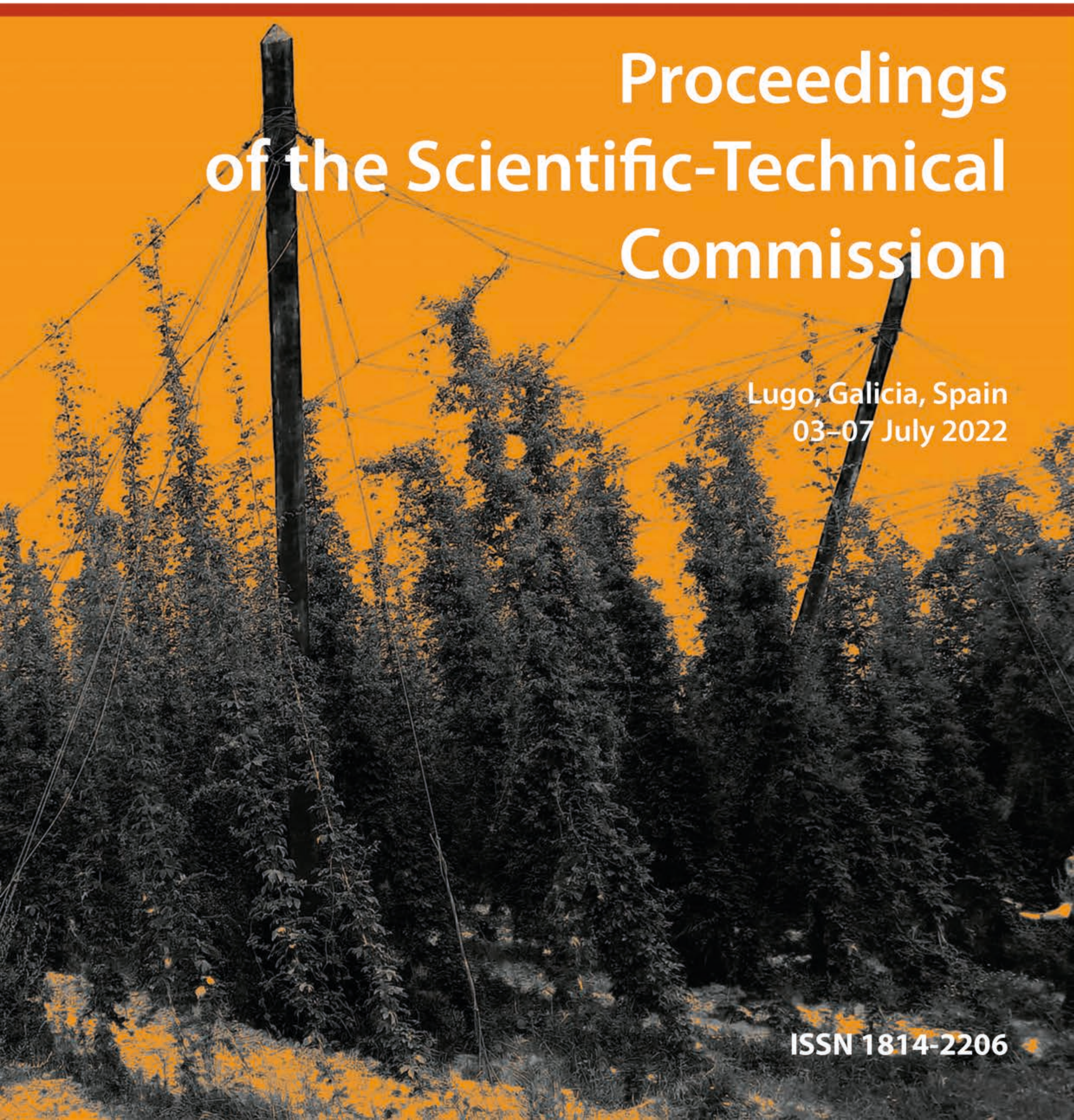
International Hop Growers' Convention I.H.G.C.

Proceedings of the Scientific-Technical Commission



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Organic hop garden (cv. Elixir) near Seebach, Alsace, France, in July 2021

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Foreword

Thirteen years have passed now since the Scientific-Technical Commission (STC) of the International Hop Growers‘ Convention (IHGC) has met for the hitherto first time in Spain. In June 2009, the meeting had been convened in Spain’s most important growing region, León, under the label of ‘Scientific Commission’ then. From a personal viewpoint, this was also my very first professional visit to Spain, and I have never had the occasion to have another look at Spanish hop growing since. So, it was with great excitement from my side when I first met two Spanish fellows from Santiago de Compostela, who were also involved in hop research, during the 2017 STC meeting in Austria. When I had got notice of the attendance of two colleagues from Galicia, I instantly had developed the plan to misuse this new contact for another STC meeting in another small but traditional hop growing area of Spain and – what can I say – this plan worked out perfectly. Consequently, with a delay of one year due to Covid-19, in 2022 the STC can finally follow the generous invitation by Javier J. Cancela (University of Santiago de Compostela) as convener and host to meet again, long overdue, in person in the wonderful city of Lugo in Galicia. ¡Mil gracias, Javier!

Due to the perfect organization of the 2022 STC conference by Javier and his team, the international community of hop scientists is facing another conference highlight under the auspices of the International Hop Growers’ Convention and 54 participants from ten hop-growing nations have registered. Altogether, 31 talks have been submitted and will be presented during six scientific sessions – hop breeding; organic hops; entomology; hop cultivation and management; phytopathology; and hops, aroma, and brewing. A poster session presenting 10 posters will round the meeting off. In addition to the scientific agenda, I am especially looking forward to the social events that add the salt to the *gazpacho* of a scientific conference. The mid-conference excursion to the Galician hop fields and to the Museo Estrella Galicia will give us the opportunity to learn much more about this small but 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, Hopfenverwertungsgenossenschaft HVG, Hopsteiner, Hopsteiner España, and Lutega, supports the mission of the STC and facilitates the participation of many scientists who otherwise would have not been able to participate.

In closing, I assume that all of us have suffered a lot from the pandemic years and the connected phase of Zoom and MS Teams, when video conferences became a sometimes convenient (and for all controllers desirable, because cheap), albeit definitely unsatisfactory substitution to an old-fashioned face-to-face meeting. Human communication is so much more than exchanging looks with a stamp-sized image on a screen! Therefore, I wish all participants a fruitful and pleasant meeting with many interesting personal discussions and encounters that will strengthen international cooperation and networking. In that context, nothing is more effective than having a beer together with colleagues… ¡Bienvenidos a Galicia!

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

I: Hop breeding

Hop cultivation in Galicia: history and future

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Abstract

After WWI, in 1915 the first testing of hop cultivation began in Spain. This initiative was promoted by José María Rivera Corral, the founder of the Brewery Hijos de Rivera (HdR) situated in La Coruña, Galicia. The good results obtained were the reason for expansion of the cultivation throughout the North of Spain and HdR played a very important role as a promoter of hop cultivation in this region 1930s. From 1946 onwards, there was an incessant increase in the cultivation of hops in Galicia, reaching in 1963 a harvested maximum of 240.369 kg on an area of 236 ha under crop. From that year began an unstoppable decline due to various reasons, like people from the rural moving to the cities, not very well adapted cultivars, political decisions, etc. Finally, hop cultivation in Galicia ceased completely in 1982.

In 2004, the enterprise Hijos de Rivera decided to recover hop cultivation in Galicia as a tribute to its founder. The main objective was making a special edition beer to commemorate the 100th anniversary of the company. Currently, HdR manages five hop gardens with a total acreage of 13.62 ha and has tested around 15 different cultivars. Main challenge is to reduce the effects of the climate change that is beating very hard in Galicia by testing new cultivars and new agronomic practices.

Key words. Hops, Galicia, Spain, history

World War I effected that Germany, a country that had supplied the Spanish brewing industries almost entirely, could not supply the needed hops anymore. Therefore, Spain had to import inferior quality hops from North America at exorbitant prices. Besides, there was an increase in beer consumption, which led to the establishment of new factories in Spain, with an according increasing demand for hops.

An initial spark for the beginning of hop cultivation in Galicia was a trip of the agricultural engineer, Don Leopoldo Hernández Robredo, Director of the Agricultural Farm of A Coruña between 1904 and 1928, to the English county of Kent in order to buy cattle of selected breeds. This trip allowed him to observe the hop fields of Kent and relate the edaphological and climatological conditions of its cultivation to those of the Galician environment that he knew and where he had already observed hop growing wildly. Thus, he became interested in cultivating hops and he thought about the convenience of starting his tests expecting a result similar to the one he had observed in Kent.

The first trials were carried out with cuttings of the English Golding variety at the A Coruña Agricultural Farm; giving excellent results in terms of product quality and performance of the plants. This showed that it could be a beneficial crop for both farmers and brewers, who came to consider the exploitation of the necessary area to be supplied. Under these circumstances the idea of turning Galicia into the first hop-producing area in Spain further matured.

However, once the war was over, trade between the different countries was restored, and foreign hops were imported again to Spain. This was favored by the fact that the Spanish currency, the *peseta*, had a very favorable exchange rate compared to other currencies and most within the interested beer industry did not deal with the matter again. However, Hernández Robredo persevered with his idea and continued with the trials, carrying out his experiments and attempts to acclimatize the varieties that he had brought from England. This was done in collaboration with the owner of the brewery “La Estrella de Galicia” from La Coruña, José Mª Rivera, who also struggled to advance the regional implementation of the crop since importing hops forced him into heavy investments; therefore they began carrying out acclimatization trials in a small experimental field on a farm in the parish of Uxes, and in another plantation attached to Rivera’s brewery.

In Betanzos near La Coruña he also collaborated with a wine entrepreneur, Raúl Fernández Meas, who was interested in the crop and adopted it on a small plot of land called “El Escorial” and later on a farm of larger dimensions. From the first tests that were carried out, the enormous importance of the hop crop and the benefits that its exploitation could bring in the Betanzos region became clear. There was suitable land for cultivation, the appropriate climate, sufficient experience after the trial period and the certainty that the product obtained was of unbeatable quality. But not only the good climatological and edaphological conditions were favorable to advance the cultivation, but also the conception of the regional agrarian structures, characterized by small farms, and a high population density.

However, as a highly labor-intensive crop, hops could not be cultivated on larger areas owned by the same person. It was only profitable when grown in a family farm where the large amount of labor required was not remunerated. Hop cultivation was eminently familiar in small plots of sizes from 0.5 to 1 ha on average. However, in this period only eleven farms requested rhizomes in the Farm to begin growing hops due to two fundamental reasons: On the one hand, there was ignorance and refusal on the side of the farmers that prevented the adoption of a new, perennial crop requiring very special care, such as: pruning, installation of stakes, setting of shoots to climb, fertilization, phytosanitary treatments, picking, drying, and baling.

Figure 1. The brewery ‘La Estrella de Galicia’ in La Coruña in 1935.

All of this required high investments and a highly refined agronomic technique. And, on the other hand, because the few hops that were grown usually reached the factories in very poor condition, due to the lack of drying facilities, they were frequently rejected by brewers.

Since 1937, everything related to the cultivation and promotion of hops was subject to intervention by the Spanish state. Trade remained under state monopoly, as it had happened with other crops such as wheat, sugar beet, tobacco, and the prices were fixes by the Ministry of Agriculture.

Cultivation was promoted in those areas where the presence of wild hops had been detected. The first area, in which the establishment of hop plantations was authorized, was in the province of La Coruña. In addition, tests were initiated in the other provinces of the North and Northwest of Spain, to which the authorization to grow hops was then extended in successive years until the total quota necessary for national supply was completed. Objective was to avoid the import of 200,000 kg of hops, which meant a financial output of approximately two million gold *pesetas*.

The Sociedad Anónima Española de Fomento del Lúpulo (S.A.E.F.L.) was established in November 1945 with its registered office in Madrid, in order to promote the cultivation of hops in the three areas that the Ministry of Agriculture considered of interest: the first area in Galicia, the second in Asturias, Santander, Navarra and the Basque Country, and the third in León, Burgos, Palencia and Logroño. A company was constituted by practically all the Spanish breweries, in which no foreign capital was involved.

From Galicia, cultivation was extended to those areas where the abundant presence of wild hop plants had been confirmed. Test fields were established in Asturias, the Basque Country and León with favorable results, and from 1949 onwards the S.A.E.F.L. began working on communication and promotion, soon being accepted by farmers. The company also built in these areas the necessary facilities to carry out the industrialization process.

Figure 2. The hop processing plant in Betanzos near La Coruña.

The rate of production steadily increased. The region around León, with continental climatic conditions and irrigated cultivation, experienced such a growth that in six years from the start of cultivation, its yield exceeded the sum of the production of the other regions -— and continued its rise. Therefore, in just ten years since the beginning of hop growing in León, Spain had shifted from being an importer to a potential exporter of a product of excellent quality. However, after a period of increasing production in the 1950s and 1960s, a negative development began that culminated in the disappearance of the crop in Galicia, Asturias and the Basque Country. Only the area of León continued with hop growing since it was able to successfully adapt to the new agronomic cultivation techniques and the new mechanized methods of harvesting and drying, thereby maintaining the profitability of hop cultivation. Also, the S.A.E.F.L. promoted to rationalize and concentrate the entire Spanish hop production in this area.

In 1958 the S.A.E.F.L. cancelled the contracts with growers cultivating more than 1.000 plants to coerce them into drying their hops by themselves, connected with a high investment for equipment. A lot of growers then abandoned hop cultivation. In 1963, the Golding hops were replaced by the cvs Hallertauer Mittelfrüher, Fine of Alsace, H-3, and H-7. The according costs also triggered the activity of a lot of growers to not continue with their activity. In 1977 a virosis hit 77 % of the hop gardens. A lot of them were not renewed.

In 2004, Hijos de Rivera decided to recover hop cultivation in Galicia as a tribute to its founder. The main objective was making a special edition beer to commemorate the 100th anniversary of the company. In 2005, HdR got in contact with the Centro de Investigaciones Agrarias de Mabegondo (CIAM) and the S.A.E.F.L., seeking for advice and collaboration in order to recover hop cultivation in Galicia. An area of 1300 m^2 was planted with cv. Nugget at CIAM Research Farm, located in the heart of the area devoted to hop cultivation in fromer years. Due to the good results, in 2007 a new plot of 8700 m^2 was planted with 83 % cv. Nugget and 17 % cv. Columbus. Currently, HdR manages five hop gardens with a total acreage of 13.62 ha and has tested around 15 different cultivars. Main challenge is to reduce the effects of the climate change that is beating very hard in Galicia by testing new cultivars and new agronomic practices.

Every December since 2006, Hijos de Rivera, S.A. produces a 100 % malt Christmas Beer called: “Estrella de Navidad”, made with hop cultivated exclusively in Galicia. The production is about 2.500 hl, bottled in 33 cl or 75 cl formats, reaching a very good acceptance among consumers.

Nowadays, in Spain around 570 ha of hops are cultivated, with a total yield of *ca* 1.000.000 kg of hops for the brewing industry. The majority of hop gardens is planted with cv. Nugget.

Figure 3. Panoramic view of Galician hop growing in the Betanzos valley in the 1930s.

Figure 4. Traditional hop picking in the Betanzos valley.

Hop breeding in the Czech Republic

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Abstract

Yield and alpha acid content have always been preferred in hop breeding. In the Czech Republic, breeding was focused on the Saaz selection. Since 1990, hop breeding has only been a method of crossing. A number of aromatic and bitter hop varieties were obtained. Since 2000, hop breeding has focused on resistance to fungal diseases, *Verticillium* wilt, and the stability of agronomic and brewing traits. In recent years, the stability of qualitative and quantitative features has been preferred. The target is drought resistance. For this reason, the breeding material is tested for the stability of the traits during hop growing. Genotypes with low variability are the basis of hop breeding in the Czech Republic. We monitor drought resistance in the greenhouse using water stress. We test resistant genotypes in hop growing and breweries. Twenty-one promising genotypes were selected in 2021.

Key words. hop, *Humulus lupulus* L., resistance, yield, alpha acids, hop oils, drought resistance

Introduction

In the 1960s, hybridization started to be used in hop breeding. In 1994 Bor and Sládek were the first two hybrid varieties registered in the Czech Republic. Premiant was released in 1986. It was the variety that later replaced Bor because of its higher productivity. In 2001 the first Czech bitter variety, Agnus, was registered. Its alpha acid content is at the level of 10 % (NESVADBA 2002). Since 2004 till 2010 other six varieties (Harmonie, Rubín, Kazbek, Bohemie, Saaz Late and Vital) were registered by theHop Research Institute in Žatec (NESVADBA et al. 2013). In 2017 two other bitter varieties, Gaia, and Boomerang, were released. New aroma varieties Saaz Brilliant, Saaz Comfort, Saaz Shine and Mimosa were registered in 2019 (NESVADBA & CHARVÁTOVÁ 2020). The outcome of breeding aimed at dwarf varieties are Country, Jazz, and Blues, released in 2018 and 2019.

Hop varieties are characterized by yield, resistance to disease and pests, sensitivity to agrotechnological interventions as well as the content and composition of hop resins and oils (ČERENAK et al. 2015). The performance of hop varieties is dependent on these characteristics. However, another crucial attribute is the stability of quantity and quality parameters. There has been a lack of precipitation in the last few years, which has had a negative impact on the quantity and quality parameters of hop varieties (KROFTA et al. 2019). Therefore, research project QK21010136 entitled “Application of new hop varieties and genotypes resistant to drought in hop growing and beer brewing“ was launched in 2021. The evaluation of stability during the growing period is essential for breweries. A lack of precipitation is naturally only one of the parameters influencing the stability of these characteristics. The evaluation does not focus on the influence of weather on the content and composition of hop oils. Its objective is to evaluate the stability of the content and composition of hop oils in Czech hop varieties. It is of great importance to brewers to know which hop varieties show a stable or, in contrast, a non-stable content and composition of hop oils.

Material and methods

The creation of hop varieties is based on the hybridization method. Seeds from suitable parent components are sown in a greenhouse and young plants are tested in terms of their resistance to *Pseudoperonospora humuli* and *Sphaerotheca humuli.* Resistant and tolerant plants are planted in a breeding hop field. In the second year of cultivation, the best genotypes are selected for the second breeding stage. Upon a five-year evaluation, the best genotypes are propagated and planted three times in a testing nursery and later on as part of field and zoning experiments.

At that stage, features important for hop growing and beer brewing are evaluated in greater detail. The best genotypes are submitted for registration experiments. New hop varieties are evaluated in breeding nurseries, field experiments and piloting areas. Mother plants are always original. Therefore, the performance of some hop varieties can be lower than that of virus-free plants. At the moment, each variety is being monitored in a maintenance breeding program including a minimum of 40 plants. Every year, 10 mother plants are evaluated. Each mother plant is evaluated in terms of morphological features with regard being paid to any deviations from the uniformity of the particular hop variety.

The following is evaluated for each variety: hop yield, content, and composition of hop resins (EBC 7.4; KROFTA 2008), content and composition of hop oils (based on liquid chromatography). Each plant is harvested separately. An experimental Wolf picking machine is used for hop picking. Yield is shown in kg of fresh hops per plant (hereinafter: kg/plant). The conversion of hop yield is based on the number of plants per hectare, which amounts to 2,900 plants at a spacing of 1.14 x 3.00 m. The coefficient of dry matter in fresh hops and dry hops is 4. The following statistics were prepared: average (A), median (Med) and standard deviation (s). Relative amount of variability is used to compare a set with different levels. Resulting variability amounts are dimensionless numbers (mostly in %). This makes it possible to compare the variability of statistical features differing in measure units. Coefficient of variation (CV), showing the extent of variability in %, was used for data processing. The t-test was applied to determine and prove the difference between hop varieties. The difference of sets was determined on the basis of significance level, which shows the probability of difference of the tested sets. For example, if the significance level is determined as p = 0.01, meaning that there is a 99 % probability that the sets under review are different.

Results

Evaluation of hop varieties with resistance to Verticillium nonalfalfae in growing conditions in the Czech Republic

Based on the results, the following hop varieties were selected from the field collection:

1. Moderately tolerant: Bramling Cross (England), Savinskij Golding (Slovenia), Aurora (Slovenia), Bobek (Slovenia)
2. Tolerant: First Gold (England), Target (England), Pioneer (England), Pilot (England)
3. Resistant: Cascade (USA), Phoenix (England)

On the base of tests with hop varieties aimed at resistance/tolerance to *Verticillium nonalfalfae*, we have selected just those ones showing resistance/tolerance to this dangerous disease. Since 2011 to 2020 we evaluated yield of hops and alpha acid contents including their variability. The highest yields have the resistant varieties Target and Cascade, and the highest content of alpha acids have the resistant varieties Phoenix and Target. Phoenix shows high variability of the observed characteristics. From the group of resistant genotypes, Cascade and Pioneer show the best productivity and low variability under Czech hop growing conditions.

Figure 1. Linear regression of the hop yield decrease in cvs Bobek (left) and Cascade

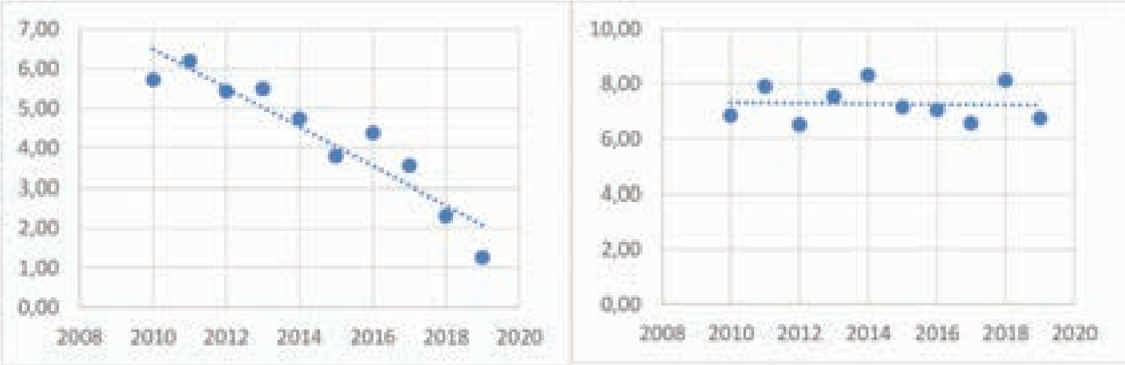

Figure 2. Linear regression of the decrease in alpha acid content in cvs Bramling Cross (left) and Premiant

Evaluation of resistance to Pseudoperonospora humuli and of the content of alpha acids and hop oils in selected genetic resources of hops

Twenty genotypes were selected for an evaluation of both primary and secondary resistance to *Pseudoperonospora humuli* as well as of the content of alpha acids and hop oils. In the category of wild hops, two wild hops from Canada and one from Belgium show resistance. Among registered hop varieties, the Czech hop varieties Kazbek and Boomerang have the highest resistance. Both wild hops from Canada have the highest content of alpha acids among wild hops, namely 4 % w/w. The lowest variability of alpha acid content in the category of wild hops was found in two wild hops from the Caucasus, one from Austria and one from Lithuania. The highest content of hop oils was determined in two hops from Canada and two from Belgium. Wild hops from the Caucasus have the lowest variability of hop oils among wild hops. Two hops from Canada and one from Belgium were selected for breeding aimed at drought resistance.

Table 1. Evaluation of resistance to primary and secondary infection by *Pseudoperonospora humuli* in selected hop genotypes, including the average content of alpha acids and hop oils

Genotype	Origin	Primary (Average points)	Secondary (Average points)	Alpha (% w/w)	Oils (% w/w)
Antler	Canada	3	3.8	4.24	0.51
Belt	USA	3	5	2.64	0.42
Blues	Czech	5	3.4	7.47	1.07
Boekhoute	Belgium	4.2	3	2.86	0.53
Boomerang	Czech	3	3.4	12.06	2.35
Fishing lakes	Canada	3	3	4.09	0.48
Francuzy	Lithuania	3	5	2.18	0,.33
Gaia	Czech	4.2	3.8	13.34	1.96
Kabarda	Caucasus	3	5	2.43	0.29
Kauno	Lithuania	4.2	5	2.21	0.47
Kazbek	Czech	3	3.4	5.42	1.16
Madame	Spain	5.8	5	2.07	0.17
N2	Czech	4.2	3	4.13	1.15
P132	Austria	4.2	3.8	2.74	0.27
Pilgrim	England	3	4.5	6.96	0.73
Poperinge	Belgium	3	3	1.99	0.49
Rhona	Switzerland	5.8	3	1.35	0.16
Sunža	Caucasus	3	5	2.17	0.15
Toses d´alas	Spain	3	5	1.87	0.29
Ursdon	Caucasus	5	3.4	1.50	0.09

Evaluation of resistance to Pseudoperonospora humuli was based on the Hop Classifier (RÍGR & FÁBEROVÁ 2000) according to a point scale and is divided into two categories:

Primary infection (occurrence of spike-like shoots):
3 – resistant (no occurrence of spike-like shoots)
5 – medium resistance (1 to 5 spike-like shoots were found)
7 - susceptible (6 and more spike-like shoots were found)

Secondary infection (damage to hop cones):
3 – resistant (no damage)
5 – medium resistance (damage below 10 % of hop cones)
7 -susceptible (damage above 10 % of hop cones)

Development of genotypes for drought resistance

We are working on a project ‘Application of new hop varieties and genotypes resistant to drought in hop growing and beer brewing’ in the years 2021 to 2026. We monitor drought resistance in the greenhouse using water stress. We test resistant genotypes in hop growing and breweries. Twenty-one promising genotypes were selected in 2021. The new Juno variety also shows drought resistance.

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Hop breeding in France: history and achievements

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Abstract

Hop (*Humulus lupulus* L.) has been grown in France in agricultural production since the beginning of the 19th century. First breeding actions were made in the 1980s to improve the old land race Strisselspalt by mass selection: the best plants in the best hop garden were multiplied.

The initial considerations about a real and own breeding program in France began in 2000. Our historical variety Strisselspalt is a fine aroma variety well adapted to the Alsatian weather conditions but with a low alpha acid level. The first idea was to cross a mother plant of Strisselspalt with males that could provide a higher level of alpha acid (first crosses in 2001).

Then, with the increasing demand for flavor hops, some other crosses were made from 2007 onwards to achieve other goals. The most recent crosses aim at the breeding of more resilient hop varieties. The choice of the crosses was made by Dr Peter Darby at the time when he was working at Wye College, UK, and he continues to be involved in this project. Male plants are still maintained by Wye Hops.

From the beginning of the program, hop gardens were provided by the Agricultural School of Obernai and greenhouses facilities by a local gardener. All the assessments, the harvest and the sampling are handled by Comptoir Agricole. Further trials are made by growers in their own hop gardens, thereby providing bigger samples to make brewing tests in several breweries.

The first cultivar released from this breeding program was Aramis in 2015, which was followed by cvs Triskel, Bouclier, Mistral, Barbe Rouge and now Elixir.

Key words. Hops, variety, breeding, Strisselspalt, Alsace

Acknowledgement

We would like to thank Peter Darby for providing scientific support during all those years and for sharing his experience. We also thank the technical team of the Agricultural School of Obernai for the farming operations.

Hop breeding in Slovenia – influence of storage on hop quality

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Abstract

Hop cones are one of the main ingredients used in the brewing industry. In the Slovenian breeding program, established 70 years ago, besides agronomically important traits included in the development of hop varieties, brewing goals play a major role as well. Today, 98 % of Slovenian hop fields are planted with own hop varieties which are covering bitter, aromatic and in last years also flavour classifications. Flavour varieties were recognised as an popular addition to classical varieties and their acreage is nowadays stable as a response of global hop market. Consequently, IHPS has developed new candidate varieties with traditional aroma, high alpha-acid content and resistance to diseases. In the disease resistance breeding, we are mainly focused on Downy mildew *Pseudoperonospora humuli*, Powdery mildew *Podosphaera macularis*, wilt *Verticillium nonalfalfae*, and during the last years to CBCVd (Citrus bark-cracking viroid). To cope with CBCVd, different strategies are in the pipeline. Selections on main diseases (Downy mildew, Powdery mildew) are at first performed at the seedling stage of the breeding program, and relationship between progeny resistance and used parental material will be presented.

Hop storage is one of the crucial things significantly influencing the hop quality. Various trials are running to discover influence of processing parameters to hop quality and its stability during ageing. In the presentation, preliminary results will be shown regarding waiting time before drying, copper content after spraying, and the shape of hop product on stability.

Key words. hop, Humulus lupulus, storage stability, selection to diseases

Steps towards marker assisted resistance breeding in UK hop

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Abstract

For the past century hop breeders have successfully implemented classical, phenotyping-based selection approaches to achieve improvements in plant characteristics such as resin content, yield, and resistances to diseases (McAdam et al. 2013). However, the identification of desirable traits using traditional selection is a time-consuming process which requires the assessment of the expressed phenotypes in the hop plants (Neve 1986). In current breeding practices resistance of breeding material to the major hop diseases Downy mildew, *Verticillium* wilt and Powdery mildew are solely assessed through artificial infection assays with no marker assisted breeding techniques being used. However, hop breeders could leverage molecular markers to bypass the phenotype-based selection methods with simple, low-cost laboratory tests on young hop seedlings which would decouple the need for extensive phenotyping from confirming disease resistance/susceptibility of breeding lines. We established a bi-parental mapping population segregating for disease resistances between cv. 'Pilgrim' and '316/1/10' for linkage map construction and QTL mapping purposes. The female genotype 'Pilgrim' is a key progenitor in the UK hop breeding programme with a complex pedigree. 'Pilgrim' carries R2 resistance to powdery mildew as well as strong resistance to *Verticillium* wilt and downy mildew diseases. The complementing male genotype '316/1/10' was derived from Alsatian 'Strisselspalt' germplasm and has been found to be susceptible to UK strains of VW disease as well as downy and powdery mildews. In total, parents and 171 individuals from the mapping population were genotyped with high-throughput DArT sequencing technology. The mapping population and parental genotypes were phenotyped for Powdery mildew R2 resistance and sex. Here we report or preliminary results on disease resistance QTL mapping and linkage map construction. The overall aim of our investigation was to generate genetic and genomic resources highly relevant to the national breeding programme through the construction of a SNP based linkage map and the development of transferrable molecular markers. This study also aims to provide a foundation for the characterisation of genetic background of resistance to pathogens for UK hop.

Key words: Linkage mapping, powdery mildew, QTL mapping, DArT sequencing

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Cryopreservation of hop pollen for hop breeding

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Abstract

The cryopreservation method is a modern method of conservation of the genetic resources of hops, eliminating the undesirable effects of biotic and abiotic stressors that represent a risk of losing valuable genotypes when grown under field conditions.

Hop breeding processes may have problems with both the availability and quality of pollen and keeping parents entering the cross or male hop plants until their quality is verified by evaluating the performance of their progeny.

This work was focused on the evaluation of the thermal characteristics of pollen with respect to its hydration in samples of hops. The water content and the following characteristics related to the behaviour of water in the biological material under ultra-low temperatures were determined: the proportion of frozen water content, melting point and glass transition temperatures. We found that the resulting thermal properties were influenced mostly by water content and, conversely, the effect of variety and plant species was not significant; the dependence of the studied parameters was a function of the water content regardless of the tested genotype. When the water content falls below 0.27 g of water per gram of dry matter, water does not crystallize and at the same time, the presence of a glass transition has always been demonstrated.

Key words. Cryopreservation, differential scanning calorimetry, glass transition, *Humulus lupulus* L., thermal analysis

Acknowledgement

This study was supported by the research project QK1910277 of the Ministry of Agriculture of the Czech Republic

II: Organic hops

Organic hop cultivation in France: 10 years from the first historic grower to the 'neo growers'

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Abstract

In France, hop (*Humulus lupulus* L.) is in agricultural production since the beginning of the 19th century. With the support of the ministry of agriculture and education, the first grower beginning with organic hop production was the Agricultural School of Obernai in 2012 after three years of conversion. At that time, there were 18 ha under organic regime. By 2017, the school's acreage increased to 24 ha and covers now an area of 30 ha of certified organic hops. The agricultural school was followed in Alsace by one grower finishing organic conversion in 2017 and three others between 2018 and 2020. The organic hop acreage of these five farms in Alsace has reached 76 ha in 2022.

After the boom of microbreweries, the increase of the 'consume locally' (and organically, if possible), the demand for French organic hop was higher than the supply. First organic hops outside of the historical region of Alsace/Grand Est were planted in 2016.

In 2020, 125 chiefly new organic hop growers – often referred to as 'neo growers' – were listed by l'Agence bio (French agency for development and promotion of organic farming) in France cultivating organic hops on 83 ha. Of this acreage, 60 % are still located in the historical hop growing region.

For the pelletising of the increasing organic hop production, the Alsatian cooperative (Comptoir Agricole) has invested in a new pelletising plant starting with the harvest 2021 (50 metric tons).

The Alsatian hop growers have built a team for sharing experience and regularly join the meetings of the other European organic hop growers from Germany, Austria and Belgium. Despite the high costs of manpower and the difficulties to manage pest and diseases, the organic hop growers stay positive and enthusiastic.

Key words. Hop, organic, France, sharing experience.

Acknowledgement

We would like to thank the other European hop growers for sharing their experience.

Marketing of organic hops – challenges from the viewpoint of a hop marketer

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Abstract

Of the total hops produced and marketed, organically grown and certified hops account for a very small proportion of less than one percent. At the beginning of organic hop production in the late 1980s, direct contracts between the few growers and breweries were the norm, whereas the market is now experiencing a significant increase in demand, presenting hop marketers with the challenge of including organic hops in their portfolios. The few growers cannot serve the large number and diversity of requests with direct marketing. This presents a new and complex challenge to hop marketers as the requirements for trading organic food are very strict, varying even within the EU from country to country, and organic production is subject to greater yield fluctuations than one is used to in conventional production. All of this results in increased administrative burdens for marketers at greater financial risk.

The basic requirement for selling organic produce is that the producer, the processor, and the seller themselves are certified organic. This means another service provider to be taken care of and paid for – the certification company. In at least annual organic control audits, the company to be certified is checked and, if necessary, conditions are determined that must be implemented by the next audit. It is also difficult to find acceptance within the company, since the share of organic hops in the total processing and trading volume is very small.

There is no standardized set of contracts for organic hops as in the conventional hop trade in Germany, where the ‘Deutsche Hopfenwirtschaftsverband’ is continuously working on the further development of the Hop Supply Contract and its General Agreements. Special requirements, such as the producer's current annual organic certificate, clarification of required pesticide analyses, necessary markings on the goods, and declarations on the papers, etc., must all be specified in individual contracts. Particularly in times of digitalization, in which work is done with automated templates, this is a large effort compared to the low volume of goods.

The same applies to the sales side, where the existing contracts do not yet provide for any organic specific content, for example in the case of complaints due to potential pesticide residues. The most frequent case here is the assumption of costs for processing and clarifying the receipt of organic status, which in turn must be handled by the buyer's and seller's certification companies as well as under-deliveries due to increased yield fluctuations.

In the quantity planning and allocation of purchased organic hops, a high degree of accuracy is required since there are no uniform regulations for the permitted maximum residue levels, neither internationally nor EU-wide. The situation is aggravated by the fact that there are hardly any legal anchors, but rather experience and guideline values from industry associations are often used; this leads to uncertainties or misleading expectations on the part of those involved. Since quantities are expensive and scarce, the marketer has little chance to replace quantities that, due to residues, have become unusable for certain countries.

Organic hops are also subject to greater risk in import and export and require increased attention, as irregularities or omissions in export and import declarations can not only lead to a delay or fine, as in conventional trade, but can mean the deprivation of the organic status of the product, almost devaluing a very expensive product for the marketer.

Key words. Organic hop, marketing

Acknowledgement

This work provides insight into the applied practice of organic hop marketing. Special thanks therefore go to all colleagues at BarthHaas GmbH & Co. KG.

III: Entomology

Efficacy of the predatory mite *Neoseiulus californicus* (McGregor) against the two-spotted spider mite *Tetranychus urticae* Koch in a hop garden

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Abstract

In 2015, we studied the efficiency of the predatory mite *Neoseiulus californicus* (McGregor) for suppression of the Two-spotted spider mite *Tetranychus urticae* in a field experiment at a hop plantation. We randomly arranged four treatments in a three-block experiment. Fungicides were used in all treatments; insecticides were used in all treatments except the predatory mite treatment and acaricides were used in only two treatments. A single inundative release of the mite *N. californicus* was carried out on 4 July. On four different dates (10 June, 17 July, 29 July, and 9 August), we counted the eggs and the mobile stages (larvae, nymphs and adults) of the two-spotted spider mite in all four treatments. In the treatment with the predatory mite, we established the fewest eggs and mobile stages of *T. urticae* 14 days after the release of the predator. The selected acaricides in our research acted in a primarily ovicidal manner, but we did not detect satisfactory effects on the mobile stages of the spider mites. This result suggests the emergence of resistance of two-spotted spider mites to the acaricides hexythiazox and abamectin. Our research established comparable effects of the predatory mite *N. californicus* and acaricides, and further improvement of the efficiency would require release of the natural enemy into a hop plantation in mid-June, followed by a second release three weeks later. The costs of acaricide use in our experiment were from 12.7-fold (two sprayings of hexythiazox, and a single spraying with abamectin) to 17.8-fold (single treatments of hexythiazox and abamectin) lower than those of a single release of the biological control agent in question. The results of our study may represent a starting point for future research, which could achieve satisfactory results in suppressing Two-spotted spider mites in hops by repeated use of the predatory mite *N. californicus.*

Key words. two-spotted spider mite, *Humulus lupulus*, predatory mite, biological control

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Evaluation of a technical solution for the application of predatory mites in hops

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Abstract

In 2021, the first prototype world-wide for the release of predatory mites for the control of Two-spotted spider mites *Tetranychus urticae* Koch (TSSM) was developed by Koppert in The Netherlands and tested in a field trial in the German Hallertau. Released predators were a mix of *Phytoseiulus persimilis* Athias-Henriot and *Neoseiulus californicus* (McGregor). The new application method with the prototype was compared with a targeted application via bean leaves and an untreated control. As a fourth treatment, the technical application was supplemented with a second, manual release. At harvest, all four treatments had lower TSSM numbers than the untreated control, but only the plots with targeted, manual release had significantly lower numbers. In general, TSSM pressure was low in 2021 and an experimental harvest showed no significant differences in yield and alpha acid content of all treatments, including the farmer's own practice with two acaricide sprays. The technical release approach, which was not satisfactory yet in 2021 alone, will be improved and adjusted to a more targeted release in future trials – an according trial is already running in 2022.

Key words. Tetranychus urticae, spider mites, predatory mites, release, pest control, beneficials

Introduction

Seen globally, Two-spotted spider mite *Tetranychus urticae* Koch (TSSM) is by far the most prevalent arthropod pest of hops. Spider mites are generally controlled in every hop-growing region worldwide by preventative, often multiple, spraying of acaricides. However, in Integrated Pest Management a biological approach to TSSM control is first priority and suitable solutions are badly needed. Predatory mites such as *Typhlodromus pyri* Scheuten, *Phytoseiulus persimilis* Athias-Henriot or *Neoseiulus californicus* (McGregor) are able to control TSSM, either as established population in a crop or as bred, released antagonists. In hops, where established, overwintering populations of predatory mites (especially of T. pyri) are currently largely lacking, many trials with released predatory mites have already been conducted and yielded successful spider mite control (*e.g.*, VOSTŘEL 2001, 2003; WEIHRAUCH 2008; OBERMAIER & WEIHRAUCH 2019). However, one obstacle for the implementation of predatory mites in an IPM approach to TSSM control is the high amount of manual labour when releasing the beneficials, connected to corresponding costs for the farmer. Therefore, a competitive technical solution for the release of predatory mites would be a milestone for biological pest control in hops. In this study, we describe a first attempt of a technical solution for the release of predatory mites in hops as the first step of an ongoing process.

Material & methods

The study resulted from a scientific cooperation between the enterprise Koppert B.V., represented by Koppert Germany, and the Hop Research Center Hüll of the Bavarian State Research Center for Agriculture. The prototype for the technical application of predatory mites (Fig. 1) was developed and assembled by Koppert B.V. in The Netherlands and the field trials were conducted 2021 in the Bavarian Hallertau in a farmer's own field (cv. Herkules), in Dürnwind in the district of Landshut. Experimental lay-out consisted of four treatments: (i) Predatory mites released on bean leaves, on which they had been bred (17 June), (ii) technical release with the Koppert prototype (15 June), (iii) technical release (15 June) and additional manual release in Koppert Dibox distribution boxes (7 July), and (iv) untreated control.

Each treatment comprised four replications of ca 550 m^2, totaling to 16 plots with an experimental area of almost 1 ha. Released predatory mites were a commercially available mixture of P. persimilis and N. californicus. Monitoring of TSSM and predatory mites was conducted four times during the field season (21 June, 12 July, 12 August, 07 September 2021), using the standard procedure of the Hop Research Center. On 14 September, an experimental harvest was operated in one plot of each treatment, comparing yield and alpha acid contents. As a conventional standard, we chose a farmer's practical plot in the same field, which had been treated twice with an acaricide (spirotetramat and spirodiclofen) during the 2021 field season.

Figure 1. First Koppert prototype for the release of predatory mites in a hop garden. Dürnwind, Hallertau, Germany; Photo: FW (15 June 2021)

Results

The TSSM numbers recorded in the four plots were generally at very low levels until mid-August and only the last monitoring, on 7 September, yielded noteworthy values with more than 50 adult individuals per leaf in the untreated control. Treatments (i) and (iii) showed significantly lower infestation levels briefly before harvest, reaching less than 10 and 20 adult mites per leaf, respectively, on average (Fig. 2).

During the experimental harvest, no significant differences in yield between the treatments with released predatory mites, the untreated control and the farmer's own practice with two acaricide sprays was recorded (Fig. 3). With 547 kg /ha, the alpha yield was lowest in the acaricide-treated rest of the garden, compared to the four experimental plots, which reached between 623 and 572 kg/ha alpha.

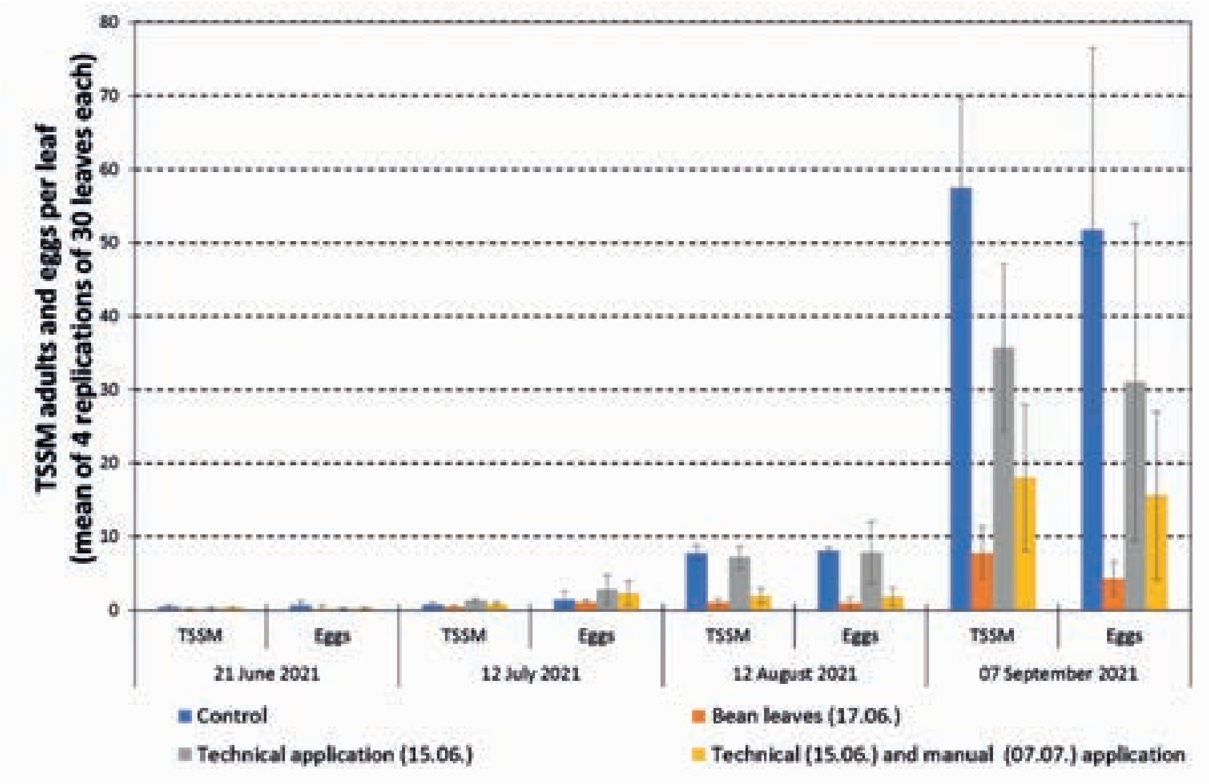

Figure 2. Effect of various release methods of predatory mites (Neoseiulus californicus, Phytoseiulus persimilis) on the development of Tetranychus urticae in a hop garden. Dürnwind, Hallertau, Germany, 2021, cv. HKS

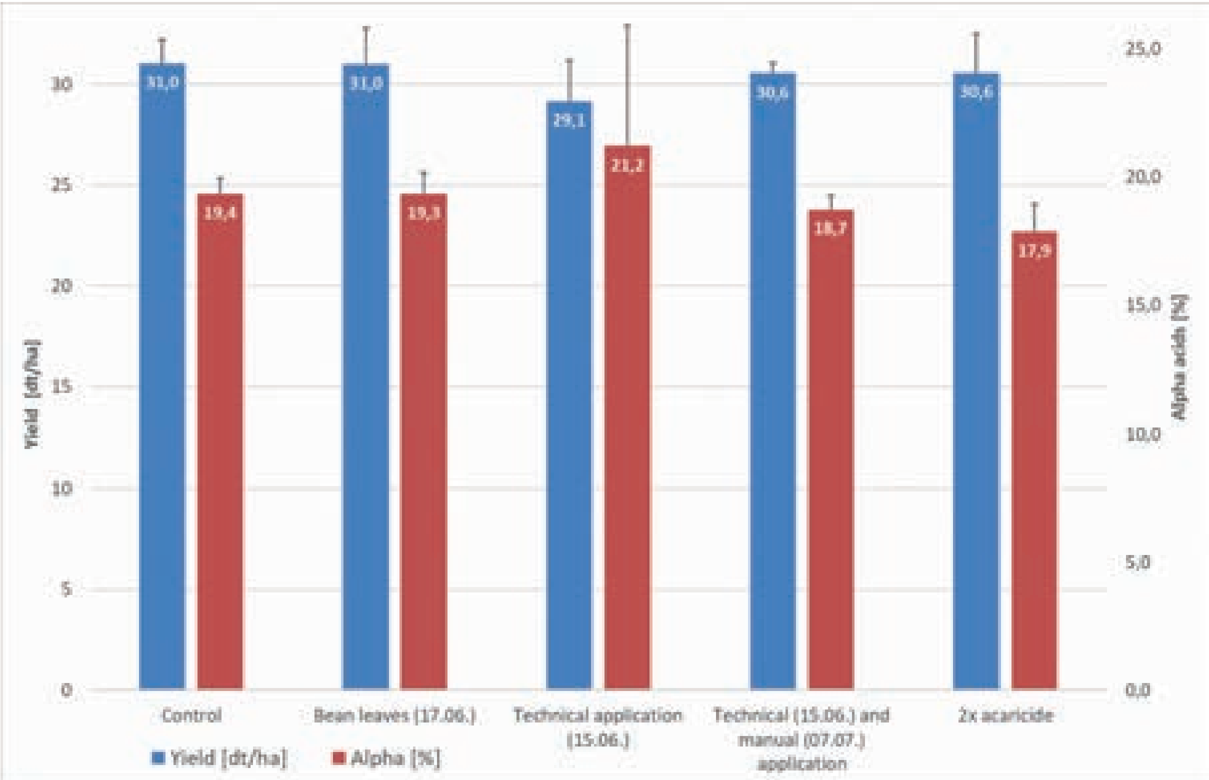

Figure 3. Effect of various release methods of predatory mites (Neoseiulus californicus, Phytoseiulus persimilis) for the control of Tetranychus urticae on yield and alpha acid content of harvested hops, as compared to the farmer's own practice using two sprays of acaricide. Dürnwind, Hallertau, Germany, cv. HKS, experimental harvest on 14 September 2021.

Discussion

Generally, the results of the 2021 trial showed that TSSM infestation during that year was low. Accordingly, the experimental harvest proved that (chemical) control would not have been necessary, as the untreated plot yielded the same (or even slightly better) alpha acid amount as the farmer's own practice with two sprays of acaricide.

At first sight, the results of what was likely the first attempt world-wide to release predatory mites in hops using a technical device as an assistance do not represent a full success. In combination with the rather late timing of the release and the according height of plants, the prototype – with three tubes at each side to blow the predators into the canopy (Fig. 1) – obviously distributed most predatory mites on the ground and not on the plants. This led to a much less dense predator population on the hop plants in this plot, compared to those two plots where the beneficials were released directly on the plants – albeit with much more manual labour – and a connected, reduced control effect in the plots with technical release.

On the other hand, the experiment proved once more that predatory mites, if released targeted and well-timed, are able to control TSSM at a similar level as chemical control can do. Thus, the control of TSSM via this biological, environmentally friendly modus operandi is probably the most promising approach for IPM in hop cultivation. The reduction of manual labour when releasing the predators via the development of a device that is better adopted to the specialty crop hops is therefore highly desirable; a well-working release method with a tractor might also facilitate the future acceptance of hop growers towards working with beneficials. In 2022, a new, slightly modified prototype has already been developed by Koppert and was used for the release of predatory mites at an earlier phenological stage. The results of the 2022 trials will however be available only later.

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IV: Hop cultivation and management

Closing the loop of biomass on hop farms by composting hop biomass after harvest

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Abstract

During hop harvest, the entire above-ground biomass of plants is removed from the field and taken to the harvest machine. While cones, presenting one third of the biomass, are harvested, dried, and packed, stems and leaves are left next to the harvest machine as a by-product. This left biomass is a valuable source of organic matter and nutrients. However, in the Slovenian reality, the problem is a synthetic polypropylene twine, which is used as a support for hop plants during growth and remains intertwined in hop biomass after harvest. It does not decompose by composting, thus aggravating the use of this biomass as raw material and at the same time posing an environmental problem.

With the introduction of 100 % biodegradable and 100 % on-site compostable PLA twine in hop production within the LIFE BioTHOP project, the hop biomass after harvest became a valuable resource for composting and material for various bio-plastic and moulded products. Focusing on the narrowest circle of circular economy on hop farms, implemented by this new opportunity, we can say that on-farm composting is the most promising in terms of utilizing biomass to close the nutrient cycle on hop farms. On-farm composting could be an efficient, cost-effective, and environmentally safe biological process for the recycling of residual agricultural biomass. It is an exothermic decomposition that depends on material mixture, moisture, volume, material composition, pH, particle size and their distribution, mixing, and aeration.

In close collaboration with hop growers from Lower Savinja Valley, which was the project's demo region, macro experiments with 675 tons of hop biomass after harvest were performed by the Slovenian Institute of Hop Research and Brewing during the three years in order to find the proper way of this biomass on-site composting. The research was focused on finding a feasible on-farm composting treatment of plant biomass to produce safe and quality compost at which also the PLA twine will degrade. Three different composting treatments were prepared and followed (with different additives at the start – biochar and effective microorganisms, no additive; covering and not covering the pile; different start particles size). The piles were regularly turned according to the temperature measurements. Samples of final, mature composts were analysed in April for nutrient concentrations, phytotoxicity and bacterial and fungal presence after seven months of composting. We were also collecting the leachate from the composting biomass during the process in order to minimize it as much as possible.

All final composts had no phytotoxic properties and were stable and ready to use in plant production, according to the radish germination index. They were nutrient-rich (in 100 g of dry matter, the average compost contained 2.7 g, 0.38 g and 1.08 g of N, P and K, respectively) and proved being biostimulative as soil amendment, taking the cress germination test into consideration. They were stable in terms of respiration rate, growth, and germination tests. The hygienisation standard was met by all piles as all of them had temperatures over 55°C for more than 14 days. Elevation of temperature in all piles indicates that at least 15 tons of start amount of hop biomass (this is the biomass from approximately one hectare of hop field) is enough to start composting process.

Results have shown that hop biomass after harvest has great potential for composting. The best results were observed when having small start particle size (<5 cm) and when we used the whole biomass for composting (leaves and stems), not only stems. Composting the whole biomass after harvest is more efficient than composting stems alone, as it provides more nutrients and less empty spaces, thereby generating a longer thermophilic phase, which is crucial for PLA twine degradation and hygienisation as well.

Considering leachate, it is recommended that a semipermeable membrane is always used during the maturation phase as well as during any heavy precipitation expected in the thermophilic phase (in the first 2 months). There was a strong linear correlation between the amount of precipitation and leachate quantity (0.86), NH_4 leached amount (0.87), and total N leached amount (0.92), namely, if the pile was not covered. The composting procedure had a significant impact on the quantity of the NH_4 leached amount. The majority of the NH_4 was lost in the second month of composting. The maturation phase was the most critical for NO_3 loss since it had the highest amount of leached NO_3 and the greatest variances among the composting protocols.

LIFE BioTHOP project is financed by the EU LIFE Programme, Slovenian Ministry for Environment and Spatial Planning, Municipalities of Lower Savinja Valley and Association of Slovenian Hop Growers. BioTHOP Consortium forms a transnational partnership, comprised of seven partners from five EU Member States: Slovenia, Portugal, Spain, Germany, and Czech Republic.

Adopting the Leaf Wall Area (LWA) system to hops

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Abstract

Dose expressions for the application of plant protection products in two-dimensional field crops and in high growing crops such as hops were specified with kg or l per ground area. In case of three-dimensional high growing crops the target leaf area, which should actually be protected, can differ from the ground area, which is just used to grow these crops. To express this target area the concept of the Leaf Wall Area (LWA) was defined. With the EPPO Guideline PP1/239 the biological efficacy assessments for plant protection products in hops must be executed using the leaf wall area system. For hops numerous problems had to be faced until the LWA-system was ready to use in the official plant protection product testing in the hop research center in Hüll. In this article the crucial problems and the main concerns are briefly presented.

Key words: Leaf wall area, Leaf surface area, harmonization of plant protection products dossiers

Introduction

Since 1 January 2020, application dossiers for plant protection products in high growing crops are only accepted when the trials were executed according to the Leaf Wall Area (LWA) concept. In Germany for hop since 2018 the documentation for the LWA parameters in efficacy trials had to be collected to convert the data, when the LWA concept is established. The EPPO Standard PP 1/239 (2) has been updated to PP 1/239 (3) and was published on 24 February 2021.

Dosage expression models for hops

Three step model (current system in practice)

The current used three step model for dose expression in hops is based on the ground area. The scaling of needed plant protection products depends on the current state of plant development. There are a few examples in early stages when hop is treated as single plant in the hop garden, or for hop stripping, but when it comes to the use of the power sprayer the three-step model is executed. The steps are 75 % of trellis height, before flowering, and beginning of flowering. Practical experience has shown that plant protection in hop is crucial after the beginning of flowering.

Leaf Wall Area

The Leaf Wall Area is defined by a fixed formula: $LWA = \frac{1\text{ha}}{\text{R}} * LWH * 2\ Sides$

(LWA = Leaf Wall Area [m²], R = Row spacing [m], LWH = Leaf Wall Height [m])

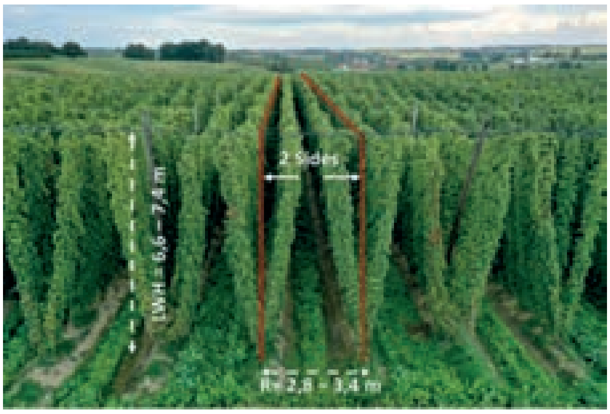

Figure 1. Hop garden with visualized parameters of the LWA formula

Most parameters of the linear formula are already set with the construction of the hop garden and its training type.

Hops in this context

Hop is one of the fastest growing plants. After hop training, in the beginning of May it usually reaches full height (7 m, depending on the hop garden height) at the end of June. According to the LWA formula, in which the treated plant height is the only variable factor, the crop officially reached its full LWA at this point. At this moment (BBCH 39) the vegetative plant growth has not been finished. Most hop varieties start to develop their typical habitus after reaching full height of the hop garden, when the growth of the lateral shoots accelerates.

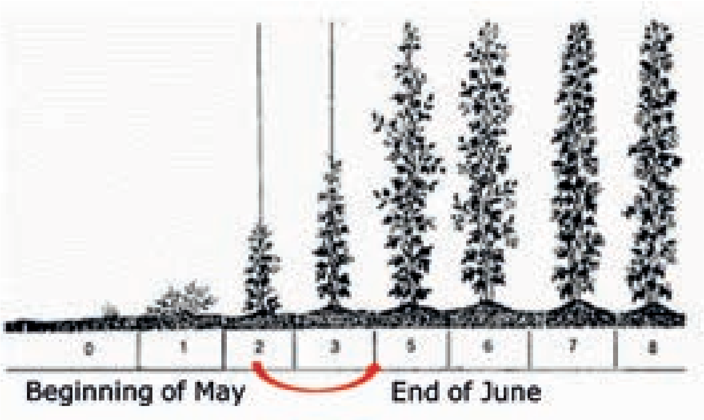

Figure 2. Development of hops with major BBCH stages

Leaf surface development

To cope with the problem of the unsimilar development of plant height and plant habitus, the surface area of two extremely differing cultivars (Herkules and Perle) was measured by digital picture analysis. This data was used for a better understanding what is happening in the hop garden.

Main hop training systems

In Germany the most common hop training system is the wide spacing trellis. In this hop yards the plants are trained in V-shape which means that one hop plant has two bines with usually three shoots each. In Germany most of the hop yards are seven m high measured form the soil level. The hill which is formed in the period from May until July is not considered. The relevant range of row spacing is between 2,8 m to 3,2 m. Usually the rows of older hop yards tend to be narrower. The wide spacing trellis can be built in different ways, which is not relevant for LWA calculation.

The outdated former training system is called narrow-gauge trellis. This training type was also 7 m high but with a smaller row spacing of 1.4 to 1.5 m. In these hop yards every plant has just one bine. These hop yards were later converted to “Normalanlagen” by grubbing every sixth row to form beds of five planting rows surrounded by two spraying lanes. For the calculation of modern dosage system such as LWA these training systems seem to be insignificant.

Results

Comparison of LWA and leaf surface area development in hops

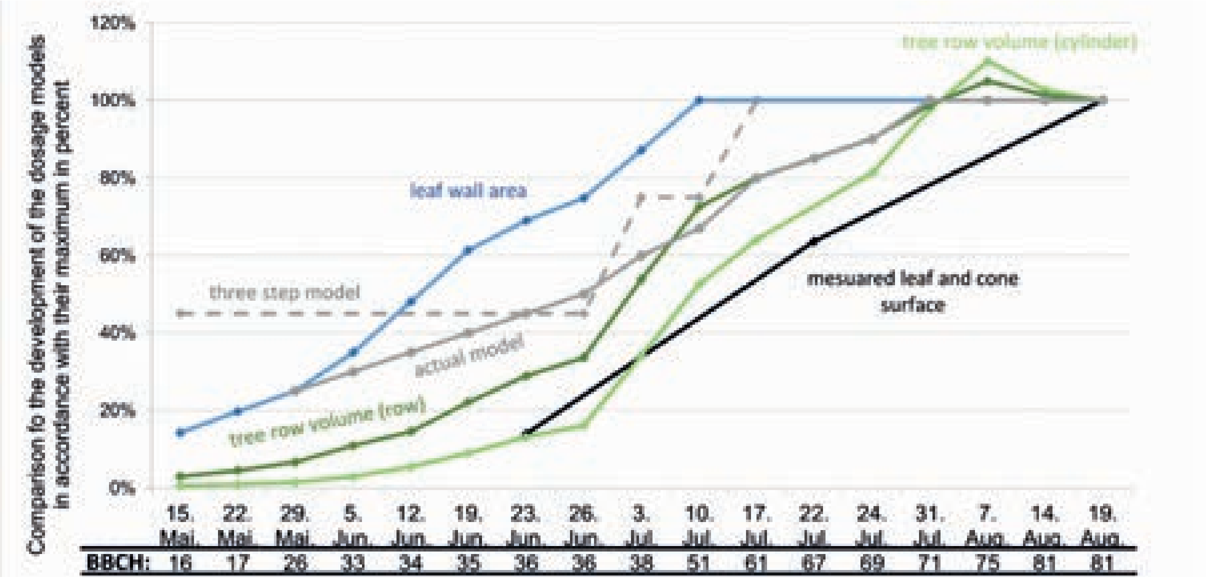

Figure 3. Comparison of the development of the dosage models in accordance with their maximum in percent

The graphs of the dose expression in Figure 3 are shown regarding the different ways to calculate the recommended dosage. The grey line shows the current three-step model farmers use for applying plant protection products. The black line shows the development of the hop plants' surface, which actually has to be protected. The two green lines show the increase of the tree row volumes one time calculated as hedge row; and the other time calculated as isolated cylinders. The line graph shows the growth of the leaf wall area during the vegetation period. The LWA increases fastest because the only variable in the calculation is the height of the shoots, and its maximum is reached when the hop starts to get to the top of the trellis in the end of June/beginning of July. In the volume models the diameter of the hop plant is besides the growth height also an important factor. The height and diameter of a hop plant increase simultaneously, but the maximum diameter is reached after the growth in length has ceased. As a result, the increase in volume is slower compared to the LWA-model using only plant height as parameter.

The measured plant surface is the last graph to reach its maximum and it possibly is still slightly increasing until harvest happening approximately four weeks after the last measurement.

Validation of the LWA concept by remote sensing

Table 1. Validation of the LWA calculation by remote sensing of two different cultivars

	cv. Herkules		cv. Perle	
	D2	**D3**	**D2**	**D3**
area per training [m^2]	6.7	6.7	4.1	4.3
area ha [m^2]	26300.8	26173.3	18432,2	19055,7
% of LWA	140.3	139.6	98.3	101.6

The figure above shows the cross section measured by a remote sensor on a drone and later the surface was calculated for one side of the V-training and must be doubled for a compare with the LWA.

For the first measurement the hop plants were too small to be detected by the sensor correctly and the collected data was not usable. Therefore, only data of the measures taken place later in the vegetation period is shown. Comparing the LWA and the area measured by remote sensing we get nearly 100 % for cv. Perle, which is optimal, and around 140 % for cv. Herkules. The measurement error is higher for the cv. Herkules because the side shoots are growing together making it impossible for the sensor to distinguish between two plants growing next to each other.

Estimated LWA range in German hop production

Table 2. LWA formula cross table of possible parameter combinations in German hop production in wide spacing trellis with V-shaped training

m²		LWH = Leaf wall height [m]				
		6,6	6,8	7	7,2	7,4
R = row spacing [m]	2,8	47.143	48.571	50.000	51.429	52.857
	2,9	45.517	46.897	48.276	49.655	51.034
	3,0	44.000	45.333	46.667	48.000	49.333
	3,1	42.581	43.871	45.161	46.452	47.742
	3,2	41.250	42.500	43.750	45.000	46.250
	3,3	40.000	41.212	42.424	43.636	44.848
	3,4	38.824	40.000	41.176	42.353	43.529

covering most of the hop production in Germany

common modern most likely factor combination

The LWA formula cross table shows the possible and the most common factor combination according to the definition of the parameters visualized in 'Material and methods'.

Conclusion and outlook

Within the process of adopting the LWA concept to the efficacy testing in the Hop Research Center in Hüll, a lot of internal and external considerations were made, different definitions of parameters were tested, and even other theoretical dose expressions were compared. As a result, we found that the LWA concept does not fit perfectly to the growth behavior of hop. LWA concept reaches its maximum early in the vegetation.

However, if comparing the current three-step model to what is actually performed by the German hop farmers, there is already a very good adjustment to what we consider adjusted plant protection. Speaking for pure efficacy testing adopting the LWA concept is presumably no big adjustment, if the definitions are clear. The point when new registrations with LWA concept must be introduced to the farmers could be a challenge, but it is crucial for the correct application of plant protection products.

Dossiers for plant protection products in hops must be submitted in accordance with LWA concept. The formula for LWA is fixed. It would be possible to add a crop adjustment factor, but it seems impossible to add a universally working factor for all varieties, training forms, countries, and crop management systems in hop. As official test facility for plant protection in hops we assume that it would be best to stick to the basic LWA formula and to simple definitions of parameters.

It seems crucial for the harmonization of plant protection within the EU member states if all hop growing nations would have the same understanding of the definition of the parameters of the LWA formula. We suggest a discussion and a common approach within the EU Commodity Expert Group (CEG) 'Minor Uses in Hops'.

Acknowledgement

The Bavarian State Research Center for Agriculture (LfL) working group IPZ 5b Plant protection would like to thank the working group IPZ 5a Hop cultivation and production techniques (Johann Portner, Andreas Schlagenhaufer, Stefan Fuß), Tobias Berger and various representatives of the national authorities, Julius Kühn-Institut (JKI) and Federal Office of Consumer Protection and Food Safety (BVL).

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Nutritional analysis in leaves of organic and conventional hops: 2020 season

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Abstract

Soil analyses show the presence of available nutrients but not the level of assimilation or availability by a crop. The study of macro- and micronutrients in the leaves around flowering is a key aspect for good management of fertilization practices. In this study, the evolution of nutrients from flowering to harvest has been carried out in two hop gardens in Galicia (cv. Nugget) during the 2020 season. The organically managed plantation showed slightly lower levels in all parameters analysed in leaf blade and petiole. Conventionally managed plants showed high values of Mn due to the application of phytosanitary products. In general, deficiencies of P were observed in blades but not in the petioles. However, for Zn in both cases there was a deficiency, which can generate problems in the formation of the floral cone and therefore in the final production. The evolution of nutrients between flowering and harvest showed that most of the elements remain stable, indicating that the plant is in balance between the input and extractions made during that period. However, in the case of the organic plot there was a reduction in the N and Mg content during the study period.

Key words. Macronutrients, micronutrients, petiole, blade, sufficiency range

Introduction

Hop nutrition studies addressing the concentration in leaves are scarce (NEVE 1991). The assessment of the nutritional status of a plant simply consists of comparing a sample with a standard, i.e., plants that present an adequate amount and proportion of nutrients, allowing high yields and good visual appearance. There is little information in the literature addressing the nutritional status of hops (SIRRINE 2016; AFONSO, 2022). Hop producers need to know which are the adequate values of nutrients in the plants to obtain a sustainable and adequate production in each phenological stage, highlighting micronutrients such as boron, zinc, and iron (VERHOEVEN et al. 2021).

One of the terms used to define the relationship between nutrient concentration and production or growth is the sufficiency range (JONES 1991), which defines the adequate range in concentration for normal growth. Nutrient concentrations outside the sufficiency range will either result in deficiency or toxicity. No tissue standards are available for hops (AUSTIN 2012), why it is essential to build a database to define nutrient sufficiency ranges for future fertilization.

Therefore, the aim of the current study was to assess the reliability of leaf blade and petiole diagnosis of cv. Nugget under conventional and organic management, by means of monitoring nutrient concentrations in blade and petiole tissues throughout a growing season. Moreover, the study aimed at establishing the reference values suggested by other authors at global level. The current study was conducted using data from a single season, despite the great variability caused by the year effect. Our work provides a first approach to assess the nutritional status of cv. Nugget in Galicia (Spain).

Material and methods

During the 2020 season, samples of hop leaves (1.5–2.0 m) of cv. Nugget were taken from conventionally and organically managed hop plots in Spain. Material was collected at 'Lagazós', Lalín (Pontevedra), in organic production since 2014, managed by Kalinka Lagazos S.L., with a plant spacing of 1.5 m and 3 m between rows, totalling to 2222 plants per hectare (42˚39´ N, 08˚06´ W, 612 m. a.s.l.). In addition, plant material was collected at the 'Presedo' plot, Abegondo (A Coruña), planted in 2008 and conventionally managed by the LUTEGA cooperative, with the same planting framework as in Lalín (43˚12´ N, 08˚16´ W, 166 m. a.s.l.).

Samples of 25 leaves (blades plus petioles) were collected per plot at several dates evenly spaced (**July 5**, July 19, **August 1**, 15 and **28**), three times in organic treatment (**in black**) and five times in conventional treatment (*ca* 15 days between samples). Leaf blades and petioles were separated, washed with tap water, and rinsed with distilled water, oven-dried (Dry Big, J.P. Selecta, Barcelona, Spain) at 70˚C for 48 h. Then they were ground with a disc mill, sieved through a 1-mm mesh, and finally stored at room temperature to be analysed. Nitrogen (N) concentration was determined by oxidizing the sample (KALRA 1998) and then quantifying the gas produced during this combustion using a thermal conductivity detector (TruSpec CHNS, Leco, St. Joseph, MI, USA). For chemical analysis of the other nutrients, 1 g of sample was calcined at 500˚C for 8 h and subsequently wet-digested with 1 mL of deionized water and 5 mL 2 M HCl. Phosphorus (P), potassium (K), calcium (Ca), magnesium (Mg), iron (Fe), manganese (Mn), zinc (Zn), and boron (B) concentrations were determined with inductively coupled plasma-optical emission spectroscopy (Optima 4300DV, PerkinElmer, Norwalk, CT, USA). Deionized water was used for all dilutions. Concentrations were expressed in terms of % (g kg^{-1} x 10^{-1}) for N, P, K, Ca and Mg, and mg kg^{-1} for micronutrients.

Results

Tables 1 and 2 show the average results of the contents of blades and petioles of the different samples taken in the Lalín and Abegondo plots during 2020 season of cv. Nugget. The results show the existing differences for the different crop management, conventional and organic. The fertilization strategies and the phenological phase in which the samples were taken is crucial to understand the variation that is obtained throughout the season, from flowering to harvest. A general tendency to maintain the concentrations throughout the campaign can be observed (Table 1), or to a clear reduction in the concentration as occuring in the case of N and Mg in the organic plot.

Table 1. Mean nutrient concentration in leaves (blade) (% or g kg^{-1} x 10^{-1}, macronutrients; mg kg^{-1}, micronutrients) at different sampling dates in 2020

Date	Jul-05		Jul-19		Aug-01		Aug-15		Aug-31	
Plot	Con	Org	Con	Org	Con	Org	Con	Org	Con	Org
N	3.645	3.837	3.618	-	3.514	3.015	3.562	-	3.981	2.863
P	0.235	0.249	0.251	-	0.208	0.187	0.210	-	0.258	0.234
K	1.552	1.389	1.853	-	1.695	1.407	1.364	-	1.730	1.889
Ca	2.238	2.239	2.667	-	2.755	2.184	3.081	-	2.942	2.399
Mg	0.391	0.770	0.468	-	0.459	0.707	0.419	-	0.411	0.527
Fe	76.882	54.684	89.341	-	61.32	48.513	64.586	-	65.583	41.831
Mn	128.303	74.138	224.260	-	213.935	80.948	225.518	-	139.515	81.319
Zn	11.818	9.363	13.902	-	9.023	5.951	8.985	-	8.369	7.783
B	12.603	15.789	14.718	-	19.724	14.904	19.422	-	15.873	17.921

Table 2. Mean nutrient concentration in leaves (petioles) (% or g kg^{-1} x 10^{-1} for macronutrients; mg kg^{-1} for micronutrients) at different sampling dates in 2020

Date	Jul-05		Jul-19		Aug-01		Aug-15		Aug-31	
Plot	Con	Org	Con	Org	Con	Org	Con	Org	Con	Org
N	1.698	1.753	1.755	-	1.422	1.185	1.722	-	2.116	1.290
P	0.126	0.173	0.157	-	0.116	0.108	0.134	-	0.120	0.125
K	3.322	3.713	3.952	-	3.675	3.482	3.692	-	3.099	2.940
Ca	1.426	1.258	1.971	-	2.194	1.423	2.361	-	1.458	1.318
Mg	0.283	0.555	0.469	-	0.566	0.815	0.675	-	0.426	0.628
Fe	83.594	31.383	85.206	-	36.421	21.676	49.801	-	58.410	21.276
Mn	257.525	123.136	424.394	-	434.663	227.849	559.441	-	238.112	229.714
Zn	11.306	28.839	29.050	-	25.981	49.578	36.364	-	22.379	41.653
B	26.105	31.116	33.498	-	37.507	36.472	36.758	-	25.912	31.541

Figure 1 shows the dynamics of the concentration of elements (N, P, Mn, and Zn) in blades, in the two plots studied. There are lower levels than those reported in the literature (dot lines), in the case of P and Zn, and excessive in the case of Mn. It is worth mentioning that the sufficiency ranges values refer to the limbo plus petiole set, so lower values may be masking this aspect.

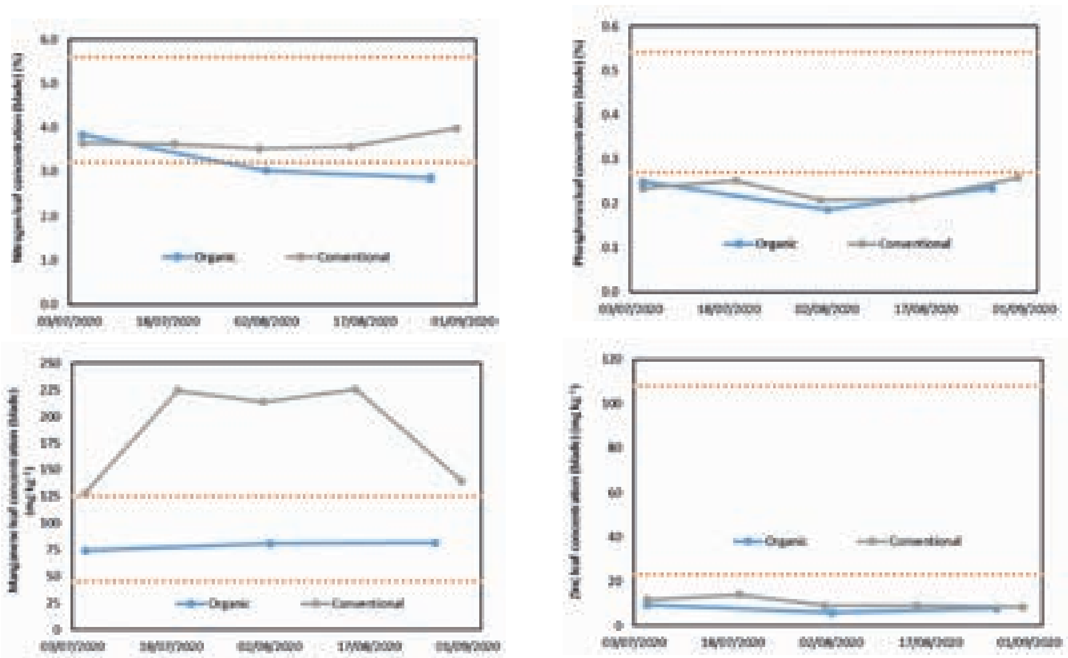

Figure 1. Dynamics of elements in hop leaves (blade) during the 2020 season. Dotted lines represent minimum and maximum ranges.

Discussion

Concentration of nitrogen, one of the main nutrients, is in the sufficiency range. In the conventional plot, the values were similar to those determined by AFONSO et al. (2021) for plants with very good vigour. However, in the organic plot the N values in blades were lower than those shown for plants with weak vigour, but similar to values given by AUSTIN (2012) in July. VERHOEVEN et al. (2021) establish a threshold to N-NO3 concentration in petioles of 4,000 ppm, with deficiency at levels below that value.

In our study, N values are around 1,185 and 2,116 ppm (Table 2), which would mean a deficiency in nitrogen, which however was not detected at blade level.

It stands out that of all elements analysed in blades, P and Zn remain at levels below the sufficiency range established by BRYSON et al. (2014) and AFONSO et al. (2021). Although these authors have worked with analysis of whole leaves, as can be seen in Table 2, the concentrations of Zn in petioles are relatively high, meaning that both plots were within the range of sufficiency. SIRRINE (2016) gives a normal range of Zn concentration in petioles from 24 to 50 ppm, which is obtained in the organic treatment but not in conventional plot. In relation to the P concentration in petioles, values of more than 10 ppm are considered sufficient as found in our study plots with 10.8–17.3 ppm (Table 2). For Zn, BOAWN (1965) found that values below 12 ppm can cause visual symptoms and would affect final yield. For Mn concentration in blades, there is a clear influence in the conventional plot of the phytosanitary treatments applied with values higher than 125 ppm during all the measurement days (BRYSON et al. 2014). However, in the organic plot, the values are within the set sufficiency range. SIRRINE (2016) establishes an optimal range in petioles between 25 and 150 ppm prior to the plant reaching full trellis height, which is observed in the organic treatment (Table 2).

The interaction between the different elements can lead to difficulties in their assimilation, not allowing the desired production to be achieved. The amounts of K and Ca in petioles are higher than the optimal values, which can cause difficulties in the development of the plant as well as later limiting the growth and development of the cones. The values obtained in this study are limited to one season only, why the study should be extended to successive years, making the nutritional status of the plant possible deficiencies and excesses actually known. This will facilitate to adequately manage the nutrition of hop plants even using fertigation systems.

Acknowledgements

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Improving hop plant nutrition and yield with *Bacillus velezensis*

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Abstract

In the Andean Patagonia region of parallel 42º hop cultivation together with tourism are important economic activities. Every year it becomes necessary to improve agronomic practices based on a more environmentally friendly management. This research was conducted in a hop yard located in El Bolsón (NW Patagonia, Argentina), to assess the product "RISE P", a root inoculant with a high concentration of the Bacillus velezensis IT45 strain of rhizosphere bacteria. Trials at two different scales were proposed, evaluating the impact of this plant growth promoting rhizobacteria (PGPR) on both underground biomass production in nursery pots, and field cone yield. At nursery-scale the relative growth obtained between the initial rhizomes weight and the final weight of underground biomass in the inoculated pots presented a positive relative growth of 105 %, while in the control pots (not inoculated) was 43 %. At field scale, the hop mature rows inoculated had an average cone yield 9.4 % higher than the non-inoculated control rows. Regarding the analysis of plant tissue, the nitrate concentration was lower in the inoculated sample, while the phosphorus and iron content were higher. No important variations were detected in the measurement of cations (K, Ca, and Mg) between the leaf tissue from control and inoculated plants. The results of this study allow us to infer that the use of this PGPR in hops has a phyto-stimulant effect. Nevertheless, to unravel the mechanisms of action at the physiological level of the plant, further analyses are required.

Key words. Hop nutrition, PGPR, Lallemand Plant Care, B. velezensis, Patagonia, Argentina

Introduction

In the Andean Patagonia region of parallel 42º the first hop farm began in 1956 and there are currently about 140 hectares in commercial production. Hop cultivation is restricted to a few valleys with low incidence of winds. The landscape value and the growing interest of tourism in hops and beer make this region a unique place in Latin America. Every year it becomes more necessary to improve agronomic practices based on a more environmentally friendly management. In this context, the traditional use of fertilizers must be re-analyzed and the study and implementation of plant growth promoting rhizobacteria (PGPR) is gaining interest.

Some soil bacteria harbor mechanisms to promote plant growth, which include biocontrol of plant pathogens, phyto-stimulation, nutrient mobilization, and stresses protection. These microbes, also so-called Plant Probiotic Bacteria, applied as biofertilizers in crop production, constitute a possible solution to enhance hop nutrition and yields, while taking care of the soil and thus developing a more sustainable agriculture. From this approach, it becomes necessary to understand that agronomic practices should be in favor of the natural cycle of nutrients, which are not only influenced by physical variables (pH, humidity, temperature), but also soil biology plays a fundamental role. In terms of crop nutrition management, for sustainable agriculture it is necessary to reduce dependence on chemical products and avoid basing annual fertilization programs on strict balance models.

In the study region, small valleys are characterized by deep soils and high organic matter content. However, from a systematic monitoring carried out for more than 15 years in soils of several hop yards, a decrease in the percentage of organic matter was detected. Agronomic practices have perhaps relied too much on chemical fertilization and excessive tillage to encourage mineralization during spring cold conditions. Based on this negative trend, in recent years a series of trials with PGPR have been initiated in different hop yards.

These studies represent valuable background to generate a possible positive impact on crop production and demonstrate the potential of new biotechnological tools that can be complemented with traditional nutrition practices to work in integrated management. At the same time, the interannual continuity of this type of tests in systems that are in commercial production allows learning and improving techniques in the design of agricultural trials with scientific method. In Argentina there are very few products with registration enabled for the cultivation of hops.

Lallemand Plant Care business unit (belonging to Lallemand Inc.) works in development of microbiology and fermentation for the agricultural world and specializes in plant bio protection and bio fertilization. Among the main inoculants developed and marketed by Lallemand, arouses a particular interest the product "RISE P", a root inoculant with a high concentration of the *Bacillus velezensis* IT45 strain of rhizosphere bacteria. Most strains are capable of solubilizing $Ca_3(PO_4)_2$ and producing plant growth hormone of indole acetic acid (IAA). There are trials with proven positive effects as PGPR in crops of economic importance (horticultural, oilseeds, legumes), but there are no records of use in hop plants. Sustained growth in the last decade of the hop industry in Patagonia, beyond its small scale, generates particular interest in some hop growers to incorporate new technologies, which encourages large companies such as Lallemand to focus on this crop.

Materials and methods

This research was conducted in a hop yard belonging to "Patagonia Lúpulos Andinos S.R.L.", located in El Bolsón (Río Negro Province, NW Patagonia, Argentina). The family business owns six farms, with a total of 82 ha growing eight commercial hop varieties (2 from USA, 3 from Europe, 1 from Australia and 2 local). Trials at two different scales were proposed to evaluate the impact of PGPR both on underground biomass production as well as the cone yield in the field.

Nursery trials

In August 2020 a nursery-scale trial was set up using Australian hop rhizomes of cv. Victoria. Initially, 90 labeled pots were filled with 1 L of a specific substrate called "A1 - HIGH PERFORMANCE" produced by the company "Cía. de Minas Magri y Gallardon S.A.", located in El Hoyo (Chubut Province, NW Patagonia, Argentina). The substrate has *Carex* peat, volcanic sand, dark soil and worm compound. Basically, it is a naturally fertilized substrate with a wide use spectrum (slightly acid pH). Rhizomes were selected by size (length < 10 cm) and thickness (fairly uniform), placing one per pot and recording the initial weight of each individual rhizome. Irrigation and trellising tasks were handled manually, with almost daily assistance, under well-controlled conditions.

Each pot constituted an experimental unit, assigning the inoculation treatment randomly to 50 % of the pots. The inoculation with "RISE P" was carried out by applying 0.3 grams of product per pot. In order to achieve it with precision, a 5 % (w/v) dilution was made in deionized water. A total of 3 identical inoculations were carried out with intervals of 60 days (August, October, and December). Although plants did not reach flowering, the vegetative development was satisfactory. They were maintained with good irrigation and manual weed control until the growth cycle was completed (autumn 2021). The response variable of the trial was the wet weight of underground biomass (remains of the original rhizome, crown, and roots). Each plant was pruned almost at soil level, then removed from the labeled pot and carefully washed with water to remove all the substrate. Thus, a differential was obtained between the initial weight (Iw) and the final weight (Fw) so the relative growth (RG) is calculated as RG=(Fw-Iw)/Iw. For statistical purposes, the effect of treatment on RG was evaluated. Data were analyzed using generalized linear models, considering a Gamma distribution of the variable. This was chosen since the variable does not present a normal distribution and is inflated at zero (it has a lot of data close to zero). Free software R 4.2.0 was used. Differences between treatments were considered significant at $p<0.1$.

Field trials

In the late spring of 2021, the field-scale trial was designed and set up, in an adult hop yard cultivated with the American cv. Nugget (12 years old). The experimental site was a 3-ha plot (41°53'48.5"S 71°30'39.5"W; elevation 468 m.a.s.l.). Plant spacings were 0.8 x 2.8 m (4.464 plants/ha) and the plot was irrigated by surface drip. The lateral pipes were equipped with in-line non-compensated emitters (2 L/h) spaced 50 cm in the crop row, thus 1.6 emitters per plant.

While this is not the highest quality site in El Bolsón, is adequate to achieve acceptable yields. However, some plots have suffered from poor irrigation management for years (before the installation of drip irrigation), and there was a negative impact on soil fertility for growing hops. Soil texture at this site is classified as silt loam, with some proportion of clay (less than 20 %). According to apparent density measurements and considering a depth of 50 cm, water storage capacity was calculated in 175 mm. Soil reaction is slightly acidic to neutral (pH 6.3). These are allophanic soils (derived from volcanic ash and positive to the Fieldes test), so the availability of phosphorus in certain environments is critical, even though it is not a main nutrient for hops. In all the soil samples of the farm, values below 20 ppm of this nutrient were recorded (Olsen et al. 1954). The original geologic material ("loess") ensures an adequate supply of soil potassium (above 250 ppm).

Prior to inoculation, treated and control rows were randomly assigned, recording the row number in each case. The inoculation was carried out in a very simple way, taking advantage of the fertigation system and the calculated dose of the product resulted in 0.1 grams per plant. Except for the time of application of the treatment, the plot received the same agronomic management throughout the growing season. Other than a few days of excessive heat in spring (mid-November), and possibly having experienced slight hydric stress for a short period, growing conditions were normal. There were no severe pest and/or disease attacks and the crop developed normally. Towards the end of March, when the cones were at their optimum point of maturity (22 % dry matter), the harvest was carried out with an Alleys picking machine Nº 7 and the total fresh (wet) cone weight per individual row was recorded. Data were analyzed by analysis of variance (ANOVA). Free software R 4.2.0 was used. Differences between treatments were considered significant at $p<0.05$.

Plant tissue analysis

At harvest time, Nugget plant tissue (leaf and petiole) was collected and a composite sample of the rows of each treatment was obtained. Each sample was divided into two and while a subsample of each treatment was dehydrated and sent for analyses, the others were processed fresh, obtaining the sap immediately. Phenylsulfonyl acid was used to measure nitrates ($N\text{-}NO_3$), while the methodology used for P, K, Ca, Mg, and Fe were by digestion with nitric and perchloric acid; and then spectrophotometry (RICHARDS 1993). For the measurement of sap, the Horiba LAQUAtwin Nitrate and pH Pocket Meters were used. After taking the pH reading and before measuring nitrates, a 1:4 dilution (5 ml sap: 20 ml water) was made.

Results

Nursery trials

The relative growth (RG) obtained between the initial rhizomes weight and the final weight of underground biomass in the inoculated pots presented a positive relative growth of 105 %, while in the control pots (not inoculated) was 43 %. As can be seen in Figure 1, there is a significant statistical difference (t-value=3.81; p=0.06).

The inoculated pots presented a greater mass of adventitious roots, and in some experimental units it was very notorious. Taproots of variable length and vigor were observed in both treatments, which surely generates a direct impact on the RG response variables and a non-normal data distribution.

Field trials

The mature Nugget hop rows inoculated with *B. velezensis* had an average cone yield 9.4 % higher than the non-inoculated control rows. Figure 2 shows the significant differences between treatments (F value=13.2, p=0.02).

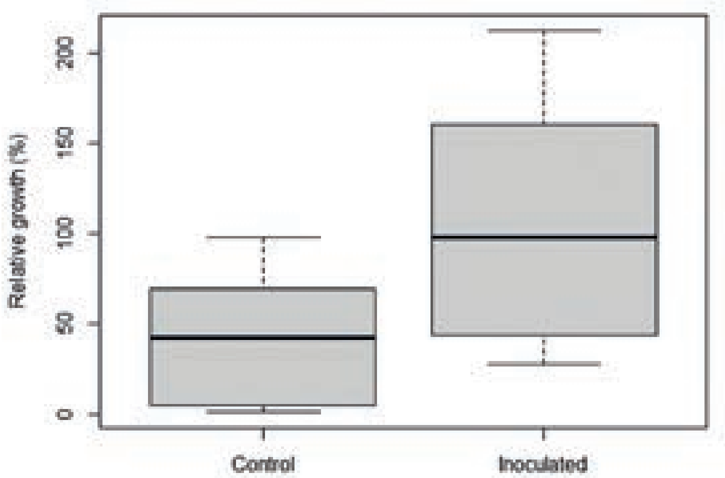

Figure 1. Relative Growth (%) of underground biomass between treatments

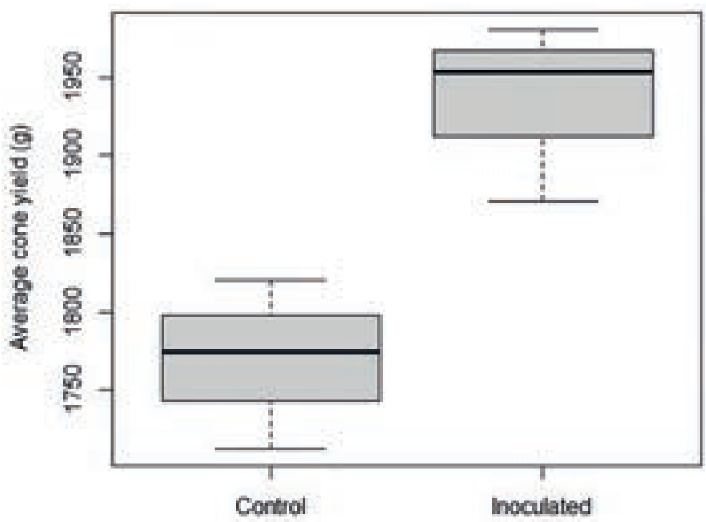

Figure 2. Average cone yield (kg/ha) between treatments

In converting measured yields from wet cone to dry flower, a theoretical multiplication by 3.6 could be assumed. In this way, the theoretical average yield values would be 1935 kg/ha for the inoculated rows and 1769 kg/ha for the control rows. Visually, no differences were observed in terms of vegetative vigor or leaf coloration.

Plant tissue analysis

As can be seen in Table 1, the nitrate concentration was lower in the inoculated sample, while the phosphorus and iron content were higher. No important variations were detected in the measurement of cations (K, Ca, and Mg) between the leaf tissue from control and inoculated plants.

When the measurement technology available on the farm was used, the nitrate measurement (Table 2) was consistent with the laboratory results, being the nitrate concentration lower in the inoculated sample. The pH measured in each pure sap sample was practically neutral in both cases.

Table 1. Elemental analysis on leaf tissue

Nutrient	Units	Control	Inoculated	Variation
NO_3	mg/kg	1722	1282	-26%
P	%	0.12	0.17	42%
K	%	1.72	1.67	-3%
Ca	%	2.83	2.82	0%
Mg	%	0.37	0.36	-3%
Fe	mg/kg	245	474	93%

Table 2. Horiba LAQUAtwin Nitrate and pH Pocket Meters

Nutrient	Units	Control	Inoculated	Variation
NO3	ppm	1800	1400	-22%
pH		6,9	7	1%

Discussion and conclusions

The treatment of hop plants with the product RISE P showed positive and statistically significant results. In this work, RISE P was evaluated in two different varieties of bitter hops, in two different stages and using unequal doses. The action of *Bacillus velezensis*: Increased the growth of underground biomass in nursery pots; improved the yield of cones in adult commercial hopyards and increased the concentration of phosphorus and iron measured in leaf tissue at harvest time. The results of this test allow us to infer that the use this PGPR in hops has a phyto-stimulant effect although the mechanisms of action at the physiological level of the plant require further explanation.

It would be interesting to evaluate over time the importance of the implementation of these technologies based on microorganisms to be able to measure the improvement of soil biological parameters, as opposed to the exclusive use of chemical fertilizers. The evaluation of nutritional parameters and agronomic yields in systems that include these biotechnology products would allow an economic evaluation against traditional management. The results of this study encourage us to perform future new tests in different productive conditions (different doses, crop stages, varieties, soils). New knowledge regarding the use of these biological products will favor the transition from traditional management to a more sustainable integrated management.

Acknowledgements

Our thanks to all people in our young hop growing country who work to improve the industry by adding more knowledge every day. A special acknowledgment to Florian Weihrauch, Chairman, Scientific-Technical Commission I.H.G.C. for inviting us to this STC Meeting and motivating us to carry out the disclosure of our trials in Patagonia. Our thanks to Lallemand Plant Care (Lallemand Inc.) and Cía. de Minas Magri y Gallardon for providing the specific inputs necessary to carry out these studies. Special thanks to Verónica Chillo (CONICET) and Andrea Trochine (IPATEC, CONICET-UNCO), for collaborating with the data analysis, helping with the writing, and sharing their knowledge and goodwill.

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Nitrogen dynamics in hop soils

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Key words. Nitrogen efficacy in hop, soil nitrate nitrogen content, fertilising of hops

Introduction

In the Hallertau in Germany, hops are a very densely cultivated specialty crop. Because such intensive cultivation of hops comes with an according high nutrient demand, nitrogen fertilizer requirements are also correspondingly high. Higher fertilizer rates often result in increased nitrate levels in the soil, especially on farms with additional applications of organic fertilizers. After harvest, hops obviously no longer absorb any residual nitrogen in the soil. The remaining loads of nitrate nitrogen in soil can only be partially reduced from the nitrogen uptake of intermediate cover crops. Any remaining mineral nitrogen can shift in the soil and can also lead to nitrate leaching.

Objective

As part of a larger project, the nitrogen dynamics in hop soils were investigated at 21 hop farms all around the German hop growing region Hallertau. For this purpose, intensive soil N_{min} tests were conducted in spring, fall and winter. This involved determining the nitrogen requirements for these plots and recording their actual amounts of N fertilization. The data was summarized into an operational nutrient comparison. This allowed for an estimate of nitrogen shifts in the soil and of depletion potentials during the growing season, for different farm types, with different fertilization systems, on different soils. It also allowed for the development of possible approaches to optimizing nitrogen management in hop cultivation. The objective was to optimize operational processes for optimal yields and qualities, while still observing and complying with the specifications of the Fertilizer Ordinance as well as protecting clean water resources

Methods

For each of the 21 farms, three plots were selected. The 63 sub-areas reflect the actual range of varieties grown in the Hallertau. They also represent a wide variation of operating and fertilizer systems. N_{min} sampling was carried out at the start of the vegetation period in March and after harvest in October to record the remaining nitrogen levels in the soil as well as during dormancy in winter to identify possible N shifts in the soil. The plant-available nitrogen in the form of ammonium and nitrate was examined up to a soil depth of 90 cm. Each sample was divided into three 30 cm deep soil sections to better determine the displacement in the different soil layers. Each farm received individual advice on fertilization strategies. All nitrogen fertilizer applications were recorded in terms of timing and quantity.

During the first harvest in 2018, cones and residual plants were sampled at the farms to calculate the exact nitrogen removal from the soil. Because the exact amounts of cones and bine shreds at harvest time could only be approximated in these working farms, such sampling was abandoned in the succeeding two years. Instead, various hop gardens with the most important varieties cultivated in the Hallertau were harvested at the hop research centre in Hüll with great precision. This allowed for a more precise determination of the parameters for cones and bine chop as well as for the entire plant, for different varieties, at different yield levels. With the help of this data, the nitrogen removal as well as the accumulation of bine shreds could be re-assessed for a greatly expanded range of varieties, as a function of the cone yield.

Results

The trial years 2018-2021 provided extensive insights into the nitrogen dynamics in hops. Based on 10 sample periods, the distribution of N_{min} contents in the respective soil layers can be shown as a function of the sample dates (Fig. 1). The higher N_{min} levels in the upper 30 cm in the fall are striking, in both relative and absolute terms. The decline of these levels until spring can be explained by the N uptake of intermediate cover crops. However, nitrogen shifts into deeper soil layers – especially when there was plenty of precipitation in the fall and winter – cannot be ruled out as a cause either. In addition, strong annual fluctuations in N_{min} levels were evident.

Further analysis reveals that the N_{min} content depends on the variety cultivated in the respective sample plots. It also shows that aroma varieties have higher N_{min} levels than bitter varieties do have. Because the new Hüll aroma varieties and old landraces were sampled on only a small number of plots, the data does not allow for an evaluation of their variety - specific N_{min} content (Fig. 2). Differences in N_{min} content between aroma and bitter varieties were particularly pronounced in the fall samples. The differences can be explained by a more extended root system and a resulting higher N-removal by bitter varieties near harvest time. In addition, as part of the fertilization documentation, we discovered that, in the past, growers did not always make a distinction between varieties and different yield levels in N fertilization routines. However, a new approach of differentiating between varieties and site-specific yields in N fertilization is considered essential for optimizing N fertilization in hops. More interesting considerations about N_{min} levels depending on soil type or different types of farms and fertilising practices in hops were made in this study.

Acknowledgement

This study was part of a research project funded by HVG Germany.

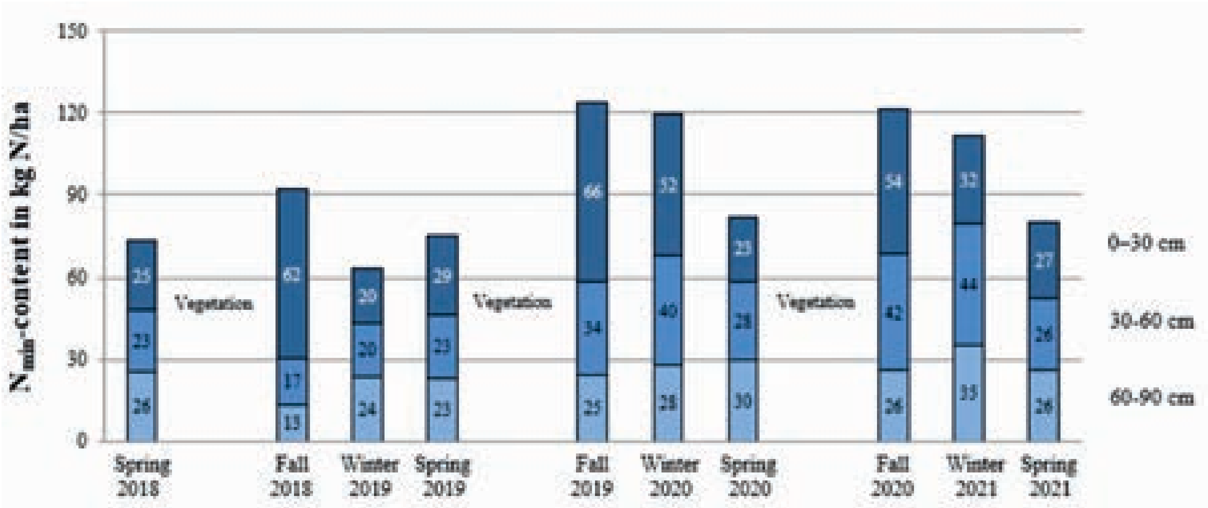

Figure 1. N_{min} levels across all sampling dates during 2018 to 2021, broken down by soil layers (0–30 cm, 30–60 cm, 60–90 cm)

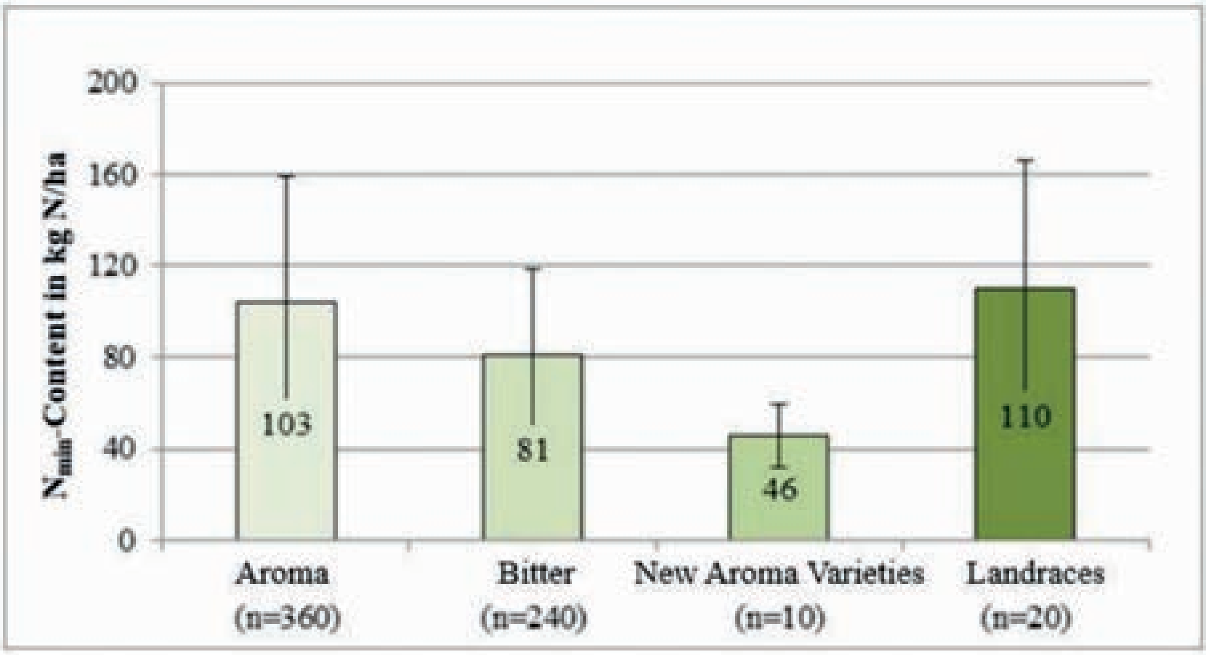

Figure 2. Mean N_{min} levels across all sampling dates during 2018 to 2021, broken down by variety groups

Exploring the use of electric current for weed control in hopyards

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Abstract

Attempts to use electricity as a weed control methods date back to the 19th century, but a renewed interest in this method of weed control has occurred in response to concerns with pesticide residues and pesticide resistance. Electric weed control (EWC) systems generate a high voltage current applied directly to the target plant via foliage contact and conducted downward through the roots. As the current passes through the plant, electrical resistance generates heat; resulting in the vaporization of cellular water, cell membrane rupture and plant death. Plant sensitivity to electric current is related to plants' greater electric conductivity; herbaceous tissue has greater electric conductivity than woody tissue. Hop shoots are herbaceous, but its woody root system leads us to hypothesize that with proper timing and rate, EWC may be used selectively to remove weeds and hop foliage without damaging the perennial root system. This study evaluates the response of selected weed species and hops (*Humulus lupulus*) to electric current. A field study was conducted in a one-year-old 'Cascade' hopyard in Corvallis, OR. Current is generated by a commercial unit (EH-30 Thor, Zasso, Switzerland) driven by the tractor's power take-off. The unit employs electrodes in continuous contact with soil or plant foliage. Equipment settings and duration of treatment, speed of operation influence the energy applied. We have identified three timings against which to evaluate EWC in hop production: (i) crop emergence, when emerging hop shoots are chemically pruned to suppress disease and weeds, (ii) basal foliage removal in spring and summer, and (iii) dormant treatments to control weeds. EWC was applied at two speeds, 0.4 and 2 km h^{-1} and 30 kW nominal electrical power. Initial results indicate excellent foliar removal of hop foliage and weed when applied at these speeds. However, EWC stunted crop growth compared to carfentrazone (35 g ai ha^{-1}) and nontreated controls. Initial results of this ongoing study indicate the potential of EWC in hopyards.

Key words. Physical weed control, electricity, herbicide

Introduction

Increasing interest in sustainable food systems has led to the expansion of organic and sustainable agriculture worldwide (WILLER et al. 2020). These fundamental changes in food systems require novel technologies to ensure high crop quality and productivity, including novel nonchemical weed control solutions. Electric weed control (EWC), which works by passing an electric current through the target species to heat cellular water, can play an important role in nonchemical weed control. EWC does not disturb the soil, may improve soil health and reduce erosion (BRODIE et al. 2018; SAHIN & YALINKILIC 2017). EWC does not leave residues in the crop or soil and could provide growers greater flexibility to produce and commercialize their crops. EWC technology, first patented in the 1880s, is an effective weed control tool (BRODIE 2018; DIPROSE et al. 1984; KOCH et al. 2020; REISER et al. 2019; SAHIN & YALINKILIC 2017) although most recent and historic research efforts have focused on row crop agriculture. Consequently, there is limited information describing how electric weeding technology will perform in perennial systems like hops (*Humulus lupulus*).

Weed control in hops relies on soil tillage and herbicides, but alternatives to replace or augment these weed control practices are needed to satisfy the demands of the changing global market. EWC is a technology that may replace both tillage and herbicide use in hop production. However, no information on hop response to EWC is publicly available. Electric weed control (EWC) technology is not new, although its use as a tool in agriculture remains underexplored. The first electric weeders were patented in the 1880's (VIGNEAULT & BENOÎT 2001).

In the 1970s and 1980s, EWC successfully to control weeds and bolting sugar beets in that crop (DIPROSE et al. 1985; WILSON & ANDERSON 1981) (EWC acts by passing an electric current through the target species, vaporizing cellular water, disrupting cell membranes, and causing cell death. The electric power needed to kill a plant is inversely related to the dielectric resistance of the target plant, as described by the following equation:

E = V2*(ECp)*1/(tc)

where E is the energy in joules. The energy transferred to a plant is related to the voltage (V), the plant electric conductivity (ECp), and the duration of contact with the electrode (tc) (adapted from VIGNEAULT & BENOÎT 2001).

Plant electrophysical properties are species-, tissue-, and stage of development dependent; the response will be plant-specific at a given amount of electrical energy applied. By manipulating the energy applied, voltage, and exposure time, the natural differences in plant electrical conductivity can be exploited to achieve desired weed control goals, with minimum crop damage. The development, optimization, and adoption of a new and effective weed control technology will provide hop producers with expanded and diversified vegetation management and crop canopy manipulation options.

Hop producers remove basal foliage to control weeds and suppress foliar diseases like Powdery mildew (*Podosphaera macularis*) (GENT et al. 2016). This project explores the use of EWC in hops.

Material and methods

The equipment: A 105 hp tractor will be used in this experiment (JD5100GN). Electric current will be generated by an EH 30 Thor unit (Zasso, Switzerland) (Figure 1). A PTO-driven generator (30 KVA) producing 240 V and 30 A is the power source.

The generator is connected to a transformer that can vary voltage from 5 to 12 kV. The transformer has a tap lever used to manipulate electric current, by making connections on the transformer winding points to change electric current and voltage output.

The high-voltage electric current is transferred to cables at the front of the tractor, where two 0.6 by 0.5 m applicators are located on each side of the tractor.

Each applicator is equipped with electrodes that maintain contact with plant foliage or soil throughout the process. Energy applied can be changed by changing the speed of operation.

Figure 1. Electric weed control system EH 30 in a hop yard in Hubbard, OR, Spring 2021.

A replicated field study was initiated in April 2022 in a one-year-old ‘Cascade’ hopyard located on a Chehalis silt-loam soil located at the OSU Lewis Brown Research Farm in Corvallis, OR. Plants are 0.75 m apart in the row with 3.3 m between the rows and have been provided surface drip irrigation. The study is organized as a randomized complete block with four replicates. Each replicate consists of six hop plants. Electricity is applied at two speeds of operation, 0.4 or 2 km ha$^{-1,}$ and four timings, (i) crop emergence, (ii) in season, (iii) dormant, (iv) crop emergence, in season, and dormant. A nontreated control and an herbicide standard were included as references.

Results

This study is ongoing, and at the time of writing, only the crop emergence treatment timing has been conducted. EWC treated hops were 35 cm in height eight weeks after treatment and were significantly smaller than nontreated (55 cm) and standard (72 cm) controls (Figure 2 left). It is important to note that this was the height of plants that survived treatments. EWC also reduced survival (45 to 65%) compared to 91 and 100 % for the nontreated and standard treatments, respectively (Figure 2 left).

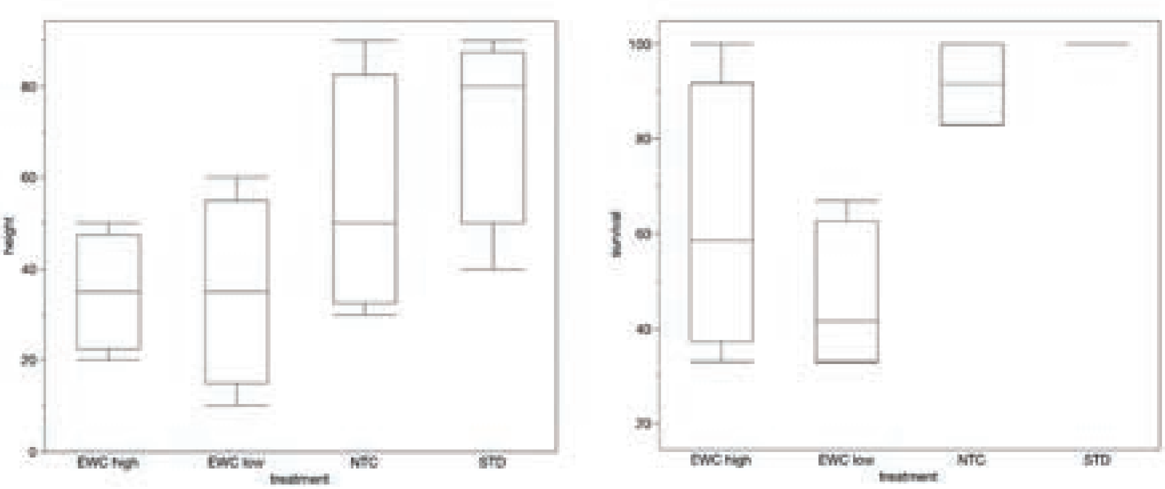

Figure 2. One-year-old ‘Cascade’ hop bine height (left) and survival (right) eight weeks after electric weed control (EWD) treatments application in Corvallis, OR. Box plots (n=4) are presented. Abbreviations. EWC high - electric weed control applied in high energy (0.4 km/h), EHC low - electric weed control applied at low energy (2 km/h), NTC - nontreated control, and STD - standard.

Acknowledgment

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Systemic Acquired Resistance of hop plants against spider mites - a keystone of future plant protection in hops?

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Abstract

The Two-spotted spider mite *Tetranychus urticae* (TSSM) is a major pest of cultivated hops which, in the event of severe infestation, can lead to significant losses in quality and yield, and even total crop loss. Acaricides are regularly used worldwide to protect hop plants. Since heavy spider mite infestations are favoured above all by dry, warm weather, it can be assumed that this polyphagous pest will gain further importance in Germany because of climate change. Weather-related problems caused by spider mite infestation in hops are already increasing and are leading to more frequent use of acaricides; in consequence, also resistance of spider mites to registered pesticides is increasingly being observed.

Over a period of five years, the presented research project will investigate the extent to which heavy spider mite infestation of an individual hop plant reduces its susceptibility to spider mites in subsequent years. The presumed underlying mechanisms are usually summarized under the generic term 'induced resistance'. Induced resistance describes the increase in the natural resistance of an individual plant without altering its genetic constitution. In the interaction between hop plants and spider mites, we are presumably dealing with Systemic Acquired Resistance (SAR), the defense reaction of a plant against a pathogen. SAR is a type of plant immune system and is also referred to as plant memory. When a plant is confronted with a pathogen, a reaction is initiated in the plant based on biochemical processes that protect the whole plant when the pathogen attacks again. The project will also provide information on the underlying mechanisms by analyzing the constituents of both infested and not infested hop leaves.

The results of the project will be used to develop findings for plant protection in practice, which should contribute to a significant reduction in the use of acaricides in the cultivation of cultivated hops – ideally towards a situation in which acaricide use is the exception rather than the rule. The minimum objective is the waiving of one acaricide treatment per hectare of hops per year on average, *i.e.*, to save one treatment on 20,000 hectares per year in Germany.

In the first year of the project, 20 German hop farms were successfully recruited as cooperation partners for the project (16 in the Hallertau, 4 in Tettnang). These provide a total of 31 hop yards, which means that five to ten practically managed hop gardens have been found for each of the four different hop varieties selected for the project (Hallertauer Tradition [10], Herkules [10], Spalter Select [6], and Tettnanger [5]; see Fig. 1, 2). In every garden, the two plots "untreated" against TSSM and "sprayed", each with approx. 500 m², were defined to be observed over the entire duration of the 5-year project. For this purpose, these plots were marked with wooden boards and in the center of each plot a sampling area was marked directly on the plants. In addition, the exact position of the plots was recorded so that they could be found again and re-marked in subsequent years. All plots were monitored for TSSM infestation four times in 2021 and in one garden of each cultivar an experimental harvest was conducted.

Preparations for the pot trial began at the end of January 2022, after the hops had been exposed to a sufficient cold stimulus in the field. Pre-planting of >200 rhizomes of each of the four cultivars was done, and in week 12, half of the plants of each cultivar was deliberately exposed to artificial high spider mite infestation. The other half was kept free of infestation with acaricides. By early May, the infected plants were already extremely heavily infested. Both cohorts of all four varieties serve as a basis for further trials, whether for observing future infestation in the field or for further investigations into the extent to which SAR is further transported during vegetative propagation of the hop plants.

In the first year of the project, no relevant spider mite population built up either in the German hop-growing areas in the Hallertau or in Tettnang. As a rule, no significant damage occurred, and it was barely possible to detect different TSSM infestation in either the untreated or the sprayed practice plots of the experimental gardens. The experimental harvests likewise did not record any differences between 'treated' and 'untreated' (Fig. 3). Also, no differences regarding TSSM susceptibility could be found between the four hop cultivars considered.

The pot trials started as planned in spring 2022.

Key words. Two-spotted spider mite, *Tetranychus urticae*, induced resistance, Systemic Acquired Resistance, pest control

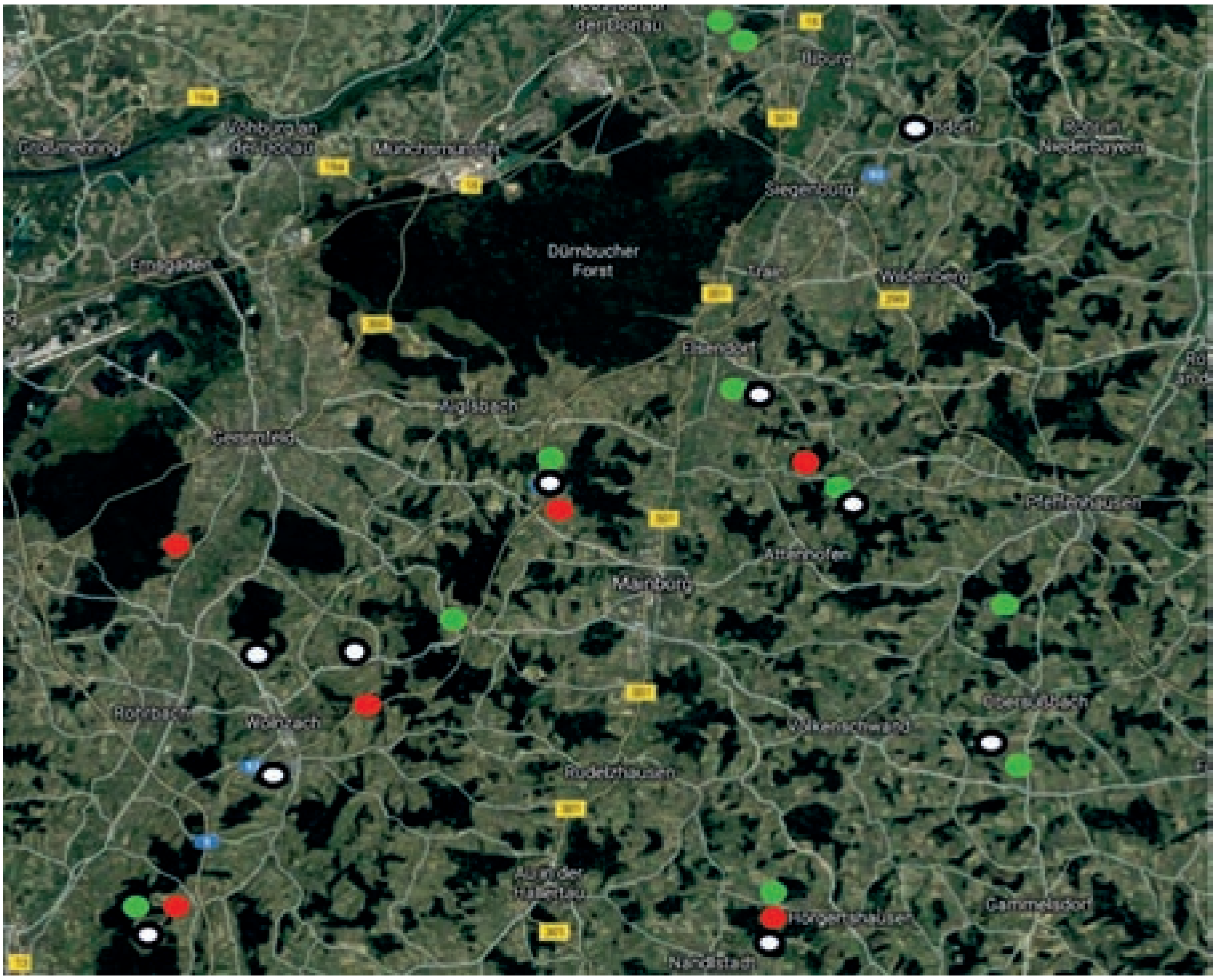

Figure 1. Situation of the project's 26 experimental hop gardens in the Hallertau, Germany. Black/white – cv. Herkules (10), green – cv. Hallertauer Tradition, red – cv. Spalter Select (6).

Figure 2. Situation of the project's five experimental hop gardens (cv. Tettnanger, blue) in Tettnang near Lake Constance, Germany.

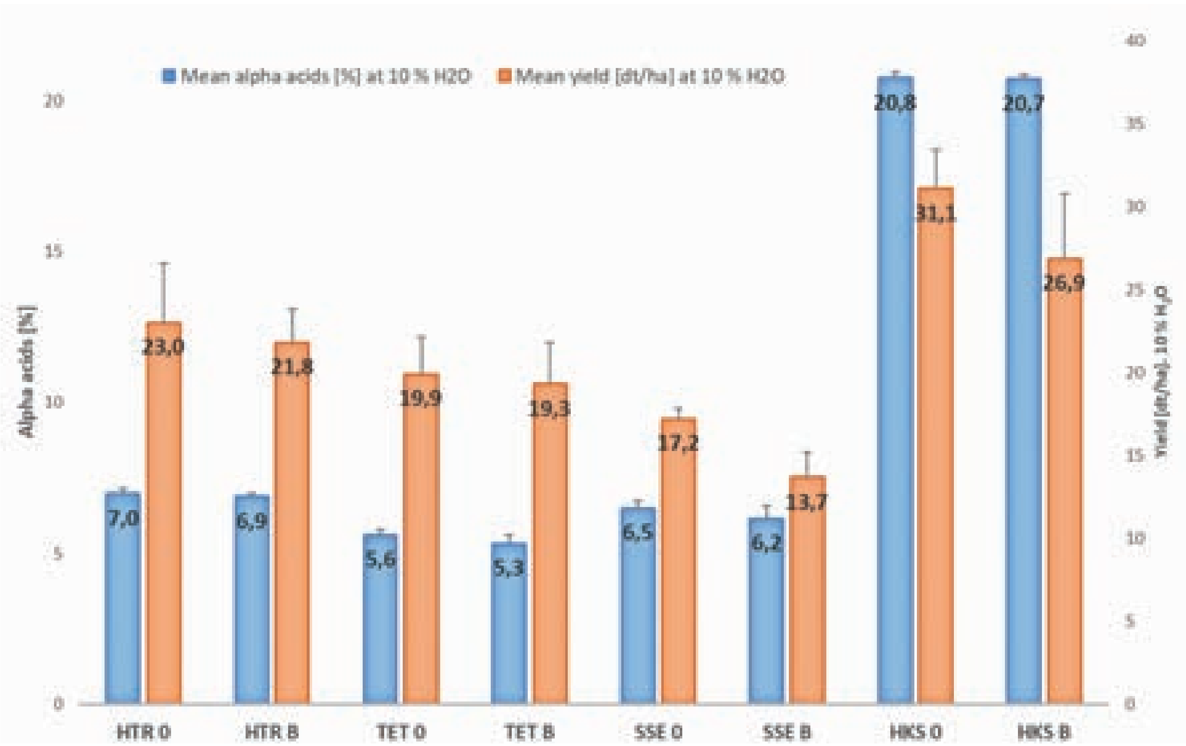

Figure 3. Results of the experimental harvests 2021, comparing alpha acids and yield in the TSSM-treated (B) *vs* -untreated (0) plots of the four cultivars, Hallertauer Tradition (HTR), Herkules (HKS), Spalter Select (SSE), and Tettnanger (TET).

V: Phytopathology

Verticillium: Thermal treatment of hop waste – bioassay using the indicator plant eggplant

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Key words. *Verticillium* wilt, *Verticillium nonalfalfae*, hop waste, thermal treatment, indicator plant, eggplant, *Solanum melongena*

Abstract

Verticillium wilt of hops, caused by *Verticillium nonalfalfae*, is an increasing problem although hop varieties show better tolerance. Hop waste, which is returned to the hop yards freshly after harvest and without being stored, has a major potential for infecting the hop plants. The infection potential of hop waste can be reduced by storage. In this trial, the possible reduction potential of the fungus is to be estimated by thermal treatment of the hop waste by using the indicator plant eggplant. It was found that a longer storage of the hop waste can significantly reduce its infection potential.

Introduction

An increased frequency of *Verticillium* wilt has been observed in some regions of the Hallertau during the last 15 years. This disease in hops is mainly caused by the soil fungus *Verticillium nonalfalfae* and rarely by *Verticillium dahliae* (EFSA 2014).

Research work on the wilt problem in hops was resumed at the Institute for Crop Science and Plant Breeding in 2008. In addition to the so-called mild wilt races of *Verticillium nonalfalfae*, aggressive races were also detected in the Hallertau for the first time during this project. Due to natural selection on wilt-tolerant hop variants, these more aggressive fungus races developed. These lethal races induce evident wilt symptoms and plant death in hop varieties classified as wilt tolerant.

In commercial yards there is often a mix of mild and lethal races present. As the usual plant protection strategies fail to stop the fungus, hop growers face increased economic loss. The goal of this project is the implementation of hygienisation and redevelopment measures for wilt disease caused by *Verticillium*.

Since the first observation of aggressive *Verticillium* strains in Germany, a continuous spread of the infested area has been observed in the Hallertau growing region. The pathogen is a soil-borne fungus with a broad host spectrum. It can survive in the soil for up to five years in the form of an infectious permanent mycelium and cannot be controlled directly.

Contaminated hop waste should generally not be spread in hop yards. Because specialised hop farms often lack other options due to hops being the only crop on the farm, hop residues must be returned to hop yards. To reduce the risk of new infestations and to not worsen the *Verticillium* problems on a farm, hygienisation is necessary. Thermal treatments are considered an option. Because of the legal framework in Germany, these thermal treatments cannot be extended at will. Therefore, different periods of storage time on the infectiousness of the hop waste were tested with eggplant used as an indicator plant.

Material and methods

Bioassay using the indicator plant eggplant

Eggplant (*Solanum melongena* L.) has been identified as a suitable indicator plant for hop wilt, as it is also susceptible to this pathogen (ANGELOPOULOU et al. 2014; ELMER & FERRANDINO 1994). Furthermore, it is suitable for a potting system in the greenhouse and quickly develops typical wilt symptoms. With the help of eggplants, for example, possible hygienisation measures can be tested for their effectiveness against the *Verticillium* fungus.

In this trial, infectious hop waste was exposed to thermal composting for different periods of time. Thereafter, the treated hop waste was mixed into potting soil and eggplants were planted on top. These eggplants had initially been cultivated in non-infectious potting soil.

During plant growth, parameters such as growth height, vitality and the expression of wilt symptoms were assessed. The development of the eggplants in the different experimental variants can be used to indirectly determine whether there is infectious *Verticillium* material in the plant pots and thus also whether the hygienisation was successful. In addition, some eggplants were examined in the laboratory via real-time PCR to validate the results (MAURER et al. 2013).

Thermal treatment of hop waste

Fresh hop waste is highly infectious and can infect the hop or other host plants very easily. However, the potential for infection cannot be determined straightforward, as high temperatures may occur in the composts during longer storage. This degrades the DNA and makes it more difficult to detect the *Verticillium* fungus. There are also some substances in the hop waste that cause an inhibition of the PCR reaction.

With the help of the bioassay on eggplant, the infection potential of the hop waste can be estimated. Results show that the infection potential of the *Verticillium* fungus in hop waste can be reduced drastically by storing it for four to seven weeks. In practice, however, to ensure that all layers are sanitised it is essential that the crop residues are shifted regularly. Only the high temperatures that develop inside the hop waste pile result in sufficient degradation or inactivation of the fungus and thus in a significantly lower infection potential of the hop waste. In this trial, compost boxes were used to simulate the inside of a hop waste pile.

Figure 3. Healthy control variant without hop waste and plants with wilting disease caused by infected hop waste (left); Compost box for hop waste storage (right).

Infected hop bines were taken from a *Verticillium*-infested hop yard as starting material and chopped. Depending on the experimental variant, the material was stored in the boxes for 1, 2, 4 or 7 weeks. In addition, two storage densities were tested. In the loose variant, the material was loosely filled in. In the other variant, the material was compressed.

As a control, fresh, non-hygienised material was frozen to keep the degradation processes caused by microorganisms or other changes as low as possible.

After storage, the hop waste was mixed into the planting soil of the eggplants. In addition to a control variant without the addition of hop waste, eight other variants were created. These can be distinguished based on their infection potential and the degree of compaction. Each of the experimental variants was performed on 15 plants.

Results and discussion

There were clear differences in the variants: the non-hygienised control showed the clearest disease symptoms of the plants, up to the death of the eggplants. In the composted material, the longer deposits (4 and 7 weeks) showed only marginal symptoms and appeared almost identical to the non-infected control. Thermal treatment for longer periods of time seems to be more effective. The storage of one to two weeks also showed success as the plants exhibited only little damage due to wilt disease.

Overall, the infection potential of hop waste could be significantly reduced by composting the hop waste over a longer period. The thermally treated variants performed better than the untreated variants. The level of the healthy control was however not reached. Nevertheless, a clear improvement in the fitness of the plants can be observed. This suggests that the material should be stored for a longer period, if possible.

Nevertheless, it is not advisable to return hop waste from infected plants to the hop yards. If in practice it will be necessary to spread these residues in hop yards, the risk of *Verticillium* infection can be reduced by thermal sanitation. In this study it was not possible to determine if a longer period of storage time will reduce the infection potential to zero.

Acknowledgement

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Genome-wide association study for Powdery mildew resistance in Common hop (*Humulus lupulus* L.) germplasm resources

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Abstract

Hop production, while affected by several pests and diseases, is routinely affected by the local, regional, and intercontinental spread of Powdery mildew, caused by *Podosphaera macularis* (Wallr.) Braun & Takah. Major losses are due to planting susceptible hop varieties or planting formerly resistant varieties in the presence of novel virulent pathogen strains. The Pacific Northwest (PNW) is the primary region of hop production in the United States, with substantial increases in acreage during the past two decades. Importation of infested plant material, subsequent pathogen dispersal, and novel virulence have contributed to the recent breakdown of resistance. Thus, identifying and exploiting novel alleles for powdery mildew resistance breeding remains one of the most effective means of combating this disease. To determine the genetics of powdery mildew resistance and help facilitate development of powdery mildew resistant hop varieties, our primary objective was to identify molecular markers associated with powdery mildew resistance. First, we assembled a panel of 1,152 wild hop accessions from the USDA – National Plant Germplasm System that included *H. l.* var. *lupuloides*, *H. l*. var. *lupulus*, and *H. l.* var. *neomexicanus*. Then, we phenotypically characterized the panel for resistance to an isolate possessing *VB35* and genotyped the panel using genotyping-by-sequencing (GBS). We found 1, 45 and 2 accessions from *H. l.* var. *lupuloides*, *H. l.* var. *lupulus*, and *H. l.* var. *neomexicanus* respectively that exhibited complete resistance. We used 33,956 markers from the GBS data, showing that individual taxa clustered separately. The combined phenotype and genotype data were used to conduct a genome-wide association study and we identified 10 QTL located on six chromosomes that were associated with resistance. These markers may be useful for selecting Powdery mildew resistant genotypes in breeding programs.

Key words. Hop, genetics, disease resistance, hop powdery mildew, GWAS

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Control of Downy mildew (*Pseudoperonospora humuli*) with the help of *Pythium oligandrum* (Polyversum) and *Pongamia pinnata* (Rock Effect New).

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Abstract

The spectrum of plant protection products that can be used to cope up with harmful organisms in hop cultivation is getting still narrower. Based on the European Food Safety Authority (EFSA) conclusions, the European Commission proposes non-renewal or restricted renewal of many of the present registered and practically used fungicides in the control of the most dangerous disease, downy mildew (*Pseudoperonospora humuli*). "European Green Deal" and its instrument "Farm to Fork Strategy" suppose reduction of synthetic pesticides use by 50 % till 2030. Under such situation it is critical to look for adequate alternatives. Bio-fungicide *Pythium oligandrum* (Polyversum), and botanical pesticide *Pongamia pinnata* (Rock Effect New) seem to be promising agents for not only organic hop protection but for IPM strategies as well.

Key words. IPM, organic hop growing, downy mildew, *Pythium oligandrum*, *Pongamia pinnata*, botanical pesticide, bio-fungicide, biological efficiency, strategies.

Introduction

Hop protection against pests and diseases is based nearly entirely on the application of synthetic pesticides. Nevertheless, their number is endangered by EFSA proposals of non-renewal or restricted renewal to the SCoPAFF. IPM strategies are getting compulsory for crop growers within EU including hops, which say that synthetic pesticides should be used only if there is no other option, meaning if there is not adequate substitution. In organic hop growing we are dependent nearly entirely on copper fungicides to control Downy mildew. However, the fortune of copper within plant protection is very uncertain. If considering Maximum Residue Levels (MRLs), *i.e.*, the highest amount of active ingredients allowed in hops exported to the country of destination and which is differs a lot within the world (DUŠEK et al. 2021), there is no wonder that hop growers are worried and it is up to us to search for alternative ways. In the control of Two-spotted spider mite *Tetranychus urticae*, the predatory mite *Typhlodromus pyri*, can support native acarophagous predators and help to re-establish seriously disturbed natural balance in both organic hop growing and IPM (VOSTŘEL 2019). Similarly, the release of predatory mites *Galendromus occidentalis* and/or *Neoseiulus fallacis* for the management of spider mites is recommended in hop yards in Washington and Oregon in USA. Selective pesticides that have minimal negative impact on natural enemies, including predatory mites, are used (O'NEAL et al. 2015). Some natural phenolic compounds, including *Pongamia pinnata* show good antifungal efficacy against significant pathogenic and toxinogenic fungi (ŽABKA & PAVELA 2013). *Pythium oligandrum,* a non-pathogenic soil-inhabiting oomycete, colonizes the root ecosystem of many crop species. Whereas most members of the genus *Pythium* are plant pathogens, *P. oligandrum* distinguishes itself from the pathogenic species by its ability to protect plants from biotic stress in addition to promoting plant growth (Benhamou 2012). Good efficiency of both *P. pinnata* (product name 'Rock Effect New') and *P. oligandrum* (Polyversum) was observed in experimental hop yards in Saaz hop growing region in 2019 (VOSTŘEL 2021).

Material and methods

From 2019 to 2020, Polyversum was applied in the registered rate of 0.25 kg/ha in the following Bohemian hop gardens: Libočany, Nesuchyně, Blšany, Soběchleby (Saaz hop growing region) and Liběšice and Brozany (Úštěk hop growing region). Each experimental hop garden was divided into two parts; one was sprayed completely with Polyversum and the other with synthetic fungicides, which are registered in the Czech Republic and commonly recommended for practical use against Downy mildew.

In 2020 and 2021 a part of each experimental hop garden was divided into three plots: Polyversum, synthetic fungicides and the combination of Polyversum (applied under lower to medium infection pressure) and synthetic fungicides (applied under higher infection pressure). The experimental area was from 0.5 to 2.0 hectares. Water volumes were adapted to the actual habitus of hop plants (BBCH). Number of applications during the season varied between five and ten, according to infection pressure, locality, and tolerance of the variety. The field trials were established in early spring to control primary infection. Last treatments were carried out in August, several days before harvest.

Symptoms of the disease were assessed by EPPO Guidelines for the Efficiency Evaluation of Fungicides – *Pseudoperonospora humuli* /PP 1/3(4)/. Cones were sampled shortly before harvest. Five hundred cones were evaluated in four replicates, meaning a total of 2,000 cones from each experimental plot. For this purpose, the following scale was used: 1. Without damage, 2. Light damage (1-5 bracteoles damaged), 3. Medium damage (up to 50 % of the cone area), 4. Severe damage (more than 50 % of the cone area). Besides, alpha acid contents were measured as well.

Research on potential uses of products based on plant extracts in hop protection against harmful organisms is a part of the five years' project, which was started in 2019. Within this project Rock Effect New was applied in 1.0 % concentration in an experimental hop garden (cv. Premiant) to control Downy mildew in 2020 and 2021. The experimental area amounted to 0.3 ha. Water volumes were adapted to the actual habitus of hop plants (BBCH). Evaluation of hop cone damage was carried out according to EPPO Guidelines for the Efficiency Evaluation of Fungicides – *Pseudoperonospora humuli* /PP 1/3(4)/.

Results and discussion

It is known that *P. oligandrum* induces systemic resistance to Fusarium crown and root rot in tomato plants (BENHAMOU et al. 2001). A proteinaceous molecule produced by *P. oligandrum* also induces resistance to *Phytophthora parasitica* (PICARD et al. 2000) and expresses putative effectors during mycoparasitism of *Phytophthora infestans* (HORNER et al. 2012).

As good efficiency of *P. oligandrum* had been confirmed to primary infection of Downy mildew in Bohemian hop gardens, field trials were carried out since 2019 till 2021 to find out efficacy of this species to secondary infection of the disease. Polyversum applied in the registered rate of 0.25 kg/ha during the season in the intervals common for synthetic fungicides showed good biological efficiency under lower infection pressure in 2019 (Fig. 1), which was comparable to the efficacy of conventional fungicidal sequences routinely used in practical hop protection. In the trials carried out in 2021 and 2022 Polyversum was a part of a fungicide sequence. On the contrary to 2019 favourable weather conditions led to high infection pressure of DM. Under such conditions Polyversum was recommended to be applied under lower to medium, whereas synthetic fungicides were applied under higher infection pressure. The results show that such a strategy is possible to control Downy mildew even under weather conditions suitable for the pathogen. Noteworthy is also its positive effect on the content of alpha bitter acids (Fig. 2).

Rock Effect New also showed good efficacy on Downy mildew during field trials in 2020 and 2021, the years with high infection pressure if applied in 1.0 % concentration in the common fourteen days intervals (Fig. 3). Its liability consists in high rate, which is necessary for this purpose. On the other hand, it also showed good side effect on Two-spotted spider mite and Damson hop aphid. No phytotoxicity was observed during the trials.

Acknowledgement

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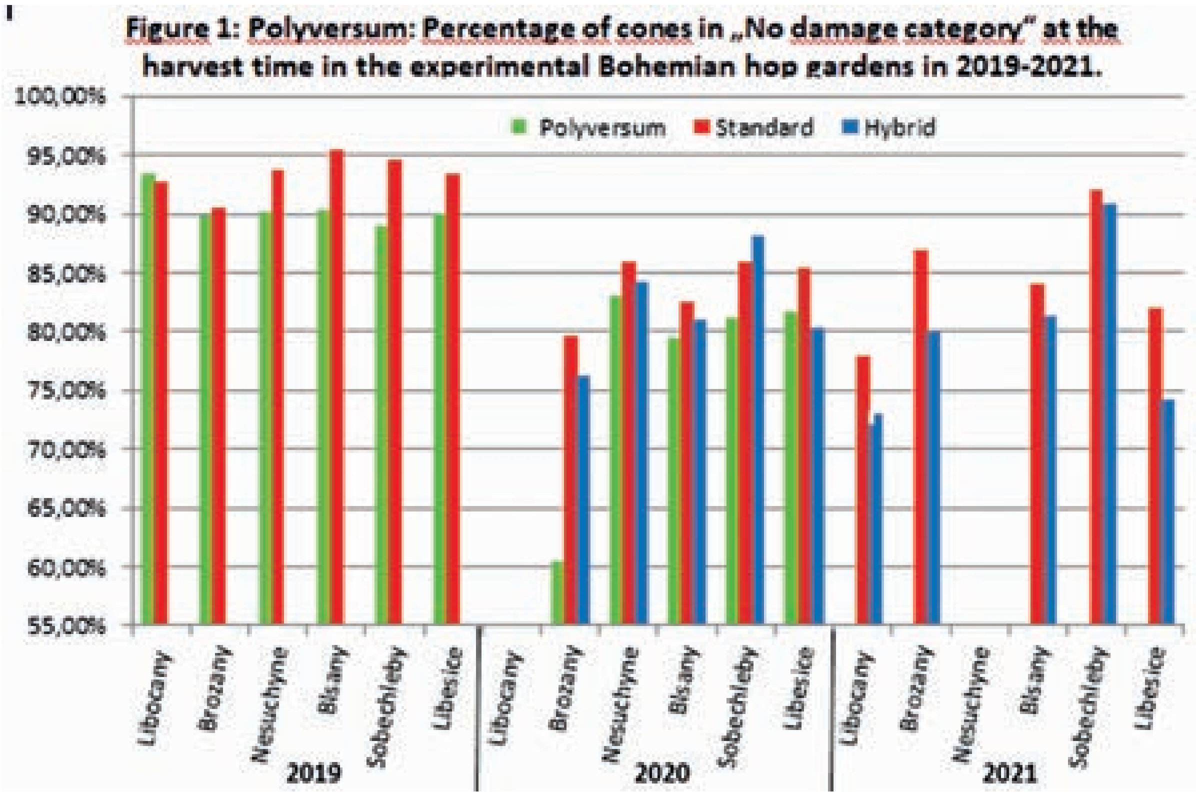


Figure 2: Polyversum: Contents of alpha bitter acids at the harvest time in the experimental Bohemian hop gardens in 2019-2021.

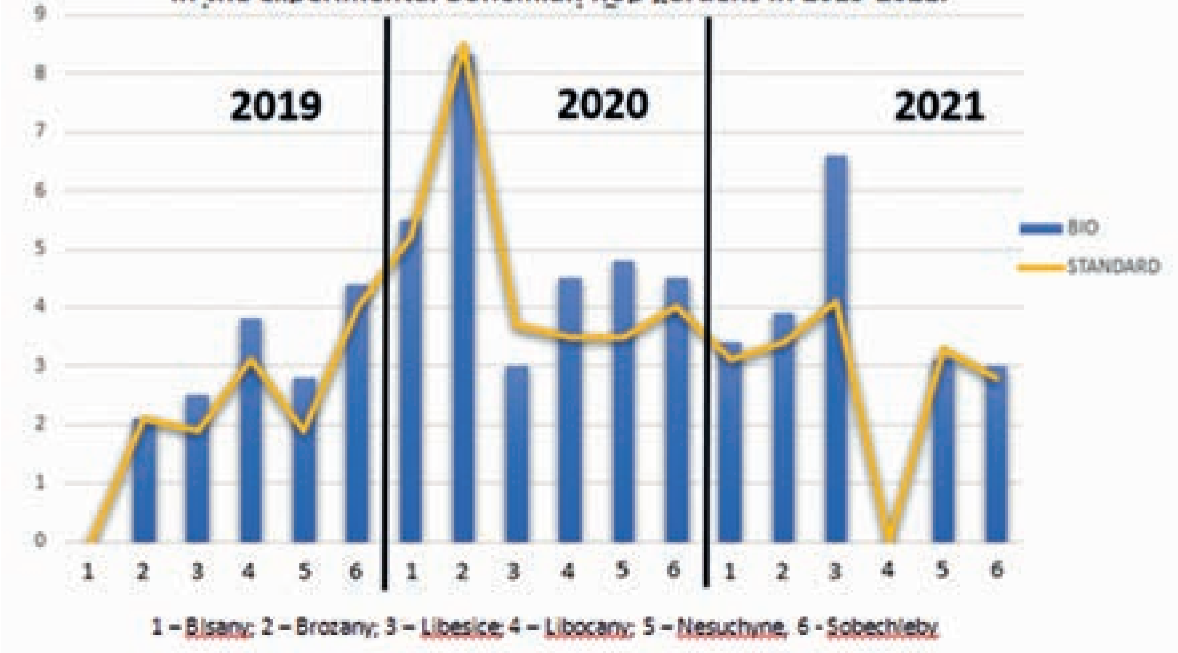
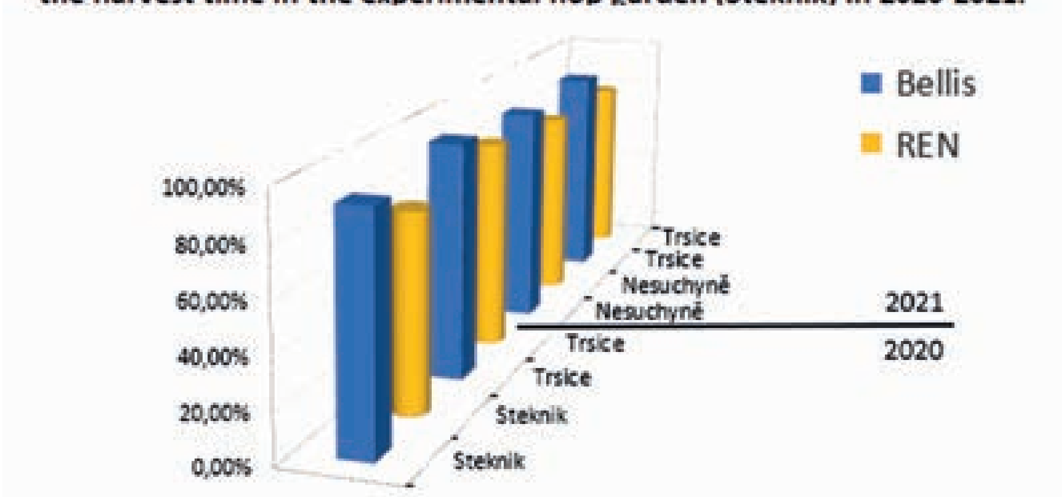


Figure 3: Rock Effect New: Percentage of cones in „No damage category" at the harvest time in the experimental hop garden (Steknik) in 2020-2021.



Tiafenacil – a new herbicide in hops

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Abstract

Powdery mildew (*Podosphaera macularis)* in hop is among the most economically damaging diseases in the Pacific Northwest. Management is multifaceted including removal of basal foliage during the growing season to reduce disease inoculum pressure. Basal foliage in hops is removed using two or three basal-directed applications of postemergence herbicides like paraquat or carfentrazone during the summer season. These basal treatments also target weeds including kochia (*Kochia scoparia*) and Italian ryegrass (*Lolium perenne* spp. *multiflorum*). Alternatives for paraquat are needed to cope with increasing regulatory restrictions on pesticide use and export markets. Tiafenacil is a protoporphyrinogen oxidase inhibitor with foliar activity on dicot and monocot plants. We tested tiafenacil weed control efficacy and hop tolerance in four locations between Oregon and Washington in 2020 and 2021. Treatments included one or two applications of tiafenacil at the 50 g ai ha^{-1} (expected field rate) and 100 g ai ha^{-1} compared with a nontreated control and a carfentrazone reference treatment at 35 g ai ha^{-1}. The initial application was made when hop was 0.9 to 1.8 m tall and the second 30 d later. Tiafenacil improved hop basal foliage removal and Italian ryegrass control compared to carfentrazone while providing similar control of kochia. Better weed control results were observed when treating bines 1.8 m or less. Tiafenacil did not affect hop bine height or crop injury. Combined analyses of four sites showed no effect of application timing, number of applications, or herbicide rate on crop injury or hop fresh weights. However, both carfentrazone and tiafenacil reduced cone yield by 20% compared to nontreated. This yield reduction by both herbicides likely resulted from applications made to small hop bines (<1.8 m) and high variability in hop growth. The results indicate that tiafenacil is a promising option for hop basal shoot control and weed control. Additional work is needed to evaluate hop tolerance to tiafenacil over multiple growing seasons.

Key words. Basal shoot, weed control, herbicide.

Introduction

Hop (*Humulus lupulus*) is an herbaceous perennial plant with twining stems that regrow vigorously from the root system every year. Despite hop's vigorous growth, weed competition can significantly reduce yield, ranging from 69% yield loss to complete crop loss (DELAHUNTY & JOHNSTON 2015). The current weed control practices in the Pacific Northwest (PNW) region of the United Stated consist of cultivation between the planting rows and chemical control within the planting rows. Herbicides are also used to remove hop basal foliage to suppress powdery mildew disease (*Podospheara macularis)* (GENT et al. 2008). Despite the reliance on chemical weed control, very few herbicides are available for hops in the US (MORETTI 2022), and availability is further limited by export or regulatory restrictions as in the case of paraquat. The hop industry has a critical need for new herbicides to replace paraquat uses.

Tiafenacil is a foliar active herbicide with contact and nonselective activity in broadleaves and grasses (PARK et al. 2018). Its mode of action was classified as a protoporphyrinogen IX oxidase inhibitor (PPO). The PPO inhibitors prevent formation of protoporphyrin IX, which in turns accumulates in the cytoplasm, forming greater levels of reactive oxygen species that results in cell membrane damage and plant death (DAYAN & DUKE 2010). Tiafenacil is active at low application rates and has reduced human toxicity. The objective of this study to determine hop tolerance to tiafenacil and weed and basal leaf control in hop yards.

Material and methods

Four field studies were conducted in mature commercial hop yards in Oregon and Washington in 2020 and 2021. The hop yards in Oregon were located on silt loam soils, and the location in Washington in a fine loamy soil. Commercial growers managed the hop yards up to training. The experiment was initiated when hop bines were 0.8 to1.8 m in height. Treatments included tiafenacil applied at 50 and 100 g ai ha^{-1} (active ingredient) applied twice, first two weeks after hop training, and again six weeks after training. Some treatments were applied at both timings. Treatments were applied to each side of the hop row as a banded-directed application to the lower part of the hop plant, with a CO_2 pressurized backpack sprayer calibrated to deliver 187 l ha^{-1} at 275 kPa. The spray boom was equipped with three TeeJet AI-11002 nozzles spaced at 0.5m and set 0.5 m above the target. Assessments included crop height (m), visual estimates of crop injury (%), and basal leaf control (%). Whenever present, weeds were evaluated, including kochia (*Bassia scoparia* L.), and others. Hop was harvested by mobile harvester when cone dry matter content was 25 to 28 %. Cones were dried to 10 % moisture and weight per plant was recorded and averaged within a plot. Treatment plots were arranged in a randomized complete block design with four replicates. Each experimental unit included three hop plants. Data were subjected to analysis of variance; means were separated by Turkey's test. Contrasts were designed to compare application timing, number of applications, and herbicide injury.

Results

Basal foliage control with tiafenacil was more effective than with carfentrazone, and no signs of crop injury or reduction in plant height were observed throughout the growing seasons (Fig. 1). Tiafenacil at 50 g ai ha^{-1} controlled 80 % of the kochia present at one of the sites, and it was not different than carfentrazone at 35 g ai ha^{-1} (70 %) (Fig. 2 left). Contrasts combining data from multiple studies showed no effect of application timing, number of applications, or herbicide rate on crop injury or hop fresh weights. However, carfentrazone and tiafenacil reduced cone yield by 20 % compared to nontreated (Fig. 2 right).

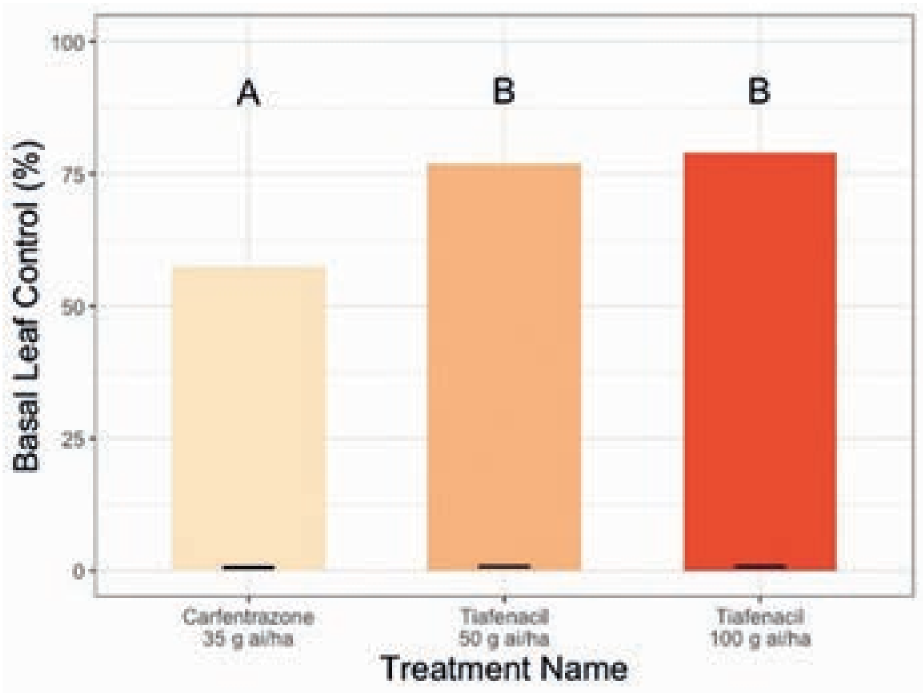

Figure 1. Basal foliage control with carfentrazone or tiafenacil at 50 and 100 g ai ha^{-1} evaluated seven days after treatment. Means are averaged over four locations (n=16) and means followed by different letter are statistically different based on Tukey's test (P<0.05).

Discussion

This research indicates that tiafenacil is an effective herbicide to remove basal foliage, improving foliar removal compared to current standard treatment. The improved removal of basal foliage is likely to benefit powdery mildew suppression (GENT et al. 2008). Although tiafenacil did not cause observed injury to the hop bines, we documented a yield reduction when a combined analysis was done. Yield reduction was also noted on carfentrazone-treated plants, an herbicide currently used in the US. This suggests that basal foliage removal was performed when hop bines were too small. The high variability in hop growth within a field may also have affected results. Further work must be done to confirm crop tolerance over multiple seasons and to document its impact on disease suppression.

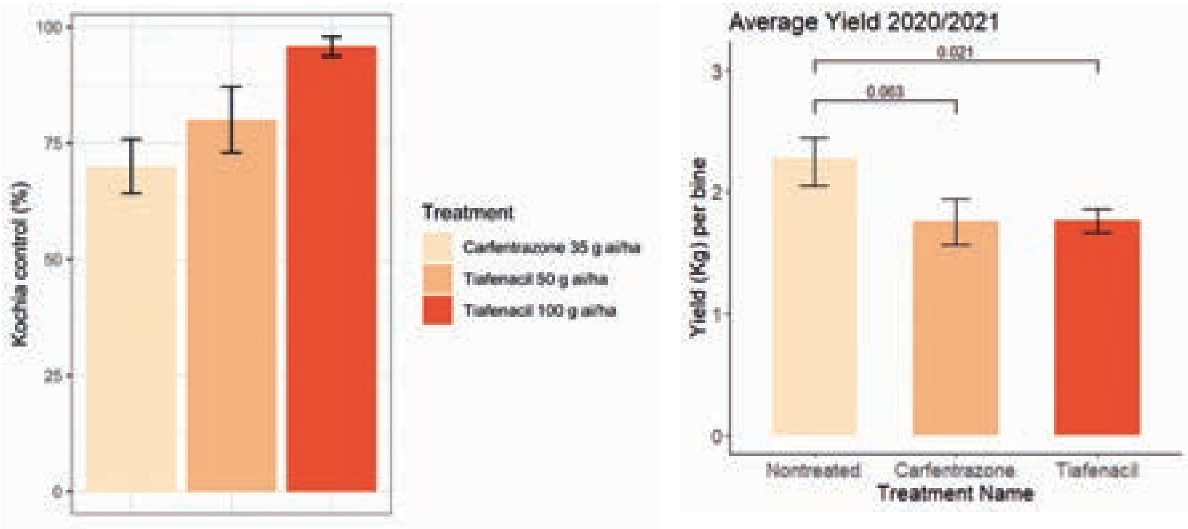

Figure 2. Kochia control 14 days after treatment with different postemergence herbicides in a hopyard in eastern Washington, USA during the summer of 2020 (Left). Contrasts comparing untreated control to carfentrazone and tiafenacil treatments show a 20 % reduction in yield associated with herbicide treatments (right). Error bars represent the standard error of the mean.

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Stability and transmission of Citrus bark cracking viroid (CBCVd) with water on hop (*Humulus lupulus* L.)

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Abstract

In recent decades, water quality in crop production was threatened by different environmental impacts like the contamination with plant pathogens. Because of a more difficult water access and water scarcity, crop production with irrigation systems or in soilless cultures, using closed or open hydroponic systems, has been increasing worldwide. Those types of crop production can be sourced from surface water supplies such as ponds, lakes, rivers, and reservoirs. As such, the water used can harbour microorganisms causing diseases including viroids, which can cause many symptoms on plants and lead to production loss. Existence of plant pathogens in environmental waters is important for causing water pollution and dissemination among agricultural areas. Different water supplies could be pathway for many water-borne plant pathogens, such as Citrus bark cracking viroid (CBCVd), which causes agressive symptoms on hop (*Humulus lupulus* L.). CBCVd spreads mainly by mechanical means such as residues of plant sap of infected plants on tools and organic residues on hop fields. Unknown pathway of viroid spread is spread with water supplies. In our study we investigated the stability of and the infection with CBCVd in water samples. In a first experiment we tested the higher dilution of CBCVd in water samples that could be detected with real time RT-PCR. In a second experiment, we tested weekly the presence of CBCVd in water. Both experiments were confirmed with diagnostic analysis (real time RT-PCR) and then via mechanically infected testing hop plants of cv. Celeia.

Possible import routes and sequence variations of the Citrus bark cracking viroid in German hops

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Abstract

Due to high-quality bitter hops, fine-flavoured aroma hops and approximately one-third of the global hop harvest, Germany is the most important hop-growing country for the international beer industry. The pathogen Citrus bark cracking viroid (CBCVd) was detected for the first time in 2019 in hops in Germany. Until now it remains unclear how CBCVd was initially introduced to the largest German growing region, the Hallertau. There are two possible explanations; (i) the German outbreak might result from infected planting material imported from Slovenia, where CBCVd is present, or (ii) the CBCVd infection is based on residues of citrus fruits, which can be hosts for CBCVd.

To further investigate the actual cause of events leading to CBCVd infections in Germany, we examined the full-length sequences of CBCVd from samples from the hop viroid screening in 2020 of the Bavarian State Institute for Agriculture and from fruits of local grocery stores close to hop production sites. Because citrus can be a host for CBCVd, we analysed mainly citrus fruits including oranges, grapefruits, lemon, lime, easy peelers, and more rare citrus species. To get a good overview of the changing assortment in stores in relation to countries of origin, we collected samples at 7 dates between summer 2021 and summer 2022. The analysis of fruits from the grocery stores showed that CBCVd was detectable in 8% of the total samples, with citrus sample sequences differing from the hop variant sequences. More than half of the CBCVd positive samples derived from lemon (*Citrus limon* L.), the other samples were either from oranges, easy peelers, or grapefruits. There was no clear association between CBCVd detection and the origin of the fruit sample. The results of the hop viroid screening showed that there were at least 13 CBCVd variants in the Hallertau, whereas 41% were identical to the originally described Slovenian variant (KM211546). The larger of the three CBCVd outbreak areas also showed the highest sequence diversity, which might be based on silent mutations. At first glance these results suggested that the German outbreak was related to the Slovenian outbreak. The three locations and sequence variability further suggested that CBCVd had been present undetected for several years. However, viroids can quickly undergo a process called host adaptation, thus citrus variants might have adapted from citrus to hops over time. This possibility will be addressed in the future using infection experiments.

Introduction

Viroids are the smallest known pathogens and can cause severe symptoms in plants. Hop (*Humulus lupulus* L.) is known to be a host plant for four viroids, of which the citrus bark cracking viroid (CBCVd) causes the most severe symptoms. CBCVd was first detected based on heavily stunted hop plants in Slovenia in 2007 (JAKŠE et al. 2015). In 2019 it was also detected in the Hallertau, which is the largest continuous hop growing region in the world (JULIUS KÜHN-INSTITUT 2019). Besides stunting, CBCVd leads to a reduction in quality, lower yields and even death of infected plants within a few years (RADIŠEK & BENKO-BELOGLAVEC 2016). While CBCVd is a threat to profitable hop cultivation, it is either tolerated or even actively used as dwarfing viroid in citrus production (VIDALAKIS et al. 2010; BAR-JOSEPH 1993). This fact has led to the hypothesis that the primary infection of hop plants in Slovenia is somewhat based on CBCVd positive citrus plant residues (RADIŠEK & BENKO-BELOGLAVEC 2016).

Since citrus is not produced in Central Europe, the most obvious import route of citrus is global trade through grocery stores. Furthermore, citrus fruit residues might end up in hop gardens by careless consumers, through compost or citrus-based products. Consequently, the aim of this study is to quantify the potential of CBCVd infected fruits in grocery stores in the hop growing region were the German outbreak occurred. Additionally, we compared the sequences of CBCVd-variants in hops in the Hallertau with sequences from grocery stores. Since viroids exist as quasispecies with high mutation rates up to one mutation per 1000 nucleotides, their sequences can vary within a species or individuum but also quickly undergo host adaptation (BRASS et al. 2017; LÓPEZ-CARRASCO et al. 2017). Nevertheless, sequence comparisons did help to reconstruct possible infection routes in the past (SANO et al. 2001) and the results presented here are a starting point for discussing the two most likely hypotheses for the origin of the CBCVd infection in German hops.

Materials and methods

Sample preparation and RNA extraction

Citrus samples were collected from local grocery stores in the Hallertau at approximately two-month intervals starting in late summer of 2021 until summer of 2022. This was done in order to account for the changing assortments of grocery stores and therewith get fruit from different producing countries. The following fruit was sampled based on availability: lemon, lime, orange (navel orange, cara-cara orange, newhall, salustiana, tarocco), easy peeler (clementine, tangerine, satsumas), grapefruit (grapefruit and pomelo), citrus diverse (bergamot, cumquat). All samples were processed at the University of Hohenheim within two days after purchase. Citrus fruit was peeled with a blade to collect the flavedo, because this tissue showed higher RNA yields compared to the albedo (data not shown). Then the fruit peels were stored at -30°C until RNA extraction.

For the RNA extractions the Monarch Total RNA Miniprep Kit (New England Biolabs, Ipswich, USA) has been used following the manufacture's guidelines. Approximately 100 mg of each sample was homogenized by grinding using liquid nitrogen. Thereafter, RNA purity was tested by analysing 2 µl with the spectrophotometer (Nanodrop 1000, ThermoFischer, Waltham, USA). The samples were stored at -80°C until further use.

The RNAs from hop plants were provided by the Bavarian State Institute for Agriculture (LfL). The samples derived from the CBCVd survey conducted in 2020. The RNA extraction and PCR analysis have been conducted by an external service provider following the protocols described earlier (SEIGNER et al. 2020). The CBCVd positive samples have been collected at the LfL and transported to the University of Hohenheim on dry ice, then stored at -80°C until further processing.

PCR-Analysis

The citrus samples were analysed by reverse transcription – duplex – real-time quantitative PCR (RTRTqPCR) with primers for the identification of CBCVd and the internal control nad5 (SEIGNER et al. 2020; Table 1). For every RNA sample two volumes 0,2 µl and 1 µl were analysed to account for possible inhibitory effects from the sample matrix.

The RNA samples of CBCVd positive as determined with RTRTqPCR either in our lab or at the LfL, were reverse transcribed and amplified in a one-step reaction (RT-PCR) with the QIAGEN OneStep RT-PCR Kit (QIAGEN, Hilden, Germany). In order to receive two complementary amplicons, *i.e.*, amplicons with overlapping ends, the primers CVd-IV-F1 and CVd-IV-R1 as well as CBCV_1 and CBCV_1B were used (Table 1). A subsequently conducted gel electrophoresis resulted in a single band. To account for the small sequence length the gel was made with 1,5 % agarose (Bioproducts SeaKem® LE, Rockland, USA) and stained with 5 µl peqGREEN DNA/RNA Dye for a 100 ml gel (peqlab by VWR Part of Avantor, Darmstadt, Germany).

Table 1. List of primers and probes used in this publication

Name	Sequence	Direction / Fluorophore	Reference
CVdIV_qPCR_F	GGAACAGGAGCTCGTCTC	Sense	(SEIGNER et al. 2020)
CVdIV_qPCR_R	CAAGAGTTGTATCCACCGGG	Antisense	
CVdIV_qPCR_P	CATCGCTGGCTCCACATCCG	FAM	
NAD5-Menz_F	GATACTTCTTGGGGCTTCTTGTT	Sense	(MENZEL et al. 2002)
NAD5-Menz_R	CTCCAGTCACCAACATTGGCATAA	Antisense	
NAD5_P	AGGATCCGCATAGCCCTCGATTTATGT	HEX	(BOTERMANS et al. 2013)
CVd-IV-F1	GGGGAAATCTCTTCAGAC	Sense	(BERNAD & DURAN-VILA 2006)
CVd-IV-R1	GGGGATCCCTCTTCAGGT	Antisense	
CBCV_1B	GTTGTTCCTCCCAGGCTTGT	Sense	this publication
CBCV_1	CAAGAGTTGTATCCACCGGG	Antisense	(HAGEMANN et al. 2021)

Sequencing

CBCVd positive samples from citrus fruit (n=14) and from the Hallertau hop survey (n=56) were sequenced by Sanger sequencing. Therefore, the amplicons of the RT-PCR reaction were prepared with the Exo-CIP Rapid PCR Cleanup Kit (New England Biolabs, Ipswich, USA) with 3 µl each of Exo-CIP A and B, 5 µl of sample and 10 µl of deionised water, since this gave the best results for sequencing. The samples were then sent to Eurofin Genomics (Ebersberg, Germany) for the Eurofins Supreme Tube Sanger Sequencing service. The received sequences were then trimmed based on low quality and primers used in the corresponding PCR reaction. The trimmed sequences were then assembled de novo with the Geneious assembler (Geneious Prime® 2022.1.1, Biomatters Ltd, New Zealand) and the resulting consensus sequence was verified by BLAST at NCBI (ALTSCHUL et al. 1990) and used for sequence comparisons with the MAFFT alignment tool at the following settings, determination of algorithm set to auto, score set to 200PAM, gap opening penalty set to 3, offset value set to 1, automatic determine sequence direction was activated (KATOH et al. 2002). The resulting alignment was then used as input for the Randomized Axelerated Maximum Likelihood (RAxML) phylogenetic analysis tool (STAMATAKIS 2006).

Result and discussion

Citrus fruit survey

The results of the citrus fruit survey showed that indeed CBCVd had entered Germany with the route of global trade. The total number of 8 % CBCVd fruit samples make clear that certainly not every fruit is infected but there was a substantial chance for the presence of CBCVd especially in lemons, where the percentage of infected fruit was 19 %. Six of the CBCVd positive fruit samples came from Turkey, three from Spain, three from Israel, and two from Italy. Because of the small sample size this finding should not be interpreted as if those countries were a major source of CBCVd, but others are not. However, we concluded that if CBCVd is present in a citrus producing and exporting country, this might be a source for infected imported fruits.

We also tested grapes and melons for CBCVd. But since they are not known to be CBCVd-host plants, therefore it was not surprising that the results were negative (data not shown). It was interesting to note that lemons in contrast to limes and cumquats did not show infections despite their close evolutionary relation and despite reports that lime and cumquats actually can be hosts for CBCVd (RADIŠEK & BENKO-BELOGLAVEC 2016; WU et al. 2018). However, this might be coincidental or based on the lime species, since in the risk assessment of Radišek, limes were referred to as *Microcitrus warburgiana*, while the fruit in the German grocery stores is more likely to be *Citrus × aurantifolia.*

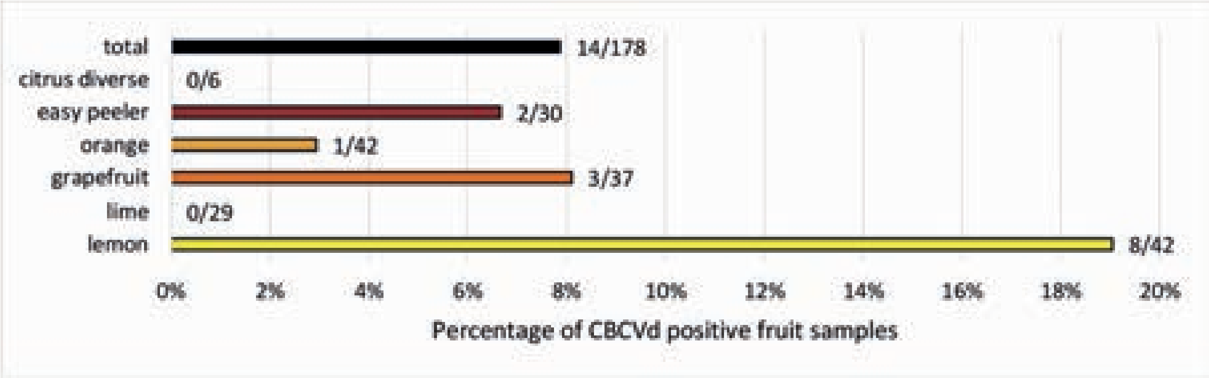

Figure 4. Percentage of CBCVd positive fruit samples analysed by RTRTqPCR. Bar labels indicate the total sample size and number of positive samples.

Pathways from grocery stores to the field

Currently, only hypotheses are available to explain the pathway of CBCVd from grocery stores to the field. The most probable theory for the introduction of CBCVd into Slovenia is that a mechanical transmission from citrus fruit waste took place in the past. This theory is supported by the observation that the hop garden, where the first outbreak was detected, was partly placed on a former illegal deposit for household waste and waste from a fruit distribution centre (RADIŠEK & BENKO-BELOGLAVEC 2016). The line of reasoning thus could be that the infested citrus fruit went from the grocery either directly to the deposit (old fruit that was not sold, and other citrus waste) or first to the user (private households, restaurants, processing) and then to the deposit. The location of the hop garden on a former waste deposit was a coincidence that significantly increased the risk of transfer of the viroid to hops. The transfer of the viroid from waste deposits or from compost piles where citrus fruit or peel have been discarded to hop gardens without a direct connection between these areas seems less probable, but would need further investigations. However, the use of compost containing citrus or hop waste to directly fertilize hop gardens on the other hand seems to be more probable though. So far, the introductions of the viroid to Germany that have happened seem to be more related to the Slovenian outbreak.Theories that infections resulted from discarded citrus fruit or peel by workers in hop gardens or by consumers seemed less probable due to the very low amount of infectious material. However, also this pathway cannot be fully excluded.

CBCVd variants in German hops

A total of 56 full-length sequences could be received from the Hallertau CBCVd survey samples. Seven sequences are unique, and 6 occurred in 2 or more Hallertau hop CBCVd isolates (Table 2). The most abundant variant A1a (41 % of cases) corresponds to the originally published variant from Slovenia KM211547 (JAKŠE et al. 2015). As expected, based on preliminary data (L. Seigner pers. comm.) a share of the sequences (59 %) belongs to new, previously unpublished hop CBCVd variants. We grouped those variants based on sequence variations in the central conserved region, whereas the group A has no variation compared to KM211547. The groub B has the sequence “AAGT” instead of “GAAA” at position 223-226, while the group C has the sequence “AAAA”, and group D a deletion resulting in “-AAA” at the same position, respectively. It is important to know that Sanger sequencing will only identify the quantitatively dominant variant out of a given sample, while deep-sequencing analyses have shown for CBCVd and other viroids that typically more viroid sequence variations exist in an individual infected plant, but those variants are typically less abundant compared to the dominant sequence (JAKŠE et al. 2015; BRASS et al. 2017; TESSITORI et al. 2013).

Table 2. Number of CBCVd variants found in three different hop growing areas in the Hallertau.

Area	Size	CBCVd variants												
		A1a	A1b	A2a	A2b	A2c	A2d	B1a	B1b	B2	C1a	C1b	C2	D
A	large	18		6	1	1	1	5		4	6	1	1	1
B	small	1												
C	small	4	1					3	2					

Nevertheless, the fact that apart from the A1a variant, other variants developed to be the dominant variant show that there are either silent mutations or that host adaptation is involved. If silent mutations have no negative effect on viroid reproduction, they will occur at equal frequency. This seems to be the case for most of the CBCVd variants in the Hallertau and is in line with experiments with the citrus dwarfing viroid (CDVd). It has been shown that over 25 years the same CDVd variant was dominant in individual trees of trifoliate orange, while each tree had indented subvariants (TESSITORI et al. 2013). In contrast to those results the B1a variant of CBCVd did occur at two different production areas in the Hallertau and has been detected in 8 independent samples. The CDVd study also showed that viroid variants can adapt to new hosts within only one year. This host adaptation has also been shown for hop stunt viroid (HSVd) in Japan. The HSVd is widely present in grapevine in Japan and elsewhere (SANO et al. 2001). When HSVd was discovered to be the causal agent of a hop stunting disease in Japan the sequence analysis showed a new hop adapted variant, but it could be shown experimentally that the HSVd grapevine variant will quickly evolve in the hop variant when infected to hop plants (SANO et al. 2001). This finding has been interpreted as evidence that HSVd "jumped" from grape to hop, causing a new disease, similarly to the "jump" of CBCVd from citrus to hops in recent years. This host adaptation might also occur on the cultivar level. However, currently the data are not available for German CBCVd hop variants, but it would be interesting to know if the variants A2a and B2 or C1a are specific for a certain cultivar. In future experiments the cultivar adaptation will be assessed along with the important search for viroid tolerance in hops.

CBCVd variant comparison

The results of the phylogenetic analysis are shown in Figure 2. Based on the examination of the branches we constructed five clusters considering biology and sequence similarity. To justify those clusters, we calculated the average sequence deviation relative to the Slovenian CBCVd hop accession KM211547. The pistachio variant of CBCVd was set as an outgroup because it had 57 nucleotide differences or in other words was only 78 % identical to the hop variant. According to viroid taxonomy this would even justify a discussion if this variant was a distinct viroid (DI SERIO et al. 2014). Apart from pistachio, the first group (purple) only contained variants from Chinese citrus fruits, which have not been detected in our citrus survey. Within the second cluster of hop variants (green) the sequence variation is low with on average 4 nucleotide differences between the variants. The closest accession between the hop cluster and the third citrus grapefruit cluster (black) had 5 nucleotides different between A1a and MG457797, which was also a citrus fruit from China. This small difference could make host adaptation more likely. However, the different biological background was used to justify the clustering. Within the third cluster grapefruit and lemon samples showed to be CBCVd positive, so this might be a possible origin for CBCVd hop variants. The fourth cluster (red) stands out by being more distant to the other CBCVd variants. Again, this cluster contained mainly Chinese citrus accessions but also accessions from Cuba and Egypt. None of those accessions have been found in Germany. The fifth cluster (grey) contained the most CBCVd accessions from very different origins and fruit species. This cluster contained most of the variants found in German grocery stores. In contrast to the diversity of sample type, sequence variation was relatively low with 10 bases difference on average. The highest difference found between these citrus samples and the hop variants was 9 nucleotides, so this might as well be a possible source of CBCVd infection.

In future experiments, the infection of hops with CBCVd-citrus variants will be conducted to find out if there are similar host adaptation processes possible as described for HSVd (SANO et al. 2001).

Concluding remarks

These studies have demonstrated that plant material causing viroid infections can be found in grocery stores. Depending on what happened with the resulting citrus waste after consumption, there could be a risk of introducing viroids (and other pests or pathogens) to their host plants provided a transfer is possible (availability and proximity of the host plants, effective transmission, suitable climate etc.). The highest risk appears to stem from a direct connection between the area, where the infected material has been deposited, and the hop garden, as observed in Slovenia. Another risk for hop growing might be the use of untreated infected hop harvest residues. A direct disposal of citrus fruit or citrus waste by individuals, however, is probably of a lower risk.

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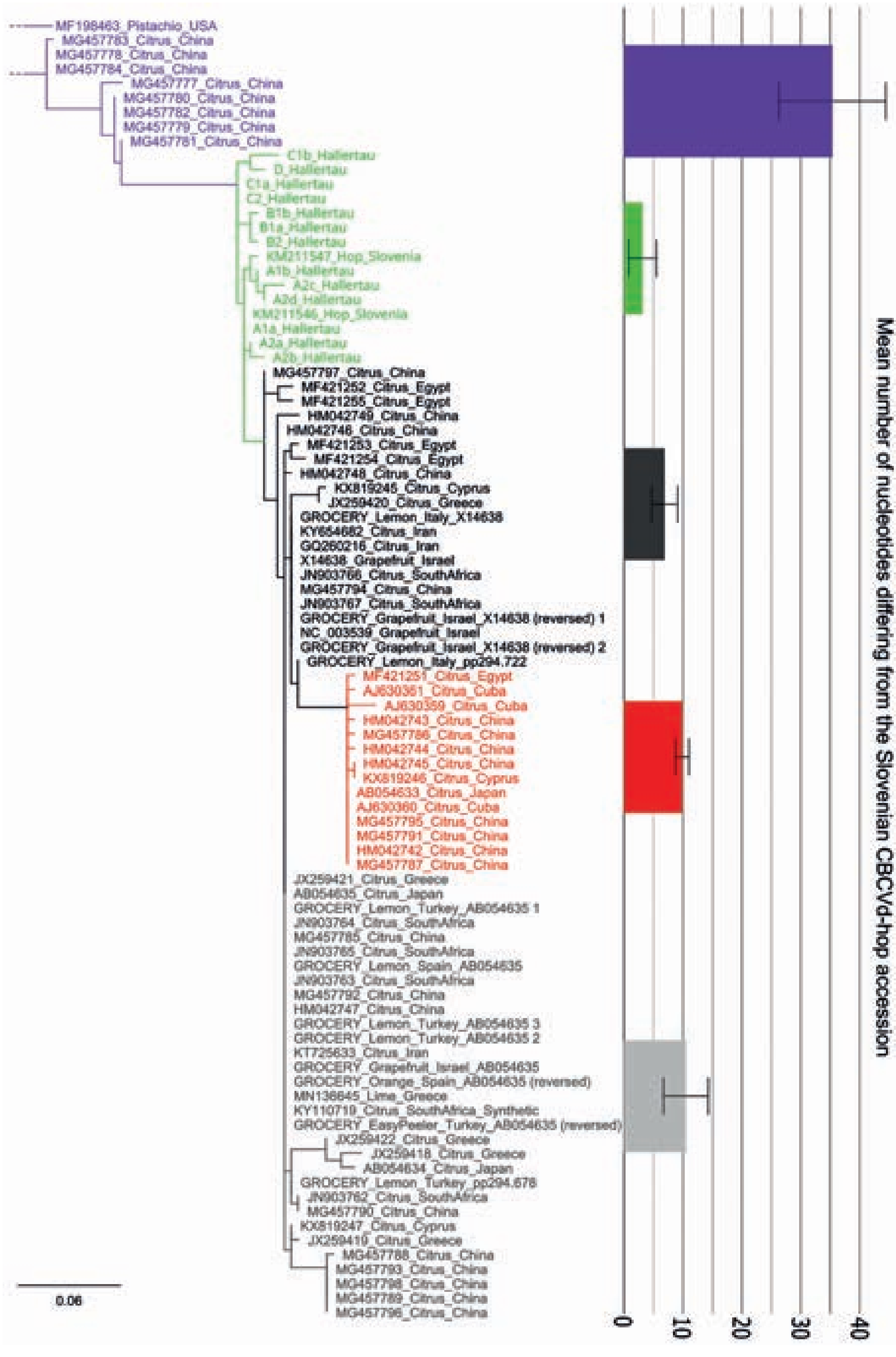
Figure 5 (next page). Left – Phylogenetic analysis of CBCVd variants (1) from the NCBI database with their accession number, species and contry of origin, (2) from the CBCVd survey in the Hallertau with the variant number labeled “Hallertau”, and (3) the results of the citrus fruit survey indicted by the label “Grocery” followed by either the accession or PCR number if no accession matches at 100% pairwise identity. **Right** – Mean value and standard deviation of the number of nucleotides differering from the Slovenian CBCVd-hop accession KM211547 for each of the CBCVd clusters indicated by different colors.



MF198463_Pistachio_USA
MG457783_Citrus_China
MG457778_Citrus_China
MG457784_Citrus_China
MG457777_Citrus_China
MG457780_Citrus_China
MG457782_Citrus_China
MG457779_Citrus_China
MG457781_Citrus_China
C1b_Hallertau
D_Hallertau
C1a_Hallertau
C2_Hallertau
B1b_Hallertau
B1a_Hallertau
B2_Hallertau
KM211547_Hop_Slovenia
A1b_Hallertau
A2c_Hallertau
A2d_Hallertau
KM211546_Hop_Slovenia
A1a_Hallertau
A2a_Hallertau
A2b_Hallertau
MG457797_Citrus_China
MF421252_Citrus_Egypt
MF421255_Citrus_Egypt
HM042749_Citrus_China
HM042746_Citrus_China
MF421253_Citrus_Egypt
MF421254_Citrus_Egypt
HM042748_Citrus_China
KX819245_Citrus_Cyprus
JX259420_Citrus_Greece
GROCERY_Lemon_Italy_X14638
KY654682_Citrus_Iran
GQ260216_Citrus_Iran
X14638_Grapefruit_Israel
JN903766_Citrus_SouthAfrica
MG457794_Citrus_China
JN903767_Citrus_SouthAfrica
GROCERY_Grapefruit_Israel_X14638 (reversed) 1
NC_003539_Grapefruit_Israel
GROCERY_Grapefruit_Israel_X14638 (reversed) 2
GROCERY_Lemon_Italy_pp294.722
MF421251_Citrus_Egypt
AJ630361_Citrus_Cuba
AJ630359_Citrus_Cuba
HM042743_Citrus_China
MG457786_Citrus_China
HM042744_Citrus_China
HM042745_Citrus_China
KX819246_Citrus_Cyprus
AB054633_Citrus_Japan
AJ630360_Citrus_Cuba
MG457795_Citrus_China
MG457791_Citrus_China
HM042742_Citrus_China
MG457787_Citrus_China
JX259421_Citrus_Greece
AB054635_Citrus_Japan
GROCERY_Lemon_Turkey_AB054635 1
JN903764_Citrus_SouthAfrica
MG457785_Citrus_China
JN903765_Citrus_SouthAfrica
GROCERY_Lemon_Spain_AB054635
JN903763_Citrus_SouthAfrica
MG457792_Citrus_China
HM042747_Citrus_China
GROCERY_Lemon_Turkey_AB054635 3
GROCERY_Lemon_Turkey_AB054635 2
KT725633_Citrus_Iran
GROCERY_Grapefruit_Israel_AB054635
GROCERY_Orange_Spain_AB054635 (reversed)
MN136645_Lime_Greece
KY110719_Citrus_SouthAfrica_Synthetic
GROCERY_EasyPeeler_Turkey_AB054635 (reversed)
JX259422_Citrus_Greece
JX259418_Citrus_Greece
AB054634_Citrus_Japan
GROCERY_Lemon_Turkey_pp294.678
JN903762_Citrus_SouthAfrica
MG457790_Citrus_China
KX819247_Citrus_Cyprus
JX259419_Citrus_Greece
MG457788_Citrus_China
MG457793_Citrus_China
MG457798_Citrus_China
MG457789_Citrus_China
MG457796_Citrus_China
0.06
0
10
20
30
40
Mean number of nucleotides differing from the Slovenian CBCVd-hop accession

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Studying cytosine methylation in genomic DNA of viroid-infected hop plants (*Humulus lupulus* cv. 'Celeia')

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Abstract

Abiotic and biotic factors cause changes in host DNA methylation, which in plants is largely mediated by an RNA-directed DNA methylation (RdDM) mechanism. Viroid infections have been shown to affect DNA methylation dynamics in various plants. The aim of our study was to determine the cytosine methylation level (5-mC) in the genomic DNA of hop plant (*Humulus lupulus* cv. 'Celeia') infected with different viroids and their combinations. The adapted HPLC-UV method proved to be suitable for this purpose, and thus we were able to estimate for the first time that the cytosine methylation level in viroid-free hop plants was 26.7 %. Interestingly, the observed level of cytosine methylation was highest (31.4 %) in hop plants infected only with the Hop latent viroid (HLVd), suggesting that cytosine methylation of hop plant genomic DNA depends on the viroid species infecting the hop. On the other hand, the lowest cytosine methylation (23.7 %) was observed in hop plants infected with Citrus bark cracking viroid (CBCVd), HLVd, and Hop stunt viroid (HSVd), indicating a synergistic effect of the three viroids infecting hop plants simultaneously. To improve our understanding of DNA methylation dynamics in hop plants, we identified three DNA methylases (HlDNMT, HlCMT, and HlDRM) and one DNA demethylase (HlDME) in the hop draft genome and compared their gene expression between viroid-free and viroid-infected hop plants using RT -qPCR. Most importantly, we observed upregulation of all four genes in hop plants infected with all three viroids, again suggesting a synergistic relationship between the three hop-infecting viroids.

Key words. Hop plant, viroids, CBCVd, HLVd, HSVd, DNA methylation, 5-mC, DNA methylase, DNA demethylase, gene expression profile

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

The influence of Hop latent viroid (HLVd) infection on secondary metabolite contents in hop cones

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Abstract

Hop latent viroid (HLVd) occurs worldwide in all hop growing regions without any visible symptoms on infected hop plants. It was found that HLVd infection changed the content and the composition of secondary metabolites in maturated hop cones. We evaluated influence of HLVd infection on the content and the composition of secondary metabolites in dried cones of cvs Saaz, Sládek and Premiant during three harvest years. We confirmed that reduction of alpha bitter acids content has been the most noticeable effect of viroid infection for all cultivars studied. Alpha bitter acid content has been in close correlation with xanthohumol content, which was also reduced by viroid infection.

Key words. hop, Humulus lupulus, Hop latent viroid, HLVd, bitter acids content, xanthohumol, essential oils

Introduction

Hop latent viroid (HLVd) is a single-stranded, circular infectious RNA of 256 nucleotides (HADIDI et al. 2021a). HLVd infection has been reported worldwide from all hop growing regions, contrary to only local outbreaks of three other viroids: Hop stunt viroid (HSVd), Apple fruit crinkle viroid (AFCVd) and Citrus bark cracking viroid (CBCVd), which have also been found in hop plants (PATZAK et al. 2021a).

Although HLVd-infected hop plants are symptomless, infection leads to a significant reduction in cone yield and bitter acids content in hop cones (BARBARA *et al.* 1990). The yield was lower from 8 to 35 % for infected plants of hop cultivars (cvs) Wye Challenger and Omega, respectively. The content of alpha bitter acids was reduced by 15 and 30 %, while the content of beta bitter acids was slightly higher. Follow-up experiments with Wye Challenger showed 11 % yield reduction, 11 % reduction of the content of alpha bitter acids and 8 % of increment of the content of beta bitter acids (ADAMS et al. 1991). The reduction of alpha bitter acids content due to HLVd infection ranged from 20 to 50 % within English cvs and was genetically dependent (ADAMS et al. 1992). Similar results were found for cvs Saaz, Premiant (reduction of 14.8 to 40 %; PATZAK et al. 2001, 2021a), Sládek (28.2 %), Agnus (8.8 %; PATZAK et al. 2021a), Aurora (18 %; RADIŠEK 2017), Sybillla, Marynka, Pulawski and Magnat (11 to 23 %; PISTELLI *et al.* 2018). The content of beta bitter acids was slightly higher (0–5 %) for all cvs. A significant reduction of the xanthohumol content was also found for Czech cvs ranging from 8.8 to 28.2 %.

Yield loss due to HLVd infection reached in average 18.6 % for cv. Saaz (PATZAK et al. 2021a), 15 to 37.5 % for Slovenian cvs (RADIŠEK 2017) and 6.4 to 15.3 % for Polish cvs (PISTELLI et al. 2018). Viroid infection also influences the composition of essential oils in hop cones. An increasing of the myrcene content by 38 % for infected plants of cv. Wye Challenger was reported first (ADAMS et al. 1991). Similar results were found for cvs Saaz and Premiant (PATZAK et al. 2001), when the content of myrcene increased by 29 % together with both monoterpene pinene isomers (about 40 % increase) in infected plants. On the contrary, all sesquiterpenes were reduced by 4.4 to 29 % in cones of infected plants. Regarding other compounds, terpene alcohols (linalool, geraniol, methylgeranate) and epoxides had increased, and ketones decreased in infected plants. Therefore, the composition of essential oils in hop cones is genetically dependent and specific; these changes cannot be general. Trends for content of sesquiterpenes and monoterpenes (myrcene, β-pinene) were similar within Polish cvs, with exception for myrcene for cv. Sybilla (PISTELLI et al. 2018). The content of linalool in cones of infected plants was higher in cvs Sybillla, Lubelski and Pulawski, but lower in Marynka and Magnat. The content of methylgeranate was vice versa lower in infected plants of all cvs.

The composition of essential oils was not significantly influenced by viroid infection in the Czech cvs Sládek, Premiant, and Agnus, except β-pinene and geraniol in Sládek and Premiant, and linalool and methylgeranate in Sládek (PATZAK et al. 2021a).

In the present study, we continued the evaluation of the influence of HLVd infection on the content and the composition of secondary metabolites in mature hop cones of three Czech cultivars in field-grown mericlones.

Material and methods

Analysed hop plants were obtained from in vitro multi-shoot culture of mericlones (PATZAK et al. 2001, 2020). Fourteen mericlones of cv. Saaz, two of cv. Sládek and one of cv. Premiant were viroid-free. Sixteen mericlones of Saaz and one of each hybrid cultivars were HLVd-infected. Two to eight in-vitro plants of selected mericlones were acclimatized in the greenhouse and, when well rooted, put under field conditions to experimental hop gardens in the Steknik farm of the Hop Research Institute in Žatec (cv. Saaz in 2018 and hybrid cvs in 2019). All hop plants were grown under standard agronomic conditions. Hop latent viroid (HLVd) infection was analysed by molecular dot blot hybridisation using 32P[dCTP]-labelled cDNA HLVd probe (PATZAK et al. 2001). Viroid level was quantified by means of STORM PhosphorImager device and ImageQuant software (Molecular Dynamics, Chatsworth, CA, USA). RNA samples, isolated by PureLink™ Plant RNA Reagent (ThermoFisher Scientific, Waltham, MA, USA) according to protocol, were used for HLVd detection by real-time quantitative RT-PCR by QuantiTect SYBR Green RT-PCR Kit (Qiagen, Hilden, Germany) according to PATZAK et al. (2017). For chemical analyses, a minimum of 500 g of mature cone samples were collected from the end of August to early September 2019, 2020, and 2021 and kiln dried at 55°C for 8 h to a target water content of 10 %. The dried cone samples were used for chemical analyses of hop resins, polyphenols, and essential oils. Hop resins and polyphenols were determined by liquid chromatography (HPLC) with diode array detector (DAD) according to modified EBC 7.7 method (ANALYTICA EBC 1998) on the column Nucleosil C18 (Macherey-Nagel, Düren, Germany), 5 µm, 250 x 4 mm using Shimadzu LC-20A (Shimadzu Europe GmbH, Duisburg, Germany) liquid chromatograph (PATZAK et al. 2010). The flow rate of the mobile phase was 0.8 ml/min. The detection was carried out at wavelengths of 314 nm (hop resins) and 370 nm (polyphenols). Hop resins, alpha and beta acids, and xanthohumol were quantified by external calibration standards. Hop essential oils were estimated from vacuum concentrated, water distilled samples by gas chromatography (GC) on a capillary column DB 5 (Chromservis, Prague, CR, 30 m x 0.25 mm x 0.25 m film thickness) using a Varian 3400 gas chromatograph (Varian Inc., Palo Alto, CA, USA) combined with a Finnigan ITD 800 mass detector (Thermo Scientific, Waltham, MA, USA) (PATZAK et al. 2021b). Compound identification was based on comparison of GC retention indices and mass spectra with those of authentic compounds. Semi-quantitative evaluation of hop oils composition was performed based on peak areas of individual components and expressed relatively to the total integrated area of all substances involved. STATISTICA 8.0 CZ (StatSoft, Tulsa, OK, USA) was used for the evaluation of chemical analyses data by basic statistic functions. SigmaPlot for Windows v.10.0.0.54 (Systat Sowtware Inc., San Jose, CA) was used for statistical group and t-test analyses.

Results and Discussion

We analysed contents and compositions of hop resins and essential oils in mature hop cones of three Czech cultivars in field-grown mericlones during three harvest years (2019, 2020 and 2021). We confirmed previous findings (PATZAK et al. 2001, 2021a; RADIŠEK 2017; PISTELLI et al. 2018) that reduction of alpha bitter acids content has been the most noticeable effect of viroid infection. The reduction of alpha bitter acids was significant in the harvest years 2019 and 2020 for cv. Saaz (Fig. 1). In 2021, the reduction was not significant but an increase of beta bitter acids in hop cones of infected plants was significant. This could be also due to progressive re-infection during the field seasons, which statistically eliminated the number of negative samples.

For example, only one of six HLVd free mericlones (Fig. 2) have not been re-infected. Three of them were re-infected the second year and two others in the third year under field conditions. In a previous study (PATZAK et al. 2021a) it was found that the content of xanthohumol was also significantly reduced by viroid infection. A study of hop genetic resources (NESVADBA 2012) found that alpha bitter acid content is in close correlation with xanthohumol content. We found the same linear regression for cv. Saaz samples through all harvest years (Fig. 3). PATZAK et al. (2021a) found that humulone synthase (HS1 and HS2) genes for a last step of alpha bitter acids biosynthesis and O-mythyltransferase (OMT1) for a last step of xanthohumol biosynthesis were down-regulated by HLVd infection in cones of cv. Saaz. Even though these compounds are on different biosynthetic pathways, it can be assumed that their syntheses are regulated together. Their common production can be also correlated to lupulin gland development and regulation (PATZAK et al. 2015, 2021b).

The same trends for contents of alpha bitter acids for Sládek (Fig. 4) and Premiant (Fig. 6) mericlones were found. We also confirmed that viroid infection changed the composition of essential oils in cones of cv. Saaz. The content and composition of essential oils in cones of cvs Sládek and Premiant were not significantly influenced by viroid infection. Vice versa, significant differences between harvest years were found.

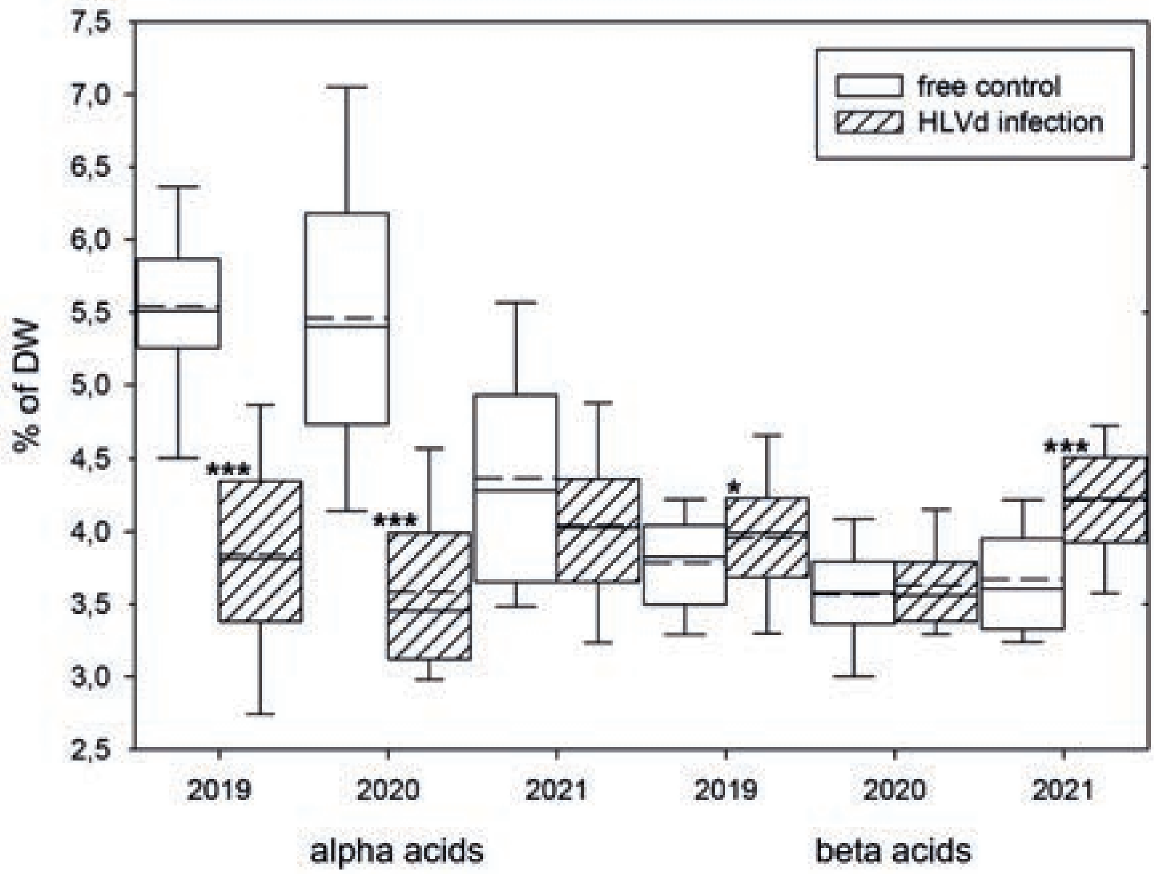

Figure 1. Box plots of the contents of hop bitter acids in dried cones of HLVd-free and infected plants of cv. Saaz in three harvest years. Probability level: * $P<0.1$; ** $P<0.05$; *** $P<0.01$; straight line: median; dashed line: average; box: 95 % percentile ± standard deviation.

Figure 2. Contents of hop bitter acids in dry cones of six HLVd-free mericlones of cv. Saaz, during three harvest years. Reinfection of mericlone plants is highlighted by red edgings.

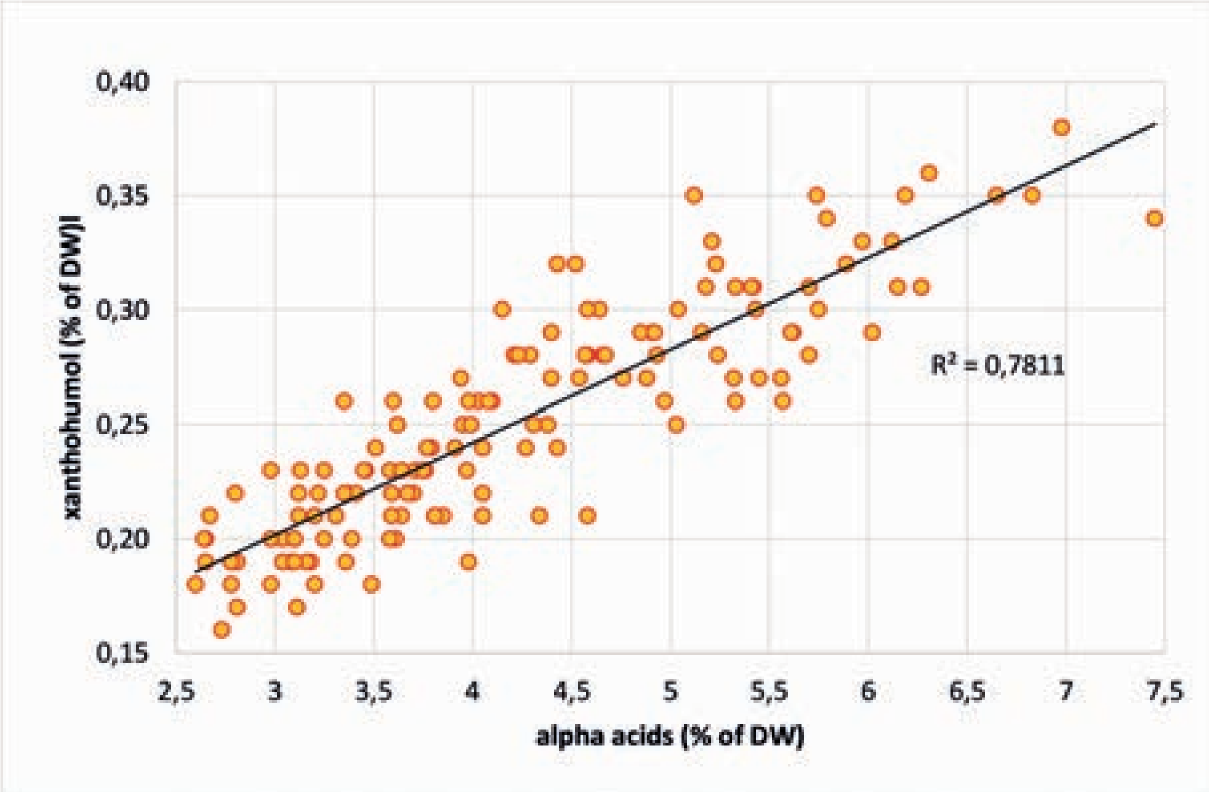

Figure 3. Linear regression between alpha acid and xanthohumol contents in cv Saaz hop cones during three harvest years.

Figure 4. Contents of hop bitter acids in dried cones of two HLVd free and one HLVd infected (red edgings) mericlones of cv. Sládek during two harvest years.

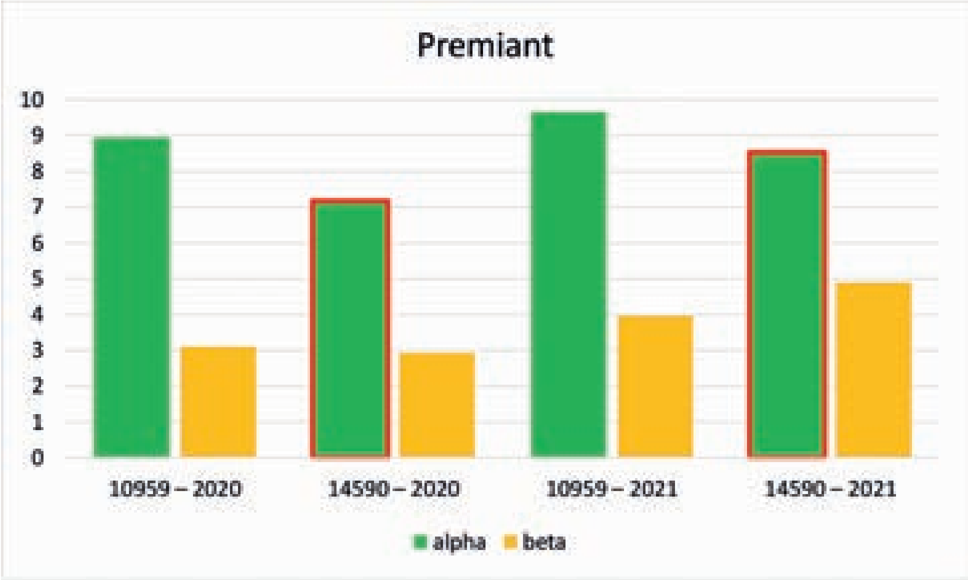

Figure 5. Contents of hop bitter acids in dried cones of one HLVd free and one HLVd infected (red edgings) mericlones of cv. Premiant during two harvest years.

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VI: Hops, aroma and brewing

Distinguishing hop varieties as either aroma or bitter hops – is this still relevant?

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Abstract

The classification of hop varieties into aroma and bitter hops dates back to 1971, a time when there were only four aroma and two bitter varieties in Germany. The two groups could be easily distinguished from each other based on analytical characteristics. Today, differentiation is difficult due to the breeding of numerous new varieties. In particular, the hop varieties used for dry hopping, often referred to as flavor hops, do not fit into the usual scheme, although they are literally 'aromatizing hops'. To change this unsatisfactory situation, it is proposed to divide the group of aroma hops into three groups, namely fine aroma hops, aroma hops and flavor hops. No subdivision is required for bitter hops.

Introduction

The European Union's "Protocol for Distinctness, Uniformity and Stability Tests" from 15 November 2006 deals exclusively with the description of hop plants and their cultivation properties. Annex II classifies hop varieties based on the 'type of use' criterion: 'aroma', 'bitter', 'high alpha', 'ornamental' and 'other'. However, this is the full extent of the information. In particular, there are no points of orientation for brewers.

Therefore, the question to be addressed is posed from the viewpoint of brewers who require guidance on how to differentiate the numerous hop varieties based upon these pre-determined groups. These considerations are focused on the cultivation of hops in Germany and are only intended as a means for initiating a discussion on the topic.

The origins of placing hop varieties into groups

First, no hops in existence contain only aroma substances or only bitter substances. This, in and of itself, reveals straightaway the difficulty in assigning hops to groups designated as 'aroma hops' or 'bitter hops'.

The categorization of hops into aroma and bitter varieties was carried out by the EEC in 1971. Aroma hops produced lower yields of bitter substances but enhanced the quality of beer, such as imparting a more harmonious bitterness and a finer hop aroma as well as increasing the drinkability, especially when added late in the brewing process. Bitter hops are added at the beginning of the boil and only convey bitterness to the beer. As the use of bitter hops became more widespread, some breweries started to advertise that they were using aroma hops, emphasizing their higher reputation. Over time, the continuous development of analytical criteria has provided tools for the differentiation of hop varieties, which may also be employed to define the limits for each group of hops.

Data for the hop varieties grown in Germany in the 1970s are depicted in Table 1. These include the four German landraces of aroma hops (Hallertauer Mittelfrüher, Hersbrucker Spät, Spalter, Tettnanger) as well as two bitter varieties, Northern Brewer and Brewers Gold. The key metrics developed over time serve as the basis for this analysis (BIENDL et al. 2014; ASSOCIATION OF GERMAN HOP GROWERS 2016).

Aroma hops were expected be elegant and fine in smell, while bitter hops were characterized as strong and aggressive often exhibiting fruity notes, *i.e.*, they are more "unconventional". Therefore, based on these descriptors, aroma and bitter hops were analytically and sensorially easy to distinguish, and their differences were clearly delineated. There were no commonalities except for linalool.

Table 1. Data for the distinguishing of ‘aroma’ and ‘bitter’ hop varieties grown in Germany in the 1970s

		Aroma	Bitter
α-acids	% w/w	3.1–4.1	7.0–9.2
β : α		1.3–1.4	0.6
Cohumulone ratio	% rel.	20–25	27–44
Polyphenols	% w/w	4.4–5.3	3.7–3.9
Total oil	ml/100 g	0.6–0.75	1.3–1.5
Linalool	mg/100 g	4–6	4–7

What changed in the last decades?

Two fundamental shifts have emerged since 2000: The first was a change in the accepted scientific opinions on some topics. Still accepted is a positive effect of low cohumulone ratio and a high beta/alpha ratio. But he importance of mono- and sesquiterpenes is neglectable because they are evaporated in the brew kettle. Older doctrines no longer applied, *e.g.*, myrcene is now no longer considered unfavorable and, likewise, farnesene as simply “good”. Especially the thiols are today essential in flavor hops for dry hopping, while their reputation was bad in the past.

The second shift was that varietal breeding has increased enormously and continues to do so, *e.g.*, from the development of five new varieties from 2001 to 2005 to 22 new varieties in the years 2016 to 2020 in Europe. The primary driving forces behind these accelerated breeding efforts comprise the search for climate and disease tolerances as well as the desire of craft brewers of new aromas for their beers. New hop varieties are now protected, making it potentially lucrative to license their cultivation. In addition to state institutions, there are private breeders.

The importance of “flavor” hops

Flavor hops, as they are known, perform additional tasks when they are used for dry hopping compared to conventional hop additions on the hot side. They are intended to impart an aroma, mostly fruity notes, as directly as possible to beer. Flavor hops serve as an agent or transmitter for aromas atypical for hops. They are literally ‘aromatizing hops’. The composition of the bitter substances and the polyphenols plays a minor role in dry hopping. Flavor hops are presently classified in the aroma group. However, this has necessitated significantly expanding the ranges for the key metrics for analyzing hop attributes.

The current situation

Table 2 shows the actual range of analytical data of aroma and bitter hops.

The numerous new aroma varieties have enormously increased the analytical range for characterizing these hops. A clearly - defined analytical delineation of aroma and bitter hops is problematic since the boundaries between the two are eroding.

The evaluation made by the breeder is decisive in determining to which group a new variety is assigned. Since aroma hops are associated with having a higher value, the breeder tends to designate a newly developed variety as an aroma hop. Objective criteria are often not required. Northern Brewer (2016) and Brewers Gold (2021) are two examples of the seemingly arbitrary “promotion” of two bitter varieties to the aroma group. There exist no scientific publications in brewing, which provide justification for this classification. If a hop was originally bred as a bitter variety – yet does not meet the expectations for alpha acid content (>15 %) and alpha yield (>500 kg/ha) it is registered as an aroma variety.

Table 2. Data for the distinguishing of ‘aroma’ and ‘bitter’ hop varieties accepted today

		Aroma	**Bitter**
α-acids	% w/w	3.1–12.0	11.3–18.6
β : α		0.6–2.4	0.3–0.5
Cohumulone ratio	% rel.	15–30	20–36
Polyphenols	% w/w	3.7–5.4	2.8–4.0
Total oil	ml/100 g	0.55–2.5	1.7–3.2
Linalool	mg/100 g	3–18	8–19

This indicates that therefore the current method of grouping hop varieties is unsatisfactory. It suffers also from a lack of transparency and reproducibility.

What alternatives are there for classifying hops in this manner?

- The current unsatisfactory and non-transparent procedure will continue.
- Hop varieties will cease to be separated into groups.
- Aroma hops, which cover a particularly wide spectrum, can be further divided into subgroups.

A proposal below using German varieties as an example:

Fine aroma varieties as a group would encompass all the old landraces, such as HAL, HEB, SAZ, SPA, TET as well as closely related varieties, such as Saphir and Spalter Select. These varieties are used for brewing superbly well-balanced, bottom-fermented styles like lagers and pilsners.

Aroma varieties, such as Perle, Hall. Tradition, Opal, Smaragd, Diamant, Aurum, Ariana and Callista can be added at any point from the beginning and the end of the boil. They can at least partially replace noble aroma varieties in many beers.

Flavor varieties possessing a pronounced fruity character are often used for dry hopping.

Bitter varieties as a group are primarily added at the beginning of the boil. Attributes such as aroma compounds or polyphenols play a subordinate role.

The current analysis data for these four proposed groups are shown in Table 3:

A concerted effort should be made to expand the criteria for aroma hops, e.g., to include attributes, such as the oxygenated fractions or the esters. Currently, analysis of the thiols is neither sufficiently reproducible nor comparable between laboratories. Data on this is urgently needed.

Table 3. Current analytical data for the future classification of hop varieties into four proposed groups (nr* – no relevance)

		Fine Aroma	Aroma	Flavor	Bitter
α-acids	% w/w	<7	6–10	<12	> 12
β : α		0.8–2.5	<0.7	<0.5	nr*
Cohumulone ratio	% rel.	<28	<32	<35	nr*
Polyphenols	% w/w	>4.0	>3.5	>3.0	nr*
Hop oil	ml/100g	<1.2	>0.6–1.5	>1.0	>1.5
Linalool	mg/100g	>3–8	>4–12	>6	nr*

Synopsis

- The distinction between aroma and bitter hops was understandable considering the limited number of varieties available up into the 1970s. In addition to obvious analytical differences, their purpose was also defined based upon when they were added in the brewing process. Aroma hops were primarily added at the end of the boil, while bitter varieties were added solely at the beginning.
- The multitude of varieties developed since then has resulted in an enormous expansion of the analytical data characterizing the attributes of hops. Breeders, in large part, determine into which group a new variety will be classified; however, they lack sufficient experience regarding, *e.g.*, the suitability of a particular variety for certain applications.
- The increasingly popular introduction of hop aroma into beer by means of dry hopping is a technique that supersedes the rules which were previously followed in the brewing industry. Hops have become the transport mechanism for “unconventional”, even unhoppy, fruity flavors.
- Continuing to observe the current method of classifying hop varieties into one of the two aforementioned groups does not represent a viable option. Two alternatives remain:
  - abolish the designation by group (either as “bitter” or “aroma”)
  - adopt a proposal to divide the aroma hop varieties into three groups: fine aroma varieties, aroma varieties and flavor varieties. Bitter varieties would belong to the same group as before.
- Varietal determination and classification should be overseen by relevant committees.

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Influence of fungal diseases on hop quality and sensory properties of beer

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Abstract

Downy mildew is caused by the fungal-like pathogen Pseudoperonospora humuli and is most severe during wet weather and mild temperatures. Downy mildew appears early in the season on emerging basal spikes. Spikes growing from infected crowns or buds may appear distorted with shortened internodes that give them a stunted appearance. The disease threatens the development of hops throughout the whole vegetation.

Powdery mildew can occur on all green tissue of a hop plant. The infection will first express on the underside of the leaf, often as pale, chlorotic spots on the upper surface. Infected cones also contain white powdery colonies within each of the bracteoles. Over time brown, necrotic lesions develop on the surface of cones.

The smell of green cones infested by Downy mildew has no off-smell, but the intensity of the smell was significantly weaker compared to healthy ones. The smell of green cones infested by Powdery mildew has an off - smell after the mold, which disappears after drying. However, the smell of hops remains weak and indistinct. The influence of fungal diseases on content and composition of the most important secondary metabolites was observed in several years, which differed in course of weather conditions during the vegetation.

Cones damaged by fungal diseases were manually separated from healthy ones in damage categories up to 50 % and over 50 % of the cone, if almost the entire cone was affected. Content and composition by bitter acids were analyzed by HPLC according to EBC 7.7 method. Hop essential oils were isolated from green cones by solid phase microextraction (SPME) and analysed by gas chromatography. Green cones were cut manually in pieces before analysis. The content of alpha acids in the infected hop cones, compared to healthy ones, decreased by up to 75 % in the case of Downy mildew and by up to 35 % in the case of Powdery mildew. The beta acid content was not so much affected. The content of hop essential oils was lower in the infected cones, proportional to the reduction in the amount of alpha acids.

The composition of hop essential oils in the hops infested with Downy mildew did not differ significantly from the composition of healthy cones. However, in the case of powdery mildew infection, significant differences were found between healthy and infested cones, especially in the composition of the oxygen fraction. Methyl esters of straight and branched chain fatty acids were practically not found in the cones heavily damaged by Powdery mildew.

Brewing tests were performed on batches of 11 % bottom-fermented Czech lager. Barley malt and bottom fermentation yeast were used for production. Sensory evaluation of beers, which was carried out by a triangular test by several tasting panels, showed that the infestation of hops with fungal diseases did not have a significant effect on the sensory quality of beers.

Key words: hop, fungal diseases, alpha acids, hop oils, beer

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The impact of climatic conditions on the biogenesis of various compounds in hops

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Abstract

Climate influence on alpha-acid content of hops is known. Systematic research on the biosynthesis of aroma substances and polyphenols in different hop varieties depending on the weather conditions are lacking. Pellet samples from big lots of 20 German hop varieties were chosen for the study. The two consecutive crop years 2015 and 2016 were suitable for a comparison. Summer 2015 was hot and dry, whereas the summer 2016 offered good conditions with enough rainfall and moderate temperatures. α- and β-acids were particularly sensitive to unfavourable conditions, in aroma varieties more than in flavor or bitter hops. However, the polyphenols were stable. The susceptibility of hop substances and varieties to climate conditions evidently is different. Brewers should take that into account when selecting hop varieties.

Introduction

Climate change is undeniable. For instance, middle daily temperature in the months June to August increased in Bavaria from 15.8°C (1961-1990) to 17.6°C (1991-2020).

The influence of climatic conditions on alpha yield in kg per hectare is reasonably well understood. Hop varieties behave very differently (Forster & Schüll 2019). As the other secondary metabolites are concerned, there are no well-established data available on β-acids, the cohumulone ratio, polyphenols (PP) and aroma compounds. This presentation deals with this issue and is a shortened version of Forster et al. (2021).

Method of comparison

Since long-term information is missing, a comparison of two years with totally different weather conditions is a suitable instrument. Crop 2015 and 2016 are good examples for a bad and a good harvest. The comparison of mean temperatures from June to August is 19.5°C (2015) *vs* 17.7°C (2016), the sum of precipitation from June to August 178 mm (2015) *vs* 334 mm (2016) and 36 hot days (>30°C) in 2015 *vs* 7 days in 2016.

Pellet 90 samples were taken from big lots, representing an average of numerous homogenized individual lots of hops. Twenty varieties cultivated in Germany in those years were selected:

- 4 landraces: Hallertauer (HAL), Hersbrucker (HEB), Tettnanger (TET), Spalter (SPA)
- 7 aroma cvs: Perle (PER), Hallertauer Tradition (HTR), Spalter Select (SSE), Saphir (SIR), Opal (OPL), Smaragd (SGD), Northern Brewer (NBR)
- 4 flavors: Mandarina Bavaria (MBA), Hüll Melon (HMN), Hallertau Blanc (HBC), Cascade (CAS)
- 5 bitter cvs: Magnum (HMG), Taurus (HTU), Herkules (HKS), Polaris (PLA), Nugget (NUG)

Analysis and calculation of the results

a- and β-acids: By means of HPLC including the cohumulone ratio

Polyphenols (PP): - total PP using an unspecific spectrophotometric method

- low molecular weight PP by means of HPLC

- xanthohumol using HPLC, analogous to the bitter acids

Aroma compounds (GC-FID): - sum of all calibrated compounds
- myrcene and a-humulene
- oxygenated fraction, linalool, sum of carboxylate esters, sum of sesquiterpene
- alcohols and epoxides of a-humulene and β-caryophyllene

The important question is: how sensitive react components of different varieties on climate conditions? How do they suffer under dryness and heat?

This information is obtained by a calculation of absolute values in % relative of 2015 against 2016:

$$\frac{\text{Ø } value\ of\ a\ substance\ in\ the\ 2015\ harvest}{\text{Ø } value\ of\ a\ substance\ in\ the\ 2016\ harvest} * 100\% = \Delta \text{ in \% rel.}$$

Results

All results in % relative of values 20215 vs values in 2016 are shown in two tables:

Table 1. Results for the bitter acids and polyphenols; XN – xanthohumol; TPP – total polyphenols; lmwPP – low molecular polyphenols. The colours indicate the sensitivity to climate from red (high) to green (none).

Variety	α-acids	β-acids	% Coh	XN	TPP	lmwPP
HAL	54	64	105	71	103	110
HEB	68	69	100	88	103	113
SPA	60	78	100	90	130	121
TET	57	73	106	87	110	99
Ø landraces	**59.8**	**71.0**	**102.8**	**84.0**	**112.5**	**110.8**
HTR	76	59	112	91	105	102
SIR	63	59	100	81	107	100
SSE	64	63	95	75	100	93
SGD	75	73	111	87	119	125
PER	51	52	110	59	107	108
OPL	84	69	106	79	111	110
NBR	40	50	106	52	136	141
Ø cultivars (w/out NBR)	**68.8**	**62.5**	**105.7**	**78.7**	**108.2**	**106.3**
MBA	79	103	97	102	86	82
HMN	82	94	99	96	91	93
HBC	75	84	95	88	90	102
CAS	69	106	92	80	102	110
Ø flavor varieties	**76.3**	**96.8**	**95.8**	**91.5**	**92.3**	**96.8**
HMG	86	84	97	85	110	110
NUG	77	89	105	88	108	124
PLA	86	75	97	100	114	133
HTU	71	73	106	85	136	152
HKS	87	79	95	98	114	151
Ø bitter varieties	**81.4**	**80.0**	**100.0**	**91.2**	**116.4**	**134.0**
Ø all varieties incl. NBR	**70.2**	**74.8**	**101.7**	**84.1**	**109.1**	**114**

Comments to Table 1:

- α- and β-acids are very sensitive to climate change, esp. those of aroma varieties.
- The cohumulone portion is not impacted (homologs of α-acids exhibited the same behavior).
- Total PP and low molecular weight PP are “green”, *i.e.*, no impact on these compounds was apparent.
- Dry, hot conditions negatively affect xanthohumol, especially in aroma hops.

Table 2: Content of some groups and individual aroma compounds from the 2015 harvest compared to those of the 2016 harvest [% rel.].

1 – Sum of all calibrated aroma compounds; 2 – myrcene; 3 – humulene; 4 – oxygenated fraction; 5 – linalool; 6 – sum of the esters; 7 – sesquiterpene alcohols; 8 – epoxides.

Variety	1	2	3	4	5	6	7	8
HAL	73	69	63	73	75	30	108	93
HEB	78	90	98	43	86	71	82	64
SPA	86	77	112	51	75	31	65	40
TET	81	63	102	67	75	50	75	88
Ø landraces	**79.5**	**74.8**	**93.8**	**58.5**	**77.8**	**45.5**	**82.5**	**71.3**
HTR	95	96	99	82	100	55	120	78
SIR	95	83	112	100	70	67	128	75
SSE	80	63	118	88	70	60	100	80
SGD	81	74	85	80	80	73	100	58
PER	72	63	77	74	75	57	108	73
OPL	80	84	66	72	100	61	94	35
NBR	58	44	76	53	74	41	84	77
Ø cultivars (w/out NBR)	**83.8**	**77.2**	**92.8**	**82.7**	**82.5**	**62.2**	**108.3**	**66.5**
MBA	110	104	131	94	100	84	113	56
HMN	78	70	94	74	100	60	98	100
HBC	107	97	117	83	125	72	91	100
CAS	94	92	110	81	80	70	114	60
Ø flavor varieties	**97.3**	**90.8**	**113.0**	**83.0**	**101.3**	**71.5**	**104.0**	**79.0**
HMG	82	84	80	79	71	76	96	63
NUG	70	58	88	70	53	63	100	64
PLA	95	103	100	66	88	60	95	45
HTU	76	67	111	72	59	83	77	40
HKS	77	64	100	63	71	57	100	67
Ø bitter varieties	**80.0**	**75.2**	**95.8**	**70.0**	**68.4**	**67.8**	**93.6**	**55.8**
Ø all varieties incl. NBR	**83.4**	**77.3**	**97.0**	**73.3**	**81.4**	**61.1**	**97.4**	**76.0**

Comments to Table 2:

- Humulene is more stable than myrcene.
- The oxygenated fraction (OF) is more sensitive than the sum of all the other compounds.
- The esters are the most sensitive of those in the OF.
- Linalool is more stable than the esters.
- Sesquiterpene alcohols are more stable than the epoxides.
- Most sensitive are landraces, most stable flavor varieties.

A comparison illustrates that bitter acids are very sensitive (72.5 %), aroma compounds a bit less (83.4 %), while polyphenols (except xanthohumol) are stable (109.1 %).

In order to get an easy overview, we combine the results of bitter and aroma compounds as following by creating two terms: “bitter potential” and “aroma potential”:

Aside from the a-acids, hops also contribute with auxiliary bitter substances to the overall bitterness of the beer, especially in late brewhouse additions. β-acids are a suitable indicator for the quantity of auxiliary bitter substances in beer (BIENDL et al. 2014).

The bittering potential of a hop variety is calculated in this manner:

Bittering potential = 4 × α-acids + 1 × β-acids

Out term of “aroma potential” of a variety is based upon the contribution of a compound/group of compounds to the hop aroma in beer in late additions or with dry hopping (BIENDL et al. 2014). The definition of aroma potential is calculated on the average of six aroma attributes: sum of all calibrated compounds; myrcene (can especially have an impact with dry hopping); oxygenated fraction (compounds with various solubility levels in beer); sum of the esters (fruity notes and conversion into aroma active ethyl esters); linalool (indicator for hop aroma in beer); and epoxides.

Table 3. Bittering and aroma potential as a ratio (% rel.) of 2015 and 2016 for all 20 varieties in this study, with the resultant mean listed. Varieties are ranked from 1 (hardly climate-sensitive) to 14 (very climate-sensitive).

Variety	Bittering potential	Aroma potential	Mean	Ranking
MBA	83	91	87	1
HBC	77	97	87	1
HMN	84	80	82	2
HMG	86	76	81	3
PLA	84	76	80	4
HTR	73	81	77	5
OPL	81	72	77	5
CAS	75	79	77	5
HKS	85	67	76	6
SIR	62	89	76	6
SGD	75	74	75	7
NUG	79	63	71	8
HEB	68	72	70	9
HTU	71	67	69	10
SSE	64	74	69	10
TET	60	71	66	11
HAL	56	68	62	12
SPA	64	60	62	12
PER	51	67	59	13
NBR	42	58	50	14

The classification of varieties according to their climate sensitivity has a wide range, from MBA and HBC (best) to NBR (worst). Furthermore, it seems that early picked varieties react more sensitively. The ratios of the various compounds in hops shift in relation to one another according to the climate conditions, especially the ratio of polyphenols to α-acids, which is double. This fact is especially interesting for practical brewers.

Synopsis

The analysis results of the poor harvest of 2015 were compared with the abundant harvest of 2016 and calculated in % (rel.). The reduced yield in 2015 compared to 2016 can be summarized as follows:

- Given the overall changes in the mean values for α-acids (-30 %), aroma compounds (-17 %) and polyphenols (constant), all 20 varieties exhibited significant differences in their reactions to fluctuations in climate conditions.
- Values for aroma compounds react differently. The most sensitive are the esters (-39 %), followed by myrcene (-23 %) and linalool (-19 %).
- Polyphenols are astoundingly stable in the face of climate change – the only aberration being xanthohumol (-16 %).
- The most climate-sensitive are the landraces followed by the aroma cultivars, the bitter varieties and the flavor varieties.
- Since the substances in the hops varied in how they reacted to the change in climate, the ratios of the various groups of compounds shifted according to the crop year, especially the relationship between the polyphenols and the a-acids. These ratios can largely be offset by adapting the enrichment process during pelletization to the changes in the climate conditions.
- Climate conditions can also cause the relationship between the bittering and aroma potential to shift, resulting in changes to the beer aroma over time, e.g., if a late addition is dosed according to the concentration of the α-acids. Brewers should take one or more aroma attributes into consideration when dosing the aroma additions at the end of the boil, in the whirlpool or particularly when dry hopping.

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Study of the volatile composition of different varieties of hops from three regions of Spain (León, Galicia, and the Basque Country)

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Abstract

Hops (*Humulus lupulus* L.) are used to aromatize beer and obtain bitter taste. Volatile composition of hop essential oil is an important tool for evaluation of hop quality. Volatile composition of different varieties of Hops from three regions of Spain (León, Galicia, and the Basque Country) were analysed. The oils obtained from hops samples were dissolved in isopropanol and the aromatic compounds were quantified by FID flame detector. Results showed that β-myrcene, β-caryophyllene and α-humulene compounds are the main aromatic compounds in all hop varieties' essential oils from León, Galicia, and Basque Country.

Key words. Aroma compounds, hop production area, essential oil, α-humulene, β-myrcene

Introduction

Hops (*Humulus lupulus* L.) are used to aromatize beer and obtain the characteristic bitter taste of the drink. In addition, hops contribute to the stability of the foam and have antiseptic properties. The use of hop cones in brewing is essential as it provides very specific characteristics due to its chemical composition (40-50 % cellulose, 15 % protein, 10 % water, between 2 and 20 % alpha acids and between 0.5 and 3 % essential oils) (STEVENS 1967).

The chemical composition of hops depends on the variety, the influence of environmental conditions, the post-harvest process, and the storage (RETTBERG et al. 2018). The main components of hops are alpha-acids, beta-acids, resins, and essential oils. Alpha and beta-acids, also known as bitter acids, are responsible for providing bitterness and represent between 5 and 20 % of the weight of mature hops, depending on the variety. Alpha-acids or humulones are specific resins responsible for bitterness. They also contribute to foaming and stability. Beta-acids or lupulones are similar resins, but with a very low bittering power (STEVENS 1967).

Hop aromas are mainly given by the essential oils contained in hops. These highly volatile compounds are present in large quantities in the different varieties. The composition of these essential oils varies greatly depending on the crop. Between 50 and 80 % are oxygenated or non-oxygenated hydrocarbons and approximately 1 % are sulfur compounds. Best known within the essential oils is myrcene, which usually constitutes almost 50 % of the compounds present and is responsible for the green, herbaceous, and resinous hop aromas.

In this work, we present the volatile chemical profiles for eight different varieties of hops: Nugget, Columbus, Cascade, Admiral, Hallertauer Magnum, Perle, Saaz and Spalt from different origins of Spain, León, Galicia, and the Basque Country.

Materials and methods

A total of 40 hop samples have been analysed, 11 from León (LE), 18 from the Lutega (LG) cooperative and 11 from Neiker (NK). The hop varieties included in the study were Nugget, Columbus, Cascade, Admiral, Hallertauer Magnum, Perle, Saaz, and Spalt. For the analysis of the aromatic compounds, the extraction of the essential oil was carried out by steam dragging with Clevenger. The oils obtained were dissolved in isopropanol and the aromatic compounds were separated by non-polar column gas chromatography and quantified by FID flame detector. An Agilent 7890A GC with FID detector and a capillary column (HP-5, 30 m x 0.32 mm id x 0.25 µm) were used for chromatographic analysis. Helium was used as the carrier gas with a constant pressure of 41.1 kPa and the injection temperature was 250°C. The oven temperature was initially held for 5 minutes at 40 °C, increased to 190°C at 5°C/min, and finally held at 190°C for 10 minutes. 1 µL of sample was injected using a split ratio of 1:50. A calibration line was prepared for each of the standards for the quantification of the samples and the quantification was expressed in µg/g of dry matter and in % of oil, g/100 ml. The aromatic compounds quantified were: β-myrcene, β-caryophyllene, α-humulene (major aromatic compounds) and α-pinene, β-pinene, limonene, methyl heptanoate, citroneol, linalool, geraniol, β-humulene (minor aromatic compounds).

Results

The GC-FID analysis of hop samples allowed the identification of 11 compounds. The β-myrcene, β-caryophyllene and α-humulene compounds are the main aromatic compounds in hop essential oils (Fig. 1). Hallertauer Magnum and Spalt showed the highest values of β-myrcene, while α-humulene was higher in the Perle variety than in others. Nugget, followed by Columbus, showed high content of β -caryophyllene.

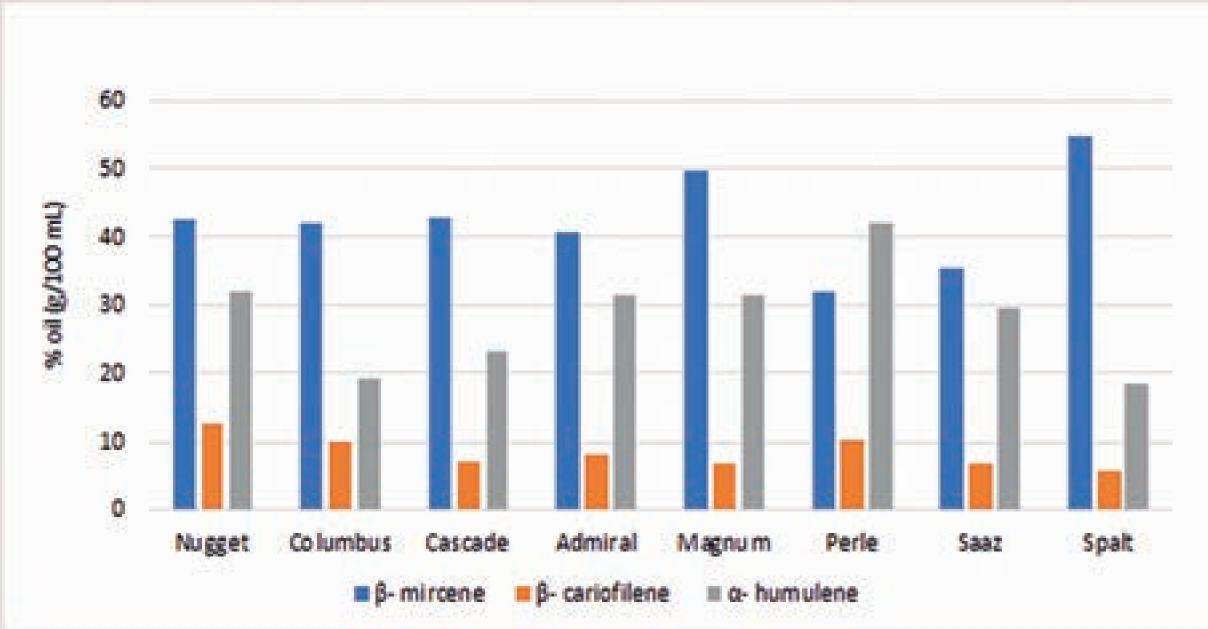

Figure 1. Major volatile compounds of essential oils from different hop varieties (% oil, g/100 mL)

Figure 2 shows minor compounds by variety of hops expressed in percentage of oil (g/100 mL). Citronellol (lemon) characterizes the Nugget, Columbus, and Admiral varieties. β-pinene shows the highest value in Hallertauer Magnum, and methyl heptanoate in Perle. The highest contents of linalool (rose) were found in Spalt and geraniol (flowery) in Columbus. Limonene (lemon) is shown in higher concentrations in Hallertauer Magnum, Admiral and Spalt. β-humulene shows the higher value in Cascade, Saaz and Spalt varieties.

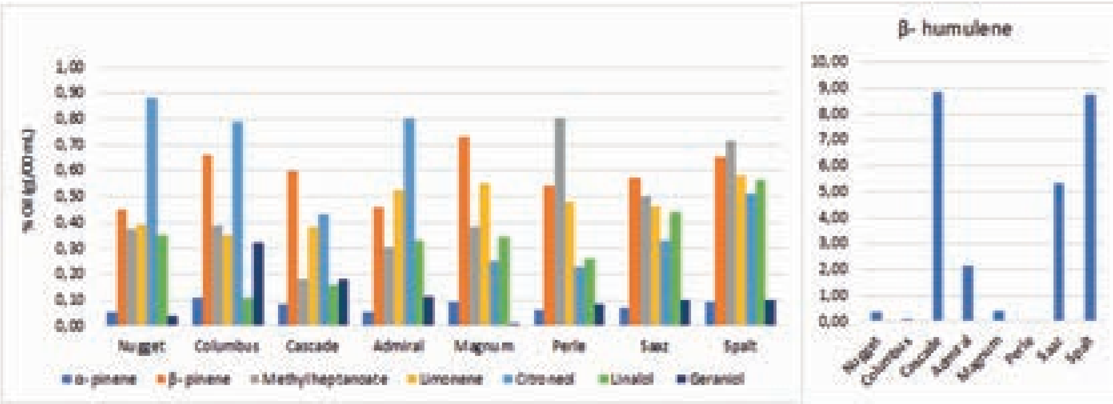

Figure 2. Minor volatile compounds of essential oils from different hop varieties (% oil, g/100 mL)

Figure 3 shows principal component analysis (PCA) to determine the differentiation between hop varieties based on their origin and volatile composition (% oil, g/100 mL). The first two main components added 62.69 % of the variance, 35.67 % and 27.02 % in PC1 and PC2 respectively. Nugget from the three different origins (LG, LE and NK), Perle-NK and Admiral-LG sited in negative side of PC1 were characterized by citronellol, α-humulene and β- caryophyllene. Cascade from NK and LG, Spalt-NK, Magnum-NK were sited in positive side of PC1 and characterized by α-pinene, β-pinene and β-myrcene. However, Cascade-LE showed the lowest values of volatiles. Columbus-LE, sited on the positive side of PC2, showed high values of geraniol and β-humulene.

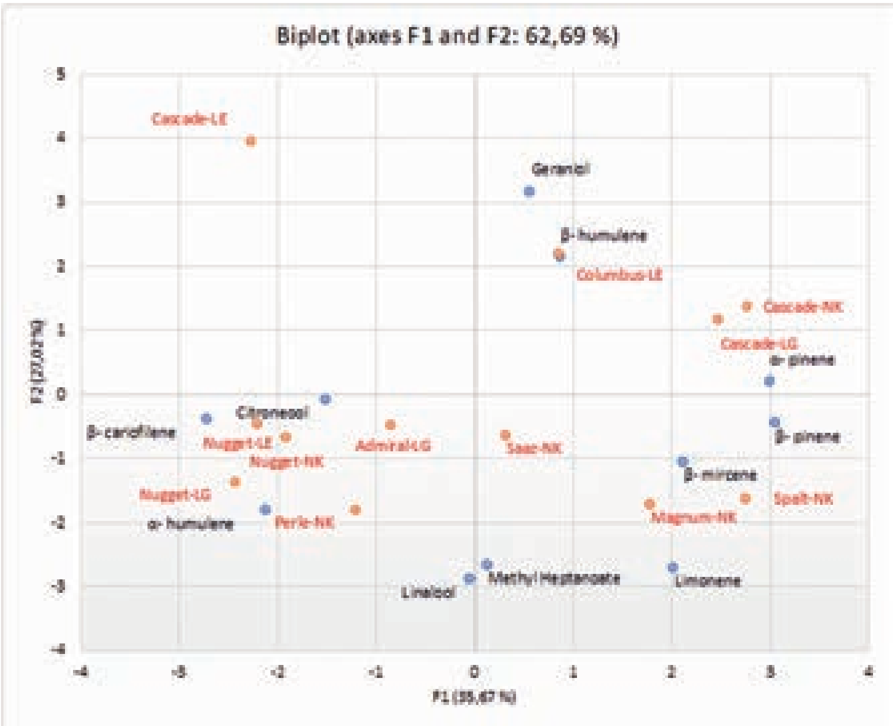

Figure 3. PCA of the volatile compounds of the essential oils of different hop varieties from different geographical areas (% of oil, g/100 mL)

Discussion

Essential oils represent the essence of the plant, meaning its distinctive aroma. Clustering analysis of essential oils from 25 different hop varieties has shown that the highest amounts, accounting for 47.1 to 89.3 % of the oils, are represented by β-myrcene, α-humulene, β-caryophyllene, caryophyllene oxide, and humulene epoxide II (KASKONAS et al. 2016). This is almost consistent with our study where the major volatile compounds of essential oils from different hop varieties were β-myrcene, β-caryophyllene, α-humulene, accounting for 62.45 to 95.74 % of the oils. Nugget-LE, Nugget-LG and Magnum-NK showed the highest value of these three volatile compounds. Also, LIGOR et al. (2014) listed the most important components of hop aroma as myrcene, α-humulene, β-caryophyllene, and β-farnesene. However, a more recent study where comparative aroma extract dilution analysis was performed on the special flavor hop varieties Huell Melon and Polaris determined myrcene, (3R)-linalool, and 2- and 3-methyl butanoic acid as important variety-independent hop odorants and found (1R,4S)-calamenene as a new odor-active compound in hops (NEIENS & STEINHAUS 2018). The major contributor to the hop aroma from the essential oil fraction is presumably the monoterpene β-pinene found also in rosemary, parsley, dill, rose and other essential oils (AMEH et al. 2015). In this sense Cascade-NK, Cascade-LG and Magnum-NK showed the highest values of β-pinene. The content of a specific substance in essential oils not only depends on the cultivation conditions, storage, and processing, but the extraction and analytical process may impact it as well (LIGOR et al. 2014; MATSUI et al. 2016).

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Categorization and quantification of hop aroma

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Abstract

As the effects of climate change increase year by year, with prolonged droughts and an increasing number of hot days in summer, several economically important hop varieties are suffering from declining yield and lower quality. In order to ensure the supply of high-quality hops in the future, breeders need to focus on breeding climate-adapted varieties with desired aroma. Accelerating the breeding process via targeted selection of resistant genotypes containing aroma-relevant metabolites is therefore of utmost interest.

To get a better understanding of the complex hop aroma, a sensory evaluation was conducted on a population of 150 genotypes and 164 phenotypes, ranging from experimental breeding lines to commercial aroma and high alpha varieties with various genetic origin. A trained sensory panel, consisting of eleven participants, evaluated the samples in six categories (citrusy, fruity, floral, herbal/spicy, resinous and onion/garlic). The samples were prepared as a hop tea and rated by the panelists in each category whether as 0 (aroma category not detectable) or 1 (aroma category detectable). Mean values from the binary sensory evaluations were calculated for further evaluation. The principal component analysis based on the rating in the different categories showed a broad differentiation between the phenotypes. Furthermore, the loadings intended that the categories "citrusy” and "fruity” are closely linked to each other, while “onion/garlic” and “resinous” spanned their own clustering.

Subsequently, an untargeted chemical analysis of the volatile metabolomes of all phenotypes was carried out, using headspace solid - phase microextraction (SPME) followed by gas chromatography and mass spectrometry, which led to the quantification of 586 non-redundant metabolites. Further research will focus on the correlation of the chemical and sensory data to identify aroma relevant and influencing compounds in hops.

Aroma-associated volatile metabolites in hops

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Abstract

The brewing industry is constantly on the lookout for hop varieties that bring new and exceptional flavors. Germany, but especially the USA, have seen a considerable increase in the area under aroma hops in recent years (EUROPEAN COMMISSION 2020, 2021; HOP GROWERS OF AMERICA 2021). In response to the demand, hop breeders' efforts are more and more focused on the development of new varieties rich in desired aroma characteristics and reduced in prominent off-flavors.

The olfactory characteristics of the cones are mainly influenced by the highly complex composition of the hop oil, which consist of several 100 to over 1000 different substances (ROBERTS et al. 2004). The elucidation of the composition of the hop oil, especially of the aroma-promoting substances, forms the basis for targeted breeding.

Organoleptic data on six defined aroma categories was collected in a hop population consisting of 150 genotypes. In this follow-up experiment, undirected analysis of the volatile metabolomes of these cultivars was performed. Using headspace solid - phase microextraction followed by gas chromatography, 586 metabolites were quantified by mass spectrometry. A large number of metabolites correlated to the sensory categories, such as fruity, resinous or citrusy, could be determined. The highest Pearson correlation coefficients (R) exceeded values of 0.5 and were found for the onion/garlic odor trait. These results provide the basis for identification of substances causative for aroma traits in subsequent studies. Based on these findings, further research will also focus on the identification of breeding markers, through association of metabolite levels and genetic data.

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VII: Posters

The size of leaf area, a tool for environmentally friendly hop protection

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Abstract

Environmentally friendly pesticide application methods are being sought for permanent crops such as vineyards, hop gardens or orchards. One way to reduce the environmental risk of excessive spray liquid dispersion in permanent crops is to apply an amount that meets the current needs of the crop while maintaining the quality of the treatment. Compared to the area application concept, parameters based on leaf area size and stand height are much more suitable for calculating the spray liquid volume. While the height of the hop plants can be measured easily, measuring the leaf area is not that simple. Hops belong to plants with high dynamics of height increase of bine and leaf area in a relatively short time. It creates two types of leaves. Bine leaves, grow directly from the main bine, lateral ones grow from the lateral shoots.

The leaf area of the hops was measured by a direct destructive method, consisting in removing all the leaves from the plant. All hop vine leaves were first plucked by hand and weighed on analytical balances and at the same time the leaf area per unit weight (cm^2/g) was determined planimetrically. The second method was based on planimetric image analysis. The measurements took place in 7- to 14-day intervals from the end of May until the harvest in the years 2019 to 2021 on a hop garden planted with the Czech variety Premiant. The results of measurements by both methods were very similar, the differences did not exceed ± 15 % rel.

The total leaf area of the double vine, which was wound one training wire (which is the most common way of hop training in the Czech Republic), ranged from 3 to 5 m^2 at the time of hop harvest. Measurements on other varieties at harvest period showed that larger plants could have a leaf area in the range of 5–7 m^2. The leaf area of solitary bines, which also occasionally occur in hop gardens, reaches 60–80 % of the leaf area of the two-bine line.

While the longitudinal growth of hops is terminated at the time when the plant enters the generative phase (flowering and cones formation), the leaf area, especially of the small lateral leaves, is constantly growing. During the harvest season, when the hops reach full maturity, the lateral leaves make up 60 to 75 % of the total leaf area. The area of bine leaves at the end of the growing season in the lower parts of the plant often decreases due to the fall of necrotized leaves. The size of the leaf area depends on the variety and weather conditions during the vegetation. An important factor is also the occurrence of diseases and pests, which can lead to growth anomalies affecting the size of the leaf area (necrosis).

At the same time, the hop garden was monitored by a fixed wing eBee X drone, equipped with a Micasense RedEdge MX multispectral (MS) camera and a Duet T thermal camera. The Micasense RedEdge MX camera consist of five spectral bands (Blue, Green, Red, Red Edge and Nir) to calculate the structure and crop vitality. The Duet T camera comprised a thermal sensor and a reference S.O.D.A. camera in the visible part of electromagnetic spectrum (Blue, Green and Red bands = RGB). The MS and RGB image can be used to calculate the leaf area. Triangular Greenness Index (TGI) was first derived from the images and converted to a raster binary model, only two categories were detected – the green parts of the detected crops and the others (soil, litter etc.). After extracting the green parts of the hop garden, it is possible to calculate the leaf area. If the height of the crops is known, it is possible to calculate the crops volume.

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Identification of broad-spectrum resistance to Powdery mildew in wild hop germplasm resources

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Abstract

Global acreage of common hop (*Humulus lupulus* L.) has increased by nearly 50 % during the past decade to meet the continued demand of the craft brewing industry. Hop powdery mildew, caused by *Podosphaera macularis* (Wallr.) Braun & Takah., can cause substantial crop loss, leading to reduced yields and perceived quality. Wild hop germplasm resources have proved invaluable sources of novel alleles for disease resistance, chemical content, and tolerance to various pests. However, few exhaustive assessments of these genetic resources for resistance to powdery mildew have been carried out. We screened 6,732 wild individuals collected from North America and Eurasian hop populations by sequentially inoculating subsets of resistant individuals with powdery mildew isolates known to represent the extant pathogenic diversity including *VB35*, *VB3456*, *VB1235*, and *VB346WH18*. We identified seven powdery mildew resistant wild hops from Eurasia that could be used to develop future varieties conferring broad spectrum powdery mildew resistance. We were able to identify the sex of six genotypes phenotypically, which included three males and three females. These materials have been deposited into the United States Department of Agriculture – National Plant Germplasm System and will be made available for distribution.

Key words. hop, disease resistance, hop powdery mildew, germplasm resources

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Collection of hop genetic resources in the Czech Republic

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The Czech Hop Research Institute in Žatec has two rich collections of genetic resources. The first collection contains hop varieties grown all over the world in the frame of "National Program of Conservation and Utilization of Genetic Resources in Plants and Biodiversity" issued by Czech Ministry of Agriculture. There are 380 items, which represent old and new hop cultivars, which serve as donors of important characteristics within the breeding process. The second collection contains wild hops, which have been regularly collected since 1997. There are 295 items from Europe (128), North America (73) Caucasus (76) and eastern parts of Russia (18). Wild hops show genetic, chemical and phenotypic variability. It is necessary to bring wild hops from habitats into field conditions to find out if demanded features are based genetically and not just influenced by environment. Assessment is carried out at least for five years and each genotype is planted there in three replicates, which are evaluated individually. All the characteristics are transferred into the information system with the help of our classifier.

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

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

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

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

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

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

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Evaluation of content and variability of hop oils in Czech hop varieties

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Abstract

The average weight of hop oils is 0.43 to 2.28 % w/w. The hop cultivars (cvs) Saaz, Saaz Brilliant, Mimosa, Saaz Shine and Saaz Comfort have a low content of hop oils. Their maximum content of hop oils amounts to 1.1 % w/w. The average share of myrcene is between 23.42 and 45.14 % rel. Only cvs Agnus, Vital and Boomerang have a maximum myrcene share higher than 50 % rel. The average share of caryophyllene ranges between 6.19 and 13.15 % rel. Saaz Late has a wide range of caryophyllene share – from 5.39 to 15.53 % rel. The average share of farnesene is between 0.14 and 16.91 % rel. Only cvs Saaz Comfort and Saaz Shine have a maximum farnesene share of more than 20 % rel. The average humulene share has a very wide range between 2.23 and 35.79 % rel. The cvs Vital, Mimosa, Gaia and Saaz Comfort are a group with a low humulene share, which is clearly different from other hop cultivars. The average share of selinenes ranges widely from 0.97 to 33.56 % rel. Cv. Mimosa differs with its share of selinenes between 23.08 and 43.75 % rel.

Table 1. Average content and composition of hop oils in Czech hop cultivars

Cultivar	IHGC code	Weight (g/100g)	Myrcene (% rel.)	Caryophyllene (% rel.)	Farnesene (% rel.)	Humulene (% rel.)	Selinenes (% rel.)
Boomerang	BOO	2.28	45.14	8.95	0.48	20.75	0.97
Gaia	GAA	1.97	35.40	12.18	4.42	2.92	22.72
Agnus	AGN	1.83	33.45	12.21	0.34	19.30	2.99
Sládek	SLD	1.52	30.18	13.15	0.22	28.24	1.76
Harmonie	HRM	1.49	31.15	8.69	0.14	23.00	16.85
Vital	VIT	1.43	40.18	7.40	1.63	2.23	18.63
Rubín	RUB	1.23	27.27	9.03	0.17	21.89	19.13
Kazbek	KAZ	1.13	34.93	11.06	0.29	18.76	3.40
Premiant	PRE	1.12	26.97	11.89	1.57	35.25	2.61
Bohemie	BOH	1.04	29.18	8.16	1.03	21.44	10.67
Bor	BOR	0.92	28.41	11.52	0.29	35.79	2.01
Saaz Late	SAL	0.83	31.28	7.87	11.21	18.57	4.75
Saaz Comfort	SAC	0.81	28.11	8.26	16.91	3.49	16.41
Saaz Shine	SAH	0.72	23.55	11.04	12.56	29.96	2.34
Mimosa	MIM	0.67	30.55	6.19	0.90	2.89	33.56
Saaz Brilliant	SAI	0.50	23.42	9.50	14.45	22.93	4.68
Saaz	SAZ	0.43	24.37	6.87	14.04	21.84	1.46

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New aroma hop varieties in the Czech Republic

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Hop breeding in the Czech Republic mostly focuses on aroma hops. The Saaz fine aroma variety is the best-known Czech hop variety around the world. The breeding of aroma hops dates back for 160 years at least. It gives preference to aroma features, including the aroma of hop cones, the balanced ratio of alpha/beta acids and the positive impact on beer quality. The breeding of aroma hops is based on the Saaz variety. In recent years, foreign varieties or even wild hops have been used as well. In 1995, a new program for breeding aroma hop varieties with Saaz in their origin were launched, resulting in the registration of three new cultivars (Saaz Brilliant, Saaz Comfort and Saaz Shine) that show numerous features identical with Saaz. Another registered variety from this program is Mimosa, which is different. All new varieties are currently being grown under pilot conditions. Brewing tests and test brews are under way in brewery operations.

Four new aroma hop cultivars (cvs) – Saaz Brilliant, Saaz Comfort, Saaz Shine and Mimosa – were registered in the Czech Republic in 2019. All the new cvs have significantly higher yields than the traditional Saaz aroma variety. Saaz Comfort has the significantly highest content of alpha acids (5.59 %) whereas Mimosa has the significantly lowest content of alpha acids (1.90 %). Saaz Comfort and Saaz Brilliant show a variability of alpha acid content below 20 %. The other cvs, Saaz Shine and Mimosa, as well as Saaz, have a variability of alpha acid content above 25 %. Mimosa has the significantly highest content of beta acids (6.07 %). Mimosa shows the highest average cohumulone content (29.29 % rel.) whereas Saaz Comfort has the lowest cohumulone content (18.04 % rel.). Saaz Comfort and Saaz Shine have the highest average contents of hop oils (0.84 % w. and 0.75 % w., respectively). Saaz, Saaz Shine and Saaz Brilliant show the significantly highest farnesene contents (13.47 % rel., 12.50 % rel., and 12.38 % rel., respectively), which are higher than those of Saaz Comfort and Mimosa.

The results achieved in comparison with Saaz unambiguously show that these hop cultivars do not have identical parameters. They fall within the category of fine aroma hops but are not identical with the Saaz benchmark variety. The Saaz Brilliant, Saaz Comfort and Saaz Shine cvs are currently being tested in pilot experiments in Chrášťany, Stekník, Běsno, Nesuchyně and Staňkovice. For several years, all the new cvs have been tested in large and small Czech breweries. There are 200–500 kg of hops available from experimental growing areas for brewing tests. The Saaz Brilliant, Saaz Comfort and Saaz Shine cvs were planted in the fall of 2020 on two hectares.

Acknowledgement

This article was written as part of project NAZV QK21010136 entitled “Application of new hop varieties and genotypes resistant to drought in hop growing and beer brewing“ with the financial support of the Czech Ministry of Agriculture. Genetic resources are a part of “National Program of Conservation and Utilization of Genetic Resources in Plants and Biodiversity” (51834/2017-MZE-17253/6.2.1) issued by Czech Ministry of Agriculture.

Smart Hop Yard – use of real-time weather data in pest and disease prognosis

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Abstract

A network of automatic weather stations helps forecasting pest and disease pressure during vegetation, assisting farmers to effectively use pesticides in Czech hop growing regions.

Key words. Hop, weather, pest and disease prognosis, hop protection

Introduction

The Smart Hop Yard project is based on the already proven Smart Vineyard project and is based on the installation of a network of weather stations that cover all hop-growing areas of the Czech Republic. Prediction models for individual pathogens can be automatically calculated due to the obtained data. In 2021, the function of prediction model of Downy mildew *Pseudoperonospora humuli* was verified. Prediction of Hop aphid Phorodon humuli was added into the calculation in 2022. Up-to-date information on disease and pest pressure enables growers to apply plant protection products in a targeted and effective manner.

Material and methods

The service is operated by Breuss Technology, Ltd. The weather stations consist of a printed circuit board equipped with 1 or 2 AA batteries with an output voltage of 3.6 V, a data recording interval switch (15 or 30 min.), a network (Sigfox) antenna and connectors for connecting optional sensors. In the basic state, the station records air temperature, relative humidity, and precipitation. Optionally, it can be extended by other sensors, such as an anemometer, soil temperature and humidity sensor, etc. The data is sent for processing in the selected interval and is available on the website www.chytrachmelnice.cz in the dashboard within about 10 seconds after sending. Station overviews are divided according to hop-growing areas, mainly for the sake of clarity and specific environmental conditions in individual areas. Stations can be installed either directly on the hop garden construction at a height of about 6-7 meters (when installed below, the station loses signal after plants reach full growth) or on the perch near the hop garden or in the general area (up to 500 m if there are no major obstacles between the hop garden and station to secure the relevance of the data). The accuracy of the temperature and relative humidity sensor is ±0.2°C. resp. ±2 % (Bosch sensors used in the automotive industry), the rain gauges are calibrated to 0.31 mm per flip. The obtained data are collectively statistically evaluated after the growing season and presented to growers and public at seminars.

Results

Based on the weather data and data of the prognostic models, we annually evaluate statistical correlations among weather parameters, yield, alpha-acids content, altitude, etc. General statistical data show the difference in quality and quantity of hops among Czech hop growing regions as well as the yield and alpha-acids content decrease with age. Significant correlation confirming long-term observation also shows the negative effect of increasing temperature on alpha-acids content, therefore higher alpha contents were reached in localities in higher altitudes, where the temperature is lower.

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From the hop variety to the volatile compounds in beer: high-throughput screening of hops using automated micro fermentation and GC/MS

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Abstract

Hop (*Humulus lupulus* L.) plants are essential for the brewing industry. The choice of the hop variety is a key factor on the final aroma of beer due to the diversity of volatile compounds present in hop. It is therefore essential to choose the hop variety according to the flavors it will provide to the final beer. Up to now, the volatile compounds present in a hop variety can be screened by GC/MS on the raw material (cones, pellets, essential oil), identifying what is unique in it. But the different processes of beer production such as hopping method, boiling, bio - transformation or fermentation may lead to new flavors that can only be observed in beer and cannot be predicted using the volatile profile of the raw material. This can be done by screening the aroma profiles of finished beers in GC/MS, but this screening needs to produce beer for every variety tested and then at least, one fermentation by variety. This approach is very time-consuming and according to the literature, beers are generally produced in laboratory-scale fermentations of at least 2.6 L, whereas only a few mL are needed to obtain an aroma profile of beer. In this study we propose an automated micro-scale brewing platform combined with a micro-scale fermentation in 20- and 10-mL vials compatible with the extraction and analysis of volatile compounds of the beer by GC/MS.

All stages of beer production such as brewing, filtration and fermentation were carried out automatically using a modified autosampler (GERSTEL MultiPurposeSampler [MPS]). Fermentation temperature was controlled using a cooling-stack adapted to the micro-fermentation and stages were followed automatically by weight using a connected balance every 2 h. At the end of the fermentation, alcohol by volume (ABV%) was measured by GC/MS. Volatile compounds of these micro beers were screened using GC/ToF-MS after Stir Bar Sorptive Extraction (SBSE).

A pilot study was performed to produce a separate fermentation from each of five hop varieties, namely Aramis, Barbe Rouge, Columbus, Elixir, and Strisselspalt (n=3), using 3 mg of crushed malt (4 EBC) and 15 mL of water (an unhopped wort was measured at 13°P as control). Hop addition was 10 min before the end of the boiling process at 20 mg/10mL (200 g/hL); then the wort was transferred to a new vial and a lager strain from Fermentis® was pitched at 0.5 g/L. After seven days of fermentation at 18°C, the temperature of the cooling-stack was decreased to 4°C for two days to remove diacetyl (not controlled). The screening of these five fermentations showed 86 significantly different compounds between the five varieties. These data allow us to determine which volatile compounds are most present and predict which flavors they will provide to the final beer. For example, Barbe Rouge produces the most of ethyl 2-methyl butanoate – a compound that will attribute strawberry and red fruit aroma to the beer. Linalool, another key compound in beer that provides a floral aroma, is identified in greater quantities in beers brewed with Elixir, Columbus, or Barbe Rouge. Furthermore, Principal Component Analysis (PCA), separating varieties according to their concentrations of major volatile compounds in micro beer, indicated that Aramis, Elixir and Strisselspalt are closely grouped varieties that will contribute similar flavors to the micro beers. Also, it is possible to compare the volatile compounds present in micro beer from each hop variety in order to suggest if one variety can replace another or even to predict the flavors which will be imparted from new varieties not yet used in beer.

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Precision farming of indoor hydroponic hops: A revolutionary growing system

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Introduction

In 2020, Ekonoke's R&D Department identified a series of crops that currently undergo significant climate risk, with hops ranked at the top of the list. AI-backed vertical farming specialist Ekonoke is growing hydroponic hops to mitigate climate risks often associated with this key ingredient for the premium beer industry. Growing hops in a completely controlled environment could be the answer to maintaining consistently high levels of specific compounds that contribute to a more desirable flavour and aroma profile.

Our controlled environment allows us to keep out pests, fungi, or diseases, which in turn helps us to eliminate the use of pesticides. We also avoid abiotic stresses such as drought or salinity stress. Therefore, plants don't need to dedicate energy to defend themselves against the insects or diseases or to respond to these abiotic stresses and we can accelerate the growing cycle without impacting yield and quality.

Process and achievements

The research carried out involved four varieties of hops and five replicas of each one. We provide the plants with a specific nutrient solution through a recirculating irrigation system that allows us to optimize both quantity and quality of harvested hop flowers, while using 20 times less water than conventionally grown hops.

According to Simon Dusséaux, EvodiaBio, »Farming of hops generates up to 5 kg CO_2 and requires more than 2700 liters of water per kg of hops, while it uses fertilizers and pesticides«. After over two years of research, we have achieved a significant reduction in cultivation time, compared to the traditional method in the field, and are now able to obtain three harvests per year.

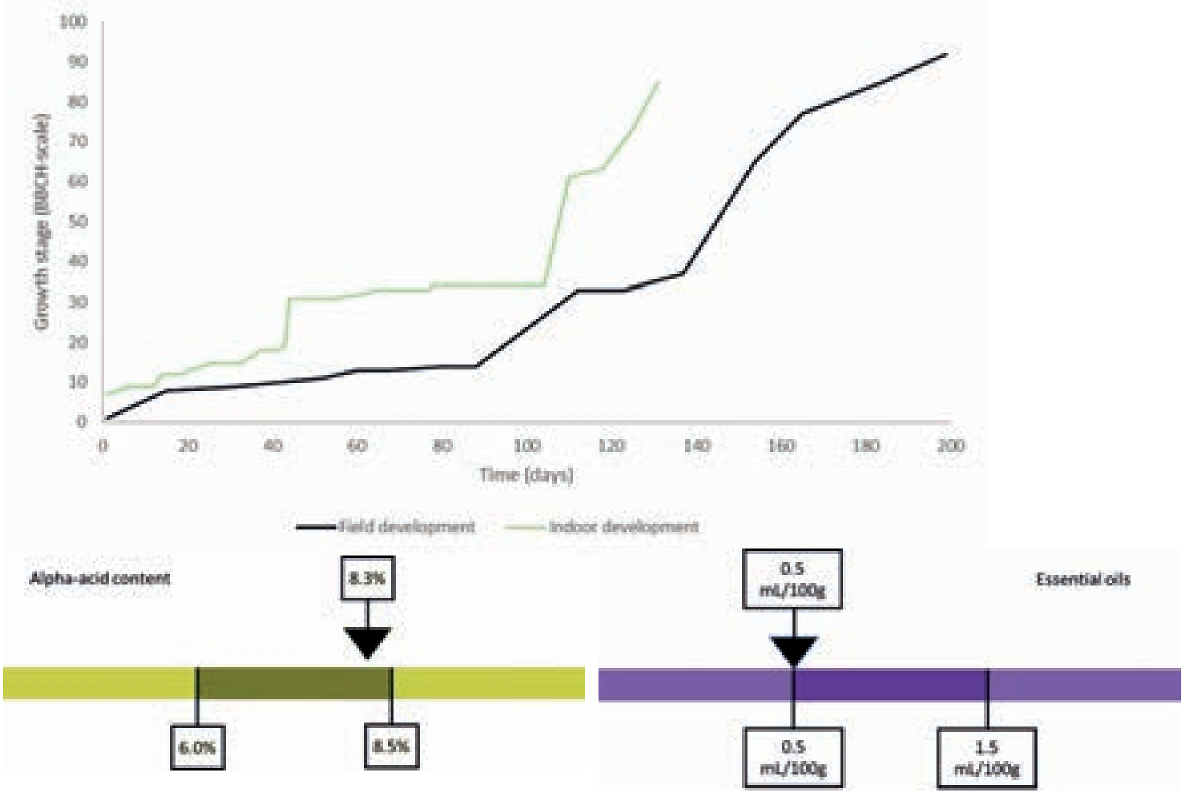
Results and discussion

The results obtained are promising in terms of the quality of soft resins, reaching, and even exceeding, the percentage of alpha-acids in some of the varieties studied. Regarding essential oils, the amounts obtained are close to the levels observed in the field. Myrcene levels are generally higher.

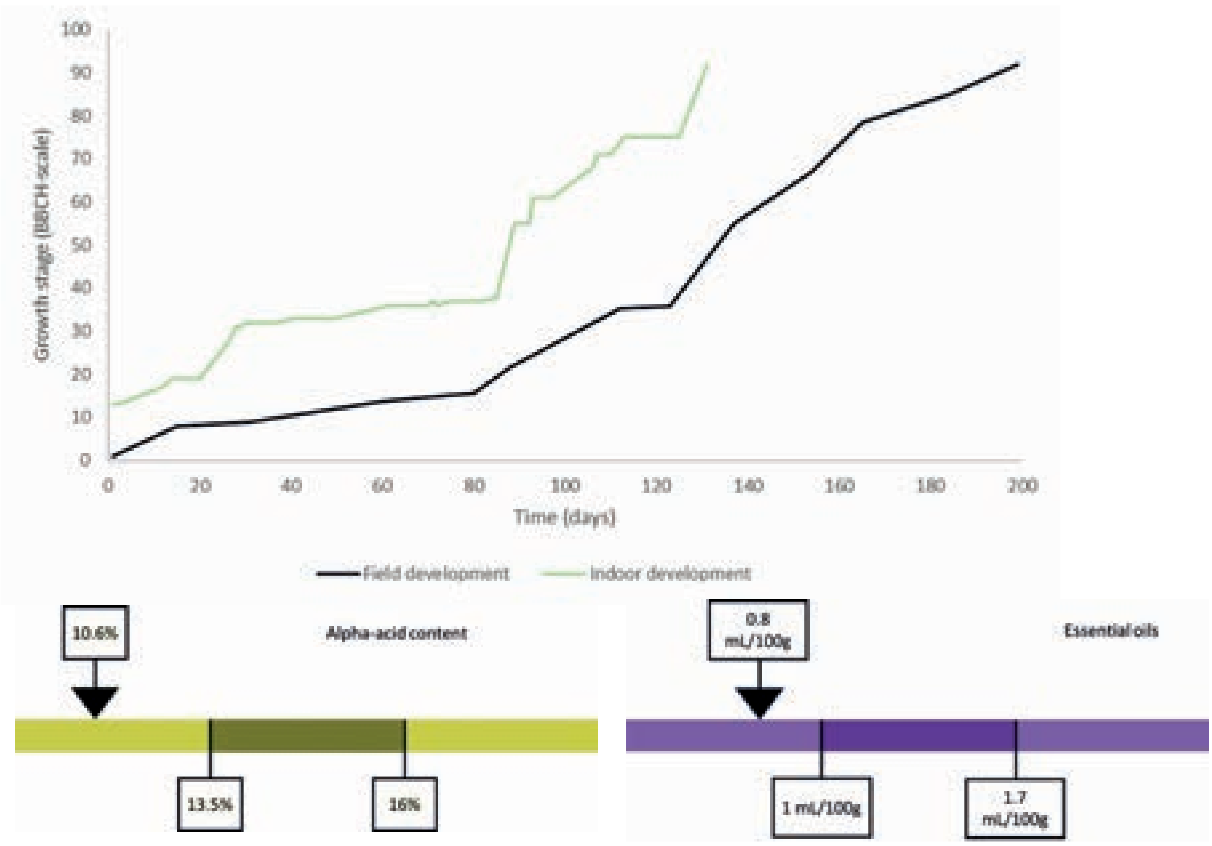
Variability of the results of the different cultivars are strongly linked to the quality of the rhizomes used for the research. Subsequent experiments are being conducted using only hop plants developed in vitro.

Data obtained from each cultivar is presented below, showing is a comparison of hop growing times between field and indoor phenological stages (according to BBCH scale); the average of alpha-acid content in harvested samples of each cultivar, where the darker yellow section represents the alpha-acid range generally obtained in the field; and the average amount of essential oil content in harvested samples, where darker purple represents the essential oil range normally obtained in the field.

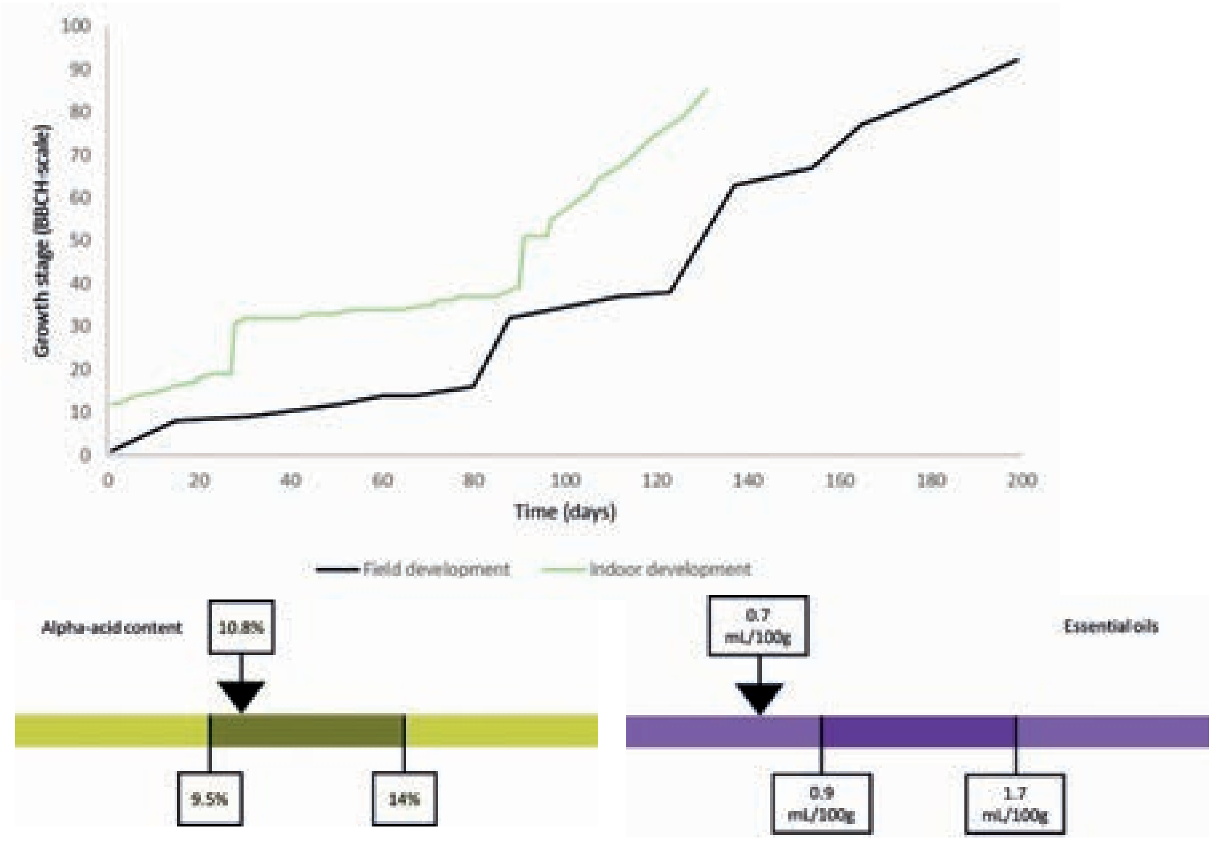
Cultivar 1



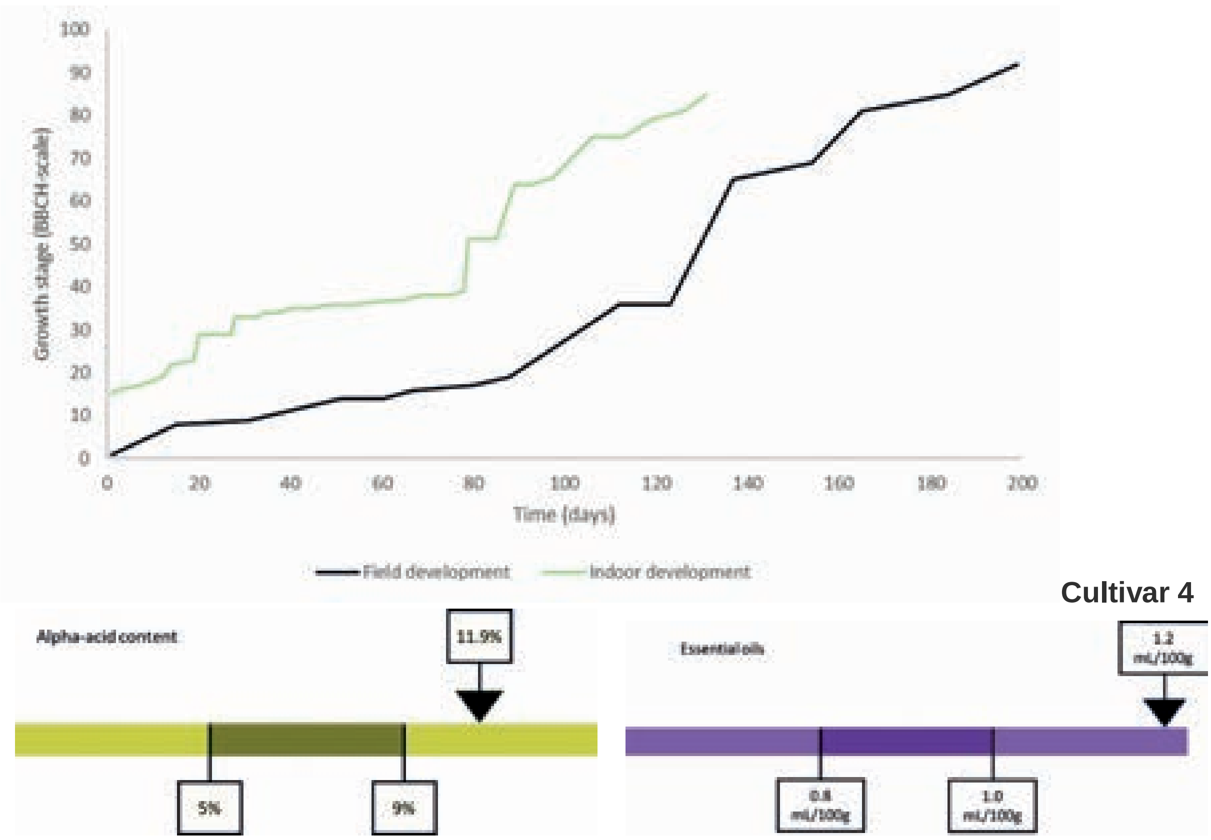
Cultivar 2



Cultivar 3



Cultivar 4



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Analysis of genetic and metabolomic diversity in wild hops collected in the French North-East region

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Humulus lupulus L. (Cannabaceae) is an iconic industrial crop of the French North-East region with an increase of area under crop since the beginning of the 19th century, connected to the development of the brewing activities.

Although hop cones, *i.e.*, female flowers, are mainly used in the brewing industry for their aromatic and bitter properties, hops are also a potential source of specialized metabolites for pharmaceuticals, cosmetics, nutraceuticals, and agronomic use. Indeed, some molecules specifically produced in lupulin glands have been reported to have either (i) antimicrobial activities such as humulone and lupulone (*e.g.*, ZANOLI & ZAVATTI 2008), (ii) anti-cancer activity such as polyphenolic compounds like xanthohumol (*e.g.*, STEVENS & PAGE 2004), and (iii) estrogenic activity with 8-prenylnaringenin (*e.g.*, FAIVRE et al. 2007). Moreover, polyphenolic compounds have been shown to play an important role in plant defense, against biotic and abiotic factors (*e.g.*, ABRAM et al. 2015).

The overall aim of this work is to assess the relationship between genetic and metabolic diversity of different wild hop accessions (48 geolocalized individuals) using fingerprinting methods (respectively microsatellites markers) and a non-targeted metabolomics approach using UHPLC-DAD-MS/MS, to carry out a detailed analysis of the metabolome present in flowers, leaves, and stems.

The work carried out within the framework of the Bio4Solutions Chair will facilitate to characterize the production of high-value-added biomolecules. The following step will be to characterize the impact of the metabolomic diversity on the ability to recruit endophytic and rhizospheric microbiota. The results collected will help to imagine and set up original strategies usable for promoting the agro-ecological transition of crop systems.

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