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

INTERNATIONAL HOP GROWERS` CONVENTION
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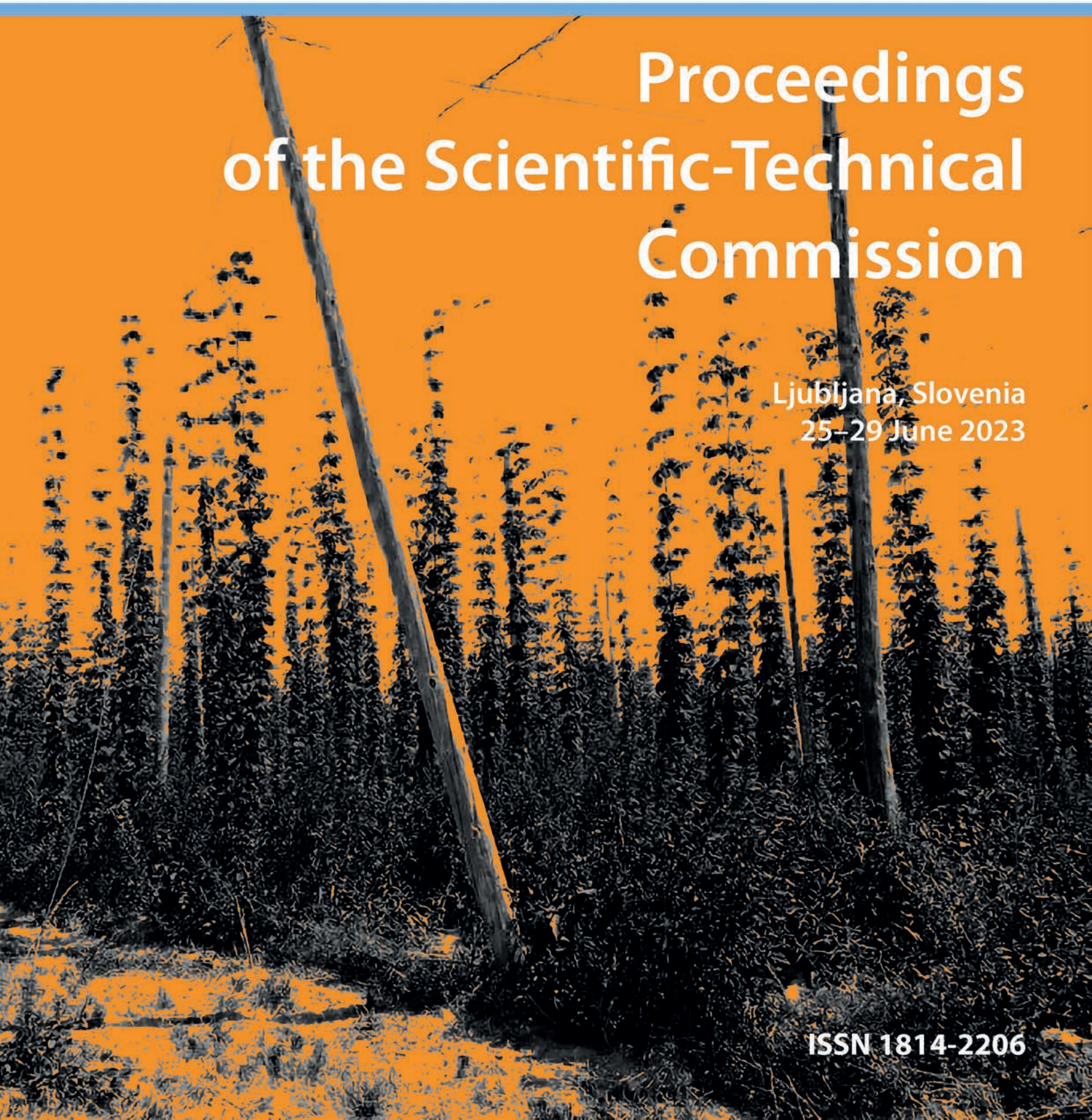

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Foreword

While considering what to write in this foreword, it occurred to me that I know no scientific expression for hop research based on Latin or ancient Greek. My own speciality, the study of insects, is called entomology, and there are many dozens of other like terms, such as ornithology or odonatology, that derive from the Greek word *lógos* (λόγος) – translated as 'word', 'reason', or 'the study of', and several dozen other meanings – as part of the name of a particular branch of learning. What is more, there is musicology or theology, and even the science and study of wine making is referred to as oenology. So, what would hop science be called then? Humululogy? Lupululogy? At least an expression that can be decently enunciated only after a few beers… So, never mind, and we can probably attribute this to the fact that hops were not much used until the late Middle Ages. However, the point I wish to make concerns hop research in our host country, Slovenia. With more than 1,600 ha, the hop acreage of Slovenia currently ranks sixth, globally; but if one considers the amount of scientific research – in terms of both manpower and output – in relation to hectares under cultivation, then Slovenia is definitely the scientific leader on Planet Hops. Admittedly, this is quite an old-fashioned approach in times where public decision-makers quite often consider the value of a three-minute spot on YouTube superior to a three-year research project, but I am convinced that the Slovenian hop industry will benefit from this outstanding effort in the following decades.

Therefore, it is now long overdue that the Scientific-Technical Commission returns to Slovenia with its biennial meeting. The last event we had in this wonderful country was in 2003, *i.e.*, two decades ago. Back then we had met in Dobrna near Žalec, organised by IHPS under the responsibility of the late Majda Virant, and old hands like myself, who knew Majda, will remember her with a smile on their faces. However, today, there is another girl in Slovenia (albeit not the only one), who knows how to put a smile on my face again and again. This is hop breeder Andreja Čerenak, guiding spirit of the local organisation team and my perfectly matched partner in the organisation of the 2023 STC meeting. Together with all the other friends in Žalec and Ljubljana, Andreja put a tremendous effort into the flawless organisation of the 2023 STC meeting. This is already mirrored in the figures connected with this event: In 2023, our meeting will include more than 80 participants from 15 hop-growing nations, delivering 36 lectures from all fields of hop research, and exhibiting 20 posters. These figures exceed by far those from any previous STC meeting and indicate the increasing worldwide acceptance of our series of STC conferences.

I am also expressing my gratitude to the main sponsors of our conference; the generous financial backing by Barth-Haas Group, Hopfenverwertungsgenossenschaft HVG and Hopsteiner supports the mission of the STC and facilitates the participation of so many scientists. Special thanks go to all local sponsors, who supported the 2023 STC meeting: Hmezad Exim, Inbarco, Slohops, Hmeljarstvo Čas, Vitahop, HopPris, Alpine Hops, and Green Gold Brewing.

Finally, I wish all attendees a fruitful and joyous meeting, with many essential personal discussions and encounters that will strengthen international cooperation and networking. Therefore, with all my heart: Najlepša hvala Andreja, Martin, Jernej, Magda, Sebastjan, and others too numerous to name; dobrodošli v sloveniji!

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

I: Hop breeding

The Slovenian hop industry: a general overview

Čerenak A.

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Hop growing has a long tradition in Slovenia as it has existed in this area for almost 1000 years, and the beginnings of industrial cultivation date back to the 19th century, when hop growing flourished. We can look with admiration at the hop growers when, 70 years ago, they came to the common realization that in order to progress in hop growing, it is necessary to develop one's own knowledge. The hop growers' decision to establish the Hop Institute was a brave one in those difficult post-war times, and it soon turned out to be necessary for hop cultivation in Slovenia. With the support of the institute, Slovenian hops gained an important place on the competitive European and worldwide market. Today, they cover almost 1700 ha, which makes us the 3rd largest producer in the EU and 6th in the world. In comparison with other agricultural plants, Slovenian hops are characterized by the fact that as many as 98 % of hop fields are planted with Slovenian varieties that were bred at the institute; 98 % of the crop are exported. The reason for such a large proportion of our own varieties is the successful breeding and the less effective introduction of foreign varieties.

As a public research, advisory and educational organization, the **Slovenian Institute of Hop Research and Brewing** (IHPS) acts for the needs of the Ministry of Agriculture, Forestry and Food, the Chamber of Agriculture and Forestry, and for the needs of domestic and foreign agribusiness industry. The institute has also project partnerships with several local and foreign scientific research and technical institutions. Its engagement in agribusiness, food processing and in rural development enables its integration into the domestic and the international agribusiness sector. At IHPS, researchers are connected to the 'Research Group for Plants, Soil and Environment', consisting of 24 scientists including nine with a doctor's degree and four doctoral students. Our research is varied and is carried out in the fields of plant production, chemistry, and biology. More specifically, we cover the fields of hops, breeding, brewing, plant protection, medicinal plants, analytical chemistry, ecology, agricultural economics, and plant nutrition. Over the past 20 years, researchers have led or participated in 75 research projects.

We are involved in three research programs led by the University of Ljubljana, Biotechnical Faculty, and one led by the Institute of Chemistry: Agricultural plants – genetics and modern technologies, competitiveness of agro-food, biochemical and biophysical-chemical characterization of natural substances, and chemistry and structure of biological agents.

Hop breeding is one of the leading fields of work at the Institute since its establishment. In Slovenia first varieties were released in the early 1970s, while in last 20 years varieties with new disease resistance and brewing characteristics were released. In the disease resistance breeding we are focused on main diseases as downy and powdery mildew, *Verticillium* wilt resistance and during the last years we are focused on studying Citrus bark-cracking viroid (CBCVd) resistance. We are following new demands in brewing industry to fulfil expectations of all stakeholders in the hop industry chain. New biotechnological approaches were developed and applied in the classical breeding procedure to increase effectiveness of the program. Hop breeding is a highly multidisciplinary field with cooperation of many of the institute's employees. In the research field, we are tightly connected with the Biotechnical Faculty of the University of Ljubljana. Planting new varieties, more resistant to diseases and with a higher good quality yield, will enable the preservation and development of the industry.

In accordance with the legislation in the field of agricultural plant seed material and plant health, we have introduced a certification scheme for **hop planting material** at IHPS. The purpose of the certification scheme is to produce quality planting material without hop pests and diseases, which cannot be prevented with plant protection products (viruses, viroids, verticillium wilt) and could cause high economic damage in cultivation. In 2007, we introduced non-viroid-nuclear stock into the regular production of certified hop plants, in which the hop latent viroid (HLVd) was eliminated.

Due to the climate changes, **hop irrigation** is becoming a very popular topic. In the last twenty years, six droughts were recorded in Slovenia, which caused major consequences in agriculture and were declared as a natural disaster. To cope with the problem, up to 80 % of hop fields are irrigated, of which 80 % have a drip irrigation system. In the last twenty years the irrigation research focus was mainly on the studies of drip irrigation technologies and its influence on the quantity and quality of the hop crop and on the development or support of decisions about irrigation – irrigation forecast. In recent research, we have also focused on modelling the distribution of water in the soil under the above-ground drip irrigation system in hop farms. In recent years our activities relate to the modernization and construction of new irrigation systems in the Savinja Valley.

With the European project LIFE BioTHOP, we introduced **circular economy** into hop growing sector. We introduced a bioplastic twine, made from renewable sources, 100 % biodegradable, recyclable and compostable for hop plants support during growth. It represents more environmentally friendly alternative to currently used polypropylene twine, which remains intertwined in hop waste biomass after harvesting hop cones and poses a problem for use of that biomass. On the other hand, hop biomass, intertwined with biodegradable twine, represents an excellent raw material for processing it into compost and biodegradable products, which can be composted after use (planting pots, bottle trays, buttons, harvester fingers …). Compost of hop biomass or from compostable products is used on agricultural land, thereby closing the circle on the farms.

In the last 20 years, the **brewing** sector in the world and in Slovenia has experienced intensive development, which we also followed at IHPS. To this end, we have completely modernized and upgraded our own brewery. For successful work in the field of scientific research, we successfully awarded laboratory instrumental equipment for chemical and microbiological analysis and special equipment in the field of brewing and hops. In response to the rapid development and demand of external stakeholders, we have established various educational programs, bringing our work closer to our target groups.

Integrated Pest Management (IPM) is a strategy formally developed in the 1950s. In Slovenia IPM solutions in hop gardens began in 1966. IPM aims at integrating and implementing complimentary pest management tactics to maintain pests at economically accept levels, while negative ecological and social impacts of pest management activities are minimized. In hop production IPM is based on biological technologies including cultural methods such as tillage, variety selection (use of pest-resistance or tolerant varieties and pest-free seedling material). In hop production in Slovenia, IPM is based on monitoring for downy mildew, powdery mildew, *Phoma exigua*, *Cercospora cantuariensis*, damson-hop aphid, hop-flea beetle, European corn borer, two-spotted spider mite, etc., on forecasting systems, good application practice of plant protection products (PPP’s), management of pesticide resistance, and avoidance of pesticides that have negative impact on beneficial arthropods. Slovenian hops are produced without herbicides.

In the last 20 years, Slovenian hop production has experienced an intensive emergence of new diseases, including the lethal form of *Verticillium* wilt and severe hop stunt disease (CBCVd). Large parts of the activities were focused on the study and management of these diseases and to the development of modern methods of integrated plant protection. In this period, we have developed a modern **diagnostic laboratory**, which is actively involved in the network of official and national plant health laboratories.

Monitoring and forecasting of harmful organisms are based on a network of agro-meteorological locations, in which measurements from meteorological stations and monitoring of plant and pest (diseases, pests, weeds) phenology takes place. We use a variety of biotechnical devices to achieve that goal. Based on the measurements, observations and use of forecasting models and modules, forecasting news are produced with recommendations for optimum plant protection. This news is provided in accordance with IPM guidelines. We are available to agricultural growers with advice and recommendations by phone and e-mail. We also respond to requests to inspect the situation on the ground.

A quality management system of **Good Experimental Practice** (GEP) was implemented to perform tests and analyses to obtain data on the effectiveness, phytotoxicity of plant protection products and biostimulants, and their impact on the quantity and quality of the crop.

The **quality management system** is a carefully prepared model of good business practices of successful organizations. Slovenian Institute of Hop Research and Brewing is accredited by Slovenian Accreditation in the field of testing (SIST EN ISO/IEC 17025: 2017). Accredited test methods are performed by the Department for Agrochemistry and Brewing and the Diagnostic Laboratory for Plant Protection. Testing fields are foodstuffs (beer), agricultural products (hops) and biological samples.

Ensuring the **hop quality** grown in Slovenia is the main task of all Slovenian hop growers who grow hops for sale. In order to obtain a certificate for the sale, their hop crop must undergo a carefully controlled certification process, which, since 2004, has been led by the Organization authorized to control and certify the hop crop at IHPS. The certificate confirms the minimum quality, geographical origin and varietal purity of the hop crop. In 2013, we added a higher value of Slovenian hops with the protected geographical indication **Styrian hops**, which indicates the geographical area and method of production of Slovenian hops and its quality. This is of great importance for the recognition of Slovenian hops on the international hop market and gives Slovenian hops added value. At IHPS, we take care of the appropriate flow of information and instructions to hop growers and the processing industry, so that together we can achieve optimal quality criteria regarding the content of hop resins and essential oils and stability during storage until sale.

The institute with its professional and advisory services acts as the centrepiece of Slovenian hop growing industry. We are all focused to enable production of high-quality Styrian hops.

Breeding hops for drought resistance

Nesvadba V., Donner P., Charvátová J. & Trnková S.

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Abstract

Hop breeding for drought resistance has a high priority in the Czech Republic. Varieties Rubín, Vital and Boomerang have low variability in hop yield and alpha acid content. Testing hops for drought resistance is by determining the rate of photosynthesis and the rate of transpiration. Older Czech hop varieties are sensitive to drought. The new varieties Ceres and Juno have good drought tolerance. There are six drought-resistant genotypes in registration trials. There are already new breeding lines with drought resistance. Wild hops are also used for breeding.

Key words. hop, Humulus lupulus L., variability, photosynthesis rate, transpiration rate

Introduction

From a historical point of view, hop breeding was focused on clonal selection in original populations of Žatec type hops. By 1993, 10 clones of Žatec hops were registered. Currently, only three clones (Osvald's clones 31, 72 and 114) are grown on an area of 4,000 ha. Since 1993, clonal selection in the Czech Republic was stopped (NESVADBA et al. 2020a) and only the crossing method was used. In 1994, the first Bor and Sládek varieties were registered after crossing. Another eight hop varieties (NESVADBA et al. 2020b) were registered from 1996 to 2010 (Premiant, Agnus, Harmonie, Rubín, Vital, Kazbek, Bohemie, and Saaz Late). As of 2017, another 15 hop varieties have been registered. The collection of genetic resources of hops is the basis of breeding in the Czech Republic (CHARVÁTOVÁ et al.1997). The high variability of wild hops (PATZAK et al.1997) has been used a lot in breeding in recent years.

Breeding goals have always been set on resistance to fungal diseases, high yield, required content and composition of hop resins and essential oils (TREFILOVÁ et al. 2022). Of course, during breeding, the stability of qualitative and quantitative traits was monitored from the point of view of brewery and grower requirements. Due to the significant increase in temperatures and often very low rainfall, there is another new perspective on hop breeding. In recent years, preference has been given to breeding new varieties resistant to high temperatures and drought (KROFTA et al. 2019). A number of new crossings have been carried out with the objective of obtaining new genotypes of hops that will be resistant to drought. For this reason, the research project QK21010136 "Application of new varieties and genotypes of drought-resistant hops in growing and brewing practice" is being addressed from 2021. As part of the project, several new genotypes are being tested for drought resistance (NESVADBA et al. 2022). New Czech hop varieties are part of the testing. The target is to create new hop varieties that will show very low interannual variability in hop yield and the content of important brewing substances.

Material and methods

Data were obtained from evaluation of breeding material and genetic resources of hops. Each plant is harvested separately. An experimental Wolf picking machine is used for hop picking. An average sample was taken from each hop variety and dried at a temperature of 56°C to reach a humidity of 7%. The content of alpha acids was determined from dry hop cones by using liquid chromatography according to EBC 7.7 (KROFTA 2008).

The basis of the assessment is statistical data. The following statistics were prepared: average (x). Coefficient of variation (CV), showing the extent of variability in %, was used for data processing. A paired t-test was applied to determine and prove the significance of difference between hop varieties. The difference of sets was determined based on significance level, which shows the probability of difference of the tested sets (MELOUN & MILITKÝ 1994). Dependence was determined by using the coefficient of determination (r2). A centuple of the coefficient of determination shows in % to what extent the value of the alpha or beta content is influenced by the age of the plants.

Testing of hop genotypes for drought resistance in the greenhouse continued in 2022. A total of 46 hop genotypes were selected. These genotypes were propagated again for the greenhouse experiments at the same time so that the plants were in the same growth phase. For measuring the physiological parameters of hops using the LCpro SD device, five plants of each genotype were propagated, planted in 5L pots in a standard growing substrate. Three plants in the most similar growth phase were selected for the measurement. The initial measurement of all genotypes took place on irrigated plants that were not stressed by drought. After the initial measurement, their irrigation was terminated and the effect of drought stress on the visual and physiological manifestations of the plants was subsequently monitored. After 10 days from the first measurement, the same plants were measured again. The rate of photosynthesis and transpiration was determined after the water and partially also heat stress (greenhouse conditions), when the plants could not compensate the heat stress by cooling down due to irrigation. Based on the measured parameters, the instantaneous water use efficiency (WUEi) was calculated. Thanks to the measured and calculated parameters, but also to the visual scoring of the genotypes, it is possible to assess which genotypes show better/worse performance parameters after exposure to water stress. Visual assessment of plants is based on points 1 (very poor) to 5 (excellent condition).

Results

High yield and alpha acid content is a very important feature for hop varieties. But the most important thing is stability over the course of 10 to 15 years (Table 1). Varieties with low variability are resistant to the external environment, *i.e.*, drought and high temperatures in recent years.

Table 1. Average yield, content of alpha acida and their variability in Czech hop varieties. ***VC** – Variance coefficient.

Variety	IHGC code	Yield (kg/plant)	Yield ***VC** (%)	Alpha acids (% w/w)	Alpha acids ***VC** (%)
Kazbek	KAZ	3.66	**36.87**	5.72	19.16
Bor	BOR	3.39	**33.61**	7.47	16.96
Gaia	GAA	3.25	27.31	13.81	**23.49**
Saaz Late	SAL	3.24	27.50	4.06	15.03
Vital	VIT	3.06	19.53	12.31	15.38
Rubín	RUB	3.01	17.31	11.38	10.05
Bohemie	BOH	2.93	**35.24**	5.48	13.12
Sládek	SLD	2.81	**31.77**	5.94	**20.79**
Agnus	AGN	2.81	29.37	10.69	14.37
Harmonie	HRM	2.79	22.76	7.00	**29.68**
Premiant	PRE	2.51	20.14	7.54	14.14
Boomerang	BOO	2.31	17.42	11.60	8.19
Saazer	SAZ	2.02	24.39	2.87	**25.38**

Table 2 shows the evaluation of plants under water stress. Evaluation is focused on photosynthesis rate (A), transpiration rate (E) and visual assessment (V). It can be seen that the new Ceres and Juno hop varieties have a higher rate of photosynthesis and at the same time a lower transpiration rate than the older hop varieties. Therefore, they also have a better visual rating. The new generation of hop genotypes has a higher resistance to water stress than all Czech registered hop varieties. Wild hops from the Caucasus are interesting.

Table 2. Assessment of plants for water stress

Genotype	End of irrigation			After 10 days of water stress		
	A (µmol $CO_2/m^2/s$)	E (mmol $H_2O/m^2/s$)	V	A (µmol $CO_2/m^2/s$)	E (mmol $H_2O/m^2/s$)	V
Kazbek	14.04	3.91	1	0.00	0.05	2
Sládek	11.02	2.35	1	0.00	0.43	3
Premiant	7.14	1.59	2	0.00	0.48	4
Rubín	6.69	1.51	1	0.00	0.45	4
Ceres	12.06	2.79	1	1.99	0.19	2
Juno	12.80	2.42	1	1.55	0.24	2
5165 ®	8.25	2.03	2	4.88	1.32	2
5304 ®	9.65	2.36	2	3.27	0.86	3
5432 ®	10.50	2.29	2	8.77	1.61	2
5461 ®	11.79	2.92	1	5.15	1.11	1
5465 ®	6.36	1.38	2	6.02	1.26	2
5559 ®	9.62	2.45	1	9.05	1.69	1
5194	5.09	1.61	2	6.44	1.97	2
5398	11.09	2.66	2	8.46	2.32	2
5646	11.23	2.72	2	8.00	1.54	3
Wild Cau	9.99	2.01	2	7.08	1.54	2

Table 3 shows the genotypes registered for registration trials. Genotypes 5165 and 5304 are of the bitter type, genotypes 5432, 5461 and 5465 are of the aroma type, and genotype 5559 is a flavor hop with a distinct fruity aroma. Bitter genotypes show an alpha acid content consistently above 10 %, such as Agnus, Rubín or Vital, and have higher yield potential. Aroma genotypes have cv. Premiant in their pedigree – with a higher content of alpha acids and an alpha/beta ratio above 2. Genotype 5559 with a specific aroma is bred from cv. Kazbek and is characterized by a balanced ratio of alpha/beta acids and a lower proportion of cohumulone than cv. Kazbek.

Table 3. Content and composition of hop resins in genotypes in registration trials

Genotype	Alpha acids (% w/w)	Beta acids (% w/w)	Ratio alpha/beta	Cohumulone (% rel.)
5165	12.30	5.13	2.40	25.10
5304	11.63	3.57	3.26	25.30
5432	7.46	4.12	1.81	24.70
5461	8.39	3.53	2.38	29.70
5465	7.36	3.62	2.03	22.20
5559	5.08	5.38	0.94	22.40

Conclusion

Evaluating dry hops is very problematic. The variability of important characters is not only affected by the weather, therefore it is necessary to carry out greenhouse experiments. A very important feature is a high rate of photosynthesis and, conversely, a low rate of transpiration. the higher the ratio between photosynthesis and transpiration, the higher resistance to drought can be assumed. It is interesting that a number of new genotypes have almost the same rate of photosynthesis and transpiration during water stress. Currently, prospective genotypes are being evaluated under field conditions in various irrigated and non-irrigated locations.

Breeding for drought resistance has a high priority in the Czech Republic. Currently, 6 prospective genotypes are being evaluated in registration trials. In the breeding material, other genotypes that have a high resistance to drought. The breeding program also includes wild hops that are resistant to drought.

Acknowledgement

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Optimizing hop production sustainability by breeding

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Abstract

Hop production in Germany is currently facing a multitude of serious challenges. Besides new pests and exploding production costs, climate change, and tightened legal standards, breweries are expecting a more sustainable production of their beer ingredients. Already a decade ago breeding efforts within the Bavarian hop breeding team focused on a strategy that could be summarized as “low input – high output”. Within this scope, significant progress in terms of the release of hop varieties enabling hop producers for a more sustainable production was achieved. New Huell aroma and high alpha varieties are characterized by lower CO_2 equivalent emissions per production unit through an increased nitrogen use efficiency and higher harvest index, thereby decreasing transportation and harvest energy needs. Due to increased resistance or tolerance to major biotic stressors such as downy mildew, powdery mildew, and verticillium wilt, chemical crop protection application can be reduced contributing to a preservation of biodiversity. High yield and alpha acid stability under adverse temperature and water supply conditions were a major focus of the newest Huell hop varieties released.

TANGO is the newest member of the Huell fine aroma varieties. The goals that were trying to be met when TANGO was bred were many. Besides being very well adapted to the changes in climate (temperature, pests, diseases), it should require less inputs (nutrients, water, pesticides) while producing higher and more stable outputs (yield, oil content, alpha content). Additionally, it should be competitive within the world hop market, and it should deliver good properties to the finished beer (good isomerization, good flavor, good stability). Already at early breeding stages the breeding line becoming TANGO was extraordinary and through rigorous testing, TANGO has successfully proven itself as a very robust hop and goes beyond in fulfilling the target goals. TANGO excels in producing a high output with low inputs and can cope with major diseases and pests while producing a high yield that can be harvested unprecedented homogenously. These characteristics make the lives of the hop grower easier and provide the brewer a reliable and more economical product.

TANGO results from a cross between cv. Cascade and a strong male Huell breeding line, which is an offspring of the fine aroma variety, Hallertauer Tradition, popular with brewers worldwide. TANGO is special because it can act as a bittering hop, a traditional aroma hop, and a flavoring hop depending on the time of addition in the brewing process. As a bittering hop, TANGO conveys similar flavors comparable to those of cvs Perle (cross-bred in 1966) and Hallertauer Tradition (cross-bred in 1978). TANGO is not intended to replace these two noble hop varieties, which are part of many brew recipes, but to complement the German aroma hop portfolio. When TANGO is added at a later stage in the brewing process it imparts notes of citrus and fruit to the beer, especially if you use top fermenting yeasts like ale yeast. This allrounder hop is most notable in Lager styles but can also be used in other styles with heavier dry hoping schedules.

The newest member of Huell-released varieties is TITAN. This bitter hop was bred based on the genetics of the currently most successful German bitter cvs, Herkules and Polaris, fortified by the most disease and pest resistant/tolerant Huell male breeding line. Thus, TITAN combines the high alpha acid yield potential and high alpha acid quality of cv. Herkules with the high alpha acid stability of cv. Polaris but with much better resistances, especially against powdery mildew. TITAN is characterized by its good cylindrical bine with an even hanging of cones from top to bottom. Its relatively small leaves reduce transpiration making this variety more water efficient then its parents with a similar relation of cone to rest-plant as cv. Polaris. Besides displaying improved resistance properties that render several crop protection measures unnecessary, this growth habit helps additionally to reduce the amount of pesticides used in hop production.

As a bitter variety besides total alpha acid yield, alpha acid quality is of major importance. Only a bitter variety with a bittering quality that can match that of cvs Herkules and Hallertauer Magnum has any chance of being used by master brewers in their recipes as a substitute hop despite its agronomic superiority. As part of a genome-based research project, a sophisticated tasting procedure protocol was developed at the Hop Research Centre to determine the bittering quality. Standardized beers were brewed with just one basic addition of hops using all the bitter hop varieties available on the market. These were then degustated and rated by an independent expert board consisting of 37 beer experts. As a result, Herkules was selected as a reference hop variety with its typical bittering quality profile. Further brewing trials were conducted based on the same standard recipe with TITAN in comparison with cv.Herkules at different breweries. The degustation of those beers by the expert board concluded that the increase in and total intensity of the bitterness were rated as being identical to that of Herkules and of same quality. Thus, TITAN provides improved agronomical properties thereby increasing sustainability of hop production to the hop producers and perfect bitter quality to the brewers.

Although these new highly adapted hop varieties already bridge to a more sustainable hop production, breeding progress must be accelerated to keep pace with changing production conditions in term of climate change and reduced crop protection. In this context, genomic selection is supposed to contribute to an increased selection gain in hop as it did in other crops. Here, we present preliminary results regarding our investigations on the implementation of genomic selection in hops. As calibration set a hop diversity panel with almost 200 selected hop varieties/breeding lines was phenotyped for over five years regarding major disease susceptibility, yield and quality in the lab, greenhouse, and field. The diversity panel was genotyped through genotyping by sequencing resulting in over 2000 SNP markers spanning the entire hop genome. Prediction accuracy based on 5 – fold cross validation ranged from 0.3 to 0.7 for major traits indicating genomic selection and genomic assisted mating to be an additional tool in hop variety development soon.

Key words. Sustainability, climate change, TITAN, TANGO

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Hop breeding in Belgium: development of regional hop varieties

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Abstract

Hop (*Humulus lupulus* L.) has been cultivated in Belgium since the beginning of the 16th century. Despite this long history of hop growing, Belgium lacks its own breeding program: hop varieties nowadays cultivated in Belgium, such as Hallertauer Magnum, Wye Target, Goldings and Challenger were developed abroad years ago. But not all varieties developed in other countries are suitable for cultivation in Belgium and Belgian hop growers frequently need to grow and test these foreign varieties under local conditions.

In the past decade there was not only a switch in the Belgian hop cultivation from bitter hops to the more popular aroma hops, but also a change in the sales market, from international trading rather to a supply chain to small and medium-sized, local craft breweries. This change triggered the demand from the hop industry and the brewing industry to develop locally bred hop varieties. Farmers want to grow disease resistant varieties (*Pseudoperonospora humuli*, *Podosphaera macularis*, *Verticillium nonalfalfae*), with a good tolerance to heat and drought. Brewers are interested in locally grown varieties with a unique aroma.

From 2017, a collaboration between different partners in Flanders (ILVO, Inagro, Vives, vzw hop, city of Poperinge), resulted in the initiation of a small-scale, participatory breeding program. Now the consortium holds a collection of 100 female plants (mainly established cultivars) and about 75 male plants (collected in the wild in Flanders). These collections are currently phenotyped for earliness and disease susceptibility and used to perform crosses, either wind pollinated or, in the near future, by manual crossing. Seeds are collected and germinated in the greenhouse. At seedling stage, seedlings are inoculated with powdery mildew, and mildew resistant seedlings are subsequently transferred to a container field for further monitoring. For two years, the plants are monitored by scientists and hop farmers for growth characteristics and sex determination. From year 3 onwards, high performing female plants are transferred to hop fields in the hop region of Poperinge (both under conventional and organic regime). Together with the farmers, the agronomic performance of these candidates is monitored during the next three years. Cones are analysed biochemically, and sensory analyses are performed, next to a *Verticillium* resistance screening. Based on all these assessments, we select candivars that are assessed for three more years in a larger set up with brewing experiments. Finally, we aim to introduce these candivars in the Belgian hop growing region.

Key words. Hop, variety, breeding, disease resistance

Acknowledgement

This breeding program is supported by Leader, the province of West Flanders, the Flemish government and ELFPO (project HopBel) and VLAIO and the European Union (HBC.2021.1079 project HopBel 2.0) in close collaboration with Stad Poperinge, vzw HOP and the involved breweries.

II: Marketing of hops

Economic impact of proprietary varieties on hop markets

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Abstract

The ownership of hop proprietary varieties, their acreage and production were compared with public varieties. Market share for each proprietary hop variety acreage and production was calculated between 2000 and 2020. The quantity of land under centralized control in the U.S. hop industry due to increased proprietary variety acreage between 2000 and 2020 was quantified. To evaluate changes to hop industry concentration and competitiveness, the Herfindahl-Hirschman Index (HHI) was used. The HHI enabled us to quantify the portion of land under oligopoly control. The HHI analysis of hop acreage and hop production demonstrated that market concentration rose rapidly between the years 2010 (0.0376 and 0.0729) and 2020 (0.4927 and 0.5394). This resulted in decreasing business competitiveness within the market during this period caused primarily by rapid consolidation of ownership during increased proprietary variety acreage and production increases.

Key words. Hop industry, varieties, market concentration, intellectual property

Introduction

Between 2000 and 2020 the proportion of patented hop varieties increased. The increase of proprietary variety acreage and production has a causal effect upon hop prices (MacKinnon & Pavlovič 2022). In the United States during that time, one variety development company, the Hop Breeding Company, grew to the point where its varieties enjoyed significant market share (MacKinnon & Pavlovič 2023).

In 2020, Germany and the U.S. produced 38 and 39 percent of the global crop, respectively, so a comparison between the two hop producing countries was reasonable. Between 2009 and 2019, the annual farmgate value of American hops increased by 282 % (USDA NASS 2020). According to IHGC country reports of the International Hop Growers' Convention between 2009 and 2019, the 70 farmers in the Pacific Northwest (PNW) received approximately $4.7 billion during that time, $2.88 billion more than the $1.87 billion the 1,087 German hop farmers received during the same period (MacKinnon & Pavlovič 2019).

The objective of the study was to evaluate changes to the hop industry area and production concentration and competitiveness with respect to the changes in proprietary varieties of hops relative to public varieties. The publication of intellectual property (IP) necessitates the use of symbols for registered trademarks, unregistered trademarks, and copyright, (i.e. "®", "™" and "©") respectively, their ownership is publicly available. The introduction of proprietary varieties, therefore, enabled the calculation of hop market share for the first time. We used the Herfindahl-Hirschman Index (HHI) to measure changes in hop industry competitiveness by way of measuring market concentration (MacKinnon & Pavlovič 2023).

Material and methods

The companies that developed proprietary hop varieties own and license the production of multiple proprietary varieties to growers (for production) and sales and distribution of those varieties to merchants in their supply chain, thereby facilitating the management of production and distribution. We calculated the percentage for each proprietary variety produced within the Pacific Northwest (PNW) by the total acreage for the PNW, *i.e.*, the total market share. We calculated the market share for each entity owning IP listed by the USDA NASS in the USDA National Hop Report (NHR) by grouping those with common ownership of patented and trademarked products (USDA NASS 2022).

We used the Herfindahl-Hirschman Index (HHI) to evaluate changes to hop industry concentration and competitiveness with respect to the changes in proprietary varieties of hops relative to public varieties. A significant portion of PNW acreage appears in two aggregate categories called “other” and “experimental”. USDA data show that in 2022 7.12 % of total acreage was categorized as “other” or “experimental”. The categories are used to report acreage and production for varieties that do not meet the three-independent-grower threshold set by the USDA. Based on historical data, we believe at least half of this acreage was proprietary.

The HHI is a method used also by the United States Department of Justice (USDOJ) to measure market concentration during mergers or acquisitions, to evaluate one competitor’s position relative to another, and to uncover potentially anti-competitive practices. The HHI values of zero to 0.1500 mean a low market concentration. Values of 0.1500 to 0.2500 are considered a moderate concentration. Values of 0.2500 and above count as high concentration. The HHI value will be low when market shares among participants are equal. The value will be high when one firm has a disproportionate share of the market (CALKINS 1989). The value of the HHI decreases as the number of firms in the market increases. Market concentration is inversely proportional to competitiveness (DEPKEN 1999). The HHI is responsive to the asymmetry of market shares and is used to evaluate changes in the competitiveness within a single industry over time or to compare one industry to another (CALKINS 1989). In our research, we adopted this method for the first time in the hop industry for the measurement of its market concentration.

The HHI Formula:

$$HHI = S1^2 + S2^2 + S3^2 + \dots Sn^2 \tag{1}$$

where:

n refers to the number of varieties in the market;

S refers to the percent market share for a variety.

Results

Calculating the Herfindahl-Hirschman Index (HHI) of the U.S. hop industry based on the market share of hop sales to brewers was a hopeless endeavor as information regarding market share based on sales of hops by merchants to breweries was proprietary information and never shared. We discovered an alternative method for measuring market share. The USDA NASS restrictions related to the reporting of proprietary U.S. acreage and production (i.e., that three or more independent producers must list acreage or production for the corresponding statistics to be reported in aggregate form) to meet the needs of this research.

Five companies comprised approximately 70 % of proprietary U.S. hop acreage and production in the Pacific Northwest in 2022 (Table 1). These variety development companies can license hop merchant companies to sell their varieties. They can license hop farms to produce their varieties. In some cases, the variety of development company ownership and the licensed merchants and farms shared common ownership. Licenses extended beyond companies in which they shared ownership. Previously independent farms were transformed into contract growers. The decision-makers for the five largest variety development companies, therefore, enjoyed a disproportionate influence in the industry and upon the market. The acreage on which a company's proprietary varieties were produced represented the market share of influence of the owners of each variety development company. The market share of influence represented a new and significant measurement possible within the industry all made possible by the growing demand for and reporting of proprietary varieties of hops.

Table 1. Five largest U.S. hop variety development companies and the market share of U.S. acreage and production of their proprietary varieties in 2022

	Variety Development Company	**Market Share of U.S. Total Acreage in 2022**	**Market Share of U.S. Total Production in 2022**
1	Hop Breeding Company (HBC)	49.05%	49.12%
2	HopSteiner	7.71%	10.78%
3	Association for the Development of Hop Agronomy (ADHA)	3.27%	3.35%
4	Virgil Gamache Farms (VGF)	3.20%	2.88%
5	CLS Farms	2.06%	2.02%
	TOTAL	65.30%	68.15%

Calculating the market share for each ownership group based on their ownership of proprietary hop varieties enabled the calculation of the market share of influence over the scarcest resource in the hop industry, acreage. Branded proprietary varieties are products that enjoy monopoly control by their very nature as patented and trademarked products. Seventy percent of the acreage, therefore, was governed by the decision-makers of five entities. Public varieties, in contrast, are available for any grower to produce.

We calculated the market share for each proprietary variety production and acreage relative to total U.S. acreage for the years 2000 through 2020. During this time, market concentration moved from low to high according to the standards set by the U.S. Department of Justice when evaluating mergers and acquisitions between competitors.

Using the HHI market share data by variety, we calculated the market share for all proprietary varieties collectively as the U.S. hop industry resembles what is referred to as a complex monopoly in the U.K. (BRENT 1993). We calculated the increase in market concentration between 2000 and 2020 of publicly reported U.S. proprietary hop varieties. The increasing HHI values between 2000 and 2020 demonstrated the changes in the degree of competitiveness in the industry (Fig. 1). The U.S. proprietary varieties used to calculate these results were the following: Ahtanum™, YCR 1, Amarillo® VGXP01, Apollo™, Azacca™ ADHA-483, Bravo™, Calypso™, Chelan, Citra®, HBC 394, Columbus/Tomahawk®/Zeus (AKA: C/T/Z®), Ekuanot™, HBC 366, El Dorado®, Eureka ™, IDAHO 7™, Idaho Gem™, Jarrylo™, ADHA-881, Loral™, HBC 291, Millenium®, Mosaic®, HBC 369, Pahto™, HBC 682, Palisade®, YCR 4, Pekko™, ADHA-871, Sabro™, HBC 438, Simcoe®, YCR 14, Strata™ OR 91331, Summit™, Super Galena™, Talus®, Warrior™, YCR 5, Zappa®.

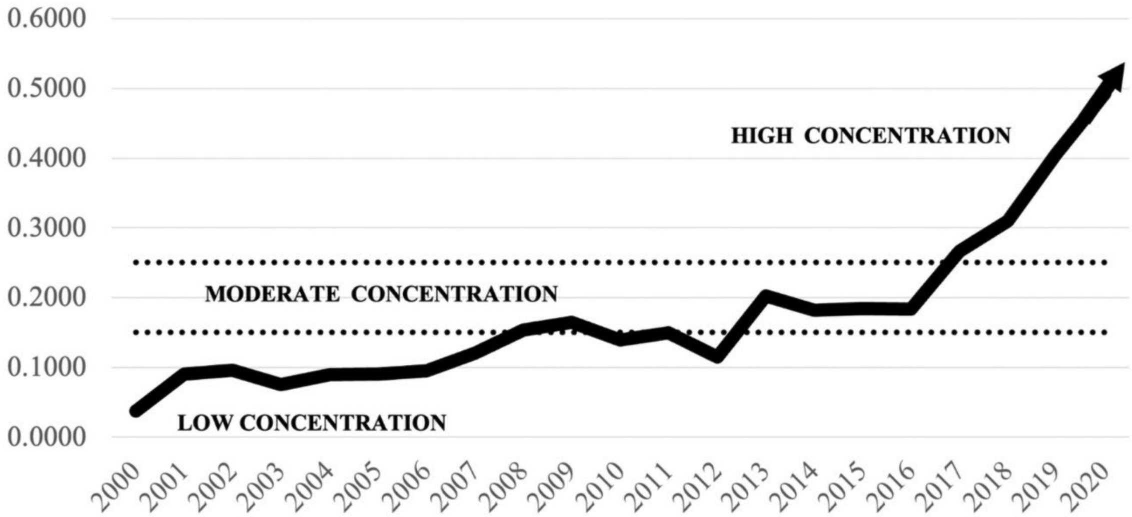

Figure 1. The HHI for total U.S. branded proprietary variety acreage 2000–2020 (own study based on USDA NASS 2022)

Discussion

Common ownership between the entities that create branded proprietary varieties, individual hop farms and hop merchant firms further increased market concentration. The individuals who own the entities that create proprietary varieties have created a competitive advantage for the merchant companies and farms in which they share a financial interest. We concluded that branded proprietary varieties, when their ownership is concentrated in few hands, reduced competition within the market and encouraged market segmentation.

We expanded the variety-specific acreage market share calculations to group those varieties that share common ownership to get a better picture of the influence of the five largest variety development companies. One company, the HBC, had a much greater share than the rest.

The HHI calculations revealed that increases in proprietary variety acreage resulted in increased concentrations of power within the industry. This resulted in reduced sector competitiveness.

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III: Entomology

The control of damson-hop aphid (*Phorodon humuli* Schrank) and two-spotted spider mite (*Tetranychus urticae* Koch) by Rock Effect New (*Pongamia pinnata*)

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Abstract

Damson hop aphid *Phorodon humuli* (Schrank) and two-spotted spider mite *Tetranychus urticae* Koch are the most dangerous pests of hop in Czech Republic. In practice they are controlled by synthetic insecticides and miticides. Nevertheless, many of them are endangered by EU restrictions. So, as to be able to cope up with these pests after "Green Deal" comes in force it is critical to look after adequate alternatives. Botanical pesticide Rock Effect New (*Pongamia pinnata*) showed good efficacy on damson hop aphid and two-spotted spider mite in field trials carried out in 2022. Nevertheless, repeated application is necessary for efficient control. No phytotoxicity was observed if applied in 1.0 and 1.5 % concentrations. Synergism with spirotetramat does not show increase in biological efficiency. Surviving aphids and spider mites can be controlled by aphidopahagous and acarophagous predators thanks to selective effect of Rock Effect New.

Key words. Damson hop aphid, two-spotted spider mite, Rock Effect New, *Pongamia pinnata*, botanical pesticides, synergism, spirotetramat, selective effect, predators

Introduction

Hop protection against damson hop aphid and two-spotted spider mite 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 Standing Committee on PAFF. Whereas use of spiroteramat, flonicamid and abamectin is time-limited, the most efficient miticide bifenezate cannot be used any more. Moreover, these pests have many generations during a year and thus there is a real danger of the development of resistance to the applied aphicides and miticides. Last but not least, IPM strategies are getting compulsory for crop growers within EU including hops, meaning that synthetic pesticides should be used only if there is no other option, it means if there are not adequate substitution. Therefore, it is necessary to look after efficient alternative strategies to be able to cope up with these dangerous pests (VOSTŘEL 2021).

Insecticides derived from naturally occurring microorganisms and natural insecticides are also important tools, which play still larger roles also in non-organic production (O'NEAL et al. 2015). Essential oils from *Foeniculum vulgare*, *Mentha piperita*, *M. pulegium*, *Ocimum basilicum* and *Pimpinella anisum* have shown very good efficacy in both contact and fumigation tests and can thus be considered an optimal source of active substances for the development of botanical insecticides against aphids (IKBAL & PAVELA 2019).

Material and methods

Rock Effect New (*Pongamia pinnata*) was applied to control damson-hop aphid (Tršice) and two spotted spider mite (Kněževes) in 2022. The trial aimed at the control of damson hop aphid was started on June 06th, the second application was carried out on June 13th. In both cases REN was applied in 1.5 % concentration by motor back sprayer Stihl SG 400. Together 128 hop plants (crowns) were treated in four replicates (each containing 32 plants).

There were four rows within each replicate and only the plants in two inner ones were assessed. Samples of 50 leaves were taken during each assessment (25 from the top of the bines, 13 from the middle and 12 from the lower parts of the bines). The evaluations of the number of aphids were carried out 3, 7, 14, 21 and 28 days after the first treatment. Numbers of aphids were compared to non-treated plot and the plot treated with the reference product Teppeki (flonicamid), which contained the same number of hop plants. At the time of the establishment of the trial the medium number of aphids counted 14.6 ex./leaf.

The field trial aimed at the control of two-spotted spider mite was started on 3 August, the second spray was made one week later (10 August). REN was applied in 1.0 and 1.5 % concentrations. The mode of application, the number of treated plants and the intervals of the checking were the same as in the case of damson hop aphid. Samples of 30 leaves were taken during each assessment (10 from the top, 10 from the middle and 10 from the lower parts of the bines). Numbers of spider mites were compared to non-treated plot, which contained the same number of hop plants. At the time of the establishment of the trial the medium number of mites counted 11.9 ex./leaf. Movento 100 SC (spirotetramat) applied in the registered rate of 1.5 l/ha and in the rate of 0.3 l/ha was used as the reference product. Mixture of Movento 100 SC (0.3 l/ha) and REN (1.0 % concentration) was used to find out if there is a synergic effect. Both aphids and spider mites were counted by a binocular in the laboratory.

Results

Increase of population density of aphids in non-treated plot is obvious only three days after treatment (Figure 1). Later decrease in numbers of aphids was observed, which was caused by numerous aphidophagous predators, especially harlequin lady bird, *Harmonia axyridis*. Biological efficacy of REN applied in 1.5 % concentration can be seen in Table 1. The difference between both applications is visible. If the repeated treatment was carried out the increase in *P. humuli* mortality amounted to 25 % (89.0, resp. 64.1 %) in the time of the last assessment, 28 days after the first treatment.

On the contrary to the trial with aphids in Tršice hop growing region, predators were not numerous enough so as to be able to cause the decrease of population density of spider mites in the trial in Kněževes (Žatec hop growing region). Number of spider mites increased steadily during the trial and 28 days after treatment it reached 90 ex./leaf (Figure 2). Even though acarophagous predators were not as efficient like the aphidophagous ones, they were observed during each assessment not only in non-treated plot but in the plots treated with REN as well. Larvae of the lady bird *Stethorus punctillum* were dominant among the predators, followed by staphylinid beetle *Oligota* spp. and acarophagous gall midge *Feltiella acarisuga.* Predatory bugs of the family Anthocoridae were less numerous.

Table 1. Biological efficiency of Rock Effect New (REN) and flonicamid (Teppeki) on damson hop aphid, Tršice 2022

PPP	Rate	Biological efficiency in % (number of days after treatment)				
		3	7	14	21	28
Non-treated		0.0	0.0	0.0	0.0	0.0
Teppeki	180.0 g/ha	95.1	96.3	98.3	98.8	99.2
REN (1x)	1.5 l/ha	50.0	75.5	70.9	67.5	64.1
REN (2x)	1.5 l/ha	50.0	75.5	90.3	89.4	89.0

Biological efficiency of REN on *T. urticae* is shown in Table 2. Similarly, to the field trial with aphids, also in this trial there is a big difference (> 30 % in the last assessment) between the plots treated only once and those ones treated twice. On the other hand, the difference in *T. urticae* mortality is surprisingly low if we compare 1.0 and 1.5 % concentration of REN in both one and repeated applications. Biological efficacy of the mixture of REN (1.0 %) and Movento 100 SC (0.3 l/ha) is insufficient.

Table 2. Biological efficiency of REN and spirotetramat (Movento 100 SC) on two-spotted spider mite, Kněževes 2022

PPP	Concentration resp. rate/ha	Biological efficiency in % (number of days after treatment)				
		3	7	14	21	28
Non-treated		0.0	0.0	0.0	0.0	0.0
REN (1x)	1.0 %	44.6	49.5	57.2	58.5	52.2
REN (1x)	1.5 %	49.7	53.2	62.3	64.6	56.8
REN (2x)	1.0 %	42.1	51.2	89.7	87.4	86.9
REN (2x)	1.5 %	48.7	52.9	92.6	89.1	89.0
REN (1x) x Movento	1.0 %, 0.3 l	54.4	59.9	68.4	74.2	73.7
Movento	1.5 l	84.6	98.9	98.5	97.7	97.3
Movento	0.3 l	46.2	46.5	57.9	56.4	55.1

Discussion

Repeated applications of the extract from *Sapponaria offcinalis* were found to significantly reduce the numbers of two-spotted spider mites on tomato and cucumber leaves, and their counts remained significantly lower compared to untreated plants throughout the observation period of 140 days (PAVELA 2017). Similarly, to this trial, on the base of above-mentioned results, we can conclude that Rock Effect New is efficient enough to control both damson hop aphid and two spotted spider mite if applied twice in seven days' intervals. Surviving aphids and spider mites can be controlled by predators, which are not killed by REN. No symptoms of phytotoxicity were observed during the trial. No synergism between REN and spirotetramat was confirmed.

Acknowledgement

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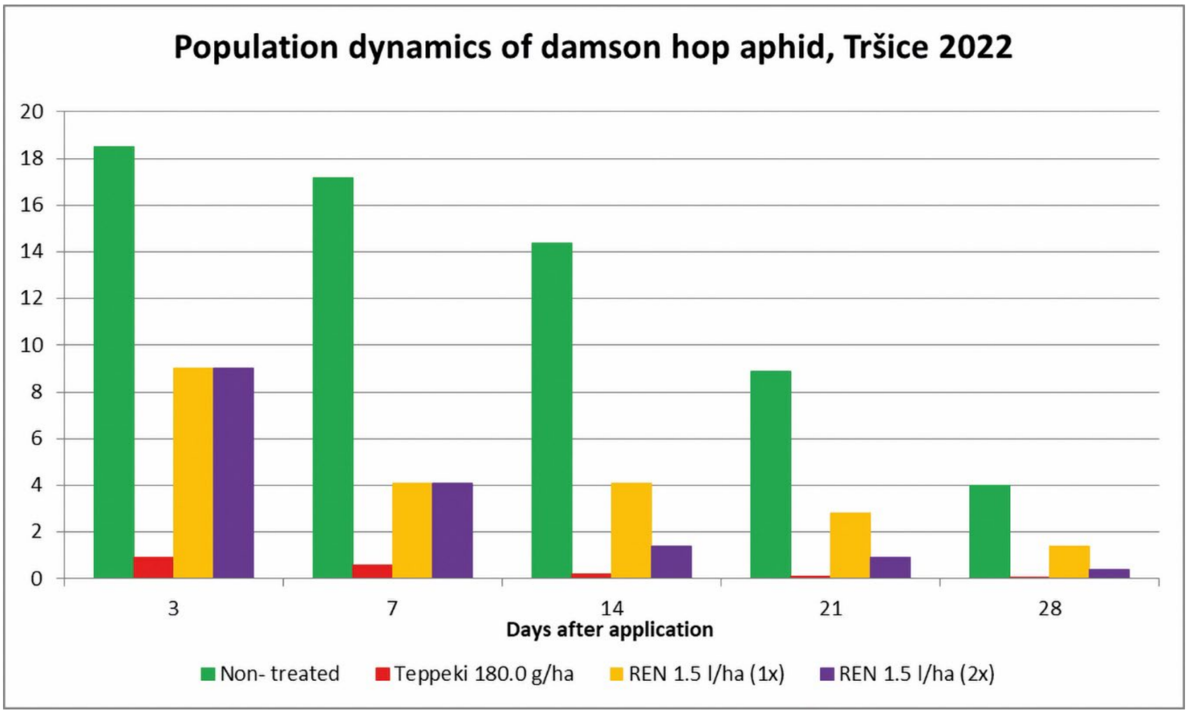

Figure 1. Population dynamics of damson hop aphid, Tršice 2022

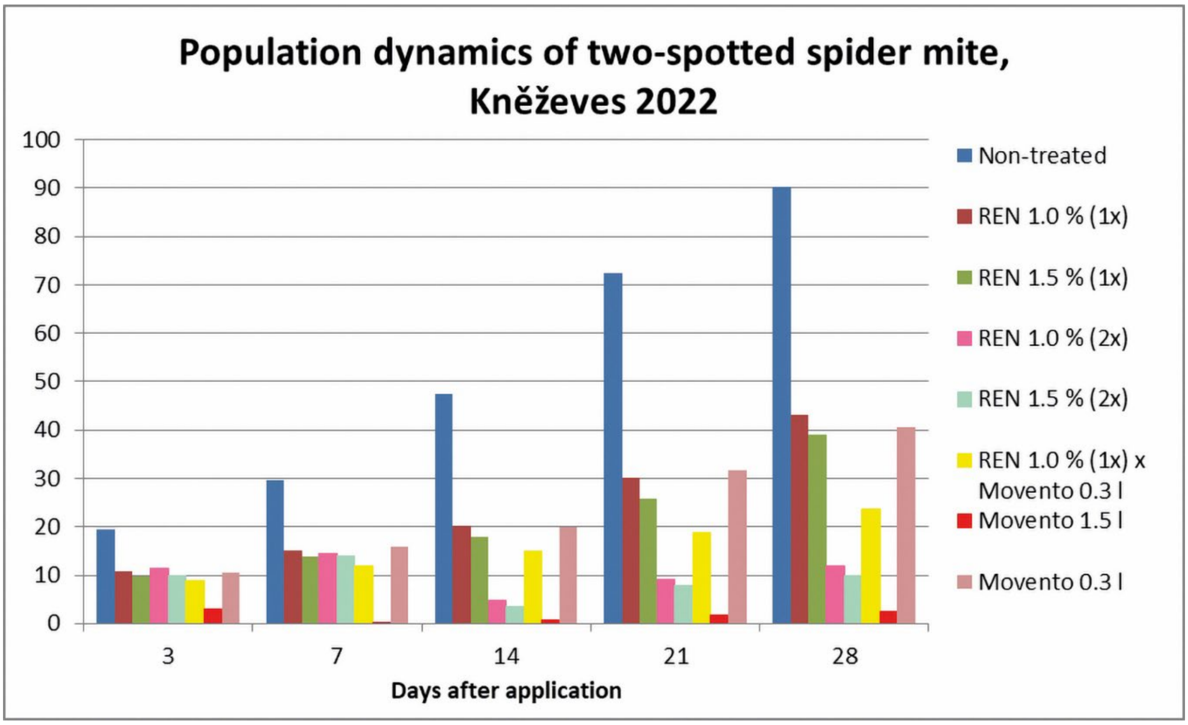

Figure 2. Population dynamics of two-spotted spider mite, Kněževes 2022

Is the reproductive performance of female European corn borer, *Ostrinia nubilalis*, a useful method in biological control?

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Abstract

In Slovenia, the European corn borer (ECB), *Ostrinia nubilalis* (Hübner) (Lepidoptera, Crambidae) is an important pest causing economic damage mainly to hops (*Humulus lupulus* L.) and corn (*Zea mays* L.) but is also often found on many other vegetables and ornamental plants. In Slovenia, ECB has two generations per year on hops, with the larvae of the first generation causing the most damage to hop stems and the larvae of the second generation damaging leaf stems and cones. Hop plants can be completely destroyed, especially in dry and hot seasons (RAK CIZEJ et al. 2012). ECB causes direct damage by piercing of different parts of the plant and indirect damage, as in the part where the larvae are active, *e.g.*, on the panicle, fungi are installed, which can also produce mycotoxins.

Knowledge about ECB biology is very important for Integrated Pest Management (IPM) and even more so for biological crop protection. In general, IPM emphasizes the selection, integration, and implementation of complementary pest management tactics to maintain pests at economically acceptable levels while minimizing the negative ecological and social impacts of management activities. For a successful integration in ECB control, the following parameters are very important: the size of the ECB population, the timing of egg-laying, the emergence of the first larvae, and the occurrence and frequency of mating, as measured by the presence of spermatophores in the female.

The most effective method for monitoring ECB adults is the use of the classical light trap, with which both males and females can be caught. In hop gardens or corn fields it is difficult to find eggs laid by ECB females, especially in the early stage of the season when the adult population is still low. The technique of determining the presence and status of spermatophores in females can help. Egg-laying or the appearance of immature stages should be monitored along with adult activity. In this regard, population levels can be measured precisely by the presence of spermatophores in the bursa copulatrix of females, which is a good indicator of ECB mating activity (FADAMIRO & BAKER 1999). Dissecting ECB females to count spermatophores, as an estimate of mating reduction, is a more direct method than examining oviposition.

The purpose of the present study was to test whether the reproductive performance of ECB females by checking the presence of spermatophores is a useful method in IPM control. A laboratory method was preliminarily introduced to verify the mating activity of ECB females and the time when the first larvae are expected to appear. In the future, it will be tested whether the proposed method is useful in practice, especially for the placement of interventions with *Bacillus thuringiensis* products against the early stages of ECB larvae.

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Final adjustments for the technical application of predatory mites in hops

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Abstract

In 2021 and 2022, first prototypes world-wide for the release of predatory mites to control two-spotted spider mites *Tetranychus urticae* Koch (TSSM) were developed by Koppert in The Netherlands and tested in field trials in the Bavarian Hallertau. Released predators were a mix of *Phytoseiulus persimilis* Athias-Henriot and *Neoseiulus californicus* (McGregor), and a release of *P. persimilis* alone. The new application method was compared with a targeted application via bean leaves and an untreated control. At harvest, all four treatments had again lower TSSM numbers than the untreated control. In general, TSSM pressure was low in 2022 and an experimental harvest showed only a significant difference in alpha acid yield between control and the technically released mix. It became also clear that the mixture of both predators achieved better results than *P. persimilis* alone. The technical release approach, which was not satisfactory yet in 2021 alone was improved and adjusted to a more targeted release in 2022. In future trials the fine tuning of ther method will be improved – an according trial is already running in 2023.

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

Introduction

Two-spotted spider mite *Tetranychus urticae* Koch (TSSM) is the most prevalent arthropod pest of hops world-wide. Spider mites are generally controlled in every hop-growing region by preventative, often multiple, spraying of acaricides. However, in Integrated Pest Management a biological approach to TSSM control is priority and suitable solutions are badly needed. Predatory mites such as *Typhlodromus pyri* Koch, *Phytoseiulus persimilis* Athias-Henriot or *Neoseiulus californicus* (McGregor) can 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 2003; OBERMAIER & WEIHRAUCH 2019). However, one obstacle for the implement-tation 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. First results of a technical solution for the release of predatory mites in hops were presented by WEIHRAUCH et al. (2022). In this follow-up study, we continued this approach with a slightly modified technical set-up.

Material & methods

The study is the result of 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. A first prototype for the technical application of predatory mites (Fig. 1) was developed and assembled by Koppert B.V. in The Netherlands in 2021

(WEIHRAUCH et al. 2022). Field trials were conducted 2021 and 2022 in the Bavarian Hallertau in a farmer's own field (cv. Herkules), in Dürnwind in the district of Landshut. Experimental layout 2022 consisted of four treatments: (i) Mix of predatory mites (*P. persimilis* and *N. californicus*) released on bean leaves, on which they had been bred (31 May), (ii) mix of predatory mites released with the Koppert prototype (2 May), (iii) *P. persimilis* solo, released with the Koppert prototype (2 May), 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. Monitoring of TSSM and predatory mites was conducted four times during the field season (2 June, 28 June, 27 July, 23 August 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 once with an acaricide (spirotetramat) during the 2022 field season.

Figure 1. The second Koppert prototype for the early release of predatory mites in a hop garden. Dürnwind, Hallertau, Germany; Photo: FW (02 May 2022)

Results

The TSSM numbers recorded in the four plots were generally at very low levels until late July and only the last monitoring, on 23 August, yielded noteworthy values with more than 60 adult individuals per leaf in the untreated control. Treatments (i) and (iii) showed significantly lower infestation levels briefly before harvest, reaching less than 30 and 40 adult mites per leaf, respectively, on average (Fig. 2).

During the experimental harvest, we recorded a significant difference in alpha-acid yield between the treatments with a technical release of the mix of predatory mites and the untreated control. All other treatments, including the farmer's own practice with one acaricide spray, were not statistically different (Fig. 3). TSSM numbers in both variants with release of the mix of predators were considerably lower late in the season than in the variant where *P. persimilis* was released alone.

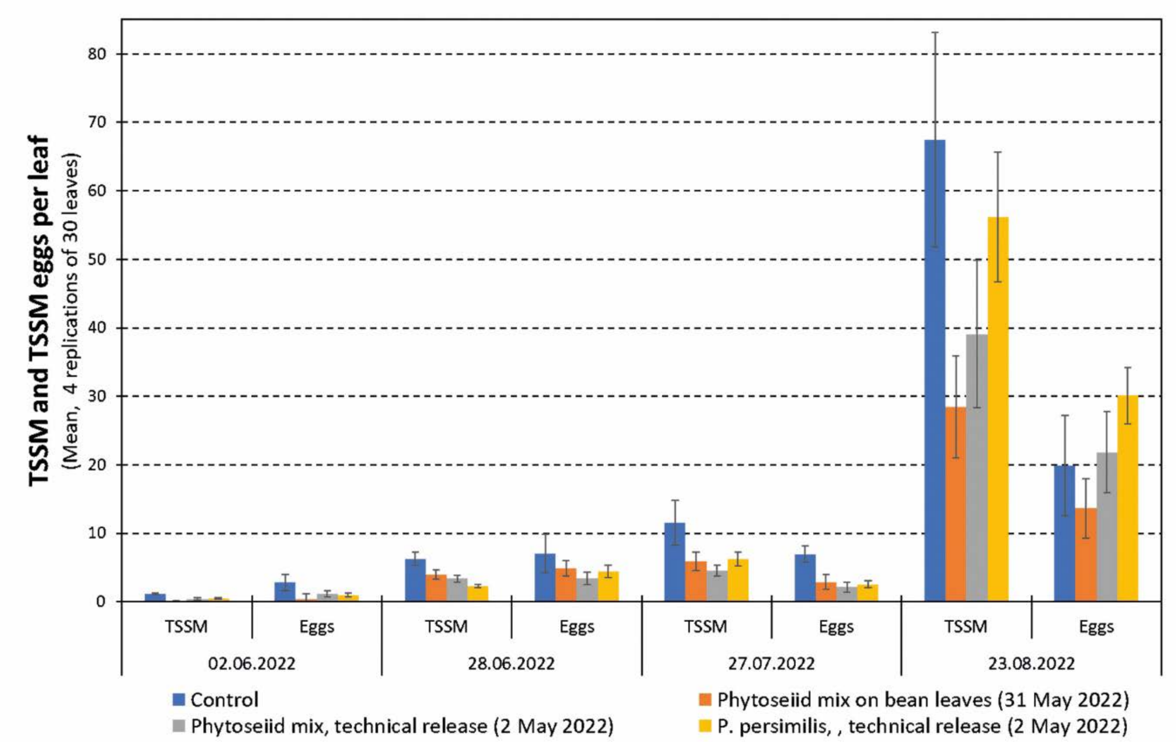

Figure 2. Effect of various release methods of phytoseiid mites (Mix of *Neoseiulus californicus* and *Phytoseiulus persimilis* vs *P. persimilis* solo) on the development of *Tetranychus urticae* (TSSM) in a hop garden. Dürnwind, Hallertau, Germany, 2022, cv. HKS

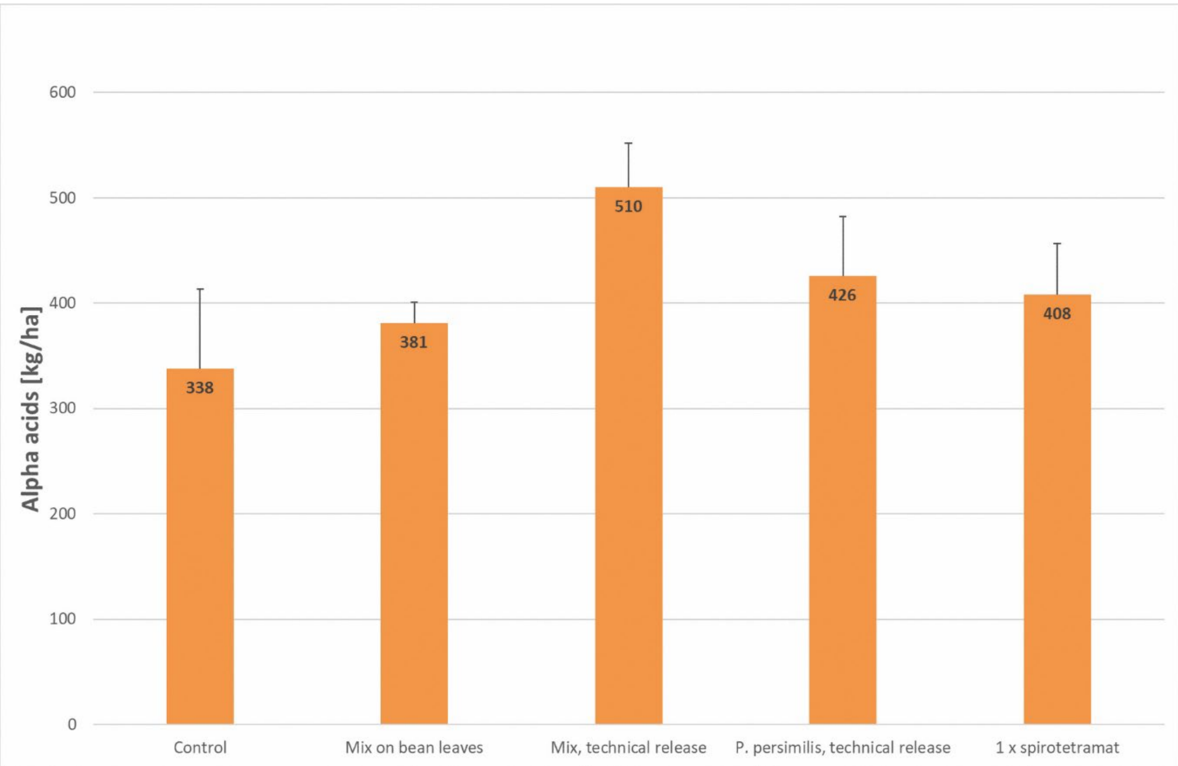

Figure 3. Effect of various release methods of phytoseiid mites (Mix of *Neoseiulus californicus* and *Phytoseiulus persimilis* vs *P. persimilis* solo) for the control of TSSM on alpha acid yield of harvested hops, as compared to the farmer's own practice (one spray of spirotetramat). Dürnwind, Hallertau, Germany, cv. HKS, experimental harvest on 12 September 2022.

Discussion

Generally, the results of the 2022 trial confirmed the positive results from 2021. Still, TSSM infestation during that year was again low. However, the 2022 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 technically is essential; a well-working release method with a tractor might also facilitate the future acceptance of hop growers towards working with beneficials. In 2023, a third, slightly modified prototype has already been developed by Koppert (Fig. 4) and was used for the release of predatory mites at a similar early phenological stage as in 2022. In 2023 this technical release method was already applied in altogether 54 ha of organic hops in Germany (Elbe-Saale, Hallertau, and Tettnang). If successful in organic hops, the technical release method could quickly be adopted by conventional growers as well.

Figure 4. The third Koppert prototype for the early release of predatory mites in a hop garden. Eichelberg, Hallertau, Germany; Photo: FW (17 May 2023)

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Herbivore-induced resistance of hop plants against spider mites – state of play

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Abstract

The two-spotted spider mite, *Tetranychus urticae*, is a polyphagous pest that attacks around 90 different crops in Germany, including hops. The damage pattern designation in hops is also known as “copper browning”. Heavily infested leaves desiccate and turn grey or copper-brown and eventually fall off. Spider mites can build up very large populations in a short time during dry, hot summers and can sometimes cause enormous losses in hop quality and yield.

Observations in recent years from various plant protection trials in the Hallertau have shown that after surviving a severe spider mite infestation, hop plants are capable to defend themselves against excessive spider mite infestation in subsequent years. The objective of this study is to investigate whether and to what extent one or two years of heavy infestation of hop plants with spider mites reduces the susceptibility of these plants to spider mites through induced resistance in subsequent years.

Therefore, field trials are being conducted on four hop cultivars: Hallertauer Tradition (HTR), Spalter Select (SSE), Tettnanger (TET) and Herkules (HKS), in 31 hop gardens (5-10 gardens per cultivar). Each experimental garden contains two plots of 500 m². One control plot, in which the spider mite can develop freely without the use of pesticides, and one practice plot, which is treated with an acaricide at least once and is as free of spider mites as possible. In the center of both plots, leaves are regularly taken and monitored for spider mites. In the hop gardens with the biggest difference in spider mite infestation between the plots, an experimental harvest of both plots is carried out at the end of the season. Yield per hectare, alpha-acid levels, and cone quality are determined and analyzed for possible differences between control and practice plot. In 2022, all hop gardens were monitored 4-5 times and two experimental harvests per cultivar were carried out for HTR, SSE and HKS, and one harvest of TET.

The year 2022 was ideal for spider mites, due to the persistent drought and heat, it was able to multiply rapidly and even reach a pest index around 4 in the control plots of the HKS hop gardens to be harvested by the end of the season. Nevertheless, in HKS, neither yield per hectare nor alpha-acid levels were affected by the heavy infestation. This was different in the harvested HTR hop gardens. Despite less infestation than HKS, the yield of the control plots was significantly lower than in the practice plots. The same was observed in one of the two harvested SSE hop gardens. The second SSE hop garden showed no impairment from spider mite infestation, and there were no differences between control and practice plot for cv. TET.

The harvested practice plots were not completely free of spider mites and thus cone quality suffered in all experimental gardens due to the strong spider mite pressure of the year, in both the practice and control plots.

IV: Cultivation and management of hops

Hop field variability: soil properties (Galicia, Spain)

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Abstract

Determination of spatial variability in soil composition is key to obtaining a sustainable hop production. For its evaluation, a random soil sampling was carried out in 2022, in a hop plot (cv. Nugget) located in Galicia (Spain), analyzing in the laboratory the classic soil parameters (pH, OM, $_{e}$CEC, P, K, etc.). In addition, the soil apparent electrical conductivity was determined at two depths (0.75 and 1.50 m). The results show the existence of a good correlation with EC_{a-H} in many of the parameters (*rho* > 0.40), highlighting the relationships with the OM and the $_{e}$CEC, which allows monitoring their evolution, without the need for soil sampling. Knowledge of the spatial variability of the soil allows for differential fertilization, correcting deficiencies/excesses that may generate unbalanced production. Non-destructive methods such as the study of EC_a, represent a competitive advantage, in terms of cost and time, compared to laboratory soil analyses, although calibration is required for each plot.

Key words. Organic matter, soil apparent electrical conductivity, soil nutrition, Nugget

Introduction

The irregularity of the intra-plot production in the cultivation of hops is due to a multitude of parameters. However, it is well-known that soil characteristics is one of the key aspects to obtain a homogeneous production (CANCELA et al. 2022). The existence of rapid, non-destructive measurements, such as the soil apparent electrical conductivity (EC_a), open up the possibility of obtaining a rapid and low-cost characterization allowing efficient handling of differential fertilization. Soil quality is an essential factor in the cultivation of hops (BIENDL et al. 2015). General studies have some relevance of focus for the fertilization of nitrogen, phosphorus, potassium, and magnesium as key macronutrients (NEVE 1991). Hop nutrient requirements vary depending on soil testing, cultivars, yield potential, and growing region. In this context, the aims of this research are i) to determine the soil property variability in a characteristic hop plot (cv. Nugget) in Galicia, and ii) to analyze the relationships between EC_a and the main physical-chemical parameters of the soil.

Material and methods

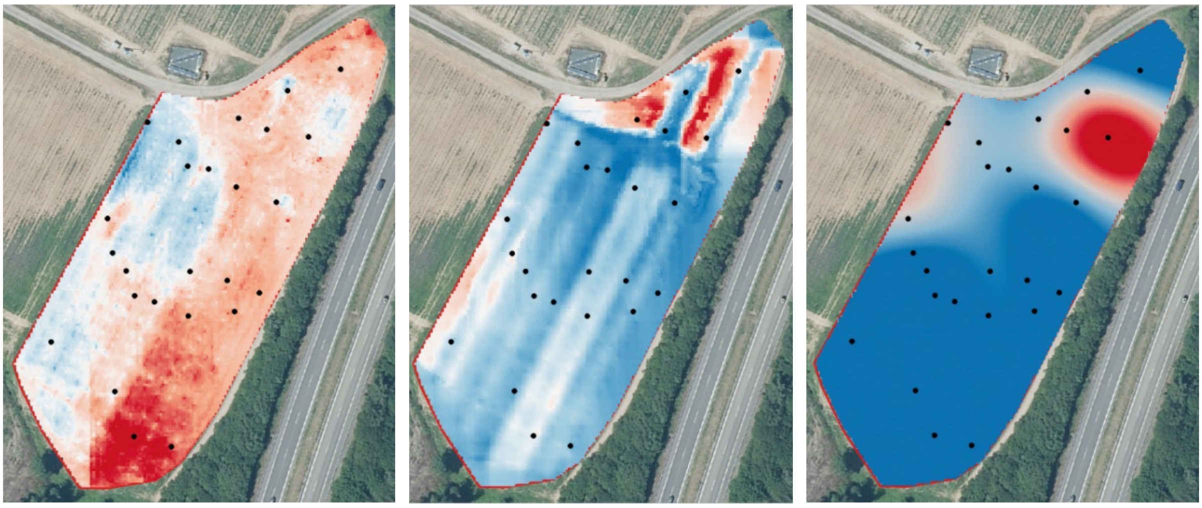
During the 2022 season, soil samples were taken from the 'Borreiros' plot, Abegondo (A Coruña, Spain), planted in 2008 and managed conventionally, with plants spaced 1.5 m within and 3 m between rows, making a total of 2222 plants per hectare (43˚13.5´ N, 8˚16.5´ W, 84 m a.s.l.). The soil is developed over a basic underlying schist layer, referred to as 'Ordenes Complejo'. The soil is classified as Cambisol (IUSS 2014), without relevant changes in the first meter of soil depth.

The annual plot fertilization program is based on annual extractions and includes the application of 400 kg ha^{-1} of nitrogen-phosphorus-potassium (NPK; 8:15:15) in March and 250 kg ha^{-1} of calcium ammonium nitrate (27 %) split into two applications (June and July). The crop annual extractions were determined based on the work carried out at the CIAM-Galicia over the last 20 years (MAGADÁN et al. 2011).

Soil apparent electrical conductivity (EC_a) data were measured on May 6, 2022, at 25 % of soil water content, near field capacity level, using an electromagnetic induction sensor (EM-38DD, Geonics Ltd., Mississauga, ON, Canada). Normally, the depth range is 1.5 m or 0.75 m, respectively, when the vertical or horizontal dipole is used. In our study, simultaneous measures of the apparent electrical conductivity (EC_a) in the horizontal ($EC_{a\text{-}H}$) and vertical ($EC_{a\text{-}V}$) dipoles were determined; for a homogeneous terrain, this reaches 70 % of the response at the upper 1.5 m in the vertical dipole, and at the upper 0.75 m in the horizontal dipole. A GNSS-RTK receiver (Hi-Target V200, Guangzhou, China) was used to find the 25 measurement points (Figure 1). Soil samples were taken from the surface horizon (0–40 cm depth) in the row for physico-chemical analysis. Samples were oven dried (40 °C) and sieved to 2.00 mm to separate the fine soil fraction. Soil physical and chemical properties were determined according to standard methods (Tan 1996). More detail about soil measures are included in Cancela et al. (2022).

Figure 1. Global location of the 'Borreiros' plot in Galicia, Spain (left), and location of the analyzed sampling points in the field (black dots; right).

A descriptive analysis of the different variables determined in the soil analysis was carried out (mean, median, standard deviation, variance, minimum, maximum, skewness, and kurtosis), including a data normality analysis (Shapiro–Wilk test), for assessing a central trend and data spread. $EC_{a\text{-}H}$ and $EC_{a\text{-}V}$, and the relationships between EC_a (both $EC_{a\text{-}H}$ and $EC_{a\text{-}V}$) and soil physico-chemical properties were assessed through the Spearman correlation coefficient, *rho* (Mirás-Avalos et al. 2020). The spatial distribution of EC_a over the study plot was evaluated via deterministic and geostatistical methods using the GSTAT software for R (Pebesma & Graeler 2019), and the estimated values of EC_a were obtained via the Random Forest regression kriging technique (RK-RF), using the GSIF package for R (Hengl et al. 2019), and mapping with QGIS v3.8 (https://qgis.org). The extraction of the median, maximum, and minimum values of EC_a (EC_a median, EC_a max, and EC_a min) was carried out considering a buffer of 2 m, using the final EC_a maps for both dipoles, and using the 'zonal statistics' command (QGIS v3.8, https://qgis.org). Mapping was carried out using regularized spline with tension, which performs surface interpolation from a vector points map using splines (Mitášová & Mitáš 1993). These maps were implemented in the geographical information system (QGIS v3.8, https://qgis.org) using the command: 'v.surf.rst'.



a) b) c)

Figure 2. Soil apparent electrical conductivity (EC_a, mS m^{-1}) maps obtained with the horizontal (a) and vertical (b) dipoles of the EM-38 equipment, and interpolated through the Random Forest Regression Kriging technique (RK-RF); and organic matter (%, c), map obtained with QGis-'v.surf.rst'. Sampling points are represented by black dots.

Results

The EC_a variation, both vertical and horizontal dipole, is greater vertically (variance >35) than horizontally (variance <5; Table 1, Figs 2a, 2b). The spatial distribution of EC_a variability shows different spatial patterns, depending on whether the data are analyzed at the surface ($EC_{a\text{-}H}$, Fig. 2a) or at depth ($EC_{a\text{-}V}$, Fig. 2b). Similar patterns were obtained by CANCELA et al. (2022). In the eastern part of the plot, the $EC_{a\text{-}H}$ values were slightly higher than the rest of the plot, corresponding to the areas with the highest vegetative development (data not shown).

Final results show that soil at the site was loam textured, with 31 %, 45 %, and 23 % sand, silt, and clay, respectively (Table 1). The soil organic matter content was 4.5% on average. The average effective cationic exchange capacity ($_e$CEC) was 9.94 (cmol (+) kg^{-1}). In global terms a higher variability of properties was obtained, as shown in Figure 2c, to organic matter (%). However, several positive or negative correlation were obtained between $EC_{a\text{-}H}$ or $EC_{a\text{-}V}$ and soil parameters, allowing us to determine the soil parameters using soil apparent electrical conductivity as a predictor variable.

Discussion

EC_a as a non-invasive technique might provide useful information for hop yard zoning. Positive significant were found with the EC_a (horizontal dipole) for pH, C, N, OM, and $_e$CEC, (*rho* > 0.40) which suggests that the determination of the EC_a can help to evaluate the nutritional status (N, OM and $_e$CEC) of the soil at a lower cost. However, the lack of correlation of the EC_a with parameters such as the percentage of aluminum saturation and P should be based on classical soil analysis. The correlations obtained are similar to those shown by CANCELA et al. (2022), so the study technique of variability in hop plots, on acid soils, can be applied reliably. Precautions must be taken, regarding the level of soil water available, at the time of data collection, to avoid deviations in the EC_a values, derived from the change in soil water content, as compared with the starting point, collected in this study.

Table 1. Statistical summary of the soil properties studied. SD: standard deviation; CV: coefficient of variation (%); Min.: minimum; Max.: maximum; eCEC: effective cation exchange capacity; Al Sat.: aluminum at saturation; ECss: electrical conductivity in the soil solution; ECa-H: soil apparent electrical conductivity in the horizontal dipole; ECa-V: soil apparent electrical conductivity in the vertical dipole.

Variable	Units	Mean	SD	Variance	Min.	Max.	Skewness	Kurtosis
pH (H_2O)	-	5.33	0.42	0.18	4.78	6.37	0.873	0.359
pH (KCl)		4.65	0.49	0.24	3.94	5.81	0.891	0.342
Ca	cmol(+) kg^{-1}	6.81	2.56	6.56	3.34	13.01	0.927	0.732
Mg		1.36	0.46	0.21	0.46	2.46	0.592	0.660
Na		0.27	0.08	0.01	0.15	0.49	0.843	1.455
K		0.88	0.37	0.14	0.15	1.70	0.409	0.115
Al		0.62	0.64	0.41	0.01	1.92	0.966	-0.489
eCEC		9.94	2.37	5.62	6.93	16.26	1.215	1.467
Al Sat.	%	7.60	8.66	74.97	0.07	27.72	1.150	0.007
P	mg kg^{-1}	67.32	19.54	382,00	35.18	102.53	-0.192	-1.009
C	%	2.60	0.90	0.80	1.55	5.33	1.373	2.203
N		0.28	0.09	0.01	0.17	0.53	1.278	1.801
Organic matter		4.49	1.54	2.39	2.68	9.20	1.379	2.235
Coarse fraction		40.82	8.62	74.37	20.2	52.5	-0.802	0.020
Fine fraction		59.18	8.62	74.37	47.5	79.8	0.802	0.020
Sand		31.28	3.18	10.13	26.0	37.0	0.304	-0.774
Silt		45.36	2.87	8.24	40.0	52.0	0.341	-0.213
Clay		23.40	1.87	3.50	20.0	27.0	-0.266	-0.653
C / N	-	9.37	0.76	0.58	7.10	10.63	-0.847	2.129
K / Mg		0.77	0.49	0.24	0.06	2.45	1.688	4.639
Ca / Mg		5.31	2.09	4.36	2.84	10.46	1.202	0.387
EC_{ss}	mS m^{-1}	19,69	6,46	-	11,01	32,50	0.807	-0.201
$EC_{a\text{-}H}$ median		9.98	2.05	4.21	6.14	16.00	0.904	2.203
$EC_{a\text{-}H}$ min		9.34	2.11	4.46	5.40	15.58	0.762	2.145
$EC_{a\text{-}H}$ max		10.69	2.24	5.00	6.81	17.90	1.315	3.892
$EC_{a\text{-}V}$ median		14.66	6.35	40.31	7.03	34.46	*1.303*	*2.497*
$EC_{a\text{-}V}$ min		12.60	5.94	35.25	4.74	30.02	1.028	1.657
$EC_{a\text{-}V}$ max		16.61	7.04	49.51	7.33	36.05	1.051	1.087

Acknowledgement

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Influence of different cover cropping systems in hops on humus balance and soil physics

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Abstract

The aim of this work was to analyse the influence of the perennial cover crop, regarding to various soil physics and humus content parameters, in comparison with a one-year cover crop. Based on these results, possibilities are to be identified as to how the concept of perennial cover crops in hops can be improved and developed further.

Key words. Perennial cover crop, soil structure, humus balance

Introduction

The influence of climate change can be observed in all areas of agriculture, including hop cultivation. That is why BarthHaas focuses on these influences to ensure the supply of raw materials. One possibility would be irrigation systems, but it will not be possible to cover 100 % of the cultivated hop area soon, especially in the Hallertau region. Therefore, it is becoming increasingly important to improve the soil structure to use it as a water reservoir, sustainably and over a wide area. That is why BarthHaas is doing trials with perennial cover crop mixtures between the hop rows.

Figure 1. Pictures of Roots in the soil of a perennial cover crop

Material and methods

Four different locations in the Hallertau region with different soil conditions were analysed: trial field A (Oberpindhart), field B (Kirchdorf), field C (Sallingberg), field D (Oberulrain). Samples were taken at five measuring points on each location and per variant "conventional" (variant 1) and "perennial cover crop" (variant 2). Two depth levels were considered: 0 – 15 cm and 0 - 30 cm. The samples were analysed in the LfL laboratory in Freising and the data statistically evaluated. In addition, a soil profile was evaluated at two locations.

Results & Discussion

The humus parameters show no clear differences between the variants due to the analysed period of three to four years. For the parameter C_{org}, the following tendencies can be identified, differentiated according to the depth levels. In the upper soil layer, the values are higher in the conventional variant, in the lower soil layer in the cover crop variant. A distinction can be made between the trial fields concerning the N_t content: Trials A and B show higher values in variant 1, trials C and D in variant 2. The C/N ratio as an indicator of the quality and age of the organic matter indicates clear differences between the fields, with values between 3.5 and 10.9. The C/N ratio is higher in the upper soil layer at all trials. At sites A and C, higher ratios are recorded in variant 1 and at sites B and D in variant 2. The C_{org} stocks calculated from the storage density and C_{org} show higher values in the catch crop variant, except for one field.

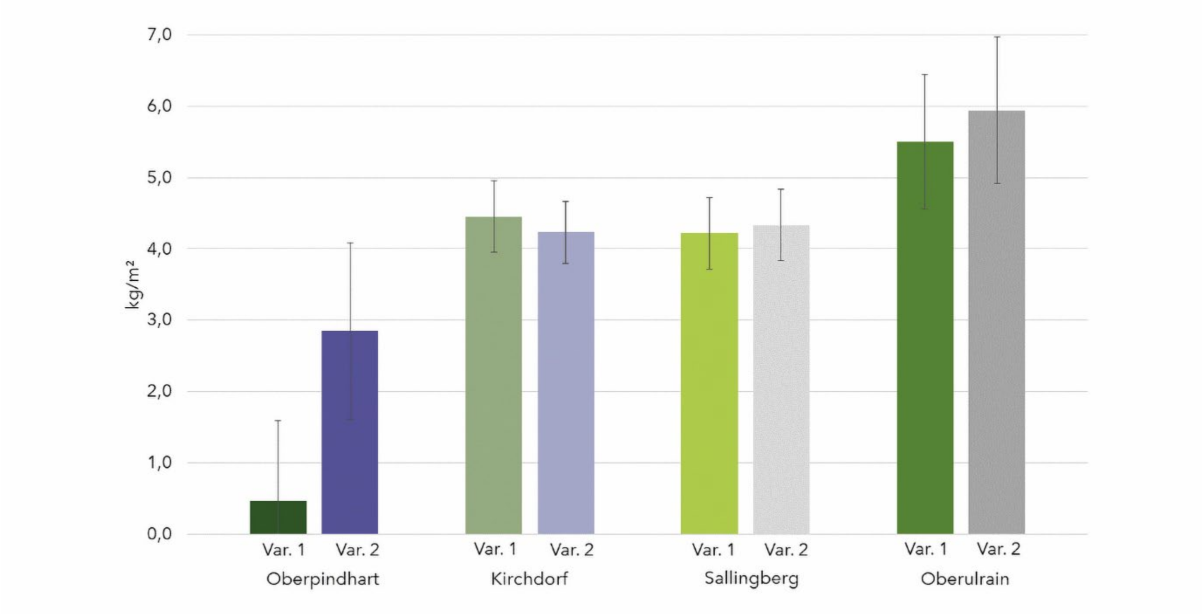

Figure 2. C_{org} stock at the four trial fields analysed at a depth of 0-30 cm

The following differences can be identified in the soil physics parameters. In the case of coarse pore volume, it is not possible to say clearly which variant is better; in general, the values are close to each other between 40 and 54 %. The upper soil layer shows higher values. The values in this layer are somewhat lower in the perennial cover crop variant. In the lower layer the values are a bit higher. The values for dry density are similar, as the two parameters are closely linked. The values in the upper soil layer are somewhat higher in the perennial cover crop variant, *i.e.*, the soil is more densely stored. And in the lower test depth, the values are somewhat lower in variety 2.

The pore distribution at the locations is divided as follows. The values for the particle volume range between 45.9 % and 59.6 %. The results are higher in the deeper soil layer. No clear difference can be found between the variants. The stagnant water percentages in the lower soil layer are smaller in variant 2, apart from location C. The higher usable field capacities are seen at the more clayey fields A and B, while the sandier fields C and D have lower capacities. With a view to the soil layers, there are contrasting results: at the upper measurement depth, the conventional variant is significantly higher, at the lower measurement depth, the cover crop variant.

The values for air capacity range widely across the trials, from 5.55 to 28.86 %. The values are consistently lower at the deeper measurement depth. In terms of the differences between the two variants, the same trend can be seen as with the usable field capacity: in the upper soil layer, the conventional variant shows higher values, in contrast to the perennial cover crop variant in the lower soil layer.

That’s interesting because this is the layer that can't be tilled and it's important to ensure good soil aeration to improve root growth and nutrient uptake by plants.

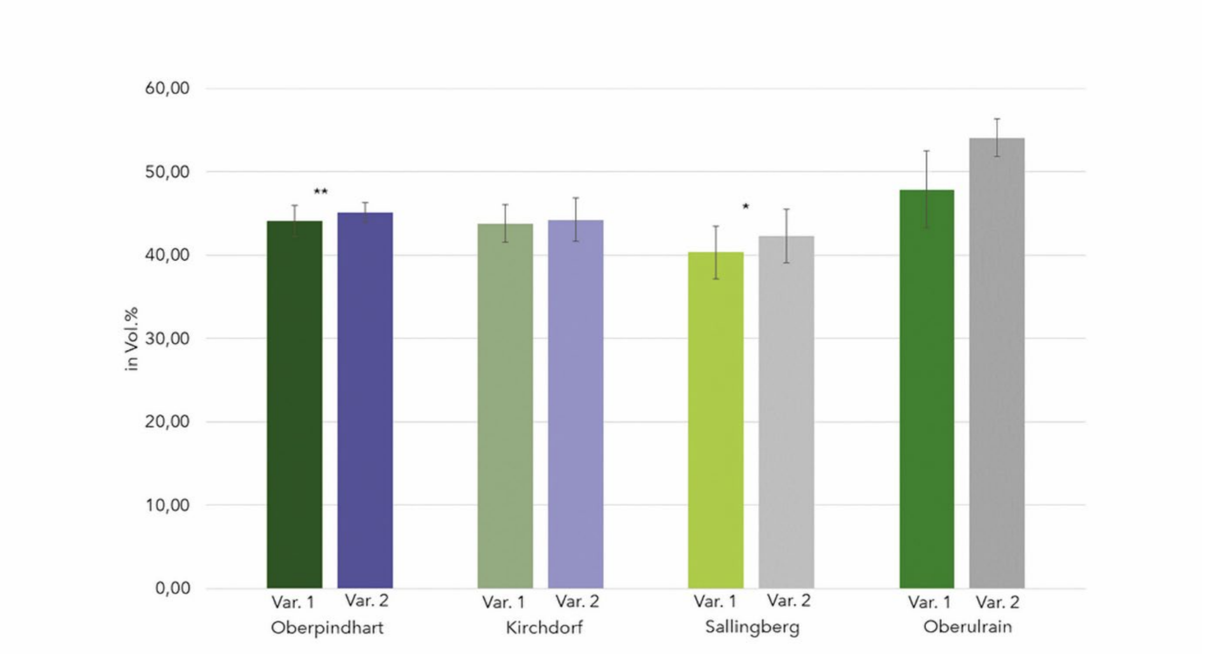

Figure 3. Coarse pore volume at the four trial fields analysed at a depth of 15-30 cm

Acknowledgement

The trials were performed by BarthHaas. The work was supported by the LfL Department of Soil Protection and Soil Fertility in Freising and the University of Applied Sciences Weihenstephan-Triesdorf (HSWT).

Irrigation of hop in the Savinja Valley: Irrigation systems

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Abstract

Hop irrigation is a subject that is gaining a lot of attention as a result of climate change. In the past twenty years Slovenia has experienced six droughts that had a significant impact on agriculture and were classified as natural disasters. In the past twenty years drip irrigation technology studies and their influence on the quantity and quality of the hop crop as well as the development or support of irrigation decision making (irrigation forecasts) have received the most attention in irrigation research. Our recent activities have involved updating, refining, modernizing, and building new irrigation systems in the Savinja Valley where our collaboration with the Ministry of Agriculture and Forestry of Slovenia (MKGP) and the Agriculture and Forestry Institute Celje (KGZS) has been excellent.

First irrigation systems in the Savinja Valley were built in the years from 1986 to 1988. After more than 30 years of operation, these systems were worn out and technologically outdated. In 2018, the MKGP, in the frame of the Rural Development Programme of the Republic of Slovenia for the period 2014–2020, published the 1st public tender for support for investments in infrastructure related to the development, modernization or adaptation of agriculture and forestry. More specifically, the operation supported technological updates of irrigation systems that are intended for multiple users. In this 1st public call, five irrigation systems from the Lower Savinjska Valley (Breg, Gotovlje, Šempeter-Vrbje, Latkova vas and The Slovenian Institute of Hop Research and Brewing, IHPS) applied for the first phase of their modernization. Applications were submitted at the end of August 2018. In December 2021, in the 2nd call, additional three applications were submitted for the first phase of modernization of irrigation systems, namely Novo Celje A, B and C. In 2022, in the 3rd call, seven irrigation systems applied for the second phase of modernization, which included all mentioned irrigation systems from 2018, including the system IHPS and the systems Pod Letušem and Šmatevž. By renewing the irrigation systems, the water consumption was reduced (no losses), pumping efficiency was increased (more efficient pumps) and electricity consumption was reduced (more efficient electric motors, frequency regulators). Now, modernized irrigation systems cover around 1,900 hectares of hops and other agricultural crops in the Lover Savinja Valley.

With this, the conditions for irrigation of hops and other agricultural crops have been established in the Lower Savinjska Valley. However, it is necessary to emphasize that there are still many challenges in the field of irrigation, as it is necessary to update the other irrigation systems that are built but not working. In addition, it is also necessary to build new irrigation systems and ensure enough water to cover irrigation needs.

In recent years, as part of EIP projects and pilot projects from the Rural Development Programme 2014–2020, IHPS established a hop irrigation forecast for a drip irrigation technology as well. Now, our goal is to cover all main irrigation areas with irrigation forecast which will increase the productivity of irrigation water use and will provide significant adaptation potential under future climate change.

Key words: Irrigation systems, hop irrigation, drip irrigation, Savinja Valley

Exploring innovative strategies for the management of hop waste biomass

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Abstract

During the hop harvesting process, the entire aboveground biomass is removed from the fields, leaving behind stems and leaves as a by-product collected next to the harvest halls. This excess hop biomass can reach up to 18 tonnes per hectare (15 tonnes on average) and is often considered waste, despite its potential as a raw material for various applications. The potential uses for waste hop biomass include bio-gas production, composting, biomass for heat production, extraction of antioxidants for use in food and medical products, and fiber production. The latter can be used as an additive in the manufacturing of biodegradable biocomposites as a sustainable alternative to conventional plastic materials.

Circular economy principles can be applied in the narrowest way through the utilization of hop waste biomass as a valuable resource for composting. On-farm composting could be an efficient, cost-effective, and environmentally safe biological process for recycling residual biomass. By returning this material back to the soil in a form of a fine quality organic fertilizer, farmers can enhance the nutrient content and organic matter of the soil, which in turn can lead to more resilient and sustainable agricultural practices. The adoption of circular economy practices can contribute to the long-term sustainability of this industry, while also promoting a more environmentally responsible and socially equitable approach to agricultural production.

Waste hop biomass produced from 1 ha of hop plantation is a source of 21–22 kg P_2O_5, 50–71 kg K_2O, 32–41 kg MgO, 5.5 kg S and 88–99 kg N. The LIFE project BioTHOP has successfully developed also two procedures for the extraction of fibers from waste hop stems, which can be utilized for two distinct purposes. Firstly, these fibers can be incorporated into biodegradable and compostable wine bottle transport packaging, offering a sustainable and environmental-friendly alternative to traditional packaging materials. Secondly, the extracted fibers can serve as an ingredient in the production of biocomposites, which can be employed for the injection of various biodegradable and compostable products. In total, BioTHOP has developed 13 such products, as demonstrated in Figure 1. At the end of their useful life, these products can be subjected to composting, in which they are degraded into water, CO_2, and organic matter. The resulting compost can then be used to return valuable nutrients and organic matter back to agricultural land.

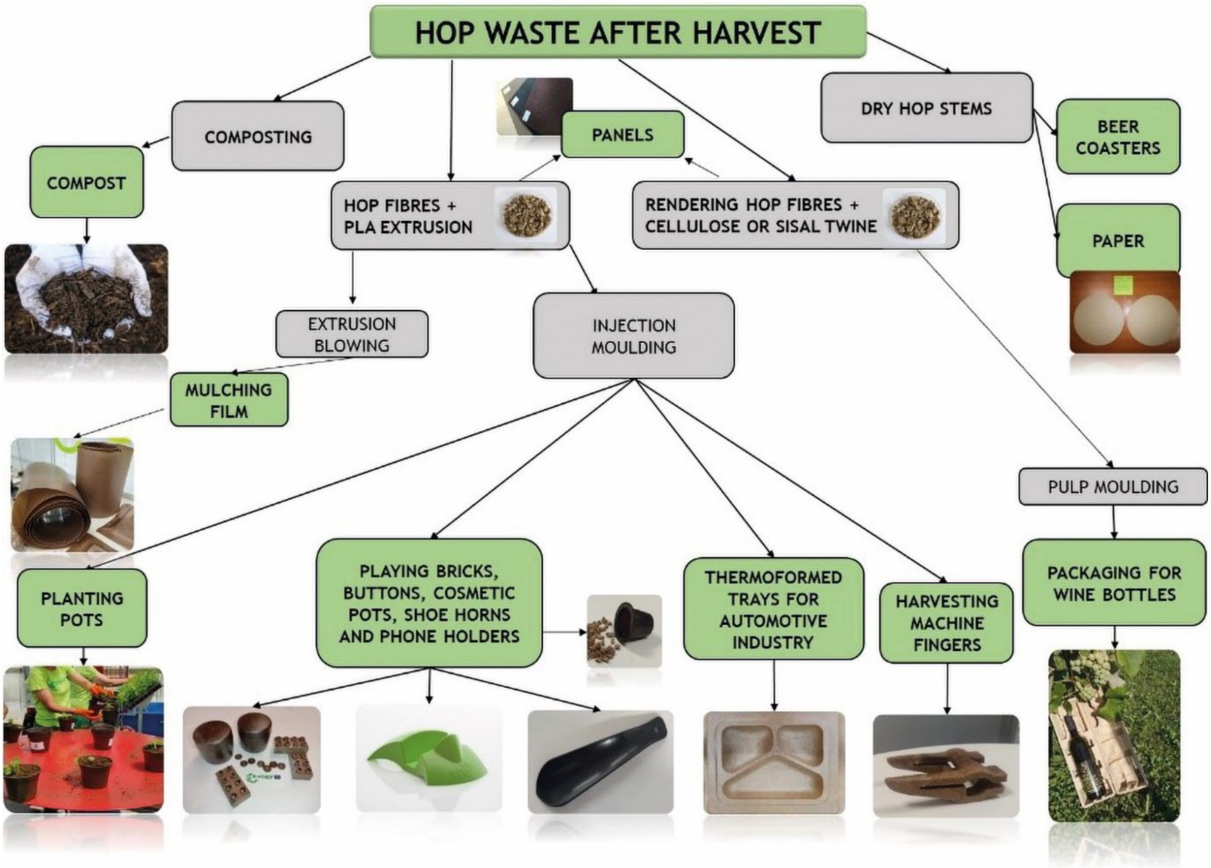

Figure 1. Possible products made from waste hop biomass (BioTHOP examples; https://www.life-biothop.eu/)

Improving hop plant nutrition and yield with plant growth-promoting rhizobacteria

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Abstract

Along with tourism, hop cultivation and brewing are mayor economic activities in the Andean Patagonia region of parallel 42º. Every year it becomes necessary to improve agronomic practices based on a more environmentally friendly management. This research was conducted in hop yards located in El Bolsón (NW Patagonia, Argentina), to further evaluate the product "RISE P" (RP), a root inoculant with a high concentration of the *Bacillus velezensis* strain of rhizosphere bacteria; and on the other hand, to assess three different bioinputs used together (MGx3) based on selected strains of *Azospirillum brasilense*, *Pseudomonas fluorescens* and *Bacillus subtilis*. Trials were proposed in different commercial hop yards, evaluating the impact of plant growth promoting rhizobacteria (PGPR) on field cone yield, and some nutritional parameters measured in leaf tissue at different phenological moments. Rows inoculated with RP had an average cone yield 12.3 % higher than the non-inoculated control rows, while rows inoculated with MGx3 had an average cone yield 13.9 % higher than the non-inoculated control rows. Regarding the analysis of plant tissue, the nitrate concentration was lower in the inoculated samples in both PGPR, while the sulfur, iron, copper, and boron content were higher in both PGPR. Phosphorus, potassium, and magnesium concentration was higher in the RP inoculated samples, but lower values were detected in the MGx3 inoculated samples when compared with the leaf tissue of the control 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, Minas Magri, Patagonia, Argentina

Introduction

In the Andean Patagonia region of parallel 42º (Argentina), there are currently *ca* 146 ha in commercial production. Hop growing 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. The traditional use of fertilizers has been 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, applied as bioinputs 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 soil biology plays a fundamental role in cycling nutrients. 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 traditional models.

In the study region, a decrease in the organic matter content was identified in soils with intensive agricultural use in the last 15 years. Based on this negative trend, in recent years a series of trials with PGPR have been initiated in different hop yards and with different strains of rhizosphere bacteria. 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 certain traditional nutrition practices to work in integrated management. In Argentina there are still very few products with registration enabled for the cultivation of hops.

Lallemand Plant Care business unit (part of 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, the product "RISE P" (RP) stands out, 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 many crops (horticultural, oilseeds, legumes), and for the second consecutive year we have evaluated RP in hops cultivation in Patagonia.

Cía. de Minas Magri y Gallardon S.A. is a family company located in El Hoyo (Chubut province, Argentina), a pioneer and specialized in the production of substrates based on *Carex* peat and bioinputs for agriculture. They carry out research and development of biofungicides, biofertilizers, and biostimulants. Among the main products developed in recent years, we found it interesting to evaluate three different rhizobacteria from selected strains of *Azospirillum brasilense*, *Pseudomonas fluorescens* and *Bacillus subtilis*. The first has been reported in agricultural crops of great economic importance with positive effects in stimulating the production of Indole-3-Acetic Acid and zeatin synthesis as mechanisms to improve plant growth (BRAMBILLA et al. 2022). *Pseudomonas fluorescens* in the plant rhizosphere produces a wide spectrum of bioactive metabolites like antibiotics, siderophores, volatiles, and growth-promoting substances (DAVID et al. 2018). *Bacillus subtilis* in many cultivated species exert a growth-promoting mechanism through the synthesis of root growth regulatory hormones; in addition to its antifungal properties related to the release of enzymes that degrade the cell wall of the fungus, to end up destroying the mycelium of the fungus. There are no records of these 3 different bioinputs (MGx3) used together in hop plants.

Materials and Methods

This research was conducted in 2 different hop yards belonging to “Patagonia Lúpulos Andinos S.R.L.”, located in El Bolsón (Río Negro Province, NW Patagonia, Argentina). This is the largest hop company in Latin America. The family business owns 7 farms, with a total of 85 hectares growing nine commercial hop varieties (3 from USA, 3 from Europe, 1 from Australia and 2 local). Both trials were carried out on sites of different soil quality but with similar design, to evaluate the impact of PGPR in the cone yield.

RP trial site

In the late spring of 2022 (end of November) a field-scale trial was designed and set up in the “Nogales” farm, in an established hop yard cultivated with cv. “Cascade” (>20 years old). The experimental site was a 3.6 ha plot (41°55'48"S 71°31'27"W; elevation 372 m a.s.l.). Plant spacings were 1.0 x 3.0 m (3.333 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 2.0 emitters per plant.

Although El Bolsón is a high-quality site, it is a plot where yield would be below the potential for that variety in that environment since the plants are old and with a certain incidence of Red Crown Rot caused by *Phomopsis tuberivora* (GENT et al. 2013). Soil texture at this site is classified as silt clay loam, and the organic matter content is 8.1 %. According to apparent density measurements (depth= 0-50 cm), water storage capacity was calculated in 225 mm. Soil reaction is slightly acidic to neutral (pH 6.1).

MGx3 trial site

In the late spring of 2022 (December 1st) a field-scale trial was designed and set up in the “Mallín Medio” farm, in another established hop yard cultivated with cv. “Cascade” (>15 years old). The experimental site was a 2.8 ha plot (41°53'56"S 71°30'41"W; elevation 463 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 (prior to 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 (<20 %), and the organic matter content is 4.8 %. According to apparent density measurements and considering a depth of 50 cm, water storage capacity was calculated in 175 mm. Soil reaction is neutral (pH 6.6).

Allophanic soils (derived from volcanic ash and positive to the Fieldes test) are found in most places of this region, so the availability of phosphorus in certain environments is critical, even though it is not a main nutrient for hops. In the fall of 2022, phosphorus measurements (Olsen) from soil samples at the MGx3 trial site showed a value of 22.4 ppm, while the results in the RP trial site yielded a value of 81.1 ppm. The original geologic material, “loess”, ensures an adequate supply of soil potassium at the MGx3 trial site (203.1 ppm) and perhaps exceeded for the RP trial site (445.3 ppm).

Prior to inoculation, treated and control rows were randomly assigned in both plots, recording the row number in each case. The inoculation was carried out in a very easy way, taking advantage of the ferti-irrigation system. The calculated dose of the product RP (2E10 CFU/g of Bacillus amyloliquefaciens IT45 strain as reported by the manufacturer), resulted in 0.06 grams/plant, following the technical advice proposed by Lallemand Plant Care. In the case of MGx3, a dose equivalent to 1.38 milliliter/plant was applied (0.46 milliliter/plant of each product, mixed). The CFU counts were carried out in mid-November 2022 and in the case of Azospirillum brasilense (1.5E8 CFU/g of INTA strain Az39) and Pseudomonas fluorescens (7E8 CFU/g of INTA strain Pf5) were made in YEM medium (yeast extract mannitol) with Congo Red. In the case of Bacillus subtilis (5,5E8 CFU/g of own strain G-101), for spore count, the sample was heated at 80 °C for 10 minutes and counted in Luria Bertani Medium (LB).

Except for the day of treatment application, each plot received the same agronomic management throughout the growing season (comparing treated and control rows within the same plot). Other than 2 early frosts (mid-February) and possibly experiencing slight stress and consequent acceleration of maturation, 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 just have passed their optimum point of maturity (25 % dry matter), the harvest was carried out with an Alleys picking machine Nº 7 in the MGx3 site and with a LCCH 2M Czech picking machine in the RP site. 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 statistically significant at $p<0.05$. Due to the low number of replicates, we also considered values of $p<0.3$ as an important trend.

Plant tissue analysis

At full bloom (31/01/2023) plants tissue (leaf and petiole) was collected and a composite sample of the rows of each treatment was obtained. Each sample was divided into 2 and while a subsample of each treatment was dehydrated and sent for P, K, Ca and Mg analyses, the others were processed fresh, obtaining the sap immediately. At harvest time plants tissue (leaf and petiole) was collected again and a composite sample of the rows of each treatment was obtained and sent for micronutrients analyses. The methodology used for P, K, Ca, Mg, S, and micronutrients was by digestion with nitric and perchloric acid; and then spectrophotometry (RICHARDS 1993). For analyzes of plant sap, the Horiba LAQUAtwin Nitrate and pH Pocket Meters were used. After recording pH and before measuring nitrates, a 1:4 dilution (5 ml sap: 20 ml water) was made.

Results

RP trials

The hop mature rows inoculated with *B. velezensis* had an average cone yield 12.3 % higher than the non-inoculated control rows. Figure 1 shows no significant differences between treatments ($F=0.7091$, $p=0.4$).

In converting measured yields from wet cone to dry flower, a theoretical multiplication by 3.5 could be assumed, considering the value of dry matter measured in the field at the time of harvest just past their optimum point of maturity. In this way, the theoretical average yield values would be 1913 kg/ha for the inoculated rows and 1704 kg/ha for the control rows. Visually, no differences were observed in terms of vegetative vigor or leaf coloration.

MGx3 trials

The hop mature rows inoculated with *Azospirillum brasilense*, *Pseudomonas fluorescens* and *Bacillus subtilis* had an average cone yield 13.9 % higher than the non-inoculated control rows. Figure 2 shows no significant differences between treatments ($F=1.49$, $p=0.2$).

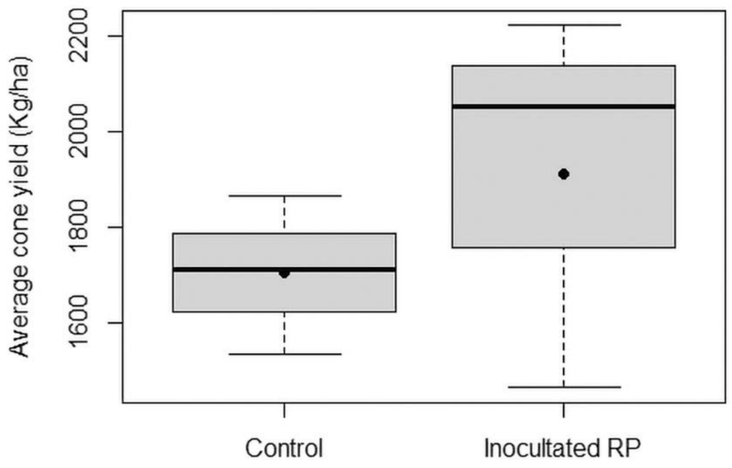

Figure 1. Box plot of average cone yield (kg/ha) in control vs inoculated with RP treatments. Central black lines represent medians and black dots means.

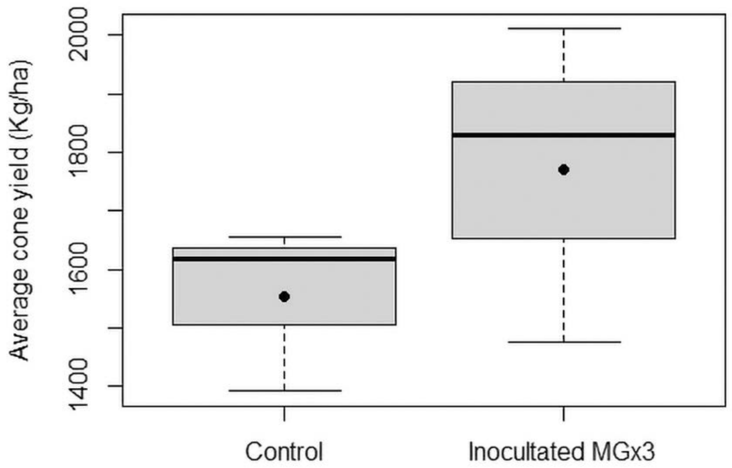

Figure 2. Box plot of average cone yield (kg/ha) in control vs inoculated with MGx3 treatments. Central black lines represent medians and black dots means.

The theoretical average yield values would be 1771 kg/ha for the inoculated rows and 1555 kg/ha for the control rows. Visually, certain differences were observed in terms of vegetative vigor or leaf coloration in favor of inoculated plants and while the value of dry matter measured in the field at the time of harvest was 24.2% for these plants, the composite sample collected from the control rows was 26.4%.

Plant tissue analysis

The sulfur, iron, copper, and boron content were higher in both PGPR. Phosphorus, potassium, and magnesium concentration was higher in the RP-inoculated samples, but lower values were detected in the MGx3 inoculated samples when analyzing the leaf tissue from control and inoculated plants (Table 1).

Table 1. Elemental analysis of leaf tissue. Ctrl – untreated control plot, Var. – Variation.

Nutrient	Unit	Ctrl	RP	Var.	Ctrl	MGx3	Var.
P	%	0.05	0.09	86 %	0.10	0.08	-17 %
K	%	2.24	3.10	39 %	2.80	2.22	-21 %
Ca	%	3.59	3.56	-1 %	4.34	3.23	-26 %
Mg	%	1.11	1.26	14%	1.34	0.83	-38 %
S	%	0.13	0.15	15 %	0.12	0.14	17 %
Fe	mg/kg	356	392	10 %	279	324	16 %
Cu	mg/kg	8.6	11.0	22 %	8.3	9	12 %
B	mg/kg	39	51	30 %	37.5	39	5 %

The measurement technology available on the farm was used to evaluate the concentration of nitrates (Table 2), being lower in the inoculated sample of both PGPR trials, in agreement with the results obtained last year in the RICE P trial (TESTA et al. 2022). The pH measured in each pure sap sample was near-neutral in both cases.

Table 2. Concentration of nitrates as measured with Horiba LAQUAtwin Nitratre Pocket Meter. Ctrl – untreated control plot, Var. – Variation.

Nutrient	Unit	Ctrl	RP	Var.	Ctrl	MGx3	Var.
NO3	ppm	2267	2033	-10 %	2700	1600	-41 %
pH		7.2	7.3	1 %	6.9	6.9	0 %

Discussion and conclusions

The treatment of hop plants with PGPR showed positive results beyond that they were not statistically significant. In this work, RP and MGx3 was evaluated in *Cascade* hops, in two different soil quality sites. The action of both *Bacillus velezensis* and the three MG bio inputs improved the yield of cones in adult commercial hop yards and increased the concentration of certain important nutrition parameters. The results of this test allow us to infer that the use these 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 continue evaluating 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 complementary study to last year (TESTA et al. 2022) 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.

Acknowledgement

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 Joaquín Rodriguez Bonnet (Patagonia Lúpulos Andinos S.R.L.) and Martín Kandrachoff (Rural Extension Agency of INTA El Bolsón), for collaborating with the field work to install and measure the trials.

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Exploring the use of electric current for weed control in hopyards

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Abstract

Attempts to use electricity as a weed control method date back to the 19th century; a renewed interest in this approach is in response to concerns about pesticide residues and resistance. Electric weed control (EWC) systems generate a high-voltage current applied directly to the target plant via foliage contact. The current is 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 tissue conductivity; herbaceous tissue has greater electrical conductivity than woody tissue. Hop shoots are herbaceous, but the woody root system leads us to hypothesize that with proper timing and rate, EWC may selectively remove weeds and hop foliage without damaging the perennial root system.

This study evaluates the response of selected weeds and hops 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 (Raiden, Zasso, Switzerland) driven by the tractor's power take-off. The unit employs electrodes in continuous contact with soil or plant foliage. Treatment placement and speed of operation influence the energy applied. We have identified three timings against which to evaluate EWC in hop production: (i) crop emergence, when 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 6 kW nominal electrical power. Initial results indicate excellent removal of hop foliage and weeds when applied at these speeds. However, EWC killed some young hop plants when applied over the top in the spring. No effect on crop growth or yield was observed.

Key words. Physical weed control, electricity, herbicide.

Introduction

Growing interest in sustainable food systems has resulted in global expansion of organic and sustainable agricultural practices (WILLER et al 2020). These transformative changes in food systems necessitate the development of innovative technologies to ensure high crop quality and productivity; innovations in nonchemical weed control are among these technologies. Electric weed control (EWC) is an innovative technology that may play a crucial role in nonchemical weed management. Notably, EWC offers several benefits, including no soil disruption and reduced erosion (Brodie et al. 2018; Sahin & Yalınkılıc 2017). Additionally, EWC leaves no residues in the crop or soil, thereby granting growers greater flexibility in crop production and marketing.

EWC technology was first patented in the 1880s, when it demonstrated its efficacy as a weed control tool (BRODIE 2018; DIPROSE et al. 1984; KOCH et al. 2020; REISER et al. 2019; SAHIN & YALINKILIC 2017). EWC acts by passing an electric current through the target species, vaporizing cellular water, rupturing cell membranes, and ultimately causing cell death.

The electric power required to kill a plant is inversely proportional 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, and it is related to voltage (V), plant electric conductivity (ECp), and the duration of contact with the electrode (tc) (adapted from (VIGNEAULT & BENOÎT 2001). EWC was successfully used to control weeds and bolting sugar beets in that crop (DIPROSE et al. 1985). Despite its historic staying-power, most recent and historical research has predominantly focused on agronomic crops, and the literature provides limited information on the performance of EWC in perennial systems like hops.

The electrophysical properties of plants vary depending on the species, tissue type, and stage of development. Consequently, the response to EWC is specific to each plant species when a particular amount of electrical energy is applied. By carefully manipulating the energy applied, including voltage and exposure time, it is possible to leverage the natural variations in plant electrical conductivity to achieve weed control objectives while minimizing damage to the crop. The development, optimization, and adoption of this novel and effective weed control technology will offer hop producers expanded and diversified options for vegetation management and manipulation of the crop canopy. Basal foliage removal is a common practice employed by hop producers to control weeds and mitigate foliar diseases such as powdery mildew *Podosphaera macularis* (GENT et al. 2016). This project aims to investigate the potential application of electric weed control (EWC) in hops, as an alternative approach to address these challenges.

Material and methods

A 75 hp tractor was used in this experiment. Electric current was generated by a Raiden unit (Zasso, Switzerland). A PTO-driven generator (6 KVA) produced 240 V and 14 A. The unit produces direct current; voltage is adjusted according to soil conditions. High-voltage electric current is delivered by a single side mounted applicator of 0.3 by 0.5 m. Changing the speed of operation changes the energy applied.

A replicated field study was initiated in April 2022 in a one-year-old cv. Cascade hopyard located on a Chehalis silt-loam soil at the OSU Lewis Brown Research Farm (Corvallis, OR). Plants are 0.75 m apart in the row with 3.3 m between rows and are under surface drip irrigation. The study is organized as a randomized complete block with four replicates.

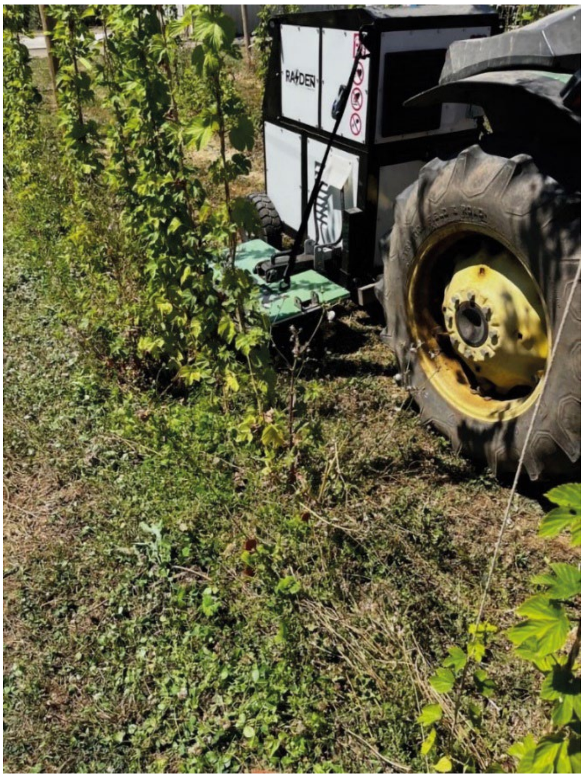

Figure 1. Raiden® electric weed control system in a hopyard in Corvallis, OR, summer, 2022.

Each replicate consists of six hop plants. Electricity is applied at one of 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

Hop survival at the end of the season was affected by treatments (Figure 2). Hop survival ranged from 45% to 75% when EWC was applied in the spring, regardless of the rate of energy used. In contrast, hop survival ranged from 82% to 100% when EWC was applied in winter or summer. The spring application was made to the growth points of emerging shoots. The dormant application did not contact hop foliage. Summer applications were made to basal foliage and could have compounded an increased sensitivity from the spring application.

When considering only the surviving plants, plant height was not affected by treatments (Table 1) and averaged 374 cm at 101 DAIT. Shoot and cone weight of the surviving plants was recorded at the end of the season, and EWC did not affect the shoot or cone weight of the surviving plants, suggesting that EWC did not cause any long-term damage caused.

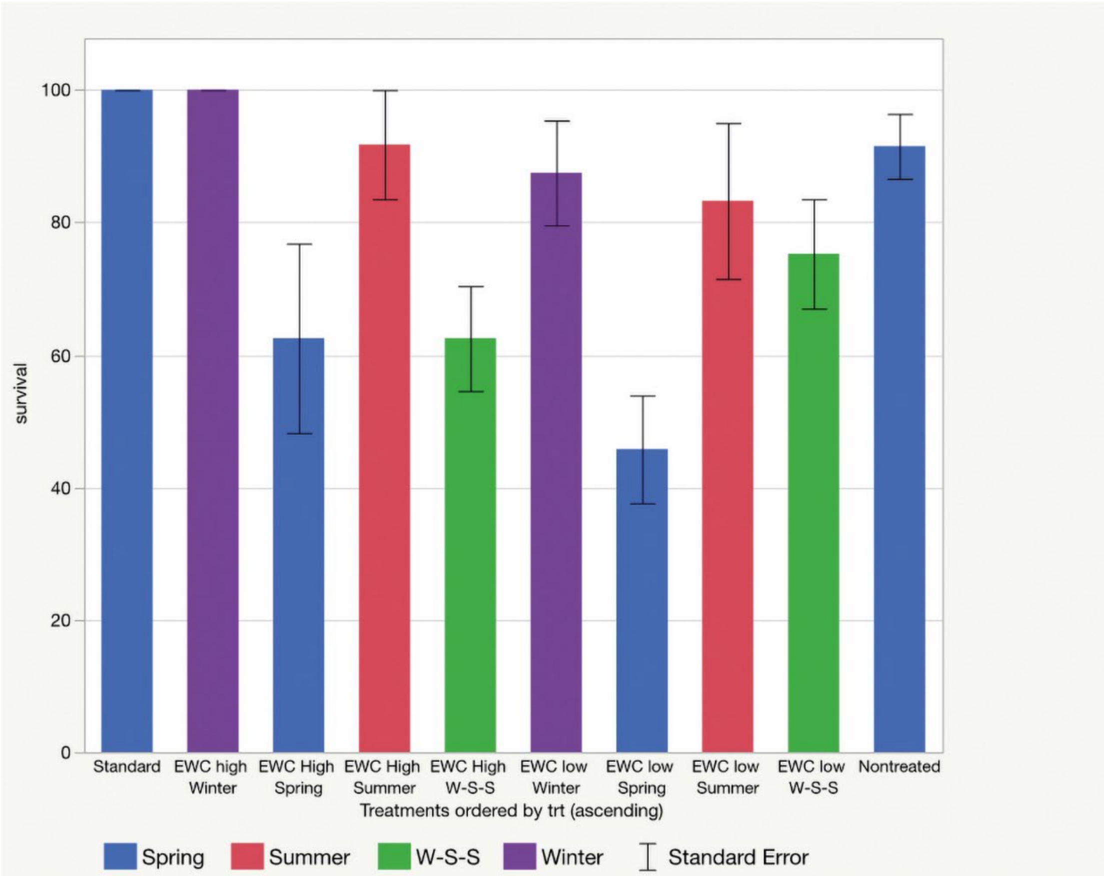

Figure 2. One-year-old cv. Cascade hop survival before harvest after electric weed control (EWC) treatments applied in winter, spring or summer in Corvallis, OR. Abbreviations. EWC high – electric weed control applied in high energy (0.4 km/h), EHC low - electric weed control applied at low energy (2 km/h). W-S-S – winter, spring, and summer treatment applications.

Table 1. One-year-old 'Cascade' hop response to electric weed control in a hopyard in Corvallis, OR, in 2022. Means followed by the same letter are not statistically different (Tukey's test, $p<0.05$). DAIT – days after initial treatment. W-S-S – winter – spring – summer.

	Treatment	Timing	Height (cm)		Shoot weight	Cone weight
			51 DAIT	101 DAIT	(kg /plant)	(kg/plant)
1	Carfentrazone	W-S-S	72.5	418.6	0.92	0.21
2	Electricity high	Winter	70.0	342.4	0.70	0.15
3	Electricity high	Spring	35.0	357.5	0.54	0.13
4	Electricity high	Summer	52.5	361.3	0.58	0.13
5	Electricity high	W-S-S	37.5	334.6	0.53	0.12
6	Electricity low	Winter	75.0	384.3	0.67	0.15
7	Electricity low	Spring	35.0	369.1	0.74	0.15
8	Electricity low	Summer	65.0	395.8	0.71	0.16
9	Electricity low	W-S-S	62.5	411.3	0.82	0.18
10	nontreated	-	72.5	365.1	0.66	0.15
	P value		NS	NS	NS	NS

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Prediction of alpha-acids accumulation in hop cones according to empiric mathematical model, general linear regression, and Cobb-Douglas production model-function

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Abstract

As the most important quality parameters of the hops the development of mathematical models for predicting of alpha-acids accumulation in hop cones is very important for planning of hop stocks in order to avoid the market stress. The aim of this study is to verify the accuracy of the empiric mathematical model for predicting the content of alpha-acids in hop cones of cv. Aurora using the linear regression, general linear regression, and Cobb-Douglas production model-function. The values of standard deviations (σ) of differences between predicted and analyzed content of alpha-acids for used methods are much lower in the comparison with the empiric mathematical model of alpha-acids prediction in the case of non-compliance with the strictly set condition for reliability of the empirical mathematical model, according to which the daily reference crop evapotranspiration (ET_0) in July ≤ 4.5.

Key words. Hop, alpha acids prediction, empiric mathematical model, general linear regression, Cobb-Douglas prediction model-function

Introduction

It is well-known that the alpha-acids are the most important quality parameters of hops on the world market. The world hop industry depends on the balance or disbalance of alpha acids caused by their offer and demand in every year. Consequently, the hop stocks are carefully planned according to available quantities of alpha acids. However, it is not easy to predict the alpha-acids content in hop cones technological maturity because of interaction between genotype of hop cultivars and environmental conditions during the growing season (PAVLOVIČ 2014). According to the results of all previous studies (SREČEC et al. 2004; 2008; KUČERA & KROFTA 2009; MOZNY et al. 2009; PAVLOVIČ et al. 2013), the quantity and distribution of rainfalls during the hop vegetation stays in positive correlation with the accumulation of alpha acids during the technological maturity of hop cones. On the other hand, higher temperatures in July and August have a strong negative impact on alpha-acids accumulation in hop cones (SREČEC et al. 2008; KUČERA & KROFTA 2009; PAVLOVIČ et al. 2013).

Development of mathematical models for prediction of alpha-acids content in relation to weather conditions during the hop vegetation was and still is in the focus of hop research, particularly because of global warming and connected global weather extremes. The overall hop supply depends not only on hop acreage but also on the weather attributes. Nevertheless, there are different approaches in mathematical modeling of alpha-acids prediction in hop cultivars to predict the hop cones yield as well as that of alpha-acids (KUČERA & KROFTA 2009; MOZNY et al. 2009; SREČEC et al. 2013; PAVLOVIČ et al. 2013).

The aim of this study is to verify the accuracy of the empiric mathematical model for predicting the content of alpha-acids in hop cones of cv. Aurora.

Material and methods

For verification of the accuracy of the empiric mathematical model for predicting the content of alpha-acids in hop cones of cv. Aurora, the empiric mathematical model reached by Eureqa Nutonian Inc. mathematical software (SREČEC et al. 2013) was used (1).

$$y = \frac{5.38w - 453 - 1.33w^2}{x} : (-10) \quad (1)$$

Where:

y – alpha-acids content in dry matter (%)

x – sum of effective temperatures (°C) from second germination after the spring pruning until technological maturity

w – sum of total rainfalls (mm) for the same period

It is also important to point out that the authors set up a condition for accuracy of equation (1), according to which the daily reference crop evapotranspiration (ET_0) in July ≤ 4.5 (SREČEC et al. 2008, 2013).

The accuracy of the empiric mathematical model (1) was estimated by using the linear regression (FREEDMAN 2005), general linear regression (ELLENBERG 1972), and Cobb-Douglas production model-function (COBB & DOUGLAS 1928).

All evaluations were done based on our own *Mathematica*-modules and were performed on the computer with a 2.90 GHz Intel(R) Core(TM)i7-75000 CPU with 16GB of RAM.

Results

First, the functional dependence of the proportion of a-acids in dependence on the amount of rainfalls w (in mm) and the sum of effective temperatures x (°C) was determined using ordinary linear regression (2) and the parameters are determined by the principle of least squares by minimizing the function (3)

$$y = a + bw + cx \quad (2)$$

$$F(a, b, c) = \sum_{i=1}^{27} [a + bw_i + cx_i - y_i]^2 \quad (3)$$

and we get the following expression (4):

$$y = 15.117 + 0.0135w - 0.00515x \quad (4)$$

and $\sigma = 1.934$ of differences between predicted and analyzed content of alpha-acids.

Second, instead of equation (1) functional dependence between the content of alpha-acids and x and w, was also determined using the regression with general parameters a, b, c (5):

$$y = a\frac{1}{x} + b\frac{w}{x} + c\frac{w^2}{x} \quad (5)$$

That means the model formally written in equation (1) is accepted, but instead of parameters

(53.8, -453, -1.33)/(-10)

we searched the new parameters (a, b, c) and the parameters are determined by the principle of least squares by minimizing the function (6)

$$F(a, b, c) = \sum_{i=1}^{27} \left[a\frac{1}{x_i} + b\frac{w_i}{x_i} + c\frac{w_i^2}{x_i} - y_i\right]^2 \quad (6)$$

and we get the following expression (7):

$$y = -67.8938\frac{1}{x} + 99.0955\frac{w}{x} - 0.12397\frac{w^2}{x} \quad (7)$$

and $\sigma = 1.931$ of differences between predicted and analyzed content of alpha-acids.

Third, instead of equation (1) and (5) functional dependence between the content of alpha-acids and x and w, was determined using the general linear regression (8).

$$y = a_0 + b\frac{1}{x} + b\frac{w}{x} + c\frac{w^2}{x} \quad (8)$$

The parameters were also determined by the principle of least squares by minimizing the function (9)

$$F(a_0, a, b, c) = \sum_{i=1}^{27}\left[a_0 + a\frac{1}{x_i} + b\frac{w_i}{x_i} + c\frac{w_i^2}{x_i} - y_i\right]^2 \quad (9)$$

and we get the following expression (10):

$$y = 1.99 - 6438.14\frac{1}{x} + 120.19\frac{w}{x} - 0.16\frac{w^2}{x} \quad (10)$$

and $\sigma = 1.921$ of differences between predicted and analyzed content of alpha-acids.

Fourth, functional dependence between the content of alpha-acids and x and w, was determined using the Cobb-Douglas production model-function (11).

$$y = Aw^{\beta}x^{\gamma}, \quad \beta, \gamma > 0. \quad (11)$$

The function (11) might be written in following form (12)

$$\ln y = \ln A + \beta \ln w + \gamma \ln x \quad (12)$$

Parameters A, β, γ were also determined and the parameters are determined by the principle of least squares by minimizing the function (13).

$$F(a, \beta, \gamma) = \sum_{i=1}^{27}[a + \beta \ln w_i + \gamma \ln x_i - \ln y_i]^2, \text{ where } a = \ln A. \quad (13)$$

and we get the following expression (14):

$$y = 787.738w^{0.518758}x^{-0.98109} \quad (14)$$

and $\sigma = 1.978$ of differences between predicted and analyzed content of alpha-acids.

Discussion

Considering the values of standard deviations (σ) of differences between predicted and analyzed content of alpha-acids, it is obvious that in all four cases the σ values are much lower in the comparison with the empiric mathematical model of alpha-acids prediction (SREČEC et al. 2013), because the standard deviation of differences between predicted and analyzed content of alpha-acids for equation (1) is $\sigma = 3.383$, for equation (4) $\sigma = 1.934$, for equation (7) $\sigma = 1.931$, for equation (10) $\sigma = 1.921$, and for equation (14) $\sigma = 1.978$, respectively. Nevertheless, this is only at the first view. Namely, the authors of empiric mathematical model of alpha-acids prediction set up a strict condition for reliability of the empirical mathematical model, according to which the daily reference crop evapotranspiration (ET_0) in July ≤4.5 (SREČEC et al. 2008, 2013). That means the extreme high values of the sum of effective temperatures and, consequently, the values of total rainfalls during the hop vegetation are excluded, because they are treated as out layers (Figure 1).

On the other hand, in this study all the values for all independent and dependent variables are included (*i.e*., every *x, w, y* data). Moreover, the accuracy of the equation (1) is confirmed in completely different environmental conditions of Savinja Valley, but only for the same hop cultivar, Aurora (SREČEC et al. 2013). That corresponds with the conclusions of previous authors that crop mathematical models are useful tools for assessing the vulnerability and response of crops to climate change if they are adequately tested against observed data during the validation process (MOZNY et al. 2009).

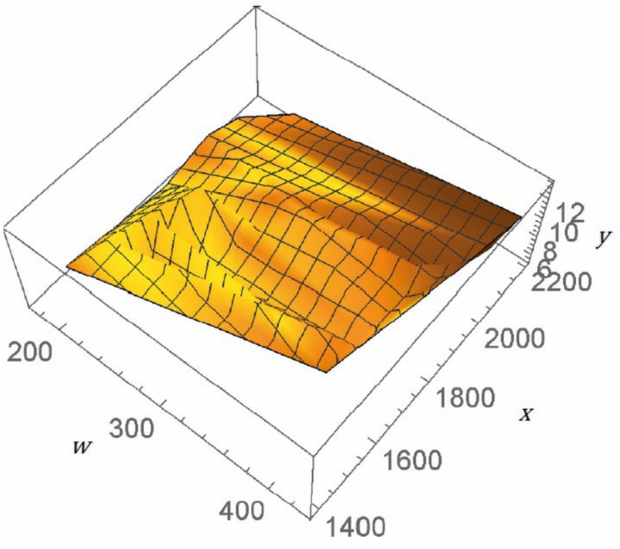

Figure 1. Interaction between accumulation of alpha-acids (*y*) regarding the total rainfalls (*w*) and the sum of effective temperatures (*x*)

Acknowledgement

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V: Viroids

News and insights from the CBCVd monitoring in Germany

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Abstract

In 2019, severely stunted hop plants became conspicuous in a field in the Hallertau region in Germany. Laboratory tests identified the causative agent as Citrus bark cracking viroid (CBCVd), a pathogen originating from *Citrus* species and first reported to cause severe stunting disease in hops in Slovenia in 2015. From 2020 to 2022, an extensive monitoring effort was carried out in Germany. The aim was to discover infection sites and to estimate the extent of spread of CBCVd in the Hallertau region. Farms were included into the monitoring program based on spatial proximity of their acreage to previously known infection sites and by evaluation of aerial images of the respective fields that were screened for visible signs of pathogen infestation. Additionally, random samples were taken from the suppliers of the biogas plant Hallertau. From 2020 until 2022, approximately 4000 ha of hop fields were surveyed systematically with drones and by walk-through. CBCVd-infected hop plants were detected in fields of 13 farms. Currently, 41 plots with a total of 110 ha are affected. The monitoring in 2022 detected fields with a very low number of symptomatic plants and farms in which CBCVd was found to still be limited to a single hop garden. Based on our observations, it can be assumed that the spread of CBCVd in the Hallertau is currently well recorded. Alongside monitoring, comprehensive counseling of the farmers on field hygiene measures was carried out with a recommendation for extensive grubbing of symptomatic plants and their surroundings. Nevertheless, the CBCVd-infested area is currently growing slowly but steadily. Future monitoring activities would be especially beneficial for farms that are currently not affected and in which CBCVd can be detected at an early stage after introduction. The aim of this project is to offer practical advice on the control of CBCVd and to keep the infection pressure within the Hallertau region at the lowest possible level.

Key words. Hop, Humulus lupulus, Citrus bark cracking viroid, stunting disease

Acknowledgement

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Epigenome analysis of CBCVd-infected hop plants

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Abstract

Viroids are small, infectious RNA molecules that lack a protein coat and rely on host factors for replication. In plants, certain viroids such as CBCVd can cause significant economic losses due to the severe diseases they induce. A growing body of research suggests that viroid-derived RNAs are involved in the pathogenesis of these diseases by modulating host factors at various regulatory levels, ultimately leading to changes in gene expression, metabolism, and phenotype. Recent studies have also highlighted the potential role of viroid-induced DNA methylation changes mediated by the RNA-directed DNA methylation pathway (MARTINEZ et al. 2014; CASTELLANO et al. 2015, 2016; SEČNIK et al. 2022). To shed further light on the putative role of DNA methylation in viroid pathogenesis, we performed an epigenome analysis of CBCVd-infected hop plants. Our results show that CBCVd infection does not cause global changes in hop plant DNA methylation, but rather exerts subtle and methylation context-dependent effects on specific genomic regions. We identified 172 hyper- and 146 hypo-methylated protein-coding genes that were enriched in pathways related to RNA transcription, as well as pathogen interaction pathways such as MAPK signaling and LRR genes. Taken together, our study provides new insights into the complex interplay between viroids and host factors and underscores the importance of considering epigenetic mechanisms in the context of plant-viroid interactions. Our findings raise intriguing new questions about the mechanisms underlying viroid-induced changes in DNA methylation and pave the way for future investigations aimed at unraveling the molecular basis of viroid pathogenesis.

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

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Transmission of Citrus bark-cracking viroid with soil and roots

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Abstract

Citrus bark cracking viroid (CBCVd) is a circular RNA molecule with 283–286 nucleotides that causes aggressive symptoms on hop such as dwarfing, stunting, and rotting of roots. The viroid is mostly transmitted mechanically by exposing plants to contaminated tools and machinery used during the cultivation of hop gardens. CBCVd can also be transmitted by foliar contact between plants or by infected plant remains in hop gardens. The purpose of the presented research was to determine the possibility of CBCVd transmission with soil and intertwined roots of infected and healthy hop plants. In first experiment, infected hop plants were planted in artificially infected soil. In the nonsterile experiment we sampled garden soil, prepared it by sieving, and planted it with infected hop plants. In the sterile soil experiment, we sampled garden soil and autoclaved it to eliminate all microbiological elements, and then planted infected hop plants. All hop plants were grown for two months, removed, and the soil was sieved to eliminate organic infected particles. Healthy hop plants were then planted, after four months, we analyzed all plants on the presence of CBCVd with real time RT-PCR. Testing was also repeated after plant dormancy. In second experiment, we sampled rhizosphere and soil nearby roots of CBCVd-infected plants in hop gardens. The soil was sieved to eliminate any plant remains and used for planting healthy plants, which were tested for the presence of CBCVd after two months. Sampling and testing were repeated after plants dormancy. In third experiment, we studied root transmission between CBCVd-infected hop plants and healthy plants. Each pair of CBCVd-infected and healthy plant was planted in one pot to stimulate the intertwining of roots. The upper parts of the plants were physically separated by a net to prevent any contact of greener parts. After growing season, we evaluated the rate of intertwined roots and tested plants for the presence of CBCVd before and after dormancy. In soil experiments, all plants were negative for the presence of CBCVd. Therefore, we conclude that CBCVd could not be transmitted with contaminated soil with removed plant parts. In the experiment where we investigated transmission of CBCVd by root intertwining, we found a high rate of infections since 70 % of healthy plants exhibited a positive infection with CBCVd.

Key words. CBCVd, transmission, soil, roots

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Risk of hop viroids in citrus-based plant-strengthening products

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Abstract

Germany is well-known for being the world's top bitter hop producer, thanks to its long history of consistently produced high-quality hops. However, hops are vulnerable to many pathogens, such as Citrus bark cracking viroid (CBCVd), known to have come from citrus plants. CBCVd can cause severe symptoms in hops, including heavy yield losses and destruction of the plant (EPPO 2021). In recent years, synthetic plant-strengthening agents have been restricted or banned in the EU due to environmental and health concerns, leading to an increased demand for alternative plant-strengthening agents. As a result, citrus-oil-based products have emerged as an allegedly more sustainable and environmentally friendly option. Additionally, these natural agents are biodegradable and pose fewer risks to non-target organisms, making them a safer option for farmers and consumers. However, they may also pose a risk of reintroducing CBCVd into agricultural fields.

In this study, we established a citrus oil RNA extraction protocol and used it to evaluate three citrus-oil-based plant-strengthening products for the presence of various viroids. Spiking the oils with viroid RNA showed that the oils did not inhibit the RT-PCR reaction and allowed downstream analysis. Furthermore, none of the tested viroids, including CBCVd, HSVd, or CEVd, were detected in the provided oils. Thus, these oils may have a low risk of containing hop pathogenic viroids. These findings may interest hop farmers, those involved with the hop and brewing industry, along with authorities concerned with the safe use of citrus-oil-based products in agriculture.

Introduction

Viroids are single-stranded RNA molecules that cause various plant diseases, including the citrus bark cracking viroid (CBCVd), which is known to cause severe symptoms in citrus plants. However, CBCVd can also infect hops (*Humulus lupulus* L.), a significant crop in the German and international brewing industry. CBCVd causes stunted growth, reduced yield, and even death in hop plants within a few years (EPPO 2021). The first instance of CBCVd in Europe was reported in Slovenia in 2007 (JAKŠE et al. 2015), followed by its discovery in Hallertau, the world's largest continuous hop-growing region, in 2019 (JKI 2019).

In addition to CBCVd, hops are susceptible to various diseases, such as powdery mildew, downy mildew, aphids, and spider mites. Traditionally, synthetic plant protection agents have been used to safeguard hops against multiple pests and diseases. However, in recent years due to environmental and health concerns, synthetic plant-strengthening agents have been limited or prohibited in the EU. To address this problem, there is a need to explore sustainable and eco-friendly plant-strengthening agents. In this study, we investigated citrus-oil-based products that have recently emerged as a promising alternative to synthetic products because they are biodegradable and pose lower risks to non-target organisms. Furthermore, adopting natural plant-strengthening agents and other integrated pest management techniques can lead to more sustainable agriculture in the future.

However, using natural plant-strengthening agents may reintroduce CBCVd and other viroids into agricultural fields as these agents may contain nucleic acids of infectious agents. Therefore, monitoring and regulating the usage of citrus-oil-based plant-strengthening agents is crucial to minimise the risk.

Consequently, in this study, we established an RNA extraction method from citrus oil and used it to evaluate three citrus-oil-based plant-strengthening products for the presence of various viroids. The experiment of spiking oils with viroid RNA revealed that the oils did not hinder the RT-PCR reaction and enabled further analysis of the samples. Moreover, none of the tested viroids, including CBCVd, HSVd, or CEVd, were detected in the provided oils, indicating a very low possibility of these oils containing hop pathogenic viroids. These findings have significant implications for the hop-growing community, stakeholders in the brewing industry, and government agencies responsible for regulating the use of citrus-based oil products in agriculture.

Material and methods

This study aimed to analyse and compare three samples consisting of about 500 ml of a citrus-oil-based product and to validate the RNA extraction method. In addition, three different commercial olive oil samples originating from different regions were also analysed.

Sample preparation. An RNA reference was prepared by adjusting the RNA concentration to 50ng/µl from RNA extracts from CBCVd-infected hop plants. Aliquots of all three oils were used directly or mixed with 6% or 94% of CBCVd-containing RNA for sample spiking and recovery analysis. The RNA reference, the oils, and the oil-RNA mixes were incubated for one hour, one day, or one week at room temperature under constant rotation at 40 rpm in darkness. Then, the samples were either directly analysed or purified with two RNA extraction methods. The first RNA extraction was performed starting from ~ 100 µl of the liquid sample using the Monarch Total RNA Miniprep Kit (New England Biolabs, USA) as described earlier (HAGEMANN et al. 2021a).

The second RNA extraction method utilised the NucleoZol kit from Macherey-Nagel GmbH & Co. KG to isolate total RNA from a liquid sample, using approximately 100 µl of the starting material. The preparation of the sample followed the same procedure as the first RNA extraction method, with one reference at 50 ng/µl and three oil aliquots, either used directly or mixed with 6% or 94% of CBCVd-containing RNA (50 ng/µl). The individual tubes of pure oil and spiked samples were also incubated on a rotator for one hour, one day, and one week, followed by extraction.

The results of the comparison between the two RNA extraction protocols indicated that the NucleoZol kit was the superior choice for RNA extraction from oil samples. This is likely due to the presence of guanidine isothiocyanate and phenol in the kit, which can denature proteins, disrupt lipid membranes, and solubilise lipids, ultimately resulting in the release of RNA into the aqueous phase. These findings suggest that the NucleoZol kit may be a highly effective option for RNA extraction from challenging samples, such as those with high lipid content. The reverse transcription quantitative PCR analysis (RTqPCR) was conducted in 1µl duplicates based on the procedure described by SEIGNER et al. (2020) with modifications (HAGEMANN et al. 2021b).

The following viroids were tested in pure oils according to primers and probes described earlier; citrus bark cracking viroid (CBCVd), citrus exocortis viroid (CEVd), and hop stunt viroid (HSVd). NADH-dehydrogenase 5 (NAD5) was used as plant RNA-based internal PCR control (IPC), as described in SEIGNER et al. (2020). Additionally, a cytochrome oxidase was used as a second IPC to validate the NAD5 results. In case of ambiguous results, the analysis was repeated, and additionally, selected RTqPCR amplicons were sequenced (GATC service (at) Eurofins Genomics).

For the samples of concern where the RT-qPCR reaction curve suggested a product was generated, sequencing results confirmed that such curve trajectories are not viroid amplicons but by-products of the reaction that can be formed with low efficiency in the absence of matching templates (CBCVd). Since no RNA was extracted from the pure oils, we adopted a cell debris enrichment procedure from nucleic acid extraction successfully performed for olive oil (BUSCONI et al. 2003). As we did not extract RNA directly from the pure oils, we successfully employed a cell debris enrichment procedure for nucleic acid extraction, as previously described for olive oil. Therefore, 40-45 g of the oil samples were centrifuged with an ultracentrifuge at 4°C, 48.384 x g, for 30 min. The supernatant was discarded after centrifugation. Then the pellet was dissolved in 500 µl NucleoZol and transferred to a 2 ml tube. The resulting pellets have also been extracted with the NucleoZol kit as described above.

Results and discussion

The oil RNA spiking experiment shows that the citrus oils do not inhibit the RTqPCR analysis, irrespective of the extraction method or all types. However, considering the recovery of RNA in comparison with the RNA reference shows that the NucleoZol RNA extraction is most effective for the task of extracting RNA from citrus oils. Furthermore, none of the tested viroids, CBCVd, HSVd, or CEVd, and no plant RNA was detected in any of the provided oils; thus, we assume that the oils have a low risk of containing hop-pathogenic viroids. In fact, despite two extraction methods and one pre-extraction enrichment, no RNA could be recovered from any of the three oils.

The remaining risk is estimated as low, especially compared to the risk of viroids from infected citrus fruit residues used as fertiliser. Also, from our experience, it is not easy to get viroid infections even from injecting viroid-infected plant sap or purified RNA into the petiole if the conditions are not optimal. Optimal conditions would be high humidity and high temperatures during the growth phase of the host plant (ŠTAJNER et al. 2019). Using viroid-free citrus material for cold pressing reduces the risk of viroid contamination even further since the initial material is free from viroids, which means the final product will likely be free from viroids. However, our research on citrus fruits from various sources suggests that most citrus plants carry at least one type of viroid (HAGEMANN et al. 2023). Thus, we assume that viroids are likely to have entered the oil in principle but either degrade or have been filtered out through oil storage or processing.

In summary, no viroid or other RNA was found in the tested oils. However, to lower the residual risk, we recommend testing further batches of the citrus-based product with the NucleoZol RNA extraction and RTqPCR for at least two citrus viroids and two plant RNAs prior to test application. If they show to be viroid-free and RNA-free despite different batches, we conclude that there is a very low risk from the tested citrus-oil-based products to transmit viroids that might infect hops.

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Long read sequencing of viroid RNA

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Abstract

Viroids are small, non-coding RNA molecules that replicate via a rolling circle mechanism. Viroids from the family *Pospiviroidae* replicate through an asymmetrical pathway, producing more-than-unit length intermediates in the first step (FLORES et al. 2017). The detection of these intermediates in different viroid species has traditionally relied on outdated methods such as gel electrophoresis and blotting. In recent years, long-read sequencing technologies have revolutionized the study of genomes (TOMBÁCZ et al. 2016) and transcriptomes (BALÁZS et al. 2017), as well as being able to generate reads that extend over repetitive regions or regions of low complexity. In this study, we utilized the Nanopore sequencing platform to detect and quantify linear forms of viroids, focusing on three different viroid species infecting hop plants. Despite some limitations of the Nanopore platform, our results demonstrate the suitability of long-read sequencing for viroid research. We were able to successfully count the number of viroid intermediates and identify potential pivot points for the development of novel diagnostic and quantification methods for viroids in biological samples. Overall, our study highlights the potential of long-read sequencing to advance our understanding of viroid biology and pathogenesis. Future studies using this technology could provide valuable insights into the replication and evolution of viroids as well as their interactions with host plants.

Key words. Viroid replication, LRS, nanopore, viroid intermediates

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

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VI: Phytopathology

Plant pathogenic fungi under inspection: The *Verticillium nonalfalfae* case report

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Abstract

Fungi of the *Verticillium sensu stricto* genus are a group of plant pathogenic soil-borne fungi capable of infecting a diverse range of agriculturally important plants and crops. The primary host of *Verticillium nonalfalfae* is hop (*Humulus lupulus* L.), grown extensively in Slovenia, a growing region contributing roughly 2.5 % to world production. Regrettably, in 1997, the first infections showing lethal wilting symptoms were observed in Slovenian hop fields (RADISEK et al. 2003). With the lack of suitable treatments, the disease management relies on strict sanitary measures and breeding new resistant hop cultivars. The key in fast response to hop infections is accurate species and pathotype identification, as hop is the host of less virulent and highly virulent *V. nonalfalfae* isolates as well as of *Verticillium dahliae* (INDERBITZIN et al. 2011). Several molecular markers were developed to distinguish the two species and the two *V. nonalfalfae* pathotypes (INDERBITZIN et al. 2013; RADIŠEK et al. 2004), based on unique sequences amplified with the PCR approach.

We tested the usefulness of the short sequence repeats (SSR) for *Verticillum sensu stricto* species identification. Additionally, loop-mediated isothermal amplification or LAMP was used to develop novel rapid identification method, enabling pathogen detection immediately after isolation on hop fields (JESENIČNIK et al. 2023). Also, the conserved RNA interference core components were used in phylogenetic analysis of various filamentous fungi, including three *Verticillium* sensu stricto species, where the fungi were accurately differentiated based on protein sequences of Dicer-like, Argonaute and RNA-dependant RNA polymerase (JESENIČNIK et al. 2019).

The SSR marker survey was performed using the MIcroSAtellite (MISA) tool on the sequences of *V. alfalfae* VaMs.102 isolate (GenBank accession GCA_000150825.1). The 61 newly designed primer pairs were tested on 16 *Verticillium sensu stricto* isolates, acquired from the culture collection of the Slovenian Institute of Hop Research and Brewing. Twelve primer pairs, showing the highest polymorphisms, were chosen for further analysis. For the LAMP primer design, genomes of *V. nonalfalafe* isolate T2 (GenBank accession GCA_002776445.1) and *V. dahliae* isolate Vdls17 (GenBank accession GCA_000150675.2) were aligned and regions, unique for *V. nonalfalfae,* were imported to the Primer Explorer V5 software. All LAMP reactions were performed using the WarmStart LAMP Kit (NEB). In the RNAi-associated phylogeny, the protein sequences of several ascomycete and basidiomycete fungi, acquired form the UniProt database, including the newly identified *V. nonalfalfae* RNAi core proteins (JESENIČNIK et al. 2019), were aligned using MUSCLE algorithm, implemented in the CLC Genomic Workbench software, and the phylogenetic three was constructed with the Neighbor-joining Kimura model.

The MISA analysis revealed 628 sequences of *V. alfalfae* VaMs.102 contain SSR repeats, where the most frequent were dinucelotides, representing 52.9 % of all SSRs. The identification analysis was performed on 94 isolates from the culture collection. The locus, designated as 3632 (Table 1), produced 237 bp long allele in *V. nonalfalfae*, while in the *V. alfalfae* 252 bp long allele and in *V. dahliae* 235 bp long alleles were amplified. The locus 886 produced 168 bp long allele only present in *V. dahliae* species. For differentiation of *V. alfalfae* and *V. nonalfalafe* species the locus 3111 was detected, producing a specific 213 bp long allele only present in *V. alfalfae*.

Table 1. Newly identified highly polymorphic SSR loci

SSR locus designation	SSR motif	Length (bp)	Comment
598	(AG)16	177	*V. albo-atrum* differentiation: combination of 2756 and 3507, absence of 1556, 598, 886
886	(TG)25	160	*V. dahliae* specific 168 bp allele
2756	(GT)17	226	*V. albo-atrum* differentiation: combination of 2756 and 3507, absence of 1556, 598, 886
3507	(GA)17	197	*V. albo-atrum* differentiation: combination of 2756 and 3507, absence of 1556, 598, 886
2390	(CT)17	145	*V. longisporum* differentiation: combination of 2390 and 1556
3111	(GA)24	200	*V. alfalfe* specific 213 bp allele
1556	(CT)16	130	*V. longisporum* differentiation: combination of 2390 and 1556
3632	(GA)15	230	*V. nonalfalfe* specific 237 bp allele

For the LAMP reaction, six specific primer pairs were developed and used for identification and differentiation of *V. nonalfalfae* and *V. dahliae* species. For accurate species identification, the combination of newly designed 1) Vna_Vd2 primers for the differentiation of *V. alfalfae* and *V. albo-atrum* species from *V. nonalfalfae* and *V. dahliae*, 2) an optimized Vna_Vd1_1 and Vna_Vd1_2 protocol for the differentiation of *V. nonalfalfae* from *V. dahliae*, and 3) PG2_1 primers for the differentiation of the highly virulent and less virulent pathotypes of *V. nonalfalfae* were necessary to determine the *V. nonalfalfae* isolates. The listed primer combinations, used in specific order, enable the fast diagnostic analysis of unknown *Verticillium sensu stricto* isolates, which are primarily hosted by hop.

Key words. *Verticillium sensu stricto*; *Verticillium nonalfalfae*; molecular markers; SSR; LAMP

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The defensive role of secondary metabolites and small RNAs during hop-Verticillium interactions

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Abstract

Hops are grown for use in the brewing industry and, recently, also for the pharmaceutical industry. Severe Verticillium wilt caused by the phytopathogenic fungus *Verticillium nonalfalfae*, is one of the factors influencing yield loss in hops (*Humulus lupulus* L.).

Phenolic compounds are involved in plant responses to various biotic and abiotic stress factors, with many studies suggesting their role in defense mechanisms against fungal pathogens. Soilborne vascular pathogen **Verticillium nonalfalfae** causes severe wilting and consequent dieback in a wide range of economically important crops, including hops. In this study, we investigated the differential accumulation of phenolic compounds in the susceptible cv. Celeia and the resistant cv. Wye Target during the pathogenesis of **Verticillium** wilt. In the roots of the resistant cultivar, a significant increase in total flavanols was detected at three days post-inoculation (dpi), suggesting a possible role in preventing fungus spread into the stems. The accumulation of phenolic compounds was less pronounced in the stems of the resistant cultivar since, compared to the latter, significant increases in flavonols at 3 and 15 dpi and hydroxycinnamic acids at 6 dpi were observed in the stems of the susceptible cultivar.

MicroRNAs, 21- to 24-nucleotide long non-coding RNA molecules, mostly regulate gene expression at the post-transcriptional level. They can modulate various biological processes, including plant response to fungal pathogens. In our study, 56 known and 43 novel miRNAs were identified. After inoculation of susceptible and resistant hop cultivars with *V. nonalfalfae* their expression levels in roots were evaluated. We found five known and two novel miRNAs that were differentially expressed in the susceptible cultivar and six known miRNAs that were differentially expressed in the resistant cultivar. Differentially expressed miRNAs targeted 49 transcripts involved in protein localization and pigment synthesis in the susceptible cultivar, whereas they are involved in transcription factor regulation and hormone signaling in the resistant cultivar. Upregulation of miR160a in the resistant cultivar could suppress auxin response factors, leading to stabilization of Aux/IAA repressors and consequently downregulation of auxin signaling pathway. Similarly, upregulation of miR319c-f may lead to suppression of genes that inhibit root growth (Teosinte branched 1), branching, and vascular tissue development in the resistant hop cultivar during *V. nonalfalfae* infection, thereby activating subsequent processes that lead to successful defense against the invading pathogen.

Key words. *Humulus lupulus; Verticillium nonalfalfae*; biotic stress; phenolic compounds, plant–pathogen interactions; high-throughput sequencing

Acknowledgement

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Identification of hop cultivars tolerant to *Verticillium* wilt

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Abstract

Verticillium wilt, caused by the fungal pathogen *Verticillium nonalfalfae*, is an increasing problem in hop growing areas. Breeding efforts resulted in tolerant hop varieties with better tolerance to lethal *Verticillium* strains. However, the process of selection and identification of new varieties is time-consuming and takes several years. In order to provide valuable knowledge for breeding and practical farming, trials are carried out over several years in hop yards contaminated with lethal strains of the *Verticillium* fungus. This project supports the breeding programme at the Hop Research Center in Hüll and aims to identify varieties tolerant to *Verticillium* wilt.

Key words. *Verticillium* wilt, *Verticillium nonalfalfae*, tolerance, varieties, cultivars

Introduction

An increased frequency of *Verticillium* wilt has been observed in some parts of the Hallertau during the last 15 years. *Verticillium* is a soil-borne fungus with a wide host spectrum. It is able to survive in the soil for up to five years in the form of an infectious permanent mycelium and cannot be controlled directly (RADIŠEK et al. 2006). Hops is predominantly affected by *Verticillium nonalfalfae* and rarely by *Verticillium dahliae* (EFSA 2014).

Research work on the wilt problem in hops was resumed at the Hop Research Center in Hüll in 2008. In addition to so-called 'mild' wilt strains of *Verticillium nonalfalfae*, aggressive strains were detected in the Hallertau for the first time during this project (SEEFELDER et al. 2009). Since the first observation of aggressive *Verticillium* strains in Germany, a continuous spread of the infested area has been observed. These more aggressive or lethal fungal strains developed due to the natural selection on wilt-tolerant hop variants. They induce evident wilt symptoms and plant death in hop varieties previously classified as wilt-tolerant. In commercial yards there is often a mix of mild and lethal races present. As the standard plant protection strategies fail to stop the fungus, hop growers face increased economic loss. The goal of this project is to identify hop cultivars that are tolerant to lethal *Verticillium* strains.

Material and methods

Since 2016, different hop genotypes have been tested for their tolerance towards *Verticillium* at three sites with different soil properties during five growing seasons. The hop yards are managed by farmers and the plants are assessed for visual symptoms of the fungus at regular intervals by scientific staff. Each genotype is grown in three replicates with 7 to 8 plants per replicate. Each plant is trained to two hop strings with three bines per training wire (Fig. 1).

As a first step, the plants in the trial field are screened for *Verticillium* by qPCR analysis (MAURER et al. 2013). Furthermore, a detailed assessment is carried out to determine how the fungus is distributed within the hop yard. After grubbing, each plot is assigned a score and replicates of the newly planted test varieties are arranged in a way that each genotype is grown on both a heavily infested site and a less infested site.

The planting material is obtained from the Hop Research Center in Hüll and is free of *Verticillium*, viroids, and viruses. After planting, care is taken to ensure that all hop plants are able to develop well. In the following trial years, diseased and dead plants are not replanted.

In each hop yard, well-known cultivars such as Herkules (tolerant) and Hallertauer Tradition (susceptible) are planted in addition to the genotypes that are to be evaluated. These plants serve as comparison and allow better assessment of the susceptibility of the new varieties to wilt. At the end of each season, a wilt index indicating the susceptibility of the genotype relative to the tolerant cultivar Herkules is calculated. Each genotype with an index <1.0 is considered tolerant to lethal *Verticillium* strains.

Figure 1. Susceptible (left) and tolerant (right) hop genotypes in our trial field infested with lethal Verticillium strains

Results

Many new varieties of the Hop Breeding Department in Hüll are tolerant to wilt and therefore highly suitable for practical cultivation (Table 1).

Discussion

Our variety garden provides a unique opportunity to test new genotypes for their tolerance towards wilt under natural conditions and standard cultivation techniques. While artificial infection trials in the greenhouse can provide a faster, cheaper and space-saving statement, infection rates are usually low, and plants can only be observed over a short period of time. If the possibility of in-field testing exists, this advantage should be used. To avoid potential uneven spread of the fungus in the soil and the according different infection pressure at each planting site, all plant material is chopped on the soil after the harvest. This ensures high and even infection pressure in the trial field.

As we are not testing male plants to avoid pollination of surrounding fields, the tolerance of the male hops neds to be estimated on basis of our observations of female ancestors in the pedigree. Since it takes time for new varieties to enter the hop market and brewers to adapt their recipe, at the beginning the cultivated area is usually low. The variety garden gives our breeders knowledge about the Verticillium tolerance prior to the launch of a new variety and hop growers can be properly advised.

Table 1. Results of identification of hop cultivars tolerant to lethal Verticillium strains (site 3); all cultivars scored <1.0 are tolerant to lethal wilt strains. The varieties tolerant to the lethal Verticillium strains are marked in green

	cultivar	2020	2021	2022
1	Hallertauer Tradition	2,07	2,16	1,17
2	Target	0,14	0,49	0,32
3	Herkules	1,00	1,00	1,00
4	Polaris	0,41	0,71	0,66
5	Ariana	0,43	0,25	0,16
6	2003/067/020	0,00	0,10	0,00
7	Xantia	0,27	0,14	0,01
8	2002/047/011	0,07	0,30	0,10
9	2010/008/124	0,73	1,44	0,38
10	2010/075/078	1,11	1,91	1,32
11	2010/080/728	1,45	1,44	1,13
12	Tango	0,02	0,35	0,13
13	Titan	0,41	0,80	0,82
14	2011/071/116	1,84	2,47	1,97
15	2012/017/119	1,93	1,85	1,71
16	2013/017/107	0,75	1,80	1,08
17	2013/064/007	0,11	0,35	0,11
18	2015/021/035	1,27	1,75	1,22
19	2015/052/004	0,02	0,56	0,10
20	2015/054/057	0,50	0,39	0,23
21	2015/054/069	0,02	0,51	0,12
22	2015/058/058	0,05	0,71	0,31
23	2016/041/051	0,07	0,73	0,10
24	2016/041/071	0,14	0,61	0,34
25	2016/063/013	2,61	2,22	1,89
26	2017/087/002	0,25	1,54	1,72

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Management of *Verticillium* wilt of hop by using green manure crops

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Abstract

One of the major limiting factors in hop production is *Verticillium* wilt, caused by the soil-borne fungus *Verticillium nonalfalfae*. Management of the fungus is particularly difficult due to its production of dark resting mycelium, formed from melanised hyphae, which can persist in the soil for several years. Efficient soil sanitation is usually based on at least four-year crop rotation using non-host plants; however, such a lengthy measure is not compatible with demand for rapid re-establishment of hop production. Green manures of various crop plants, particularly from the Brassicaceae family and genus *Sorghum,* have shown to reduce the inoculum potential of some soil-borne diseases and could be effective in the suppression of *Verticillium* wilt in many plants. Both groups of these plants act through a biofumigation effect, which is attributed to the release of volatile compounds during degradation of plant metabolites such as glucosinulates and cyanogenic glucosides that are toxic to a pathogen. Incorporation of green manure in soil also contributes to increased microbial biomass and changes in soil microbial community due to the organic matter provided, which may influence disease suppression as well. In our study, we evaluated the efficacy of various *Sorghum* and *Brassica* plants used as green manure crops aimed at the suppression of *V. nonalfalfae* in soil. Before incorporation into the soil, plants were assessed for their biofumigation potential based on measurements of their biomass and cyanide or isothiocyanates content. Efficacy evaluation was based on a nylon membrane bag (NMB) assay, which consists of placing artificially prepared fungal inoculum in a bag made of nylon filtration membrane. Immediately after the treatment, NMBs were placed in soil into depth of 20 cm and after the exposure period retrieved to analyse survival of the fungus. In addition, changes in total fungal and bacterial population were analysed as an indicator of influence of green manure treatments on soil microbial activity. The results showed that majority of green manure treatments had higher efficacy than the untreated soil; however, none of the treatments eliminated the fungus in nylon meshes completely. All green manures significantly increased bacterial and fungal soil population, which probably contributed to the suppression of *V. nonalfalfae* in treated plots. The highest efficacy was confirmed in *Sorghum*-based green manures that showed up to 70 % reduction of fungal infection potential. *Sorghum* plants also developed higher level of biomass than *Brassica* plants and were less susceptible to pests, especially in juvenile stages. Chemical analyses revealed high variability among plants regarding their cyanide or isothiocyanates content; however, no correlation was found with fungal suppression efficacy.

Key words. *Humulus lupulus*; *Verticillium nonalfalfae*; soil health

Acknowledgement

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Coupling an epidemiological and economic model to optimize management of hop powdery mildew at the landscape level

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Abstract

Developing sound management strategies for plant diseases requires consideration of aspects of pathogen spread, the effectiveness of control measures, and the resulting economic costs. This is exemplified with hop powdery mildew in the western United States, one of the most challenging and expensive diseases affecting the U.S. hop industry. In this environment, primary inoculum of the pathogen can be limiting to epidemic development due to the absence of the asicergous stage of the fungus. However, the disease can spread rapidly and damage crop yield and quality due to the density of hop yards, the susceptibility of varieties that are widely grown, and the potential for long-distance dispersal of the pathogen. We developed an epidemiological model that estimates disease development and spread taking into account the fungicide effects, and the expected crop damage. We then coupled this epidemiological model to an economic model of expected revenue and costs. The coupled model was parameterized using data collected from a census sample of commercial hop yards in Oregon during 2014 to 2017, including the monthly incidence of plants with powdery mildew, fungicides and other disease control measures applied by growers, and estimated revenue depending on the severity of powdery mildew on the cones. We introduce this model and demonstrate its application in simulating epidemics and their associated costs due to factors such as the frequency of primary inoculum and its spatial location, the proportion of the crop planted to resistant cultivars, and market conditions. The model can be further extended to apply optimal control theory for epidemics, quantify the effectiveness of cooperation in area-wide disease management, and answer questions that cannot be easily addressed through field experimentation.

Key words. Disease ecology, epidemiology, modeling, powdery mildew

Volatile-mediated signalling modulates resistance in neighbouring plants

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Abstract

Volatile organic compounds (VOCs) are known as signalling molecules in plant communication. They are released upon abiotic and biotic stresses and the volatile signature is specific for the type of stress. Recent studies suggest that VOCs can act as signalling molecules in plant defence and induce resistance in distant organs and neighbouring plants after herbivore attack or pathogen infection. However, research focused mainly on the function of VOCS against pests on crops, like *Phorodon humuli* (damson-hop aphid) or *Tetranychus urticae* (two-spotted spider mite), but knowledge is lacking on the volatile-mediated interaction during the infection with biotrophic fungi, like powdery mildew (*Podosphera macularis* on hop).

We found volatile-mediated modifications of the plant–pathogen interaction by using the model organism barley (*Hordeum vulgare* L.) and its interaction with powdery mildew (*Blumeria hordei, Bh)*. Pre-exposure with physiologically relevant concentrations of (*Z*)-3-hexenyl acetate, a common green leaf volatile derived from the lipoxygenase pathway, resulted in induced resistance against infection with *Bh* and an upregulation of defense-related metabolites, like hordatines. (LAUPHEIMER et al. 2023). Exposure experiments with methyl salicylate, the volatile methyl ester form of salicylic acid, also led to induced resistance in the recipient plant. Both volatile compounds are naturally present in the volatile bouquet of barley plants. Mechanically damaged barley emitted mostly green leaf volatiles, while *Bh*-infected barley showed a higher abundance of terpenes (e.g., β-caryophyllene) and benzenoids (e.g., methyl salicylate). It might be interesting, if and how these observations of resistance modulation in a monocot plant could be transferred to a dicot plant like hop.

For hop (*Humulus lupulus*), VAN DEN BOOM et al. (2003) identified the volatile profile of mechanically damaged and spider mite-damaged hop leaves. Methyl salicylate was highly abundant in leaves infested with spider mites compared to mechanical damage or uninjured control leaves. In the mite-infested blend, β-caryophyllene also occurred in small amounts. Interestingly, (*Z*)-3-hexenyl acetate and its corresponding alcohol (*Z*)-3-hexenol, was also detected in the volatile bouquet of hop, but mostly abundant in undamaged control samples. This emission pattern is similar to what we found in the *Bh*-infected barley profile. These results show that hop can produce defence-related signals by itself, and some studies already tested the ability of VOCs to control pests.

A behavioural study with spring migrants of the damson-hop aphid using an olfactometer showed a direct repellent effect of methyl salicylate, while (E)-2-hexenal and β-caryophyllene attracted the aphids (CAMPBELL et al. 1993). But volatile signals are also known in tri-trophic interactions by attracting key predators. Field studies showed a reduced mean seasonal number of *T. urticae* in MeSa exposed plots using a slow-release dispenser. In the same plots, the number of *Stethorus* sp. was significantly higher than in control plots (WOODS et al. 2011). Assessment of aphids in the same plots showed no effect of methyl salicylate.

To date, the mechanisms behind induced resistance in barley are not fully understood. An antifungal effect of VOC on the powdery mildew development is unlikely, as rather the number than the size of the developing pustules was reduced. From earlier studies, it appears likely that this observation can be explained by a reduced success in initial cell wall penetration and haustoria formation, the pivotal step in powdery mildew pathogenesis and crucial for further development and reproduction (EICHMANN et al. 2008). *Blumeria hordei* and *Podosphaera macularis* are phylogenetically related and the mechanism of invading the host tissue is similar. We speculate that VOCs could be exploited as potential plant protection measures to supplement future agronomic or horticultural practices. For example, as a key compound in induced resistance and systemic acquired resistance (SAR) to fungal, bacterial, or pathogen attack and volatile treatments, methyl salicylate might be an option as additive or synergist to optimize plant protection strategies.

Key words. Volatiles, barley, powdery mildew, hop

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VII: New frontiers in hop cultivation

Actual issues of hop growing in Russia

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Abstract

At present, there are certain problems in hop growing in Russia including issues of hop cultivation, research in the field of breeding, in vitro culture, viral infection of plantations, and implementation of investment projects. Now the interest of scientific and educational government organizations and business in these issues is increasing. After 30 years of negative dynamics of the hop industry, there are trends in the formation of competence centers for the study, breeding, and treatment of hops in the Chuvashia and Altai regions.

Introduction

Hop growing and processing of hop products are an important component of the economy of developed countries. Over the past decades, there has been a gradual increase in the area and gross harvest of hops in the world, while at the same time in Russia there was a negative situation with the hop cultivation. The purpose of this study was to analyze the key elements of the hop industry in Russia and assess the prospects for development in the coming years.

Materials and methods

Our analysis is based on statistical data on the development of hop growing in Russia, official documents from open sources, public speeches by government officials, scientific publications, and the results of own scientific experiments conducted in 2020-2023. The main method was system analysis, in experimental studies – in vitro clonal micropropagation, DAS-ELISA immunological test kits (BIOREBA AG, Switzerland; Loewe Biochemica GmbH, Germany), real-time PCR using the QuantStudio 5 DNA amplifier (Thermo Fisher Scientific, USA) and diagnostic kits «NPK «Sintol» LLC (Russia). RNA isolation from plant samples was performed using a DiamondDNATM kit (Russia).

Results and discussion

The Russian Federation (RF) as the successor of the Soviet Union inherited a developed hop growing industry. In 1990 the area of hop fields in the RF was 4,500 ha, but over the next 30 years the development rate of hop growing dramatically decreased and the area under crop reduced by more than 30 times (Fig. 1). The Institute of Hop Growing, which was engaged in the breeding of hops and the development of new equipment and machinery, was reorganized and existing hop growing experimental stations, including the one in the Altai Republic, were closed. The main region of hop cultivation was and remains the Chuvash Republic (Chuvashia), where a catastrophic decline was likewise observed: the area of fruiting hop fields in the region decreased from 2,923 to 182 ha, and the gross hop harvest by almost 10 times (IVANOVA & DEMENTIEV 2019). The major reasons for this are the deterioration of the material and technical support of hop growing enterprises, the lack of processing capacity, the absence of effective specialized equipment and planting material.

The necessary requirements for hop products in the RF amount to at least 7.5–8 k tons of hops per annum and are supplemented by imports, the volume of which in 2021 was 3.6 k tons, while the key suppliers are EU countries (mainly DE, CZ), and the USA. Domestic hop production covers about 2 % of the required volume, demonstrating the significant dependence of brewers on import.

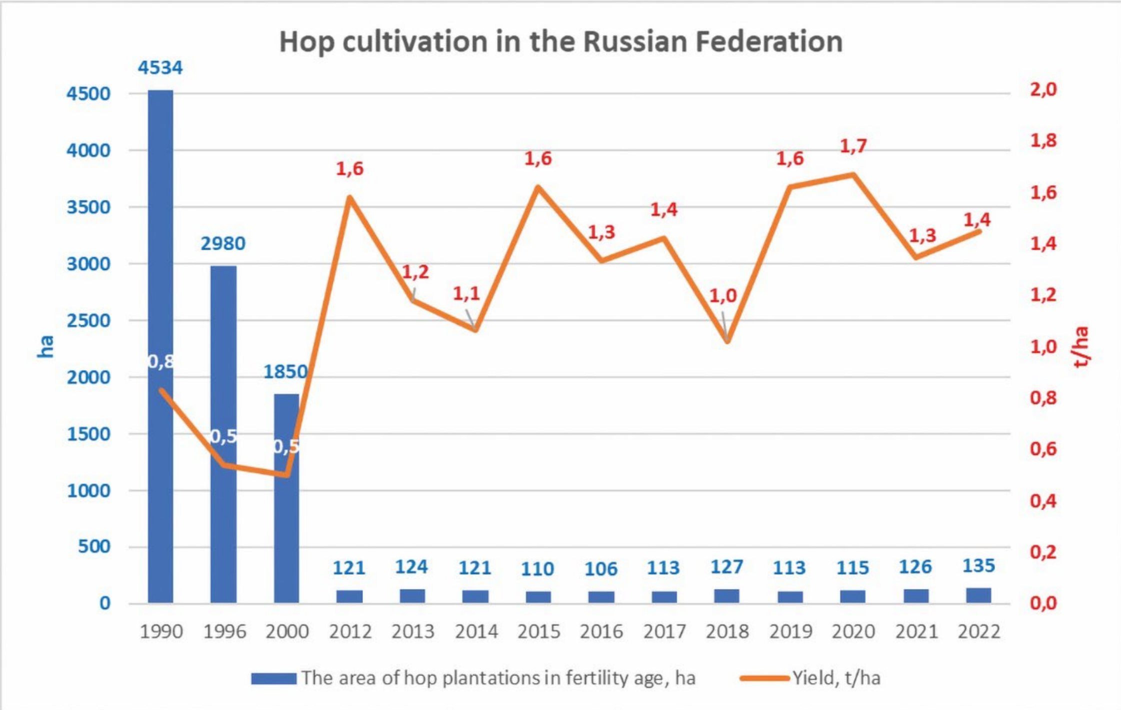

Figure 1. The area of hop cultivation and the yield per hectare in the Russian Federation, 1990-2022

Nevertheless, the climatic conditions of the RF are quite suitable for hop growing. The main production of hops is concentrated in Chuvashia and small plantations are in the following regions: Altai, Mari El, Lipetsk, and a new region is in the Krasnodar. The northernmost growing region is located at the 56th parallel north, and the southernmost at 44th. parallel north.

In recent years there has been an increasing interest of agricultural producers in the cultivation of hops. However, the revival of the hop growing industry requires not only serious investments, but also providing enterprises with planting material, specialized machinery, including domestic production, and modern resource-saving technologies (PUSHKARENKO et al. 2018).

In Chuvashia, hop growing is included in the list of priority areas for the development of agricultural production in the republic. In the Chuvash Research Institute of Agriculture (ChRIA), a collection of hops is maintained, which is on par with the collections of the world's leading research institutions. From a collection of 250 samples of wild and varietal hops from various regions of Russia and 17 foreign countries, hops are selected by the clone selection method and planting material is being grown (FADEEV et al. 2014; IVANOVA & DEMENTIEV 2019).

The state register of breeding achievements approved for use and cultivation in Russia includes 13 cultivars of domestic breeding: Druzhny, Mikhailovsky, Krylatsky, Sumer, Civil, Podvyazny, Favorite, Flagman, Fakir, Pharaon, Feodal, Forward, and Salampi. The list has grown to 26 varieties in 2023, due to foreign breeding achievements.

According to ChRIA, if the necessary conditions are created, the production yield of hops in Russia can be provided at international level with 1.8–2.0 t/ha, since the potential maximum productivity of modern domestic varieties exceeds 3.0 t/ha (OSIPOVA et al. 2022).

In recent years, there has been a positive trend in the erecting of new hopyards, with 80 ha in 2022 and another 100 ha planned in 2023. Since the autumn of 2021, a hop growing project in the Krasnodar region has been implemented by the Grainrus LLC, which intends to erect hop trellis on an area of up to 1,000 ha. MBB Group, the Russian representative office of the Czech company Hakus a.s., is going to grow hops in the Voronezh region. In case of successful implementation of the project, the region will produce 1.8-2.3 k tons of hops per annum.

In 2020, the Charysh Agricultural Consumer Cooperative began implementing an investment project to revive hop growing in Altai. Powerful mountain chernozems with increased fertility, a significant amount of rainfall and the absence of extreme heat and cold allows the formation and preservation of bitter substances and aromatics of hops. In addition, northern hop plants are less exposed to pests, and therefore do not require a significant number of chemical treatments.

Among the major issues of the recovery of domestic hop growing is a critical shortage of high-quality planting material and the education of qualified personnel. Chuvash State Agrarian University (ChSAU) has resumed training in the specialty "Hop growing", and more than 40 specialists have been qualified for three years. Also, scientists of the ChSAU together with specialists of Agrodiagnostica LLC (Moscow) have developed test systems for the main viral and fungal diseases of hops: Hop mosaic virus, Arabis mosaic virus, Hop latent virus, *Verticillium*, and Hop downy mildew.

Since 2020, Altai State University has been conducting studies of clonal micro-propagation of domestic and foreign varieties. More than 20 genotypes have been studied, the dependence of the breeding efficiency of varieties has been shown, effective biotechnological schemes of breeding in vitro culture have been selected (KHLEBOVA et al. 2021). The adaptation of regenerants on hydroponics has been tested, the technology of recovery of samples from viral infection using apical meristem culture, thermo-, and chemotherapy is being worked out (BROVKO et al. 2021; MIRONENKO et al. 2021).

The work was carried out in 2022 in industrial plantations in the Altai Republic. Testing for the presence of 13 types of viruses and one viroid was carried out on five varieties (Bryansky, Podvyazny, Smolisty, Northern Brewer, and Hallertauer Magnum) in the amount of 142 samples using DAS-ELISA and RT-PCR in real time. The total infection of the material was 66.0%, reaching 100% in cvs Bryansky, Podvyazny, Smolisty, and Hallertauer Magnum. Infection by viruses that pose the greatest potential threat to hops – PNRSV (Prunus necrotic ringspot virus) and HpMV (Hop mosaic virus) – amounted to 5.9 and 9.5 %, respectively, and in cv. Podvyazny it reached 36.4 %. SLRSV (Strawberry latent ringspot virus), PVY (Potato virus Y), PLRV (Potato leaf roll virus) and PVS (Potato virus S) were most prevalent with an incidence of 40.5, 63.2, 73.5 and 82.4 % respectively. Mosaic viruses PVY and PVS were diagnosed on hops for the first time in the world, Tobacco ringspot virus (TRSV) for the first time in the Russian Federation. TRSV occurs in 46.7 % of plants of cv. Smolisty. Potato virus X (PVX), potato virus A (PVA), and potato spindle tuber viroid (PSTVd) were not detected. The proportion of plants affected by one of the 14 studied pathogens was 8.2 %. The prevalence of associations of three or more viruses in the pathogenic complex of most genotypes was revealed (KHLEBOVA et al. 2022).

To modernize the hop growing industry and reach a more efficient level of production in 2018-2019, a project was initiated in Chuvashia to develop and manufacture the first domestic hop-harvesting and drying complex. The MX-300 AM "Civil" hop harvester is at the stage of industrial testing. The new three-tier conveyor dryer XC-400 is being successfully operated. Granulators manufactured by Nagema and Probst are efficiently used with subsequent hermetic packaging in an inert gas environment. Wolf hop harvesting complex with a stationary harvester and a dryer has been installed in the Krasnodar. Also, to support hop farming, subsidies are provided from the budget of Chuvashia to compensate of part of the costs of new trellises and care for perennial plantings, reconstruction of hop trellis, and technological modernization of manufacturing.

To implement the concept of hop growing development in Chuvashia until 2025, a five-party agreement was signed in the spring of 2023, aiming at developing innovative technologies in the hop industry, technical re-equipment, strengthening human resources, improving highly productive hop varieties, and exchanging experience. The parties to the agreement were the Cabinet of Ministers of the Chuvash Republic, the ChSAU, the ChRIA, and business representatives ("Volga Combine Plant", "Tekstilmash").

Altai State University continues research together with the ChRIA within the framework of the project "Genetic resources of wild and cultivated hops (*Humulus lupulus* L.): screening, conservation, use in breeding", supported by the State Russian Scientific Foundation for the period up to 2026.

Conclusion

Hop growing is one of the most labor-intensive branches of plant cultivation, and in recent decades in Russia it has been characterized by complex logistical support, a decrease in production and economic indicators. The decline of the hop industry caused by the instability of the political and economic situation in Russia at the end of the last century, the decline in the volume and quality of breeding, all this led to almost total dependence of the brewing industry on imported supplies of hop products. The preservation and replenishment of the genetic collection of hops of domestic and foreign varieties, the introduction of modern biotechnological and molecular genetic methods into scientific research, the development of its own technological base of production indicate the actualization of issues, both at the level of state institutions and at the business level. In general, there is a tendency to form competence centers for the study, cultivation, and processing of hops in Chuvashia and Altai regions. State support for various areas of hop growing allows us to hope for an acceleration of the pace of development of the hop industry.

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An overview on Argentinean hops: from industry focused varieties to re-purposing old hops into new trends

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Abstract

When brewers think about southern hemisphere hops, they usually think of Australian, New Zealand, and maybe South African hops. Argentina is not a main actor for this category, even though its hop industry began almost 80 years ago. This is maybe due to the lack of technology, few varieties, small cultivation area, low alpha production and/or little hop-exports. Local craft beer is close to getting 5 % of the beer market share, but local hops were considered low quality or less interesting by brewers, until macroeconomic factors pushed to re-discover them. For the last few years, Argentinean hops have been in the spotlight again: hop growers improved their technologies while brewers, putting aside their prejudices, experimented with modern hopping techniques, finding new features in old hops. In this manuscript, we briefly describe the Argentinean hop history and current trends, from the very beginnings to modern craft beer usage.

Key words. Argentina, Patagonia, *Humulus lupulus*, Mapuche, Traful, Nahuel.

Beginnings and industrial era: hop production settlement, pushing towards alpha

The first mention of hop introduction to Argentina is from Welsh immigrants in 1865 bringing rhizomes to be used as ornament, to bake hop bread and small-scale brewing (KAMINKER 2019; MATAMALA & MARTINEZ 2013). Likewise, German immigrants also brought rhizomes to El Bolsón during the beginning of the 20th Century (MATAMALA & MARTINEZ 2013). In 1928, to supply the local market, local beer industry players laid out the first experimental fields in Mendoza and Buenos Aires Provinces. By the 1940s, Cervecería y Maltería Quilmes (CMQ) started growing hop trials leaded by Leopoldo Leskovar, a Slovene agronomist researcher and member of the Technical Department of CMQ. By 1947, two additional experimental fields were laid out in Mendoza and Río Negro Provinces, with the latter selected for further development. In 1949, hop production in Río Negro High Valley and Neuquén was established. The same occurred almost 600 kilometers to the south in 1956, when the Slovene immigrant, Antonio Sinojov, established the first hop fields in Lago Puelo (Chubut Province), while another Slovene, Vojteh Budinek, began hop growing activities in El Bolsón (MATAMALA 2014). During the first years, Spalt, Semsch, and Tettnang hops were tested, with Spalt acclimating better, even though alpha acid yields were reduced over the years. With that in mind, CMQ established a research program to perform adaptive experimentation of different varieties to Argentina's environment, introducing and testing varieties in different regions: Buenos Aires Province, Río Negro High Valley, and El Bolsón. The hop list for those evaluated by 1976 is detailed in Table 1.

The better acclimated cvs were Late Cluster, Cascade, Bullion, Brewers Gold, Atlas, Neoplanta, and Vajvodina (LESKOVAR 1978). Best performance regions back then still are the main regions today: Río Negro High Valley (Parallel 39º) and El Bolsón/Lago Puelo region (Parallel 42º). In this latter region, 80 % of all Argentinean hops are currently grown.

Historically, based on Leskovar's book and reports by IHGA, USA-Hops and BarthHaas, Argentina counted 250 ha for hop production by 1949, reaching an early maximum of 340 ha by 1978 and a historical maximum of 350 ha by 1994. In contrast, the historical minimum reached below 170 ha in the year 2000, after the 1990s. During this decade, macroeconomic politics in Argentina vigorously promoted imports over local industrial production, and big players shifted to alpha-acids imports over local hop production. The general trend was always stationary until the early 1990s and then decreasing to current values: from the initial more than 300 ha to the current less than 200 ha. The total hop growing area in 2022 was 194 ha with a yield of 314 mt. Area evolution is +21% as compared to the year 2018, with 47 ha for alpha yielding 85 mt, -28 % compared to 2018. As for aroma, 2022 records reported 131 ha (+37% compared to 2017), yielding 240 mt production (+65% compared to 2018) (LESKOVAR1978; HGOA 2021; BARTHHAAS 2022; IHGC 2023).

Table 1. Hop varieties produced or in experimental phase by 1976 and 2022. Adapted from LESKOVAR (1978) and authors' personal surveillance. Notes: * – formerly Yugoslavia; ** – for 1976 reports, hops produced on less than 1 % of total acreage were in experimental phase.

Hop variety	Origin	Cultivated area (% of total acreage)	
		1976	**2022**
Ahil	Slovenia*	<1**	-
Atlas	Slovenia*	0.6	-
Aurora	Slovenia*	<1	-
Bačka	Serbia*	<1**	-
Brewers Gold	England	2.4	-
Bullion	England	0.6	2
Cascade	USA	12.8	52
Dunav	Serbia*	<1**	-
Fuggle-Bolson	UK	<1**	-
H7 Leonés	Spain	<1**	-
Late Cluster	USA	7.6	-
Mapuche	Argentina	-	10
Nahuel	Argentina	-	7
Neoplanta	Slovenia*	<1**	-
Nugget	USA	-	13
Pride of Ringwood	Australia	4.3	-
Río Negro Spalter	Germany	69.7	<1
Slovenian Golding	Slovenia*	<1**	-
Styrian Goldings-El Bolsón	Slovenia*	<1**	-
Traful	Argentina	-	1
Victoria	Australia	-	10
Vojvodina	Serbia*	<1**	
Willamette	USA	-	<1
Others	-	-	3

Going towards the craft

Argentina started growing only three varieties in the 1940s, Tettnang, Semsch, and Spalt; however, only the latter acclimated well to local conditions. During the late 1970s, Cascade was selected as the best-performing variety after industry-wise trials. Total hop production in Argentina by 2021 was around 320 mt with imports corresponding to 70 % of the total domestic hop demand. During 2021-2022 there have been 750-800 mt of hops (or its equivalent in hop extract instead of pellet) imported, showing a clear increasing trend, with 69 % of it for industrial brewing, which in turn uses 70 % of total local hop production. By the beginning of the 21st century, the craft industry demanded special hops. But unfortunately, during the 1990s the cultivated hop area had decreased by around 50 %, leading hop growers to maintain their low production levels and few varieties, with lack of investment and without proper maintenance. Then the machinery in many farms became obsolete or inefficient. Even with a growing demand, hop growers could not afford the quality standard that craft brewers demanded, related to technology and varieties. By this reason, hop growers opted to adapt classic varieties to get “new world” profiles: Cascade, Bullion, Victoria, Traful, Mapuche, and the newly developed cv. Patagonia Red are used in modern ways. Curiously, also Spalter was part of this re-branding: it was re-introduced to produce European lagers.

Currently produced varieties

Nowadays, several varieties are produced in Argentina. The main production region remains the same, where several hop growers and CMQ have hop fields. Current varieties inherited the genetic pool explained before; in the list we find cvs Spalter, Cascade, Nugget, Bullion, Victoria, Hallertauer Magnum, and Willamette. In the 1980s, as an evolution of the national hop-growing plan, CMQ associated with John I. Haas to establish a breeding program. As an output from this program, three cultivars arose, Mapuche, Traful and Nahuel (TESTA et al. 2019). Main work was done in CMQ experimental fields in 1984 in Fernandez Oro, Río Negro Province (-39° S, -67.9° W).

A study case

In the year 2022 a series of single-hop beers were launched using locally produced hops. To systematically evaluate their different impact in beer, we set a tasting trial based on previous experiences (LEGISA & MENGONI 2021). Beers were produced at IAINE Brewery (Buenos Aires, Argentina). For each single-hop beer, the grain bill was oriented to an American Pale Ale profile. A general rule for hopping was: 5 IBUs boil addition 60 min, Whirlpool: 3g/L, 85°C 40 min, and dry hop: 5 g/L when worth reached a gravity of 1.020-1.015. Cultivars Cascade, Victoria and Bullion were used. This analysis is part of a wider study evaluating all the hops produced in Argentina (ongoing project).

It would be unfair to directly compare foreign varieties to the equivalents in Patagonia since, as in the wine industry, *terroir* effect is described for hop production (VAN HOLLE et al. 2017, 2021). When these experimental Iaine beers were released, public acceptance was good, showing that if these hops are used in modern ways, special flavors could be obtained matching some current trends in public taste. Regarding the hop evaluation: ARG-Cascade was described as citrusy, sweet, floral, herbal, and hearty with balanced aroma and flavor. As for the comparison with catalog information, we recorded a diminished global impact and no full correlation between the specific characters. What regards cv. Bullion, the tasting panel found it predominantly citrusy, stone fruity, tropical fruity, and moderately sweet with some herbal and earthy hints. Again, aroma and flavor showed a balanced profile. Compared with catalogs, Bullion showed a similar profile.

Nowadays and due to this repurposing, Bullion is praised by this stone fruit flavor obtained when used in late additions. Finally, for cv. Victoria, the tasting panel found a citrusy, sweet, stone fruit, tropical fruit, and herbal profile, again balanced in aroma and flavor. In Figure 1 we summarize the results. In comparison with available data in hop catalogs, profiles are quite different. This global divergence with catalogs was expected regardless of hop origin, as we have shown before (LEGISA & MENGONI 2021).

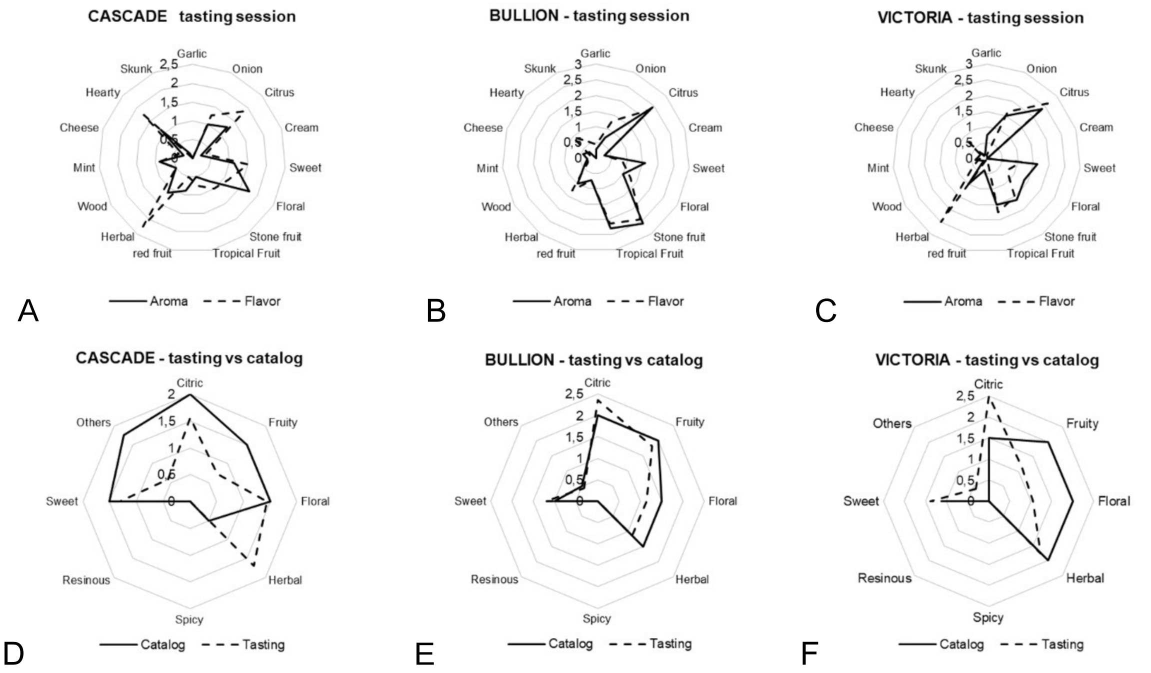

Figure 1. Spider graphs showing aroma and flavor recorded during our tasting session (A, B and C) and comparison between tasting session hoppy profile and catalog information (D, E, F)

New trends for old hops

As mentioned earlier, hop repurposing evolved along with recent formal academic research on hop chemistry and hoping techniques derived from modern beers. In retrospective, evidently several economic crises in Argentina have forced innovation into the brewing industry. General speaking about uses of local hops, when it comes to selection and application, most used hops are cvs Cascade and Nugget, perhaps because they are best available. Since hop growers are just a few, it is possible to select which variety to acquire based on each's grower technology. On the addition techniques: the general trend for use is late hop additions besides the obvious (cvs Hallertauer Magnum and Spalter for boiling) and for active fermentation addition and cold side, cvs Cascade and Bullion. In summary, new trends in hop usage is based on increasing loads in late hop additions, WH, DH-high-krausen and cold-DH. The vegetal fraction is still present due to processing, so the brewers need to take care of chlorophyll effect; due to machinery update this is expected to change in the next years. Also, recent knowledge showed that grain bill composition could contribute to flavor retention in the case of hazy IPAs, so this is another way by which local hop flavor is improved (MAYE & SMITH 2018).

Final remarks

The global trend for hop evolution during the last 15 years was towards craft interests; on the contrary Argentinean hop growers could not fully answer them. When craft demand started growing, the strategy was to give a new use of some non-modern varieties for modern-aroma purposes. The craft industry growth in the period 2015-2018 was around +30 % per year, showing that a potential market is out there. However, the recent recession and pandemic related crisis led to a new semi-stationary phase. Nevertheless, hop imports numbers show that the market is feasible: local craft needs more hops. Anyways, as a hint for the future, one of the hop growers in El Bolsón recently released new aroma varieties, product of field findings and not of a controlled breeding program (LÚPULOS PATAGÓNICOS 2023). Also, CMQ announced that they will duplicate their production area in their hop fields in Río Negro High Valley by 2025, while new regions have arisen as possible future production: Mar del Plata and Coronel Vidal (Buenos Aires Province), Villa Regina, Chimpay, and Viedma (Río Negro Province). In the last five years, many small trials have been settled in northern provinces like Mendoza, San Luis, Santa Fe and Salta. Therefore, the hop landscape in Argentina is promising; we just need stability: for the country and for the hop cones.

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Could hops become a Mediterranean crop? Opportunities and constraints of their cultivation in Italy

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Abstract

The global rising popularity of craft beers pushed many brewers, researchers, and farmers to explore the possibility of growing hops out of their traditional production areas. This trend is still underway in North and South America as well as in Europe. As an example, in the European Union (EU), the expansion of the Italian brewing industry has increased the demand for raw materials, with positive effects on Italian agricultural production, including hops. However, many structural, cultural, and agronomic issues hinder the development of a steady and efficient commercial hop production in Italy. Particularly, new hop growers lack information about the best agronomic practices to be adopted, because results from public research activity on basic hop agronomy are very few and discontinuous as well.

Key words. Hops, Mediterranean environment, Italy

The complicated history (and present) of hop cultivation in Italy

Italy can be considered a new growing area for the cultivated hop, even if wild hops (*Humulus lupulus* L.) thrive in many regions of the country (PIGNATTI 2017). Hop plants naturally grow on hedgerows and bushes along riverbanks as well as climbing up trees, fences, electricity posts or telephone poles in the Italian countryside (Fig. 1). Documents exist attesting the satisfactory outcomes achieved in the cultivation of hops by the agronomist Gaetano Pasqui (first) and the businessman Militone Moretti (later), between the end of the 19th and the beginning of the 20th century, in northern and central Italy. Similarly, one hundred years later, scholars from the University of Tuscia reported promising results on yield and quality aspects of hops obtained from the first open-field trials in northern Latium (D'ANTUONO et al. 1989). However, as anyone can easily imagine, hops did not have good fortune in the country of wine and their cultivation never really took off.

Figure 1. Wild hops growing in Tuscany (Viterbo), central Italy.

The Italian craft brewing revolution (FASTIGI & CAVENAUGH 2017) was needed to encourage many Italian brewpub owners and microbrewers to establish their own local hop garden and to make hop cultivation a current topic again. In 2020, ca 52 ha were used by 109 farms for hop cultivation in Italy, thus resulting in an average 0.5 ha hopyard (https:// innovaluppolo. crea.gov.it). A large proportion of the farms (almost 57 %), sharing 67 % of the total hop acreage, are located in northern Italy, 35 % in the central regions (28 % of the total acreage), and just 8 % in southern Italy including the islands (<5 % of the total acreage). Even if these data provide a credible summary of the hop spread in Italy, fragmented and limited information indicate that hops currently cover an area much higher than 52 ha, probably the double.

Despite this renaissance in hop cultivation and promising indications about yield potential (1-2.5 t ha^{-1} of marketable cones), the Italian supply chain of hops is struggling to develop, mainly because of a major knowledge gap at its beginning. In other words, while we (in Italy) are sufficiently aware of the composition and quality of the Italian hops, the same cannot be said about agronomic performances and plant requirements under different management and environmental conditions. Although in this last decade, we have laboriously got some information regarding the adaptation to the Mediterranean pedo-climatic conditions of different commercial cultivars as well as the cone and shoot production under real field conditions (ROSSINI et al. 2016; RUGGERI et al. 2023), the effects of agronomic practices and trellising systems on yield and quality of hops are mostly unknown.

Organic farming: more than a option for the Italian hop growers

Italy is one of the leading countries in the European Union (EU) for both the area farmed organically and the share of the utilized agricultural area (UAA) under organic regime. In Italy, organic certification was found to be the fourth (of thirteen) most important beer attributes for craft beer drinkers, following taste, fermentation process and color (LERRO et al. 2020).

This could sufficiently explain the importance of the organic segment within Italian hop production and its marked increase since 2016. However, this is just one side of the coin. In fact, while organic farming represents an option for many hop growers around the world, it is nearly inevitable for Italian farmers. Indeed, since no chemicals are yet registered for plant protection of hops in Italy, the only discriminating factor between organic and conventional hops is the use of chemical fertilizers. Our experience suggests that the production of organic hops is feasible, but it needs a deep knowledge of all agronomic strategies able to i) protect the plant, ii) increase soil fertility, and iii) boost plant growth and productivity without relying on agrochemicals. Moreover, plant requirements in different phenological phases should be known to timely apply the needed agronomic practices. All these competences are very hard to find in the small-scale Italian hop production system, where managers of hop yards are often brewers or pub owners and not professional farmers.

The challenge of Mediterranean hops

The growing popularity of craft beer, the increasing interest in natural medicine and the climate change scenario could add a new paragraph to the history and geography of the global hop (KOPP 2014), especially in the Mediterranean environment. However, structural constraints and absence of registered pesticides heavily hinder the progress of cultivation in these emerging zones. One of the major issues for new hop growers is to find harvesting and processing equipment that is affordable and scaled appropriately for their activity (Fig. 2). Currently, the best profitability seems to be provided to the small hop yards (<1 ha), mainly cultivated for the internal use of craft- and agribreweries.

On the other hand, growing hops for the market often exposes farmers to the risk of an extreme variability of prices. The major opportunities and constraints a beginner would face choosing to grow hops in Italy are listed in Table 1.

A more practical and inclusive approach should be adopted at national level to support the still weak hop supply chain. Particularly, projects including all the experts and stakeholders of hop production (from academy to industry, from agronomy to post-harvest processing) should be financed to overcome constraints associated with the introduction of a new crop. Otherwise, a full hop culture will never develop in Italy as it was until now.

Figure 2. Hop-picking machine customized for an agribrewery having also a small hop yard (0.3 ha) in the Marche Region, central Italy.

Table 1. Main opportunities and constraints for new hop growers in Italy.

Opportunities	Constraints
Making the brewery production distinguishable from other craft beers by using Italian hops	Lack of information about phenology and plant requirements under Mediterranean climatic conditions
Having the possibility to brew a 100 % local beer, with *terroir* impressed by specific pedoclimatic conditions	A little knowledge about hop agronomy in the Mediterranean environment and the optimization of agronomic practices
Diversifying beer production and thus increasing competitiveness of breweries, by using different hop genotypes, recipes, and raw materials	No data about trellising systems alternative to the standard high trellis
Widening the options of Italian farmers for diversification of farming systems	The lack of proper and registered technical tools
	Finding harvest and post-harvest equipment
	The absence of an extensive and specialized consulting system helping farmers who want to face this new challenging crop

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Analysis of climate change scenarios and associated impact on the development of hop production in the South-West of France

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Abstract

In the South-West of France, in Nouvelle Aquitaine hop production is developing to supply the increasing number of local breweries. American cultivars are mainly planted and as the growing season is characterized by hot summers and water deficits, irrigation is developed, and agricultural practices are being adapted to local weather conditions to ensure economically viable yields and quality. With temperature increase and rainfall reduction, climate change will exacerbate water deficits and extreme conditions for hop production. This study analyzes the main climatic evolution to be expected in short-, medium- and long-term following RCP emission scenarios and the impacts on hop production.

Key words. Climate change, hop production, sustainability

Introduction

According to the last IPCC report (IPCC 2022), temperatures over land in 2011-2020 are already 1.5°C higher than 1850-1900. Of all climatic records, 2022 is so far the hottest year and climate change effects are already a reality with uncertainties on future agricultural production and water availability. Among the perennial crops, climate change impact on grapevine worldwide and in France is today well-studied (QUÉNOL 2014). Regarding hop production, the impact of climate change and adaptation strategies remain unevaluated.

Some studies analyze the relationship between weather and climate characteristics and hop phenology and yield. A negative correlation between the increase in growing degree days and cone yields and quality has been observed (SREČEC et al. 2004, 2008; ROSSINI et al. 2016). With extreme weather conditions, as in 2003, high temperature accumulation can lead to early flowering before the end of vegetative growth (SREČEC et al. 2004). Furthermore, high evapotranspiration during cone formation in July and hydric stress are also negatively correlated to cone yield and quality (SREČEC et al. 2008).

The impact of extreme weather conditions differs with hop varieties (ROSSINI et al. 2016; DARBY et al. 2017; MARCEDDU et al. 2020) and agricultural practices can be adapted to face more extreme weather conditions during the growth cycle: pruning calendar, irrigation, variety choice, etc. Nevertheless, adaptation and mitigation strategies need to be anticipated and necessitate detailed research about the impact of climate change on hop production.

In France, the number of breweries is rising, with a total of 2513 breweries in 2022, of which 271 are in Nouvelle Aquitaine (GILLARD 2023). As a result, hop production is growing in France to supply local breweries and is counting new production areas as the South-West of France. Climatic conditions are similar to the US states of Oregon and Washington, and American cultivars are mainly planted in this area. Hop yards are subjected to hot summers and water deficits and therefore adaptation to climate change is vital to maintain and develop hop production. This work is focused on the analysis of climate change impact in the region Nouvelle Aquitaine.

Material and methods

Climate change scenario data

The work was conducted using climate simulations at high resolution (8 km^2) based on the Representative Concentration Pathways (RCP) scenario downloaded on the DRIAS data platform (SOUBEYROUX et al. 2021). Among the available datasets, the product CNRM-CM5 Aladin63 V3, produced in 2020, is used in this study. The following variables were selected: Minimum temperature of the day, maximum temperature of the day, daily rainfall, and daily potential evapotranspiration following FAO method (ALLEN et al. 1998). Those variables are simulated for the historical period (1976-2005) and 2006-2100 for three emissions scenarios: RCP2.6, RCP4.5 and RCP8.5

Seasonal metrics calculation

To describe hop growing season characteristics and their evolution with climate change scenarios, three bioclimatic indices were calculated (Table 1).

Index	**Equation**	**Detail**
Growing Degree Days	$\sum_{01/04}^{15/09} \frac{Tmax - Tmin}{2} - Tbase$	Tbase = 5°C
Dryness index	$\sum_{01/04}^{03/09} P - ETa$	With $ETa = Kc * ETp$ using hop Kc from FAO
Number of extreme temperature events	$\sum_{01/04}^{15/09} (Tmax > 35)$	Count the number of days where maximum temperature is above 35°C

Results and discussion

Bioclimatic indices evolution and variability

Figure 1 details the evolution of the calculated bioclimatic indices on a pixel located near Agen in Lot-et-Garonne, where 5 ha of hop are planted. For the historical period, the median of growing degree days reaches 2150 and the simulation suggests an increase around 2300 in the near future. In medium and far future, the calculation is around 2400 with RCP2.6 and RCP4.5, and around 2500 and 2700 in medium and far future, respectively, with the RCP8.5 model. As an example, in 2022 GDD accumulation reached 2330°, similar to what is expected in the 2011-2040 period. From near future and for every emission scenario, seasons warmer than 2022 can also be expected because 2022 GDD is equivalent to the median values and variability is high around the median. Associated with stabilized or decreasing annual rainfall amounts, growing seasons in warmer conditions will increase hop evapotranspiration and water demand increasing water deficit and irrigation needs. In all scenarios and all periods, irrigation needs are simulated to be higher. Nevertheless, the higher the emission scenario, the higher is the water deficit. The same tendency is expected regarding the rise in extreme hot temperature events during the season. Maximum temperatures above 35°C are expected to be more frequent and more intense.

The evolution of average growing degree days and dryness index for some periods and scenarios are presented in Figures 2 and 3. Only RCP4.5 is shown for near future as all the scenarios are similar for this period. For far future, RCP 4.5 and 8.5 are presented because they represent respectively an expectable scenario given actual policies and a worst-case scenario. The southern part of the region is mountainous, so rainfall amounts are currently higher and cumulative temperatures lower. The North-East part is also under the influence of the Massif Central mountains leading to lower growing degree days and higher rainfall amounts for historical period. Average growing degree days for a given period and scenario will increase homogeneously over the region. For the dryness index, South and North-East of Nouvelle Aquitaine present supplementary water for hop production for historical period. The rest of the area is between 0 to -100 mm on average for historical period. Dryness will increase for all the region and higher altitude areas may be more affected by rainfall reduction so dryness might deepen in a higher amount.

Sustainability and risks for hop production in Nouvelle Aquitaine

Climate change in Nouvelle Aquitaine, according to RCP, is characterized by an increase in temperatures, rainfall reduction and increase in extreme temperature events. This agrees with the tendency observed in weather measures of the last two decades and simulations using SSP in the last IPCC report (IPCC 2022).

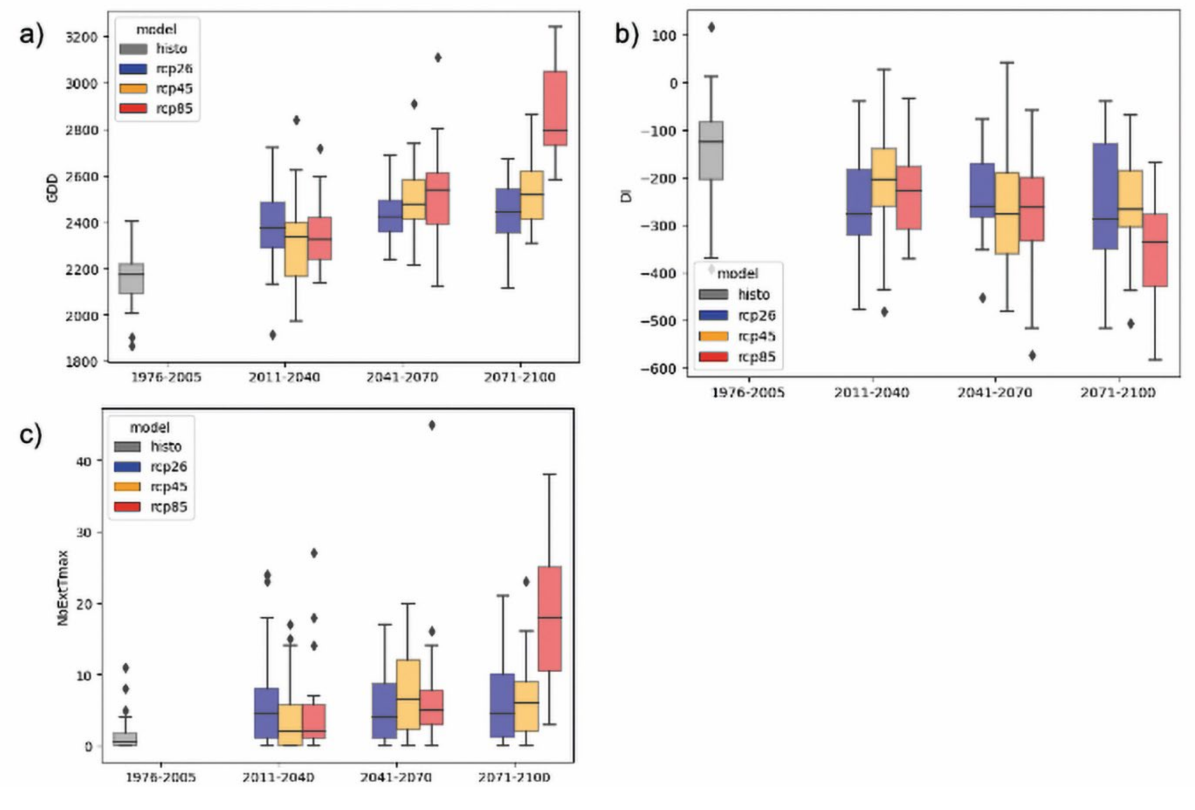

Figure 1. Bioclimatic indices evolutions in Lot-et-Garonne by period and climate change scenario a) Growing degree days, b) Dryness index, c) Number of extreme temperatures events

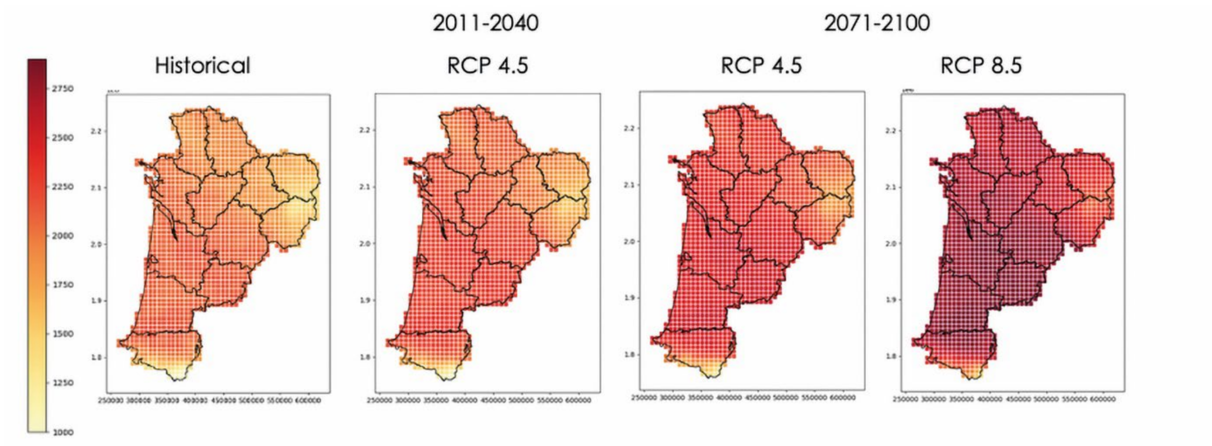

Figure 2. Growing degree days maps over Nouvelle Aquitaine for historical period, near future and RCP 4.5 scenario, far future and RCP 4.5 and 8.5 scenarios

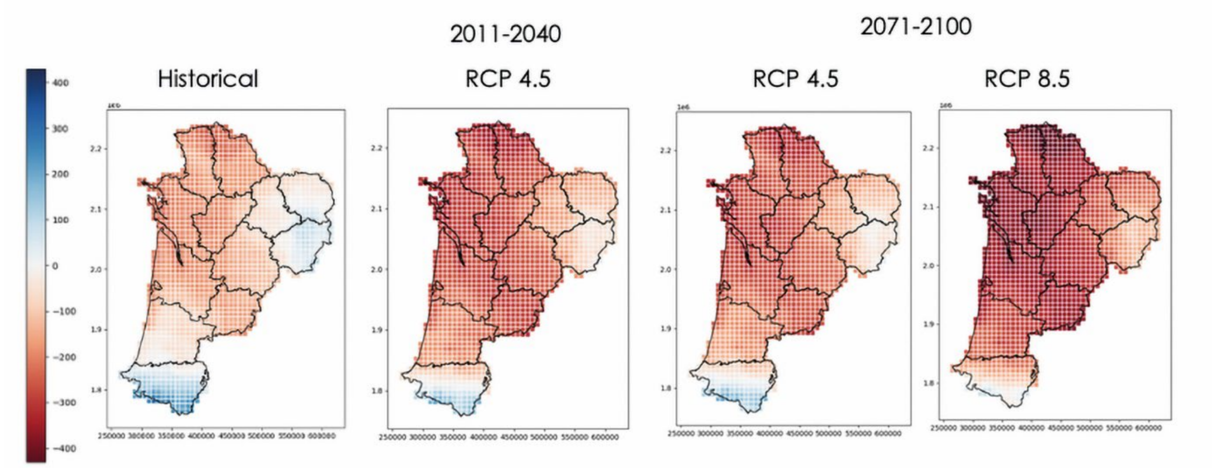

Figure 3. Dryness index maps over Nouvelle Aquitaine for historical period, near future and RCP 4.5 scenario, far future and RCP 4.5 and 8.5 scenarios

For hop production, an increase in temperature is also associated with a rise in GDD during the season. As a response, pruning date adaptation might be sufficient in the first place to avoid precocious flowering and variety change might be anticipated in the second place. To choose the most adapted variety, more detailed research dedicated to variety response to weather conditions is necessary. Besides, as higher temperatures are also associated with more frequent extreme temperature events, decrease in hop production and quality can be expected as in 2022. In the South-West of France, hop varieties responded differently to the extreme conditions of 2022, which emphasizes the research need in variety physiological response to temperatures. Finally, hop yards in Nouvelle Aquitaine are already irrigated. But dryness worsening encompasses national water management policies and cannot be managed by an increase in water consumption only. Improving irrigation management and strategies, incorporating new water sources such as recycled water, developing new varieties more tolerant to hydric stress can help in maintaining hop production in Nouvelle Aquitaine. Furthermore, extending this study to other regions or countries might help in apprehending climate change impact on hop production at a larger scale and ensure hop production sovereignty.

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VIII: Hops and aroma

Identification and genetic regulation of aroma relevant compounds in hops

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Abstract

As prolonged droughts and hot summers become more frequent, several economically significant hop varieties are suffering from declining yield and lower quality. This poses a significant challenge to the brewing industry, which relies on a steady supply of high-quality hops to produce consistent and desirable flavors in their products. To address this challenge, breeders need to develop climate-adapted hop varieties with desirable aroma traits. This requires a focus on accelerating the breeding process through targeted selection of resistant genotypes that contain desired aroma-relevant metabolites.

To obtain a better understanding, which metabolites contribute to a certain aroma expression of hops, a first 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). A Best Linear Unbiased Predictor method was used to calculate the sensory score for each sample. This experiment was followed by another sensory evaluation. In the second run, 297 genotypes and 321 phenotypes were evaluated in four categories (citrusy, fruity, resinous, onion/garlic) by 15 panelists. The principal component analysis (PCA) based on the rating in the different categories showed a broad differentiation between the phenotypes in both years. However, the pattern of the PCA remains almost the same.

Subsequently, an untargeted chemical analysis of the volatile metabolomes of all phenotypes of both years was conducted, using headspace solid-phase microextraction followed by gas chromatography and mass spectrometry. To identify aroma-related metabolites contributing to a particular aroma, several correlation analyses were performed, and a set of potentially causative metabolites was identified. This selection was then used as the basis for a genome-wide association study to find metabolite-associated single nucleotide polymorphisms.

From the olfactory signature of hop cones to the aroma profile of beers: screening of volatile compounds by GC/MS

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Hop (*Humulus lupulus* L.) is an essential plant for the brewing industry. The choice of the hop variety and the way to use it in the brewing process are the key factors to obtain the final aroma of the beer. This ''aroma signature'' of a specific beer is largely due to the diversity of volatile compounds it contains. These volatile compounds can be determined by gas chromatography-mass spectrometry (GC/MS) analysis of extracts prepared from hop cones (SHARPE & LAWS 1981). However, the volatile compounds of these cones undergo significant chemical changes during the brewing process as well as biotransformation during fermentation (KING & DICKINSON 2003).

For this reason, the volatile compound profile of hop cones does not strictly reflect the specific "olfactory signature" of a beer. In practice, to find out the influence of a hop variety on the final aroma of a beer, it is necessary to produce a brew, then to make the fermentation and the analysis of its aroma profile by GC/MS. Even in a pilot brewery, this process requires a minimum of 20 L of beer per hop variety to be tested. This approach makes the screening of many hop varieties difficult, or even impossible, to set up for different reasons of time, cost, and space.

At Twistaroma, we have already shown that the analysis of volatile compound profile by GC/MS can be performed with only a few milliliters of beer. Therefore, we developed a miniaturized and automated brewing and fermentation system on a platform called AMBERS (Automated Mini BEeR Screener) via the use of a modified Multi-Purpose Sampler (MPS, Gerstel) allowing the production of more than 120 mini beers of 5 mL in a single batch.

In this study, we analyzed the cones of 14 hop varieties by SPME-GC/ToF-MS. These included five French varieties, viz. Aramis, Barbe Rouge, Elixir, Mistral, and Triskel, and nine varieties of foreign origin: Cascade, Centennial, Chinook, Citra, and Mosaic from the USA, and Hallertau Blanc, Huell Melon, Mandarina Bavaria, and Solero from the Hallertau. The corresponding mini-beers made from these 14 varieties by SBSE-TD-GC/ToF-MS were compared with the respective aroma profiles. We detected more than 229 compounds in the hop cone extracts and 244 compounds in the mini beers.

Several important results were obtained from this study. Firstly, molecules such as geraniol, which have a floral odour descriptor, were detected both in the hop cones and in the resulting mini-beers. They are therefore essential markers of the raw material and the final product. This indicates that the identification and quantification of these compounds in hop cones can provide a detailed understanding of what happens to them in the beer. Secondly, we also observed that some compounds, such as caryophyllene or beta-myrcene, which are very present in hop cones, are almost totally absent from mini-beers made from these hops. Moreover, these compounds have only a slight influence on the final aromatic composition of the beer under our laboratory conditions. Then, some compounds, such as isoamyl acetate (an ester with a banana aroma), come exclusively from the yeasts used in our experiments and are not associated with the presence of hops.

Finally, after statistical analysis (Metaboanalyst), we identified 26 volatile markers in hops that correlate with the concentration of linalool, which is a quality marker in beer. Fourteen markers were positively correlated with the concentration of linalool in the mini-beers, while 12 were negatively correlated.

Our work shows that it is possible to choose one hop variety rather than another according to the desired aroma profile of a beer, based on analytical aroma chemistry data. In the long term, the idea is to be able to predict the organoleptic quality of beer via the chemical characterization of hop cones. To conclude, this work also opens an interesting perspective for hop breeding, since it can help breeders of new varieties in their mass selection programs, thanks to the powerful tool called AMBERS platform.

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IX: Diseases and hop quality

Things aren't quite what they used to be? Availability of pesticides for use in hops in Germany in the 21st century

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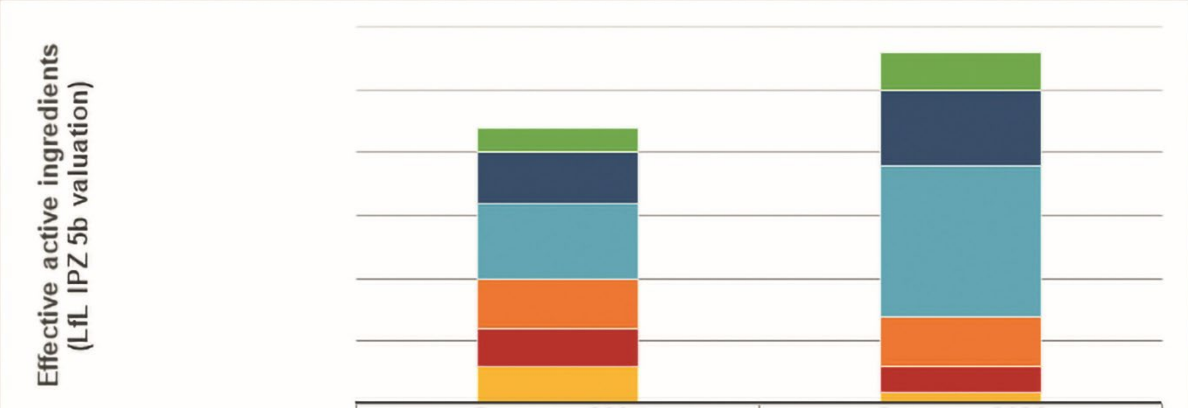
Several pests occur in hops (*Humulus lupulus* L.) during the field season. In the past, this occasionally led to complete crop failure. For this reason, in addition to preventive and non-chemical measures, the availability and use of plant protection products have always been a very important production factor of yield stability and quality assurance in German hop cultivation. As early as 1928, in his book "Hopfenbau und Hopfenbehandlung", FRUWIRTH (1928) wrote that plant protection products (PPPs) such as potassium salt of natural fatty acids are used in hop growing. Easy and highly effective pest control became possible with the introduction of chemical-synthetic pesticides (*e.g.*, phosphoric acid compounds) around 1950. Chemical-synthetic plant protection has been used in hop growing ever since. However, because of EU Regulation 1107/2009, during the past 10 years the EU has adopted the strictest PPP registration rules in the world (PM/LEY 2023). This also affects the registration or re-registration of highly effective active substances, especially for field applications. For example, the EU Commission terminated the outdoor use of the neonicotinoids imidacloprid (Confidor® WG 70) and thiamethoxam (Actara®) in 2018 (BVL 2018). Both products had already been registered in hop production for years and thiamethoxam in particular was an extremely important component for the control of soil pests such as the alfalfa snout weevil *Otiorhynchus ligustici.* To this date, no substitute product for Actara® has been approved for use in German hop production. It seems that the situation for German hop growers is getting progressively worse. But is this truly the case or are appearances deceptive? In the following, the current registration situation in German hop production is compared with that of 2001.

The data in Figure 1 indicates that fewer effective active substances were registered in hop production in 2001 than in 2023. However, a closer look shows that fewer insecticides are registered today. The situation is particularly critical in the case of soil pests: since the removal of Actara®, there is no approved plant protection product available for either wireworms (*Agriotes spp.*) or alfalfa snout weevil.

In Germany in 2001, damson hop-aphid *Phorodon humuli* could be controlled with three active ingredients. From 2019 – 2021 only Teppeki, with the active ingredient flonicamid, was available for hops. In 2022, the active ingredient spirotetramat (Movento® SC 100) was registered under Article 33. Therefore, there are currently two effective ingredients registered for aphid control. The registration of spirotetramat was expected around 2010, according to documents of IPZ5b. Especially after the removal of Confidor® WG 70 and Plenum® 50 WG, an approval of Movento® SC 100 would have been very important from a technical perspective. The fact that Teppeki was the only registered product against the hop aphid for many years was neither reasonable regarding to the development of resistances nor was it sufficient for the control of the hop aphid in every season. Therefore, multiple emergency registration for 120 days for Movento® SC 100 were necessary. After expiry of the registration for Movento® SC 100 on 30th April 2025, only the active ingredient flonicamid will again be available for aphid control. The product Sivanto® Prime (flupyradifurone) is still not approved for field use in Germany. To our knowledge, no other products are currently in the registration process for aphid control in hop production.



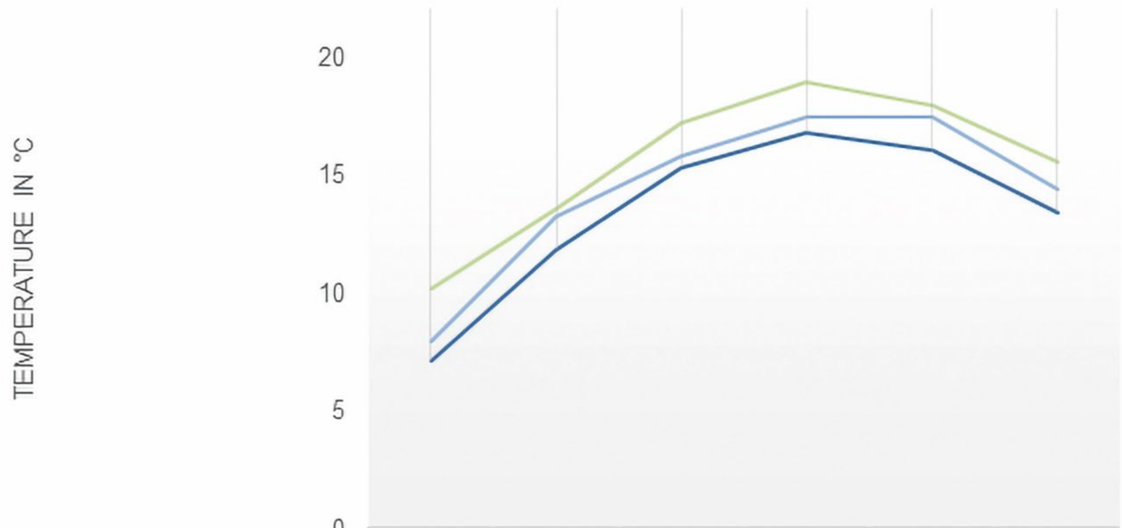
	Germany 2001	Germany 2023
defoliation	2	3
powdery mildew	4	6
downy mildew	6	12
twospotted spider mites	4	4
aphids	3	2
soil pests	3	1

Figure 1. Number of effective active ingredients registered in 2001 (LBP 2001) compared to 2023 (LFL 2023). The efficacy was evaluated by LfL, IPZ 5b (Crop Protection Working Group). This evaluation is based on field trials, experience, scientific data, and information. No distinction was made between chemical-synthetic, chemical, or biological active ingredients.

The number of active ingredients for the control of two-spotted spider mite *Tetranychus urticae* is similar comparing the situation in 2001 and 2023. However, due to higher temperatures, as shown in Figure 2, the pressure of *Tetranychus urticae* has also increased. The higher the temperatures, the higher the reproduction rates of two-spotted spider mites.

In recent years, powdery mildew *Sphaerotheca macularis* has become a much more important pathogen in hop production. The most important German cv. Herkules is susceptible and particularly requires many applications, which is certainly also due to the fact that no curative active ingredients are available. This means that preventive applications must be made continuously. For these preventive applications, there are currently just six active ingredients and five products available. These are three chemical-synthetic products and two chemical ones – sulfur and Kumar (Potassium bicarbonate). Experience shows that in general the protective effects of our available products last 10 to 14 days. The effect of sulfur and its duration is very depending due to weather conditions. In certain areas and for susceptible varieties the available active ingredients and the number of permitted applications is already insufficient to produce high-quality hops.

For hop stripping, three active ingredients are currently approved. In 2001, the German hop growers could only use two different products to defoliate the first meter of the bine. But one of the three active ingredients, which are registered now, has a very low US Maximum residue level (MRL) and the other two active ingredients require mixing partners like nutrient solutions to achieve the desired effect.



	April	May	June	July	August	Average
25-year Ø (1961 - 1985)	7,1	11,8	15,3	16,8	16,0	13,4
15-year Ø (1986 - 2005)	7,9	13,2	15,78	17,4	17,4	14,3
15-year Ø (2006 - 2020)	10,1	13,5	17,2	18,9	17,9	15,5

Figure 2. Comparison of monthly mean values of different periods, weather station Hüll

Global warming has not only negative effects on the plant protection situation. For example, downy mildew *Pseudoperonospora humuli* is much less of a problem in hot, dry summers than it is in cool, rainy summers. Regarding to active ingredient availability, the situation has improved significantly compared to 2001.

In summary, now absolute more effective active substances are available in hop production in Germany as compared to 2001. A differentiated look reveals major difficulties and a loss in the number of products with a good efficacy.

Changing climatic conditions lead to a changing pest and disease situation also in hop production. Considering the pathogen situation individually, the number of registered plant protection products in 2023 can be assessed as follows: Downy mildew has a good to sufficient supply situation by now, equally to hop stripping. For hop aphids, the situation is still adequate with two approved products, as well as for the two-spotted spider mite with four products. For soil pests and powdery mildew, the situation is no longer satisfactory. But even though the raw number of registered products in different crops is frequently discussed by experts and the society, this approach seems not to be suitable to describe the problems and needs of modern agriculture and hop growing. If an important, effective and for its use very appropriate product is cut sometimes it is necessary to have more than one additional application or more than one candidate for substitution to cope with the removal. It depends on the efficacy of the substitution candidate.

Unnecessary applications of plant protection products must be reduced as far as possible, no matter if the product is listed as chemical-synthetic, chemical, or biological. With all honorable actions to reduce the use of plant protection it remains crucial that it is still possible to perform an integrated pest and disease management to produce high-quality hops. Otherwise, yield supply safety and quality matters can become more and more under pressure, which inevitably leads to a less competitive German hop production especially on an international level.

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How do diseases influence hop quality?
Part 1: Influence on the quality characteristics of hops

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Hops are exposed to pests and diseases that are combated in conventional agricultural practices with synthetic chemical pesticides. Plant protection agents of the future, however, will not be highly effective “cudgels”. Their improved compatibility with the environment will be offset by their limited efficacy. As long as there are no resistant hop varieties, hop cultivation has on the one side to use pesticides and on the other side therefore to tolerate moderate disease and infestation levels in the future. As part of beer brewing orthodoxy, diseased and infested hops are thought to negatively impact the quality of beer. This rationale is anchored in the fact that a slightly musty odor is evident in some hops heavily infested with powdery mildew *Sphaerotheca macularis* or downy mildew *Pseudoperonospora humuli*. The focus of our research was to determine the effects of massive disease infestations on the compounds in hops and on the resultant beer quality.

Different approaches were taken to collecting samples in crop years 2018 to 2021:

- A total of 46 infested lots were selected from the crop years 2018 and 2019. The hops were separated manually into only green, uninfested cones and brown, infested cones.
- The Bavarian State Research Centre for Agriculture (LfL) regularly conducts testing on plant protection agents which are in the approval process. In this process, a portion of the hop bines in a plot are specifically treated against a disease, while another portion remains untreated. This ensures that the infestation is attributable to a defined pest. Eight comparisons for the crop years 2019 and 2020 were available.
- Three uninfested lots and three lots heavily infested with powdery mildew were sampled from crop year 2020 and compared. For this year, it was also possible to carry out large-scale processing of uninfested and heavily powdery mildew infested Herkules lots, converting the hop cones into pellets and subsequently into CO_2 hop extract.
- In some instances, hops from crop year 2021 exhibited an extensive downy mildew infestation yielding four comparisons.

All samples were analyzed for bitter substances, while aroma substances, polyphenols, mycotoxins, and chlorophyll were measured in selected samples. In addition, 17 comparisons of uninfested and infested hops were carried out in trials through the production of strongly late-hopped and dry-hopped beers.

The results from the research trials can be summarized as follows:

- Separation of the 46 samples into green and brown cones revealed no differences in α- and β-acids or the HSI (Hop Storage Index) values. Four comparisons of the hop aroma compounds also yielded identical results. No peaks occurred in infested samples which might indicate the formation of aroma compounds brought about by infestation.

- The samples collected by LfL for the 2019 and 2020 crop years were grouped by the type of infestation: hop aphids, spider mites, downy mildew, and powdery mildew. Variations in the concentrations of bitter and aroma compounds among the infested and uninfested samples were negligible. Additional “infestation peaks” could not be detected in the aroma-chromatograms. The particularly hazardous aflatoxins and ochratoxin A were not present in any of the samples. Tenuazonic acid was detectable at harmless concentrations in the mildew-infested hops. However, the chlorophyll content is lower by 20-70 % because of infestation.
- Heavily mildew-infested and uninfested bitter varieties (2020 crop year) from commercially available lots showed a 45 % degradation of chlorophyll, on average. Ochratoxin A and aflatoxins were not detectable in any of the samples. Furthermore, there were no significant differences in bitter or aroma compounds.
- Herkules lots, which were heavily infested with powdery mildew, as well as uninfested lots were processed into pellets and then into CO_2 extract. Aflatoxins and ochratoxin A could not be detected in either group of pellets, but tenuazonic acid could. The chlorophyll content was 40 % lower in the infested pellets. Although the batches were not from the same lots, the two sets of pellets and the extracts prepared from them were almost identical in terms of bitter and aroma compounds.
- Four comparisons of heavily downy mildew-infested batches with uninfested ones exhibited a chlorophyll loss of one-third. Mycotoxins were not detectable in any of the samples. No noteworthy differences were detected in either the bitter or aroma compounds.

Conclusion

Grouping the 62 comparisons of the hop cone samples, the LfL trials and all other lots, the following picture emerges:

- In 28 cases, green and brown samples were indistinguishable.
- In 15 cases, the values for green samples were higher than for brown ones.
- In 17 cases, the opposite was true.

Hops heavily impacted by disease and infestation with pests exhibited no measurable damage to the relevant hop compounds compared to uninfested hops. Only intensive brewing trials will be able to elucidate the influence of diseases on hop quality.

How do diseases influence hop quality? Part 2: Influence on beer quality

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The comparison of heavily infested and not infested hop cones, partial lots and commercial lots lead according to Part 1 of this study to the following conclusions:

- The secondary metabolites of hops that can be measured in our laboratories (bitter substances, aroma compounds, and polyphenols) do not significantly suffer from an infestation with pests or diseases.
- Occasionally infested hops show an “off-flavor” but no additional aroma substances are detectable. Still open is the question whether thiols could be made responsible for that.
- Any type of infestation damages the chlorophyll by 20 to 70 %. Even though the cones/hops/pellets can merely be described as brown, there is obviously still enough residual chlorophyll for metabolic processes and so for the biogenesis of aroma and bitter substances as well as polyphenols.
- As the analytical data did not leave any indicators for a degradation, only brewing trials could yield some insights. Although it has been expected that in the absence of analytical differences in the hop samples the usual procedures could hardly show any differences of the degree of infestation in the beer, the beers have been analyzed thoroughly. Consequently, the sensory evaluation of the beers has been paramount.

At the beginning of the four-year study the expectation prevailed that beers produced with infested hops can be distinguished from the ones made with uninfested hops, which did not prove true.

17 comparisons of uninfested (“green”) and infested (“brown”) hops in beers were produced at the 2-hl pilot brewery in St. Johann with following characteristics and examinations:

- Bottom-fermented, strongly hopped pale lagers. In order to detect possible sensory anomalies due to foreign odors in the “brown” batches, late hop additions at the end of the boil and in the whirlpool were the rule, often combined with dry hopping.
- Beer analysis included IBU and HPLC of bitter components, total polyphenols, linalool, and foam.
- Three sensory evaluations:
    - Triangle or three glass tests in direct comparison of green and brown beers; one of the beers is presented in two glasses, the other in one glass. First, the taster must select the sample that is different from the other two. Tasters who match the samples correctly, were asked to answer questions on preference and reason for a preference (*e.g.*, quality of bitterness or aroma).

- Tasting according to a modified scheme of DLG (German Agricultural Society): most interesting is an average of seven sensory attributes and the quality of bitterness in points 1-5 and at least the preference ranking according to a statistical test of Kramer.
- Tasting using a CMA (German Central Marketing Association for Agricultural Products) scheme for hop aromatic beers with the evaluation of five characteristics, *i.e.*, fruity, floral, citrusy, green-grassy and hop-spicy (in 1-10 points).
- Additional queries were made of the participants regarding any unusual, strange or unpleasant sensory impressions in the aroma or flavor of the beers.

Conclusion

The results can be summarized as follows:

Beers brewed with heavily infested hops showed no clear defects compared to uninfested hops, despite when using high dosing rates in late and dry hopping. Nevertheless, some comparisons showed a tendency to favor beer brewed with green hop lots in sensory tests. In conclusion, given that severe disease infestation does not cause detectable deficiencies in hop compounds and beer quality, it follows that moderate infestation will also not exert negative effects on beer. However, at this time, these results do not reflect the doctrines generally accepted by those in the world of brewing.

The current objective in combating disease in hops with pesticides is producing green hops with no visible signs of infestation or disease. Maybe the future goal of disease control will be to maintain the quality of the relevant secondary metabolites especially in the lupulin glands and, in particular, that of the lupulin itself to the greatest extent possible and not simply to retain a green color in the hop cones.

X: Posters

Viruses, viroids, and pathogenic fungi of hops

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Abstract

The biodiversity of all organisms associated with their ecosystems and agricultural diversity is the basic source of productivity of agricultural systems. Among other things, they allow their adaptation to changing environmental conditions and maintaining their basic functions.

The basic objective of the national program of the preservation and use of genetic sources of microorganisms and small animals of economic importance (NPGZM) is to ensure long-term preservation of genetic sources of plants, animals, microorganisms, and small animals important for nutrition and agriculture. This is done in accordance with national legal regulations, international obligations and needs of users of genetic resources and for sustainable development of agriculture, adaptation to change climate and maintaining the quality of rural space. The NPGZM is governed by Act 148/2003 and Decree 458/2003, associates 22 collections for 13 organizations including Crop Research Institute v.v.i. in Prague, which coordinates the activities of NPGZM.

Collection of pathogens of hops (SPCh) plays an important role in the context of biodiversity conservation of selected pathogens and serves also as a collection of positive controls for diagnostic and research activities.

In the isolated greenhouse, 46 hop plants containing ApMV, HMV, HLV, HLVd, and HSVd and their mixed infections were retained in 2022. Cultivation in nutrient medium in Petri dishes is carried out by 18 isolates of *Verticillium nonalfalfae* and 3 isolates of *Verticillium dahliae*, 170 isolates of pathogens are maintained in culture in vitro, 74 isolates are maintained in tubes over calcium chloride, 251 isolates are preserved in dried form, and 190 isolates are stored by lyophilisation.

The data are transmitted to a central database, which is managed by the Crop Research Institute v.v.i. in Prague (www.vurv.cz/mikroorganismy/).

Acknowledgment

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The CBCVd research project in the Hallertau

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Abstract

Citrus bark cracking viroid (CBCVd) is a pathogen in hops that is currently found in Slovenia, Germany, and Brazil. Symptoms include severe stunting, reduced internode length, small leaves, and abnormally formed cones. Ultimately, CBCVd causes a major reduction in yield an alpha acid content and can lead to plant death. CBCVd belongs to the family of Pospiviroidae and is a single stranded, self-replicating circular RNA that adopts a rod-like conformation. Despite being only approximately 284 nucleotides in length, CBCVd causes major symptoms by silencing of host genes. Pospiviroidae replicate in the nucleus by taking advantage of the hosts transcriptional machinery and can be found in all parts of the plant. Therefore, they are easily transmitted via plant sap by cultivation techniques. Since plants cannot be cured once they are infected, spreading of the disease can only be limited by grubbing of infected plants. A large monitoring programme in the Hallertau region revealed that CBCVd is limited to a few farms and currently is assumed to spread slowly. To keep the infection pressure in the region on the lowest possible level, it is important that affected farms take effective countermeasures at an early stage after first detection of the pathogen. This research project aims to establish evidence based practical guidelines for best agricultural practice following the discovery of CBCVd in a hop garden. Over the course of three years, adapted cultivation and disinfection techniques will be tested for their efficacy to prevent CBCVd transmission. Furthermore, we aim to find out how long hop cultivation must be paused after clearing of a heavily infested field in order to avoid re-infection from remaining plants or root parts. Finally, a variety garden with hop varieties of diverse genetic background will be planted and monitored for differences in symptom development after CBCVd infection to identify potential tolerant or resistant cultivars.

Key words. Hop, *Humulus lupulus*, Citrus bark cracking viroid, CBCVd, stunting disease

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Suspension cultures for interaction studies of hop (*Humulus lupulus* L.) and CBCVd

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Abstract

Hop (*Humulus lupulus* L.) is a perennial plant, important to the brewing industry. Hop production is threatened by various pathogens, including viroids. Slovenian hop production has recently been threatened by the newly discovered Citrus bark cracking viroid (CBCVd), which was also recently confirmed in Germany. The CBCVd viroid causes a severe hop stunt disease (JAKŠE et al. 2015). Despite many years of studies on viroids, their pathogenesis is still not fully understood (NAVARRO et al. 2021). Most hop cultivars, including the most grown Slovenian cultivar Celeia, are highly susceptible to infection with CBCVd. However, a recent resistance screening revealed that in cv. Styrian Wolf CBCVd infection could not be confirmed even after several rounds of artificial inoculation. Tissue and suspension cell cultures have proven to be an important tool to facilitate basic research in plant science as they provide homogeneous plant material in a controlled environment (MUSTAFA et al. 2011). With their implementation, we can also skip the half-year dormancy of hop plants. They are suitable for the study of viroid-cell interactions, mainly because the environmental and growth conditions can be easily controlled and standardized. We have successfully established viroid-free callus cultures of two hop cvs, Celeia and Styrian Wolf. The viroid-free callus was used to prepare suspension cultures. Dimeric in vitro transcripts were used to infect the viroid-free callus to produce CBCVd-infected suspension cultures. Strand-specific RT-qPCR was used to confirm the presence of CBCVd RNA strands with positive and negative polarity. We were able to detect the negative strand of the CBCVd viroid in suspension cultures of both hop cultivars, indicating that infection with the in vitro transcript of CBCVd was successful even in cv. Styrian Wolf. We sought to determine the effects of CBCVd infection on cell viability and biomass accumulation. No differences in cell viability and biomass accumulation were observed between viroid-free and CBCVd-infected cell suspension cultures. By daily sampling of biomass from infected suspension cultures of cv. Celeia, we determined the occurrence of negative polarity of the viroid strand. The results indicate, that in the suspension cultures, the synthesis of the negative strands of the viroid CBCVd begins at approximately day 15 post-infection. We believe hop suspension cultures could be a useful tool for further studies on the molecular mechanisms and effects of CBCVd infection in hops.

Key words. viroid; CBCVd; plant cell cultures; pathogen-host interactions; resistance

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Evaluating resistance, tolerance, and sensitivity of tissue culture hop cultivars to CBCVd

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Abstract

Three different viroids are threatening Slovenian hop gardens: hop latent viroid (HLVd), hop stunt viroid (HSVd), and citrus bark cracking viroid (CBCVd). Recently, field observations and testing in Savinjska valley in Slovenia have led to the impression that hop varieties can be divided into three groups based on their susceptibility to CBCVd: the largest group comprises sensitive varieties, followed by tolerant varieties, and finally, the most interesting group comprises resistant varieties that do not develop symptoms and cannot be infected with viroids using currently established methods.

In this study, we evaluated the susceptibility of 13 hop cultivars towards CBCVd. The cultivars were maintained in tissue cultures, which ensured their uniformity and prevented any pre-existing infections. We also developed a method for sterile artificial inoculation, which allowed us to control the timing and quantity of the viroid inoculum, thus minimizing any experimental variation.

The cultivars were inoculated with CBCVd transcripts and monitored for seven weeks. Disease severity index (DSI) was assessed regularly to quantify the symptoms. After seven weeks, the CBCVd infection was confirmed by performing RT-qPCR (GUČEK et al., 2019) on samples from the upper four leaves of each cultivar which developed after the infection.

Our results showed that all 13 tested hop cultivars were susceptible to CBCVd infection. Interestingly, our evaluation of the CBCVd infection did not reveal any significant difference in the DSI among the 13 tested hop cultivars. However, RT-qPCR analysis suggested varying levels of CBCVd infection between cultivars. To confirm these findings, we plan to use qPCR with primers designed to specifically anneal to the negative viroid strand, which will provide a more accurate evidence of CBCVd replication and accumulation.

In addition to CBCVd, we are also conducting similar experiments to assess the susceptibility of hop cultivars to HLVd and HSVd. The results of these studies will provide valuable information on the susceptibility of Slovenian hop cultivars to these viroids, which pose a serious threat to hop production. It will also provide information on suitability of tissue culture experiments for viroid research in hops. The findings can aid in the development of effective viroid management strategies, including breeding and can ensure the long-term sustainability of hop production.

Key words. Viroid, CBCVd, transcript, hop, tissiue culture

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Diagnostic methods for detection of hop viroids

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Abstract

Incurable plant diseases caused by viroids can limit hop production and quality and result in significant losses. Such an example is citrus bark cracking viroid (CBCVd) which, since its first finding in 2007, affected nearly 500 ha of Slovenian hop yards. Implementation of strict phytosanitary measures significantly reduce infection potential, however CBCVd infections are still present and cause constant threat to the hop production. An important factor in preventing its spread is the use of rapid and reliable diagnostic tools. Due to the nature of viroids, which are non-coding naked RNAs, their detection is limited only to RNA level. Viroids are commonly detected by hybridisation, PAGE, RT-PCR, Real-time RT-PCR, RT-LAMP, and other methods. They enable reliable identification of viroids but, due to the spread of multiple viroid infections, they can be hindered because of the presences of several different viroids in one sample. For detection of hop viroids, we developed and validated multiplex RT-PCR (mRT-PCR), single and multiplex RT-qPCR (mRT-qPCR) assays. The assays appeared to be robust, reliable and suitable for screening of hop viroids on a large-scale to prevent further spread of disease. In addition, we are developing CRISPR/Cas-RT-RPA assay that has the advantage of being applicable in field-based scenarios, as the tests require minimal sample preparation and are performed at constant temperature without the use of sophisticated equipment. The use of CRISPR system (clustered regularly interspaced short palindromic repeats) represents great potential in plant diagnostics, due to its sensitivity, specificity, speed, flexibility, robustness and ease of use. CRISPR in combination with RPA (recombinase polymerase amplification) and LFA (lateral flow assays) allows fast detection, which is also suitable for field use. The main goal of our project is to develop the CRISPR/Cas-RT-RPA method for the detection of CBCVd viroid, which will allow fast detection, without prior RNA isolation, while being sensitive and suitable for field use. The project will represent a major advance in hop research as well as plant diagnostics in general. The method will be used for fast detection of new outbreaks, testing of hop seedlings, evaluation of cultivar resistance and epidemiological studies. This will limit the further spread of the disease and mitigate the economic damage in hop growing.

Key words. diagnostics, CBCVd, CRISPR/Cas-RT-RPA, lateral flow assay, field use

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User-friendly and rapid pathogen detection in high-throughput sequencing data as demonstrated for hop viroids

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Abstract

The number of regulated plant pathogens is constantly rising and changing due to international trade, invasive species, and global warming. Furthermore, modern highly sensitive methods such as high-throughput sequencing (HTS) make it possible to detect several pathogens in parallel in a single analysis. However, the bioinformatic analysis of HTS data requires the user to have computer coding skills and, considering the file size and demanding algorithms, access to high-performance computing. To overcome those constraints, the user-friendly Microbe Finder (MiFi®) web-based tool has been developed at Oklahoma State University (ESPINDOLA & CARDWELL 2021). Here, we compare the e-probes based MiFi tool, with mapping of raw and cleaned reads to reference genomes and (q)PCR data. We compare the sensitivity, discuss limitations, and estimate the time investments per analysis. Our study object is hop (*Humulus lupulus* L.) either infected or uninfected with the citrus bark cracking viroid (CBCVd), the hop latent viroid (HLVd) or the hop stunt viroid (HSVd). In particular, CBCVd is leading to production losses for the European hop industries, especially in Slovenia and Germany. However, HLVd is affecting hop quality and HSVd is a production constraint in the US, but also a potential thread for European hop producers as well.

The results show that the curated viroid e-probes are highly specific and indeed qualify as a quick method for pathogen detection from Illumina and Ion torrent sequencing data. Furthermore, our e-probes did work with data derived from the sequencing of total RNA, double-stranded RNA, or small RNA. A constraint of the MiFi® tool might be that the raw data needs to be uploaded to non-European servers for the analysis and that the cloud storage is limited. Therefore, we also show alternative but computationally more demanding fast data analysis methods. In any case, using fast in silico detection pipelines is a promising approach once established for several pathogens and considering that sequencing cost might further drop in the future.

Key words. Plant pathogen diagnostics, user-friendly tools, next-generation sequencing

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Mycoviruses in *Verticillium* species

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Abstract

Verticillium sensu stricto is an anamorphic genus within the pathogenic plant fungi of Ascomycetes that causes verticillium wilt, one of the most harmful vascular diseases that affects numerous crops worldwide (KLOSTERMAN et al. 2009; INDERBITZIN et al. 2011). Among *Verticillium* species, *Verticillium nonalfalfae* is particularly important for hop production, as it may cause destruction of the entire plantation upon infection with the lethal pathotype (KEYWORTH 1942; RADIŠEK et al. 2006). Mycoviruses are omnipresent viruses, that infect fungi. Some of them have been reported to decrease the virulence of their fungal host, a phenomenon called hypovirulence. In *V. dahliae* four mycoviruses have been found so far: *Verticillium dahliae* chrysovirus 1 (VdCV1), *Verticillium dahliae* partitivirus 1 (VdPV1), *Verticillium dahliae* magoulivirus 1, and *Verticillium dahliae* RNA virus 1 (VdRV1) (GAO et al. 2022). The only known mycovirus infecting *V. albo-atrum* is Verticillium albo-atrum partitivirus 1 (VaaPV1) (CAÑIZARES et al. 2014). Our preliminary results, based on small RNA sequencing and RT-PCR analysis, indicate that VaaPV1 naturally infects *V. nonalfalfae*, highly virulent isolate T2. Its impact on fungus virulence is under investigation.

Key words. *Verticillium* spp., mycoviruses, hop

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First report of *Sclerotinia sclerotiorum* (Lib.) de Bary on hop in Poland

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Abstract

In June 2021, wilted hop plants were observed in Lublin Province, Poland. Preliminary diagnosis was made based on disease symptoms, *i.e.*, wilting, wet rot of the lower part of the shoots and the presence of black sclerotia. Pure cultures of the fungus were obtained from the infected shoots and subjected to mycological analysis. Then fungal DNA was extracted, and pathogen identity was confirmed by markers differentiating *Sclerotinia* spp. and sequencing of ITS region. The pathogen was identified as *Sclerotinia sclerotiorum*. Inoculation of young hop plants with the obtained isolate confirmed its pathogenic character. This study documents the first case of *Sclerotinia* wilt on hops in Poland as well as in Europe.

Key words: *Humulus lupulus*, pathogen detection, *Sclerotinia sclerotiorum*

Introduction

Sclerotinia sclerotiorum (Lib.) de Bary is a very destructive plant pathogen with a broad host range including many crops (BOLTON et al. 2006). It can survive and accumulate in the soil in resting forms (sclerotia), which may be the source of infection of plants grown subsequently in the same field. So far, the impact of the *S. sclerotiorum* on hop has been described in North America. The disease symptoms appear in late spring under cold and humid weather; they develop mostly on shoots, in the parts close to the soil surface or just below it (KROPF et al. 2012). Infected shoots and leaves wilt and dry out. *S. sclerotiorum* infection on hops is favoured by tissue damage especially when damaged plant parts are covered with soil (MAHAFFEE et al. 2009). The disease can lead to a gradual destruction of hop plants in the plantation. The aim of the study was to confirm the pathogenic nature of the isolates obtained from diseased hop plants in Poland and to provide species identification of these isolates using molecular methods.

Material and methods

Pure cultures of the fungus were obtained from hop shoots with symptoms of necrosis and decay by multiple transplanting of mycelium on potato dextrose agar medium with tetracycline hydrochloride. Isolates were classified into the genus *Sclerotinia* on the basis of macro- and microscopic observation of the mycelium using taxonomic keys. Species identification was carried out using molecular methods. First, markers differentiating *Sclerotinia* species were amplified using primers designed for calmodulin gene: SScadF1 and SScadR1 (BATURO-CIEŚNIEWSKA et al. 2017) and β-tubulin gene: TU1, TU2 and TU3 (VLEUGELS et al. 2012). Then DNA from the fungal isolates was amplified using ITS1 and ITS4 primers (WHITE et al. 1990) and sequenced by means of Big Dye Terminator v.3.1 chemistry and 3500 Genetic Analyzer. The obtained DNA sequences were compared with sequences of *Sclerotinia* spp. available on GenBank. To confirm the pathogenic character of the obtained isolates, inoculation tests were performed on young hop plants under controlled conditions.

Results and summary

Detection of single PCR bands with both primer combinations (SScadF1/SScadR1 and TU1/TU2/TU3) was consistent with results reported earlier for *S. sclerotiorum* (BATURO-CIEŚNIEWSKA et al. 2017). Comparative analysis of the obtained ITS sequences with sequences of *Sclerotinia* spp. from GenBank confirmed the assignment of our isolates to *S. sclerotiorum*. The pathogenicity of these isolates was also confirmed as inoculated young hop plants showed wilting symptoms similar to these observed on the plants from which the isolate was obtained. *S. sclerotiorum* was reisolated from inoculated young hop plants.

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Evaluation of the use of *Beauveria bassiana* for the control of two-spotted spider mite in hop plantations in El Bolsón (Argentina)

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Abstract

The two-spotted spider mite *Tetranychus urticae* Koch is recognized as a major pest in hop cultivation worldwide. The traditional management of the pest relies on the use of acaricides, but the appearance of resistance calls for the search for alternatives. Integrated pest management strategies incorporating pathogenic fungi have great potential and promise not to affect local populations of natural enemies. In particular, *Beauveria bassiana* is widely used for the management of various pests in different crops, although it is necessary to evaluate the efficacy of applications under low ambient humidity conditions and the possibility of finding viable application doses and frequencies for growers.

Thus, our objective was to evaluate the efficacy of two doses of commercial *B. bassiana* (Bb-Protect®, Andermatt) in reducing the abundance of two-spotted spider mites in hopyards in El Bolsón (Patagonia, Argentina). The effect of the product on the population of natural enemies of the two-spotted spider mite was also evaluated. The trial consisted of three treatments: no pest management (control), application of Bb-Protect® at 150 gr/ha (T1) and application of Bb-Protect® at 300 gr/ha (T2), both every 15 days. The treatments were installed in commercial cv. Cascade hopyards with a hop density of 3.333 plants/ha. Weekly, we counted live spider mites and natural enemies on three leaves per plant, and 20 plants per treatment.

The main results show that the application of Bb-Protect® significantly reduced the number of spider mites per leaf on hop plants compared to untreated plants. The protective effect was significant for two weeks, regardless of the dose. The second application (15 days later) served to keep populations with low abundance. Even after four weeks, when the population showed exponential growth, the plots treated with *B. bassiana* had significantly lower pest abundance. Regarding natural enemies, two potential predators of two-spotted spider mites were identified: *Anystis* sp., a predatory mite of the Anystidae (Acari); and a micro-coleopteran of the Staphyliniade (rove beetles). There were no significant differences in the abundance of *Anystis* sp. or staphylinid beetle individuals between treatments throughout the trial. However, in general, the abundances of beneficials were low in all treatments.

We conclude that the use of *B. bassiana* in hop plantations in El Bolsón (Patagonia, Argentina) was effective in reducing the population abundance of the two-spotted spider mite. The effectiveness was maintained for at least two weeks, enabling the use of biweekly applications. Moreover, there were no significant differences between treatments with different doses, showing the efficacy of bi-weekly applications at the lower evaluated dose (150gr/ha). Finally, the application of the entomopathogenic fungus *B. bassiana* did not have a significant negative effect on the abundance of potential natural enemies of the two-spotted spider mite, such as *Anystis* sp. (Acari: Anystidae) and rove beetles (Coleoptera: Staphyliniade).

Exploring the potential of CRISPR/Cas genome editing in Slovenian hop cultivars

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Abstract

Hop cultivation and production are threatened by many diseases such as CBCVd (Citrus bark cracking viroid) and the soil fungus *Verticillium nonalfalfae*, which result in low plant yields and quality and thus lead to economic loss. One of the possible solutions is breeding resistant cultivars. In the last decade, genome editing, especially CRISPR/Cas9 technology, has become the method of choice to introduce precise genetic modifications and develop disease resistant cultivars (ZHU et al. 2020). To date, there is only one genome editing protocol for hops (AWASHTI et al. 2021). Therefore, we aimed to develop different approaches for CRISRPR/Cas9 vector delivery into hop cells. In our first approach, we developed a method for the isolation of protoplasts from hop leaves. We tested different concentrations of enzymes and used the optimized enzyme composition for isolation of protoplasts from different Slovenian hop cultivars. The highest yield ($8{,}0 \times 10^5$ protoplasts per g of leaves) and viability (86 %) of the isolated protoplasts was achieved in cv. Celeia. For our second approach, we first induced the formation of callus from leaves, internodes, and petioles from hop. Then, callus was transformed with *Agrobacterium tumefaciens* (*A. t.*) and different plasmids carrying reporter and selection genes. Using PCR analysis, we confirmed the presence of transgenes in up to 81 % of transformed calli, which shows that hop callus is suitable for transformation with *A. t*. In the future, we will use our established protocols to modify miRNA genes involved in the defense mechanism of hops against the fungus *V. nonalfalfae* using CRISPR/Cas9.

Key words. Genome editing, CRISPR/Cas9, hops, transformation, protoplasts

Acknowledgement

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The application of hydroponic in clonal micropropagation of hops

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Abstract

Biotechnology methods in the propagation of hops have several advantages over the traditional method. Rooting and adaptation of microplants to ex vitro conditions are critical clonal micropropagation steps for most plant species. Regenerants are characterized by weak activity of the stomatal apparatus, low photosynthetic ability, insufficiently developed chlorenchyma and a small amount of cuticular wax. A more universal and simple approach to adaptation is needed, the application of which will be equally successful both for single valuable individuals and for their large-scale replication.

Test tube plants of hop cvs Hallertauer Taurus, Spalter Select, Marynka, Sumer, Civil, Mikhailovsky, Podvyazny, and Flagman served as the material for the study. Rhizogenesis was induced on MS and ½ MS media supplemented with 20 g/L glucose and various concentrations of auxins IBA or NAA (0.5-1.5 mg/L). Rooted regenerants were adapted in various ways: in an artificial climate by transplanting into a substrate (perlite:peat in a 1:1 ratio) and into seedling cassettes (vermiculite:perlite in a 1:1 ratio) in hydroponic installations for 14-16 days, with subsequent transfer to the phytotron (Fig. 1). In the first five days, the humidity was increased by spraying three times a day.

Direct transplantation of plants from a test tube into the substrate in the hydroponics gave the most successful adaptation of regenerants with a survival rate of 95-100 %. Assessing the morphological parameters of plants testified their good condition and high growth potential.

Figure 1. A tidal type of hydroponic installation with a light period of 16/8 hours and hop plants before, during and after adaptation

Czech bitter varieties of hops

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Abstract

In the years 2011 to 2022, the Czech hop cultivars Agnus, Rubín, Vital, Gaia and Boomerang were evaluated in terms of their hop yield. The highest hop yield was recorded in cvs Gaia (2.99 kg/plant) and Vital (2.92 kg/plant). Boomerang had the lowest yield (2.11 kg/plant). The lowest variability of yield was determined in Vital (19.74 %) and the highest in Boomerang (33.29 %) and Agnus (32.98 %). Vital demonstrated the lowest decreasing trend of hop yield over a period of 12 years. Rubin showed the highest decreasing trend.

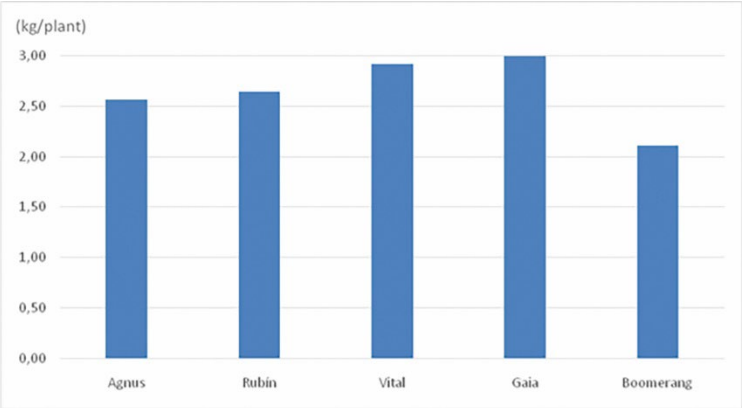

Figure 1. Average hop yield (2011–2022)

Czech bitter varieties were evaluated between 2010 and 2022. Hop varieties have an average content of alpha acids between 10.24 % w/w (Agnus) and 13.73 % w/w (Gaia). As to variability, all bittering hops have a good stability of alpha content because variability is below 15 %. All varieties show a decrease in the content of alpha acids over a period of 13 years of hop cultivation. Vital, Boomerang and Gaia have an alpha/beta acids ratio below 2. Agnus and Uran have an alpha/beta acids ratio slightly above 2. Rubín and the 5303 genotype have the highest alpha/beta acids ratio, namely above 3.

Table 1. Average content of alpha acids and its variability (2010 – 2022)

Parameter	Agnus	Rubín	Vital	Gaia	Boomerang
Average (% w/w)	10.24	11.33	12.26	13.73	11.83
Coefficient of variation (%)	13.00	9.29	9.58	9.66	11.34

Acknowledgement

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Genetic diversity of European wild hops in a historical context

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Abstract

In the early Middle Ages, wild and cultivated hops (*Humulus lupulus* L.) were found in many parts of central and northern Europe and they have been used for the preparation of beer since the 9th century. The names of traditional landraces refer to the regions and places of origin from this time. Because of these landraces have been cultivated up to now, we would like to know about their influence on wild hop populations in Europe. DNA molecular genetic methods are the most appropriate technology for this purpose. We used 16 microsatellite EST-SSR loci for cluster and PCA genetic diversity analyses of 218 wild hops and 44 landraces. Genetic diversity analyses confirmed previous results that North American wild hops are different from European germplasm and their germplasm is the origin of the old American cv. Late Cluster. It was also proved differences for wild hops from the Caucasus. Landraces from Saaz and Hersbruck were historically the most important for genetic diversity of European wild hops. Our analyses showed that the old cv. Fuggle originated in wild hops from northern France. It can be supposed that active transfer of hop plants in Europe took place in the 14th century and subsequently also in the 19th century.

Key words. wild hop (*Humulus lupulus*), landrace cultivars, expressed sequence tag-simple sequence repeat (EST-SSR), genetic diversity, cluster and PCA analyses

Introduction

In the early Middle Ages, wild and cultivated hops (*Humulus lupulus* L.) were found in many parts of central and northern Europe and they have been used for the preparation of beer since the 9th century. The names of traditional landrace cultivars refer to the regions and places of origin from this time. Because of these landraces have been cultivated up to now, we would like to know about their influence on wild hop populations in Europe. DNA molecular genetic methods are the most appropriate technology for this purpose. In our study, we used EST-SSR loci for an evaluation of genetic diversity within world wild hops and landraces

Materials and methods

The young leaf or cone samples of 218 wild hops and 44 landraces have been collected since 2005 (PATZAK et al. 2010a, b; NESVADBA et al, 2015; 2020; DUCROCQ et al., 2022). DNA was isolated from samples by CTAB method and molecular analyses of 16 microsatellite loci were carried out according to PATZAK et al. (2017). Dendrogram was based on Jaccard's similarity coefficient of 156 EST-SSR polymorphic molecular markers, determined by Neighbor-Joining (NJ) method of Unweighted Pair Group Method with Arithmetic means (UPGMA) in DARwin v. 5.0.155 (Dissimilarity Analysis and Representation for Windows, http://darwin.cirad.fr/darwin) software, visualized by Geneious Pro 4.8.2 (Biomatters Ltd., Auckland, New Zealand) software. The principal coordinate analysis (PCoA) was also conducted by DARwin software based on a genetic similarity/dissimilarity matrix. Three-dimensional graph was visualized with SigmaPlot for Windows v.10.0.0.54 (Systat Software Inc., San Jose, CA).

Results and discussion

In this study, we used 16 microsatellite EST-SSR loci (PATZAK et al. 2017) for cluster and PCoA genetic diversity analyses (PATZAK & HENYCHOVÁ, 2018) of 218 wild hops and 44 landraces. Genetic diversity analyses (Figs 1, 2) confirmed previous results that North American wild hops are different from European germplasm (PATZAK et al. 2010a, b) and their germplasm is in origin of old American cultivar Late Cluster. It was also proved differences for Caucasus wild hops (PATZAK et al. 2010b). Saaz and Hersbruck landraces were historically the most important for genetic diversity of European wild hops. Our analyses showed that old cultivar Fuggle originated in wild hops from northern France. It could be supposed that active transfer of hop plants in Europe took place in the 14th century and subsequently also in the 19th century.

Acknowledgement

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Figure 1. Dendrogram of genetic distances of 218 wild hops and 44 landraces revealed by unweighted pair group method with arithmetic means (UPGMA) and Neighbour-Joining (NJ) clustering based on the Jaccard similarity coefficient determined by 156 EST-SSR polymorphic molecular markers.

Colours represent the country or region of origin: green – Czechia, blue – France, violet – Swiss, yellow – Spain, azure – Sweden, brown – Denmark, olive – Caucasus, orange – USA, pink – Canada, red – landraces.

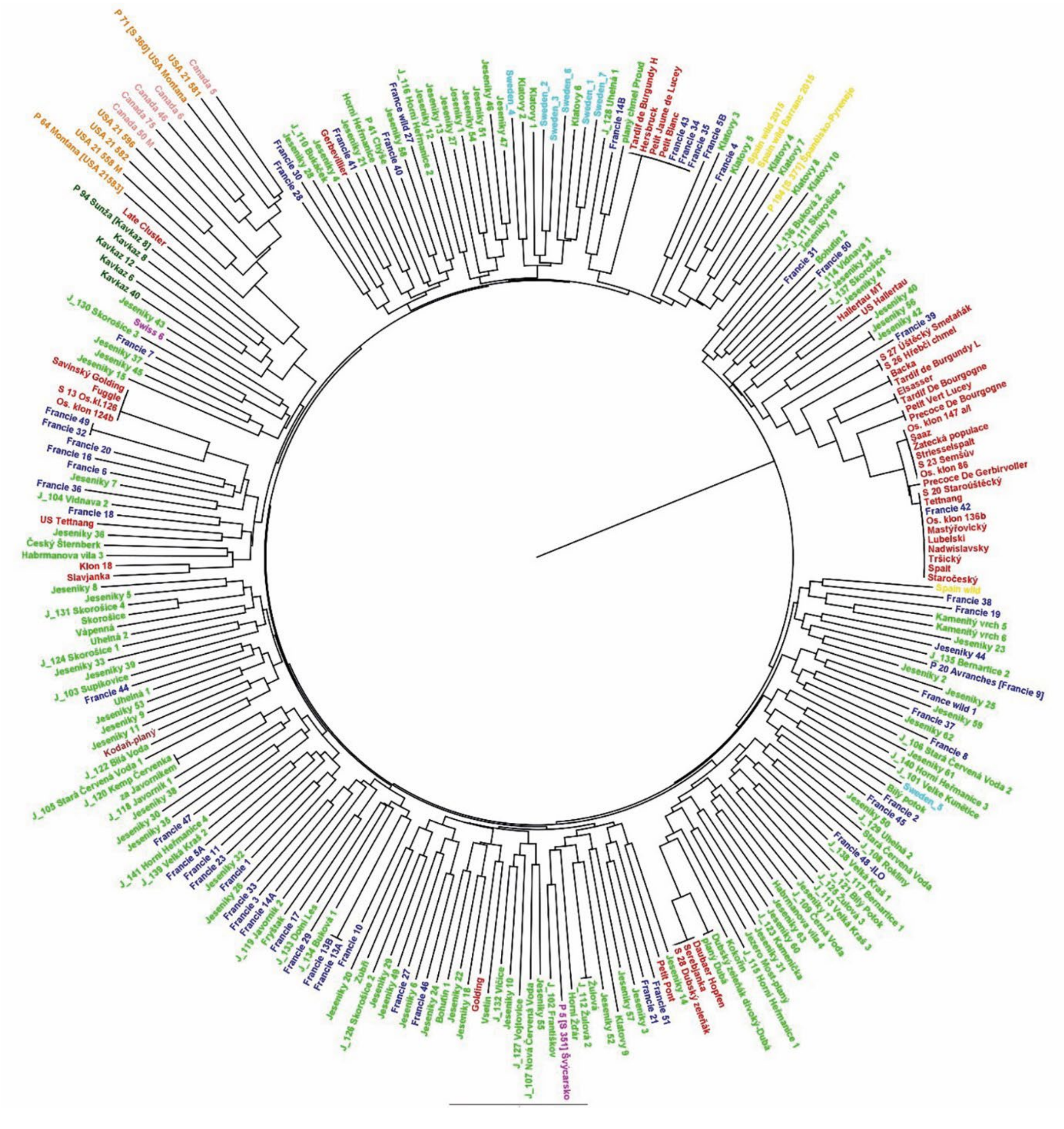
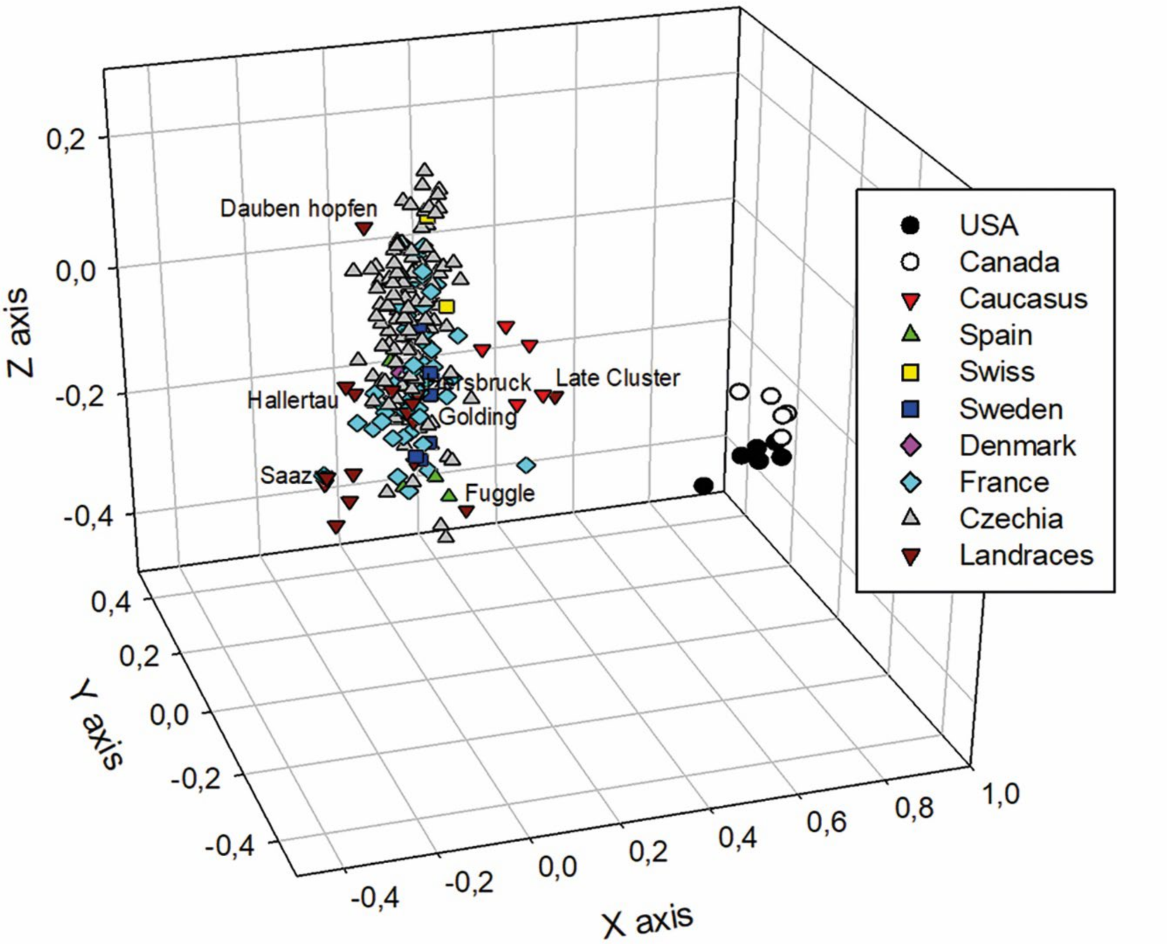


Figure 2. Principal coordinate analysis of 218 wild hops and 44 landraces revealed by DARwin (Dissimilarity Analysis and Representation for Windows) v. 5.0.155 (http://darwin.cirad.fr/darwin) based on 156 EST-SSR polymorphic molecular markers. Colours and signs represent the country of cultivar origin. x, y and z axes are the first, the second and the third principal coordinates.



The first haplotype resolved hop genome assembly and its impact on breeding

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Abstract

A haplotype resolved chromosome-scale assembly which is a basic requisite for any genomic study is still lacking in hop. We combined PacBio HiFi and HiC reads to generate a chromosome-scale and haplotype resolved genome assembly of cv. Apollo, a high-alpha bittering variety that also lends grapefruity, citrusy, and resinous notes. The 2.61 and 2.47 Gb haplotype sizes match with the estimated haploid genome size of 2.5 Gb. Higher BUSCO scores (98.4 %: HAP1; 97.46 %: HAP2) and very low (<2 %) switch errors confirm assembly completeness. Comparative analyses revealed higher divergence (~3 %) and identified large structural variations such as a ~80 Mb inversion, between the haplotypes.

To study the effect of the high divergence on recombination, a linkage map was constructed using F1 progeny (cv. Apollo x male) applying a pseudo-test-cross strategy. We identified limited to no recombination events between North American and European hop. As a conclusion of this genetic assessment and based on the new genomic resources, the breeding strategy in hop needs to be redefined.

We further used next-generation sequencing (NGS) data to study diversity within wild and cultivated hop accessions. Our results are in concordance with earlier studies and support the distinctness of North American accessions while we also identified two distinct groups within Eurasian wild accessions

Estimation of hop water consumption for irrigation optimisation using soil moisture data from capacitance probes

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Abstract

In the South-West of France, in Nouvelle Aquitaine hop production is developing to supply the increasing number of local breweries. Growing seasons are characterized by hot and dry summers associated with risk of water deficit for hop production. Hydric stress during flowering and cone formation is associated with a reduction in yield and quality (HNILIČKOVÁ et al. 2009). As 75 to 80 % of hop water consumption occurs after mid-June (EVANS 2003), irrigation compensates rainfall deficits in dry areas. Hop water needs vary with phenological stage, variety, and weather conditions. Several studies analyzed hop water consumption at different stages and crop coefficients to estimate hop evapotranspiration (ALLEN et al. 1998, FANDIÑO et al. 2015; GRAF et al. 2019).

In order to develop reliable technical reference for irrigation monitoring in Nouvelle Aquitaine and to improve water usage for hop production, capacitance probes were installed in 10 irrigated hopyards located in different locations in Nouvelle Aquitaine. In each field, five probes are installed every 10 cm from depths of 5 cm to 55 cm. Data were collected along the 2022 season and soil moisture values were recorded for each probe. Daily hop water consumptions calculated vary during the season in function of potential evapotranspiration, phenological stage, soil type, variety, and irrigation strategy. Some producers maintain soil moisture to a comfortable level for hop with frequent water supplies. In this case, water consumption and yields are higher. Those first results confirm that hop irrigation strategy is correlated to hop yield potential. Irrigation optimization needs to be further investigated in order to define irrigation schedules according to the availability of water resources and yield objectives. With this approach, new hop crop coefficients adapted for the area can also be defined.

Key words. Irrigation monitoring, hop water demand

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Water erosion in hop gardens and its reduction by cover crops

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Abstract

Water erosion is a serious problem in hop gardens on sloping land when traditional systems of cultivation are used. The solution can be inter-row cover crops, which effectively reduce erosion processes. Results in this paper were obtained using rainfall simulator. Cover crops have a soil conservation effect already one month after sowing. When they are fully grown, they can reduce soil loss by up to 80 % compared to conventional technology. Cover crops are also a source of organic matter that improves the physical and biological properties of the soil. If they are in good physiological condition, they suppress the growth of weeds. Flowering species attract a wide range of insects, enriching the biodiverstiy in hop gardens.

Key words: hop (*Humulus Lupulus* L.), extreme precipitation, cover crops, soil loss

Introduction

As a result of global climate change, droughts and torrential rains are becoming more frequent. If the soil is dry and compacted, it is unable to infiltrate large amounts of water from rainfall. Short-term and intense rainfall thus causes soil erosion and irreversible soil loss (Fig. 1). This is a serious problem because one centimetre of soil takes a long time to form. From an erosion point of view, the large-scale cultivation of wide-row crops is a major problem. One of these plants is hop, due to the large distance between the rows (about 3.0 m). Other problems are the frequent use of tractors and the insufficient plant cover in the traditional way of hop cultivation. If the hop garden is on a slope, erosion often occurs during heavy rainfall. Along with soil particles, soil nutrients are carried away in surface runoff. Planting vegetation between the hop rows has proven to be an effective soil conservation technique. The main objective of this paper is to quantify soil loss in conventional cultivation compared to soil conservation technology with different types of cover crops in the inter-rows of hop gardens.

Material and methods

Research field experiments were carried out in the year 2016-2020 near the village of Solopysky in the Saaz hop growing region. The average annual temperature in the locality is 7.0-8.5°C, the total precipitation is 450-550 mm. The experimental plots had a slopes of 17 % and 9%. The soil type was luvic cambisols. The experimental plot measured 16 m x 2.5 m. Five technologies were selected to test the soil conservation effect. The cover crops were sown in the hop garden around mid-April, depending on the current weather conditions.

- conventional cultivation (control technology)
- *Secale cereale* L.
- grass-legume mixture
- *Phacelia tanacetifolia* Benth.
- *Trifolium incarnatum* L.

Rainfall simulations on designated area were always done twice consecutively. The duration of the first simulation was 30-minute, after which a technological 15-minute break followed After the allocated time, the second (repeated) rainfall simulation with duration of 15-minute was performed. The second rainfall simulation was performed in order to simulate soil with higher moisture. The rainfall intensity was set to 1 mm/min.

Figure 1. Water erosion in a hop garden

Figure 2. Rainfall simulator

The first term of rainfall simulations was done one month after catch crops sowing (mid of May). Second term was done four weeks later (end of June). To determine the amount of soil loss from simulated area, samples of surface runoff with soil particles were taken into plastic bottles from the collecting flume. After the rainfall simulation, the amount of eroded soil in each plastic bottle was determined after filtering off the water and drying the solid portion at a temperature of 105°C for 12 hours.

Results and Discussion

The summary results of erosion losses for the first (mid of May) and the second term (end of June) of rainfall simulations are shown in Table 1. These results were obtained from 76 rainfall simulations. The soil conservation effect was already evident one month after sowing for all cover crops. This was the case for the rainfall simulation on naturally moist soil. Surface runoff was also reduced during the first rainfall simulation. By mid-June, when the cover crops had reached full growth, the soil conservation effect was much more significant. In the case of conventional technology, erosion losses were 11.8 t ha^{-1} in the first simulation, and 6.4 t ha^{-1} in the second. By sowing cover crops, erosion losses were reduced to 2.2 t ha^{-1} in the first rainfall simulation and to 1.8 t/ha in the second.

Table 1. Soil loss from rainfall simulations 2016-2020

Rainfall simulation scheme	The soil loss (t ha^{-1})					
	Ist term (mid of May)			IInd term (mid of June)		
	CC	SCT	Δ (% rel)	CC	SCT	Δ (% rel)
1. rainfall simulation - naturally moist soil	15.3	6.9	- 55	11.8	2.2	-81
2. rainfall simulation - saturated soil	6.9	5.9	-15	6.4	1.8	-72

CC – conventional cultivation; SCT – technology with cover crops

The average results of the soil conservation efficiency of the individual crop types during the growing season are shown in Fig. 3. No statistically significant differences were found between the different types of catch crop. The most important factor is therefore their presence in the inter-row, regardless of the species, but the best results were obtained with winter rye (*Secale cereale*).

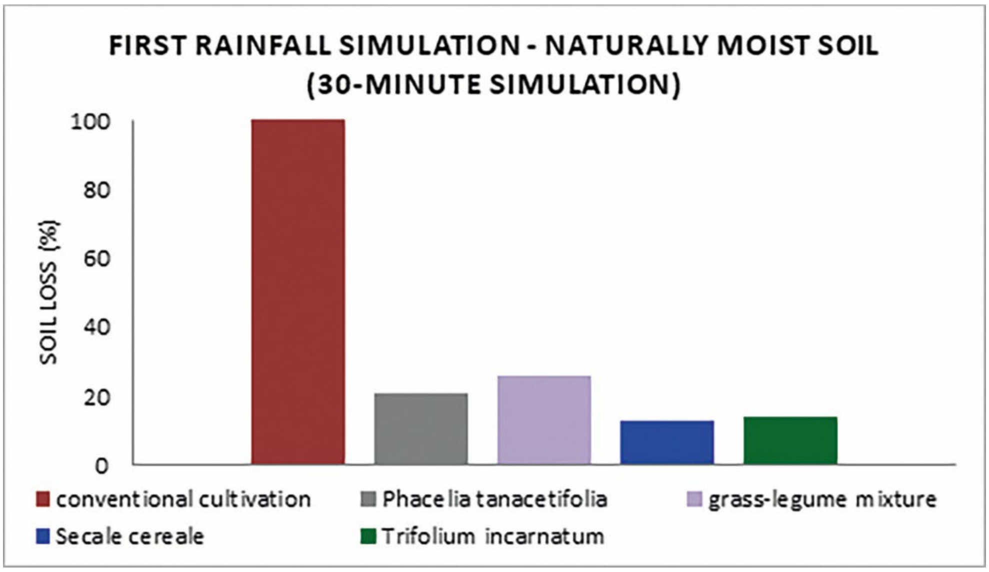

Figure 3. The average soil loss during the hop growing season for individual technologies (catch crops)

Figure 4. *Phacelia tanacetifolia* in full bloom

In the event of erosive rain, cover crops reduce water erosion by up to 80 % compared to conventional technology. In case of repeated rainfall, cover crops maintain very high soil conservation effect. In the summer period, when the probability of torrential rain is greatest, water erosion is significantly reduced compared to conventional technology. Another important feature of cover crops is that they enrich the soil with organic matter, which improving its physical and biological properties. They can also significantly suppress weed growth. Some species (*Phacelia tanacetifolia*, *Trifolium incarnatum*) flower profusely. During the flowering period they attract many species of insects, thus enriching the entomofauna in hop gardens (Fig. 4). The plant residues of cover crops remain on the surface of the soil even after the hop harvest. Thanks to this, there is a significant soil conservation effect during the throughout the season.

Acknowledgement

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Polyphenols in hop cones and hop by-products

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Introduction

Antioxidants are inherent components of plants, including vitamins C and E, carotenoids, flavonoids, and phenolic acids. These compounds possess the remarkable capacity to counteract or slow down oxidative harm attributed to free radicals. A plethora of more than 980 natural antioxidants have been identified as potential alternatives to their synthetic equivalents. Among the naturally occurring compounds, phenolic compounds are dominant. Antioxidants having phenolic groups are also the most widely used ones (NARAYANA SAIBABA 2023).

Antioxidant capacity is the crucial ability of plants to counteract or scavenge hazardous molecules, known as free radicals, that can result in cellular damage and contribute to various diseases such as cancer, cardiovascular disease, and Alzheimer's disease. Phenolic compounds can serve as antioxidants, and numerous methods have been established and outlined for assessing their antioxidant activities (HUANG et al. 2005).

Polyphenols in hop cones

Hops may be considered a natural antioxidant since the α-acids, β-acids, and xanthohumol have significant hydroxyl radical scavenging and antioxidant activities (LIU et al. 2018). The composition and amounts of secondary metabolites in hops depend primarily on the variety, which is related to their genetic potential to synthesise certain substances (GREEN 1997), on the hop growing area, as well as on the weather conditions during growth season (GREEN 1997; ČEH et al. 2007; LYU et al.,2022) and harvest time (PROESTOS & KOMAITIS 2009). Hops antioxidant constituents, mainly phenolic compounds, are free radical scavengers as shown by the DPPH assay (PROESTOS & KOMAITIS 2009). A study by LYU et al. (2022) with hop cvs Calypso, Cascade, and Cluster demonstrated high antioxidant activity with the DPPH assay. Dried hop cones contain 4–14 % polyphenols, mainly phenolic acids, chalcones, flavonoids, catechins, and proanthocyanidins (STEVENS et al. 1998). ABRAM et al. (2015) reported that cv. Aurora contains about 3.9 % polyphenols and cv. Hallertauer Magnum 2.0 to 3.0 %, while total phenolics content in the ethanol extracts from cones, related to variety, year and production location was between 0.74 and 1.73 mg CAE/mL. Antioxidant activity in extracts obtained from fresh, undried samples of hops amounted to 3.6 Trolox equivalent antioxidant activity (TEAC) (M) dry matter^{-1} and 2.32 FRAP (M) dry matter^{-1} (ADDO et al. 2023).

Polyphenols in hop plant by-products

Alternative hop plant parts such as leaves and stems, which have previously been overlooked, are garnering increasing interest as potential sources of antioxidants, antibacterial and antiviral agents, as well as for their potential in cancer chemoprevention (GERHÄUSER 2005). Additionally, hop shoots have been the subject of investigation for similar purposes.

White hop shoots

In the spring, between 15 to 40 buds emerge on the hop root system of the cultivated hop. These buds initially grow as white hop shoots if remaining below the soil surface. These sprouts can be harvested manually and consumed as a delicacy, akin to asparagus. They are considered the first hop plant by-product as they are removed during regular pruning of the top of the hop root system and are regarded as waste. VIDMAR et al. (2019) showed that the total phenolic and ferric reducing antioxidant power of white hop shoots is significantly affected by hop variety and year, with radical scavenging antioxidant potential varying according to variety. Total phenolics as chlorogenic acid equivalents (CAE) on dry mass basis varied from 0.60 to 1.80 mg/g and showed significant effects across cv. and year. White hop shoots exhibited better antioxidant properties than those reported for hop cones and leaves.

Green hop shoots

Wild hop shoots extracts contain primarily quercetin and kaempferol glycosides as the main phenolics (MAIETTI et al. 2017). The flavonoid content of these extracts ranged from 517 to 2698 µg/g fresh weight, depending on the specific growing location within northern Italy.

Hop leaves

After harvesting of hop cones, leaves and stems are left adjacent to the harvest hall. These leaves have also been found to be a source of phenolic compounds (ČEH et al. 2007). The concentrations of phenolic compounds in hop-leaf extracts correspond well to their antioxidative potential (ČEH et al. 2007). The total phenolic content in the supernatants obtained after ethanol extraction of cvs Aurora and Hallertauer Magnum from four different hop-growing regions (Žalec, Leutschach, Hallertau, and Žatec) showed that the leaves contained *ca* 3–30 times less total phenolics than the cones (ABRAM et al. 2015). Total phenolics ranged from 0.099 to 0.542 mg CAE/mL for the leaf extract and from 0.738 to 1.734 mg CAE/mL for the cones. After the harvest of each hectare of hop plants, as much as about 7.8 tonnes of leaves (fresh matter) are left as unexploited material (results of IHPS trials), offering a massive amount to consider (ABRAM et al. 2015). PROESTOS et al. (2006) determined total polyphenol content in hop leaves at 2.9 mg GA/g dry mass of raw leaves. The high antioxidant activity of hop leaves extracts was confirmed by MUZYKIEWICZ et al. (2019), who collected hop leaves from the same place during the same vegetation stage, but in different years, and statistically significant differences in antioxidant activity were confirmed. One factor responsible for these differences seems to be different climatic conditions in particular years.

MUZYKIEWICZ et al. (2019) showed that beside the technique of extraction the date of leaf harvesting may have a significant impact on their antioxidant activity. ČEH at al. (2007) reported different polyphenol content in hop leaves related to sampling time in nine investigated hop cvs. However, the most useful practise is to collect leaves after the harvest next to the harvest hall, when they represent plant waste and are already collected on a pile (ABRAM et al. 2015).

Polyphenols in beer production (by-)products

Antioxidant activity of different (by-)products in beer production, including hop pellets, spent hops, and hop extracts were also investigated. The results showed that all the hop cones products exhibited significant antioxidant activity, with the hop extracts showing the highest activity (KROFTA et al. 2008). Hop milling and pelletising did not affect antioxidant activity or the content of the polyphenol substances. Despite limited market potential, spent hops exhibit noteworthy levels of phenolic acids, presenting a promising opportunity for their valorisation.

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The expression of genes involved in xanthohumol biosynthesis and the content of this metabolite in hop cones

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Abstract

The main prenylflavonoid in hops is xanthohumol, accounting for more than 1 % of the weight of the dried cones. Xanthohumol exhibits anticancer properties and has also antifungal and antiviral, anti-inflammatory and sedative effects (PRZYBYŚ & SKOMRA 2020). It is formed from phenylalanine converted to cinnamic acid with lyase PAL. The next product in this pathway is 4-coumarate converted by ligase 4CL to coumaroyl-CoA and then to chalconaringenin in a reaction catalysed by chalcone synthase CHS_H1. Next, prenyltransferase PT1L converts chalconaringenin into desmethylxanthohumol which is methylated to xanthohumol by the o-methyltransferase OMT1 (KOCÁBEK et al. 2018) The metabolic pathway is controlled by several genes encoding enzymes and transcription factors. The activity of genes can depend on the cultivar (genotype), organ and developmental stage of the plant. The aim of the study was determining the changes in expression of genes encoding enzymes involved in xanthohumol biosynthesis and the content of this compound depending on developmental stage of cones.

Two Polish hop cvs, Lubelski (aroma) and Magnat (bitter), were grown under field conditions. Cones were collected at three dates, from BBCH 79, at which they had reached maximum size but were still immature, to BBCH 89, *i.e*., technological maturity. The relative expression of five genes (*4CL*, *PT1, OMT1, CHS_H1,* and *PAL)* encoding enzymes involved in the metabolic pathway leading to xanthohumol synthesis was determined by real time PCR with the use of specific primers (KOCÁBEK et al. 2018). As a reference gene *7SL RNA* was used. The concentration of xanthohumol was determined by HPLC in lyophilised cones.

The relative expression of genes *4CL*, *PT1* and *OMT1* of cv. Lubelski was highest at the first date and then was reduced. In turn, cv. Magnat showed essential increase of expression of genes *4CL* and *CHS_H1* at the second date (twice and over three-fold, respectively) in respect to the first date. The least changes in expression were observed for the gene *PAL*. The content of xanthohumol in cv. Magnat reached the maximum value 0.75 % of dry weight of cones collected at the second date and in cv. Lubelski 0.70 % d.w. of the cones from stage BBCH89.

In cv. Lubelski cones a high level of gene expression preceded an increase in xanthohumol content. The highest activity of tested genes was observed one month before reaching maximum xanthohumol concentration. In cv. Magnat the expression of genes increased predominantly on the second date, *i.e*. at the same time as the xanthohumol content reached its maximum level. The activity of the tested genes is regulated by transcription factors, why the expression of the genes encoding them should be measured in subsequent tests.

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Terroir effect on hop cv. Nugget volatile composition from northern Spain

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Abstract

Volatile composition of hop essential oil is an important tool for evaluation of hop quality. In this work the volatilome of cv. Nugget from three regions of Spain (Castilla y León, Galicia, and the Basque Country) were analyzed. The oils obtained from hop samples were dissolved in dichloromethane and the aromatic compounds were identified and quantified by GC-MS. Significant differences were found for forty-seven volatiles establishing a specific volatilome for cv. Nugget in relation to the geographic area of cultivation.

Introduction

Spain is the fifth largest producer of hop (*Humulus lupulus* L.) within the EU after Germany, Czech Republic, Poland, and Slovenia. However, if only the bitter cvs are considered, it ranks third with 4.2 % of the production, behind Germany (85.3 %) and Poland (5.8 %). The cultivated area for hops in Spain is around 600 hectares mainly in Castilla y León with more than 95 % of the cultivated area. Volatile composition of hop essential oil is rich in esters, monoterpenes and sesquiterpenes. These volatile compounds give beer its aromatic qualities, while α-acids are isomerized during the boiling of beer wort in iso-α-acids, which impart to the beer its bitterness. These volatile compounds are present in different quantities in the different varieties of hops and terroirs.

In this context, the present work aims at investigating the volatilome of hop oils from cv. Nugget collected in different regions of Spain: Castilla y León, Basque Country, and Galicia.

Material and methods

Hop oils were extracted by steam dragging with Clevenger in CNTA. The extracted oil was 1.3 mL/100 g on average. The study of the volatilome of Nugget from different localities was performed in ICVV. Twenty-three Nugget oils samples were analyzed in duplicate (6 from León, 11 from Galicia and 6 from Basque Country). Oil hop samples were dissolved in dichloromethane (1:200) and the volatilome was determined by GC-MS with a polar capillary column.

Results

Ninety-one volatile compounds were quantified in cv. Nugget variety belonging to different families (esters, monoterpenes, monoterpenoids, sesquiterpenes, sesquiterpenoids and others) and contributing to specific aromatic notes of this hop variety from different cultivation areas.

The total concentration of volatiles shows a trend to highest concentrations in Nugget from Basque Country, followed by Galicia and Castilla y León. A total of forty-seven volatiles showed significant differences between geographic areas. β-Myrcene and α-Humulene showed the highest concentrations in all samples, with α-Humulene being significantly higher in Basque Country than in Galicia and Castilla y León. The influence of terroir on the volatile profile of this hop variety is substantiated by our results.

Substitution of hop varieties in the context of climate change – exemplified by the traditional varieties Hallertauer Mittelfrüher, Hersbrucker Spät, Spalter, and Perle

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The climate change we have experienced so far affects both, the production yield of hops and the biogenesis of hop constituents, especially alpha acids. In this respect, however, there are major differences between varieties. Varieties that are particularly susceptible to heat and drought are Perle and traditional landraces. Compared to years with favorable weather with sufficient precipitation and moderate temperatures, the yield of alpha acids [kg alpha/ha] drops to only about 30 % of a good crop. Such large differences in difficult crop years will most likely have the effect that climate sensitive varieties will no longer be offered via long term pre-contracts. And if they are, then with a high-risk surcharge. Brewers should try to adapt to this situation and develop substitution strategies. As an example for the substitution of climate-sensitive aroma hops with more stable hops, some results of our trials are shown on this poster.

Dosed at the begin of boil, three alternatives to Perle resulted in comparable beers, namely Mandarina Bavaria, Hallertau Blanc and Ariana.

Replacing Perle in the middle of the boil succeeded with Ariana, Hallertauer Tradition, Mandarina Bavaria, Hallertau Blanc, and Opal. The latter two varieties differed in having a more pronounced aroma with a slight fruity character, which many tasters found pleasant. This shows that substitution can also have a positive effect.

For late dosages (end of boil/whirlpool), the situation is more complex; the comparison included eight varieties: the traditional landraces Spalter, Hallertauer Mittelfrüher, and Hersbrucker Spät, and the newer varieties Callista, Diamant, Hallertauer Tradition, Saphir, and Hüll Melon, with the following results:

Looking at the three groups of aroma substances in the beers (linalool, esters, sesquiterpene oxides), Hüll Melon lies at the edge of the analytical ranges in each case. Hersbrucker Spät, Saphir and Diamant are in the middle, which could indicate a balanced aroma. Hallertauer Mittelfrüher and Spalter have low values.

The newer varieties, especially Callista, impart a fruitier hop aroma. Hersbrucker Spät seems to go well with Saphir, Spalter with Diamant and Hallertauer Mittelfrüher with Hallertauer Tradition.

For climate-sensitive hop varieties, this can be taken home as fact:

Brewers will have to look for suitable alternatives soon and not "someday".

Key words: Brewing, hops, climate change, substitution

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