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

INTERNATIONAL HOP GROWERS' CONVENTION
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Hop garden with machinery in the Lower Savinja Valley, Slovenia, in July 2019

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Foreword

Considering Germany accounts for one third of the world's hop cultivation, it may seem somewhat remiss the Scientific-Technical Commission (STC) of the International Hop Growers‘ Convention (IHGC) is seldom hosted in my home country. The last meeting of the former Scientific Commission in Germany was convened 18 years ago, in 2007 in the Tettnang region around Lake Constance. However, as the 2025 STC meeting will be my last as STC Chairman, it was my earnest desire that this congress should be convened in my native Bavaria, and a logical, easy solution would of course have been to go to the well-known Hallertau. On the other hand, many of you will be aware that for their biennial meetings the STC is ever seeking to showcase comparatively unknown destinations, albeit with strong hoppy connections. And – what can I say? There is a location that perfectly meets my desiderata – a small traditional hop growing area situated in Bavaria! Consequently, the STC can proudly announce that we accept the generous invitation of Frank Braun (HVG Spalt, supported by his kindred spirit Sandra Pogue) as convener and host to meet again in person, and for the first time in one of the World's most traditional hop growing regions, Spalt in Franconia.

With the perfect venue for the 2025 STC conference having been located by Frank and Sandra, the international community of hop scientists can look forward to another splendid conference under the auspices of the International Hop Growers' Convention. As I write, 76 participants from 15 nations have registered. Altogether, 33 talks have been submitted and a poster session presenting 15 posters will round off the meeting. In addition to the scientific agenda, I am especially looking forward to the social events that enrich a scientific conference. The mid-conference excursion will take us on a visit of the city of Spalt and then to the organic hop farm of my old friend, Markus Eckert in Hersbruck, where we will end the day with a barbecue and surely some beers. This will be a perfect opportunity to network and to make new acquaintances on 'Planet Hops'. I for one consider the friendly personal international exchange between scientists from all parts of the world extremely important. Personal human contact and getting to know each other, disparate nationalities and cultures notwithstanding, must be upheld in times when dictators, autocrats, and self-proclaimed sultans and Sun Kings plunge the World into chaos. The need for conviviality and mutual understanding and respect among all peoples was never so pressing.

We also express our gratitude to the sponsors of our conference; the generous financial backing by Barth-Haas Group, Hopfenverwertungsorganisation HVG, and Hopsteiner which supports the mission of the STC and facilitates the participation of many scientists who would not otherwise have been able to participate.

I close with a few personal words. I share the fate of all of us – I am getting older and will retire in a few months' time. For that reason, I will be handing the baton of the STC Chair to someone else during this meeting, and I hope that he or she will receive the same support from you as I have during the past decade to keep the STC running. I've been part of this Commission for almost 30 years now and had the honour to act as it's Chair for a decade; this part of my life resulted in some of the most valued personal relationships I have experienced, which often became real friendships. I am truly grateful to have had this opportunity and wish you all and my successor the best for the future.

Dr Florian Weihrauch

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

Welcome

Noblesse oblige – hop growing in Spalt

Braun F.

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A journey to Middle Franconia

What is Franconia? The Germanic tribe Franks (meaning bold) formed in the 3rd – 4th century, uniting several Germanic peoples. The Frankish kingdom soon started expanding and after their victory against Alamannia in 496 the expansion went on – on a large scale. Karl der Große, or Charlemagne, reigned over an empire including nearly all of today’s France, entire Benelux, Austria, Switzerland, large parts of Germany, Italy and Slovenia, and even parts of modern Spain, Croatia and Czechia. Franconian language is still present in the Germanic dialects in Lorraine, northern Alsace, northern Baden, northern Württemberg, Luxemburg, Rhineland, Franconia, southern Hesse, southern Thuringia, and southern Saxony. Nowaday’s Franconia comprises the Bavarian districts of Upper, Lower and Middle Franconia, north-eastern Württemberg, and southern Thuringia as well as a few municipalities in Hesse.

Today, there are two hop growing regions in Franconia: Spalt (growing region Spalt) and Hersbruck (since 2004 officially part of the Hallertau growing region). The cultivation of hops in southern Germany dates back very long. For the 9th century, many documents exist. One important address is the monastery St. Emmeram in Regensburg, to which the Franconian hop village Spalt belonged at that time. As early as the 8th century, a hop field there is mentioned in the St. Emmeram histography.

In contrast to its eastern neighbours, the Frankish kingdom was Christian. The kings, and especially Karl der Große, felt responsible for the catholic church. The monastery of St. Emmeram played a major role in the Christianisation of Bohemia; this historical connection also explains the genetic proximity of the Spalter and Saazer hop varieties.

Initially, hops were used for medical purposes. We all know that by 1516 hops were an important ingredient in southern German beers. We do not know exactly when hops entered beer brewing, but we can tell that the monastic brewing significantly promoted its use in beer. For long, there were no hop cultivation regions, but the cultivation was widely spread over the whole country, close to the farms, using selected, locally found wild hops – hence the term ‘hop garden’ was coined. There was no difference in hop field percentage comparing the north, middle and south of Germany. The preferential strains thus became land varieties.

As early as the late Middle Ages, Spalt hops were marketed in Germany and abroad via the North German Hanseatic League. Even back then, traceability played a certain role – at least in ensuring that the hops had a reputable origin. Back in 1538, Philipp von Pappenheim, then Bishop of Eichstätt, granted Spalt the oldest known hop seal in the world. By the 19th century, Franconia and Württemberg had the highest density of hop fields within Germany. In Franconia these were mainly located in Altdorf, Hersbruck, and Spalt. The further development was very much southbound: Aischgrund became an important Franconian hop growing region before it was suddenly shut down by political order in the 1930s. The Hallertau grew constantly and got a boost after World War I, when other growing regions had to struggle with a lack of financial power – the heritage traditions and the resulting ownership structures being one big issue which led to the decline of most other regions.

The up-and-coming southern German brewing industry of the 18th and 19th centuries led to the above-mentioned considerable shift in hop cultivation towards the south, but also to a growing demand for imports from Bohemia – then part of Austria-Hungary. The political mercantilism that was common at the time pressed on increasing domestic hop cultivation. The Electorate of Bavaria, for example, promoted the cultivation of hops as early as the 17th century, particularly in the Upper Palatinate, and in many cases made it compulsory. As of the second half of the 18th century, the then newly founded Munich Academy of Sciences had to deal with hops – for example elaborating quality and agricultural guidelines.

It was probably the annexation of Nuremberg by Bavaria in 1806 – as a reward for joining the Confederation of the Rhine (Rheinbund) – that gave hop growing in Bavaria a decisive boost. At that time, Nuremberg was already the centre of the hop trade and sourced its hops either from its own territory, from Hersbruck and Spalt, or from Bohemia and the Upper Palatinate. The role of Nuremberg as the world hop trade centre suddenly ended in the late 1930s with the terror of the national socialists as most of the hop merchants were of Jewish descent.

In Spalt, situated 30 km south of Nuremberg, hops do well. The region profits from a mild climate with comparatively low precipitation (*ca* 625 mm p.a.) and extended sunshine (minimal 1300 hours p.a.). Hop growing doesn’t take place in huge monocultures but is widely spread over the country, only using perfect soil, meaning deep soils, easy to warm up, with light to middle heavy keupers. That´s the reason why Spalt doesn´t need to use lots of plant protection agents.

The traditional land variety, Spalt Spalter, is a superfine noble hop that made the region world-famous. The purest classic aroma the planet has to offer is found in Saazer and Spalter. These are the top nobles, apt for whirlpool additions in high-end Pilsners. Other fine land varieties grown in Spalt are Hallertauer Mittelfrüher and Hersbrucker Spät. Their benefit is not the really pure classic aroma but there are other assets. Hallertauer offers (especially in whirlpool addition) a distinct floral aroma, and Hersbrucker stands for nearly unrivalled harmony (nearly unrivalled because cv. Saphir is in that range, too). There are breeds that are close, for example cvs Spalter Select, Saphir, and Diamant.

In Germany there are currently more than 40 hop varieties grown commercially on at least one hectare. The powerful high alpha cv. Herkules makes up half of the German crop – and its share is still growing. The classic aroma cvs Perle and Hallertauer Tradition represent almost one quarter, with a downward trend. Spalt however has positioned itself quite differently. Spalt growers are very much concentrating on fine aroma. Noble hops (Spalter), fine land varieties (Hallertauer Mittelfrüher, Hersbrucker Spät) and fine breeds (Spalter Select, Saphir, Diamant) represent more than 50 % of the acreage, and all classic aroma varieties including Perle, Hallertauer Tradition, Opal, Smaragd, Hallertauer Gold, and Hersbrucker Pure even 80 %.

The variety Spalt Spalter has been granted a "protected origin" label by the EU. The European Commission thereby grants special protection to the Spalt growing area, as the origin of the superfine aroma variety. Situated south of the City of Nuremberg, the town of Spalt is famous for noble hops since medieval times. The Spalt growing area, named after the town of Spalt, is covering today an acreage of around 360 hectares. Most farms, besides of hop growing, have also other areas of economic activity which results in a rather small structured nine hectares average growing area per farm. In the course of the dissolution of the former growing region Jura (1986), the major part merged into Hallertau except Kinding, which joined Spalt.

The individual range of varieties produced in Spalt requires an individual marketing according to the conviction of the Spalt growers. This task is performed by HVG Spalt eG. It is one of two German hop producer organisations recognised by the EU. The remaining 39 Spalt hop growers are its members, and therefore owners. All other German hop growers are organised in HVG Germany eG, situated in Wolnzach in the Hallertau. In contrast to the latter, the small coop in Spalt markets almost all its members' hops.

Figure 1. The heritage building "Hopfengut Mühlreisig", built around 1746, is probably the most important and typical Central Franconian hop farmhouse. It is a half-timbered construction over solid ground floor, with five-storey gable, four-fold pitched gable roof, and with drying slits. Today it is part of a hop farm north-east of Spalt. Photograph: A. Hub (16.xi.2006)

Middle Franconia does not get lots of rain, it never did. This is why the hop growers in the region have been used to water-binding methods for many decades. The current shift in precipitation makes it necessary to organise for irrigation, though. At present, there are about 65 % of the hop fields in the Spalt region under irrigation – all of them using water from the farms' own wells. However, the water rights might expire in the foreseeable future due to the decline in groundwater. HVG Spalt has therefore taken the initiative and has been pushing a large irrigation project since 2015. It is now on the home straight – first pumpings are expected to take place in 2027. The water board that has already been set up will draw its supply exclusively from surplus surface water – on the one hand from the Bavarian water transfer system, *i.e.*, lake Brombach, and on the other from rainwater retention basins.

Spalt is a quite special town. Compared to others it is small, with just 5000 inhabitants. But the history of the medieval stronghold can still be seen, especially parts of the surrounding wall and its towers as well as the tithe barn and the toll houses. Many of the well-preserved town houses in Spalt and corresponding buildings nearby reflect the long-standing tradition of hop growing. In the old times hops used to be dried and stored in typical 4–6 floor buildings (Fig. 1). Spalt calls itself a hop and beer town, and this is very true. Spalt has the only municipally owned brewery in Germany. The mayor is also the brewery director. The century-old brewery is using modern equipment, and it is very successful proven by the annual output growth. The beers are bursting with noble flavour and incredibly harmonious bitter taste. The inhabitants of the region are open, modest, hospitable and happy. *Honi soit qui y pense à la bière*!

In Germany's brewing industry, Spalt is also known for the "Spalter Rohstofftag", an event organised jointly by the two Bavarian Brewers' Associations and HVG Spalt and annually held in mid-October in Spalt. The very first hop harvest results are announced here as well as those of the brewing barley harvest and the respective laboratory malting trials.

I: Hop breeding

Breeding new drought-tolerant hop genotypes and their application in practice

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Abstract

Global climate change affects, among other things, the cultivation of raw materials for the production of beer. The presented project QK 21010136 solves this issue by selecting new hop genotypes tolerant to drought, suitable for growing in dry areas without irrigation. Greenhouse water stress tests were carried out after the end of irrigation and after 10 days of water stress. The results are verified in field trials with and without irrigation. Genotypes with higher drought tolerance were found within the breeding genotypes. As of 2022, two new bitter type genotypes (5165 “Uran” and 5304), three aromatic type genotypes (5431, 5461, 5465) and fine aroma (5559) are in registration trials. Currently, it has been possible to obtain new prospective drought-resistant genotypes and breeding genotypes. All drought tolerant genotypes are tested by hop growers and simultaneously in breweries. New genotypes have a high and stable yield without irrigation The results of the beers show that the new genotypes are suitable for a wide range of beer styles. In 2025, registration of new drought-tolerant hop varieties, which are already known to Czech breweries, is expected. Thanks to direct cooperation with breweries, an increase in demand for these varieties has been recorded, which leads to a gradual increase in plantings of growing areas without irrigation. The Uran variety is grown on 1 ha even before registration.

Introduction

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 aim 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, a number of new genotypes are being tested for drought resistance (NESVADBA *et al.* 2022). New Czech hop varieties are part of the testing. The objective 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 the evaluation of breeding material. Each genotype is harvested separately (minimum 100 plants). An experimental Wolf harvester is used for hop harvesting. An average sample was taken from each hop variety and dried at 56°C until a moisture content of 7% was reached. The alpha bitter acid content was determined from dry hop cones using HPLC (EBC 7.7; KROFTA 2008).

The aroma of hops was evaluated by a group of 40 people (research, hop growers, brewers). The rating is on a scale of 1 (low intensity) to 10 (high intensity). The aroma of beer was evaluated by a tasting panel at the Research Institute of Brewing and Malting Prague.

Results

Table 1 shows the genotypes registered for registration trials. Genotypes 5165 and 5304 are of the bitter type, genotypes 5432, 5461 and 5465 are of the aromatic type, and genotype (fine aroma) 5559 has hoppy with a distinct fruity aroma. Bitter genotypes have an alpha acid content consistently >10 %, such as Agnus, Rubín or Vital, and show a higher yield potential. Aromatic genotypes belong to the Premiant variety group, with a higher content of alpha acids and an alpha/beta ratio of >2. Genotype 5559 with a specific hoppy aroma is in the Kazbek variety group and is characterized by a balanced ratio of alpha/beta acids and a lower proportion of cohumulone than cv. Kazbek.

Table 1. Content and composition of hop resins in new genotypes in registration trials

Genotype	Alpha acids (% w/w)	Beta acids (% w/w)	Ratio alpha/beta	Cohumulone (% rel.)
5165 (Uran)	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

Table 2 shows several parameters of the new genotypes, especially of selected substances of content. Only Uran has a high proportion of farnesene and the lowest proportion of caryophyllene.

Table 2. Composition of the aroma of hops in new genotypes

Genotype	Weight (g/100 g)	Myrcene (% rel.)	Caryo-phyllene (% rel.)	Farnesene (% rel.)	Humulene (% rel.)	Selinenes (% rel.)
5165 (Uran)	1.92	39.51	2.87	7.27	5.32	0.79
5304	1.63	24.21	10.41	0.33	22.20	22.04
5432	0.69	30.39	13.04	<0.10	36.02	2.05
5461	2.56	45.24	6.93	<0.10	7.07	16.41
5465	1.83	39.68	10.73	<0.10	26.33	11.93
5559	1.53	50.01	20.21	<0.10	25.94	2.14

Table 3 shows the composition of the hop aroma. Only Uran has a high proportion of spicy and woody aromas. The other genotypes have a hoppy aroma, which is complemented by spicy (5304 and 5461), floral (5432) or fruity (5465, 5559).

Table 3. Composition of the aroma of hops in new genotypes

Genotype	Hoppy	Citrusy	Fruity	Floral	Grassy	Spicy	Woody
5165 (Uran)	4.2	3.9	2.7	2.5	3.5	5.1	5.0
5304	4.5	3.3	3.9	3.8	3.5	4.5	3.6
5432	4.5	2.7	3.7	3.9	2.7	3.1	2.4
5461	4.8	4.2	4.1	4.0	3.0	4.5	3.6
5465	4.8	4.0	4.2	4.1	2.7	3.8	2.5
5559	3.8	2.6	3.4	3.1	2.6	2.7	2.4

Figure 1 shows the character of the aroma in beer of the two bitter types, figure 2 of the three aroma and figure 3 of the fine aroma hop genotypes. Saaz was evaluated within the genetic resources. Each genotype has a significantly different character, which is used for different beer styles. The rating given is in the beer style of IPL. Genotype 5165 is characterized by a spicy aroma. Genotype 5461 is very interesting as it has a great variability of hop aroma. Genotype 5559 exhibits a combination of Saaz and Kazbek aroma.

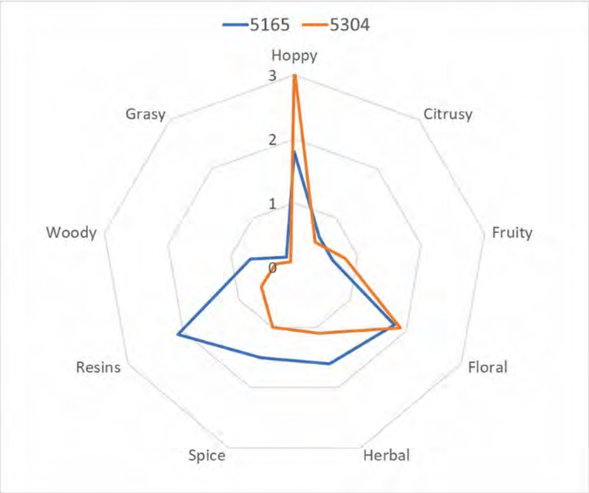

Figure 1. Character of hop aroma in beer (bitter type: 5165, 5304)

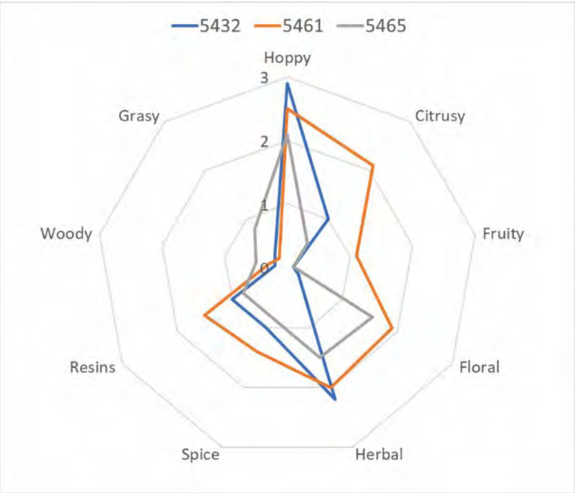

Figure 2. Character of hop aroma in beer (aromatic type: 5432, 5461, 5465)

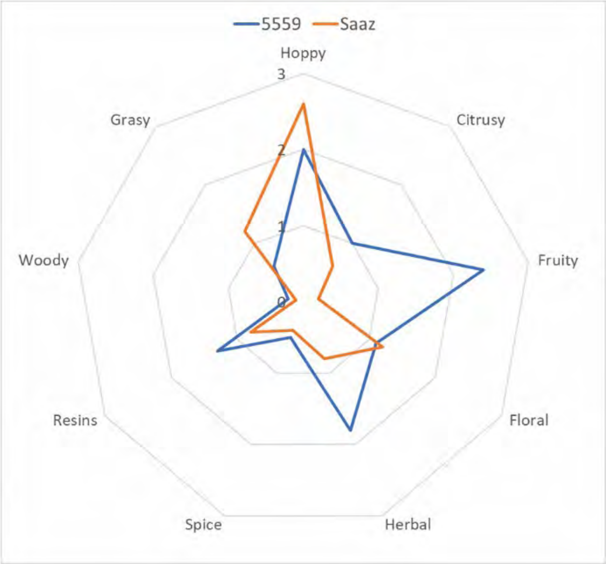

Figure 3. Character of hop aroma in beer (fine aroma type: 5559)

Home brewers are frequently used for brewery evaluation. Promising genotypes are given away for free at brewing competitions. We get a lot of results from different beer styles and hop batches. This cooperation has been going on for seven years. We support home cooking competitions in the Czech Republic and Slovakia. Now we are also interested in Germany (Saxony). These results are then applied to craft brewers and then to breweries.

Genotype 5165 is named Uran and is already used in breweries, for example:

- **Chroust/Falkon 13° Continental Fresh Hop IPA.** Beer with European hops from the cooperative batch of Chrousta and Falkon. The Eris hops from the Žatec Hop Institute were supplemented with the new Czech Uran hops and the German Huell Melon. The result is a very drinkable and pleasantly aromatic modern beer with notes of citrus, yellow melon and a specific aroma and spiciness that only green hops can provide.
- **LOUKA 11° URAN IPL.** India Pale Lager brewed after a year in Louka. The brewery returns with a popular lager hopped by a Czech nobleman called Uran. You can enjoy the hops in abundance, because the beer was hopped in an amount of 11 g / liter.

Uran has been included in the “Pivní vize” (Beer vision) brewing competition for the second year in a row; it is used in various beer styles. This competition is organized thirty times a year in collaboration of the Hop Research Institute Zatec and the Research Institute of Brewing and Malting Prague.

Conclusion

Genotype 5165, already registered under the name Uran, is the most promising genotype. It is grown on 0.5 ha and used in 12 breweries. Very promising are also the genotypes 5461 and 5559, which will be registered at the end of 2025. They are currently being tested in craft breweries and on four hop farms. All new genotypes have a hop yield of 2.2 to 3.0 t/ha. These three new varieties will be planted on hop farms on 1–2 ha in 2026 to expand brewing tests.

Acknowledgement

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Hop breeding in Belgium: development of regional hop varieties

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Abstract

Despite Belgium's long history of hop cultivation, there exists no regional hop breeding program. The changing climate however forces Belgian hop growers to search for new varieties that are adapted to the cultivation conditions in Belgium, including good disease resistance and wind tolerance. Moreover, the strong brewing industry in Belgium, existing out of many large scale and small micro-breweries, is continuously searching for new and unique aromas from hop varieties that are preferably cultivated in Belgium.

From 2017, a collaboration between different partners in Belgium (ILVO, Inagro, Vives, vzw hop, Stad Poperinge) resulted in the initiation of a participatory breeding program on hop. During the first years, breeding work focused mainly on the agronomic characteristics (growth, hop cone production, …) of the new candidate varieties including the development of powdery mildew and Verticillium susceptibility tests under controlled conditions. The different steps of the breeding program were optimized. The technique of targeted crossings in crossing cells was refined and additionally, molecular markers (DANILOVA *et al*. 2006; ČERENAK *et al*. 2019) were used for early-stage selection of female plants. The growth and development of candidate varieties were monitored on trial plots in hop fields at both Inagro and hop farms (conventional and organic), while the hop cones were analyzed yearly on alpha and oil content. About 60 different candidate hop varieties are today on several hop fields in the hop region of Poperinge.

Since 2024, the breeding program enters a new stage with more extensive studies on brewing properties of the most promising candidate varieties. Based on sensory evaluations of the hops with brewers and brewing experts, along with the alpha acid content and oil content analyses, a selection of about 10 of the most promising candidate hop varieties was made. In spring 2025, aroma profiling analyses of these hops are scheduled, together with the first brewing trials. With these trials, we started the final selection step to the introduction of a new authentic Belgian hop variety.

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.

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Enabling genomic prediction for abiotic stress tolerance in hops

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Abstract

In Germany, hop (*Humulus lupulus* L.) is an economically and culturally significant crop with its compounds being essential for brewing as well as for pharmaceutical and food industrial applications. Recent years have shown that hop production is jeopardized by prolonged soil water deficit and that varieties that are less affected by adverse climatic conditions while maintaining high quality are crucial to secure local hop production.

Genome-based breeding methods offer the advantage to predict phenotypic performance even for unregularly occurring environments such as drought or excessive heat enabling a stabilization of selection. We established genomic selection in the Huell breeding program for basic traits within diverse genetic material (LUTZ *et al*. 2023) and across sexes. Based on these results, we initiated a follow-up project. The goals of this new project are

i) to establish genomic prediction models for yield stability and water use efficiency to accelerate breeding progress in hops,
ii) to identify central genes contributing to yield stability, water use efficiency and cone compounds and functionally characterize their allelic diversity and effects, and
iii) to pinpoint specific breeding goals in terms of cone compounds for specific end uses.

Five F_1 (pseudo-F_2) populations with a common male crossing partner and an association mapping panel, genotyped by targeted genotyping-by-sequencing and a newly developed 15k SNP (single nucleotide polymorphism) chip, will be used for estimating the magnitude of genotype-by-environment interaction in replicated field trials at three locations. Plants will be phenotyped under contrasting water availability as well as under conditions enabling the observation of maximum yield potential. The targeted drought stress scenario will simulate severe water shortage around hop flowering as this scenario was the most frequent in major hop growing regions during the last two decades. Phenotyping will be done for several plant developmental, yield related and physiological traits including carbon isotope discrimination. Introducing carbon isotope discrimination in hop breeding is supposed to enable the indirect assessment of water use efficiency in the field. Furthermore, the composition of bitter acids, aroma-oils and polyphenols in hop cones will be analyzed.

Based on genotypic and cone compound information, the 200 most diverse genotypes will be selected and used for brewing of standardized non-alcoholic beers for sensory assessment. The hop used for these beers will vary solely in their genetic relatedness or the water available during hop growth enabling the link between hop cone chemistry and sensory perception in one product of hop application. The genotype-by-environment interaction of the hop compounds under control and drought stress and the implication in terms of the quality for the end products will be analyzed to check if there is a trade-off between drought stress tolerance and hop quality for brewing. Based on genotypic data, different statistical approaches dependent on the genetics underlying the trait will be applied to predict breeding values of possible selection candidates before their field performance is assessed.

In hop breeding, abiotic stress tolerance and quality traits essential for the utilization in the end product can only be assessed in late stages of the selection process on a limited number of genotypes. Adapting existing predictive breeding models to the particularities of hop breeding will enable the selection for those important traits in early selection stages. Thereby, selection gain in hop breeding will be increased and product development in line with changing environmental conditions will be enabled. For traits influenced by few genes, e.g., resistances, genome-wide association mapping (GWAS) will identify causal genomic regions harboring possible candidate genes to develop markers for use in breeding. Thus, the project will contribute to a better understanding of genome structure variation in hops and improve SNP quality for future research and breeding efforts.

Acknowledgement

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Hop predictive breeding: new genomic pipeline to support the selection of hops

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Abstract

A limiting factor in hop breeding is the ability to screen very large number of new progenies in field trials, which results in a low selection intensity and often a lack of genetic variations. The development of tools to pre-select thousands of new plantlets prior to the hop-garden stage could facilitate the breeding of hops. The ambition is to make a radical change in the way hop is bred today using cutting-edge predictive methods already used for decades in most field crops and vegetables. We present here the work that was initiated by InterHoublon over the past four years and showcase the new genomic-based tools recently developed to assist hop researchers and breeders. A brand-new Illumina Infinium XT 5K SNP array was developed and launched to perform genetic diversity analysis on a large hop collection. Through GWAS, marker-trait associations (MTAs) were identified for alpha-acid content and male-sex determinism. Cross validation using predictive equations based on genome-wide markers shows that a genomic selection (GS)-based approach for the selection of high alpha-acid content would outperform conventional selection.

Introduction

The use of modern breeding technics such as marker-assisted and genomic selection proved to be efficient for several decades in plant and animal breeding (BERNARDO 1994; MEUWISSEN *et al.* 2001). Common practice is to use those genomic-based tools to support plant breeding decision, especially to predict crosses and to get rid of the worst early-stage samples before moving to field assessment. Breeding a new hop variety takes roughly ten years and suffers from poor selection intensity due to limitation in field testing where typically a few thousands of lines are evaluated while tens of thousands of lines in field crops are annually tested. Besides, because of its dioecious reproduction that limits the expression of recessive deleterious variations, the selection of males for quality traits only expressed in female plants is only possible through the evaluation of their daughters, that is, general combining ability (GCA) assessment, which is costly, often unprecise and not efficient since only a very few male candidates can be tested. With the use of genomics, it becomes possible to predict the genetic merit of male plants for female-specific traits and to shorten the breeding cycle length, which results into genetic gain increase. To achieve this in hops, there is a need to develop an affordable high throughput genome-wide marker testing tool, which is currently not available.

Having an optimized breeding scheme in hops could offer new perspectives to deliver more rapidly high-quality varieties that would address the demand of growers and brewers. This is becoming particularly important with the impact of climatic changes in hop cultivation, which will increasingly suffer from disease pressure, drought and heat stresses and the perspective to have a decrease of alpha acids in the coming decades (MOZNY *et al.* 2023).

We report here the development and examples of analyses of a first Illumina XT SNP array in hops performed on a large collection of cultivars, wild plants and breeding populations. We show that its use will facilitate breeding steps, such as cross prediction, marker-trait association discovery, marker-assisted selection, genomic selection and quality control.

Material and methods

Hop collection

A large panel consisting of 2,502 commercial varieties, wild hops, accessions from ssp. *neomexicanus* and F1 connected breeding populations was assembled for the subsequent genetic analysis. Among those, 34 naturally occurring male wild hop plants collected in France, Germany, Austria and Italy and from private gardens were also used for the identification of sex-associated markers. For certain commercial varieties, plants were unavailable for purchase. Cones or pellets were directly sourced from hop merchants.

Phenotypic data

Alpha- and beta-acid contents were retrieved from public databases for a subset of 97 commercial varieties. Dried cones collected from F1 breeding populations and check varieties in 2023 and 2024 were sent to ITEIPMAI for the quantification of cohumulone, n+adhumulone, colupulone and n+adlupulone, the major alpha and beta acids in hops, respectively. The assessment was made by HPLC under the EBC 7.7 method on 100g of dried material.

Development of a hop Illumina XT 5K SNP array

Public expressed sequence tag and whole genome sequencing data were retrieved from NCBI (https://www.ncbi.nlm.nih.gov/sra) and HopBase (HILL *et al.* 2017; https://hopbase.org/). The most represented cultivars were Apollo, Cascade, Hallertauer Magnum, Nugget, Phoenix, Saaz, Shinshuwase, Strisselspalt, Target, Hallertauer Taurus, Teamaker, and the male line 21422M. SNP detection was performed with SequenceServer (PRIYAM *et al.* 2019) and QIAGEN CLC Genomics Workbench. Cascade alleles were set as the reference allelic versions and SNP positions were defined according to the Cascade Dovetail Hi-C assembly (PADGITT-COBB *et al.* 2023). A total of 5,674 single nucleotide polymorphisms (SNPs) was selected for the synthesis of the Illumina Infinium XT array.

Fingerprinting

Genomic DNA was extracted from dried leaves, dried cones or pellets of 2,502 hop accessions. All samples were marker tested with the new Hop Illumina XT 5K SNP array available at SGS Institut Fresenius, TraitGenetics Section and used to create a cluster file subsequently applied for the SNP calling analysis.

Genetic analyses

Graphs, basic statistics, variance component analysis, multidimensional scaling, discriminant analysis of principal components, linkage disequilibrium, minor allele frequencies, GWAS, genomic model fitting, and cross validations were all performed in R using the following packages: adegenet, BGLR, dplyr, GAPIT, ggplot2, lme4, rrBLUP, tidyr.

Results

SNP selection and basic statistics of the first hop Illumina XT SNP array

Through BLAST analyses, 5,674 single nucleotide polymorphisms (SNPs) were isolated from sequence alignments using DNA sequences from numerous hop cultivars. SNP positions were assigned according to the last Cascade genome assembly (PADGITT-COBB *et al.* 2023). Of all SNPs, 98 % were synthesised to build an add-on set to create the first Illumina Infinium XT SNP array in hops using the Bead-Based Microarray Technology, of which 79 % turned out to be functional. This success rate for a first SNP array is rather acceptable and expected since the large majority of SNPs has not been targeted before. The genome coverage is relatively even with 580, 362, 299, 359, 341, 293, 884, 392, 423 and 421 for Chr1_scaf_1531, Chr2_scaf_19, Chr3_scaf_76, Chr4_scaf_24, Chr5_scaf_172, Chr6_scaf_77, Chr7_scaf_73, Chr8_scaf_49, Chr9_scaf_191 and X_scaf_1533, respectively. Chr6_scaf_77 contains the fewest number of SNPs (293) while Chr7_scaf_73 has the most (884), a pattern that was also observed by PADGITT-COBB *et al.* (2023). The markers are evenly spread on the genome with one SNP every 0.87 Mbp on average. The larger gap spans 16.7 Mbp on Chr3_scaf_76.

2,502 hop samples were analysed with the SNP array to build the first cluster file. Most SNPs show a classical 3-cluster output consisting of one heterozygous and two homozygous groups. Complex scoring was also observed with up to five clusters suggesting the amplification and detection of common polymorphisms at different loci on the genome. Some markers were monomorphic. A total of 3,182 SNPs were defined as informative markers after filtering and removal of candidates with a minor allele frequency (MAF) <0.005 and a percentage of missing value >10 %. We replicated 76 biological samples across different fingerprinting projects (232 samples) to assess the repeatability of the SNP calling, which reaches 99.9 %.

LD and diversity landscape of the hop genome

We first performed an analysis of linkage disequilibrium (LD) to estimate the haploblock structure of the hop genome. Overall, we observed for most of the chromosomes huge LD blocks spanning sometimes several hundreds of Mbp. To assess nucleotide diversity, we looked at the MAF and observed heterozygosity (Ho) across the different chromosomes. Chr3_scaf_76 and Chr8_scaf_49 show the lowest diversity, while on average all the other chromosomes display good MAF and high Ho patterns.

Identification and confirmation of previous sex-associated markers

The use of molecular marker to sort out female and male young plants is becoming a basic tool for breeders during early-stage selection and to allocate the right resource to the right genetics. GWAS performed on the hop panel shows very strong association for four SNPs, which are all in complete linkage disequilibrium and provide, at least with our current small set of male samples, 100 % correlation with the phenotypes. Among the four SNPs, one maps on Chr3_scaf_76 according to the Cascade genome, but most likely there is a Y chromosome-specific detection for that locus. Three other markers correspond to the previous markers hPb-366371, hPb-719005 and 1533_377039504, SM1 (ČERENAK *et al.* 2019; CLARE *et al.* 2024).

Genetic architecture of alpha-acid content

We quantified the alpha-acid content on 281 accessions from harvest 2023 and 2024. Variance component analysis using mixed model suggests a high heritability (h2) of 0.71. GWAS output shows that the genetic architecture of alpha acids in hops is complex and not controlled by a few major genes.

Based on our data, two additive quantitative trait loci (QTL) were identified, accounting for 25 % and 14 % of the total phenotypic variation. The high alpha allele of the QTL with the largest effect has a MAF of 0.36, however only 7 % of the material harbours a homozygous state from the 2,502 accessions of the hop panel. Columbus, Herkules and Zeus are the only varieties being fixed for the high alpha acid allele at both QTL.

Performance of a GS-based breeding programme

The same alpha-acid content data analysed in the GWAS were used to fit a genomic selection (GS) predictive model. A classical GBLUP yields to a high predictive accuracy of 0.69, which could open opportunity in breeding to improve genetic gain (ΔG) of alpha acid. Phenotypic selection accuracy, that is, the square root of h2, is about 0.84, which suggests that phenotypic selection (PS) would still outperform GS (0.84>0.69). However, GS would make a clear difference in terms of efficiency at early stage because of both female and male genetic merit can be estimated. This has a big impact on genetic gain since in a GS-based scenario early-stage female & male prediction can be considered plus the estimation of the late-stage female. Assuming a selection intensity of 50 %, a 2-stage GS-based programme would have ΔG of 4.18, while ΔG of a conventional programme would peak at 1.28.

Discussion

The new hop Illumina XT SNP array opens new opportunities for discovery projects but also for assisting hop breeders. Redesigning the selection scheme in hops is possible today with the prospects of such a tool supporting breeding decision. We expect an immediate impact on selection intensity, leading to a substantial genetic gain, especially for traits that cannot be measured on both female and male components. The first GWAS and GS outcomes are promising, and more work is needed to tackle other important agronomic and quality traits. Monitoring current and future genetic diversity space via fingerprinting will also have an impact on the success of the breeding programmes. In the future, we can also envision to predict crosses using meiotic-recombination simulations and GS models. Finally, marker testing could also be of interest in the field of quality control to guaranty the conformity of the production lots.

Acknowledgement

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II: Genomics

Uncovering the genetic background of hop resistance to *Verticillium* wilt

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Abstract

Hop (*Humulus lupulus* L.) breeding aims to improve brewing quality and agronomic performance by targeting resistance to diseases such as Downy mildew *Pseudoperonospora humuli*, Powdery mildew *Podosphaera macularis*, *Verticillium* wilt *Verticillium nonalfalfae*, and Severe hop stunt disease, caused by *Cocadoviroid rimocitri* (CBCVd) (Čerenak 2023). The symptoms of *Verticillium* wilt in hops caused by *V. nonalfalfae* vary depending on the pathogenicity of the fungal strain and the sensitivity of the cultivar. Susceptible hop cultivars can suffer from severe symptoms (e.g., leaf chlorosis and necrosis) and also complete dieback of rootstock caused by a highly virulent strain of *V. nonalfalfae* (Radišek *et al.* 2026). The resistance to *Verticillium* wilt, caused by soil-borne fungi, remains the least explored among these due to its complex pathogenicity tests, its occurrence limited to a few growing areas and scarce tolerance availability in hop gene pool. Given the known challenges of hop breeding due to plant traits – dioecism, genetic heterogeneity and a long juvenile phase – accelerated selection by high-throughput sequencing is becoming crucial.

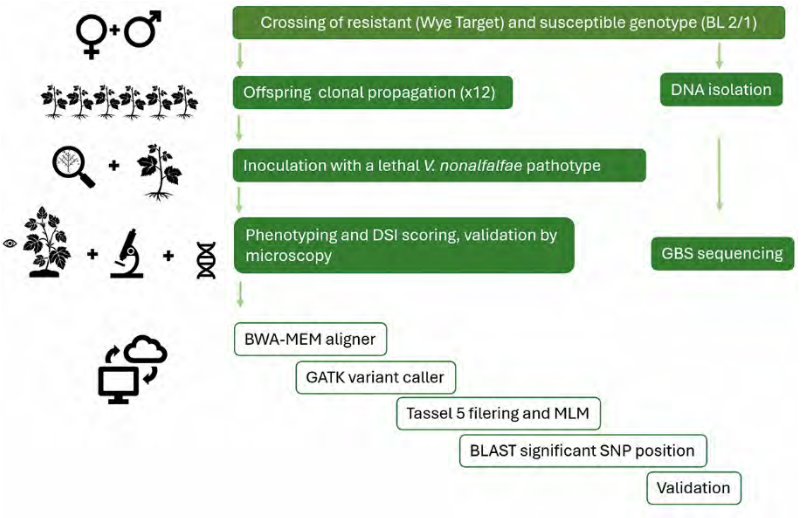

Figure 1. Workflow of the presented study.

The present study continues and expands upon the findings from the family previously analyzed by Jakše *et al.* (2013), which involved a cross between the resistant English variety Wye Target and the susceptible Slovenian wild male hop BL 2/1. Each genotype, represented by 12 clones, was inoculated with a lethal *V. nonalfalfae* pathotype and disease severity was scored weekly on a scale from 0 (no symptoms) to 5 (81-100 % leaf area affected). Disease severity indexes were determined for 120 genotypes.

To create a link between phenotype and genotype, the genome-wide association study (GWAS) was performed using genotyping-by-sequencing (GBS) approach to detect the variants. Variant calling was performed using GATK (Genome Analysis Toolkit), while PCA (Principal Component Analysis), kinship and mixed linear modelling (MLM) analyses were performed in Tassel 5. Highly significant single nucleotide polymorphisms were mapped on the genome, and those directly affecting predicted genes were matched with the BLAST database. Different sets of SNP positions were used to create the prediction models using "ranger" and "rrBLUP" packages in R.

The majority of genotypes have a score of 1 (36), indicating 1-20 % leaf surface with symptoms. The highest frequency of genotypes at scores 0 and 1 may indicate inheritance of several dominant resistance alleles. For the GWAS study, we used two genomic files that were filtered to contain approximately ~27 kbp and 36 kbp of single nucleotide polymorphism (SNP) positions. The average distance between SNPs is ~95 kbp for the first file and ~68 kbp for the second file. After MLM analysis, we found several individual regions on different chromosomes that are statistically associated with resistance or susceptibility scores. For both genomic files, the most prominent region is at the end of chromosome 1, located on and near the WAK2 genes, a cell wall-associated kinase 2 that may play a signaling role in the response to *V. nonalfalfae*. The region identified on chromosome 6 in genomic file 2 as being associated with resistance by the MLM analysis was also recognized as a QTL in a previous study by JAKŠE *et al.* (2013). Based on the 1000 SNP positions with the smallest p-values after MLM analysis, we performed a random forest model and were able to predict the phenotype with a correlation of 88 %. By testing the predictions with other models, we will compare their accuracy and apply them to a set of breeding genotypes.

Acknowledgement

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Investigating the genetic architecture of *Verticillium* wilt resistance in UK hops

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Abstract

One of the most threatening hop diseases in the UK is *Verticillium* wilt (VW) is caused by the various pathovars of the soil-borne pathogen *Verticillium nonalfalfae*. In its lethal form VW can cause complete plant death and there are no curative methods available to tackle the disease. Resistance to VW has been identified in the Wye breeding germplasm and genetic resources have been harnessed to develop varieties such as Wye Target, Pilgrim, and Phoenix with tolerance to VW. However, the characterisation of cultivar resistance status takes a long time, and the process involves laboursome wilt pathogenicity assays which can only be carried out late in the breeding timeline.

Understanding the genetic components controlling resistance to VW is essential for the development of molecular markers which allow hop breeders to identify VW resistance in the germplasm without the need for excessive phenotyping. The UK breeding programme holds high quality wilt resistance data for hundreds of the hop accession present within the Wye Hops collection, which provides invaluable resources for the implementation of genetic informed breeding strategies. In this study, we identified a panel of 547 diverse individuals with well characterised resistance status to VW. These individuals were genotyped to enable Genome Wide Association Study (GWAS) analysis. Markers were mapped to the 'Cascade' physical map to identify locations of VW resistance alleles. This study reports our preliminary results on the genetic mapping of VW resistance in hop.

Developing powdery mildew resistance in hop through CRISPR-mediated mutagenesis of *MLO S*-genes

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Abstract

Hop (*Humulus lupulus*) faces substantial yield and quality losses due to powdery mildew (PM), caused by *Podosphaera macularis*. Targeting susceptibility (*S*) genes, such as *Mildew Locus O* (*MLO*), via CRISPR-mutagenesis, offers a promising strategy for durable PM resistance. We aimed to develop a transformation and CRISPR/Cas9 editing system in hop to enable targeted mutagenesis of *MLO S*-genes. First, we characterized *MLO* homologs from the 'Cascade' assembly, focusing on Clade V *MLO*s which are commonly associated with disease susceptibility. Guide RNAs targeting two hop *AtMLO12* homologs were delivered to *in vitro*-derived tissue of 'Fuggle' via *Agrobacterium*-mediated transformation. After 6–12 weeks on regeneration media, a small percentage of marker positive edited shoots were recovered. Many edits were small indels causing premature stop codons and truncation of the C-terminal calmodulin-binding domain. To date, at least six independent CRISPR-edited transformation events have been recovered. Preliminary phenotyping of *in vitro*–derived leaves revealed variable resistance and pleiotropic effects, including chlorosis, early senescence, and reduced vigor. These findings support our hypothesis that these *AtMLO12* homologs play a role in powdery mildew susceptibility and demonstrate the feasibility of genome editing in hop. Ongoing work aims to minimize pleiotropy while maintaining *mlo*-mediated powdery mildew resistance.

Introduction

Powdery mildew causes significant economic losses by reducing both yield and cone quality. To mitigate these impacts and avoid issues related to export compliance with fungicide maximum residue levels, we are exploring the use of nullified susceptibility genes (*S*-genes) as a strategy to confer broad-spectrum resistance to powdery mildew. *S*-genes were first discovered in barley in 1942, when powdery mildew resistance was observed following X-ray mutagenesis. This resistance was subsequently linked to loss-of-function mutations in the *Mildew Locus O* (*MLO*) gene, an *S*-gene necessary for host susceptibility (JØRGENSEN 1992). Functional *MLO* alleles confer host susceptibility to compatible pathogen interactions, while loss-of-function (nullified) *mlo* alleles result in incompatible interactions and resistance. The haplotype-phased 'Cascade' reference genome contains at least 20 RNA-seq supported *MLO* homologs, four of which fall within Clade V—the clade most frequently associated with broad-spectrum resistance upon gene nullification (Table 1) (KUSCH *et al.* 2016). Functional characterization of these putative *S*-genes is needed to definitively establish their role in powdery mildew susceptibility in hop.

Prior to functional characterization, establishing a robust transformation, regeneration, and genome-editing protocol is necessary, as hop is notoriously recalcitrant.

Previous studies report that regeneration rates via *de novo* shoot organogenesis vary widely between genotypes (*e.g*., BATISTA *et al.* 1996; LIBERATORE *et al.* 2020). There are sparse reports of successful transformation, regeneration, and editing in hop with those demonstrating efficiency rates between 0 and 3 % (AWASTHI *et al.* 2021; SIMS 2022). In this work, we aim to establish a protocol for transformation, editing, and regeneration in hop. We design, mobilize, and deliver CRISPR/Cas constructs with guide RNAs targeting putative *MLO S*-genes via *Agrobacterium*-mediated transformation. We also report ongoing evaluation of the resulting transgenic lines through phenotyping for PM resistance and plant vigor, alongside allele-specific genotyping of the targeted loci.

Table 1. Putative Arabidopsis MLO S-gene homologs in hop.

Gene ID	Scaffold	Start	AA Length	UniProt Homolog
HUMLU_CAS0068957	Scaffold_49	230084079	552	MLO12_ARATH
HUMLU_CAS0050357	Scaffold_77	171879632	530*	MLO6_ARATH
HUMLU_CAS0048594	Scaffold_77	62805110	533	MLO12_ARATH
HUMLU_CAS0050448	Scaffold_77	17509059	605	MLO6_ARATH

*One haplotype of HUMLU_CAS0050357 appears to have a 5’ truncated variant in Cascade (345 AA).

Material and methods

MLO homologs in hop were identified by BLASTing amino acid sequences of *Arabidopsis thaliana MLO2*, *MLO6*, and *MLO12* against the ‘Cascade’ Dovetail genome assembly (PADGITT-COBB *et al*. 2023). Candidate sequences were further validated based on predicted protein domain architecture, presence of conserved motifs, and RNA-Seq expression profiles. Guide RNAs were selected using Geneious Prime's “Find CRISPR Sites” tool, prioritizing candidates with high predicted activity and low probability of off-target effects (DOENCH *et al.* 2016). These candidate gRNAs were delivered within a CRISPR-Cas9 system as outlined in Figure 1.

Results

To date, at least six independent transformation and editing events have been recovered, with five lines successfully surviving the acclimation process. All edited plants were produced with a guide designed to target the two AtMLO12 clade V homologs (Table 1) and thus four loci. The edited lines displayed a range of genotypes, including biallelic heterozygous, biallelic homozygous, monoallelic, and chimeric variants, predominantly featuring small insertions or deletions (<5 nucleotides) occurring a few bases from the PAM site. Many of the observed edits introduced a premature stop codon between amino acid positions 310 and 370 (out of 533 or 552 total), resulting in a truncated protein that lacks the C-terminal calmodulin-binding domain—an essential region for MLO function. Some lines presented pleiotropic effects *in vitro* including early senescence, chlorosis, stunting, and reduced vigor (Fig. 2C). Preliminary inoculations of *in vitro*–derived leaf disks with *P. macularis* isolates revealed a spectrum of phenotypic responses, ranging from complete resistance to marginal reductions in disease severity (*e.g*., Figs 2A, B).

Discussion

This study demonstrates the successful targeted mutagenesis of two *AtMLO12* homologs in hop, resulting in a spectrum of powdery mildew resistance phenotypes across edited lines tested *in vitro*. The observed increase in resistance in some lines supports the role of these *MLO* homologs as susceptibility genes in hop, consistent with findings in other species. Since all phenotyping data currently originate from *in vitro*–derived tissue, we anticipate differences in susceptibility once plants are in greenhouse or field conditions.

The observation of pleiotropic effects also highlights the trade-offs involved in *mlo*-based resistance. As C-terminal calmodulin binding on MLOs is thought to play an autoinhibitory role in calcium transport, it is possible the pleiotropic effects we are observing are due to unregulated Ca^{2+} movement. Unregulated Ca^{2+} influx can disrupt cellular homeostasis by overactivating calcium-dependent signaling pathways, including those involved in defense, oxidative stress, and programmed cell death. In the absence of calmodulin-mediated gating, excessive calcium may lead to constitutive activation of immune responses, resulting in ROS accumulation.

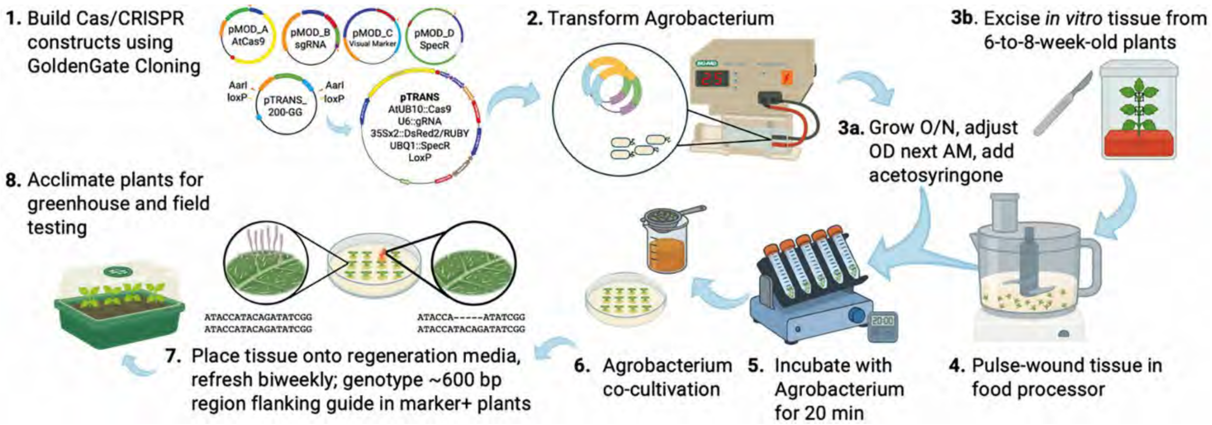

Figure 1. General approach for transformation and CRISPR-Cas9 editing in hop. **(1)** CRISPR constructs were assembled via Golden Gate cloning using customized components based on the Voytas Lab plant bioengineering toolkit (ČERMÁK et al. 2017). Constructs were verified by Nanopore sequencing, **(2)** transformed into *Agrobacterium* strain AGL-1, and **(3a)** cultured overnight at 28°C with shaking at 125 rpm. After centrifugation, AGL-1 cultures were resuspended in induction media and adjusted to OD_{600}=0.6. **(3b)** Tissue from 6–8-week-old *in vitro*-grown 'Fuggle' hop plants was excised and then **(4)** pulse-wounded in induction medium using a food processor. Tissues were then **(5)** incubated with *Agrobacterium* on a rocker for 20 minutes. Then, tissues **(6)** were transferred to a co-cultivation medium for 3 days. After co-cultivation, explants were washed, briefly dried, and placed on regeneration medium. **(7)** Explants were transferred biweekly onto fresh media and maintained at 24°C under broad-spectrum fluorescent lighting (40 $\mu mol\ m^{-2}\ s^{-1}$) with a 16-hour photoperiod. DNA from DsRed-positive shoots was amplified (~600 nucleotides surrounding PAM site) and sequenced via Sanger and Nanopore sequencing. Editing efficiency was assessed on haplotypes ± 20 nucleotides from the guide PAM site using the CRISPR tool in Geneious and with DECODR (https://decodr.org/). Susceptibility to PM was assessed by inoculating leaf disks (1 cm diameter) from the youngest fully unfurled leaves of *in vitro*-grown edited and wild-type plants using a settling tower. Percent disease severity was assessed 10 days later using a PM trained computer vision model. **(8)** Edited plants were acclimated to greenhouse conditions over a 3-week period following root development *in vitro.*

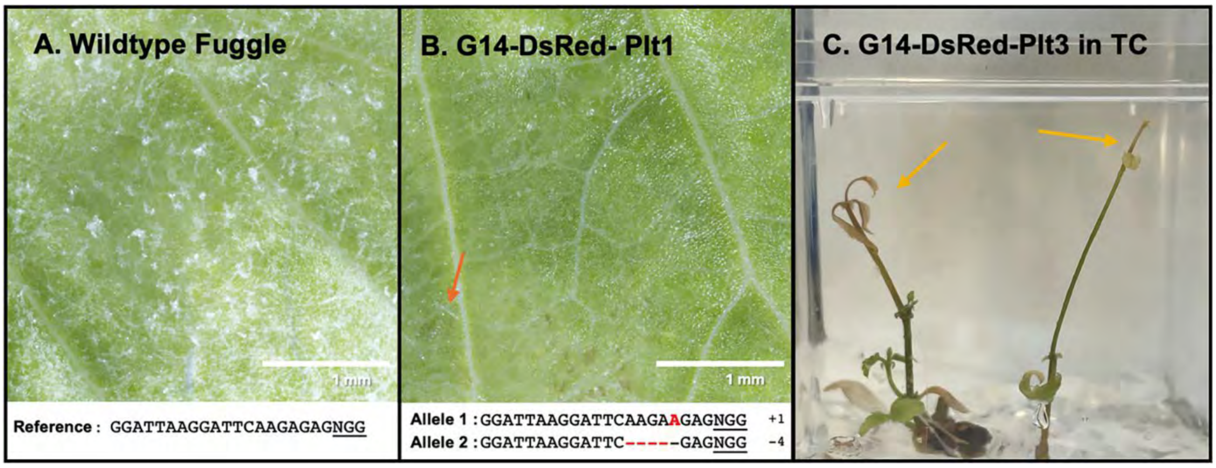

Figure 2. A, B – Representative comparison of PM susceptibility between wild-type 'Fuggle' and a biallelic heterozygous KO-*MLO* line 10 days post inoculation. Limited sporulation is present on the edited line (red arrow), a recurring observation with inoculated *in vitro* leaf disks from edited lines. **C** – Example of early senescence as a potential pleiotropic effect in tissue culture (TC), more frequently observed in certain edited lines (yellow arrows).

Acknowledgement

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III: Phytophatology

Status report on *Verticillium* research 2025

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Abstract

Verticillium wilt, caused by the fungal pathogen *Verticillium nonalfalfae*, is an increasing problem in hop growing areas. Lethal races, which have spread over the last 15 years, infect all known hop varieties and lead to high yield losses. For this reason, a research project on *Verticillium* wilt was started in 2017. The aim of the project is to identify tolerant cultivars, obtain an overview of the spread of the lethal races, and to develop suitable assessment methods and practical solutions for crop management for farmers.

Introduction

Verticillium, a soil-borne fungus, is responsible for wilt disease in hops and is an economically relevant pest due to its high damage potential. In Germany, hops have been cultivated on an area of 20,289 ha in 2024 (DHWV 2024). According to current estimates, *Verticillium* is present on approximately 40 % of this area.

This disease of hops is mainly caused by *Verticillium nonalfalfae* and rarely by *Verticillium dahliae* (EFSA 2014). The pathogen exhibits a wide host spectrum and is capable of long-term survival in soil for up to five years in form of an infectious permanent mycelium. So far, no effective direct control measures have been identified.

Research work on the wilt problem in hops began at the Institute for Crop Science and Plant Breeding in 2008. In addition to the so-called mild wilt races of *Verticillium nonalfalfae*, more aggressive races were detected in the Hallertau. Following the first observation of aggressive *Verticillium* strains in Germany, a continuous spread of the infested area has been observed in the Hallertau growing region.

These more aggressive fungus races developed due to natural selection on wilt-tolerant hop variants. The lethal races induce evident wilt symptoms and plant death even in hop varieties previously classified as wilt-tolerant. In commercial hop yards, mild and lethal races are often simultaneously present. As the usual plant protection strategies fail to stop the fungus, hop growers face increasing economic loss.

The aim of the project was to develop practical measures to effectively combat the new lethal strains in future. The scientific advice and on-farm research generated a better understanding of the pathogen and enabled the implementation of control strategies in practice. New technical possibilities and research results were and are developed as a strategy to combat hop wilt in co-operation with farmers.

Material and methods

This project included field, greenhouse, and laboratory trials. The plants were classified visually according to the severity of their disease symptoms and always assessed following to a previously defined scoring. The visual assessments were verified by qPCR analyses. These analyses were carried out by the hop breeding research group.

Key results and conclusion

Three field trials were set up to identify wilt-tolerant cultivars. Each hop garden contained lethal *Verticillium* races. Known cultivars were used as references to assess the relative susceptibility of the hop varieties; cv. Hallertauer Tradition was classified as susceptible and cv. Herkules as tolerant.

In order to ensure the economic efficiency of heavily infested hop gardens, remediation trials were set up. The diseased hop plants were grubbed, and the plot was used for several years to produce cereals only. The regrown weeds and hops were regularly removed. This remediation strategy was successful. Certified planting material from a tolerant cultivar showed no wilt symptoms.

Diseased plants must be eliminated without delay and replanting the same location should be avoided to prevent further pathogen persistence.

Infected chopped bines must be stored for as long as possible. The pile must be turned approximately after four weeks, and the temperature and oxygen content must remain high to ensure effective decontamination.

A range of management practices, such as the removal of infected plants and appropriate handling of harvested bines, must be rigorously applied in hop production. Control of *Verticillium* wilt is a long-term process spanning several years and is crucial to mitigate substantial economic damage to growers.

Acknowledgement

The Bavarian State Research Center of Agriculture (LfL) working group Plant Protection in Hop (IPZ 5b) would like to thank the working group Breeding in Hops and the group Hop Cultivation and Production Techniques for supporting the project. This study was funded by Bavarian State Ministry of Food, Agriculture and Forestry, and Erzeugergemeinschaft Hopfen HVG e.G., Wolnzach, Germany.

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Development of new approaches using beneficial microorganisms to prevent *Verticillium* wilt in hops

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Abstract

Verticillium wilt is one of the most significant diseases affecting hop plants in Slovenia and other hop-growing countries where lethal strains of *V. nonalfalfae* are present. Disease management primarily relies on phytosanitary measures, the planting of resistant varieties, and soil sanitation. In our study, we investigated the potential use of microbial biological control agents (BCAs) isolated from the hop rhizosphere and the xylem of hop plants against *V. nonalfalfae*. The strain selection process included an in vitro dual-culture plate assay and in planta efficacy testing conducted under controlled conditions in growth chambers. For field testing, the selected BCAs were used to enrich hop compost, which was then applied to *Verticillium* wilt-contaminated fields. Initial results showed that the selected strains could be promising BCA candidates for the prevention of *Verticillium* wilt in hops.

More details to this study can be found in the papers by GALLEGO-CLEMENTE *et al*. (2023) and GHORESHIZADEH *et al*. (2025).

Acknowledgement

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Mycotoxin production in *Fusarium sambucinum*, the primary causal agent of Fusarium canker in United States hop yards

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Abstract

Fusarium canker, caused by *Fusarium sambucinum*, is an emerging threat to the United States hop industry. Disease symptoms, including canker, girdling, and wilting, ultimately result in yield and quality loss in hop yards. *F. sambucinum* is also a causal agent of dry rot in potato and belongs to a group of important mycotoxin producers in the *Fusarium* genus; however, previous research into the pathogen is limited. *F. sambucinum* isolates from potato produce toxic Type A trichothecenes including diacetoxyscirpenol (DAS) and T2-toxin, yet isolates derived from hop pathosystems have not been characterized. Our objectives were to investigate *in vitro* trichothecene production among a collection of *F. sambucinum* isolates derived from hop bines and potato tubers, as well as the genetic variation in trichothecene biosynthetic gene clusters. To address the objectives of this study, isolates of *F. sambucinum* derived from hop and potato were sequenced using whole-genome sequencing and were tested for trichothecene production using GC-MS. Results revealed chemotypes in *F. sambucinum* based on host origin, which vary in production of DAS. The majority of hop-derived isolates did not produce DAS while potato-derived isolates consistently produced DAS. A conserved deletion in the *TRI13* gene was identified amongst hop-derived *F. sambucinum* isolates that did not produce DAS, providing a potential marker for chemotype. Mycotoxins are key factors in plant pathology, affecting both pathogenicity and food safety; therefore, understanding *Fusarium sambucinum* mycotoxin production is essential for advancing research on *Fusarium*-caused hop diseases.

Introduction

Fusarium sambucinum is an important member of the genus *Fusarium* being the type species and causing important plant diseases such as dry rot of potato, cone tip blight of hop, and Fusarium canker of hop (GAMS *et al.* 1997). Fusarium canker is becoming a greater problem for hop growers in the Pacific Northwest region of the United States, which encompasses Oregon, Washington, and Idaho. A higher incidence of canker has been observed in recent years, particularly in certain hop varieties. Symptoms of the disease include canker, girdling, and swelling of bines near the crown of plants, which ultimately lead to wilting and dieback of entire bines (Fig. 1) (OCAMB & BIENAPFL 2009). In fields with a high incidence, this can lead to hop yield and quality losses for growers.

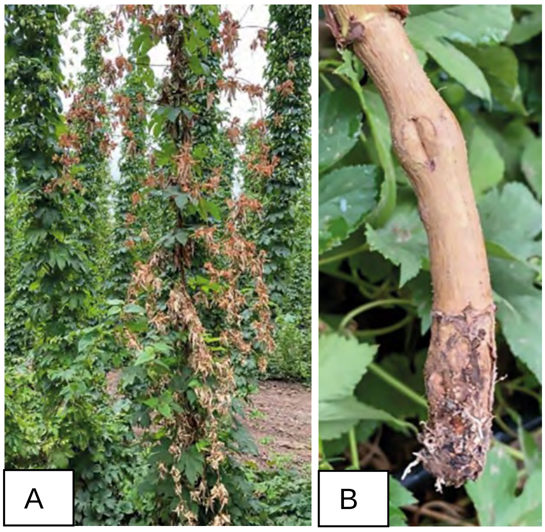

Figure 1. Disease symptoms of Fusarium canker. **A** – Wilted hop bines; **B** – a cankered and girdled hop bine.

Members of the *Fusarium sambucinum* species complex (FSSC) are known to produce several important mycotoxins including trichothecenes, a group containing some of the most impactful *Fusarium* toxins. The biosynthetic pathway of various trichothecenes is well-defined due to the relevance of trichothecene toxins to mammal health. Two toxic Type A trichothecenes are diacetoxyscirpenol (DAS) and T2-toxin, which have both been reported in other isolates of *F. sambucinum* (LARABA *et al.* 2021). Trichothecene biosynthetic gene clusters in *Fusarium* generally consist of a primary gene cluster of 12 to 14 genes, with two to four additional genes located at different loci, often on different chromosomes (PROCTOR *et al.* 2018). Unfortunately, due to a lack of historical research associated with *F. sambucinum,* there was not a thorough model of the trichothecene biosynthetic gene cluster in the species and production of trichothecenes in hop-derived isolates. To address the unanswered questions, we investigated *in vitro* trichothecene production among a collection of *F. sambucinum* isolates derived from hop and potato as well as genetic variation in the trichothecene biosynthetic gene clusters.

Material and methods

Isolates of *Fusarium sambucinum* were isolated from the crown of diseased hop bines exhibiting symptoms of Fusarium canker. Outgroup isolates were isolated from diseased potato tubers. Identifications of isolates were initially made based on morphological features. Samples suspected to be *F. sambucinum* were sequence confirmed using the translation elongation factor 1α (tef1α) barcoding region and aligned against a known database of Fusarium tef1α sequences, FusariumID, using BLAST (TORRES-CRUZ *et al.* 2022).

To determine mycotoxin production phenotypes, representative isolates of *F. sambucinum* were grown for one week on both sterilized rice and 20 mL of yeast extract peptone dextrose (YEPD) broth. Two different culture media were used to capture potential variation in metabolite production based on growing conditions. Liquid cultures were extracted with 8 mL of ethyl acetate and vortexing. Rice cultures were physically agitated to break up clumps of mycelia and rice while extracting with 10 mL ethyl acetate. 1 mL of the extract from each YEPD or rice culture was carefully transferred to a glass vial.

Glass vials were placed on an Agilent 7693A Autosampler connected to an Agilent 8860 gas chromatograph fitted with a HP-5MS column and an 5977B mass spectrometer detector for the automated GC-MS runs. Helium was used as the carrier gas, with a 20:1 split ratio and a 20 mL/min split flow. The column was held at 150°C for 1 min following injection, heated to 280°C at 30°C/min and then held for 7.7 min. ChemStation and Mass Hunter software programs were used to analyze data from each run. Peaks were identified by comparison to compounds in a NIST library and in a library of standards including trichothecenes and other *Fusarium* metabolites.

Low coverage genomes were obtained for each isolate using Illumina sequencing in addition to two high quality and coverage reference genomes using PacBio sequencing. The gene *TRI13* was identified in each genome using local BLASTn and aligned using MAAFT version 7.450 in Geneious Prime (Katoh & Standley 2013). *TRI13* sequences from *F. poae*, *F. venenatum*, and *F. sporotrichioides* were downloaded from NCBI Genbank (accession numbers: XM_044850437.1, XM_025735078.1, and AF330109.2) for evidence of the *TRI13* gene ancestral state.

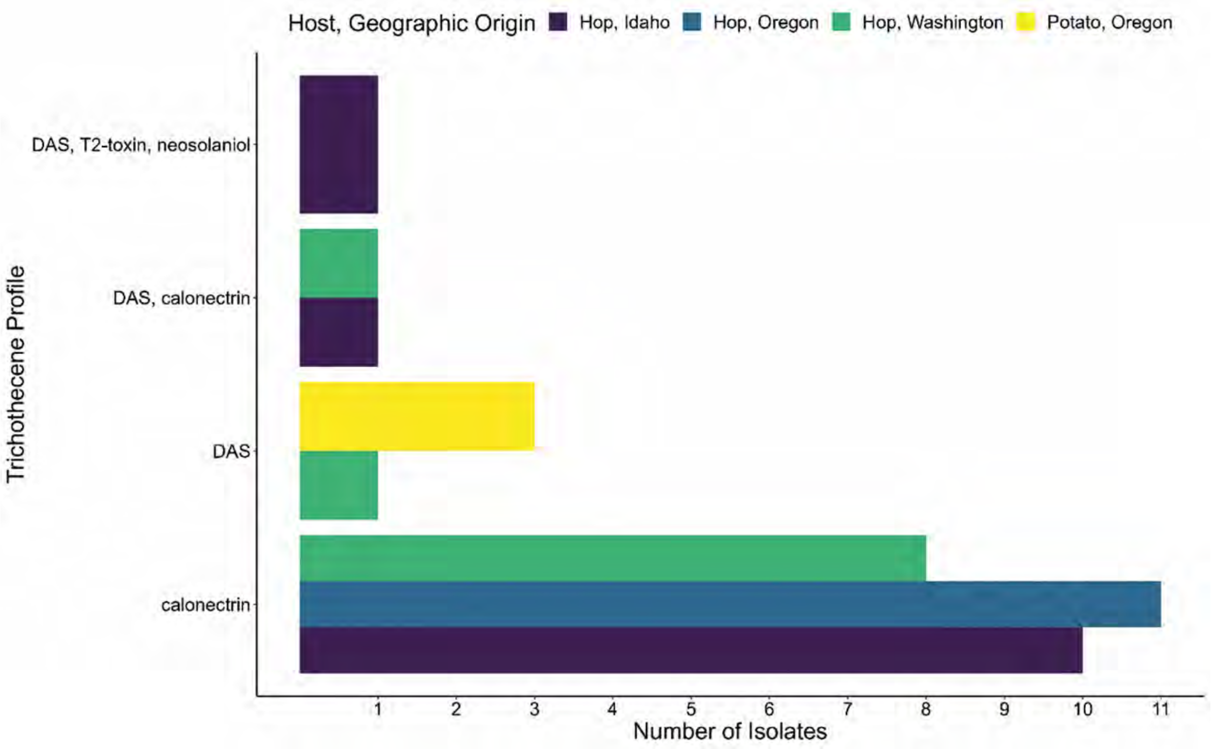

Figure 2. Distribution of measured *F. sambucinum* trichothecene profiles based on original host and geographic location. The top three trichothecene profiles include notable toxins DAS, T2-toxin, and neosolaniol.

Results

There were four general trichothecene profiles identified within the 36 tested isolates (Figure 2). There was no difference in trichothecene production in rice- and YEPD-grown cultures; therefore, only data from YEPD cultures is shown here. Of the tested isolates, 29 hop-derived isolates had trichothecene profiles that included calonectrin variants but not DAS. One hop-derived isolate produced DAS, T2-toxin, and neosolaniol. Two hop-derived isolates produced low levels of DAS while also producing calonectrin variants. The three potato isolates tested primarily produced DAS in addition to one hop-derived isolate. Trichothecene profiles did not vary substantially based on geographic origin of the tested isolate.

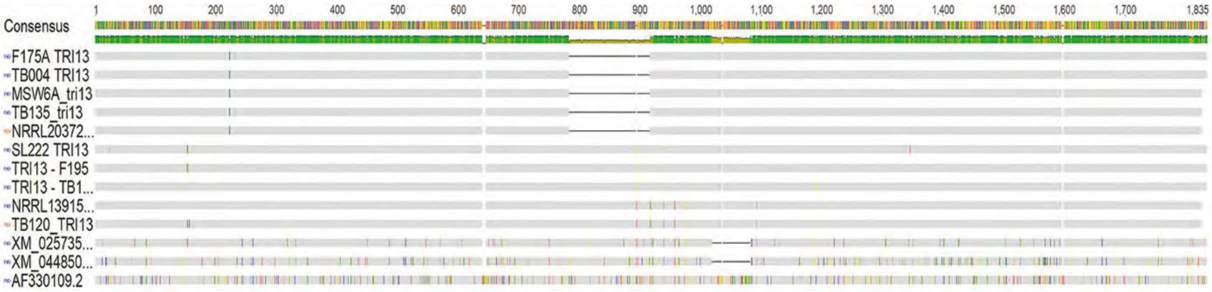

Figure 3. *TRI13* nucleotide alignment. All isolates are *Fusarium sambucinum* except numbers 11–13 which are *F. venenatum, F. poae,* and *F. sporotrichioides*, respectively. Isolates number 1–4, 8, and 10 are all hop-derived originating in the Pacific Northwest, USA. Number 5 is a hop-derived isolate originating from Poland. Numbers 6 and 7 are potato-derived. Number 9 is a cabbage-derived isolate.

Based on the results of the GC-MS experiments, specific trichothecene production genes, or *TRI* genes, were investigated more thoroughly. Specifically, *TRI13*, which codes for a cytochrome P450 monooxegenase, was a focus due to the key role it plays in the formation of DAS from the compound calonectrin. In gene alignments of the *TRI13* region in tested isolates, a deletion between position 783 and 916 was consistently identified in isolates that did not produce DAS (Figure 3). Evidence for the variant being a deletion is provided by aligning the region to *TRI13* sequences of three closely related species, *F. venenatum, F. poae,* and *F. sporotrichioides.* None of the three related species contains a deletion in *TRI13,* which indicates the hop-associated *TRI13* variant is a deviation from the ancestral state of the gene.

When assessing the *TRI13* gene in a larger collection of isolates, all hop-derived isolates exhibited the deletion in *TRI13* with the exception of two isolates: one that exhibited a trichothecene profile of DAS, T2-toxin, and neosolaniol and one that exhibited a trichothecene profile of DAS (Figure 2). A sequenced isolate originally derived from a hop yard in Poland, NRRL 20372, did have the deletion present in *TRI13*. Isolates derived from every other host tested (beet, brassica, corn, gorse, potato, and scotch broom) all had an intact *TRI13* gene (Table 1).

Table 1. Number of *Fusarium sambucinum* isolates with a deletion in the *TRI13* gene versus those with an intact *TRI13*, by host.

Host	**Intact *TRI13***	**Deletion in *TRI13***
Hop	2	142
Potato	10	0
Beet	1	0
Cabbage	1	0
Corn	1	0
Gorse	1	0
Scotch broom	1	0

Discussion

Through this research, we have begun to understand *Fusarium sambucinum* trichothecene production and diversity as it relates to hop. By directly measuring trichothecene production, a previously unidentified *F. sambucinum* chemotype was revealed. While not currently common in Pacific Northwest hop yards, *F. sambucinum* is capable of infecting cones, which has the potential to present new problems for growers. The non-DAS producing chemotype was consistently only associated with hop-derived isolates, which is positive for hop growers and brewers, as the majority of *F. sambucinum* isolates found in hop yards are not capable of producing the most toxic trichothecenes. Isolates derived from other hosts, along with two hop-derived isolates exhibiting the DAS-producer chemotype, is consistent with other research investigating trichothecene production. Two isolates exhibiting a different chemotype than others collected from hop yards may be explained by the presence of other hosts in the yard, such as from cover crops that are sometimes planted between rows or previous plantings. While preliminary data generated in our lab suggest a level of host preference among *F. sambucinum* isolates, it is possible that an isolate derived from another host could produce Fusarium canker symptoms in hop, even if the dominant population in a yard is a different chemotype.

The non-DAS producing phenotype is consistently associated with a single genotype in our experiments, providing a method to predict chemotype in *F. sambucinum*. Interestingly, among a large sample size of isolates, one was originally derived from hop in Poland, NRRL 20372. NRRL 20372 did have the deletion, indicating a chemotype consistent with *F. sambucinum* populations in the United States. Future research directions of this work could include assessing a greater number of hop-derived isolates from hop yards outside of the US for the phenotype and conserved genotype associated with non-DAS production. Eventually, understanding evolutionary dynamics of *F. sambucinum* on a global scale may provide broadly applicable insights into Fusarium canker. Additionally, research into *F. sambucinum* infection and mycotoxin production on cones is a natural follow-up to this research. Discovering a new chemotype in *F. sambucinum* is a large step toward understanding this important pathogen and the Fusarium canker pathosystem.

Acknowledgement

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IV: Hops and climate change

Use of reclaimed water for hop irrigation in Galicia and Castilla-León, Spain

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Abstract

The use and production of reclaimed water is regulated in Spain by Royal Decree 1085/2024, establishing agriculture (irrigation) as one of the possible uses. Within the I-ReWater project (Interreg SUDOE), two pilot actions were carried out in Abegondo (Galicia) and Cubillos del Sil (Castilla-León), irrigating with reclaimed water from an urban treatment plant during the 2024 season. The water status of the crop was monitored as well as hop quality and production. To guarantee the safety of water quality, analyses of the irrigation water (reclaimed and conventional) were carried out. The presence of nutrients in the reclaimed water did not affect hop cone yield in both action pilots, similar to other measured parameters, without significant differences between treatments, including qualitative aspects. The quantity of alpha and beta acids was similar in the hop cones irrigated with conventional resources (well or irrigation canal). Moreover, no variations were observed in the composition of essential oils. In global terms, reclaimed water to irrigate hops could be a safe water resource during drought periods, when no conventional water resources are available. First results show that no effects to production and quality were detected. Other micro-contaminants with possible presence in reclaimed water will be analyzed during the coming seasons.

Introduction

Climate change has generated the need to search water resources to cover hop water requirements during the growing season. In this way, policies to achieve a reliable water management are establishing innovative procedures based on “circular water management”, increasing water efficiency and water-saving, including the treated wastewater in the urban wastewater treatment plants (HUERTA *et al.* 2015). Regulation on minimum requirements for water reuse for agricultural irrigation entered into force in 2020 (REGULATION (EU) 2020/741), which was transposed into Spanish regulations in 2024 (ROYAL DECREE 1085/2024).

The objective of this study is to demonstrate the feasibility of using reclaimed water in hop cultivation in two regions of Spain, Galicia and León, in 2024. Two experimental irrigation treatments were implemented: well or canal water, versus a mix of conventional water with reclaimed water. The following aspects were evaluated: i) the plant water status and its response to irrigation; ii) yield assessment; and iii) the effects on hop cone quality (acids and essential oils).

Material and methods

The two pilot plots are located in Presedo-Abegondo (Galicia) (PRE) and Cubillos del Sil (Castilla-León) (CDS) (Fig. 1). PRE is characterized by Atlantic climate, with dry hot summers, and CDS by a subhumid continental Mediterranean climate, with dry, short and hot summers. In both cases, the hop variety is Nugget, established in 2008 in PRE and in 2022 in CDS.

The drip irrigation dose is 0.89 mm h^{-1} (PRE) and 1.47 mm h^{-1} (CDS), applying in 2024 a total irrigation of 91 mm and 283 mm in PRE and CDS, respectively, from June to early September. The amount of reclaimed water applied through irrigation was 40 mm (PRE) and 167 mm (CDS), representing about 50% of total water applied next season.

Two irrigation water treatments were implemented: conventional water (PRE: from a well; CDS: from an irrigation canal) and a water mix (reclaimed water and conventional water). Three replicates were implemented per treatment and plot, with a total of 14 plants per experimental unit. All measurements were taken in each experimental unit (e.u.). BBCH scale was determined weekly from the end of May to harvest, considering the average value of three plants per e.u. (ROSSBAUER *et al.* 1995). The well or canal and reclaimed water was sampled, to determine analytical parameters (pH, SS, BOD_5, EC, phosphorum, nitrates, and ammonia nitrogen) in a laboratory during the irrigation period, to obtain the final amount of nutrients applied with the irrigation water. Moreover, microbiological parameters were measured (nematode eggs, *Legionella* spp., *E. coli*, and *Taenia* spp.) to determine compliance with water quality (Class C) as established by legislation (ROYAL DECREE 1085/2024).

To monitor plant water status, leaf water potential (Ψ_l) measurements were taken at solar noon every 7–10 days using a Scholander chamber (PMS-600, PMS Instrument, USA) in three healthy leaves per e.u., at 1.5–2.0 m above the ground. Moreover, stomatal conductance (g_s) was measured weekly with a porometer (SC1, Decagon Devices,USA) in market leaves from July to August. In order to control the irrigation volume applied to both treatments and plots, measurements of soil water content were determined weekly from May to August, using a TDR-100 (Campbell Scientific, USA) with stainless steel rods (60 cm) (FANDIÑO *et al.* 2019).

At harvest time – PRE: 28.viii.2024, CDS: 02.ix.2024– a representative complete plant per e.u. was analysed by separating all cones manually from stems and leaves. Total green yield per plant was measured and two samples of 500 g (dry weight) were transported to Campus Terra facilities in Lugo. All hop cone samples were dried to a humidity of 10 % or higher. To determine alpha and beta acids and for quantification of essential oils (EBC 7.7 and EBC 7.10, respectively), one sample of dried hop cones (100 g sample^{-1}) was sent to an external laboratory and stored in optimal conditions until the final analysis.

Figure 1. Hydrographic limits (red lines) and location of Actions Pilots plots (blue points). Left: Abegondo (Galicia); Right: Cubillos del Sil (Castilla-León)

Results

The phenology of the two pilot actions was similar for both irrigation treatments (Fig. 2), with an advance of 15–20 days in CDS to reach BBCH stage 61 (flowering), compared to PRE. In the Galician plot, an earlier harvest was required due to a severe fungal disease outbreak during August. Plant water status measures with Ψ_l and g_s (Fig. 3), showed similar trends to Ψ_l in both treatments and plot, with a slightly higher values (-6.5 bar) in Galicia at the end of July. In relation to stomatal closure (g_S) lower values were measured during the whole growing season in CDS, without significant difference between mix and canal irrigation treatments. However, in Galicia, higher values were measured in well treatment plants in August. This aspect is related with the higher relative humidity and disease effects at the end of season.

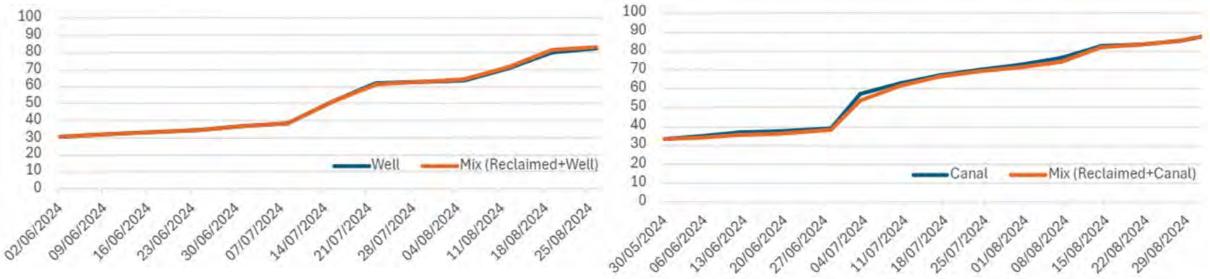

Figure 2. BBCH stage development in both action pilot during the 2024 field season. Left: Abegondo (Galicia); right: Cubillos del Sil (Castilla-León)

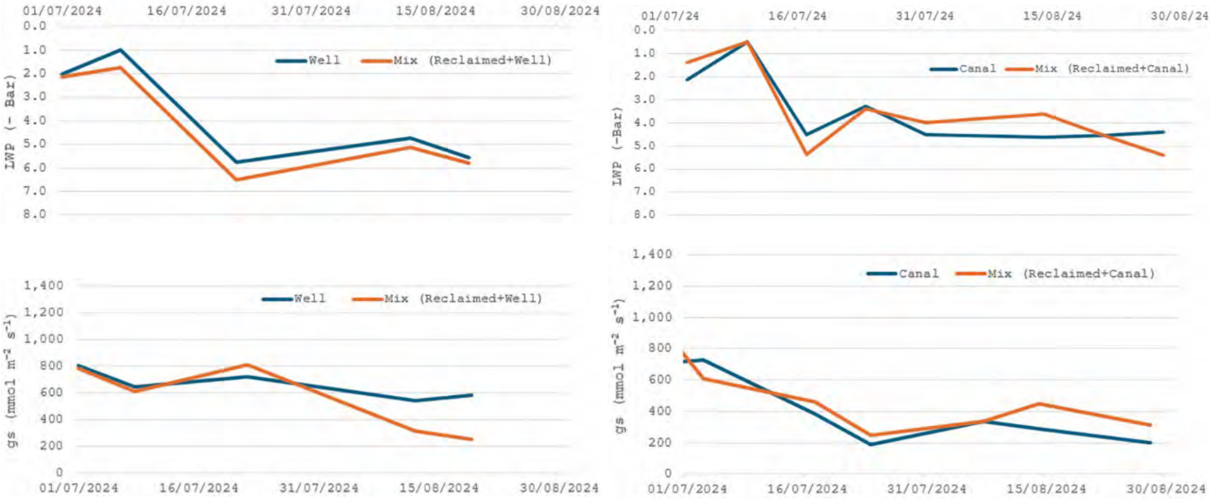

Figure 3. Dynamics of leaf water potential (above) and conductance stomatal (below) during the 2024 field season. Left: Abegondo (Galicia); right: Cubillos del Sil (Castilla-León)

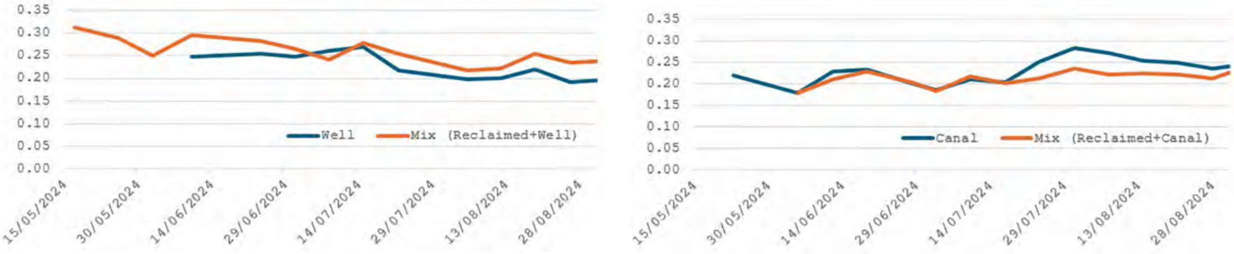

Figure 4. Dynamics of soil water content (m^3 m^{-3}) during the 2024 field season. Left: Abegondo (Galicia); right: Cubillos del Sil (Castilla-León)

Dynamic of soil water content was similar to both treatments, with higher average values of SWC in PRE (0.25 m^3 m^{-3}) than in CDS (0.22 m^3 m^{-3}), related mainly to soil conditions (Fig. 4).

In global terms, yield was similar in both treatments in PRE and CDS, but a higher production was obtained in CDS (Table 1). Drying processes were irregular, with differences between treatments, especially in Galicia. No significate difference was obtained for quality parameters, but a trend to higher α-acids, β-acids and myrcene was determined in mix-irrigated treatments.

Table 1. Yield and quality parameters in Galicia (PRE) and Castilla-León (CDS).

Plot & Treatment	Dry cones (kg ha^{-1})	Humidity (%)	α-acids	β-acids	Essential oils (ml /100g)	Myrcene (% Total oils)
PRE-Well	860.7	14.7	9.83	3.75	1.03	46.4
PRE-Mix	748.1	18.5	10.35	3.90	0.89	47.5
CDS-Canal	2579.9	13.0	10.27	2.67	1.42	61.5
CDS-Mix	2677.5	13.4	10.70	2.80	1.62	63.8

Discussion

The use of reclaimed water, mixed with conventional irrigation water, proved to have neither short-term impact on the plant's water status nor on yield and quality parameters of the final product. Especially relevant were the nutrients added. The contribution of nutrients, ammonia nitrogen and nitrates, was different in both plots compared to the control (well or canal). In PRE, there was an input of 1 kg/ha in the irrigation water for both NH_4-N and NO_3-N, while in the case of CDS, 2 kg/ha of ammonium nitrogen and 5 kg/ha of nitrates were added. The higher nutrient inputs in the mix treatment could be responsible for an increase in the amount of alpha and beta acids as well as the higher production per hectare in CDS. The PRE plantation was severely affected by a fungal disease outbreak, why the production and quality results should be viewed with caution. In summary, the use of reclaimed water offers good expectations in terms of production and quality, although the safety of this resource's quality and handling is high. Other micro-contaminants in the reclaimed water should be analyzed to determine a possible risk of soil degradation, or accumulation effects in a long-term way, avoiding negative effects on the crop.

Acknowledgement

This study was supported by the project 'Sustainable management of water resources in irrigated agriculture in the SUDOE area (I-ReWater-S1/2.5/E0136)' co-funded by the programme INTERREG SUDOE. Especial thanks to GESTAGUA to facilitate reclaimed water resource in both study areas. Thanks to all entities involved in the experiment: LU.TE.GA., Concello de Carballo, SAT Lúpulos de León, Lúpulos del Bierzo, and Concello de Lugo.

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Enhanced stress tolerance in the hop varieties 'Saaz' and 'Hersbrucker Spät' by heat acclimation

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Abstract

Climate change, particularly global warming, has adversely affected hop production. Precipitation shows a positive correlation with hop yield, while high temperatures during summer exhibit a negative correlation with α-acid content, underscoring the need for new approaches to improve stress tolerance in hops. This study investigated the potential of heat acclimation, which has been reported in other plant taxa as a mechanism in which prior exposure to sublethal heat stress confers tolerance to subsequent high-temperature stress, as a method to enhance stress tolerance in existing hop varieties. In this study, we used cvs Saaz and Hersbrucker Spät as experimental materials. The results suggest that heat acclimation alleviated reduced growth due to high-temperature or drought stress. In addition, field trials conducted in Japan have indicated that heat treatment of hops may confer tolerance to complex environmental stresses.

Introduction

Climate change such as global warming has negatively impacted hop production. Especially, it has been reported that precipitation shows a positive correlation with hop yield, while high temperatures during summer exhibit a negative correlation with α-acid content (DONNER *et al.* 2020), highlighting the need for new approaches to enhance stress tolerance in hops. This study focuses on heat acclimation, a mechanism that has been reported in other plants, whereby plants can survive sublethal temperatures by being exposed to mild levels of temperature stress beforehand. In *Arabidopsis thaliana*, it has been reported that a heat acclimation treatment involving exposure to 37°C for one hour can confer heat tolerance for up to three days under conditions of 43.5°C, which would otherwise be lethal. Additionally, heat acclimation has also been associated with changes in the epigenetic modifications of upstream regulatory genes involved in stress responses, resulting in increased expression of downstream stress-response genes (YAMAGUCHI *et al.* 2021). However, reports on heat acclimation in hops are scarce, and it remains unclear whether hops possess the capacity for heat acclimation or whether protocols established in model species can be adapted for hops.

Therefore, we applied heat acclimation treatment to hops using the previously reported protocol. The result suggested that hops subjected to heat acclimation may retain heat tolerance under high-temperature conditions at the laboratory scale. Therefore, the aim of this study was to evaluate whether heat treatment can improve heat tolerance in existing hop varieties under outdoor field conditions.

Material and methods

Plant materials and growth conditions

Humulus lupulus cvs Saaz (SAZ) and Hersbrucker Spät (HEB) were used in this study. In vitro plants were prepared as described by HIRAKAWA & TANNO (2022). Plants were cultivated in 1/2 Murashige and Skoog (MS) medium supplemented with 2 % (w/v) glucose in an incubator at 20°C under a 16 h light/8 h dark photoperiod. Plants were subcultured every 1.5 to 2 months.

Heat treatment

Heat treatment was applied during the propagation of hop axillary buds, following the protocol described in HIRAKAWA & TANNO (2022). In vitro plants were incubated in 1/2 MS liquid medium supplemented with 2% glucose at 25°C for six weeks, in contrast to the standard propagation temperature of 20°C. Following this treatment, axillary buds were cut and rooted from each stem explants. Stem explants that had developed roots were subsequently transferred to cell trays containing soil and grown in an incubator at 20°C.

Heat tolerance assay

Heat-treated plants were grown in an incubator at 30°C for 28 days. Heat tolerance was evaluated based on the plant height and leaf chlorophyll content.

Drought tolerance assay

Heat-treated plants were subjected to drought stress by withholding water in an incubator at 20°C for 10 days, followed by a re-watering period of 7 days. Drought tolerance was assessed based on the survival rate and leaf chlorophyll content.

Measurement of chlorophyll content

Chlorophyll content was measured as described by YAMAGUCHI et al. (2021). Two leaves from the first node of the hop plant were placed in 1 mL *N*, *N′*- dimethylformamide (DMF) in 1.5 mL microtubes and incubated at 4°C for 24 h. After incubation, the absorbance of the extract was measured at 646.8 nm and 663.8 nm using a spectrophotometer. Total chlorophyll content was calculated using the formula: Chlorophyll $a + b$ (μM) = $19.43\,A_{646.8} + 8.05\,A_{663.8.}$

Field trials

Heat-treated plants were grown in a greenhouse under natural photoperiod conditions for 4–5 weeks. Subsequently the plants were transplanted to two field sites in Iwate Prefecture, Japan, between April and May. Stem elongation, plant weight at the time of cone harvest, and α-acid content of the cones were evaluated.

Measurement of α-acid content

Harvested cones were dried at temperatures below 60°C for more than 6 h. The α-acid content of the dried cones was measured in accordance with EBC 7.4 and 7.5 methods.

Results and discussion

Heat-treated hops were grown under high-temperature conditions of 30°C for 28 days. The treated plants exhibited significantly greater height and chlorophyll content compared to the untreated controls (Fig. 1). In a separate assay, heat-treated plants were subjected to drought stress by withholding water for 10 days, followed by a 7-day re-watering period. The treated plants showed significantly higher survival rates and chlorophyll content than the untreated plants (Fig. 2). These findings suggest that the heat treatment enhances both heat and drought tolerance in hop plants.

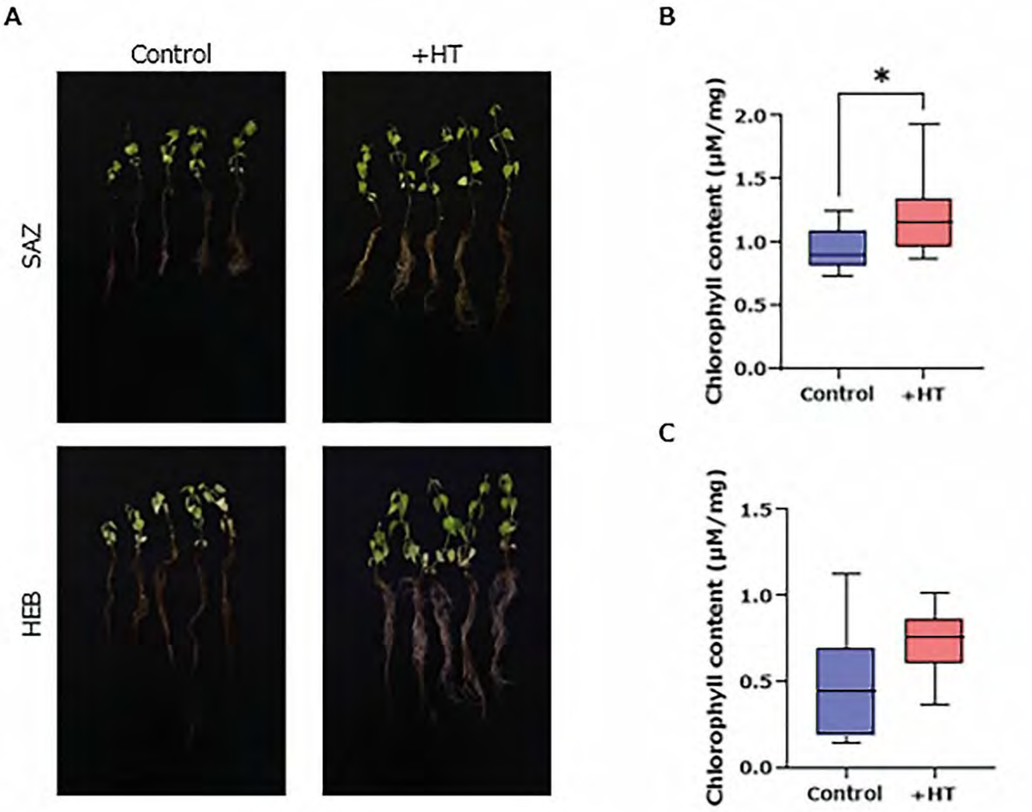

Figure 1. Heat treatment enhances heat tolerance in hops. **A** – Representative images of hop plants under control conditions (left) and heat treatment conditions (+HT, right); above cv. SAZ, below cv. HEB. Plants were grown at 30°C for 28 days. **B** – Saaz; **C** – Hersbrucker Spät. Box-and-whisker plot of chlorophyll content in control and heat-treated plants. The plots indicate the sample minimum (lower whisker), lower quartile (bottom of box); median (line within box), upper quartile (top of box), and sample maximum (upper whisker). Statistical significance was assessed by Student's *t*-test (*$p < 0.05$; $n = 10$).

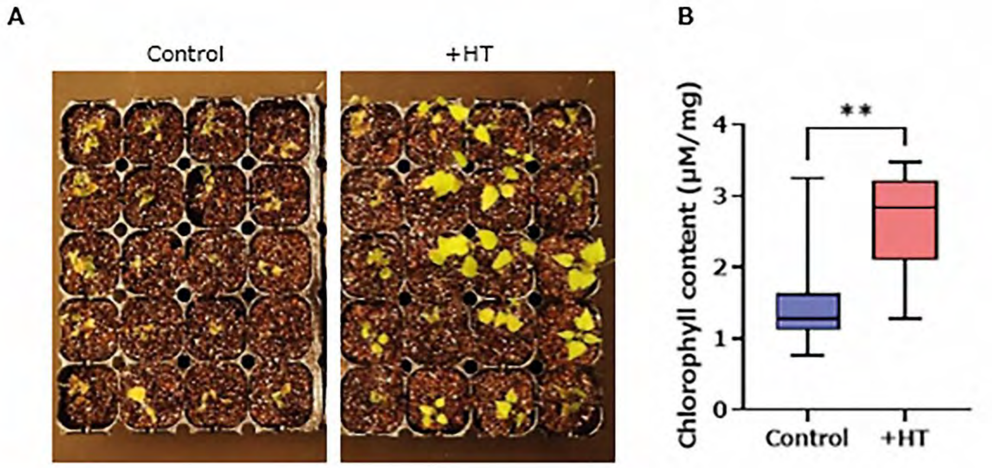

Figure 2. Heat treatment enhances drought tolerance in hops. **A** – Representative cv. SAZ plants under control conditions (left) and heat treatment conditions (+HT; right). Plants were subjected to drought stress by withholding water for 10 days, followed by a 7-day recovery period after re-watering. **B** – Box-and-whisker plot of chlorophyll content in control and heat-treated plants. Statistical significance was assessed by Student's *t*-test (**$p < 0.01$; $n = 10$).

Heat-treated hop plants were transplanted and cultivated until cone harvest in Iwate Prefecture, Japan. In the first year, the height of heat-treated plants was significantly higher than that of untreated plants by the second week after planting. The results of the second-year trial will be reported in the presentation.

Thus, applying heat treatment by cultivating hop axillary buds at a mild high temperature of 25°C for six weeks during the propagation process may confer tolerance to complex environmental stresses under field conditions. Future studies will continue to monitor growth performance in the third year and beyond. Additionally, further examination will be conducted to determine whether the resistance conferred by heat treatment in hops is mediated by the same mechanisms underlying heat acclimation or by distinct processes (manuscript in preparation).

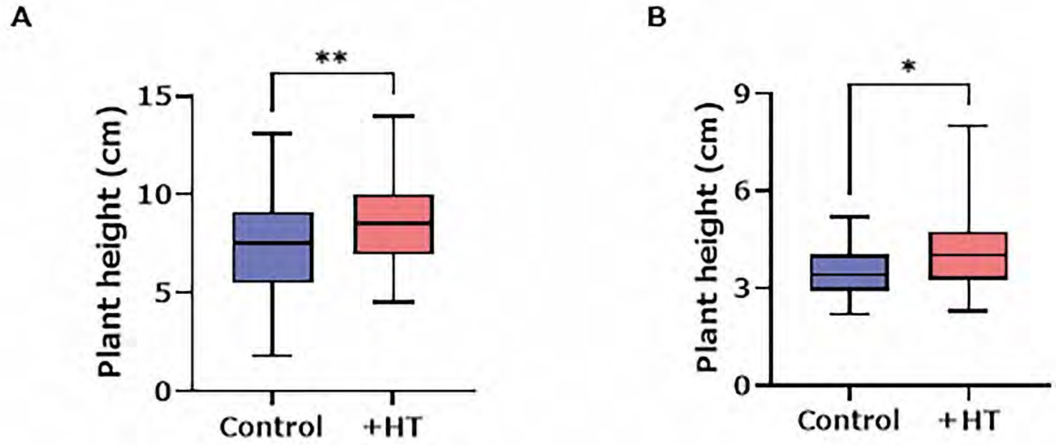

Figure 3. Heat treatment may enhance vegetative growth in the first year after planting. **A** – cv. SAZ; **B** – cv. HEB. Plants were grown in Iwate Prefecture, Japan. Box-and-whisker plots of plant height in control and heat-treated hop plants (+HT). The plots show the sample minimum (lower whisker), lower quartile (bottom of the box), median (middle line), upper quartile (top of the box), and sample maximum (upper whisker). Statistical significance was assessed by Student's *t*-test (**p* < 0.05, ***p* < 0.01; (A) n = 57, (B) n = 28).

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Hop yields and alpha acids content modelling at global scale using machine learning algorithm

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Abstract

Hop is cultivated worldwide in a large variety of pedoclimatic conditions. Despite hop breeding and agricultural practices optimisation, hop yields and hop quality yearly vary mainly influenced by weather conditions, pests and diseases. Nevertheless, the effect of weather conditions on hop yields and quality potential is largely unknown at global scale. However, this knowledge is determinant to estimate the potential of a new hop growing region and evaluate climate change consequences on hop production. In this work worldwide historical datasets from BarthHaas reports about hop yields (since 1961), alpha acids concentrations (since 1997) and weather data from weather stations located in associated hop growing regions were combined. Using a machine learning algorithm, a yield model and an alpha acids content model were developed. The analysis of the models highlights weather features which contribute positively or negatively in yield determination and alpha acids synthesis.

Introduction

Hop yield and alpha content are the two main determinants for hop quantity and quality worldwide. The impact of weather conditions during the growing season is well known and observed through the yearly variability of hop yield and alpha acids content for a given variety in a given location (ZATTLER & JEHL 1962; KROFTA *et al.* 2017; FORSTER *et al*. 2021). From this observation several studies were conducted to model hop yield and alpha acids content using meteorological data (SREČEC *et al.* 2008; KROFTA & KUČERA 2009; PAVLOVIČ *et al.* 2010; MORTON *et al.* 2017; FORSTER & SCHÜLL 2020; FORSTER *et al*. 2021; MOZNY *et al*. 2023). Their results revealed that warm growing seasons are correlated with reduced alpha and dry growing seasons with reduced yields (SREČEC *et al*. 2008; FORSTER *et al.* 2021; MOZNY *et al.* 2023). Those research were mainly conducted over European hop growing areas (SREČEC *et al.* 2008; KROFTA & KUČERA 2009; PAVLOVIČ *et al.* 2010; MORTON *et al*. 2017; FORSTER *et al*. 2021) or using European data to train the model and then extend it at a global scale (MOZNY *et al*. 2023).

As a result, the impact of meteorological conditions during the hop growing seasons on yield and alpha acids content is unknown on a global scale. Furthermore, the use of irrigation, to compensate for the lack of rainfall during summer in some growing areas, offers new assumptions to investigate other factors that favor or reduce alpha acids production. In this context, this work is focused on the analysis of global yields and alpha acids dataset associated with weather data to better understand the relationship between hop production and quality with meteorological factors worldwide.

Material and methods

Hop production and quality dataset

In order to build a global, homogeneous and reliable dataset from the BarthHaas reports (available at https://www.barthhaas.com/en/) were selected. Hop yields per hop growing region were registered since 1961 from the hop acreage and production table available in each report. Since 1997, details about average alpha acids content per hop growing region have been integrated and were used in this study.

Meteorological data

For each hop growing region, Global Summary of the Day (GSOD) data were downloaded from NOAA website selecting local weather stations. The station selection followed two criteria. First, the station selected must be inside the hop growing region or close. Then only stations with continuous and reliable data were selected. The dataset contains for each station the following variables:

- name of the station
- Station location (latitude and longitude)
- Mean, Min, Max temperatures of the day in F°
- Total precipitation of the day in inches

Processing

In order to limit the biases due to hop varieties and hop agronomical characteristics in each region worldwide, yield yearly variation and alpha acids yearly variation were calculated as follow:

$$X_{difference} = X_{year} - Mean(X)_{period}$$

Meteorological data were preprocessed to convert each column into universal system units, missing data were corrected and then the following features were calculated:

- Sum of ETp calculated using Hargreaves equation (Hargreaves & Samani 1985) during the growing season
- Growing degree days during the growing season and yearly
- Average temperature of the growing season
- Number of days with T_{max} >35° during the growing season
- At a monthly step and yearly step, min temperature, max temperature, total rainfall

The growing season corresponds to the period from 01.iv–30.ix. In order to homogenise data from the northern and southern hemispheres, months from the southern hemisphere were converted to the corresponding ones in the northern hemisphere.

The final dataset was randomly split into training and testing datasets using 70 % of the dataset for training. A random forest regressor model was then optimized and built in order to accurately predict yields yearly variation and another one to predict alpha acids yearly variation. The best parameters for each model were optimised using a range of possible parameters and 10-fold cross validation.

Results and discussion

Yield model

Figure 1 details the performance of the model on the testing dataset. In order to better visualize the relationship between predicted yield difference and the actual yields, the yield differences values were converted back to yields with the adding of the average yield values over the period for each point.

The predicted results are well correlated to the actual observed yields on the testing dataset. Nevertheless, a high dispersion can be noted leading to a RMSE of 0.29. Among the main features contributing to the model, ETp, GDD, mean temperatures during June, July and August are retrieved. High GDD and ETp in June are associated with better yields whereas high mean temperature and high minimum temperatures in August are associated with reduced yields. Also, it is interesting to highlight that several features from winter and spring are also in the top 20 list of features and that most features contribute similarly to the model. This suggests some impact of global climatic conditions of a given hop growing region or an impact of the vernalization conditions. Further investigations are necessary to evaluate these hypotheses.

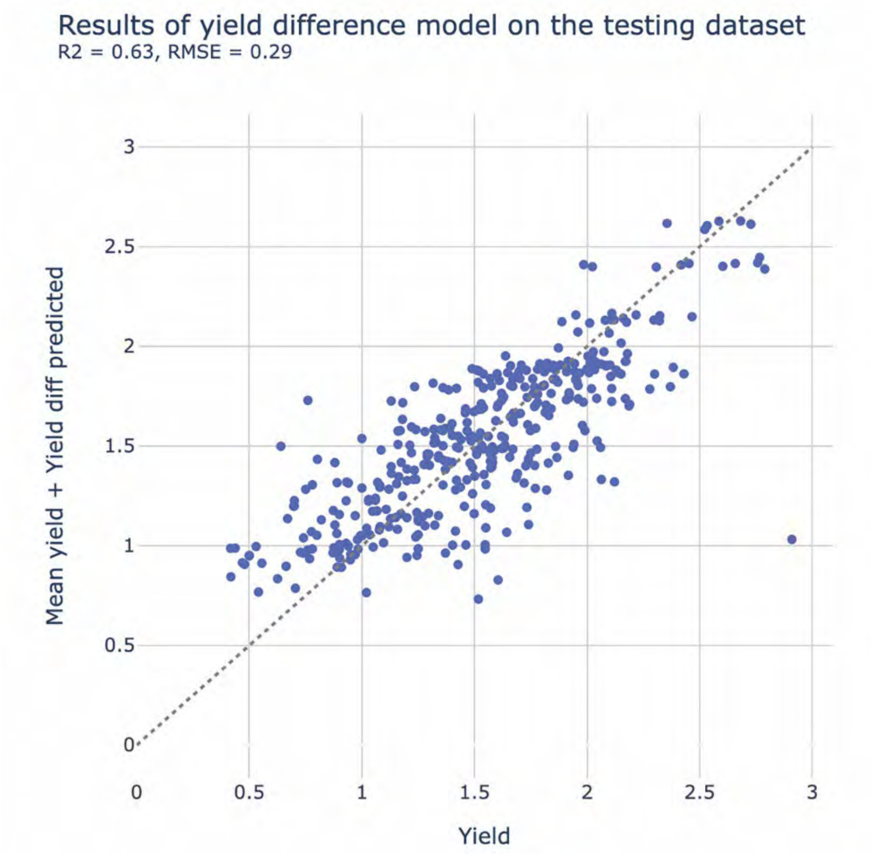

Figure 1. Yield difference model performances on the testing dataset

Alpha acids model

Figure 2 details the performance of the alpha difference model on the testing dataset. In order to better visualize the relationship between predicted alpha acids difference and the actual alpha acids values, the average alpha acids for each point were added to the predicted alpha acids difference values to convert them back to alpha acids content. The predicted results are highly correlated to the actual observed alpha acids on the testing dataset with a R^2 value of

0.91 and a RMSE of 0.93. The dataset is reduced compared to the dataset about yield, so climatic evolution bias is limited in this case. The main features contributing to the model indicate that moderate to high min temperature and GDD in May, high GDD and ETp in August, high rainfall amounts in March and April favour positive alpha acids difference. On the contrary, high GDD, mean temperature and T_{max} temperature in September, mean temperature in December and rainfall amounts in August are associated with negative alpha acids difference.

Those highlights suggest that rainy early spring associated with a warm beginning of the growth period (May), a hot end of summer (August) but cooler end of season (September) is positive to alpha acids synthesis. The negative impact of rainfall in August disagrees with previous studies. It might indicate that dryness in that particular month is tolerated but further analysis is required. Nevertheless, these observations remain at global scale and varietal difference are not distinguished as well as the difference between aromatic and bitter varieties.

Conclusion

This work highlights the potential of machine learning algorithm to predict hop yields and alpha acids content. The use of annual difference in hop yield and alpha acids content is relevant to reduce the biases related to variety and agronomical strategies but require a large historical dataset to build a liable average during the period. The results are mostly consistent with previous studies. Furthermore, it is an introductive work, and further analysis is necessary to well understand the feature contribution to the model, the impact of weather conditions in the definition of hop yield and alpha acids content.

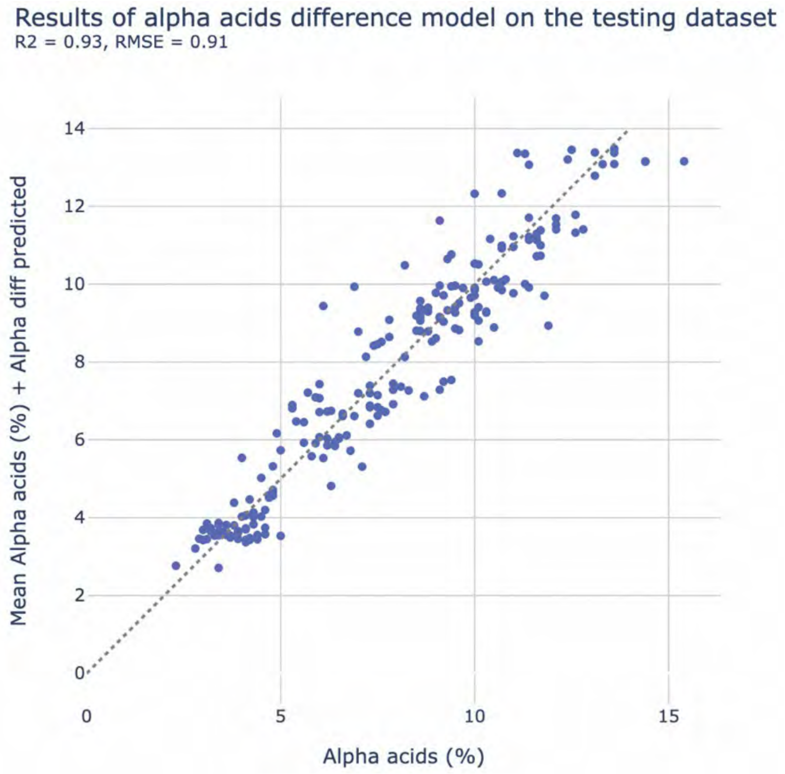

Figure 2. Alpha acids difference model performances on the testing dataset

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Hop'50: Designing resilient hop production systems in response to climate change

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Abstract

The experimental organic hop field at Obernai (France, Alsace) provides a unique opportunity to design innovative and resilient agroecological organic systems for hop production. The Hop'50 project aims to develop and assess two contrasting cropping systems – one agroecological, "Low-Tech," inspired by the natural riparian ecosystem, and one "High-Tech," artificialized, to face short-term climate stresses. These systems were established to address the main climatic, environmental, and socio-economic challenges of Alsatian and French hop production. This study outlines the experimental setup and evaluates the systems' resilience based on agronomic, environmental, and economic indicators. The project is part of broader research initiatives which investigates agroecological strategies to reduce copper use while enhancing resilience to climate change.

Introduction

Climate change is increasingly impacting the Alsace region, altering the environmental conditions essential for hop cultivation. Recent climatic trends have highlighted a significant rise in average annual temperatures, accompanied by a decrease in precipitation and an increase in the frequency and intensity of heatwaves and drought events. Between 2019 and 2023, climatic observations in Obernai revealed a consistent pattern of rainfall deficits and temperature anomalies, with annual temperatures exceeding the historical average by 1.4°C and precipitation levels falling below the norm in four out of five years.

These climatic changes have direct consequences on hop production. Elevated temperatures, particularly during the growing season, have been associated with a reduction in yield and a decline in the quality of hop cones, notably in the content of alpha and beta acids, which are essential for brewing. Extended periods of drought and high night-time temperatures have further exacerbated water stress, disrupting the physiological development of the hop plants and compromising harvest outcomes.

The vulnerability of hops is amplified by the monocultural systems predominant in Alsace, which lack the ecological buffers of natural ecosystems. This increased exposure to climatic stresses necessitates the development of adaptive strategies to ensure the long-term viability of hop production in the region.

Material and methods

The systemic experimentation approach (HAVARD *et al.* 2017) implemented in the Hop'50 project follows a six-step methodology designed to evaluate innovative cropping systems under real farming conditions. The process began with a comprehensive diagnostic of the local hop production context, identifying key agronomic, environmental, and socio-economic challenges.

Based on this analysis, two contrasting systems were co-designed with stakeholders, following a participatory approach:

i) **Low-Tech Agroecological System**: Agroforestry plots integrating hedgerows and cover crops, coupled with extensive sheep grazing, aiming to reproduce riparian ecosystem services without synthetic inputs.

ii) **High-Tech Artificialized System**: Hop yards equipped with anti-hail shade nets, combined with overhead drip irrigation to mitigate abiotic stresses linked to heat and drought events.

Both systems were developed as coherent prototypes integrating technical interventions, decision rules, and resource management strategies. The experimental setup was established at EPLEFPA Obernai, ensuring representativeness of the regional pedoclimatic conditions. A multi-year field trial was then conducted, with continuous adjustments of decision rules to account for unforeseen biotic and abiotic factors. The evaluation phase relies on the collection of agronomic, environmental, and economic indicators to assess the feasibility, performance, and sustainability of each system. Finally, the knowledge generated is valorized through stakeholder engagement and scientific dissemination to support climate-resilient hop production.

These systems were selected based on a SWOT analysis and multi-criteria evaluation aligned with agronomic, environmental, and socio-economic objectives. The key objectives include improving climate resilience, reducing copper usage, preserving soil fertility, and maintaining economic viability for hop growers.

The following data are collected:

- Microclimatic monitoring
- Agronomic performance (yield, alpha acid content, plant health)
- Soil and plant biodiversity
- Economic viability

Acknowledgements

This work was supported by the Kronenbourg Foundation, the French Ministry of Agriculture, and the Grand Est Region.

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V: Hop viroids

Evaluating CBCVd resistance in hop through artificial inoculation, grafting, and transcriptomics

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Abstract

Hop (*Humulus lupulus* L.) cultivation faces significant threats from *Cocadviroid rimocitri*, previously known as Citrus bark cracking viroid (CBCVd). Field observations in Slovenia suggest that hop cultivars exhibit varying levels of susceptibility to CBCVd, ranging from highly sensitive to tolerant and resistant varieties.

In this study we evaluated the resistance and susceptibility of various hop cultivars to CBCVd. Tissue cultures enabled controlled viroid inoculation, ensuring uniformity and eliminating pre-existing infections. The inoculated plants were monitored for seven weeks, with disease severity indices (DSI) assessed regularly and infection levels confirmed via RT-PCR. Additionally, pot experiments were conducted on selected cultivars by grafting CBCVd-infected cv. Celeia hop seedlings onto the rootstocks of viroid-free cvs Styrian Wolf, Styrian Cardinal, and Celeia. To investigate the genetic basis of resistance, RNA was extracted from infected and non-infected samples at several time-points and sequenced on the MGI-T7 platform, yielding between 20.3 M and 40.3 M reads per sample. Bioinformatics analyses using the CLC Genomics Workbench identified differentially expressed genes between resistant and susceptible cultivars.

Our findings provide crucial insights into CBCVd resistance mechanisms in hops and identify genetic markers for breeding resistant cultivars for sustainable hop production and viroid management strategies.

Early detection of CBCVd infection in hops

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Abstract

Codadviroid rimocitri, also known as Citrus bark cracking viroid (CBCVd), is a single-stranded, self-replicating, circular RNA. CBCVd belongs to the family of Pospiviroidae, which replicate in the cell nucleus and are present within all parts of the plant. In hops (*Humulus lupulus*), symptoms of CBCVd infections include severe stunting, reduced internode length, chlorosis and abnormally formed cones. Ultimately, CBCVd causes a major reduction in yield and can lead to plant death. Hop cultivation involves numerous techniques that involve plant contact like crowning and training, facilitating CBCVd transmission from plant to plant. CBCVd infections are persistent and cannot be chemically treated. To prevent the spread of the disease, grubbing of infested plants is necessary. However, CBCVd infections can remain latent and visible symptoms appear in general quite late during the season around mid-July. Detection of infected plants as early as possible and ideally before the emergence of visual symptoms, would be an advantage in fighting the disease. Since chlorosis is one of the earlier symptoms of CBCVd-infected plants, we investigated whether reduction in chlorophyll levels can be technically measured before the appearance of visible symptoms. In this study, chlorophyll contents of CBCVd-infected and control plants of cv. Perle were measured in a hop field with a SPAD meter before and after emergence of visible symptoms. Infection status was validated through qPCR analysis. Our results demonstrate that while chlorophyll depletion is a non-specific symptom of disease, it can serve as an objective measure and an early indicator of CBCVd-infection. Alongside corresponding technical advancements, future measurements may offer a practical method for earlier detection of CBCVd-infected plants in the field.

Acknowledgement

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VI: Entomology

Biological control of the European corn borer, *Ostrinia nubilalis*

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Abstract

The European corn borer (ECB), *Ostrinia nubilalis* (Hübner) poses significant challenges to hop production in Slovenia, requiring effective management strategies. This study highlights the importance of timing in ECB management, in particular by monitoring the flight of adults in fields using light traps – both conventional and automated – adapted to different microclimatic conditions. Degree-days (DD) model, spermatophores examination, combined with visual inspection of hop can help predict the emergence of the first eggs and larvae, facilitating timely intervention. Biological control measures focus on the use of natural enemies. Micro-organisms such as *Bacillus thuringiensis*, targets the larvae, and/or macro-organisms such as the parasitoid wasp *Trichogramma brassicae*, targets the eggs. These integrated approaches aim to optimise pest control while minimising environmental impact.

Introduction

Ostrinia nubilalis (Hübner) (Lepidoptera: Crambidae) is a major pest of hops and maize, reducing the number of healthy ears of maize and hop cones. In Slovenia, two generations can be observed on hops, and the larvae of both generations burrow into hop stems and cones. Their pheromone in Slovenian maize and hop fields is both E strain. Eggs and larvae can be observed directly on maize. After the third larval stage, ECB begins dispersing to neighbouring plants, posing a major threat to hops in the vicinity of maize. The larval feeding behaviour causes the heads of hop bines to dry out slowly during their growth phase, leading to the decomposition of the bine above the larval entrance hole. ECB can also cause secondary infection of crops by pathogens, in the pathway opened into the vascular system.

As ECB overwinter as larvae, mechanical removal of crop residues helps to reduce the summer population, but due to their wide host range, ECB remain a severe threat to hops. Integrated Pest Management (IPM) measures already exist on maize, and have shown great effectiveness for over 40 years, such as inundative releases of *Trichogramma brassicae*, a parasitoid wasp that lays eggs in the ECB egg mass present on the back of maize leaves (HOFFMANN *et al.* 2002, 2006; RAZINGER *et al.* 2016). Distributed by many crop protection companies, *T. brassicae* appears at different developmental stages to better adapt to the ECB's broad oviposition period. A second natural enemy is widely used against moths: *Bacillus thuringiensis* (Bt), highly effective against ECB in maize (TAN *et al.* 2011). Bt targets the ECB larval stage, keeping the second generation below a damage threshold. Both IPM methods require close monitoring of the pest to position release/treatment in the most suitable windows to keep second-generation ECB at acceptable levels.

Nocturnal insects are generally monitored using a conventional light trap, consisting of a bulb and chloroform, which tracks the flight of ECB adults throughout the season (RAK CIZEJ & TREMATERRA 2017). Depending on population dynamics, in particular the study of the first appearance of females and the peak of all adults in the trap, it is possible to determine oviposition windows on which control treatments can be applied (RAZINGER *et al.* 2016), which can be described even more precisely with an additional study of spermatophores (RAK CIZEJ *et al.* 2024).

Nowadays, new smart traps are being developed to reduce energy consumption and the burden of daily checks. Thanks to self-cleaning and Internet connection, farmers can receive EBC catch records directly on their monitors. A less reliable method of monitoring the population can be achieved with thermal time, also known as degree-days. Precise ECB life stages can be assessed with the sum of mean daily temperature above a minimum threshold (e.g, 10°C for most species, ECB included) and a phenological description table based on accumulated degree-days (IVEZIĆ *et al.* 2023; MAIORANO 2012).

Material and methods

Monitoring ECB with light traps

ECB was monitored in the hop growing region of Savinja (Slovenia) with traps placed at Roje near Žalec (D48/GK; X:5123080, Y: 511464) surrounded mainly by hops and occasionally by other crops such as maize.

Two different light traps were used for ECB monitoring. The first was a conventional light trap set 1 m above the ground, with a 160 W mercury bulb (HBO), switched on at night from 21:00 to 06:00 h. The light trap, fitted with a mechanism that added 35 to 40 ml of chloroform in the evening (when the light was on) to stun insects attracted by the light, was emptied daily and its contents identified in the laboratory. The second was a Trapview smart trap with three bulb wattages (9 W alone, 15 W alone and 15 W + 4 W combined), which trapped nocturnal insects on a self-cleaning rolling belt, whose contents were photographed daily and sent to an online web page for analysis.

Biological control of ECB

Two organisms were used to control two different stages of ECB: *Trichogramma brassicae* and *Bacillus thuringiensis*. *T. brassicae* eggs, preserved in a biodegradable capsule, were manually dispersed on 28.vi.2024 in a corn field surrounded by hop gardens to target BCE egg masses, laid under the leaves during the 2024 season.

Bacillus thuringiensis was sprayed twice a year directly on 81.0 % (in 2023) and 88.0 % (in 2024) of the total surface area of hop fields in Slovenia, respectively, to target larvae developing in bines and flower cones. Two products were used at the discretion of hop growers: Agree WG (*Bacillus thuringiensis* ssp. *aizawai* sev GC-91) and Lepinox plus (*Bacillus thuringiensis* ssp. *kurstaki* sev EG2348).

Visual assessment of maize

In Roje near Žalec, one field of maize was surveyed on 05-09-2024 after second-generation of ECB larvae to assess the efficacy of the treatment with *T. brassicae*. Every 3 rows, 10 spots consisting of 10 plants along the row were observed and the number of ECB per plant and their larval stage was recorded.

Results

Adult flight of ECB

The number of adult ECB increased annually, with 1042, 1230 and 1874 adults caught in the conventional light trap in 2020, 2021 and 2022, respectively. In the first and second years of the semi-annual Bt treatment, 2023 and 2024, only 243 and 551 adult ECB were caught in the conventional light traps, representing a massive reduction in the ECB population (Fig. 1).

ECB catches in 2024 in classic light traps were significantly higher than in smart traps, irrespective of lamp wattage (Fig. 2, left). Peak adult flights for the second generation of ECB corresponded for the conventional light trap and the 15W and 9W smart trap, while the 15W+4W smart trap was delayed by 10 days (Fig. 2, right).

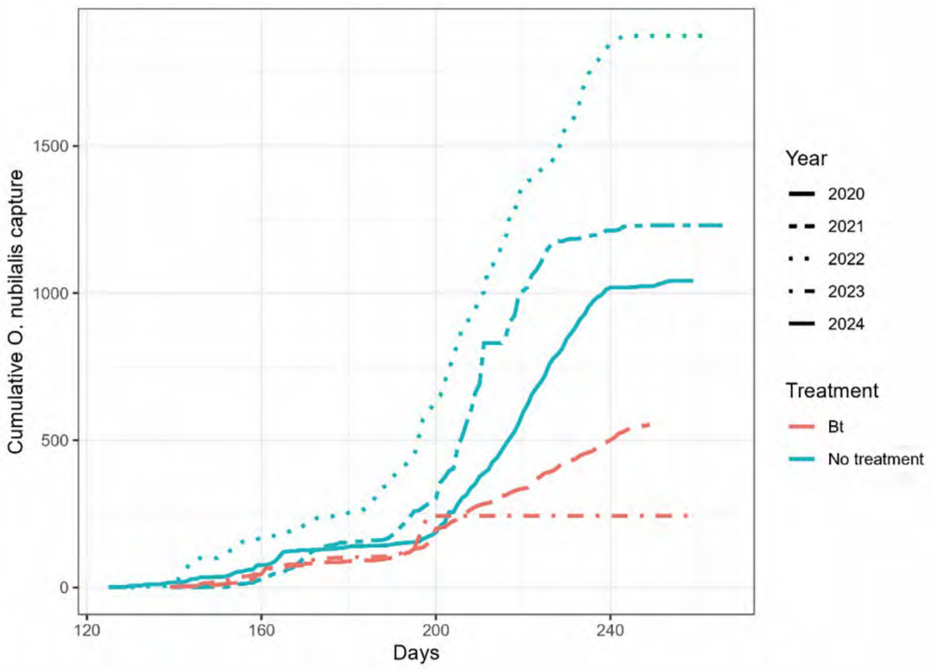

Figure 1. Cumulative capture of *Ostrinia nubilalis* in conventional light trap at Roje near Žalec from 2020 to 2024, without treatment (No treatment) before 2023, and with two *Bacillus thuringiensis* (Bt) treatments per year in 2023 and 2024 in hop yards.

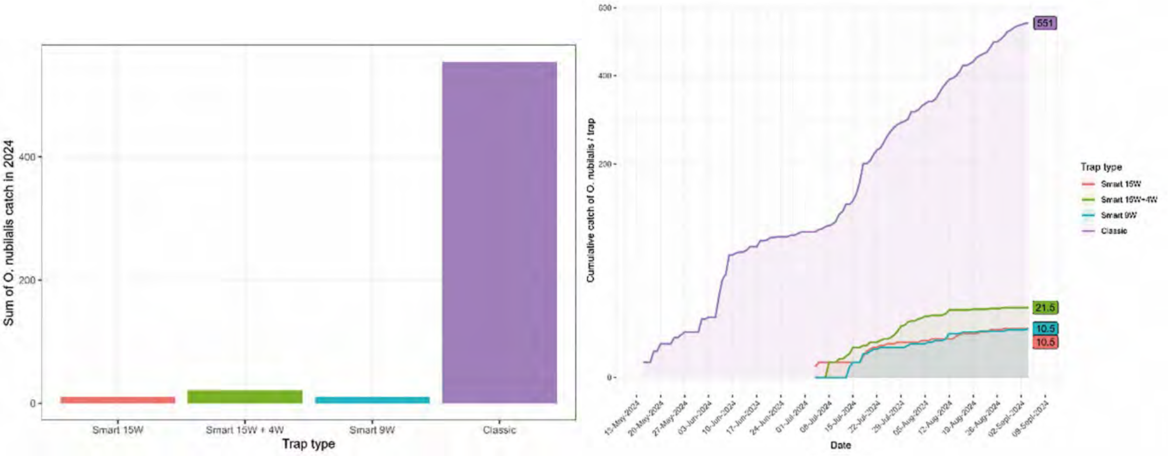

Figure 2. Yearly (left) and Cumulative (right) captures of *Ostrinia nubilalis* adults in different light traps (**9W**, **15W**, and **15W + 4W**: Trapview smart traps with 3 different wattage lamps: 9W, 15W and 15W + 4W; **Classic**: Light trap with 160 W bulb and chloroform) in Roje near Žalec in 2024. Final values in label stand for the total yearly catches per trap.

Visual assessment

Second-generation ECB larvae were found homogeneously in the maize fields, despite the heterogeneity of the field environment, surrounded by corn to the north and east, and hops to the south and west of the trial (Fig. 3). A slight trend can be observed between north and south, with slightly fewer individuals near the southern road; however, ECB larvae are still found in all maize rows.

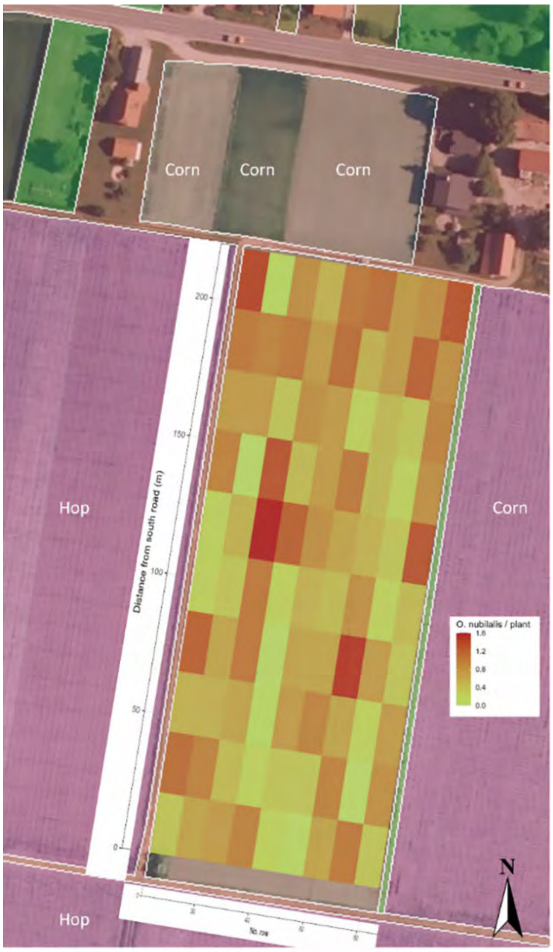

Figure 3. Heatmap of the number of second-generation *Ostrinia nubilalis* larvae per plant in a corn field surrounded by hops. Roje near Žalec, 05.ix.2024.

Discussion

Biological control methods against ECB applied to hops have given excellent results on a large scale, with treatment with *Bacillus thuringiensis* twice a year directly in the hops and could offer great protection in hop fields practicing integrated pest management (IPM). On the other hand, the local distribution of *Trichogramma brassicae* in maize fields has not been shown to be effective in controlling ECB.

Biocontrol methods against ECB applied to hops have given excellent results on a large scale, with semi-annual *Bacillus thuringiensis* treatment directly in the hops, and could offer great protection in hop fields practicing integrated pest management. On the other hand, the local inundative release of *Trichogramma brassicae* in maize field surrounded by hops has not been shown to be effective in controlling ECB. One of the reasons is that the application was done too late and only once, whereas two applications are usually needed.

Trichogramma brassicae has been used for over 40 years to control ECB in maize, but under our conditions, no control, mummies or parasitoid wasps were observed, while a huge quantity of damaged maize canes was found. Late release or poor storage of eggs could be responsible for the low efficacy of this tried-and-tested treatment (CHIHRANE & LAUGÉ 1996). Another source of control failure could stem directly from the environment of the crop, surrounded by ECB primary or secondary hosts (i.e., maize and hops), making management of this highly migratory pest very difficult (BOURGUET et al. 2000), particularly with parasitoid wasps not adapted to the difference in plant structure between maize and hops (ROMEIS et al. 2005).

ECB monitoring using smart traps can improve the efficiency of farmers and technicians in identifying peak adult flight, but some variability can still be observed between smart and conventional traps, particularly regarding the timing of captures. Capture density is extremely low in smart traps compared with conventional light traps and could be responsible for the false peak observed in smart traps, through an intentional selection of ECB that are more sensitive to smart trap lights.

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This research was supported by Ministry of Agriculture, Forestry and Food in the framework of the project IPM. Boštjan Hrustl provided technical assistance in the field assessment. We also appreciate the willingness of the hop growers to adopt biological crop protection measures in their hop yards and enable us to access their fields for research.

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Herbivore-Induced Resistance in hop cultivation: Reducing acaricide use through plant defence mechanisms

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Abstract

The two-spotted spider mite *Tetranychus urticae* is a major pest in hop cultivation, capable of causing severe yield and quality losses, particularly under warm and dry conditions that are increasingly prevalent due to climate change. The five-year project "InduResi" investigates the potential of herbivore-induced resistance in hops as a sustainable strategy for spider mite control. Field and greenhouse trials were conducted across 31 commercial hop gardens in southern Germany using four hop cultivars: Hallertauer Tradition (HTR), Spalter Select (SSE), Herkules (HKS), and Tettnanger (TET). In each garden, paired control (untreated) and practice (acaricide-treated, conventional) plots were established.

Monitoring over multiple seasons revealed cultivar-specific responses to spider mite infestation. While HKS showed no significant losses even under high infestation levels, HTR and SSE control plots often displayed reduced yields. Preliminary results from pot experiments and HS-GC/MS analysis of leaf volatiles indicate that induced resistance may be associated with changes in secondary metabolite profiles, particularly involving methyl salicylate. However, the expression and consistency of this resistance appear to be influenced by environmental conditions and initial infestation intensity.

Despite interannual variability, several trial sites demonstrated persistent suppression of mite populations in previously infested plots, suggesting a priming effect. The integration of these findings into practice could reduce acaricide use by at least one treatment per hectare annually, representing both an economic and ecological benefit. Further research is ongoing to elucidate the molecular basis of resistance and to validate its field applicability under varying climatic scenarios.

Materials and methods

Beginning in 2021 and continuing through the 2025 growing season, field experiments have been conducted in collaboration with 20 commercial hop farms across the Hallertau (n=16) and Tettnang (n=4) regions in Germany. A total of 31 experimental hop gardens were included, covering four cultivars: Hallertauer Tradition (HTR), Spalter Select (SSE), Herkules (HKS), and Tettnanger (TET) (Fig. 1, left). In each garden, two adjacent 500 m² plots were established. A control plot, where Tetranychus urticae was allowed to develop without acaricide intervention and a practice plot, managed with standard industry treatments including at least one acaricide application annually. Each plot included a central assessment area, from which data were regularly collected (Fig. 1, right). Spider mite infestation was assessed 3–5 times per growing season by collecting 30 leaves per plot (10 each from lower, middle, and upper canopy). Mites, eggs, and natural enemies were counted under magnification. The infestation index (Befallsindex, BI) was calculated for each leaf using a modified scale from WEIHRAUCH (2003), ranging from 0 (no infestation) to 5 (>1000 mites or eggs).

At the end of each season, experimental harvests were conducted in selected gardens showing clear differences in infestation levels between plots. Four batches of 10 bines per plot were harvested and processed to assess the yield, alpha acid content, alpha acid yield and cone quality. Controlled pot experiments were established in 2022 and 2023. Over 200 rhizomes per cultivar were propagated. Half of each cohort was artificially infested with spider mites; the other half was kept mite-free using acaricides. These plants were later transplanted into field plots or maintained under greenhouse conditions for further observation.

In 2023, leaf samples from 90 potted plants were freeze-dried and analyzed using Headspace Gas Chromatography-Mass Spectrometry (HS-GC/MS) to quantify volatile organic compounds potentially involved in induced resistance. Chemical profiles were evaluated using MetaboAnalyst 6.0 and compared across cultivars and treatments. Statistical evaluation was conducted using R (version 4.2.2 and later 4.3.3).

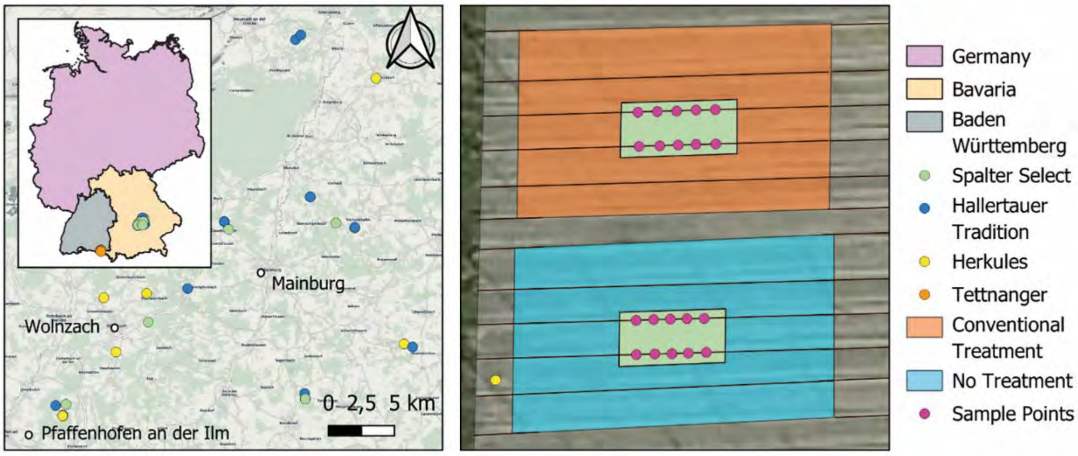

Figure 1. Geographic locations of the 31 experimental fields in Germany (inlay) and the Hallertau (left), and experimental design (right).

Results

Spider mite infestation varied substantially across project years, driven by weather conditions. The dry and hot summer of 2022 led to widespread and severe infestations, with mean infestation (BI) values of 1.03 in control plots, compared to 0.58 in treated plots. In contrast, 2021, 2023, and 2024 were characterized by cooler, wetter conditions, resulting in minimal pressure and >85 % mite-free leaves across all treatments (Fig. 2).

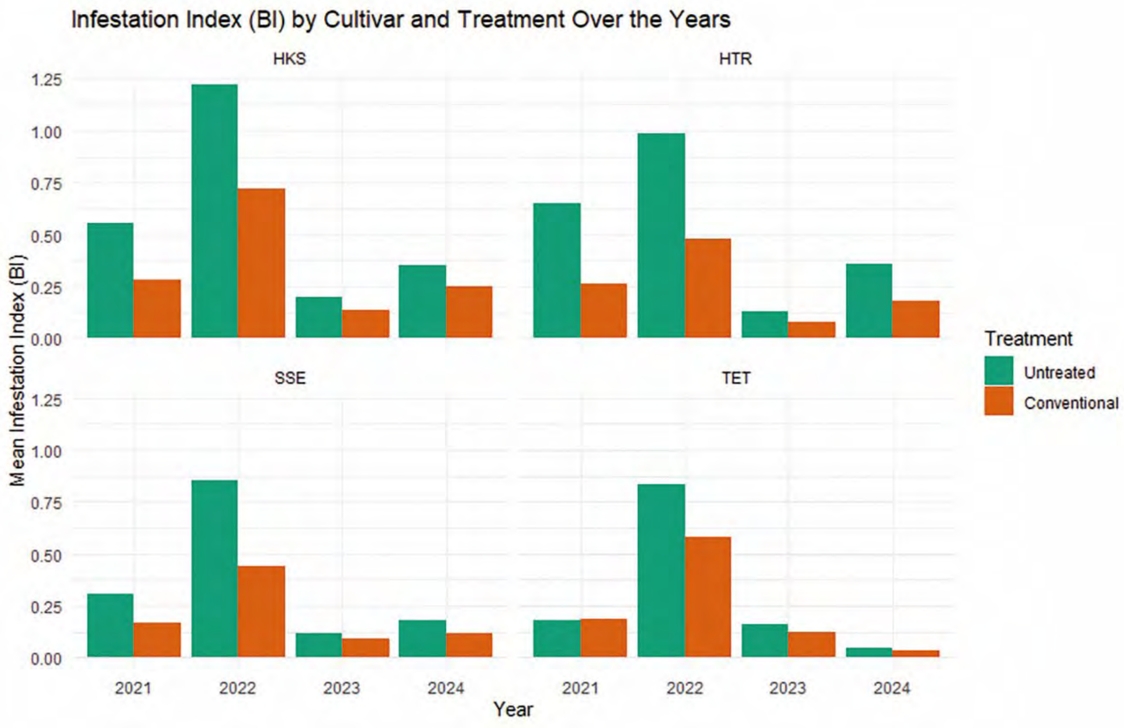

Figure 2. Mean Infestation Index (BI) per year by cultivar and treatment.

Yield effects were cultivar-specific and linked to infestation levels (Fig. 3). In 2022, Hallertauer Tradition (HTR) and Spalter Select (SSE) showed significantly reduced yields in untreated control plots compared to practice plots. However, Herkules (HKS) consistently maintained yield and alpha-acid content even under BI >4. Across all cultivars and years, no significant alpha-acid depression was observed; in some cases, mild infestation corresponded with slightly elevated alpha-acid levels.

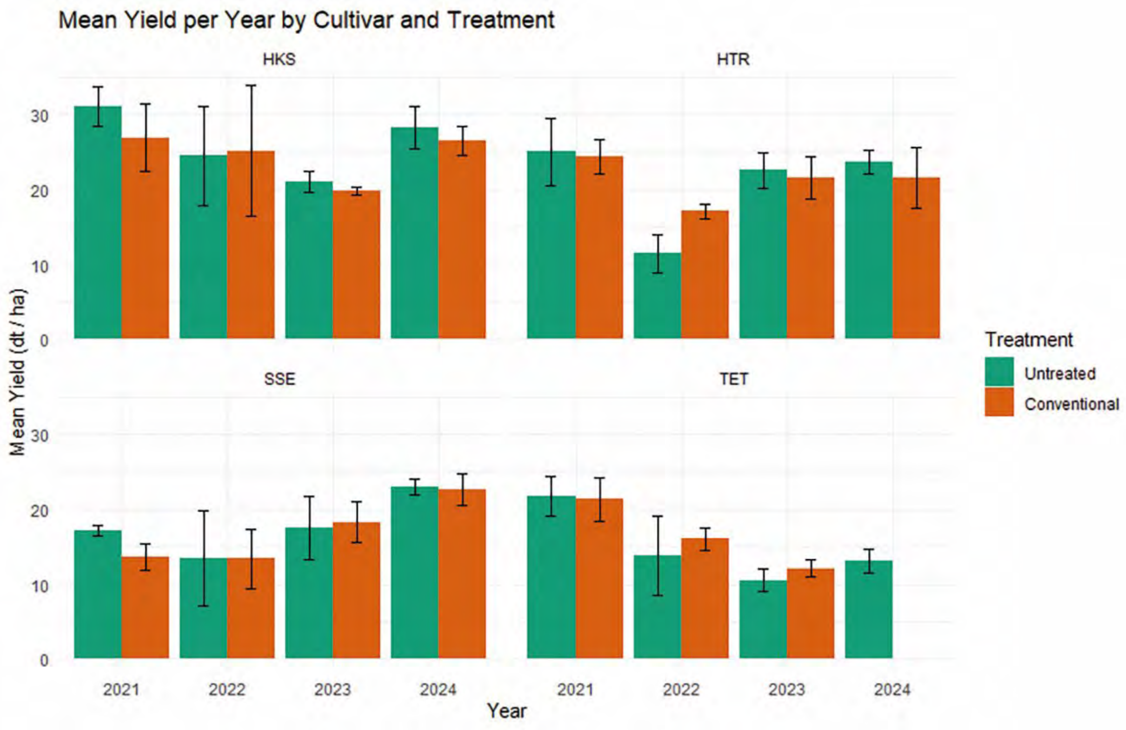

Figure 3. Mean yield (dt/ha) per year by cultivar and treatment.

Cone damage was quantified in harvested plots and categorized from healthy to severely damaged. In 2022, up to 67 % of cones in HTR control plots showed severe mite damage, compared to <10 % in other cultivars. In low-pressure years, cone damage remained below 10 % across all treatments.

Multiyear monitoring at several sites showed no evidence of infestation amplification in years following severe spider mite damage – contradicting growers' concerns. At some sites, BI values in previously infested plots dropped significantly in following years, indicating a potential priming effect.

Potted plant experiments confirmed cultivar-specific chemical defense traits. HS-GC/MS analysis of secondary plant metabolites in leaves revealed unique compound signatures in SSE and HKS, including methyl salicylate, which may relate to resistance mechanisms. Field-transplanted pre-infested plants showed no mite avoidance (therefore excluding antixenosis), but volatile differences support the hypothesis of herbivore-induced priming.

Discussion

While preliminary results suggest cultivar-specific responses to spider mite infestation and possible induced resistance, no definitive conclusions can be drawn at this stage. The project is ongoing and final analyses will follow the 2025 season. Interpretation remains limited by strong interannual variability in mite pressure, which is closely tied to weather conditions. This variability complicates the assessment of consistent resistance effects. Furthermore, the HS-GC/MS method used to analyze secondary metabolites may not detect all relevant compounds. For example, this method would have been unsuitable to detect quercetin, a flavonoid linked to spider mite resistance in other plants (Li et al. 2025). Broader metabolomic approaches could be considered in future work.

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Technical application of predatory mites in hops: Biological spider mite control *ante portas*!

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Abstract

In 2021, the first prototype world-wide for the release of predatory mites in hop culture to control two-spotted spider mites *Tetranychus urticae* Koch was developed by Koppert B.V. in The Netherlands and tested in a field trial in the German Hallertau. In subsequent years, the application machine was stepwise improved and detail questions were answered by more field trials until 2024. In general, TSSM pressure was low in these years and a yearly experimental harvest showed no significant differences in yield and alpha acid content of all treatments, including the farmer’s own practice with acaricide. As an outcome of our investigations, an optimized device for the release of predatory mites (a mix of *Phytoseiulus persimilis* and *Neoseiulus californicus*) in hops was introduced by Koppert in 2025 and used again in a field trial. In addition, the new technical application was tested already by 26 interested German hop farms on an area of altogether 162 ha in 2025.

Introduction

Two-spotted spider mite *Tetranychus urticae* Koch (TSSM) is by far the most prevalent arthropod pest of hops. Hop growers world-wide are generally controlling spider mites by preventative, often multiple, spraying of acaricides. However, in Integrated Pest Management a biological approach to TSSM control and suitable solutions are of first priority. Predatory mites such as *Typhlodromus pyri* Koch, *Phytoseiulus persimilis* Athias-Henriot or *Neoseiulus californicus* (McGregor) are able to control TSSM, either as established population in a crop or as bred, released antagonists. In hops, where established, overwintering populations of autochthonous predatory mites are currently largely lacking, many trials with released predatory mites have already been conducted in the past decades and yielded successful spider mite control (*e.g.*, VOSTŘEL 2001, 2003; WEIHRAUCH 2008; OBERMAIER & WEIHRAUCH 2019). In most of our own trials in the past 20 years, the efficiency of the predatory mites was comparable to that of an acaricide. However, one obstacle for the implementation of predatory mites in an IPM approach to TSSM control was the high amount of manual labour when releasing the beneficials, connected to corresponding costs for the farmer. Therefore, a competitive technical solution for the release of predatory mites would be a milestone for biological pest control in hops. In this paper, we summarize the evolution of a technical solution for the release of predatory mites in hops during the past five years 2021-2025, which was accompanied by annual efficacy trials (*cf.* WEIHRAUCH et al. 2022, 2023). In the meantime, this game-changing biological control method appears to be in step with actual practices of a hop farm.

Material & methods

The entire research project resulted from a scientific cooperation between Koppert B.V. in The Netherlands, represented by Koppert Germany, and the Hop Research Center Hüll of the Bavarian State Research Center for Agriculture. The first prototype for the technical application of predatory mites (Fig. 1) had been developed and assembled in 2021 by Koppert.

In subsequent years, this machinery (later named ‘Natutec Drive’) was improved and modified stepwise (Fig. 1). Field trials were conducted 2021-2024 in the Hallertau in two farmer’s own fields (both cv. Herkules), first in Dürnwind and later also in Eichelberg. Experimental lay-out always consisted of at least three treatments that remained unchanged during all five years: i) Predatory mites released manually on bean leaves, on which they had been bred, ii) technical release with the Natutec Drive, and iii) untreated control. Each treatment comprised four replications of *ca* 550 m^2, totaling to 16 plots with an experimental area of almost 1 ha. Released predatory mites were a commercially available mixture of *P. persimilis* and *N. californicus*. Monitoring of TSSM and predatory mites was conducted four or five times during each field season. Annually in mid-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, acaricide-treated plot in the same field.

Figure 1. Evolution of the technical release machinery for predatory mites in hops 2021-2025. Above, left: Dürnwind, 15.vi.2021; right: Dürnwind, 02.v.2022; below, left: Dürnwind, 15.v.2024; right: Eichelberg, 21.v.2025. Photos: FW

Results

In general, TSSM pressure was low in all four project years evaluated so far, except 2022, when pest pressure was higher in most regions. Even though, the annual experimental harvests, including 2022, never showed significant losses in yield and alpha acid content of all treatments with predatory mites, compared to the farmer's own practice with acaricide. The latter comprised two sprays (spirodiclofen, spirotetramat) in 2021 and one spray (spirotetramat) in 2022-2024, respectively. Harvests in 2021, 2022 and 2024 even yielded higher alpha results in plots with predatory mites, compared to those sprayed with acaricide (Table 1).

Table 1. Alpha acid yield (kg ha^{-1}) determined in experimental harvests 2021-2024 at two project sites (cv. Herkules; DÜW – Dürnwind; EBG – Eichelberg) with technical application of predatory mites to control TSSM in the Hallertau, Germany.

Harvest date	Untreated control	Predatory mite mix	Acaricide-treated
14.ix.2021 (DÜW)	600 ± 28.1	623 ± 162.5	547 ± 58.1
12.ix.2022 (DÜW)	338 ± 76.0	510 ± 41.9	408 ± 48.6
13.ix.2023 (DÜW)	461 ± 37.8	473 ± 58.6	479 ± 47.0
19.ix.2024 (DÜW)	560 ± 25.0	554 ± 30.9	495 ± 18.8
19.ix.2024 (EBG)	382 ± 37.8	388 ± 58.6	382 ± 47.0

Synopsis of the research project

For almost four decades, the Hop Research Center in Hüll has been looking for solutions for the practical and effective use of beneficial insects for pest control in the field. After a lot of trial and error and numerous unsuccessful attempts, it became clear that the use of insects proved to be of little use – as almost all of them reach the flying stadium at some point in their lives and are correspondingly mobile. As food opportunists, insects such as ladybugs, lacewings or hoverflies always appear naturally in the hop garden anyway when there is sufficient food to be found, *i.e.*, aphids or spider mites. On the other hand, they disappear just as quickly when there is no more prey on the plants. However, the situation is somewhat different with predatory mites for the control of spider mites. Both are not insects but belong to the arachnids and are therefore purely pedestrian. Accordingly, predatory mites that have been bred and then released largely remain where they are to be deployed. In addition, many predatory mite species like *N. californicus* can survive for some time on spruce pollen or grass pollen as a substitute food source if they do not find any spider mites as prey.

In years with normal levels of spider mite infestation, the technical use of predatory mites has proven to be competitive with chemical-synthetic plant protection. In the long term it can be equated with one acaricide treatment. In many years, this means that standard prophylactic spider mite spraying is no longer necessary. In years with a very strong increase in TSSM populations, it may be sufficient to react with a later application of acaricide. The following key findings were obtained during our tests as fundamental elements of the method:

Ideally, a mixture of the two predatory mite species *N. californicus* (30-40 %) and *P. persimilis* (60-70 %) is used to control the mites. The latter, red-coloured species, thanks to its speed and efficiency, can very quickly act as a "fire brigade" to clean up spider mites if their population is not too large. However, if there is no more prey available, *P. persimilis* won't remain on the plants for long and will disappear.

The role of *N. californicus*, on the other hand, is more that of a “secret service”, operating inconspicuously in the background and cleaning up the remains – and it can also survive well on the hop plants with substitute sources of food such as pollen. The optimum application rate has proven to be 100,000 beneficial mites per hectare, *i.e.*, around 25 predators per training.

Figure 2. Details of the technical release method for predatory mites in hops. Above, left: Infilling of the mite mix with sawdust as a carrier in the Natutec Drive funnel; right: application in the field; below, left: detail of application; right: control unit for the Natutec Drive device in the operator’s cab; Eichelberg, 21.v.2025. Photos: FW

A decisive factor for successful control is the timing of the application. As a general rule, the spider mite infestation on the hops should not be too far advanced and the upper bine areas in particular should not yet be infested. For this reason, we have released beneficial mites very early in recent years, from around the beginning to the middle of May. The ideal growth stage is BBCH 31-32, *i.e.*, 10 to 20 % trellis height. Preferably, the hops should already be manually defoliated and trained (Fig. 2). The hops should be hilled up either before the predatory mite application or at least one week later, to give the predators the opportunity to find and colonize the hop plants. As with all work in the spring, every year is different, of course. The driving speed when using the Natutec Drive for application should be at 4.0-4.5 kph. With this driving speed, the release of beneficials is executed more rapidly than spraying a pesticide, and one filling of the Natutec Drive funnel with predatory mites is sufficient for *ca* 2 ha of hops.

The acceptance of the presented method among growers – both conventional and organic – is already increasing. In a pilot project of the 'Hopfenring' to establish the method in practice, predatory mite release was already tested on 20 farms in 2024 on 119 ha altogether. In 2025, there were 26 farms interested in testing the method on 162 ha altogether (Hallertau 51 ha, Spalt 2 ha, Tettnang 72 ha, Elbe-Saale 37 ha). Considering all the benefits of the release of predatory mites, both for growers or operators as well as for the environment, we are confident that this game-changing biological control method will become standard in hop cultivation within the next few years – biological spider mite control *ante portas* !

Acknowledgement

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VII: Residue management in hops

Development of a method for determining dislodgeable foliar residue (DFR) values in hops to support risk evaluation

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Abstract

Regulatory requirements for assessing non-dietary, systemic exposure potential as part of the risk assessment for plant protection products gained relevance for hop production with release of the guidance document on the assessment of exposure of operators, workers, residents and bystanders in risk assessment of plant protection products (EFSA *et al.* 2022). The guidance first issued by EFSA (2014) included outdoor application scenarios and a calculator, while the 2022 update added more scenarios and revised values, enhancing risk assessment for workers. In order to calculate the risk to workers following plant protection applications, it is necessary to evaluate the dislodgeable foliar residues (DFR). In the absence of such an evaluation, the calculation is based on a crop-independent default value. At the time of publication of the guidance document by EFSA et al. (2022), no methods for determining DFR values in hop cultivation, particularly concerning harvest operations, were available. Therefore, the Hop Research Center, together with the Federal Office of Consumer Protection and Food Safety (BVL) and the German Federal Institute for Risk Assessment (BfR), developed a method to assess DFR values in hops.

The initial experimentation on the DFR in hops was conducted in the years 2023 and 2024. The product Funguran® progress (active ingredient: copper hydroxide) was chosen due to its properties enabling a high recovery rate. During the trials, two applications in a hop garden with the variety Herkules were conducted at the maximum application rate. Before and after the applications as well as seven days after the final application, leaf samples (400 cm^2) were collected and washed with an aerosol solution twice. The wash solution was then analyzed using atomic absorption spectroscopy. This procedure enabled the measurement of washable residue levels accumulating on plant leaves under real application conditions. The measured values were lower than the default value of 3 µgm cm^{-2}, which is used when no specific data are available for the respective active ingredient.

The collected data provide a basis for exposure estimation and enable the refinement of existing exposure models and more accurate risk assessments. Besides securing the approval of plant protection products, this approach allows for more practical adjustments to application regulations that might otherwise disrupt agricultural activities and ensures the long-term improvement in the safety assessment of workers in hop cultivation.

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Natural Deep Eutectic Solvents as a greener alternative to traditional extraction methods for pesticide residues in hop products

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Abstract

This study demonstrates the potential use of NADES for the extraction of pesticide residues in hop products. The method was proposed in response to the need for alternative, more environmentally friendly approaches to detecting residual substances in hops. For this reason, four betaine-based and choline chloride-based solvents were selected as optimal for the determination of 11 pesticides with varying polarities in hops. Solid-liquid extraction, followed by analysis using gas chromatography coupled with tandem mass spectrometry (GC-MS/MS), was selected for the identification and quantification of the target compounds. To improve the results and enable a more accurate comparison of extraction efficiency, the method conditions were optimized. Various extraction conditions were tested, including NADES selection and their ratios, the ratio of added water to solvent, the amount of extraction mixture added to the sample, and the identification of matrix effects during gas chromatography. This study was conducted with the aim of evaluating the use of certain NADES for pesticide extraction from hop pellets and proposing optimized conditions for the best results. The proposed variations of NADES represent only a subset of the various possibilities that could be investigated in the development of a method meeting the principles of Green Chemistry.

Introduction

Pesticides have become an integral part of modern agricultural practices, particularly in crops such as hops. However, both the pesticides themselves and the organic solvents traditionally used for their laboratory analysis pose risks to human health and the environment. In recent years, Natural Deep Eutectic Solvents (NADES) have emerged as a promising green alternative for sample preparation and extraction. Composed of naturally derived components such as sugars, amino acids, and organic acids, NADES are characterized by low toxicity, biodegradability, and tunable solvent properties. Their application in pesticide residue analysis offers a more sustainable and environmentally friendly approach, in line with the principles of green chemistry (CARASEK *et al.* 2024).

Based on earlier studies (RODRIGUEZ-RAMOS *et al.* 2024), it appears likely that natural deep eutectic solvents can be used for the analysis of pesticide residues in food and plant matrices. Several studies have focused on developing suitable multipurpose methods with high reproducibility and sensitivity (SOCAS-RODRIGUEZ *et al.* 2022, HRISTOZOVA *et al.* 2025) Nevertheless, none of these methods have yet been able to replace the widely used QuEChERS method, which successfully combines high yield, reliable reproducibility, rapid sample preparation, and broad applicability across a wide range of compounds.

To the best of our knowledge, there are no published studies on the application of NADES for monitoring pesticide residue concentrations in hop products. The aim of this project is to provide new insights into extraction methods and to propose a technology that aligns with the fundamental principles of green chemistry. Future studies employing this technology could provide valuable insights into the development and application of green extraction techniques for routine pesticide analysis in hop-based products.

Material and methods

To obtain a better understanding of which factors can affect the extraction and NADES behavior in the hop matrix, a series of experiments were conducted. The following samples were selected: raw hops with no applied pesticide protection during the growing season, pre-ground and stored in the dark in a freezer. For each sample there was always a duplicate.

Analyzed compounds included azoxystrobin, bifenazate, boscalid, dimethomorph, flonicamid, fluopicolide, hexythiazox, lambda-cyhalothrin, metalaxyl, metrafenone, quinoxyfen, and the internal standard TPP (IS).

Based on previously published studies, the combinations of betaine with lactic acid (BET + LA), betaine with polypropylene glycol (BET + PPG), choline chloride with lactic acid (Ch-Cl + LA), and choline chloride with polypropylene glycol (Ch-Cl + PPG) were selected as the most promising NADES solutions for testing.

Firstly, the most suitable NADES type and sample-to-solvent ratio were evaluated. Hop samples of varying weights (±0.5 g and 1 g) were spiked with pesticides and internal standard, extracted using different volumes (2, 4, and 6 mL) of NADES solutions, and subsequently re-extracted with cyclohexane, followed by analysis using GC-MS/MS.

Subsequently, the betaine and polypropylene glycol solution was tested at different molar ratios (1:3, 1:4, 1:5, and 1:6). The optimal combination was evaluated based on the amount of extracted pesticides as well as the yield percentage.

Different water contents in the extraction solution were also investigated in this study due to their impact on the viscosity and polarity of the system. Water was added at volume ratios of 10 %, 20 %, 30 %, and 40 % to the NADES combinations of BET + PPG at molar ratios of 1:3 and 1:6 (n/n).

Finally, the volume of the re-extraction solvent (250, 500 and 750 ul) was also tested due to the matrix effect, which influenced the purity of the supernatant and the ease of its collection.

Results and discussion

Four betaine-based and choline chloride-based solvents were tested for the determination of 11 pesticides commonly used in hop protection. Solid-liquid extraction followed by GC-MS/MS was chosen as the extraction method. The method conditions were optimized by selecting the best-performing option for each parameter, including the type of NADES, molar ratio of NADES components, water content in the solution, sample-to-solvent ratio, and re-extraction solvent volume. The influence of the hop matrix effect was assessed by comparing the results from blank hop samples prepared using the QuEChERS method, blanks with added NADES, and solutions of pesticide standards in acetonitrile at appropriate concentrations.

Several important results were obtained from this study. Firstly, betaine-based deep eutectic solvents demonstrated better performance in terms of recovery and sensitivity during the identification and quantification of pesticides compared to choline chloride-based solvents. Figure 1 presents the number of pesticides extracted from raw hop samples using GC-MS/MS analysis for each of the four NADES combinations.

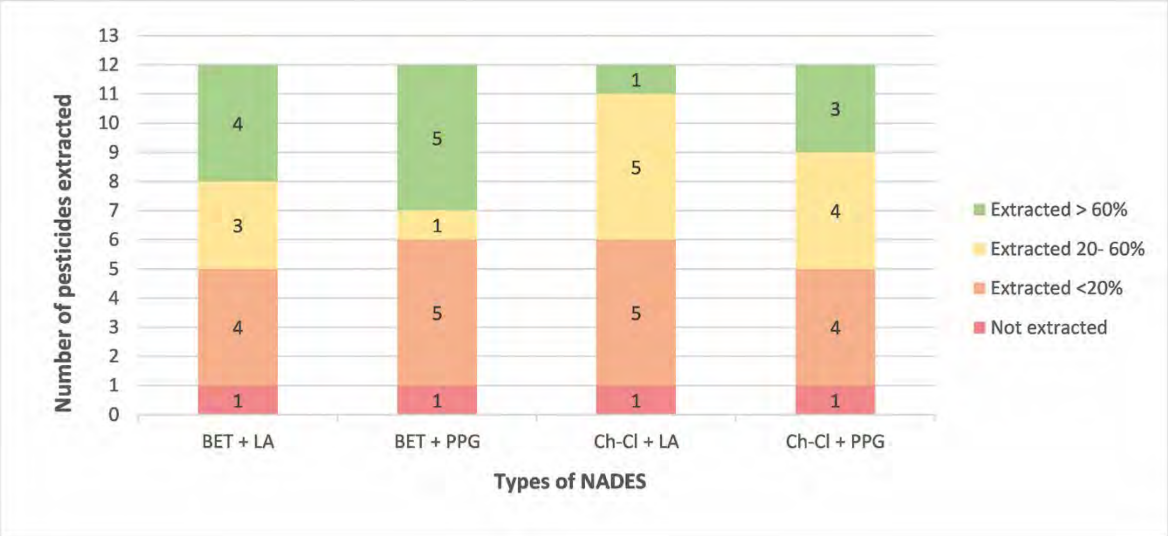

Figure 1. Comparison of pesticide recovery during extraction by different types of NADES (molar ratio 1:4, n/n, percentage of water 80:20, v/v)

The most favorable results were obtained with the combination of betaine and polypropylene glycol at a molar ratio of 1:3, with the addition of 20 % water. Table 2 reflects these findings. The same combination with 30 % water also showed satisfactory results, though with slightly lower recovery percentages. It is worth mentioning that working with the two aforementioned combinations requires thorough dissolution of the components during preparation, followed by proper tempering of the solution, as the high concentration of betaine may lead to recrystallization.

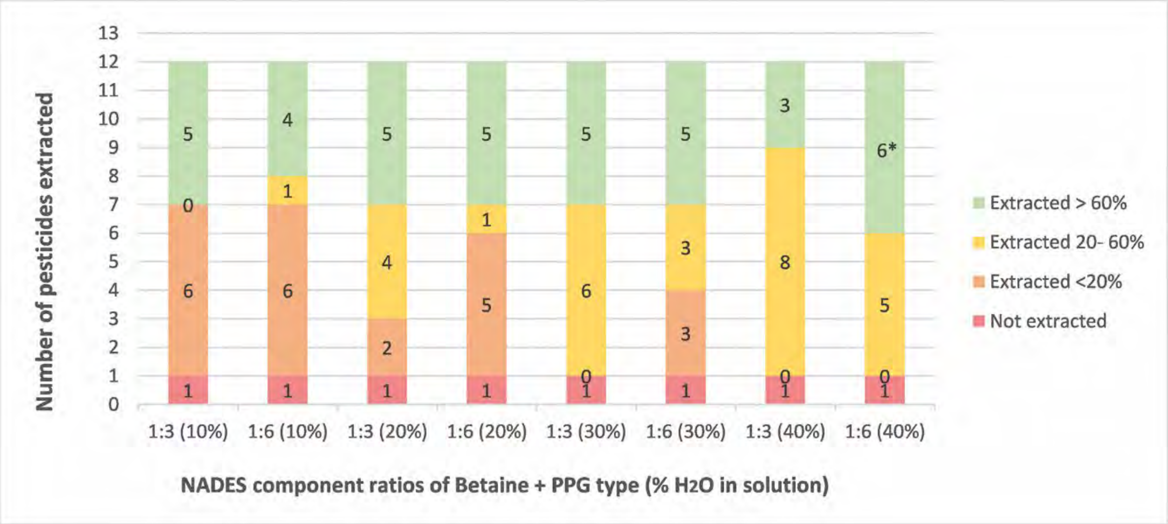

Figure 2. Comparison of two BET+PPG ratios with different water additions (molar ratio 1:3 and 1:6, n/n, percentage of water 10, 20, 30, 40%, v/v). The results for BET + PPG 1:6 (40 % of water) are marked with an asterisk (*) because the recovery of some compounds ranged from 110 to 228 %, indicating a significant impact of co-extracted impurities.

In summary, NADES can be used for the extraction of pesticides from the hop matrix in future applications. Compared to the commonly used QuEChERS method, natural deep eutectic solvents offer advantages in terms of safety, simplicity, and, in some cases, cost and time efficiency. However, the proposed method still requires improvement, as it is not universally effective for all types of pesticides—for examlpe due to differences in polarity. For future research, testing a wider range of NADES combinations could help identify a more versatile formulation. Additionally, the potential use of these solvents for analyzing other components in hops under different conditions should also be explored.

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

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Fate of pesticide residues in conventionally and dry-hopped beers

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Abstract

Pesticides, despite restrictions, still play an important role in protecting crops against diseases and pests, especially in cases of calamitous outbreaks. Beer producers closely monitor the pesticide content of raw materials in their quality management systems. This study is aimed at determining the residue content of pesticides currently used in hop protection in the Czech Republic in bottom and top fermented beers, hopped in several regimes.

The experimental beers were brewed in an experimental brewery (50 litres). Organically grown hop was spiked with 25 pesticides (fungicides, insecticides, acaricides) to a level of 10 ppm. Samples of spent hops, yeasts, hopped wort, young beer, and final beer were taken during the production process. Sample preparation of hops and liquid matrices were prepared by citrate-buffered QuEChERS method. Pesticides residues were extracted by acetonitrile. Final cleanup was carried out using dispersive solid-phase extraction. LC-HR-MS/MS analysis was carried out using Dionex UltiMate 3000 UHPLC system, detection was done by high resolution mass detector (QTOF Impact II, Bruker). The lager beers were hopped in three ways: i) whole amount of hops at the beginning of the wort boiling, ii) whole amount of hops 10 minutes before the end of the wort boiling, and iii) quantity of hops divided into three equal batches – at the beginning of the wort boiling and further in the 40th and 80th minute. Pesticide concentrations in lager beers ranged from 0.001 to 0.037 mg/l, depending on the properties of the active substance. The hopping regime had no demonstrable effect on the concentration of residues in the final beer. Dry-hopping of lager beers was tested in another batch of beer in two modes, addition of hops (300 g/hl) to the main fermentation and addition of hops to the lagering (300 g/hl). Higher concentrations in the beer were found after the addition of hops to the lagering phase (up to 0.021 mg/l), when the effect of ethanol was already evident. Residues of some pesticides (cymoxanil, pyraclostrobin) in beer were detectable only after application of dry hopping.

In top-fermented beer (ale) with different fermentation technology (type of brewing yeast, fermentation temperatures), the concentrations of pesticide residues were on average slightly higher than in lager when they ranged from 0.001–0.075 mg/l. The highest concentrations were measured for the metabolite spirotetramat (spirotetramat-enol). In dry-hopped beers, the concentrations of the pesticides increased by up to 75 % (imidacloprid, flonicamid, acetamiprid). For some pesticides, the concentration in beers after dry-hopping did not change (metrafenone, dimethomorph, hexythiazox, quinoxyfen).

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Transfer of hop pesticide residues into beer during brewing process

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Introduction

In conventional hop production, hops are treated with plant protection products throughout the growing season to counteract pests and fungal infections. But pesticide application raises concerns about the transfer of pesticide residues (PRs) into beer during brewing. Reliable data on the transfer of hop pesticides to beer during brewing are rarely available (Dušek *et al.* 2018; Kippenberger *et al.* 2014; Schmidt *et al.* 2007; Walsh *et al.* 2016). They do not necessarily cover all relevant active ingredients, nor all aspects of beer production. The aim of this work is to gain a more systematic understanding of the transfer of PRs from hops to beer.

The transfer behavior of plant protection products from hops into beers can be estimated from their solubility in water and the partition coefficients in n-octanol-water (K_{ow} or logP values), which include the solubility in octanol and water (Sangster 1997). However, the assessment of the transfer behavior of PRs requires extensive preliminary investigations and an exclusive consideration of the K_{ow} value may lead to incorrect conclusions.

When assessing the extent to which a PR from a raw material is transferred into a final food product, it is customary to determine and specify a processing factor (PF), which is calculated according to formula 1 (BfR 2023). However, the determination of a PF does not consider a very important variable such as the amount of raw material used. For an accurate calculation of residues in beer, the dosage rate of hops is crucial. Without this information, the amount of pesticide residues in the used raw material cannot be deduced from a detected residue in the beer. Hop dosages can range from 30 to 3000 g hops/pellets per hectolitre (hL), depending on the type of beer. This wide range of hop quantities in a beer from clearly shows that a PF does not allow a sufficient estimation of pesticide residues in brewing processes. Alternatively, the determination of a transfer rate (TR) of residues by considering the hop dosages in beer might be a more appropriate method (Formula 2).

$$\textbf{Formula 1:}\quad \textbf{Processing factor (PF)} = \frac{\textbf{Residue in food [mg/kg]}}{\textbf{Residue of the used raw material [mg/kg]}}\P$$

$$\textbf{Formula 2:}\quad \textbf{Transfer rate (TR) of a pesticide residue (PR)[\%]} = \frac{\textbf{PR in beer}\left[\frac{\textbf{mg}}{\textbf{kg}}\right]}{\textbf{used amount ofPR}\left[\frac{\textbf{mg}}{\textbf{kg}}\right]}\ x\ \textbf{100}\P$$

A major problem in tracking pesticide residues in brewing trials is finding hop samples that contain a sufficient number of active ingredients and a relevant amount of residues. A spiking technique was employed to simulate elevated pesticide concentrations of relevant active ingredients on hops, with its accuracy assessed against originally contaminated samples. The aim of this work was the investigation of TRs of relevant pesticides. The method of spiking was tested for its suitability to track residues from hops to beer throughout the brewing process. Both tanks and kegs were used for beer production. In addition, filtration and timing of hop addition during beer production, where a reduction of residues was expected, were analysed.

The essential parameters of hopping, including dry hopping, were conducted at a 2-hL research brewery with proven good reproducibility (GAHR *et al*. 2016). These results may contribute to a more comprehensive understanding of their transfer behavior, which is crucial for ensuring food safety in beer production.

Results and discussion

The following results were obtained from a total of 78 tests on 98 wort and beer samples. The detailed results can be found in the original paper (FORSTER *et al.* 2024). We found a significant variability in the transfer rates (TR) of pesticide residues (PRs) from hops to beer, ranging from 0 % to more than 70 %. This variability largely depends on the solubility of individual active ingredients. Steps like fermentation, agitation during dry hopping, centrifugation, and filtration have only a minimal impact on TRs.

The spiking method, while not OECD-compliant, offers a practical approach for studying the transfer behavior of PRs, especially for compounds that are challenging to detect in originally contaminated samples. This new approach of spiking samples is effective for obtaining comprehensive and reliable results, as it eliminates the need for time-consuming searches for suitably contaminated samples.

We categorized the 21 relevant active ingredients into four groups based on their solubility and TRs: not soluble, poorly soluble, medium soluble, and good soluble. The average TR of medium to good soluble PRs is around 20 % in tanks and 40 % in kegs, likely due to more intensive mass transfer in smaller units. Hopping in hot wort results in slightly higher TRs than dry hopping, with no significant effect of boiling time on the TR of moderately to good soluble substances. Also, pesticides dissolved in wort do not decrease significantly during fermentation and storage. Only poorly soluble compounds can be reduced by centrifugation or filtration, while more soluble active ingredients remain largely unaffected.

These results provide valuable insights into the behavior of PRs during brewing and contribute to a more comprehensive understanding of their transfer, which is crucial for ensuring food safety in beer production.

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VIII: Cultivation & management of hops

Model and demonstration project: Humus formation in hop cultivation

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Abstract

In the soil, humus provides a variety of beneficial properties for plant cultivation. By increasing the amount of plant-available water as well as by providing a higher water infiltration rate, it improves soil water balance. Furthermore, humus can bind nutrients and thus prevent them from being washed out. Due to root exudates or humus turnover by soil life, those nutrients are released again in plant-available form. Also, organic carbon benefits the soil particle aggregation and the soil life. Extended periods of drought as well as heavy rainfall events will become more frequent in the face of climate change (AUERSWALD *et al.* 2019). In light of these changes, the properties of humus will even be of increasing importance for hop farmers.

In addition, soil represents the largest terrestrial carbon storage of approximately 1,500 gigatons, by far exceeding the amount of carbon in vegetation and atmosphere (SCHARLEMANN *et al.* 2014). Scientific studies suggest that, due to climate change, the increase in soil temperature and thus in seasonal activity of soil microbes might raise the decomposition rate of organic matter, even though there remain uncertainties for this prediction (KÜHNEL *et al.* 2020; RIGGERS *et al.* 2021; GARCIA-FRANCO *et al.* 2024). This would result in a general loss of soil humus concentrations, leading to an emission of CO_2 as well as a reduced adaptation to climate change for the farmer. However, by adopting humus formation measures, the organic carbon levels can be conserved and even increased to a limited extent (WIESMEIER & BURMEISTER 2022). By utilizing this potential, carbon dioxide from the atmosphere can be captured by plants and stored in the soil as organic carbon, limiting climate change. This gains importance as consumers and the production chain make demands for a climate neutral hop production. With increased sequestration of carbon by humus formation, unavoidable greenhouse gas emissions along the hop cultivation and the subsequent processing chain could be compensated.

Therefore, the model and demonstration project ‘Humus formation in hop cultivation’ was funded by the German Federal Ministry of Food and Agriculture (BMEL). The project began in 2024 and will end in spring 2030. At the end, a possible extension for up to two more years will be decided upon. The aim of the project is to establish practical long-term measures for humus formation in hop yards by implementing them in ten demonstration farms across Germany’s hop-growing regions. The project is carried out in a collaboration of the Bavarian State Research Center for Agriculture (LfL) and the Hop Ring. In the summer of 2024, hop farms across Germany were able to apply to become one of the ten model and demonstration farms. The selection was based on a questionnaire, providing information regarding the farm, soil properties, management and the motivation for the application. A representative distribution across Germany’s hop-growing regions was ensured, with six of the farms being located in the Hallertau, two in Tettnang, one in Spalt and one farm in the Elbe-Saale region (Saxony).

For each farm, three experimental plots were identified on which humus-promoting measures are to be conducted. Adjacent standard plots provide a comparison with unchanged management. In the fall and winter months of 2024, an extensive soil analysis of the experimental and standard plots was performed to determine the initial soil condition, including soil texture, bulk density, amount of trace nutrients and amount of organic carbon. Subsequently, the humus concentration in the soil was calculated by bringing the amount of humus in the probe in relation to the bulk density. Further soil tests at the end of the trial period enable a quantitative assessment of humus formation by the implemented measures. In addition, N_{min}-tests are performed annually in spring and after the harvest during the trial period to identify the fertilizer requirement and to detect a possible loss of nitrogen due to leakage.

Following the elaboration of suitable measures with and for the individual farms, these are now to be implemented from 2025 onwards. A detailed overview of these measures can be found in the manuscript ‘Practices for humus formation in hop cultivation’ of Simon Arnold. The project is scientifically supported by the Julius Kühn-Institute (JKI) and the Thünen Institute. A quantitative assessment of humus formation by the varying measures as well as a calculation of the climate balances of the demonstration farms will be performed by the JKI. Additionally, the economic factors of the measures will also be examined by the scientists of the Thünen Institute, aiding hop growers in their decision-making and planning.

Workshops and field days are to be organized on the model farms to disseminate successful, humus-accumulating measures among the hop growers. Together with the other humus model and demonstration projects for apple production, viticulture and vegetables, a website is to be designed for further spread of information regarding the projects and humus formation. Regular exchange between scientific support and the projects ensures the exchange of ideas and a close coordination. In addition, the communication between the model and demonstration farms is to be promoted in order to encourage exchange of knowledge and to increase the practicability and effectiveness of the measures.

Figure 1. Hop garden with dense catch crop. Photograph: JP

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Nutrient uptake of current German hop varieties

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Introduction

During the years from 2017 to 2020, various research projects on the subjects of fertilization and irrigation of hops were carried out by the Bavarian State Research Center for Agriculture at the Hop Research Center in Hüll. As a part of the projects, a large number of plots of various trials were harvested precisely every year. Trial harvests at the Hop Research Center included not only determination of cone yield and alpha acid content, but also fresh and dry matter of cones and residues as well as dry matter content and nitrogen content. All samples that were collected over the years have now been used for extensive analysis of macro- and micronutrient contents of hop in the two fractions of cones and residues, respectively. This also allows to determine the absolute nutrient removals per hectare, broken down into cones and harvest residues from current hop varieties in Germany. In order to obtain results that are comparable with practice, only samples from plots that were fertilized demand-oriented were examined.

Material and methods

The tests included cone and residue samples from 128 trial plots harvested in Hüll in 2017, 2018, 2019, and 2020. The samples came from 12 different locations in the central Hallertau comprising three different varieties (Herkules, Perle, Hallertauer Tradition). During the trial harvests, the fresh and dry matter yields of the cones and the residues were determined. At the same time, representative cone samples were taken from the cone belt and residue samples from the waste belt to determine the dry matter content, the nutrient content, and the alpha acid content. The samples were first weighed wet and then dried at 60°C to a residual moisture content of approx. 10 % and reweighed. For the nutrient content analysis, the residue samples were separated from the wire, then coarsely ground with a hammer mill and then finely ground with a centrifugal mill (sieve: 0.5 mm). The cone samples only had to be ground once. When nutrient analysis was made in the laboratory, the residual moisture was determined at 105°C and the test results corrected for the water content. This procedure makes it possible to determine exact removal rates for all the nutrients both in the cones and in the residues.

A total of 258 samples were analysed for the respective nutrient content in the dry matter using the following methods.

Dumas combustion method according to VDLUFA method book:
Sulphur, nitrogen, carbon.

ICP OES (optical emission spectrometry):
Calcium, potassium, copper, iron, manganese, sodium, magnesium, molybdenum, nickel, aluminium, phosphorus, cobalt, zinc, boron.

Results

For all macronutrients and micronutrients examined, average nutrient removal rates were determined for the different varieties. The results were compared with the current state of knowledge and the recommendations for fertilizing rates. The study provides interesting information and shows that the constant introduction of new nutrient-efficient varieties such as Herkules means that the removal rates and recommendations need to be constantly adapted. Deviations from the current state of knowledge should be incorporated into the current recommendations. The data basis for fertilizer regulation for hops in Germany should also be adapted in order to enable doing nutrient calculations with correct figures. Therefore, a sufficient data basis was created with the study, especially for the environmentally relevant nutrients nitrogen and phosphorus.

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Optimizing spatially variable information acquisition for smart hop management

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Abstract

Georeferenced data play a critical role in advancing precision agriculture, and their importance is rapidly growing in the context of hop cultivation. Currently, GPS-based systems are employed or under evaluation for essential tasks such as hop planting, tramline guidance, and training. Accurate and automated georeferencing of individual hop plants is foundational for enabling future innovations, including robotic systems for site-specific fertilization and yield monitoring. This study evaluates three ground-based methods for georeferencing hop plants and investigates their operational effectiveness. In parallel, it explores the integration of this georeferenced field information with remote sensing data to enhance decision-making and yield forecasting in hop production. While current approaches typically establish a one-to-many relationship – connecting field-level yield data with diverse satellite-derived spectral observations – early results are highly encouraging. Sentinel-2 imagery from 2018 to 2023 was analysed for a farm in Weikenhausen (Hallertau, Germany), focusing on the widely cultivated hop varieties Hallertauer Tradition (HTR), Perle (PER), and Herkules (HKS). Among the evaluated spectral indices, the Red Edge Inflection Point (REIP) slope from May to July and the REIP value on the 75th day of growth demonstrated the highest correlations with annual yield, reaching up to 86 %. These findings underscore the strong potential of combining high-resolution georeferenced plant data with satellite-based vegetation indicators to support precision hop farming and improve yield predictability.

Introduction

Precision agriculture (PA) has revolutionized crop management by leveraging technologies such as GPS, GIS, and remote sensing to optimize inputs and enhance yields. In hop cultivation, the adoption of PA practices remains limited, despite the crop's economic significance and the benefits of site-specific management. However, advancements in sensor technologies and data analytics now offer new opportunities for implementing PA in hop farming. Georeferencing, *i.e.*, associating data with specific geographic coordinates, is fundamental to PA and, when integrated with remote sensing, enables monitoring of crop development and yield prediction at multiple spatial scales. This study evaluates different methods for georeferencing hop plants and assesses the potential of Sentinel-2-derived REIP metrics for predicting yields.

Material and methods

Georeferencing methods

• GPS-Assisted Planting System: This method uses a precision planting system developed by the Wagner company (Friedelsheim, Germany), which records the x and y coordinates of each hop plant during the planting process. Relying on GPS guidance with sub-meter accuracy, it interfaces seamlessly with tractor-mounted planters and enables automatic generation of planting maps for efficient means of registering new plants into a GIS database.

• UAV-Based Imaging: Drones equipped with high-resolution RGB cameras acquired detailed aerial imagery of hop gardens, processed using photogrammetric techniques (Structure-from-Motion and orthomosaic generation) to detect plant positions. Image interpretation, via object detection algorithms or manual digitization in GIS, offers sub-decimetre spatial resolution. This is ideal for irregular layouts or mature plantations where GPS data may be missing or outdated, with processing costs averaging *ca* € 200 per hectare.

• RTK-GPS Surveying with GIS Interpolation: In established hop gardens with aligned rows, RTK-enabled GNSS recorded precise positions of selected outer rows, using centimetre-level RTK accuracy. Intermediate plant positions were interpolated using GIS tools, assuming regular spacing, enabling high spatial accuracy with reduced fieldwork – suitable for both linear and curved configurations and for updating existing maps.

Sentinel-2 data analysis

Following MANAKOS et al. (2025), Level-2A Sentinel-2 surface reflectance products were acquired from the Copernicus Data Space Ecosystem. To ensure pixel alignment across bands, the red edge bands (B5, B6, B7) at 20 m spatial resolution were resampled to 10 m to match band B4, resulting in a unified 10×10 m grid (100 m² pixels). Only cloud-free scenes from May to July were retained. The Red Edge Inflection Point (REIP) was computed for each pixel using the surrogate formula: REIP = 700 + 40 * ((((S-2(B4) + S-2(B07)) / 2) – S-2(B05)) / (S-2(B06) - S-2(B05))).

This index, sensitive to chlorophyll content and canopy structure, was calculated for each scene during the growing period. For each variety and year, pixel-wise REIP values were averaged across all relevant fields. The resulting time series was interpolated across the 92-day growing window (01.v.–31.vii.) to estimate REIP values at specific time steps. Yield data were available only as one value per variety per year. Two REIP-based indicators were derived: i) REIP Slope (May–July): The rate of increase in average REIP over the growing period. Ii) Day-Specific REIP Value (75th growth day): The interpolated REIP value around mid-July, aligned with key phenological stages.

Pearson correlation coefficients were computed between the REIP indicators and observed average yields per variety per year. The time window that yielded the most robust and consistent correlations across all three varieties was selected for further interpretation. Additionally, anomalies such as the hailstorm event on 20.vi.2022 were factored into the interpretation of outlier behaviour in the REIP progression.

Results

Georeferencing outcomes

• GPS-Assisted Planting: This method provided immediate geospatial data integration and proved highly efficient, particularly for new plantings.

Field trials conducted at a hop farm in Ettling (Hallertau, Germany; 08. and 10.iv.2023) confirmed the system's ability to generate reliable point data for each plant location (Fig. 1). The provision of coordinate data from Wagner is typically offered at no additional cost to the grower, making this an attractive, scalable solution.

- UAV-Based Imaging and Photogrammetry: Aerial surveys were conducted using a DJI M350 drone equipped with a DJI Zenmuse P1 high-resolution camera. Flights were carried out on 06.v.2025. The imagery was processed by geo-konzept GmbH (Fig. 2). Validation was performed by remeasuring 20 sample plants using a GNSS S580 receiver from geo-konzept with CoGIS software. Results confirmed the method's suitability for high-resolution mapping.

- RTK-GPS Surveying and GIS-Based Interpolation: This approach is particularly suited for established hop fields with regular row spacing and offers a balance between precision and data acquisition efficiency. Full validation is ongoing.

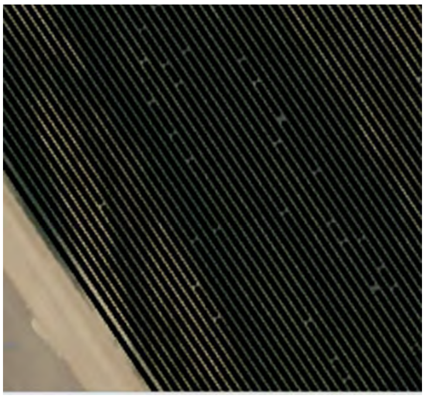

Figure 1. Georeferenced hop plants with the system of the Wagner company (hop farm in Ettling, 08.iv.2023 and 10.iv.2023)

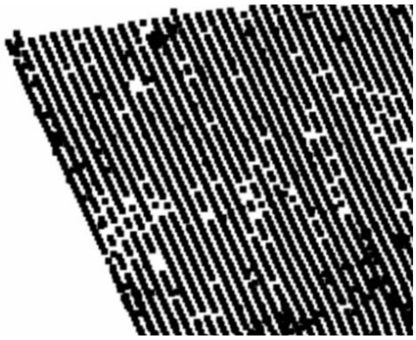

Figure 2. First results of georeferencing hop plants with a UAV and image processing (hop farm in Weikenhausen, 06.v.2025)

The combination of these methods enhances the overall accuracy and usability of the georeferencing data. Each method serves a specific operational niche: GPS-assisted planting is ideal for new installations; UAV-based imaging provides detailed spatial context and is effective for dense or irregular plantations; RTK-GPS surveying with interpolation offers a rapid approach to updating large fields with minimal flight or equipment overhead.

A slight year-to-year variation in sprouting locations was observed, caused by minor positional shifts in perennial plant re-emergence. However, this inaccuracy is within an acceptable tolerance range for most applications, including robotic deployment. For autonomous navigation and task execution in hop gardens, not only the hop plants but also static infrastructure elements such as support poles and ground anchors require georeferencing. Initial evaluations have included these elements, and further improvements are anticipated as the methods mature.

Sentinel-2 REIP Analysis

Analysis of Sentinel-2 data from 2018 to 2023 for the hop farm in Weikenhausen revealed consistent and interpretable patterns in the Red Edge Inflection Point (REIP) values across the growing seasons. For all three varieties, average REIP values increased steadily from early May to late July, followed by a stabilization phase into August. As indicated in MANAKOS et al. (2025), three main temporal REIP trends were identified: i) Low and variable REIP values in early May, typically representing less than 50 % of the seasonal fluctuation range; ii) a continuous upward trend in REIP from May through July, indicating increasing canopy vigour and biomass accumulation; and iii) a plateau of REIP values beginning in late July and persisting into August, corresponding with the crop's maturation phase.

Correlation analysis between the REIP slope from May to July and annual average yield showed moderate to good associations: HTR: r =0.84, PER: r =0.77, HKS: r =0.61. Interpolation of REIP values over the 92-day growth period (01.v.–31.vii.) enabled the identification of day-specific indicators. The 75th day of growth, typically in mid-July, consistently yielded the strongest correlation to average yield across all varieties: HTR: r =0.86, PER: r =0.69, HKS: r =0.76. This mid-July point was also observed to mark a critical breakpoint in the seasonal REIP increment, reflecting the transition from vegetative to reproductive stages. All years exhibited this trend consistently, except for 2022, where a significant hail event on 20.vi.2022 resulted in a temporary reduction in canopy vigour and suppressed the expected REIP rise.

Discussion

This study suggests that integrating ground-based georeferencing with satellite-based remote sensing provides a strong foundation for advancing precision hop farming. Accurate spatial localization of hop plants enables targeted field interventions and supports the development of autonomous systems. Vegetation indices such as the Red Edge Inflection Point (REIP), derived from Sentinel-2 imagery, have proven effective for monitoring crop development and forecasting yield. The results underscore the critical importance of July for hop yield formation, coinciding with the development of sidearms and flowers – two key components of yield potential (LfL 2024). This phenological insight aligns with agronomic findings that the upper sections of hop bines are particularly yield-determining in common German varieties (ENGELHARD *et al.* 2011). The ability to detect these yield-relevant stages as early as May, using REIP trends, highlights the added value of remote sensing over traditional, ground-based assessments, which generally produce useful results only from June onward (STAMPFL 2021; KUMHÁLOVÁ 2021). Although REIP values exhibited some fluctuation into August – particularly during the plateau phase – no consistent post-plateau decline or rebound, as reported in some other crops, was confirmed in hops. The plateau behaviour was consistently observed across all three cultivars (HTR, PER, HKS), reinforcing the importance of focusing spectral analysis on the pre-August growth window. The correlations between REIP indicators and annual yield were encouraging, particularly for mid-July values and extended May–July slopes. However, the reliance on coarse yield data, limited to one average yield value per variety per year, significantly limited the predictive power and spatial resolution of the analysis, restricting the ability to explore intra-field yield variability.

To address these limitations, future research should pursue: i) Georeferenced yield measurements at sub-field or individual vine level to enable accurate validation and calibration of spectral indices; ii) integration of in situ sensor data, such as soil moisture and canopy temperature, to contextualize satellite-derived trends; iii) fusion of data across platforms – UAVs, Sentinel-2, and ground sensors – to improve both temporal frequency and spatial detail; and iv) application of AI and machine learning algorithms, contingent on spatially detailed, multi-season yield datasets. The full-field georeferencing of hop gardens, which is already standard practice in viticulture, should be more widely adopted in hop production to support the scaling of automation technologies, such as robots for targeted fertilization or selective harvesting. These technologies are especially valuable in environmentally sensitive areas (*e.g*., water protection zones), where precise management is essential. When combined with high-resolution geospatial data, they form the basis for evidence-based, site-specific crop management strategies in hop cultivation. To fully realize this potential, continued investment in data acquisition, agronomic modelling, and interdisciplinary research will be essential (MANAKOS *et al*. 2025).

Conclusions

This study demonstrates the feasibility and potential of combining ground-based georeferencing with Sentinel-2-derived Red Edge Inflection Point (REIP) analysis to support the development of smart, data-driven hop management systems. The methods evaluated enabled precise spatial localisation of hop plants and delivered meaningful correlations between remotely sensed spectral indicators and annual hop yield, particularly for the cvs HTR, PER, and HKS. However, the study also highlights key limitations, particularly regarding the availability and granularity of yield data. Addressing these challenges through expanded, georeferenced ground-truth data collection and the integration of *in situ*, UAV-based, and sensor-derived datasets will be crucial for enhancing model robustness. The eventual application of machine learning techniques and data fusion frameworks could significantly advance predictive accuracy and operational scalability.

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Detection and evaluation of water stress of Czech hop varieties using remote sensing

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Abstract

Detection of physiological plant stress caused by biotic or abiotic factors in a hop garden is a crucial issue for carrying out agrotechnical interventions to achieve the desired quantity or quality of production. Satellite or UAV images can be used quite reliably to detect the condition of the hop garden or individual crops in all essential parts of the spectrum already commonly used in agriculture. The presented study aims to assess the usability of thermal images and the CWSI index derived from them for detecting water stress of varieties under organic and conventional management in 2023. The information is supplemented by spectral indices obtained from UAV images: NDVI, GNDVI, CIR, SAVI and NDRE as indicators of structure, vitality, health and chlorophyll content. The SAR indices derived from Sentinel 1 satellite images: DPDD, DPSVI, IDPDD and VDDPI were also used. The results showed that CWSI can be best explained in the flowering period (July) by up to 38 % using NDVI, and up to 76 % using DPSVI. Regarding varieties, organic Premiant was explained by 65 % using NDVI (structure and health), while only 50 % for organic Saaz. For conventional hops, NDVI explained CWSI from 53 % (Agnus) and 60 % (Premiant) to 63 % (Sládek).

Introduction

Satellite or UAV remote sensing is currently a very powerful tool for early detection of the physiological and structural condition of agricultural crops. Detection of physiological plant stress caused by biotic or abiotic factors in hop gardens is a crucial issue for carrying out agrotechnical interventions to achieve the desired quantity or quality of production (COMBA *et al*. 2015). In general, remote sensing is applied in three methods depending on the platform used – satellite, aerial, and terrestrial. Aerial photography using Unmanned Aerial Vehicles (UAV) equipped with multispectral or thermal camera is currently an available source of data if it is necessary to obtain data from a larger area with very high spatial resolution and therefore with high accuracy (VELUSAMY *et al*. 2022). The vegetation indices are often used for monitoring vitality, structure, chlorophyll content and biophysical processes of plants associated with their health. With climate change, the detection of water stress in crops is increasingly being addressed. The Crop Water Stress Index (CWSI; IDSO *et al.* 1981), usually calculated from UAV thermal images, is used to monitor water stress and can also be used to derive the irrigation rate (COSTA *et al.* 2020). Satellite images can provide images in all parts of the electromagnetic spectrum usable in agriculture, however, in very coarse spatial resolution. The optical images (visible, NIR, SWIR and thermal) are often influenced by cloud cover. On the other hand, microwave (SAR) images are available but the information obtained from images is very hard to interpret. SAR images are mostly used for detection of roughness (structure) and water content (SOSA *et al.* 2021).

As is generally known (*e.g.*, RYBÁČEK 1991), water content in hop crops is crucial for achieving high yield and quality of production and the Žatec (Saaz) region is one of the driest in Europe. Therefore, the aim of this study is to assess the usability of thermal images and the derived CWSI index for detecting water stress of varieties in organic and conventional farming in 2023 using spectral indices in the optical and microwave parts of the spectrum as commonly available remote sensing sources.

Materials and methods

The experimental hop gardens were located in Stekník (Czech Republic), a production and experimental farm of Hop Research Institute Co., LTD (HRI). The study comprised two hop gardens with different cultivation – one organic with cvs Saaz and Premiant, and the other conventional with cvs Agnus, Premiant and Sládek. Drip irrigation system was used in both hop gardens.

The experimental hop gardens were scanned by eBeeX fixed wing drone with MicaSense RedEdge multispectral (MS) camera and DuetT thermal camera (senseFly SA, Cheseaux-Lausanne, Switzerland) from early plant growth (May) to harvest (August). UAV was equipped with a built-in RTK-PPK functionality with accuracy up to 4.8 cm. This service was provided by CZEPOS (GNSS Permanent Station Network of the Czech Republic services at Czech Office for Surveying, Mapping and Cadastre) – VRS.MAX-CZEPOS (master – auxiliary stations) with correction format of RTCM 3.1.

The UAV missions were carried out under the same conditions, from 11:00 to 14:00 h CEST, to eliminate shadows and, at low wind speeds, to eliminate moving of the crop. The flight height over the organic hop garden was 80 m above elevation data and 73 m over the conventional hop garden to reach 5.5 and 5 cm spatial resolution for images of MicaSense Red Edge camera, and 11 cm spatial resolution for images from Duet T thermal camera at 84 m flight altitude. The image overlaps were 75 % (lateral, longitudinal) for MS camera and 80 % (lateral) – 85 % (longitudinal) for thermal camera to reach very accurate results. MicaSense Red Edge camera consists of 5 spectral bands: Blue with central wavelength of 475 nm and range of 20 nm, Green 560 (20) nm, Red 668 (10) nm, Red Edge 717 (10) nm and Near Infrared (NIR) 840 (40) nm; Duet T thermal camera 7.5-13.5 µm.

Image analysis comprised data preprocessing in eMotion SW and further data analysis in Pix4D and QGIS SW. Photogrammetric procedure was carried out for orthophoto and NDVI spectral index calculation. Threshold of 0.3 NDVI value was set to separate green part of crop from bare soil. The spectral indices were calculated from multispectral images: Normalized Difference Vegetation Index (NDVI) and Soil Adjusted Vegetation Index (SAVI) for structure, vigor, health of crop detection, Green Normalized Difference Vegetation Index (GNDVI), Normalized Difference Red Edge Index (NDRE), and Chlorophyll Red Edge Index (CIR) for chlorophyll content detection. The details about spectral indices and methodology are given in ŘEŘICHA et al. (2025). CWSI was derived from thermal images for the individual terms of canopy scanning for water stress detection.

The SAR indices were derived from Sentinel 1 satellite images downloaded from the Copernicus Open Access Hub (https://scihub.copernicus.eu/). SAR data processing consisted of several steps as follows: range-doppler terrain correction with bilinear interpolation then applying radiometric normalization, and speckle filtering using gamma map filter.

Selected SAR indices were calculated: Dual-Pol Diagonal Distance (DPDD), Dual Polarimetric SAR Vegetation Index (DPSVI), Inverse Dual-Pol Diagonal Distance (IDPDD) and Vertical Dual Depolarization Index (VDDPI) for structure and water content detection. The details about SAR indices are given in SOSA et al. (2021). The data were then statistically processed in the data analysis software system Statistica, version 13 (http://tibco.com.).

Results and discussion

The one-year results were obtained in the vegetation period 2023 from our study area. Table 1 shows the usefulness of optical and SAR indices for general monitoring of hop plants during the main phenological phases (longitudinal growth, flowering and cones development). The results show that the CWSI of the hop stand can be explained by SAR indices up to 75 % using the DPSVI index in the growth phase of flowering. DPSVI can explain the CWSI in the phenological phase of cone development up to 65 %. The essence of SAR indices is based on the fact that they are sensitive to different degrees of surface roughness and moisture. Of the optical indices, in particular NDVI, NDRE and CIR can explain the CWSI from 35–38 %, also in the phenological phase of flowering.

Coefficients of determination between the CWSI and selected spectral optical and SAR indices for the whole vegetation season 2023 and individual varieties are given in Table 2. The results showed that the CWSI for organic Saaz was explained 65 % by NDVI (structure and health), while only 50 % for organic Premiant. In the case of organic Premiant, the NDRE and CIR indices were more significant (56 and 54 %).

Both the NDRE and CIR indices work with the Red Edge spectral band. As stated by XU *et al*. (2019), the Red Edge band in the wavelength range of 670–760 nm is characterized by a greater sensitivity to changes in plant tissue and indicates various physical and chemical parameters of vegetation, such as chlorophyll a and b content, leaf cell structure, and hydric status. The variability of the hop stand in later stages can be more and longer influenced by biotic and abiotic factors and, therefore, the Red Edge indices appear to be more sensitive to capturing these changes as also followed from our study.

ŘEŘICHA *et al*. (2025) mentioned in their study that GNDVI is significant in the early stages as an index capable of capturing the chlorophyll content in the leaf tissue and corresponds to the ability of photosynthesis quality and viability of the hop stand. In later stages, structural indices (NDVI and SAVI) are important because they can better reflect plant vitality expressed by plant volume. This statement is in accordance with our study.

In conventional hops, NDVI explained CWSI from 53 % (Agnus) and 60 % (Premiant) to 63 % (Sladek). In conventional hops, disease pressure was not as significant as in organic hops, as shown by higher values of the GNDVI index as an indicator of chlorophyll content in leaves.

SAR indices generally did not show any useful results in evaluating varieties throughout the growing season. Our results suggest that optical spectral indices are more suitable for general evaluation of varieties, in contrast to CWSI evaluation in individual phenological phases, where SAR indices were more significant.

The results obtained can be applied in determining the efficacy of cultivation management and agrotechnical interventions, including hop irrigation. This statement agrees with the study by ALI *et al*. (2021). They concluded that IR-based thermal imaging helps to identify sensitive plants at the beginning of drought stress or to determine stress tolerance in different varieties.

This statement is valid also for hop management, especially for water stress detection in hop garden and irrigation dose settings. Even though our contribution deals with varieties typically grown in the Czech Republic, the methods used are transferable to other geographical areas because cultivation methods there do not differ significantly from those in the Czech Republic.

Table 1. Coefficients of determination (R^2) between CWSI and other variables in the main phenological phases of 2023: longitudinal growth (LG), flowering (FL), and cone development (CD).

Variables	NDVI	GNDVI	CIR	SAVI	NDRE	DPDD	DPSVI	IDPDD	VDDPI
CWSI LG	0.21	0.15	0.14	0.06	0.14	0.08	0.10	0.08	0.00
CWSI FL	0.38	0.21	0.35	0.11	0.37	0.56	0.75	0.56	0.14
CWSI CD	0.21	0.08	0.11	0.01	0.10	0.59	0.65	0.59	0.04

Individual variables are described in the text above.

Table 2. Coefficients of determination (R^2) between CWSI and other variables for the whole vegetation season 2023 and individual varieties. PRE$_{bio}$ – cv. Premiant, SAZ – cv. Saaz, both under organic management; AGN – cv. Agnus, PRE$_{con}$ – cv. Premiant, SLD – cv. Sládek, all under conventional management

Variables	NDVI	GNDVI	CIR	SAVI	NDRE	DPDD	DPSVI	IDPDD	VDDPI
CWSI PRE$_{bio}$	0.50	0.43	0.54	0.27	0.56	0.00	0.00	0.00	0.11
CWSI SAZ	0.65	0.52	0.56	0.40	0.59	0.08	0.08	0.08	0.10
CWSI AGN	0.53	0.53	0.53	0.45	0.52	0.26	0.18	0.26	0.32
CWSI PRE$_{con}$	0.60	0.59	0.54	0.48	0.55	0.20	0.10	0.19	0.08
CWSI SLD	0.63	0.56	0.56	0.52	0.58	0.13	0.14	0.13	0.00

Individual variables are described in the text above.

Acknowledgement

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IX: Marketing

Marketing of hops for craft industry in Argentina

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Abstract

This study compiled unpublished data on local hop production and hop imports in Argentina over the past 11 years. It categorizes the data based on its intended use, distinguishing between craft beer and large-scale brewing companies. The findings reveal trends in the local hop market and domestic hop cultivation, highlighting an increase in demand for hops from the craft breweries, which reached an all-time high in 2018. Changes in Argentina's macroeconomic environment, coupled with the pandemic, prevented the exponential growth from continuing. However, demand for hops (both locally produced and imported) from the craft beer industry over the last four years has been around 33 % in volume (tons of pellets) and around 42 % in FOB (Free on Board) value. To enhance the analysis, a comparison was conducted with the hop demand and beer production curves of the U.S. craft breweries, examining consumption proportions. A peak in demand was observed in 2019, with lower and possibly stabilized values after the pandemic, coinciding with what is observed in Argentina. The popularity of local craft breweries is no longer a novelty, and they are known to prioritize the use of high-quality ingredients. In contrast, large-scale brewing companies continue to rely on both local and imported hops, often prioritizing cost and consistency over unique flavors.

The study highlights the evolving landscape of the Argentine beer market, showing the impact of the craft beer movement on the hop import and resale business, local agriculture and the brewing industry as a whole.

Introduction

Over the past 15 years, the craft beer market in Argentina has significantly expanded, driven by consumers' increasing preference for unique, high-quality, and locally produced flavors. This movement mirrors the craft beer boom that occurred in the United States two decades earlier. As a result, Argentina has seen a corresponding rise in microbreweries and local hop cultivation, supporting both the brewing industry and regional agriculture.

Notably, the term 'craft beer' lacks formal definition within the Argentine Food Code; it is typically associated with independent production, small-scale operations, artisanal methods, and a focus on quality ingredients. By comparison, the United States provides a more structured definition through the Brewers Association, which classifies a craft brewery as small (producing fewer than 6 million barrels annually) and independent (with less than 25 % ownership by a non-craft alcoholic beverage company). This organization has maintained detailed records since the early 1970s, documenting the evolution and quantifying key data of this significant sector of the U.S. food industry.

The economic impact of Argentina’s craft beer boom is undeniable, having led to the establishment of near a thousand small and medium-sized breweries. This emerging industry has created thousands of direct and indirect jobs across all 23 provinces and the Autonomous City of Buenos Aires. Simultaneously, it has boosted the hops business, which for more than half a century was treated almost as a commodity, primarily demanded by large-scale brewers and requiring little differentiation.

Nevertheless, the Argentine craft brewing sector remains largely informal and lacks consistent statistical reporting, making it difficult to accurately evaluate its development and current status.

Objective and methods

The objective of this study was to compile unpublished data (tons and FOB value) regarding hop production and imports in Argentina between 2014 and 2024, distinguishing between usage for craft versus industrial beer production. Based on this data, and assuming an average hop dosage of 1 pound per beer barrel, estimates of annual craft beer production volumes were derived. Additionally, a comparative analysis with U.S. craft brewing trends was performed to identify similarities and differences in beer and hop market development. Reports published by Hopsteiner, Barth-Haas, and the Brewers Association were utilized, along with unpublished data compiled over several years.

Results

Domestic hop production in Argentina between 2014 and 2024 increased by 26 % in volume, accompanied by a 17 % expansion in cultivated area, with notable variation in the share demanded by the craft beer sector (Fig. 1). In 2014, only 10 % of domestically produced hops were allocated to craft brewers; by 2018, this figure had reached a historical peak of 33 %, and over the past four years, it has averaged 30 %.

In terms of FOB value, the craft sector accounted for 44 % of the locally produced hop market at its peak in 2018. Craft breweries clearly demand greater specificity in quality, variety, and packaging, and are willing to pay a higher price for it. The volumes of hops exported by Argentina are not significant, which simplifies the analysis presented.

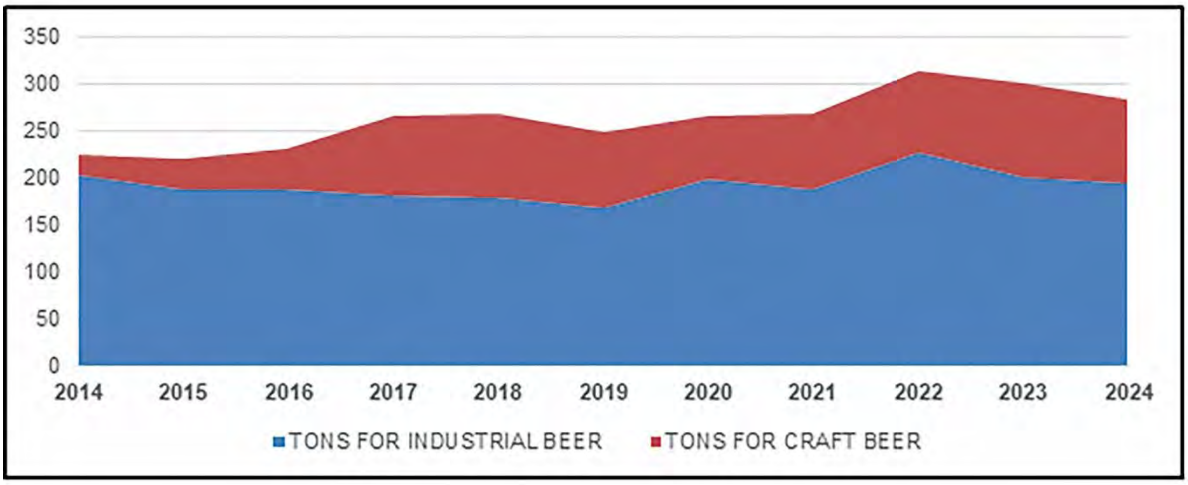

Figure 1. Domestic hop production and usage destination in Argentina (tons)

Hops imported into Argentina each year exceeds domestic production. A detailed analysis of data from the Customs system makes it relatively easy to distinguish hops imported by large industrial brewing groups, as these operations typically involve high volumes. This allows imports to be classified into three categories, enabling a focused analysis of the craft brewing sector (Table 1). It becomes clear that while some imports were acquired directly by craft breweries, after 2016 the majority were handled by local distributors, many of whom owe their existence to the exponential growth of the craft beer industry in Argentina.

While in 2014 around 4 % of imported hops were used by the craft sector, in 2017 the share rose to almost 43 %; and after the pandemic, it remained at around a third. In FOB value, craft breweries accounted for 56 % of demand at the pre-pandemic peak, reflecting a preference for higher-quality hops and privately owned varieties (which are more expensive than public ones).

Table 1. Breakdown of hops imported to Argentina (proportions based on tons)

IMPORTING COMPANY	2014	2015	2016	2017	2018	2019	2020	2021	2022	2023	2024
INDUSTRIAL BREWERIES	97%	95%	90%	57%	58%	59%	78%	66%	71%	64%	65%
CRAFT BREWERIES	3%	3%	5%	12%	11%	7%	1%	2%	2%	5%	3%
RESELLERS/DISTRIBUTORS	1%	1%	5%	31%	31%	34%	21%	32%	27%	31%	32%

By compiling annual data on hops domestically produced and imported in Argentina, it is possible to obtain a comprehensive estimate of the proportion allocated to craft beer production. During the period analyzed, the craft beer industry in Argentina exhibited remarkable changes in hop volume demand (Fig. 2).

The findings reveal an all-time high in 2018, when craft breweries demanded 40 % of Argentina's total hops regarding tons, and 51 % if analyzed in FOB value. Nevertheless, changes in Argentina's macroeconomic environment, coupled with the pandemic, prevented the exponential growth from continuing. Demand for hops from the craft beer industry over the last four years has been around 33 % in tons and around 42 % in FOB value.

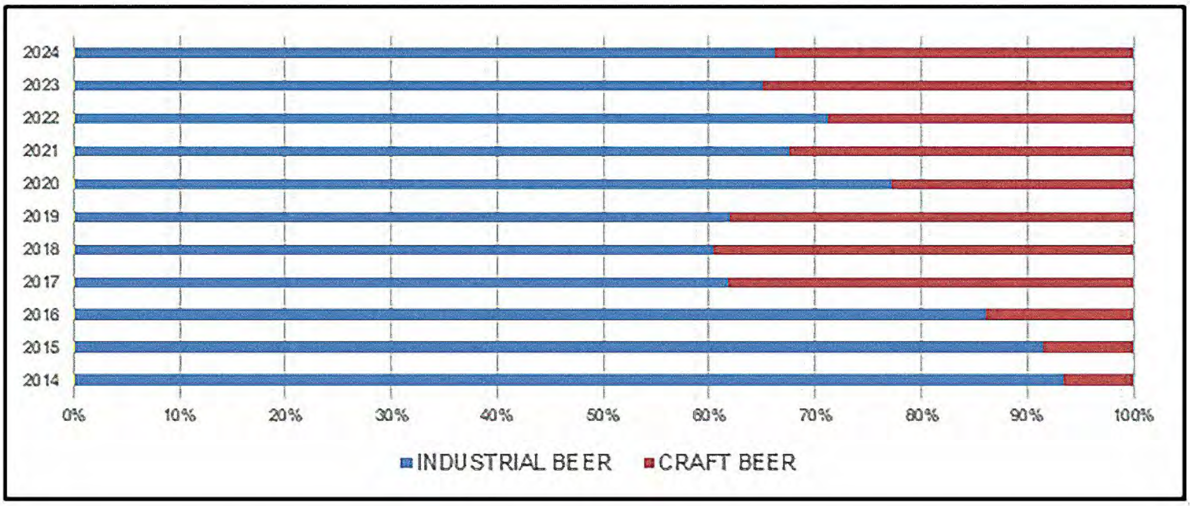

Figure 2. Hops demand in Argentina (proportions based on tons)

Unlike the United States, Argentina lacks reliable statistical records on annual craft beer production, neither official data nor figures eventually published by brewers' associations nor industry chambers are reliable. As a result, it becomes necessary to generate estimates drawn from surveys, isolated publications, and case studies of established craft breweries with systematized data that are willing to share it. Assuming for craft beer in Argentina an estimated average hopping rate of 1 pound per beer barrel (equivalent to 3.87 grams per liter), it is possible to estimate production volumes. This approach offers valuable insight that is otherwise unavailable in Argentina. By integrating these estimations with publicly available data on the global beer production volumes by country, the share of industrial and craft beer in Argentina is determined. When the resulting curve is overlaid with that of the United States, notable similarities in the observed trends become apparent (Fig. 3).

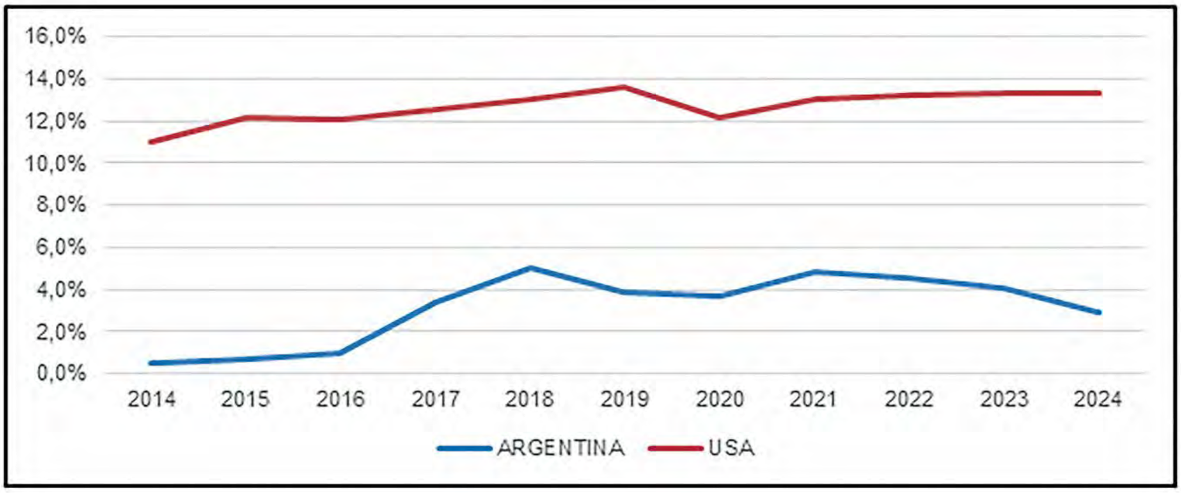

Figure 3. Craft beer share (%) in Argentina and the USA from 2014 to 2024.

The U.S. craft beer market was less affected by the pandemic and recovered quickly, showing a more consistent trend. Notably, prior to the arrival of COVID-19, most Argentine craft beer was sold on draft, with only a few breweries having developed canning or bottling operations. As a result, the abrupt implementation of lockdown measures had a disproportionately severe impact on craft beer consumption in Argentina, as numerous breweries were compelled to halt sales for extended periods.

In terms of beer production, the craft segment in Argentina accounted for just 0.5 % in 2014, compared to 11 % in the United States, peaking at 5 % in 2018 but declining to 2.9 % by 2024, while the U.S. market remained stable at 13.3 %. In 2024, following a change in government in December 2023, Argentina implemented a major economic policy shift that significantly reduced its previously high double-digit monthly inflation. However, the resulting price adjustments impacted overall consumption, and as a non-essential good, craft beer was particularly affected.

Conclusion

The craft beer movement in Argentina has profoundly reshaped the hop market, increasing demand for both locally produced and imported hops. Although economic challenges and the COVID-19 pandemic tempered the growth trajectory, craft breweries have established a stable and significant share of the market. Their focus on ingredient quality and the growing consumer interest in unique, locally produced flavors set them apart from industrial breweries, whose operations prioritize profitability and product consistency. Overall, the Argentine craft beer sector continues to evolve, fostering local agriculture, supporting regional economies, and influencing consumer trends, thus contributing to a broader cultural shift toward beer appreciation.

X: Indoor hop cultivation

Metabolomic profiling of indoor hop during cone development

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Abstract

Lupulin in hop cones has a rich composition in phenolic compounds, including α- and β- acids and essential oils that are rich in monoterpenes and sesquiterpenes. The brewing value of hops is determined by the α-acid content, which contributes to bitterness and the composition in essential oils that define beer aroma. The metabolomics of different hop cone varieties and their contribution to beer quality are well documented. However, the metabolic profile during hop cone development has not yet been documented. Our aim was to analyze the metabolic profiles of the bitter variety Nugget and the aroma variety Sladek. Cones at three phenological stages were subjected to an untargeted analysis based on an hydroalcoholic extraction followed by ultra-high performance liquid chromatography-high resolution mass spectrometry. The annotated metabolome dataset was generated by the software Compound Discoverer 3.3. The datasets were compared by different multivariate statistical approaches to identify chemical markers that may correlate with the phenological stage and physiological development. Our prospect is building up a model that allows monitoring cone quality development and forecasting for harvest management in order to maximize yield and quality.

Introduction

Common hop *Humus lupulus* L. is cultivated mainly for beer production. The female inflorescence, the hop cone, is added to the wort for beer bitterness, aroma, and antiseptic properties. The growing popularity of craft beer and the emerging interest for natural medicine is widening appealing scenarios for the use of hop varieties. Hop cones contain lupuline glands located at the base of the bracts that produce bitter phenolic compounds, including prenylated chalcones such as xanthohumol, α-acids (humulones), β-acids (lupulones) and essential oils, rich in monoterpenes and sesquiterpenes, which are responsible for the hoppy aroma of beer.

The brewing value of hops is determined by the α-acid content and the composition of essential oils that confer a unique aromatic profile. Crop metabolomics, the analysis of the pool of metabolites that are present in the plant in a given moment and tissue, is emerging as a powerful tool for precision agriculture, where the goal is to optimize management for enhancing crop yield. Metabolomics studies can provide chemical signatures for plant responses to environment or development, offering insights that were unachievable with traditional agricultural methods (HAO *et al.* 2025). The metabolic profile has been exhaustively characterized for many hop varieties (IKHALAYNEN *et al*. 2022; NEZI *et al.* 2022) and their contribution to beer quality is also well established (PAGUET *et al.* 2024). However, the composition of lupulin throughout the cone development has never been characterized yet. The aim of this study was to screen the metabolomics associated with cone development and quality for a comprehensive analysis that allows selecting compounds that are good as markers to monitor cone development for forecasting. The ultimate goal is optimizing management for harvest quality enhancement.

Material and methods

Two hop varieties that differ in their secondary metabolite profile were selected for the study: Sladek (SLD), which has a diverse aromatic profile and low α-acids, and Nugget (NUG), with a simple aromatic profile and high α-acid content. Twenty-four plants of each variety, obtained by clonal *in vitro* micropropagation, were cultivated in independent growth chambers equipped with a heating, ventilation and air conditioning (HVAC) system and CO_2 enrichment. Nutrition was provided with a recirculating hydroponic irrigation system and mineral composition was adapted to the four developmental stages. Lighting was provided artificially with dimmable LED lamps, adjusting the intensity and photoperiod to each stage. Plants were grown in 12 L pots with perlite substrate. Several main stems were trained to climb on a bioplastic twine until they^{o} reached 7 m in height, when flowering was induced by modifying the grow room environmental conditions. Plants were sampled for flowers in three different secondary growth stages (SGS-69, SGS-79 and SGS-89) according to the hop BBCH scale. Flower samples were later classified according to quality parameters (α-acids % and oil %) in range (R) when compared to the field, under range (UR) and over range (OR), which defines nine treatments in this study. Sixty flowers were collected from at least 6 different plants, frozen at -80, lyophilized and milled. Fifty mg of sample were then extracted using methanol:water 80:20 (v/v) for 10 min at 800 rpm followed by 10 min of centrifugation at 4355 g. The extract was evaporated by N2 flux and resuspended in 500 µL of 50 % acetonitril, 0.1 % formic acid.

In order to avoid instrumental drifts, quality controls were prepared for each study (QC study) and for the method (overall QC). Samples were processed in an ultra-high performance liquid chromatography system coupled to Orbitrap-type high-performance mass spectrometry (UHPLC-MS/Orbitrap) and chromatographic column (type C18) Thermo Hypersil GOLD (100x2.1 mm, 1.9 µm). The acquisition method used was full MS/data dependent MS2 with inclusion list. Sample order was randomized for each study, and the corresponding QCs were run at the beginning and end of the study. Data from non-targeted metabolomics was processed by Compound Discoverer 3.3 software (Thermo Fisher Sci.). Workflow involved retention times data alignment, detection of unknown compounds and clustering of compounds. The elemental composition of all detected compounds was predicted on these data. Regions without compound data between chromatograms were filled, and background noise was removed using blanks. Detected compounds were annotated using the mzCloud and ChemSpider databases. A classification of the results obtained in ChemSpider was performed using the mzLogic algorithm. The annotated compounds were filtered according to the error of annotated exact mass (≤ 5 ppm), the absence of background signal from the blanks, the existence of an MS2 spectrum and an area intensity higher than 5-106. The annotated compounds were filtered out and tentatively identified.

The data matrix was subjected to principal component analysis (PCA), partial least squares discriminant analysis (PLS-DA), and one-way analysis of variance (ANOVA) with a Tukey post-hoc test and a Benjamin-Hochberg correction. The p-value, adjusted p-value, the ratio of the areas between groups of samples, and the log 2-fold change were calculated. Molecular networks were generated to visualize compounds that might be structurally related.

Results

The metabolomic profiles of the SLD and NUG hop samples unequivocally differentiates the three quality ranges (UR, R and OR) in each of the secondary growth stages (SGS-69, SGS-79 and SGS-89). A variety of metabolites have been identified among samples, such as alpha/beta acids (humulones and lupulones) and polyphenols such as flavanols (catechin, epicatechin and procyanidins B1/B2/B3/C1), flavonols (quercetin, rutin), glycosylated flavonoids (myricetin-3-O-beta-D-galactopyranoside, quercetin-3,7- diglucoside, luteolin-7-O-malonylglucoside), prenylflavonoids (xanthohumol, 8-prenylnaringenin, 6,8-diprenylnaringenin) and phenolic acids (chlorogenic acid, ferulic acid). The structural relationships between these compounds can be visualized in the generated molecular network maps drafted for each growth stage and variety. Figure 1 shows the network drafted for PS-9 in SLA). In addition, the loadings resulting from principal component analysis (PCA) have allowed the selection of the tentatively identified compounds that had the highest relative abundance in each quality range. Figure 2 shows the loading graph for PCs 1 and 2 for PS-69 in SLA. Partial least squares differential analysis (PLS-DA) allows the selection of those compounds that differentiate between the quality ranges studied for each stage, highlighting as potential biomarkers flavonols such as quercetin in SLA or flavonoids such as procyanidin B1 in NUG. Figure 3 shows the S-plot that relates analyte correlation with principal component and its covariance for PS-69 in SLA. Finally, analysis of variance (ANOVA) allowed us to evaluate significant differences in relative abundances between quality ranges for each compound tentatively identified, allowing us to select those with the greatest differences in quality ranges based on their log2-fold change value (Fig. 4).

Conclusions

The study has identified candidate markers for quality forecasting in two different hop varieties. These compounds will be validated by detailed studies. An extension of this study that includes more varieties would be beneficial to find markers that are universal.

Acknowledgement

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XI: Hops, aroma, and beer

Characterizing the influence of hop microbiota on volatile organic compound production in traditional and organic agricultural systems

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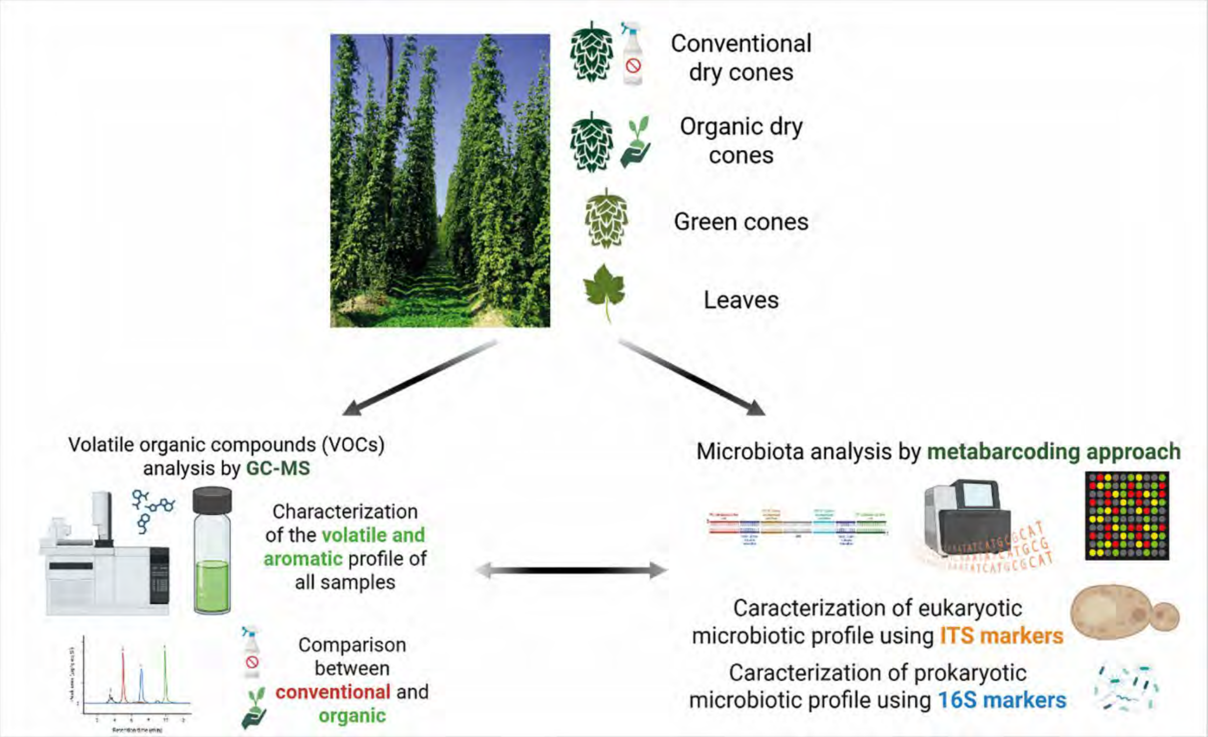
Graphical abstract



Introduction

Hop (*Humulus lupulus* L.) cultivars are essential for the brewing industry, especially for their rich content in volatile organic compounds (VOCs) that play a key role in defining beer aroma. With the rise of microbreweries and an increasing demand for locally sourced ingredients, together with the emerging idea of regenerative agriculture, farming practices must adapt to this evolving landscape. In this context, the first organic hop farm in France emerged in 2012 to meet the growing demand for organic hops. In recent years, conventional farming aimed at shifting towards agroecology-oriented practices in different places. Within this framework, a detailed characterization of hop as a holobiont is required, to refine our understanding of the role of its microbiota, and the impact of the variety or farming practices on its quality.

Material and methods

We conducted an analysis of the microbiome associated with hop cones and leaves as well as their corresponding volatile organic compounds (VOCs). Different hop samples were collected from hop gardens, including leaves, green hop cones, and dry hop cones, under either organic or conventional farming methods. Microbiota of five hop cultivars (Aramis, Brewer’s Gold, Elixir, Nugget, and Strisselspalt) were analyzed using a metabarcoding approach, which involves the amplification and sequencing of fungal and bacterial marker genes (ITS and 16S). A fully automated solid-phase microextraction coupled with gas chromatography–mass spectrometry (SPME-GC/MS) method was developed for the analysis of VOCs.

Results and discussion

Across all samples, a total of 441 Amplicon Sequence Variants (ASVs) affiliated to eukaryotic microorganisms were detected. The relative abundances of the phyla *Ascomycota* and *Basidiomycota* were compared, revealing that *Basidiomycota* generally dominated across samples. However, in the organic dry cone samples, both phyla were present in comparable proportions. Alpha and beta diversity were assessed to evaluate microbial diversity. Alpha diversity, reflecting species richness and evenness within individual samples, was higher in the organic dry cones, indicating a more diverse fungal community structure at the sample level. In contrast, beta diversity, which captures differences in community composition between samples, showed that conventional dry cone samples formed a distinct cluster, separated from other sample types, suggesting a distinct fungal community structure. A focused analysis on dry hop cone samples from both organic and conventional systems was performed to investigate variety-specific differences. A total of 388 ASVs were detected exclusively in dry cone samples. Among these, the most abundant species were *Cladosporium victoriae* (12 %) and *Alternaria herbarum* (9 %).

Following the analysis of the eukaryotic microbiota, a similar approach was applied to characterize the prokaryotic community. A total of 4984 amplicon sequence variants (ASVs) were identified across all samples. The dominant bacterial phylum was *Proteobacteria* in most samples. In leaf samples, however, bacteria affiliated to *Firmicutes* were the more abundant phylum. The third most prevalent phylum, *Actinobacteriota*, was particularly abundant in all Brewers Gold samples. Alpha diversity did not significantly vary between sample types or hop varieties. In contrast, beta diversity analysis revealed that green cones and organic dry cones clustered together, suggesting a shared microbial composition. In comparison, conventional dry cones and leaves formed distinct groups, indicating a specific microbiota for these sample types. Focusing on cones, all 4984 ASVs were detected in both organic and conventional dry cone samples. Taxonomic classification could be resolved to the genus level, with *Massilia* (5 %), *Pseudomonas* (3 %), *Sphingomonas* (3 %), *Nocardioides* (3 %), and *Corynebacterium* (2 %) being the most abundant genera.

Complementary to this microbiota analysis, SPME-GC/MS analysis was conducted to assess VOCs. A total of 423 compounds were detected and identified (match factor > 80). The VOCs profiles were markedly different between leaf and cone samples. In leaves, 227 compounds were identified, and ANOVA analysis showed 121 compounds differed significantly between varieties, highlighting the varietal influence on volatile profiles. In dry cone samples, 328 compounds were detected, with 194 compounds significantly differing among hop varieties. Principal component analysis (PCA) showed clustering by variety rather than farming method, suggesting that varietal identity exerts a stronger effect on VOCs composition.

However, when data were grouped by cultivation method, 29 compounds differed significantly between organic and conventional farming, indicating a secondary but measurable influence of farming practices on the volatile profile of dry hop cones.

Conclusion

In this study, an integrated approach was developed to characterize both the eukaryotic and prokaryotic microbiota, as well as the volatile organic compounds (VOCs), present in various hop sample types (leaves, green cones, organically and conventionally grown dry cones) and varieties (Aramis, Brewers Gold, Elixir, Nugget, and Strisselspalt). We show here that the composition of microbial communities differs according to the cultivars and farming methods. Moreover, our results suggest that the farming method may influence the concentration of specific VOCs in hop cones. Overall, our findings highlight the complex interplay between hop variety, sample type, and agricultural practices in shaping both microbial communities and the volatile profile of hops. To our knowledge, this is the first study to jointly investigate microbiota and VOCs composition in hop cones across different cultivars and cultivation methods. Further research is needed to confirm these observations across a broader dataset and to explore the potential implications for agronomic performance and aromatic quality of hops.

From field to glass: evaluating Slovenian hop varieties for sensory properties in different brewing styles

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Abstract

In hop breeding, agronomic traits such as yield and disease resistance are fundamental. However, the flavour and aroma of hops are equally important as they play a significant role in determining the sensory profile of the final beer. Notably, these sensory attributes vary according to specific beer production methods, highlighting the importance of a comprehensive approach to hop breeding programmes that integrates both agronomic performance and organoleptic quality. With the aim of creating a database on the influence of using different Slovenian hop varieties in various beer styles, we have conducted initial analyses of the bitterness content, basic physicochemical parameters of the beer, and its aromatic profile, which is one of the key factors in consumer decision-making or beer acceptance. In addition, physicochemical characteristics were coupled with sensory analysis to give a complete picture of each variety's performance. Furthermore, our study revealed that different hop varieties exhibit distinct responses to various brewing methods. In general, some hop varieties are more suited for kettle hopping, while others perform optimally when used for dry-hopping. These findings are pivotal for brewers, as they provide essential insights that facilitate the selection of the most appropriate varieties based on the desired sensory profiles.

Acknowledgement

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The hop labyrinth:
Finding a brewer's path through new varieties

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Abstract

In recent years, the approval of new hop varieties has increased significantly. While there was approximately one new variety per year approved in the EU between 1990 and 2000, this number rose to four per year from 2010 to 2024. Additionally, there are numerous national registrations, particularly in the Czech Republic and Slovenia. The main factors driving these developments are the following (*cf.* FORSTER & SCHÜLL 2020; FORSTER *et al.* 2021, 2023):

Ongoing climate change

- Climate stability varies considerably among hop varieties
- Bitter and flavor varieties tend to be more stable than most aroma varieties
- Not only traditional hop varieties and landraces are affected, but more recently bred aroma hops are also vulnerable
- Of particular concern is, *e.g*., cv. Perle, the most widely cultivated German aroma variety

Pest management (resistance or tolerance)

- Hops are attacked by viral, bacterial, and fungal diseases, and arthropod pests such as hop aphid, hop-flea beetle, and spider mites
- Breeding efforts aim to develop resistances or tolerances against these pests
- Long-term resistance has not yet been achieved; pests overcome resistance
- Resistance milestones occur roughly every 10 to 20 years. This means that to maintain resistance, new varieties must be introduced at regular intervals.
- Tolerance requires moderate control but has a longer-lasting effect.
- Controlling pests is becoming increasingly challenging for ecological reasons

Search for new sensory profiles

There is now a wide range of flavor varieties with unique aromas, allowing brewers to customize their beers, especially through the blending of varieties.

Increased efficiency in α-acid yields

Sensible limits for increasing α-acids in hops are around 20 %. Values higher than this can complicate production, as the stickiness of the lupulin hinders smooth processing in mills, pellet presses, conveyor systems, etc.

New breeders entering the field (growers, merchants)

Historically, breeding was primarily conducted by state institutions in major hop-producing countries like the UK, USA, Germany, Czech Republic, and Slovenia. The number of breeders has now increased significantly, primarily for commercial reasons. Plant variety protection generates revenue and marketing exclusivity.

Examples of breeding challenges

- External factors (climate, diseases, pests) are now dominating breeding efforts
- Successful variety development often involves numerous failed attempts
- Sometimes, resistance is overcome shortly after a variety is registered
- This challenge is not always communicated clearly when new varieties are presented
- Should some claims, such as resistance to climate change and diseases, be framed more carefully to avoid raising unrealistic expectations among brewers?
- Are all chosen names successful?

Useful information for brewers and growers

The Bavarian State Research Center for Agriculture (LFL 2025) publishes a statistical tool titled "LfL-contribution margins and calculation data", where the following information for 19 varieties grown in the Hallertau region can be obtained (in €/ha and €/kg hops):

- Yields and income
- 14 variable individual costs and the total variable costs
- Resulting contribution margins

Further information includes the "greenhouse gas emissions" (GHG) in kg CO_2 per ha and kg hops for nine individual measures and the total production

Information on secondary metabolites

Secondary metabolites are not uniformly regulated. The description of the aroma, in particular, can vary widely. Commonly measured in the EU are:

- For bitter substances: alpha-acids, ratio beta: alpha, cohumulone ratio
- Total polyphenols
- For aroma: total hop oil and linalool

What else would be desirable, especially for aroma hops?

- Substances with high aroma potential such as esters, monoterpene alcohols in addition to linalool
- Thiols – see, *e.g.*, a recent publication by SCHMIDT *et al.* (2024)

Brewing trials

- Comparisons, even when hops are dosed at the beginning of the boil with Herkules, Hallertauer Magnum, Polaris, and Titan, show a moderate influence on bitterness and flavor, even if not statistically significant. Statistics do not tell the brewer however that no consumer can taste the difference.
- Some brewers use Perle at the beginning of the boil. Alternatives like Mandarina Bavaria, Ariana, or Hallertau Blanc are comparable. However, obvious aroma chracteristics in the hops can influence the beer in the form of different kettle hop aromas.
- The replacement of Hallertauer Tradition or Perle in the middle of the boil by Mandarina Bavaria, Opal, Hallertau Blanc, or Ariana elucidates an influence on the hop character. Beers with these substitutes are a bit more fruity and citrusy.
- In late-hopped beers (end of boil + Whirlpool), the content of linalool, carboxylic esters, and sesquiterpene alcohols + epoxides varies widely when comparing classical aroma hops (Hallertauer Mittelfrüher, Hersbrucker Spät, Spalter) with new varieties (Saphir, Callista, Diamant, Huell Melon), which has an influence on the aroma of beer.

Synopsis

The flood of new cultivars is difficult for the brewer to classify. The competition between breeders leads to clear announcements. Some of the properties are not yet evident when introduced, such as resistance to climate and disease.

Breeders and their organizations should strive to obtain uniform and meaningful core data on cultivation (yields, costs, GHG emissions) as early as possible.

More relevant information on the flavor characteristics of aroma hops would be helpful. Brewing trials should develop facts and not confirm prejudices.

What is the biggest dilemma? The breeding goals require a high number of breeding varieties. The large-scale experimental cultivations take place at optimized locations. It remains to be seen whether the results can be confirmed on „normal“ fields. Without cultivation in different areas, assessments are problematic.

Without the brewers' interest in buying, cultivation cannot be realized. Without large-scale technical experience in cultivation and brewing practice, brewers can't really achieve anything. However, large-scale brewing experiments are risky and time-consuming. We as hop companies have to accompany brewers on their journey with a well-planned experimental setup. This also includes reliable analytics. What is for sure: A 1:1 substitution is often unrealistic.

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XII: Posters

Hops and sustainability:
Life cycle perspectives from Aquitaine, France

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Abstract

The growing demand for organic hops, driven by the expansion of craft breweries in France, calls for assessing their environmental performance. This study evaluates the life cycle impacts of four organic hop production systems. Preliminary results indicate a higher carbon footprint than in conventional systems, mainly due to low yields and field operations. Nonetheless, organic systems show lower impacts in water use, eutrophication and acidification, attributed to reduced fertilizer and phytosanitary inputs. Insights are offered to mitigate the carbon footprint of such systems. Hop production systems have received limited attention from a life cycle perspective (HAUSER & SHELLHAMMER 2017). In recent years, France has experienced a surge in craft beer production, accompanied by increased hop cultivation, especially as a high value complement crop in horticultural farms in the south and west. This has positioned France as a key player in organic hop production. This study evaluates the environmental performance of these systems relative to conventional production.

An attributional Life Cycle Assessment (LCA) was conducted using a cradle-to-gate approach, following the ISO 14040 and 14044 guidelines. The assessment coverede all field operations and drying process, including machinery use, water consumption, energy consumption, agrochemical application, infrastructure, and the effects of land-use change. The environmental profile was calculated using ReCiPe v1.07 (H) characterization factors (HUIJBREGTS et al. 2017), considering impact categories such as Global Warming (GW), Marine Eutrophication (ME), Freshwater Eutrophication (FE), Terrestrial Acidification (TA), Terrestrial Ecotoxicity (TEX) and Water Consumption (WC). The functional unit was defined as 1 kg of dried hop cones (10% wet content).

Preliminary results show that low yields in organic hop production are responsible for a higher carbon footprint compared to conventional fields. Main sources of emissions are fieldwork, fertilizers and drying process. However, water consumption and impacts associated with eutrophication and acidification are considerably lower compared to conventional production.

The environemntal sustainability of organic hop systems hinges on both input types and production efficiency. Emissions from fieldwork and fertilizers highlight the need for innovation and optimized resource use. Enhancing yields, through resilient varieties or agroecological improvements, could strengthen the environmental benefits of organic production without increasing its carbon burden.

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HUIJBREGTS M.A.J., STEINMANN Z.J.N., ELSHOUT P.M.F., STAM G., VERONES F., VIEIRA M., ZIJP M., HOLLANDER A. & VAN ZELM R. 2017. ReCiPe2016: a harmonised life cycle impact assessment method at midpoint and endpoint level. *The International Journal of Life Cycle Assessment* 22: 138-147

Study of drought-related gene expression in drought-stressed *in vitro* hop plants

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Abstract

Climate change and drought causes made big fluctuations in yield and content of alpha acids in hops during last years. One of the ways to overcome drought issues is developing drought-tolerant varieties that can withstand water scarcity better together with high yield and stability of quantitative and qualitative parameters. For effective drought tolerance breeding is necessary to understand the gene regulatory mechanisms of response to drought stress. In this study, we simulated osmotic stress using different concentrations of sucrose in cultivation media for in vitro plants of two hop varieties. During the first three days of cultivation on media, we evaluated the expression levels of 32 genes involved in drought tolerance to find differences in their expression profiles. These experiments allow to identify potential drought tolerance genes which can be used as molecular markers for breeding.

This work was supported by the National Agency for Agricultural Research of the Ministry of Agriculture of CR in project QL24010353.

Hop garden experiments with an electromagnetic pulse field (EMF)

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Abstract

The aim of the project was a system for streamlining plant production and microbiological processes through an electromagnetic pulse field (EMF) which is patented by the Agency for Intellectual Property of the Republic of Uzbekistan (IAP 05796 of 2019). Due to this system, it was expected to achieve an increase in yields of plant production. High trellises commonly used in hop fields can be used as conductors of electromagnetic fields. The subject of interest on Saaz hop variety was standard monitoring of growth, evaluation of yield, alpha acids and nitrate contents and pesticide screening of hop cones.

The transmitters had been operating since beginning of June flowering until harvest (2021–2023). Based on the results from previous years, the exposure time of EMF was adjusted each year. Hop cultivation and protection were carried out the same way as control variant, the experimental plots were influenced only by the transmitters. Three-years long observation did not show any significant effect on the quantitative and qualitative indicators of Saaz hops.

Acknowledgement

This study was supported by TAČR, FW03010433 System for streamlining plant production and microbiological processes (2021–2023). HRI provided the contract research within the framework of Institutional Support of Ministry of Agriculture of CR.

Combined effect of rooting medium and biostimulant application on morphological traits of micropropagated hop plantlets

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Abstract

Starting hop plantation with uniform and disease-free plants is the first step towards a good management of the crop. Recently, studies have been conducted to develop efficient micropropagation protocols for hops (CLAPA & HÂRȚA 2021; IACUZZI et al. 2023). Conversely, the post-propagation phase is a neglected topic, especially in new growing areas. The major challenge is to obtain a well-rooted plant without excessively boosting the vegetative growth. New substrates (alternative to peat) and treatments are needed to overcome that issue and to increase the sustainability of the process. The aims of our study were i) to screen two different micropropagated hop cultivars for their response to *ex-vitro* conditions; ii) to assess the effect of biostimulant application on shoot and root development of hop plantlets after the *in-vitro* propagation period; iii) to evaluate the possibility to partially replace peat as growing substrate for hops.

This study was conducted at the University of Tuscia (Viterbo, Italy) in spring 2025. Micropropagated plants of cvs Cascade and Nugget were used to assess the combined effect of growing media and biostimulant application on shoot and root development of hop plantlets. Extracts of the seaweed *Codium fragile* and *Opuntia ficus-barbarica* were used as biostimulant agents and sprayed as soon as the plantlets were moved to a glasshouse for the *extra-vitro* phase. Woodchip compost, replacing 30 % volume of the standard substrate (peat and coconut coir), was tested as an alternative to peat-based growing media. The experiment was set up in a completely randomized design with three replicates. Survival rate, plant height, number of leaves per plant, shoot and root dry biomass were determined. Additionally, root traits were measured by using WinRHIZO™ scanning and software. All data were subjected to three-way ANOVA and means were separated using Tukey test at 95 % probability level.

One month after imposing *ex-vitro* conditions, Nugget hops showed a more developed root system than Cascade hops (+45 % in length and dry weight, P <0.01), while no significant differences were detected in shoot system and survival rate between cultivars. Biostimulant treatment was able to significantly increase both root and shoot system of hop plantlets, while no statistical differences to control were found in the survival rate (P = 0.098). In more detail, biostimulant application enhanced root length and weight by 42 and 67 %, respectively; plant height, dry weight and the number of leaves by 28, 55, and 21 %, respectively. As for growing media, no significant effect was revealed in the majority of the analyzed plant traits. Results from this study can help nursery managers in obtaining the ideal hop plantlet by using sustainable materials and treatments.

This research was conducted as activity of the PhD course in “Plant and Animal Production Sciences (XL cycle)” of the University of Tuscia.

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Bitter acid synthesis in aroma and bitter hop under reduced nitrogen fertilisation

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Abstract

Hop (*Humulus lupulus* L.) is a species grown mainly for the brewing industry due to bitter acids accumulated in cones. These compounds are formed in metabolic pathways involving numerous enzymes. The activity of the genes encoding them determines the level of the synthesis. Gene expression depends on the genotype, organ and developmental stage of the plant and may fluctuate under environmental factors.

Two Polish hop cultivars, Lubelski (aroma) and Magnat (bitter), were grown in the field under fertilisation with a standard nitrogen dose (SN) and a dose reduced by 25–30 % (RN). The cones were collected at three growing phases. The relative expression of genes BCAT1, VPS, HS1, and HS2, PT1 and PT2 was determined. The concentration of alpha and beta acids was determined by HPLC in lyophilised cones.

The reduction of nitrogen fertilisation caused only minor disturbance in gene expression and in the content of bitter acids. Most differences depended on cultivar and the maturity of cones. Therefore, reducing nitrogen dose by approx. 25 % can be recommended for Lubelski and Magnat cultivars as it does not adversely affect the quality of the hops and at the same time protects the environment from pollution by excess fertiliser.

Acknowledgement

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Hop breeding in Poland – recent achievements

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Abstract

Hop growing has a long tradition in Poland, but the breeding program began in 1949. Since the beginning of the program, more than a dozen of aroma and bitter cultivars have been released. Breeding is aimed at cultivars with high and stable alpha acid content, noble aroma and high yields as well as enhanced disease resistance.

In recent years, eight perspective genotypes have been advanced to registration trials. Field experiment was established in 2021. The new genotypes were evaluated in relation to the reference cultivars Lubelski (LUB) as aroma cv. and Hallertauer Magnum (HMG) as bitter cv. They were assessed for morphological traits, yield, bitter acids and xanthohumol content, content and composition of hop oils as well as stability of alpha acid content during storage. Based on the results, the best genotypes will be selected for registration as new hop cultivars.

New hop genotypes are characterized with good agronomic properties. The average yield in 2023 and 2024 ranged from 1560 to 3150 kg·ha^{-1}, with the reference cvs LUB and HMG reaching 1640 and 2400 kg·ha^{-1}, respectively. The aroma genotypes had a higher alpha acid content than the reference cv. LUB, while the bitter genotypes showed a similar content of these compounds as cv. HMG. The highest yield expressed in kg alpha acids per hectare was recorded by genotypes 10/53 and 10/49 – 313 and 294 kg·ha^{-1}, respectively. The stability of alpha acids content during cone storage was evaluated by the decrease in the content of these metabolites when cones were stored for 6 months at room temperature, as well as by the hop storage index (HSI). The new genotypes had a moderate alpha acids stability, after six months of storage HSI ranged from 0.33 to 0.47. The content of hop essential oils varied from 1.3 to 2.3 ml per100 g and they were characterized by different composition of key components.

The new hop genotypes show great diversity in chemical and agronomic traits. With their high yields and increased resistance to major diseases, they will fully meet the diverse needs of hop growers.

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Agroecological practices for the control of two-spotted spider mite in hopyards 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. Management of this pest in Argentina consists of traditional acaricides, but the appearance of resistance calls for the search for alternatives. Agroecological practices incorporating entomopathogenic fungi or specific organic molecules may have great potential and promise not to affect local populations of natural enemies. In particular, *Beauveria bassiana* as well as cinnamaldehyde (a naturally occurring organic compound derived from the Cinnamon tree) are 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 frequencies for growers. Thus, our objective was to evaluate the efficacy of two commercial products: *Beauveria bassiana* (Bb-Protect®, Andermatt) and cinnamaldehyde (Seican®, Betta) in reducing the population abundance of *T. urticae* in hopyards in El Bolsón (Patagonia, Argentina). The trial consisted of three treatments: traditional management (control), application of Bb-Protect® at 150 gr/ha (T1) and application of Seican® at 3600 cc/ha (T2), both every 30 days. The treatments were installed in commercial hopyards (cv. Cascade) with a hop density of 3 333 plants/ha. Weekly, we counted live and dead spider mites on three leaves per plant, and 20 plants per treatment.

The main results show that the application of both Bb-Protect® and Seican® significantly increased the number of dead spider mites per leaf on hop plants compared to plants under traditional management, while no significant differences were recorded in the number of live individuals. The protective effect was significant for four weeks in both agroecological practices. The second application (30 days later) served to keep populations with low abundance. While it was observed locally that the incidence of spider mites was not high in the summer of 2025, the plots treated with *B. bassiana* and cinnamaldehyde had significantly lower pest abundance.

We conclude that the use of *B. bassiana* and cinnamaldehyde in hopyards in El Bolsón (Patagonia, Argentina) was effective in increasing the number of dead spider mites per leaf. The effect was maintained for at least four weeks, enabling the use of monthly applications. Moreover, there were no significant differences between both agroecological practices.

Molecular markers for sex determination in early stages of the hop breeding process

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Abstract

Hop (*Humulus lupulus* L.) is a dioecious crop used in brewing and the food industry. Due to the time-consuming nature of phenotypic selection in breeding, genome-based methods are needed. Additionally, since only female plants produce cones, it is crucial to select them early in the breeding process. To detect genome-based markers, a diverse set of 190 international varieties, wild hops and breeding lines was genotyped with genotyping by sequencing (GBS). The SNP (single nucleotide polymorphism) markers obtained were used for a genome-wide association study based on the sex-phenotype. Significantly associated markers were analyzed in two validation sets. At the same time, previously published markers were tested in the diversity set and the two validation sets. The study identified two markers in the sex-determining region of the X and Y chromosomes, which, when combined, reliably predicted gender in all evaluated genotypes. This progress will help to increase the intensity of selection and enable marker-based selection for sex within a few weeks after germination.

Hop is a dioecious crop, which is not only used for brewing, but also in the food and pharma industry. Since phenotypic selection is time-consuming in the breeding process, there is need for genome-based selection methods. Furthermore, only female plants have cones, and it is important to be able to select them in early stages of the breeding process.

KASP-markers for sex determination were developed based on GBS genotyping and association mapping in a diverse set of 190 international varieties, wild hops and breeding lines and literature research. For validation, 15 promising markers were tested in 25 genotypes with known gender. Two markers predicted the sex correctly in the first validation set and where further tested in a second validation set comprising 90 breeding strains.

We were able to identify two makers located in the sex determining region of the X and Y chromosomes. The combination of the two markers was able to reliably predict the gender in in the breeding material from Hüll.

The two validated KASP markers are now compatible with high-throughput DNA extraction and were used in the breeding program in 2024. This increases the selection intensity (for female hops) and thus the selection success / generation.

Acknowledgement

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Practices for humus formation in hop cultivation –
model and demonstration project ‘Humus formation in hop cultivation’

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The humus in agricultural soils is of great importance for soil life and soil fertility, water balance, availability of nutrients and the reduction of erosion. In addition, humus in the soil binds large quantities of carbon. Objective of the presented model and demonstration project is the implementation of innovative and long-term agronomic measures for humus preservation and formation in selected hop-growing farms throughout Germany and to scientifically monitor them. More details on the project can be found in LUTZ *et al.* (2025) in this issue. The demonstration farms have a central function as multipliers and learning centers for disseminating successful practical measures. The preservation and formation of humus in hop soils is thus intended to make an important contribution to maintaining the terrestrial carbon sink.

At the beginning of the project in 2024, the regional advisory team conducted a detailed analysis of the farms to identify the individual potential for humus preservation and formation and, together with each farm, determine the measures on three trial areas with associated control plots. One focus of the measures concerns catch crop cultivation. Various variants of catch crop sowing will be tested. For example, establishing a first catch crop in summer with a subsequent winter-hardy catch crop in autumn in no-till, or a single sowing in summer with frost-sensitive and winter-hardy components. Additionally, permanent green cover will be sown on some farms. In some cases, this will be established in every row, while in others a rotation between a standard system and permanent green cover will be implemented, with one row of permanent green cover following one row of cultivated catch crop. In terms of catch crop management, the effect of rolling the catch crop will be tested. It is assumed that this will stimulate tillering and root growth of the catch crops. Furthermore, the plant residues will be pressed on the soil, allowing soil-dwelling organisms to process them immediately. Mop Grazing with various animal species will also be tried. Planned grazing includes sheep and cattle after harvest, as well as laying hens. When incorporating the catch crops, efforts will be made to do shallow rotary tillage. Incorporation will primarily be done using a rototiller. The management of harvest residues (bine chaff) will also be examined in more detail. Various composting methods will be tested, with or without other compost components. The introduction of non-local and non-farm Corg is not to be a focus of the planned measures as it represents not a humus formation but an import and will only take place where it is possible according to the requirements of the ‘Fertilizer and Material Flow Balance Ordinance’. Possible options include applying mulching material to the hop rows, spreading slurry, manure or green cuttings from cooperative farms, or adding biochar to the bine chaff compost.

The project is supported by funds of the Federal Ministry of Food and Agriculture (BMEL) based on a decision of the Parliament of the Federal Republic of Germany via the Federal Office for Agriculture and Food (BLE), grant numbers 2822HUM201 and 2822HUM202.

LUTZ M., PORTNER J., ARNOLD S. & ZINNER A. 2025. Model and demonstration project ‘Humus formation in hop cultivation’. *Proceedings of the Scientific-Technical Commission of the International Hop Growers’ Convention, Spalt, Bavaria, Germany, 29 June-3 July 2025*: 89-91

HoPVen – Enhancing hop resilience to climate change: Exploring agri-photovoltaics opportunities and risks in conventional hop growing

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Abstract

Agrivoltaics and hop growing in the Hallertau

Climate change threatens hop growing in the Hallertau region with heat, drought and extreme weather conditions. To mitigate these effects and provide income diversification, the world's first agri-photovoltaic system for hops was introduced in 2023. This study investigates the effects of agrivoltaics on growth, quality, microclimate, and pest pressure of connected hops.

A 2-hectare experimental field was created comprising two hop varieties, Hallertauer Tradition and Herkules, and three conditions: no shading, 27 % and 36 % reduced solar radiation. Sensors are constantly monitoring soil moisture and environmental data. Pesticide application was adjusted to minimize spray drift onto the photovoltaic panels while ensuring crop protection.

Yield losses ranged from 10–30 %, depending on shading and variety. Alpha acid content remained stable, while hop oil content decreased with increasing shading. Pest frequency decreased slightly in 2023, but powdery mildew pressure increased in 2024. The combined land use efficiency reached 150 % (80 % hops, 70 % electricity). Future adjustments in pesticide use are needed to optimize the system.

Water and carbon footprints in indoor hops

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Abstract

Hop plants are well known to have high water requirements. However, climate change is projected to reduce water availability for irrigation by 20 % by 2050, posing a significant challenge for hop cultivation. Indoor agriculture provides a novel approach to study hop plant physiology with greater precision and control, enabling deeper insights into their requirements for optimal growth and development. Our results show that a totally controlled environment and closed irrigation system reduces water consumption by a factor of 15, compared to outdoor cultivation. On the other hand, the present study evaluates the greenhouse gas (GHG) emissions and the carbon (C) accumulation of indoor and outdoor hop cultivation. The aim of this study is to assess the net carbon balance per kilogram of hop pellets considering life cycle assessment (LCA) data and the accumulation of C in different plant parts and biomass.

Introduction

Hops are highly demanding plants in terms of water intake, with water consumption reaching up to 50 L per day on peak days, why outdoor agriculture can pressure water resources. As water for agriculture is getting scarcer due to climate change, it is essential to optimize its usage to minimize environmental impacts. In indoor farming, water footprint is usually much lower than in outdoor farming, making it one of its key sustainability advantages. Closed-loop irrigation systems, accurately controlled by many sensors, can recycle the water previously used and reduce water wastes, increasing water efficiency. Environmental control reduces water loss through evaporation and evapotranspirated water can be condensed and transferred to the irrigation system for re-use. Climate change caused by the increase in GHG has reached alarming levels, resulting in a temperature rise of 1.5 °C above pre-industrial levels (IPCC 2018). Agriculture is a significant source of GHG emissions. However, as a consequence of photosynthesis, plants act as temporary carbon sinks, transforming CO_2 to sugars. Understanding the climate impact of cultivation practices is crucial to the development of more climate-resilient agriculture.

Materials and methods

Water footprint was calculated in two different ways. In the semi-commercial scale facility of Chantada, water balances and volumes were registered using flowmeters installed in the entrance (input water) and exit (output water) pipes of the growing chamber. In Madrid's research centre, the volume of a dripper was taken as the input water volume and the volume drained from several plants was registered as the output water volume. In both ways, the water footprint of the plant was determined as the input volume minus the output volume.

Furthermore, for the estimation of the emissions of both outdoor and indoor hop cultivation systems, a directional analysis of private sector data was performed, while for the estimation of the carbon capture, the indoor hop biomass was analyzed. The biomass was separated to roots, hop cones and a mix of leaves and stems, which were dried until they reached a constant weight.

The analysis used the Dumas combustion method (Leco CHNS-932). After measuring the percentage of C content, it was converted to CO_2 using the molecular ratio $CO_2/C = 44/12$.

Results

Irrigation water use in outdoor hop growing varies between 1230 and 3458 L per plant per growing season, depending on the climate conditions and the type of soil. Indoor hops consume on average 185 L per plant per growing cycle, resulting in a reduction of 84.95 to 94.65 % of the water usage. Concerning the carbon footprint, the GHG emissions of outdoor hop cultivation were estimated to reach 3.7 kg CO_2e per kg of dried cones, whereas the emissions of indoor hop cultivation were 1.6 kg CO_2e, or in other words 2.31 times less. In more detail, outdoor hop cultivation emissions were driven on a big part by primary processing (29.9 %), fertilizers (21.9 %), field operations (13.9 %) and energy use (24 %) while indoor cultivation benefited from the control of the emissions and the use of renewable energy. Regarding e carbon capture, while the outdoor cultivation was able to capture 0.5 kg CO_2/kg of dried cones, indoor cultivation captured 1.5 kg of CO_2/kg of dried cones, i.e. three times more due to the possibility of having three harvests per year instead of one as in outdoor cultivation. As a result, the net CO_2 balance was 3.2 kg CO_2e for outdoor cultivation and 0.1 kg CO_2e for indoor hop cultivation, which is 32 times less. In respect to CO_2 capture, biomass data showed that roots, dried cones and the rest of the aerial parts contributed to a capture of 0.779, 0.729, and 1.191 kg CO_2 respectively, reaching a total of 2.699 kg CO_2 captured per plant per cycle. Summing the three harvests per year this number transforms to 8.097 kg of CO_2 captured per plant per year.

Discussion and conclusion

Optimizing irrigation frequency and system operation may lead to a much-reduced water usage. The closed irrigation system used in Ekonoke plus the capacity of recovering part of the evapotranspirated water makes the growing system very efficient in terms of water use. Compared to conventionally grown outdoor hops, its water footprint may reach a zero net water usage in future. Furthermore, Ekonoke is already studying how grey water from beer production wastes can be filtered and purified to be used as new irrigation water. Due to the low environmental impact of hop cultivation on the brewing industry, compared to other practices, little to no information exists concerning its emissions. This study sheds light on how the indoor cultivation of hops works as a significant carbon sink, significantly reducing the emissions of hop cultivation due to the important CO_2 capture and the reduced GHG emissions, securing climate-resilient hop cultivation.

Due to the low input of fertilizers, elimination of pesticide use, renewable energy use, zero-kilometer emissions, and reduced storage needs, indoor hop cultivation exhibits a significantly reduced carbon footprint and CO_2 capture compared to conventional cultivation systems. Therefore, such practices have a significant contribution to mitigation strategies in agriculture and later to the brewing industry.

IPCC [UN Intergovernmental Panel on Climate Change]. 2018. Global Warming of 1.5°C. IPPC Special Report 15. URL (15.v.2025): https://www.ipcc.ch/sr15/

Optimizing hop waste composting: the role of additives, particle size, and temperature control in enhancing compost quality and reducing leachate

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Abstract

Composting is an aerobic biological process that transforms organic waste into stable, humus-like compost, mimicking natural decomposition but accelerating it through enhanced microbial activity. In this study, hop waste composting piles (stems and leaves together) were covered with a semipermeable membrane after the thermophilic phase until spring, which reduced leachate quantity and nutrient leached; minimal leachate and low nutrient quantities leached were observed during winter and spring. All treatments produced compost without a bad smell, had a total nitrogen content greater than 2 %, which show mature (the C:N ratio was <20) and stable organic fertilizer, and the biomass hygienisation threshold was reached.

However, there were some significant differences among them. The research indicated that when adding additives like biochar and effective microorganisms to the composting biomass, along with particle sizes of 2–5 cm, piles should also be covered during the first two months and as well as during the thermophilic phase. The control pile with no additives and particles sized 2–10 cm showed the best results, with significantly lower leachate and nutrient loss, and higher final compost quality, including nutrient content and germination index.

Proper mixing based on temperature measurements during the thermophilic phase was critical; improperly mixed piles had much lower nutrient content and Corg, significantly the lowest CFU of bacteria and fungi, and significantly the lowest parameters of the growth test.

Comparison of different methods for DNA extraction from hop cones

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Abstract

The economic value of hop cones varies significantly depending on the variety and the content of alpha- and beta-acids, which opens the door to the risk of adulteration. To ensure varietal authenticity, we previously optimized a microsatellite-based genotyping method that performs reliably on hop leaf tissue. However, its application to hop cones has proven less effective when detecting low percentages of added varieties in mixtures, probably due to the interference caused by PCR inhibitors such as polyphenols and bitter acids, which are particularly abundant in certain varieties (*e.g.*, Styrian Wolf).

To address this challenge, we evaluated DNA extraction protocols for their effectiveness on both hop cones and processed hop pellets, sourced from multiple genotypes with varying levels of inhibitory compounds. Pre-treatment with hexane was tested to reduce bitter acid content prior to DNA isolation. Three extraction methods were compared and assessed using NanoDrop spectrophotometry (purity), Qubit fluorometry (concentration), and agarose gel electrophoresis (integrity). Furthermore, the extracted DNA samples will be subjected to microsatellite genotyping to evaluate PCR amplifiability and residual inhibition.

This study aims to identify a robust extraction protocol that enables reliable DNA-based varietal identification directly from hop cones and pellets, supporting quality control and authenticity verification in the hop supply chain.

Variability of hop-derived aromatic compounds from different varieties cultivated in various regions of France

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Abstract

Hops *Humulus lupulus* L. are a pivotal ingredient in beer production, significantly contributing to its aromatic profile through volatile compounds. Cultivated across diverse regions globally, hop varieties exhibit unique characteristics influenced by their growing environments. This study explores how different growing regions impact the aromatic and bitter compounds derived from hops.

Volatile compounds in cones from three hop varieties – Aramis, Barbe Rouge, and Cascade – grown by nine producers across ten departments in France were analyzed using solid-phase microextraction coupled with gas chromatography–mass spectrometry and sulfur chemiluminescence detector (SPME-GC/MS/SCD). Alpha acids, which isomerize into iso-alpha acids responsible for beer bitterness, were quantified using Ultra Performance Liquid Chromatography coupled with Ultraviolet detection (UPLC-UV).

The study identified 150 volatile compounds per hop variety. Statistical analyses, including ANOVA tests, revealed the significant effect of terroir on these detected volatile compounds within each variety. Principal Component Analysis indicated that varietal differences have a more substantial impact on the aromatic profile than terroir effects, suggesting greater aromatic variation between varieties than among different growing regions.

Key findings include higher concentrations of geraniol, characterized by its rose and floral notes, in Cascade hops compared to Barbe Rouge and Aramis, irrespective of terroir. Sulfurous compounds like 4-mercapto-4-methyl-2-pentanone (4-MMP), associated with “cat urine” and “black currant” aromas, were more prevalent in Barbe Rouge, particularly in Bas-Rhin (67). Alpha acid quantification showed elevated levels of humulone and cohumulone in Aramis and Barbe Rouge, notably in Loiret (45) (17.87 % ± 0.82 w/w and 17.82 % ± 1.22 w/w, respectively) and in Calvados (14) (17.31 % ± 1.18 w/w for Barbe Rouge).

This research demonstrates that, while the growing region influences the aromatic profile of hop varieties, the variety itself exerts a more significant impact. These insights are crucial for brewers aiming to fine-tune flavor profiles in their beers.

Phytocenotic arrangement of *Humulus lupulus* in the south of Western Siberia

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Abstract

Mobilization and conservation of genetic diversity of cultivated plants and their wild relatives is of strategic importance for the development of new varieties and ensuring sustainable crop production. To create new high-yielding cultivars of hops and to select agricultural technologies for various natural zones of Russia, it is necessary to study the natural characteristics of the species, as well as its ecotopologic and phytocenotic arrangement in the south of Western Siberia, where its significant genetic resources are concentrated.

Plant explorations that resulted in 400 herbarium sheets and 275 geobotanical descriptions were conducted in 2023–2024 in the Altai and Krasnoyarsk territories, Omsk, Kemerovo, Novosibirsk and Tyumen provinces, and the Altay and Khakassia Republics. The search for common hop thickets in hard-to-reach and remote locations was made with a DJI Mavic 3 Multispectral unmanned aircraft. Primary classification and ordination of plant communities took into account the composition, projective coverage, and permanence of plant species. Over 900 hop growth localities, both under cultivated and natural conditions, were marked on the digitized map of vegetation for the south of Western Siberia. Based on the ideas of the European Nature Information System (EUNIS), we have developed a variant of the classification of habitats (ecotopes) of hops, based on the results of field work.

Our research has shown that the range of common hops in the south of Western Siberia is between 50° and 59° N. Its southern border runs through the lowland areas of Altai and Salair, and rarely rises to the mid-mountains. In the conditions of the Baraba Lowland and the Kulunda Plain, hops are observed only in populated areas and in places of former settlements, since its distribution is undoubtedly limited by the saline soils common here. Over the southwest of Siberia, hop presently occurs within various phytocenoses situated in the steppe and forest-steppe zones, and the southern taiga subzone of the forest zone, from low to medium mountains. Hop has a significant range of habitats, from undisturbed forest phytocenoses to urbanized areas. *Humulus lupulus* is most common, abundant and diverse in morphological characteristics in natural conditions in the eastern part of the south of Western Siberia – in the forest communities of Salair, north-eastern Altai, and floodplains of the Ob, Chumysh, Irtysh rivers and their tributaries. The cultivated part of its range was formed during Siberia's colonization (XVII–XX centuries). There are 16 natural and 13 anthropogenic ecotopes where hop can be found. In the plant communities that incorporated common hop plants, 62 species of trees and shrubs were observed to serve as supports, the active core of the cenoflora consisted of 50 plant species, and 782 higher plant species were recorded in total. A local cenotic feature of hops has been established: high occurrence in various shrub communities, most often in thickets of mesophilic shrubs of *Rosa* and *Spiraea*, *Lonicera tatarica*, *Caragana arborescens*, and *Viburnum opulus*, where the projective cover of hop populations is 50–100 %.

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