ISHS Section on Medicinal and Aromatic Plants in conjuction with ISHS Commission on Plant Genetic Resources and ISHS Commission on Fruits and Vegetables and Health presents

III International *Humulus* Symposium

Book of Abstracts

Program · Abstracts · Information

9th – 14th September 2012, Hop Research Institute Co.Ltd., Zatec, Czech Republic

Welcome

The Organizing Committee has the great pleasure of welcoming you in the **3rd International Society for Horticultural Science (ISHS) International *Humulus* Symposium,** held **9-14th September 2012** in the historic city of **Zatec (Saaz), Czech Republic**. The Symposium is supported by the **ISHS Section on Medicinal and Aromatic Plants**, the I**SHS Commission on Plant Genetic Resources** and the **ISHS Commission on Fruits and Vegetables and Health**, in collaboration with the **Hop Research Institute Co.Ltd. Zatec, Czech Republic** and **The University of Tasmania, Hobart, Tasmania, Australia**. The Symposium is held at the conference facilities of the **Hop Research Institute**.

The aim of the *Humulus* Symposium is to explore the progress being made on various aspects of hop research. The program includes lectures by **invited speakers**, **oral presentations**, and **poster sessions**. The benefits of attending the *Humulus* Symposium include an abundance of opportunities to **network**, **learn**, **build relationships**, and hear first-hand **the latest hop science** and related applications. It is a great chance for everyone to connect with colleagues and discuss the latest information. As the *Humulus* Symposium is occurring in the hop **harvest season in the Saaz hop region**, **field trips and excursions to harvesting and processing facilities** are organized for the participants. Furthermore, we visit the **Saaz Hop Museum, Hop and Beer Temple**.

We hope that participants will enjoy the time spending on Symposium and in Zatec. Above all we wish you a stimulating, productive and successful Symposium and good memories of Zatec.

conveners
Dr. Josef Patzak
A/Prof. Dr. Anthony Koutoulis

Scientific committee

Dr. Ron Beatson	HortResearch, Nelson Regional Centre, Motueka, New Zealand
Dr. Karel Krofta, Dr. Josef Patzak, Dr. Josef Vostrel	Hop Research Institute, Zatec, Czech Republic
Doc. Dr. Pavel Dostalek	Institute of Chemical Technology Prague, Prague, Czech Republic
Dr. Jaroslav Matousek	Biological Centre ASCR, Ceske Budejovice, Czech Republic
Dr. Peter Darby	Wye Hops Ltd., Canterbury, Kent, UK
Dr. Andreja Cerenak Dr. Sebastijan Radisek	Slovenian Institute of Hop Research and Brewing, Department for Plants, Soil and Environment, Žalec, Slovenia
Prof. Dr. Branka Javornik	University of Ljubljana, Biotechnical Faculty, Ljubljana, Slovenia
Dr. David Gent	US Department of Agriculture-Agricultural Research Service, Department of Botany and Plant Pathology, Oregon State University, Corvallis, Oregon, USA
Dr. John Henning	National Forage Seed Production Research Center, Department of Crop and Soil Science, Oregon State University, Corvallis, Oregon, USA
Dr. Barbara Reed	Agricultural Research Service, National Clonal Germplasm Repository, USDA, Oregon, USA
Dr. Paul Matthews	Steiner Hops Ltd., S.S.Steiner, New York, USA
Dr. Stephen Kenny	Department of Crop and Soil Science, Washington State University, Prosser, Washington, USA
Dr. Ken Eastwell	IAREC, Washington State University, Prosser, Washington, USA
Prof. Dr. Anthony Koutoulis Dr. Simon Whittock	School of Plant Science, The University of Tasmania, Hobart, Australia
Dr. Sarah Pethybridge	Botanical Resources Australia – Agricultural Services Pty. Ltd., Ulverstone, Australia
Dr. Elisabeth Seigner, Dr. Stefan Seefelder	Bayerische Landesanstalt für Landwirtschaft, Institut für Pflanzenbau und Pflanzenzuchtung, Freising, Germany
Prof. Dr. Gerd Weber	Department of Plant Breeding and Biotechnology, University of Hohenheim, Stuttgart, Germany
Prof. Dr. Jonathan Page	Department of Biology, University of Saskatchewan, Canada
Dr. Arne Heyerick	Ghent University, Faculty of Pharmaceutical Sciences, Ghent, Belgium
Dr. Ana M. Fortes	Plant Molecular Biology & Biotechnology Unit, ICAT, FCUL, University of Lisbon, Lisbon, Portugal
Prof. Dr. Jean-Marc Jeltsch	Université de Strasbourg 1, Ecole Superieure de Biotechnologie Strasbourg, Illkirch Graffenstaden, France

Local organizing commitee

J. Patzak, Hop Research Institute, Zatec, CR
J. Vostrel, Hop Research Institute, Zatec, CR
K. Krofta, Hop Research Institute, Zatec, CR

Conveners

J. Patzak, Hop Research Institute, Zatec, CR
A. Koutoulis, University of Tasmania, Hobart, Australia

Symposium venue

The main building of **Hop Research Institute Co.Ltd.** Zatec, Czech Republic

Dates

9th September (Sunday) **-14th September** (Friday) **2012**

Official language

The official language of the *Humulus* Symposium is **English**.

Opening of the registration desk

Sunday 9 September 2012	18:00-21:00
Monday 10 September 2012	8:00-9:00
Tuesday 11 September 2012	8:00-9:00
Wednesday 12 September 2012	8:00-9:00
Thursday 13 September 2012	8:00-9:00

Conference secretariat

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Hop Research Institute, Co.Ltd.
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Website: http://www.chizatec.cz/ishs-2012/
http://www.chizatec.cz/en/ishs-2012/

Registration

On-line registration has started at 1st January 2012 on Symposium web page.

Registration Fees	before 15th May 2012	after 15ht May 2012
ISHS-members	450,-€	500,-€
Non-ISHS-members	500,-€	550,-€
Students	225,-€	275,-€
One-day participants	150,-€	200,-€
Accompayning persons	225,-€	275,-€

For ISHS and Non-ISHS Members:

The registration fee includes participation in all scientific meetings, coffee and tea breaks during the meeting, lunches, excursions, symposium bag with book of abstracts, regional information and name badge, final program, and a copy of the Symposium Proceedings issued by ISHS (as a volume of *Acta Horticulturae*).

For Students:

The registration fee includes participation in all scientific meetings, coffee and tea breaks during the meeting, lunches, receptions, and symposium bag with book of abstracts, regional information, name badge, and final program.

For One-day Participants:

The registration fee includes participation in all scientific meetings, coffee and tea breaks during the day, lunch.

Lectures

The date and time of the lectures are included in the detailed scientific program. Oral presentations will be presented on computer dataprojector using PowerPoint electronic files in PowerPoint2000 or later versions. Since unexpected errors may occur during the final presentation, a computer support will be provided at the beginning of the presentation day to check the compatibility.

Posters

Poster numbers, included in the detailed scientific program, will be displayed with large printed figures on poster boards. Posters should have a maximum size of about 100 cm x 120 cm (40 inches x 48 inches), portrait. Fastenings will be provided by the organizers. Poster presenters are kindly requested to be in front of their posters during the Poster sessions.

Acta Horticulturae manuscripts

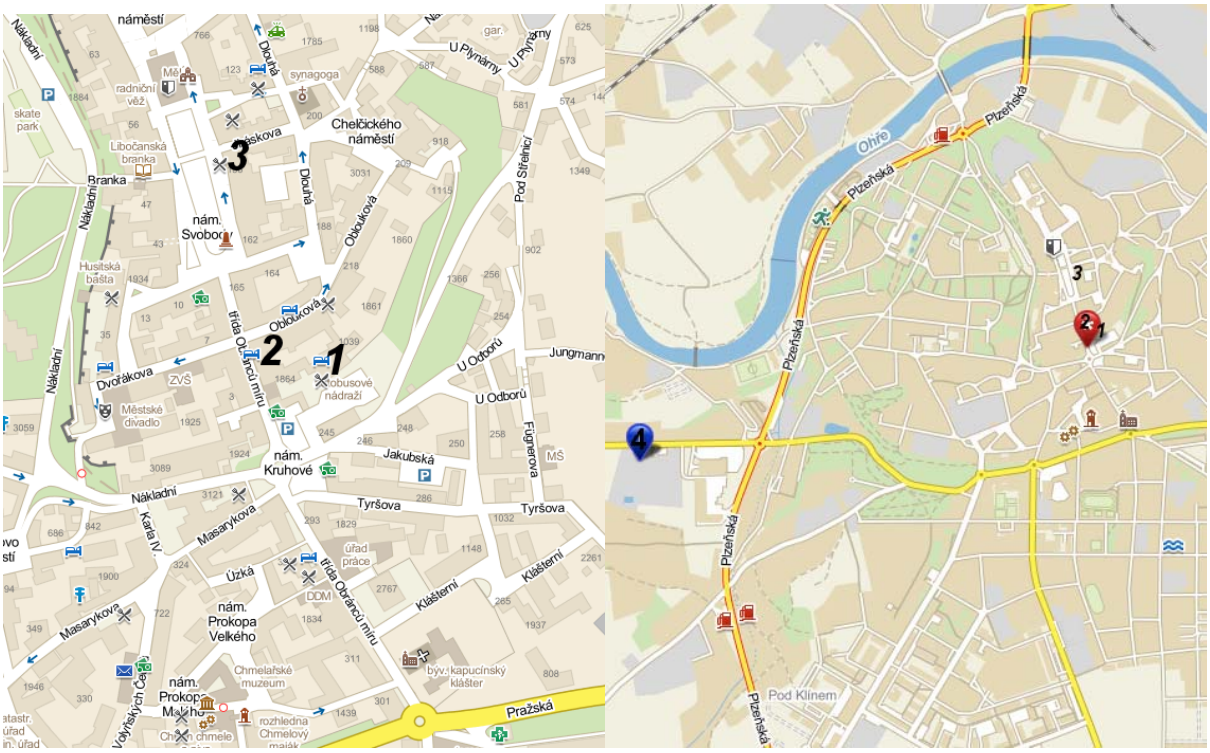
The proceedings of the III *Humulus* Symposium will be published by ISHS as an **Acta Horticulturae** volume. The willing authors are asked to submit full manuscripts during the conference for peer-review by the Symposium Scientific Committee. Reviewed manuscripts will be published post-conference by the ISHS in a volume of Acta Horticulturae. For publication guidelines in Acta Horticulturae, please follow the Authors Instructions available at http://www.actahort.org/. Each participant paying the registration fee (except guests and students) will receive a copy of the Acta Horticulturae, the Symposium Proceedings.

Internet

In the main building of **Hop Research Institute Co.Ltd.** Zatec, Czech Republic are possibility to use wi-fi connection to CHI_AULA1 and CHI_AULA2 nets by passwords **aula1** and **aula2**, respectively. For participants without computer are possibility to use computer facilities of organizers from HRI.

Accomodation and travel Information

Three hotels located in the Zatec city centre have reserved a number of rooms for participants of the ISHS *Humulus* 2012 Symposium. As a historical and touristic city, Zatec has also another accommodation options for all types of budget. Please note that the conference venue is located outside the city centre. Transport between the city centre hotels and the conference site will be organized. Every morning, bus will be place in front of hotel Cerny orel with departure time at 8:30.



1 – Hotel Cerny orel, ***2*** – Hotel Zlaty Lev, ***3*** – Hotel U Hada, **4** – Hop Research Institute

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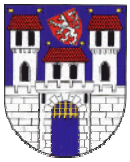

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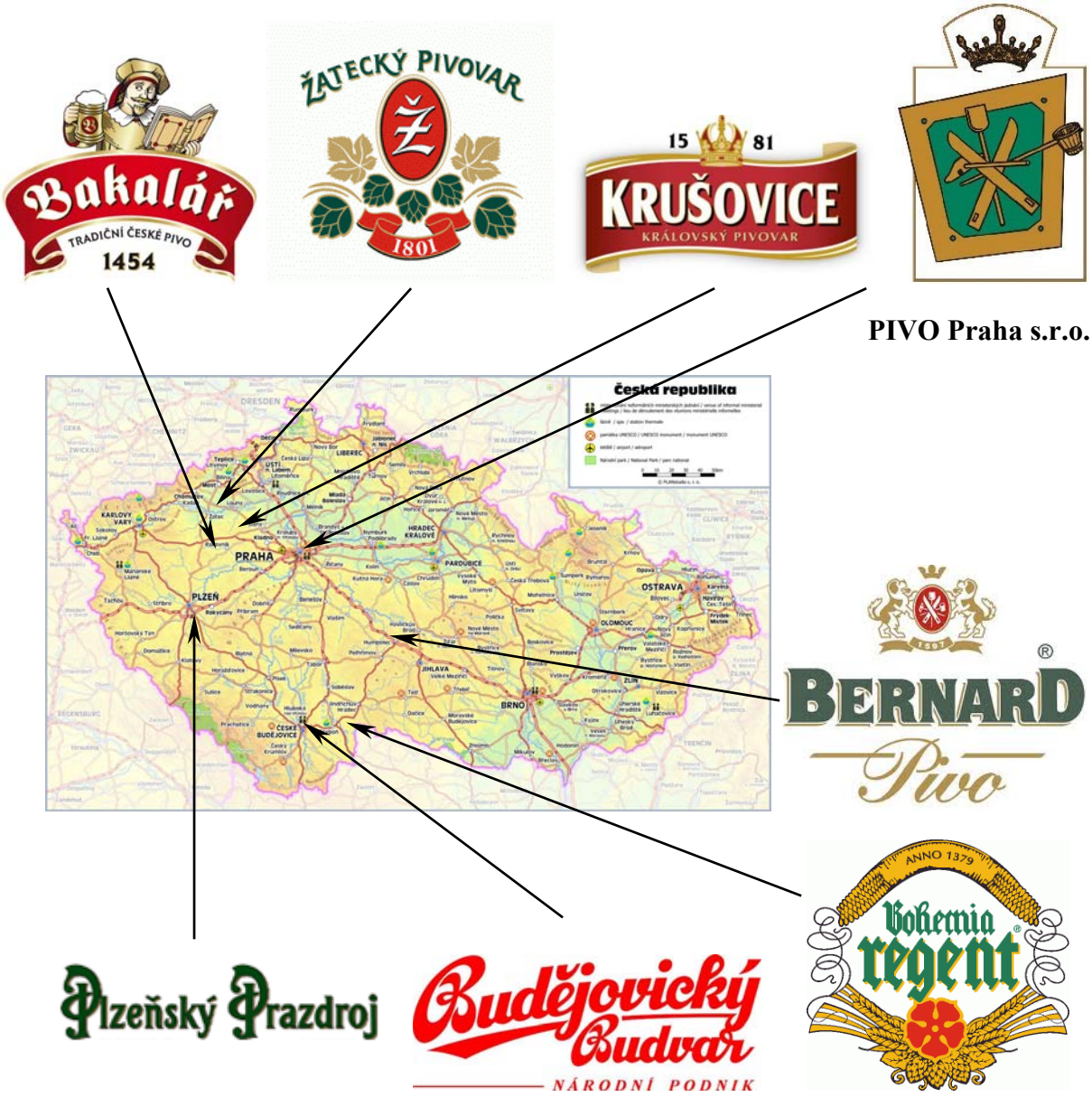
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Symposium program and abstract content

Sunday 9 September 2012

Day of Arrival - transport from Airport and hotels will be arranged
18:00-21:00 Registration
(Light refreshments, coffee, soft drinks and beer will be served)

Monday 10 September 2012

8:00-9:00 Registration
9:00-9:30 Official Symposium opening

1.Hop Breeding and Molecular Biology

Lecture's session 1: chairman A/Prof. Anthony Koutoulis, Australia

9:30-10:00 Dr. Simon Whittock – invited speaker
Hop Products Australia, Bellerive, Tasmania, Australia
„Technology applied to the development of new flavour hop varieties in Australia"

10:00-10:30 Dr. Ron Beatson
The New Zealand Institute for Plant & Food Research Limited (PFR), Palmerston North, New Zealand
„Genetic Progress in Breeding Triploid Hops for New Zealand Growing Conditions"

10:30-11:00 Coffee break

11:00-11:30 Dr. John Henning – invited speaker
National Forage Seed Production Research Center, Department of Crop and Soil Science, Oregon State University, Corvallis, Oregon, USA
„Overview of the USDA-ARS Hop Molecular Breeding Program"

11:30-12:00 Dr. Erin Howard
School of Plant Science, University of Tasmania, Hobart, Australia
„QTL analysis reveals complex genetic control underlying hop secondary metabolites in a New Zealand mapping population."

12:00-12:30 Dr. Emily Buck
The New Zealand Institute for Plant & Food Research Limited (PFR), Palmerston North, New Zealand
„Dissecting the Genetic Architecture of Hops Flavour and Aroma using a New Zealand Mapping Population."

12:30-13:30 Lunch

Lecture's session 2: chairman Dr. Josef Patzak, Czech Republic

13:30-14:15 Dr. Jaroslav Matousek – invited speaker
Biological Centre AS CR, Institute of Plant Molecular Biology, Ceske Budejovice, Czech Republic
„Molecular background putatively involved in regulation of lupulin gland metabolome - results and prospects"

14:15-14:45 MSc. Andrés Gatica-Arias
Plant Breeding and Biotechnology, Institute for Plant Breeding, Seed Science and Population Genetics, University of Hohenheim, Stuttgart, Germany
„Transgenic hop (Humulus lupulus L.) over-expressing the homologous transcription factor HlMyb3 and consequences for gene expression in the biosynthesis of flavonoids"

14:45-15:15 Dr. Katharina Häntzschel

Plant Breeding and Biotechnology, Institute of Plant Breeding, Seed Science and Population Genetics, University of Hohenheim, Stuttgart, Germany
„Biosynthesis of flavonoids and phloroglucinols: Gene expression during hop flower development.“
15:15-15:45 Dr. Gennady Karlov
Centre for Molecular Biotechnology, Russian State Agrarian University – Moscow Timiryazev Agricultural Academy, Moscow, Russia
„Application of molecular cytogenetics for the studies of genome organisation in *Humulus lupulus* and *Humulus japonicus*.“
15:45-16:15 Coffee break
16:15-17:00 Poster session I (Sections 1+2+3)

19:00-0:00 Welcome reception

Tuesday 11 September 2012

2.Plant Pathology, Virology, Entomology
Lecture's session: chairman Dr. Elisabeth Seigner, Germany
9:00-9:30 Prof. Dr. Teruo Sano – invited speaker
Plant Pathology Laboratory, Faculty of Agriculture and Life Sciences, Hirosaki University, Hirosaki, Japan
„History, origin, and diversity of hop stunt and hop stunt viroid“
9:30-10:00 Dr. Sebastjan Radišek
Slovenian Institute for Hop Research and Brewing, Žalec, Slovenia
„Outbreaks and management of hop stunt disease in Slovenia“
10:00-10:30 Dr. Jernej Jakše
University of Ljubljana, Biotechnical Faculty, Agronomy Department, Ljubljana, Slovenia
„Next Generation Sequencing as a diagnostic tool for new pathogen discovery in hops“
10:30-11:00 Coffee break
11:00-11:30 Dr. Stanislav Mandelc
University of Ljubljana, Biotechnical Faculty, Ljubljana, Slovenia
„Colonization of susceptible and resistant hop plants by *Verticillium albo-atrum* and comparative proteomics of roots and xylem fluid“
11:30-12:00 Dr. Florian Weihrauch
Bavarian State Research Center for Agriculture, Institute for Crop Production and Plant Breeding, Hop Research Center, Hüll, Germany
„Simple is Beautiful: A New Biotest for the Aphid Tolerance Assessment of Different Hop Genotypes“
12:00-12:30 Dr. Josef Vostřel
Hop Research Institute, Co. Ltd., Zatec, Czech Republic
„Protection of organic Saaz hops against two-spotted spider mite (*Tetranychus urticae* Koch) with the help of released predatory mites *Typhlodromus pyri* Scheuten“
12:30-13:30 Lunch

3.Chemistry and Plant Physiology
Lecture's session: chairman Dr. Karel Krofta, Czech Republic
13:30-14:00 Dr. Martin Biendl – invited speaker
Hopsteiner HHV, Mainburg, Germany
„Isolation of prenylflavonoids from hops“
14:30-15:00 Dr. Clinton Dahlberg

KinDex Therapeutics, LLC, Seattle, Washington, USA
„Preparation of the Unnatural Derivatives of Humulus Lupulus L. (Hops)“
15:00-15:30 A/Prof. Robert Shellie
Australian Centre for Research on Separation Science (ACROSS), University of Tasmania, Hobart, Australia
„Varietal characterisation of hop (Humulus lupulus L.) using GC-MS analysis“
15:30-16:00 Coffee break
16:00-16:45 Dr. Ana M. Fortes – invited speaker
Plant Molecular Biology & Biotechnology Unit, ICAT, FCUL, University of Lisbon, Lisbon, Portugal
„Organogenic Nodule Formation in Hop: A Tool to Study Morphogenesis in Plants with Biotechnological and Medicinal Applications“
16:45-17:15 Dr. Josef Patzak
Hop Research Institute, Zatec, Czech Republic
„Endogenous phytohormone levels in dwarf and normal hop (*Humulus lupulus* L.) plants“
17:15-17:45 Dr. Vít Gloser
Masaryk University, Faculty of Science, Department of Experimental Biology, Brno, Czech Republic
„The response of Humulus lupulus to drought: contribution of structural and functional plant traits“
Poster session I (Sections 1+2+3)
18:00-19:00 Light refreshments
19:00-20:00 Children chorus in church

Wednesday 12 September 2012

4.Hop Cultivation and Management
Lecture's session: Dr. John Henning, USA
9:00-10:00 Dr. Hiroo Matsui – invited speaker
Beer Development Department, Research Center, Suntory Liquors Limited, Osaka, Japan
„The Influence of Hop Root Age on the Quality of Hop"
10:00-10:30 Dr. Karel Krofta
Hop Research Institute, Zatec, Czech Republic
"Transpiration – an important contribution to overall water balance
in hop plantation."
10:30-11:00 Coffee break
11:00-11:30 Dr. Barbara Reed – invited speaker
USDA-Agricultural Research Service, National Clonal Germplasm Repository, Corvallis, Oregon, USA
„Cryopreservation and maintance of hop material in USDA germplasm collection"
11:30-12:00 Dr. Stefano Buiatti, Dr. Luca Pretti
Department of Food Science, University of Udine, Italy
„Hop cultivation trials in some Italian regions: alfa- and beta-acid content of hop
12:00-12:30 Ing. Josef Ježek
Hop Research Institute, Zatec, Czech Republic
„Trial growing low trellis hops in Czech Republic in 2009-2011“
12:30-13:00 Dr. Ruslan Hofmann
VLB Berlin, TU Berlin and WOLF Anlagentechnik GmbH & Co. KG, Berlin, Germany
„Energy consumption and quality control during hop kilning - latest results of an ongoing research project“

13:00-14:00 Lunch
14:00-15:00 Poster session II (Sections 4+5+6)
15:00-19:00 Excursion - Hop Research Institute, harvest and post-harvest facilities, hop museum, hop and beer temple

Thursday 13 September 2012

5.Hop, Indispensable Raw Material for Brewing

Lecture session – chairman Doc. Pavel Dostálek, Czech Republic

9:00-9:30 Dr. Jessika De Clippeleer – invited speaker
KaHo St.-Lieven, Association KULeuven, Laboratory of Enzyme, Fermentation, and Brewing Technology, Ghent, Belgium
„The origin of staling aldehydes: hop versus malt“

9:30-10:00 Dr. Thomas Shellhammer
Department of Food Science and Technology, Oregon State University, Corvallis, Oregon, USA
„Towards understanding the origin of American hop aroma in beer“

10:00-10:30 Dr. Sara Jaeger, Dr. Emily Buck
The New Zealand Institute for Plant and Food Research Limited, Auckland, New Zealand
„A sensory method developed for screening of dried hop cones for specific aroma traits“

10:30-11:00 Coffee break

11:00-11:30 Dr. Patricia Aron
MillerCoors Brewing, Milwaukee, Wisconsin,USA
„Elucidation and evaluation of hop polyphenol influence on lager beer flavor and flavor stability“

11:30-12:00 Dr. Clinton Dahlberg
KinDex Therapeutics, LLC, Seattle, Washington, USA
„Beer's Bitter Structural Chirality (Solved)“

12:00-13:30 Lunch

6.Hop, Beer and Health

Lectures / Poster session

13:30-14:00 Prof. Herbert Riepl
Organic and Analytical Chemistry, Weihenstephan-Triesdorf University of Applied Sciences, Straubing, Germany
„Screening of a commercial hop extract for substances active in neural stem cell differentiation“

14:00-14:30 Doc. Pavel Dostálek
Department of Biotechnology, Institute of Chemical Technology, Prague, Czech Republic
„Effect of xanthohumol on brewing yeast cells“

14:30-15:00 Official Symposium closing
15:00-15:30 Coffee break
15:30-16:00 Poster session II (Sections 4+5+6)

18:00-0:00 Farewell party

Friday 14 September 2012

Day of Departure or Post-Conference Tour

Post-Conference-tour (additional costs of 290,-€) will be organized to visit the most beautiful and interest places of the Czech Republic: Prague, Karlovy Vary (Carlsbad), Karlstejn castle, Pilsen Urquell brewery and beer museum.

Preliminary program

Friday - 14th September

8:00 - departure from Zatec to Pilsen
9:30 - 11:10 guided tour of brewery Pilsner Urquell
11:10 -12:00 - Pilsner Urquell brewery museum
12:00 - 13:00 lunch in restaurant Na Spilce
13:00 - departure to Karlovy Vary
14:30 - 18:00 guided tour of Karlovy Vary
18:45 - 19:45 dinner in microbrewery Chýše
20:30 - arrival to Žatec

Saturday - 15th September

8:00 - departure from Zatec to Prague
9:30 - 13:00 sightseeing guided tour of Prague
13:00 - 14:00 lunch
14:00 - 15:00 transfer to Karlštejn castle
16:35 - 18:35 guided castle tour
departure to Zatec, dinner 20:00 - 21:00

Poster session I:

1.Hop Breeding and Molecular Biology

P1: Dr. Elisabeth Seigner
Bavarian State Research Center for Agriculture, Institute for Crop Science and Plant Breeding, Hop Breeding Research, Freising, Germany
„Technique for Functional Analysis of Genes Associated with Powdery Mildew Resistance in Hops“

P2: Dr. Nikola Yakovin
Centre for Molecular Biotechnology, Russian State Agrarian University – Moscow Timiryazev Agricultural Academy, Moscow, Russia
„The use of laser microdissection for the construction of *Humulus japonicus* sex chromosome specific DNA library and cytogenetic analysis“

P3: Dr. Jakub Vašek
Czech University of Life Sciences Prague, Czech Republic
„Comparative analysis of wild hop based on genetic, chemical and morphological data“

P4: Dr. Jaroslav Matousek
Biological Centre AS CR, Institute of Plant Molecular Biology, Ceske Budejovice, Czech Republic
„Complex regulation of omt1 gene, implication of hop transcription factor HlWRKY1“

P6: Dr. Tomáš Kocábek
Biological Centre AS CR, Institute of Plant Molecular Biology, Ceske Budejovice, Czech Republic
„Functional and complementation analysis of hop genes in heterologous systems“

P7, 8, 9, 10: Dr. Jernej Jakše
University of Ljubljana, Biotechnical Faculty, Agronomy Department, Jamnikarjeva 101, Ljubljana SI-1000, Slovenia
„Hop ESTs As A Source Of Resistance Gene Analogs And Their Application In Genetic Mapping“
„Elucidaing Verticillium wilt resistance in hop“
„Molecular genotyping of worldwide hop cultivars based on microsatellite loci“
„Novel hop male markers from DArT sequences“

2.Plant Pathology, Virology, Entomology

P11: Dr. Jernej Jakše
University of Ljubljana, Biotechnical Faculty, Agronomy Department, Ljubljana, Slovenia
„Differential gene expression during interaction of resistant and susceptible hop cultivars with *Verticillium albo-atrum*“
P12: Dr. Sebastjan Radišek
Slovenian Institute for Hop Research and Brewing, Žalec, Slovenia
„Evaluation of candidate reference genes for gene expression normalization in hop using real time quantitative RT-PCR“
P13: Dr. Stanislav Mandelc
University of Ljubljana, Biotechnical Faculty, Ljubljana, Slovenia
„Comparative secretome analysis of *Verticillium albo-atrum* isolates from hop“
P14: Dr. Josef Vostřel
Hop Research Institute, Co. Ltd., Zatec, Czech Republic
„Negative effect of fungicides used in practical hop protection against downy mildew (*Pseudoperonospora humuli*) on aphidophagous coccinellids *Propylea quatuordecimpunctata* L.“
P15: Zoltán Füssy
Biological Centre AS CR, Institute of Plant Molecular Biology, Ceske Budejovice, Czech Republic
„HSVd pathogenesis involves a disbalance of hop regulatory genes“
P16: Dr. Jaroslav Matousek
Biological Centre AS CR, Institute of Plant Molecular Biology, Ceske Budejovice, Czech Republic
„Biolistic transfer of Slovenian viroid disease syndrome to Czech hop Osvald‘s72-symptoms and identification of dominant sequence upon transfer of HSVd component “

3.Chemistry and Plant Physiology

P17: Christiana Sarraf
Horticulture Research Center, Faculty of Agriculture and Food Sciences, Laval University, Québec, Canada
„Agronomic and nutraceutical potential of hops (Humulus lupulus L.) grown in Quebec“
P18: Prof. Josef Pulkrábek
University of Life Sciences in Prague, FAFNR, Department of the Plant Production, Prague, Czech Republic
„Contribution to etiology of occurrence of monecious hop plants“
P19: Dr. Jaroslav Pokorný
Hop Research Institute, Zatec, Czech Republic
„Changes in the rate of photosynthesis, transpiration and plant pigments content (chlorophylmetric units) during the vegetation of hop“

Poster session II:

4.Hop Cultivation and Management

P20: Prof. Josef Pulkrábek
University of Life Sciences in Prague, FAFNR, Department of the Plant Production, Prague, Czech Republic
„The main physiological disorders of roots covered seedling in hop“

P21: Doc. Adolf Rybka
CULS Prague, Faculty of Engineering, Department of Agricultural Machines, Czech Republic
„Analysis of activity of inclined belt conveyors with different belt structure when separating impurities from hops“

P22: Samuel Turner
Department of Crop and Soil Science, Washington State University, Pullman, WA, USA
„Cover Cropping Systems for Organic Hop Production in the Yakima Valley, USA“

P23: Ing. Josef Ježek
Hop Research Institute, Zatec, Czech Republic
„Organic hop growing in Czech Republic“

P24: Dr. Petr Svoboda
Hop Research Institute, Zatec, Czech Republic
„Production and propagation of virus free hops in the Czech Republic“

5.Hop, Indispensable Raw Material for Brewing

P25: Dr. Barbara Jaskula-Goiris
Laboratory for Enzyme, Fermentation, and Brewing Technology (EFBT), KAHO Sint-Lieven Technology, Gent, Belgium
„The use of hop-derived polyphenolic extracts to improve beer flavour and flavour stability“

P26: Dr. Karel Krofta
Hop Research Institute, Zatec, Czech Republic
„Stability of hop beta acids and their decomposition products during“
natural ageing

6.Hop, Beer and Health

Section 1:

Hop Breeding and Molecular Biology

Lectures

Technology applied to the development of new flavour hop varieties in Australia

Simon Whittock[1]*, Aina Price[2], Erin Howard[2], Anthony Koutoulis[2]

[1]Hop Products Australia, 26 Cambridge Road, Bellerive, Tasmania, 7018, Australia
[2]School of Plant Science, University of Tasmania, Private Bag 55, Hobart, 7001, Tasmania, Australia
E-mail: Simon.Whittock@hops.com.au

Abstract:
Hop production in Australia occupies a total of 500 hectares, spread across two major farms and several smaller growers. Production is based in south-eastern Australia, spread roughly evenly between Tasmania and Victoria, latitudes 37-41° south. Every hop grower in Australia relies upon varieties developed locally since 1950, in a breeding program which continues today. Since 1997, collaborative work (predominantly with UTAS) has seen the development of technologies such as in vitro colchicine induction to produce tetraploid plants, which are verified via flow cytometry. This had led to the availability of both tetraploid male and female plants, and the ability to conduct tetraploid by tetraploid crosses. An extensive database of gas chromatography-mass spectrometry data has been built up, containing information on international varieties, Australian commercial varieties, and selected material. Australian hop researchers have participated actively in the international collaboration to develop diversity arrays technology (DArT) DNA markers. Future work will include quantitative genetic analysis of large progeny trials grown in both Tasmania and Victoria, the establishment of a linkage mapping population replicated in both Tasmania and Victoria, and application and further development of DArT markers in specific roles with the new cultivar development process. Active collaboration provides hop breeding efforts in Australia with the tools necessary to develop high yielding, seedless varieties with diverse and exciting flavour potential.

Keywords: Diversity Arrays Technology, flavour, new variety

References:

Genetic Progress in Breeding Triploid Hops for New Zealand Growing Conditions

Ron Beatson*, Peter Alspach

The New Zealand Institute for Plant & Food Research Limited (PFR), Private Bag 11 600, Palmerston North 4442, New Zealand
E-mail: ron.beatson@plantandfood.co.nz

Abstract:
Breeding seedless triploid hop cultivars has been the cornerstone of the New Zealand hop breeding programme for over 50 years. Initially, the programme sought to develop high alpha type cultivars. Also, the breeding of aroma type cultivars to meet industry requirements has been a goal for the past 35 years. Since its inception, the breeding programme has successfully released 16 triploid cultivars to industry. The breeding programme, using a multi-discipline approach, continues to focus on the development of both alpha and aroma types. This paper describes the breeding procedures employed and genetic progress realised in developing seedless triploid cultivars for New Zealand growing conditions.

Keywords: Humulus lupulus, plant breeding, quantitative genetics, chemistry, statistics, population improvement

References:
RA Beatson. 2005. Genetic analysis of agronomic and chemical characters in hop (*Humulus lupulus* L) ACTA HORT 668:53-58.
RA Beatson: PA Alspach, 2009. The use of empirical breeding values to improve genetic progress in hops. ACTA HORT 848: 93-100.

Overview of the USDA-ARS Hop Molecular Breeding Program.

John A. Henning

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Abstract:
Recent improvements in massively parallel sequencing technology have resulted in molecular marker applications that dramatically improve genetic characterization of important traits in minor crops such as hop (Humulus lupulus L.). While most major crops have saturated genetic maps useful for pinpointing genetic markers tightly linked to important traits, little progress has been made in developing such a map for hop. Furthermore, marker technologies such as AFLP or SSR are cumbersome and time consuming for use in genotyping large breeding populations or genetically diverse germplasm for breeding purposes. The USDA-ARS has worked in collaboration with other scientists from around the world to develop over 1400 SNP genetic markers for use in genotyping hop accessions as well as develop a super-saturated genetic map of hop for use in high precision QTL studies. It is expected that this work will provide the genetic scaffold necessary for completion of whole genome sequence of hop.

Keywords: Genetic Map, marker, QTL mapping, Sequencing, SNPs

References:

Dissecting the Genetic Architecture of Hops Flavour and Aroma using a New Zealand Mapping Population.

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Abstract:
Hops contain a wide range of secondary plant metabolites, which are the major components of flavour and aroma of beer. However, the genetic architecture of these compounds is not well understood. The New Zealand Institute of Plant & Food Research Limited is investigating these genetic components and their impacts on flavour and aroma. We have developed a genetic mapping population, designated NxY, from a cross between 'Nugget' and a male genotype, known as 'Yugoslavian 3/3M'. In 2006 a total of 500 seedling progeny from the NxY population were planted and phenotyped for a range of compounds over the following six years. In 2011 a subset of the population were assessed for intensity of citrus aroma by a trained sensory panel. Using microsatellite and DArT markers, as part of the DArT International Hops consortium, genetic map construction and QTL mapping were undertaken at the University of Tasmania, Australia. A number of QTL for key flavour and aroma components have been identified including both bittering compounds and essential oils. We present some of our findings so far, illustrating the potential for marker assisted selection and discuss their possible impacts on the development of distinct flavour and aroma combinations.

Keywords: Humulus lupulus, linkage mapping, marker assisted selection, breeding, QTL

References:

QTL analysis reveals complex genetic control underlying hop secondary metabolites in a New Zealand mapping population.

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Abstract:
Beer derives bitterness, flavour and aroma from secondary metabolites of the hop cone. There is unexplored potential for breeding hop cultivars with unique brewing characteristics driven by particular chemical profiles. Molecular selection methods can directly target specific chemical compounds within the intricate web of biosynthesis, maturation and environmental stimuli. These methods rely on the identification of molecular markers associated with trait variation. To date, the number of compounds that affect beer quality for which marker-trait associations have been identified is low, despite the large numbers of secondary metabolites produced in hop cones. This study aimed to identify molecular markers associated with numerous key hop secondary metabolites and to improve understanding of the genetic control underlying these important brewing ingredients. Marker-trait associations were identified by quantitative trait loci (QTL) analysis using linkage maps constructed from a New Zealand mapping population in which secondary metabolites, including hop acids and essential oils, were measured. We identified QTL for many of the hop secondary metabolites examined. These QTL revealed a far more complex genetic control of hop secondary metabolites than previously thought. A number of QTL identified for different compounds were found to co-locate on the linkage map: these regions of co-location affected many of the secondary metabolites examined. QTL co-location is evidence for pleiotropy or linkage, both with implications for molecular selection methods. Selection for specific secondary metabolites associated with pleiotropic/linked loci is likely to instigate adverse changes to other secondary metabolites, impeding the development of particular secondary metabolite profiles. In this study, we also identified QTL that each influenced a single secondary metabolite. Due to their specificity, these QTL offer unparalleled potential for selection of particular hop secondary metabolite profiles. Besides application to molecular selection, this study significantly advances our understanding of the genetic mechanisms underlying variation in hop secondary metabolite composition.

Keywords: Humulus lupulus L., QTL, linkage map, plant secondary metabolite, pleiotropy, linkage,

References:

Molecular background putatively involved in regulation of lupulin gland metabolome-results and prospects.

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Abstract:
Ongoing research on the field of molecular genetics of hop is based on intensive work of various model and non-model plants, where some progress has been reached especially within last fifteen years in direction of gene regulation: e.g. functional architecture of chromatin, new classes of transcription factors (TFs) and the regulatory role of small RNAs in gene silencing. In particular, the functions of micro RNAs in regulation of TFs sketch in the complicated back loops and TFs interconnections organized in networks. Genetic background co-determining levels and composition of hop metabolome in the lupulin glands, and in particular Xanthohumol (X) production includes this complex regulation. According to our recent knowledge about the biosynthetic pathway leading to X and DMX in lupulin glands, last steps of the pathway are controlled by the chalcone synthase CHS_H1, prenyltransferase and O-methyltransferase 1. Despite relative simplicity of this pathway, quantitative character of inheritance of X and also bitter acids suggests a complexity of regulatory factors controlling the biosynthesis of these metabolites of lupulin. These genetic data are in accordance with our recent results on the involvement of several key transcription factors from Myb (M), bHLH (B) and WD40 (W) families in the regulation chs_H1 (Matoušek e.a., 2012) and omt1 gene by WRKY, W1 and silencing suppressors (unpublished). We have identified lupulin specific ternary MBW complexes (Hls-M3B2W1,: M2B2W1) strongly activating chs_H1. In addition, the results show that some TFs act in binary (Hls-M3B2: M2B2: B2W: M1W1: WRKY1W1) interactions and that the complexes are quite selective for different promoters of chs-related genes. Moreover, we demonstrated the ability of some of TFs either to moderate (bZip1 and 2) or to inhibit (HlM7) transcription from chs_H1 and omt1 genes independently. New challenging task is to analyze regulatory network of lupulin-specific TFs. Supported by GACR 521/08/0740 and by NAZV QH81052.

Keywords: gene regulation: lupulin biosythesis: transcription factors: regulatory networks: Humulus lupulus L.

References:
Matoušek e.a. BMC Plant Biol.12:27, 2012

Transgenic hop (Humulus lupulus L.) over-expressing the homologous transcription factor HlMyb3 and consequences for gene expression in the biosynthesis of flavonoids

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Abstract:
In hop flavonoids as well as phloroglucinols play an important role as flavoring compounds in beer brewing. Moreover, prenylated chalcones, like xanthohumol, have received considerable attention due to their pharmacological properties. However, their concentration in wild type plants is rather low. To enhance the production in hop, it would be interesting to modify the regulation of genes involved in the biosynthesis of flavonoids. For this purpose, stem segments of hop cv. Tettnanger were genetically transformed with the Agrobacterium tumefaciens strain EHA101 harboring the homologous regulatory gene HlMyb3 under the control of the 35S promoter. The presence of the transgene was verified by PCR. Moreover, expression of HlMyb3 was confirmed by RT-PCR in transgenic plants. Using quantitative real-time PCR, it was shown that HlMyb3 was highly expressed in transgenic plants compared to wild type plants. The effect of the over-expression of the transcription factor HlMyb3 on the expression of structural flavonoid and phloroglucinol genes, like PAL, CHS_H1, CHI, F3´H, OMT1, and VPS, as well as hop regulatory genes, such as HlMyb1, HlMyb2, HlMyb7, HlbZip1, HlbZip2, HlbHLH2, and WDR1 was examined. Transgenic plants with elevated expression of flavonoid and phloroglucinol genes were identified. Metabolic engineering using genes of homologous MYB transcription factors may open the possibility for increasing the production of secondary metabolites in hop.

Keywords: Hop, HlMyb3 regulatory gene, R2R3 transcription factors, Genetic transformation, Flavonoid biosynthesis

References:

Biosynthesis of flavonoids and phloroglucinols: Gene expression during hop flower development.

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Abstract:
Hop flavonoids and phloroglucinols are important secondary metabolites for the brewing of beer as well as for medicinal uses. During female flower development these compounds are synthesized and finally accumulated in the lupuline glands of hop cones. Detailed information about the expression levels of genes with regard to the production of these substances might help in learning more about the underlying processes. Therefore, the following steps were investigated:
1. Morphological characterization of different developmental stages of hop flowers.
2. Analysis of expression rates of relevant genes in biosynthetic pathways.
In this study, seven stages of flower development were assigned to hop of the Tettnang variety. Furthermore, material was harvested from each stage for RNA extraction. Finally, quantitative real-time-PCR reactions were performed to measure gene expression levels of relevant enzymes, for instance, phenylalanine ammonia-lyase (PAL), valerophenone synthase (VPS), or chalcone synthase (CHS).
Detailed morphological and molecular data of different developmental stages of female hop flowers will be presented.

Keywords: flavonoids, phloroglucinols, flower development

References:

Application of molecular cytogenetics for the studies of genome organisation in *Humulus lupulus* and *Humulus japonicus*.

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Abstract:
Molecular cytogenetics allows observation of nuclear genome organization at the level of chromosome and has enabled us to investigate fundamental aspects of the genome structure of a dioecious species *Humulus lupulus and H.japonicus (*Japanese hop). *H. lupulus* has the same sex determination system as in Japanese hop (X/A) but it differs in chromosome number (2n=20 in both female and male plants) and in sex chromosome systems (XX/XY). *H. japonicus* (Japanese hop) has 2n=16=14+XX for females, and 2n=17=14+XY1Y2 for males. The studies were conducted by using fluorescence *in situ* hybridization (FISH) with different probes: Arabidopsis-type of telomeric repeat, species specific subtelomeric repeats, 5S and 45S rDNA, simple sequence repeats, retrotransposons. The cloned species specific subtelomeric repeats allowed us to identify the sex chromosomes and location of pseudoautosomal regions. The FISH with *Ty1-copia* and *Ty3/gypsy* retrotransposons shows dispersed distribution through *H.lupulus* and *H.japonicus* genomes. The direct strategy for isolating sequences from sex chromosomes was also applied. Sex chromosomes of *H. japonicus* were isolated from meiotic chromosome spreads of males by laser microdissection with the P.A.L.M. MicroLaser system. The results provide the potential for identifying unique or sex chromosome-specific sequence elements. For high resolution mapping the pachytene chromosomes and combing DNA technique was used. This study should significantly further our understanding of sex chromosome organization and sex determination in this two species and could contribute to general understanding of sex evolution in plant kingdom. The authors acknowledge financial support from Russian Ministry of Science and Education (grant № П1164).

Keywords: Molecular cytogenetics: genome structure: sex chromosomes

References:

Section 1:

Hop Breeding and Molecular Biology

Posters

Technique for Functional Analysis of Genes Associated with Powdery Mildew Resistance in Hops

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Abstract:
Improving resistance to powdery mildew (*Podosphaera macularis* ssp. *humuli*) is a major objective in hop breeding. This can be achieved by marker-assisted breeding or by transgenic approaches. For both techniques, it is advantageous to have DNA sequences available with proved function in the hop-powdery mildew interaction. Therefore, a transient transformation assay for the functional characterization of resistance associated genes was established as already applied in routine in barley and wheat. In this assay, single epidermal cells of detached leaves are transformed with a GUS reporter gene construct and a knockdown or overexpression construct of the gene of interest. Subsequently, leaves are inoculated with *Podosphaera macularis* spores and three days later they are stained for GUS activity and epiphytic fungal structures. Haustorium formation of powdery mildew in single transformed cells is determined by light microscopy and points to gene function. For the establishment of an efficient transient transformation assay it was crucial to determine the most suitable hop epidermal cell type, to adapt the different parameters for the transformation process and the evaluation procedure. Proof of principle was performed by the knockdown of a putative hop susceptibility gene, which led to significant reduction of haustorium formation in transformed cells.

Keywords: hop breeding, powdery mildew, resistance associated genes

References:

The use of laser microdissection for the construction of Humulus japonicus sex chromosome specific DNA library and cytogenetic analysis

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Abstract:
Dioecy is relative rare among plant species and distinguishable sex chromosomes have been reported only in several dioecious species. One of them is Humulus japonicus. The multiple sex chromosome system (XX/XY1Y2) of H. japonicus differs from other members of the family Cannabaceae, where XX/XY chromosome system is present [1,2]. At the moment we know little about the genetics of sex determination in these species. In complex plant genomes, containing widespread repetitive sequences, it is important to establish genomic resources that enable us to focus on a particular part of the genome. The direct strategy for isolating sequences from chromosomes of interest is to separate them by microdissection [3]. Sex chromosomes of H. japonicus were isolated from meiotic chromosome spreads of males by laser microdissection with the P.A.L.M. MicroLaser system. The chromosomal DNA was directly amplified by degenerate oligonucleotide primed-polymerase chain reaction (DOP-PCR) [4]. The fast fluorescence in situ hybridization (FAST-FISH) [3] with labeled chromosome-specific DOP-PCR product as a probe has shown the preferential hybridization to Y chromosomes. Also the DOP-PCR product was used to construct a short insert H. japonicus sex chromosomes specific DNA library. The length of the cloned DNA fragments ranged from 450 to 3000 bp with average 1000 bp. The 17% of randomly sequenced clones have shown significant homology to *H.lupulus* and 88% to *C.sativa*. The 44% of sequences have shown homology to plant retroelements. It was concluded that the laser microdissection is useful tool for isolating DNA of individual chromosomes, including relatively small chromosomes of H .japonicus and to construction of chromosome specific libraries for studying the sex chromosomes structure and evolution. Results provide the potential for identifying unique or sex chromosome-specific sequence elements in H. japonicus and predict a possible success in identifying sex chromosome-specific repeated and coding regions through chromosome isolation and genome complexity reduction.

Keywords: laser microdissection, Japanese hop, sex chromosomes, FISH, DNA library

References:
1. Winge O (1929) On the nature of sex chromosome in Humulus. Hereditas 12: 53–63.
2. Sinoto Y (1929) Chromosome studies in some dioecious plants with special reference to the allosomes. Cytologia 1: 109–191.
3. Hobza R, Lengerova M, Cernohorska H, Rubes J, Vyskot B (2004) FAST-FISH with laser beam microdissected DOP-PCR probe distinguishes the sex chromosomes of Silene latifolia. Chromosome Res 12:245–250
4. Telenius H, Carter NP, Bebb CE, Nordenskjold M, Ponder BAJ, Tunnacliffe A. (1992) Degenerate oligonucleotide-primed PCR – general amplification of target DNA by a single degenerate primer. Genomics 13:718–725.

Comparative analysis of wild hop based on genetic, chemical and morphological data

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Abstract:
Collections of wild hops are valuable resources of genetic variability useful for breeding of new cultivars with demanded properties. For such purpose, it´s necessary to make their characterization and classification. Aim of our work was making a complex and comparative analysis by utilization of genetic, chemical and morphological data. Information about genetic variability was obtained by analysing 20 SSR loci. Chemoclassification was based on total compounds of alpha and beta bitter acids, essential oils, and 9 chemical substances part of previously mentioned groups. There were evaluated 13 morphological parameters. Comparison of 38 genotypes was done because of stringent criterion for full dataset of all type of markers. For statistical analysis was used NMMDS (Non-Metric Multidimensional Scaling) method and cluster analysis. Objective comparison for all kind of markers was ensured by creation of distance matrix based on Euclidean distances. Two and three dimensional models were tested in the frame of NMMDS. The quality of fitting the proper model was determined by *stress* and *alienation* parameters with combination of Shepard diagram. From this point of view chemoclassification had the best fitting quality performance, but the highest information content was obtained by microsatellite markers. On the other hand morphological characterization found the least explanatory power. Genetic analysis results separated hops into three isolated groups – American, European and Caucasian, which was confirmed by other studies also (Patzak et al., 2010). Chemical profile mixed European and Caucasian groups together. Morphological characterization was able to distinguish American and Caucasian groups, but unfortunately the many of European hops were overlapped with other groups. Three dimensional model brought excellent biological interpretation, because first two dimensions separate genetic isolated groups of hop and the third dimension distinguished two American varieties – *lupuloides* and *neomexicanus*. This result supports current knowledge about those varieties (Reeves and Richards, 2011).

Keywords: hop (*Humulus lupulus* L.), microsatellites, chemical compounds, morphology, MDS

References:
Patzak, J., Nesvadba, V., Krofta, K., Henychova, A., Marzoev, A.I. and Richards, K. 2010. Evaluation of genetic variability of wild hops (*Humulus lupulus* L.) in Canada and the Caucasus region by chemical and molecular methods. Genome 53:545-557.
Reevers, P.A. and Richards, Ch.M. 2011. Species delimitation under the general lineage concept: an empirical example using wild North American hops (Cannabaceae: *Humulus lupulus*). Syst. Biol. 60:45–59.

Complex regulation of omt1 gene, implication of hop transcription factor HlWRKY1.

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Abstract:
O-methyltransferase 1 (OMT1) that is specific for hop lupulin glands performs the final reaction step in the biosynthesis of X by methylating DMX (1). In the present work we cloned and modified promoter elements of omt1 (Pomt1) and analyzed lupulin-specific transcription factors (TFs) that have ability to control omt1 expression. Performing wide functional analyses we discovered specific omt1 stimulation by HlWRKY1 in the transient expression system- A. tumefaciens-infiltrated leaves of N. benthamiana. HlWRKY1 contains characteristic consensus sequence of WRKYGQKxxK/RxxxRRxYF/ YR/KC: it is small basic protein (pI 9.75) having MW of 16.9 kDa and its epression is strongly apecific for lupulin glands, while other WRKY homologues that we isolated from hop (HlWRKY2 and 3) are larger, more acidic proteins and not lupulin specific. Using subcloning of Pomt1 it was found that the activation by HlWRKY1 is rather complex, it requires G-box and other sequences and depends on other factors like HlWDR1, which is the component of recently characterized TF complexes in hop (HlMBWs) (2). Non of so-far characterized HlMBWs is, however, able to activate Pomt1. It was found that the strength of Pomt1 increases enormously and is quite comparable to 35S CaMV promoter, if the binary complex HlWRKY1/HlWDR1 act in the presence of silencing suppressor p19, suggesting some dependency of this activation on PTGS. This specific complex activation is true neither for other two HlWRKY2 and 3 from hop, nor for ortholog AtWRKY75 from Arabidopsis thaliana, which is mainly involved in defense and stress reactions (3). HlWRKY1 specificity shows that not only the core domain, but also N and/or C terminus, where HlWRKY1 and AtWRKY75 differ significantly, may play some role in Pomt1 activation, maybe via interaction with some other developmentally regulated protein(s). Analysis of HlWRKY1 regulation is in progress. Supported by NAZV QH81052.

Keywords: complex gene regulation: transcription factors: hop metabolome: transient expression: Humulus lupulus L.

References:
(1)Nagel, J. et al. Plant Cell 20:186–200, 2008
(2) Matoušek et al. BMC Plant Biol.12:27, 2012
(3) Agarwal, P. et al.: Mol. Biol. Rep. 38:3883–3896, 2011.

Functional and complementation analysis of hop genes in heterologous systems

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Abstract:
Several hop genes involved in the phenylpropanoid pathway responsible for the production of prenylated chalcones (e.g. xanthohumol) with significant bioactivity have been identified. These include genes encoding for chalcone synthases (CHS_H1) or o-methyltransferase (OMT) and genes encoding for transcription factors (TFs) regulating their expression. Recently, combinatorial action of lupulin specific TFs from R2R3Myb (M), bHLH (B) and WDR (W) families involved in regulation of chs_H1 genes has been described (Matoušek et al., 2012). We have identified ternary MBW complexes as well as binary interactions. To investigate the function of these TFs in vivo, we cloned the appropriate genes into Agrobacterium tumefaciens vectors to reach their stable overexpression in plants. Because the hop transformation is labour intensive and time consuming, we performed such analysis firstly on heterologous systems Arabidopsis thaliana, Nicotiana sp. and Petunia hybrida. Overexpression of the hop TF genes showed that they are able to affect plant morphology (i.e. enhanced branching for 35S:HlMyb3 in Arabidopsis, pleiotropic effects for 35S:HlWDR1 regardless the plant recipient and increased level of anthocyanins in tobacco flowers overexpressing HlbHLH2). To know more about the hop TFs function, we used Arabidopsis mutants of genes involved in similar pathways as presumed for hop genes to perform complementation study. The results showed that hop TFs genes are able to regulate the flavonoid and/or anthocyanin biosynthesis pathway. For instance, HlWDR1 gene complemented ttg1 mutant lacking trichomes and anthocyanins. HlbHLH2 gene complemented tt8 mutant, member of TTG1/TT8/GL2 complex, analogical to hop MBW complex. gl1 mutant was complemented by HlMyb1 gene, which suggests involvement in trichome formation which is also connected with anthocyanin biosynthesis in Arabidopsis. This work was supported by the Czech Science Foundation 521/08/0740, by the National Agency for Agricultural Research QH81052 and by GA ASCR AV0Z50510513. The authors thank H. Matoušková and O. Horáková for their technical assistance.

Keywords: chalcone synthase, prenylflavonoids, transcription factors, transgenosis, complementation

References:
Matoušek, J., Kocábek, T., Patzak, J., Fussy, Z., Procházková, J., Heyerick, A. (2012) Combinatorial analysis of lupulin gland transcription factors from R2R3Myb, bHLH and WDR families indicates a complex regulation of chs_H1 genes essential for prenylflavonoid biosynthesis in hop (Humulus lupulus L.). BMC Plant Biology. 12, 27.

Hop ESTs As A Source Of Resistance Gene Analogs And Their Application In Genetic Mapping

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Abstract:
Hop growers are constantly faced with outbreaks of new diseases or the appearance of more lethal pathotypes, e.g., *Verticillium* wilt in hop. Breeding programs of all hop research centers devote a lot of effort to breeding more resistant cultivars, mainly by the introduction of new sources of resistance. Structural and sequence analyses of cloned plant resistance genes (R genes) have revealed that many of them contain similar sequence motifs, even though they determine resistance to different pathogens (McHale et al., 2006). Such knowledge makes it possible to exploit available EST sequences as an alternative approach to map-based cloning, transposon tagging or the use of degenerate primer sets for PCR amplification. Recent hop transcriptome projects have contributed more than 25,000 publically available EST single pass sequences (Wang et al., 2008; Nagel et al., 2008). We conducted searches for the presence of five different *pfam* motifs characteristic of plant R genes. A total of 35 sequences with structural properties of R genes were found. BLASTP searches found the most similar annotated plant gene, which enabled us to predict the intron-exon boundaries and precise design of primer pairs. Primer pairs revealed successful amplification in the hop family 'Wye Target' X 2/1, segregating for wilt tolerance and powdery mildew resistance. Fourteen markers were mapped in the existing microsatellite and AFLP hop map. We showed that we can extend the existing genetic map with new tentative hop R genes. Efforts at mapping and identifying disease R genes in hop could facilitate marker assisted selection (MAS), which offers fast and reliable identification of resistant genotypes, as well as the discovery of new allelic variants of hop R-genes in wild germplasm.

Keywords: express sequence tags, EST, RGA, resistance gene analogs, genetic mapping

References:
McHale L et al. (2006) Genome Biol. 7: 212
Nagel J et al. (2008) Plant Cell. 20: 186–200
Wang G et al. (2008) Plant Physiol. 148: 1254-1266

Elucidating Verticillium wilt resistance in hop

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Abstract:
Fungi of the genus *Verticillium* are a major cause of yield loss in Slovenian hop orchards (Radisek et. al, 2006). Our aim was to isolate hop homologs of the tomato Ve1 gene, the role of which in resistance to *Verticillium* is well established (Fradin et al., 2009), through EST database mining and TAIL-PCR based retrieval of genic sequences. Two sets of complete coding region sequences were retrieved, designated as HlVe1 and HlVe2. SNP markers were developed for each set of sequences and their segregation was determined in pseudotestcross progeny of 'Wye Target' and a hop wilt susceptible male parent. HlVe1 exhibited linkage with the *Verticillium* resistance QTL. At the HlVe2 locus, both maternal alleles were present in all progeny, providing evidence that this locus does not account for variability in resistance to *Verticillium*. We cloned two putatively functional (HlVe1A and HlVe1B) and an additional truncated (HlVe1T) variant of HlVe1. Progeny inheriting the HlVe1A variant displayed significantly less symptom development on challenge with *Verticillium* than those inheriting HlVe1B. Phylogenetic analysis of HlVe1 and HlVe2 sequences in a set of hop cultivars and wild hops showed HlVe2 to be omnipresent, while HlVe1 locus amplification was limited to American wild hops and a limited subset of hop cultivars. HlVe1A appears to have been introgressed into the 'Wye Target' lineage from an *H. lupulus* var neomexicanus progenitor. This finding is concordant with pedigree data for 'Wye Target'. These findings suggest that HlVe1 is involved in recognition of *Verticillium* infection in resistant hop plants and confers some resistance to the pathogen.

Keywords: Ve gene, Verticilium, EST, hop wilt resistance

References:
Fradin EF (2009) Plant Physiol, 150, 320–332
Radišek S (2006) European Journal of Plant Pathology, 116, 301–314

Molecular genotyping of worldwide hop cultivars based on microsatellite loci

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Abstract:
Hop cultivar identification is of prime importance for growers, nurseries and breeders, as well as for scientists. The use of molecular markers for identifying hops has already been proven by numerous publications showing the suitability of different molecular markers for genotyping hops, such as RAPDs, SCARs, AFLPs, SSRs and DArTs. Of all the aforementioned markers, microsatellites (SSRs) are superior due to a number of positive features, including their abundance in the genome, high level of variability, which can result in several alleles at each locus, they usually amplify a single locus in a diploid genome and, most important, they are highly reproducible among laboratories. Several SSR markers for hop have been isolated in recent years, mainly for mapping purposes and selected diversity studies. The aim of this study was to evaluate a list of previously published SSR markers for identifying worldwide hop cultivars and to publish a list of loci suitable for such a purpose. A minimum set of microsatellite markers for identification is also proposed.
DNA of 137 hop cultivars, representing the currently available hop germplasm, was included in the analysis. Automated fluorescent analysis of economically labelled PCR fragments, allowing post-PCR multiplexing of 4 loci, was chosen as a suitable method. Fifty microsatellite loci were selected based on the literature and our mapping data for evaluation, and amplified in 8 diverse cultivars. Amplification profiles were evaluated based on the number of alleles, bias to produce stuttering, ease of scoring and even amplification of short and long alleles. Based on this data, a list of 23 loci was defined and amplified on the entire 137 cultivars. Various measures of variability were defined: number of alleles (total 221, average 9.6, min 3, max 17), heterozygosity (average 0.68, min 0.17, max 0.93), number of unique alleles (38) and PIC value (average 0.60, min 0.15, max 0.85). Three-allelic profiles confirmed the triploid origin of all triploid cultivars. Seven loci were able to distinguish 107 genotypes of cultivars, while the remaining loci confirmed the clonal origin of several cultivars.

Keywords: microsatellites, SSR, hop cultivars, genotyping, identification

References:

Novel hop male markers from DArT sequences

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Abstract:
Sex determination in hop is currently limited to phenotypic evaluation in the second year of growth. Earlier sex determination at the seedling stage is desirable for breeding and cultivation of hop: one approach is the use of molecular markers. A SCAR marker developed from a RAPD fragment (1) and a microsatellite marker (2) have been reported to be linked to male sex in hop. Data suggest that incomplete linkage to the male character or non-amplification in certain male genotypes can occur in some markers (3, 4). More new markers linked to sex would be helpful in programmes of marker assisted selection. Recently, 730 polymorphic markers from 92 hop accessions were discovered using diversity arrays technology (DArT) (5), which were further used in linkage studies. Six of these DArT markers showed linkage with the male phenotype and were evaluated as potential male markers. Primer pairs were designed from DArT sequences and PCR amplification conditions could be optimised for four of these six markers. PCR was carried out on 115 female and 42 male hop plants, both cultivated and wild, from a broad range of geographic locations. None of the four DArT male markers amplified in any female plant. Non-amplification in a few male plants of Japanese, North American or hybrid origin was observed when using single markers, but all males were identified by summing the results of all four markers. Furthermore, amplified fragments of all four male markers were sequenced and their identity with the original DArT sequences was confirmed. Interestingly, a length polymorphism in amplified fragments was identified when using one particular DArT male marker among some hop accessions. Sequencing identified a deletion of 50 bp mainly in American hop accessions. The four new male specific markers identified in this study could be used in hop breeding programmes.

Keywords: hop male, molecular marker

References:
(1) Polley A *et al.* 1997. Genome 40: 357-361.
(2) Jakse J *et al.* 2008. Mol Breeding 21: 139-148.
(3) Patzak J *et al.* 2002. Rostl Výroba 48: 318-321.
(4) Cerenak A, Javornik B 1999. Proceedings of the IHGC Scientific Commission, Pulawy, Poland.
(5) Howard EL *et al.* 2011. TAG 122: 1265-1280.

Section 2

Plant Pathology, Virology, Entomology

Lectures

History, origin, and diversity of hop stunt and hop stunt viroid

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Abstract:
Emergence and symptoms of hop stunt disease: Hop stunt was first emerged in Japan and recorded as "dwarf hop" or "cider-shaped hop" in the early 1950s. The disease emerged in Korea in 1988 and has been confirmed in North America in 2004 and in China in 2007. The diseased plant develops yellowish green leaves and drooped leaf petioles early–mid in the growing season and finally results in stunting of main and lateral bines. Visual stunting becomes apparent only several years after the infection. More serious are the reduction of cone size and the alpha-acid content, which is occasionally accompanied by reduction in the total cone numbers per vine. The alpha-acid content of sensitive cultivars is downed to less than a half.
Causal agent: Viroids are the smallest known pathogens and cause severe to mild diseases in economically important crops. They are single-stranded, circular, and self-replicating non-coding RNAs, with the size 250–400 nucleotides. Viroid replication is dependent on host transcriptional machinery and pathogenicity depends entirely on interactions with cellular components of the host. Hop stunt disease is caused by the *Hop stunt viroid* (HpSVd), a member of the *Pospiviroidae* family. Infection of hops by *Apple fruit crinkle viroid* also exhihits similar disease symptoms.
Origin and diversity of HpSVd: The agent was first discovered from the dwarfed-hop, but soon after, it was found to have infected cultivated grapevines, citrus varieties and stone fruits, including plum, peach, apricot, almond and Jujube. HpSVd is now considered to be a ubiquitous and genetically variable pathogen that has spread among the varieties of fruit trees cultivated worldwide. Unfortunately, all the HpSVd isolates have a potential to cause hop stunt, and current hop stunt epidemics in Japan, US, and China, may have originated from inter-specific transmission of HpSVd from cultivated grapevines to hops.

Keywords: Humulus lupulus, Hop stunt viroid

References:

Outbreaks and management of hop stunt disease in Slovenia

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Abstract:
A severe outbreak of an unknown disease was found in a hop garden (cv. Celeia) in the central part of Savinja valley in the summer 2007. Affected plants developed symptoms of severe stunting, including leaf culring, small cone formation and dry root rot. In the following years, 2008-2011, the disease was found in 17 hop gardens located in the vicinity of the first outbreak, and in 2 hop gardens located in the Koroška region, 60 km away. The majority of the affected hop gardens were planted with the variety Celeia, and the rest with the varieties Bobek, Savinjski golding and Aurora. The disease incidence varied among hop gardens from 1-30% and increased rapidly (up to 10%) each subsequent year, predominantly along plant rows. The diagnostic analysis of symptomatic plants revealed the presence of hop stunt viroid (HpSVd) (1), which has previously been reported as a causal agent of hop stunt disease in Japan, South Korea, USA and China (2,3). In order to prevent further spread, the Slovenian Institute of Hop Research and Brewing (SIHB) and the Phytosanitary Inspectorate, co-ordinated by the Phytosanitary Administration (PARS) of the Ministry of Agriculture and the Environment (MAE), carried out a monitoring survey of hop gardens, which included visual inspections of hop gardens, sampling, laboratory analysis and expert support. In addition, strict phytosanitary measures have been taken and included in Slovenian legislation to prevent further spread and to eradicate HSVd infections.

Keywords: Humulus lupulus L., Hop stunt viroid (HpSVd)

References:
(1) Radišek et al. First Report of Hop stunt viroid Infecting Hop in Slovenia. Plant Disease, 96 (4): 592, 2012.
(2) K. C. Eastwell and T. Sano. Hop Stunt. Page 48 in: Compendium of Hop Diseases and Pests. W. F. Mahaffee et al., eds. The American Phytopathological Society, St. Paul, MN, 2009.
(3) L. Guo et al. Hop stunt viroid (HSVd) newly reported from hop in Xinjiang, China Plant Pathology 57:764, 2008.

Next Generation Sequencing as a diagnostic tool for new pathogen discovery in hops

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Abstract:
Diseases are a significant factor in reducing yield quality, quantity and the production life of hop fields. Infections by 14 fungal diseases, five viruses and two viroids are or have been widespread and significant in commercial hop fields (Mahafee et al., 2009). Identification of new causal agents of diseases currently relies on a large range of different techniques. They are often successful but they suffer from significant drawbacks. They are limited when trying quickly to identify 'unknown' or new disease agents, i.e., either a pathogen infecting a new host or a previously uncharacterized pathogen. Next-generation sequencing (NGS) based technologies are likely greatly to increase the speed, sensitivity and accuracy of new pest and pathogen detection and diagnosis. This promising approach enables the use of metagenomic analysis coupled with the enormous nucleotide sequence data generation offered by NGS technologies. The use of this technology in the field of new pathogen identification has been described in relation to the investigation of human, animal and plant samples (Coetzee et al. 2010).
In 2007, the appearance of hop plants of various cultivars showing stunted vigor was reported. The stunt disease symptoms resembled those of hop stunt disease caused by hop stunt viroid (HSVd), which was later confirmed to be present in some diseased samples (Radisek et al. 2012). In order to investigate in-depth the cause of stunting, a metagenomic approach of sequence comparison produced by NGS Illumina sequencing was applied. A total of 21 M and 12M small RNA reads and 108 M and 102 M paired-end total RNA-seq reads were sequenced from healthy and diseased samples, respectively. Reference mapping and a de-novo assembly approach revealed the presence of a new viroid species present in diseased samples, which had never previously been reported in hops. We demonstrated that NGS sequencing can quickly and successfully identify a new pathogen.

Keywords: next generation sequencing, plant diseases, plant diagnostics, viruses, viroids

References:
Coetzee B et al. (2010) Virology 400 (2):157-163
Mahaffee W et al. (2009). Compendium of Hop Diseases and Pests. APS, 93 pages
Radisek S et al. (2012) Plant Disease 96 (4):592-593

Colonization of susceptible and resistant hop plants by *Verticillium albo-atrum* and comparative proteomics of roots and xylem fluid

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Abstract:
Aggressive strains of *Verticillium albo-atrum* represent a serious limitation in hop production. Molecular mechanisms of resistance to Verticillium wilt remain poorly understood, despite the considerable economic losses caused by the disease. In this study, the spatial and temporal pattern of host colonization by the fungus and host response on a proteome level were analyzed in order to gain new information about resistance in hop. Susceptible cv. Celeia and resistant cv. Wye Target were used in comparative analyses. Host colonization was monitored in roots and stem sections through a time course of 30 days. Fungal biomass was determined by measuring the amount of fungal DNA by quantitative PCR. Both spatial and temporal colonization patterns differed markedly between the cultivars. In the resistant cv., colonization was slower and restricted to the roots, while the fungus rapidly spread up the stems in susceptible plants. Proteomic analyses of roots and xylem fluid isolated from control and infected plants of both cultivars were performed with two-dimensional electrophoresis. Two defence-related proteins were up-regulated after infection in xylem fluid; however the susceptible cv. displayed a stronger response. In addition, the xylem of the susceptible cv. contained two fungal proteins, which are most likely involved in virulence. A typical and strong defence response was also observed in the roots of the susceptible cv., while the infection did not induce any changes in the roots of the resistant cv. Altogether, the resistant cv. showed a very weak response to infection on a protein level and managed to restrict fungal colonization almost exclusively to the roots. The results suggest that the resistance in cv. Wye Target is conferred by an antifungal substance, which is produced constitutively rather than induced by infection.

Keywords: Verticillium, host colonization, roots and xylem

References:

Simple is Beautiful: A New Biotest for the Aphid Tolerance Assessment of Different Hop Genotypes

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Abstract:
The breeding of cultivars tolerant or resistant to Phorodon humuli infestation is a major issue of future pest management in hops. Therefore, a practical and simple biotest to assess the aphid susceptibility of different hop genotypes is needed for the breeding routine. From 2006 to 2009 we tried to develop a method by the use of single leaves, taken from greenhouse plants of six genotypes. Those trials failed and did not yield the expected results (Weihrauch et al. 2009). Apparently the response of single leaves to aphid infestation differs significantly from entire plants, most likely due to a different biochemistry.
We therefore changed our test method and used rooted cuttings, which were planted with little soil in 2.5 l preserving jars. Similar to the former leaf tests, we investigated the susceptible cultivars Hallertauer Magnum (HM) and Herkules (HS), the aphid-tolerant or –resistant cvs Spalter Select and Boadicea, and two apparently resistant genotypes from our germplasm – the male accession "38" and the breeding line "2005/034/022" in 12 replications, respectively. Each jar was equipped with one cutting and one aphid larva with the same day of birth, closed with a sheet of gauge and then stored in a climate chamber. The enclosures were left untouched for the duration of the respective series (22-27 days). All enclosures of a series were opened synchronously and the aphids in each jar were counted. Altogether six test series were conducted during 2010 and 2011, and six more are planned for 2012. As the most offspring of one aphid in each series was produced on HM (means of 67-753) and HS (77-539), we chose HS as standard cultivar (100 %) for the relative assessment of other genotypes.
The hitherto achieved results clearly demonstrate that the development of a simple aphid biotest for breeding purposes now was successful.

Keywords: Humulus lupulus, damson-hop aphid, aphid susceptibility, aphid resistance, biotest

References:
Weihrauch F., Baumgartner A., Felsl M. and Lutz A. 2009. Aphid Tolerance of Different Hop Genotypes: First Attempts to Develop a Simple Biotest for Hop Breeding by the Use of Phorodon humuli. Acta Horticulturae 848: 125-129

Protection of organic Saaz hops against two-spotted spider mite (*Tetranychus urticae* Koch) with the help of released predatory mites *Typhlodromus pyri* Scheuten

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Abstract:
After three years of the necessary temporary period certified organic fine aroma Saazer is at disposal since August 2012. Whereas damson hop aphid (*Phorodon humuli* Schrank) is controlled with the help of extract of the tropical plant *Quassia amara* as well as by native predators, mainly aphidophagous coccinellids, predatory mite *Typhlodromus pyri* Scheuten is released to support native populations of acarophagous predators to control two-spotted spider mite (*Tetranychus urticae* Koch). The release rate of *T. pyri* depends on a locality and actual endangering by spider mites. The fact this species is able to hibernate in hop gardens and control this dangerous pest in the following years is of great importance within the project of organic hop growing in Czech Republic.

Keywords: Humulus lupulus, organic hops, damson-hop aphid, two-spotted spider mite

References:

Section 2

Plant Pathology, Virology, Entomology

Posters

Differential gene expression during interaction of resistant and susceptible hop cultivars with *Verticillium albo-atrum*

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Abstract:
The *Verticillium albo-atrum* strain with increased virulence in hop (pathotype PV1) was first discovered in the UK in 1933, followed by outbreaks in Slovenia in 1997 and Germany in 2005. This pathotype causes very severe symptoms, with rapid plant withering, and has become a serious problem in hop production. Understanding the molecular mechanism of hop resistance to Verticillium wilt might contribute to successful disease control and we therefore undertook a study of hop-Verticillium interactions on the transcriptome level in order to contribute to knowledge of the resistant mechanism. Resistant cv. Wye Target and susceptible cv. Celeia were inoculated with pathotype PV1 and stem samples were collected from infected and control plants 10, 20 and 30 days after inoculation. Total RNA isolation was followed by generation of cDNA templates, which were used for two differential expression analyses, cDNA-AFLP and GeneSnare. Around 5000 bands, ranging in size from 50-750bp, were amplified by ten primer combinations in cDNA-AFLP analysis. Differentially expressed patterns were observed for 257 transcript derived fragments (TDFs) of which 117 (45.5%) were successfully re-amplified, cloned and sequenced, obtaining 90 contigs and 43 singletons for sequence analysis. Sixty-six TDFs with altered expression pattern were amplified by 12 ACP-arbitrary primers in GeneSnare analysis. These TDFs were re-amplified, cloned and sequenced, giving a total of 84 sequences, 31 contigs and 53 singletons, with an average size of 440bp. A Blast2go algorithm revealed the homology to known genes of 90 TDFs out of a total of 217 TDFs and gene ontology categorises them by biological processes to cellular and metabolic processes, biological regulation, response to stimuli, transport and disease resistance. The expression pattern of 34 TDFs with homology to known genes and additional defence related genes of PR-1, β-1,3-glucanase (PR-2), chitinase (PR-3) and thaumatin-like protein(PR-5) were further analysed by quantitative real-time PCR on samples from a second experiment carried out in the same way as the first inoculation experiment. The expression pattern of TDFs in differential analysis and in RT-PCR analysis were similar, although RT-PCR analysis showed TDFs expression in more detail. For example, TDFs showing homology to the 14-3-3 protein and syntaxin showed stronger upregulation in resistant than in susceptible cultivars; defence related hevamine, protein phosphatasa 2C and chitin binding protein were more strongly upregulated in the susceptible cultivar and revealed a cyclic expression pattern in the resistant cultivar. Stronger and faster upregulation in the susceptible cultivar was also observed for PR-1, PR-2,PR-3 and PR-4 defence related genes, implying a stronger defence response in the susceptible than in the resistant cultivar. This study provides the first identification of candidate genes involved in the hop-Verticillium interaction and might help in understanding resistance to Verticillium wilt.

Keywords: differential analysis, host-pathogen interactions, hop, Verticillium

References:

Evaluation of candidate reference genes for gene expression normalization in hop using real time quantitative RT-PCR

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Abstract:
Real time quantitative reverse transcription PCR is an important method for discovering the function of genes. The sensitivity of the method requires the optimization of the process and any source of variation that biases the result, including sampling error, template quality and amplification efficiency, should be limited. Normalization of the data of RT-qPCR by using internal control genes (housekeeping genes) is therefore essential for the accuracy of the results. It is now known that the expression of internal control genes in different organisms varies considerably during developmental stages and under different experimental conditions. Only few reference genes have been reported for hop. In this study, twenty-three of the most widely used plant reference genes were evaluated for expression stability in hop under biotic stress conditions (infection with *Verticillium albo-atrum*). The candidate reference genes were amplified from cDNA obtained from stem tissues of susceptible and resistant hop cultivars sampled at three different time points after infection and from control plants. Validation of the reference genes was assessed by four different approaches and the most stable ones were subsequently defined and the optimal required number of reference genes determined. A combination of two reference genes, YLS8 and DRH1, resulted as the most suitable for normalization of the Q-PCR data of the gene of interest (PR-1). On the basis of the expression of the PR-1 gene, which is implicated in biotic stress, we outlined the differences between normalised and non-normalised values. The selection of the most suitable reference genes depends on the experimental conditions, and is tissue and cultivar specific.

Keywords: qRT-PCR, quantitative real time PCR, reference genes, normalization

References:

Comparative secretome analysis of *Verticillium albo-atrum* isolates from hop

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Abstract:
A broad range of extracellular proteins is employed by plant pathogenic fungi in the pathogenesis process. These proteins are critical to the success of an infection. The most important of them are cell wall-degrading hydrolytic enzymes, specific toxins and effector proteins, which cause changes in plants that facilitate infection. Verticillium wilt of hop is a vascular wilt disease caused by the colonization of xylem vessels by *Verticillium albo-atrum*. Fungal isolates differ in aggressiveness and have been classified by pathogenicity tests into mild and lethal pathotypes. In this study, we cultured six isolates (mild and lethal pathotypes originating from three countries) in a simulated xylem medium to reproduce *in vivo* nutritional conditions and therefore induce the secretion of a set of proteins similar to those secreted during plant-pathogen interaction. The secreted proteins were collected from the culture supernatants and analyzed by two-dimensional difference gel electrophoresis (2D-DIGE). Approximately 850 protein spots were reproduced among the samples, of which 191 were identified by mass spectrometry. The secretome samples contained an arsenal of various hydrolytic enzymes capable of degrading different components of cell walls (pectin, cellulose, hemicellulose and proteins). Other identified proteins include lipases, oxidoreductases, effector proteins and conserved proteins with unknown function. Comparative analysis showed that lethal isolates from all three countries secrete higher amounts of lipases, esterases, endoglucanase and rhamnogalacturonan acetylesterases than mild pathotypes. Significant differences in secreted protein levels were also found for many other proteins, but these changes were specific to individual isolates. The results lead to the conclusion that the increased level of aggressiveness of lethal isolates is a combination of mechanisms shared among isolates and mechanisms specific to each isolate.

Keywords: hop, Verticillium, secretome analysis

References:

Negative effect of fungicides used in practical hop protection against downy mildew (*Pseudoperonospora humuli*) on aphidophagous coccinellids *Propylea quatuordecimpunctata* L.

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Abstract:
Still increasing attention has been paid to the development of IPM systems within EU countries. Natural enemies play very important role in these systems. Therefore, it is necessary to use only such pesticides, whose harmfulness is generally lower and acceptable. Downy mildew (*Pseudoperonospora humuli*) is commonly controlled by fungicides applied during the season. Nevertheless, at that time numerous aphidophagous predators, especially coccinellids are present at the leaves of the treated plants feeding on aphids. Ladybird *Propylea quatuordecimpunctata* L. belongs to the most important species attacking damson-hop aphid (*Phorodon humuli* Schrank). Fungicides, which are in current use, were tested on eggs, larvae, pupae and adults of this ladybird species to determine their real effect and convenience of their inclusion within IPM systems.

Keywords: Humulus lupulus, downy mildew, fungices, ladybird *Propylea quatuordecimpunctata* L.

References:

HSVd pathogenesis involves a disbalance of hop regulatory genes

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Abstract:
Hop stunt viroid (HSVd) is a serious pathogen with symptoms ranging from metabolic to developmental changes. In hop (Humulus lupulus L.) cv. Admiral infected with HSVd-g/CPFVd we observed stunted growth, leaf rugosity and epinasty, and petiole decolouration, along with detected shifts in secondary metabolite levels. Owing to simple structure, HSVd is hypothesised to exert symptoms via not specified transcriptional silencing mechanisms. In search of markers linked with the disease, we employed quantitative expression screening of already known hop transcription factors (TFs), as well as newly identified Myb and WRKY genes. The most profound changes were detected in HlMyb5, HlWRKY1, and HlMyb3 with increased mRNA levels, while HlMyb4 and HlbHLH2 mRNA levels were markedly decreased. Importantly, HlMyb3 and HlbHLH2 were recently shown to co-operate in regulation of chalcon synthase chs_H1 gene enconding the flavonoid pathway key enzyme (Matoušek et al., 2012). Solely the disbalance of these transcription factors might influence the flux through flavonoid pathways, having biotechnological potential for future research. In addition however, bioinformatic prediction of HSVd-derived small RNA targets within publicly available nucleotide sequences was carried out, predicting chs_H1 transcript as effective target. Marked decrease of its expression was confirmed in diseased samples, but the unveiling of the precise cause(s) is still in progress. TF disbalance may therefore combine with direct chs_H1 mRNA degradation to induce secondary metabolite-related symptoms. Other predicted small RNA targets were not confirmed by expression analyses.
This work was supported by the Czech Science Foundation project GCP501/10/J018 and by the Grant Agency of the University of South-Bohemia 134/2010/P. The authors would like to thank Ing. O. Horáková, Mrs. H. Matoušková, Ing. L. Orctová, and RNDr. Jitka Procházková, Ph.D. for their excellent assistance.

Keywords: gene expression: transcription factor: hop stunt viroid: Humulus lupulus L.: phenylpropanoid

References:
Matousek J, Kocabek T, Patzak J, et al. BMC Plant Biol 2012:12:27.

Biolistic transfer of Slovenian viroid disease syndrome to Czech hop Osvald‘s72-symptoms and identification of dominant sequence upon transfer of HSVd component.

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Abstract:
Slovenian hop disease syndrome has been firstly detected since 2007. According to some symptoms it resembles viroid disease in hop. To study possible impact(s) of this syndrome to Czech hop, total RNA was isolated from symptomatic plants of Slovenian cultivar Celeia and transferred as a „naked RNA agent“ by the biolistic method using Helios GeneGun to Osvalds’s 72 hop under isolated experimental conditions. First symptoms of the syndrome were observed only after free months of post dormancy (winter) period, five months post inoculation, suggesting some „incubation“ interval necessary for disease development. Strong symptoms that appeared on cv. Osvals’72 were quite comparable to cv. Celeia: low plant fitness, strong stunting, small leaves and frequently visible mosaic, affected and weak roots were observed. Because the frequent mosaics, infected plant were checked in parallel for nine known hop viruses like ApMV-L, PNRV-L, ArMV-L, HMV-L, SLRV-L, CLRV-B, CMV-B, TNV-B, PAMV-L, however, non of these viruses was detectable. Because weak HSVd population was detectable previously in the original Slovenian samples (1), HSVd was analyzed in detail in the inoculated Czech hop and it was confirmed that spectrum of HSVd-CPFd-like variants was detectable in the leaves with clearly dominant sequence. It was found that Slovenian syndrome disease lead to some changes in expression and misbalancing of transcription factors of hop MBW complexes (for the hop TF complexes see 2). The misbalanced gene regulation is characteristic for viroid-mediated diseases. This work was supported by the Czech Science Foundation (GACR P501/10/J018).

Keywords: Biolistic inoculation: experimental disease transmission: hop viruses, Hop stunt viroid (HSVd) population: transcription factors: Humulus lupulus L.

References:
(1) Radišek et al. Plant Disease, 96 : 592, 2012.
(2) Matoušek et al., BMC Plant Biology12:27, 2012.

Section 3

Chemistry and Plant Physiology

Lectures

Isolation of prenylflavonoids from hops

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Abstract:
Hops provide a rich source of prenylflavonoids, some of which have remarkable pharmacological potential, e.g. xanthohumol which has a wide range of activities, or 8-prenylnaringenin, the most active phytoestrogen currently known. The extraction of hops with ethanol and further fractionation with supercritical carbon dioxide make it possible to separate the prenylflavonoids from other hop polyphenols and from bitter acids too. The remaining fraction ("Xantho-Extract") can be further separated using polyvinylpolypyrrolidone (PVPP) as a selective carrier for prenylflavonoids. The resulting extract (Xantho-Flav) contains up to 85% xanthohumol and still retains the full spectrum of different prenylflavonoids (more than 20 different structures). This is consequently a suitable source for isolating single compounds. Using only one step of re-crystallisation it is possible to produce xanthohumol with a purity - 90 %.
For over a decade prenylflavonoids have been tested in numerous experiments for various pharmacological activities. An overview is given on the current status achieved with extracts or single compounds produced according to the processes described above. Other than ongoing "in vivo" confirmations of xanthohumol's cancer chemo-preventive activity, a few more, completely different inhibitive reactions have been observed. In this connection xantohumol's potential for the treatment of endometriosis or metabolic syndrome could be shown "in vivo". In both cases pure xanthohumol is now being used for further investigations with the objective of starting clinical trials soon. "Xantho-Extract" (with a specific 8-prenylnaringenin content) is thoroughly tested as an alternative for hormone replacement therapy. Should there be demand for pure 8-prenlynaringenin, a synthesis from xanthohumol has been developed. The two-step chemical process (isomerisation followed by demethylation) is considered a reasonable alternative to other processes such as the (stereospecific) prenylation of naringenin.

Keywords: prenylflavonoids, xanthohumol, 8-prenylnaringenin

References:

Preparation of the Unnatural Derivatives of Humulus Lupulus L. (Hops)

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Abstract:
The alpha acids derived from Humulus lupulus L. (hops) are most known for the bittering agents they produce during the brewing process, the isomerized alpha acids. The use of concentrated extracts of the bittering acids (isomerized alpha acids and modified shelf-stable derivatives) enables a more efficient utilization, as the bittering acids are introduced as a finished product without needing further modification. As a result there have been many attempts to produce the extracts from non-conventional/synthetic means. Any previous attempts to prepare the isomerized alpha acids and the derivatives therein from totally synthetic (phloroglucinol starting material) (1) and/or semisynthetic routes (beta acids starting material) has resulted in a racemic mixture (2). The resulting bittering acid extracts are a racemic mixture of natural and unnatural enantiomers in ratios never seen through the conventional production of beer using the naturally occurring alpha acids. As a case study, the resolution of racemic humulone has been described as a method of preparation of enantiopure standards of (+) and (-)-cis-tetrahydroisohumulone. The methods of purification/resolution used include liquid chromatography, countercurrent chromatography, crystallization and precipitation. The purified cis-tetrahydroisohumulone standards were subsequently characterized by UPLC-MS/MS, optical rotation, and X-ray crystallography, thus confirming the absolute stereochemistry. In addition, the opposing enantiomers were compared in-vitro and found to demonstrate differences in biological activity.

Keywords: Countercurrent, humulone, tetrahydroisohumulone, unnatural, chiral preparation

References:
(1) Ting, P. L.: Kay, S.: Ryder, D. J. Am. Soc. Brew. Chem 2009, 67, 152-156.
(2) Ting, P. L.: Goldstein, H. Journal of the American Society of Brewing Chemists 1996, 54, 103–109.

Varietal characterisation of hop (Humulus lupulus L.) using GC-MS analysis

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Abstract:
Gas chromatography (GC) has been used for decades to analyse the volatile components in hop (Humulus lupulus L.) extracts, with modern studies almost exclusively incorporating a second analytical dimension such as mass spectrometry (MS). In the present investigation, we have employed a range of contemporary approaches for hop analysis.

Analysis of the terpenoid compounds in hop essential oil is an age-old approach for hop varietal characterisation. To this end, we will compare and contrast the use of three approaches, namely:
• High resolution GC using long capillary GC columns coupled to a quadrupole MS (GC-MS)
• Ultra-fast GC utilising short, resistively heated capillary GC columns coupled to Time-of-Flight MS (GC-TOFMS)
• Comprehensive two-dimensional GC (GC×GC)

Although essential oil analysis is very robust, it generally requires large amounts of sample and the extraction is time consuming (~4h per sample). It is also recognised that steam distillation can impart changes in oil composition. Thus we have investigated an alternative approach that draws on the experience of the metabolomics community. This approach provides new knowledge of the profile of a rarely studied fraction of hop cone extracts.

This presentation will provide an overview of the abovementioned studies and discuss the relative merits of each of the developed approaches.

Keywords: GC-MS, Hop, Characterization, Essential Oil

References:

Organogenic Nodule Formation in Hop: A Tool to StudyMorphogenesis in Plants with Biotechnological and Medicinal Applications

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Abstract:
The usage of Humulus lupulus for brewing increased the demand for high-quality plant material. Simultaneously, hop has been used in traditional medicine and recently recognized with anticancer and anti-infective properties. Tissue culture techniques have been reported for a wide range of species, and open the prospect for propagation of disease-free, genetically uniform and massive amounts of plants in vitro. Moreover, the development of large-scale culture methods using bioreactors enables the industrial production of secondary metabolites. Reliable and efficient tissue culture protocol for shoot regeneration through organogenic nodule formation was established for hop. The present review describes the histological, and biochemical changes occurring during this morphogenic process, together with an analysis of transcriptional and metabolic profiles. We also discuss the existence of common molecular factors among three different morphogenic processes: organogenic nodules and somatic embryogenesis, which strictly speaking depend exclusively on intrinsic developmental reprogramming, and legume nitrogen-fixing root nodules, which arises in response to symbiosis. The review of the key factors that participate in hop nodule organogenesis and the comparison with other morphogenic processes may have merit as a study presenting recent advances in complex molecular networks occurring during morphogenesis and together, these provide a rich framework for biotechnology applications.

Keywords: organogenic nodule, signal transduction, growth regulators, transcriptomics, metabolomics

References:

Endogenous phytohormone levels in dwarf and normal hop (*Humulus lupulus* L.) plants

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Abstract:
The cultivated hop (Humulus lupulus L.) is a short-day plant, normally grown in 5-8 m tall wirework to produce commercial yields of inflorescences known as cones. Relatively recently, dwarf hops have been developed in the United Kingdom, which have shorter internode lengths and are cultivated in low trellis systems. Phytohormones are organic substances, which regulate in small amounts plant growth and developmental processes including vegetative growth, internode elongation, flower induction, shoot formation, leaf senescence, chlorophyll production, etc. Endogenous levels of phytohormones are associated with environmental changes and various hop genotypes have different growth and flowering responses. Few years ago, Villacorta et al. (2008) characterized hormonal levels and changes associated with both vegetative and reproductive development in hop. The aim of our study was to investigate possible differences of phytohormone contents between normal and dwarf hop genotypes during development in field conditions. Samples were collected from female plants of three normal (Saazer, Sladek, Admiral) and three dwarf (First Gold, Herold, 5021) cultivars grown in experimental hopgardens of Hop Research Institute, Zatec, CR. Different tissue samples were immediately frozen by liquid nitrogen and stored at -80°C until analyses. Growth hormones (cytokinins, auxins, gibberellins) and stress hormones (abscisic acid, jasmonic acid and salicylic acid) were measured by LC-electrospray tandem-mass spectrometry in the Institute of Experimental Botany AS CR, Prague. Various plant tissues (apex buds, young leaves, old leaves, inflorescences) markedly differed in the phytohormone levels, independently on hop genotypes. Higher levels of auxins (IAA) and gibberellins (GA19, 20, 29) were detected in inflorescences suggesting these phytohormones might be involved in flowering, maturating and generative growth of plants. For cytokinins, statistically significant difference was revealed in cis-zeatin riboside-O-glucoside content between dwarf and normal hop plants. Higher levels of this cytokinin storage form were found in all tested tissues of dwarf cultivars. Slightly higher levels of abscisic acid were detected in apex buds of normal cultivars while considerably higher levels of salicylic acid occurred in apex buds and young leaves of dwarf cultivars. This work was supported by the Ministry of Education, Youth and Sports of CR in EUREKA project LF11008 (JP) and the Czech Science Foundation (P506/11/0774, PD and VM).

Keywords: cytokinins, auxins, gibberellins, abscisic acid, jasmonates, salicylic acid

References:
Villacorta, N.F., Fernandez, H., Prinsen, E., Bernad, P.L., Revilla, M.A., 2008. Endogenous hormonal profiles in hop development. J. Plant Growth Regul., 27, 93-98.

The response of Humulus lupulus to drought: contribution of structural and functional plant traits

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Abstract:
Reduction of the total precipitation in recent years in the traditional hop growing regions in the Czech Republic has negative impact on the yield of hop. One of the possible options for resolving this problem is selection of drought tolerant cultivars. However, detailed knowledge of key physiological and anatomical traits, which control efficiency of water use in hop plants is needed for selection and breeding processes. This work was aimed at analysis of importance of some processes and structural traits for water transport in hop plants. We measured the rate of transpiration, leaf water potential and the rate of net photosynthesis of hop plants in relation to water availability in soil of several hop cultivars. We also explored the anatomical traits of xylem in the stem. We analyzed potential limitations that structural and functional traits may represent for hop plants.
Plant showed decrease of transpiration rate and shoot water potential under declining water availability. We found that hop cultivars may differ significantly in some traits that underlie water use in plants such as the rate of transpiration and leaf water potential. We found that transpiration rate is in ideal evaporative conditions close to the rate of evaporation. Under normal conditions plants use only 30% of their maximum transport capacity in stem. Therefore, even the maximal degree of natural cavitation in xylem that we observed (30%) does not represent serious thread of water transport from roots to leaves.

Keywords: transpiration rate, water transport, xylem, cavitation, leaf water potential

References:

Section 3

Chemistry and Plant Physiology

Posters

Agronomic and nutraceutical potential of hops (Humulus lupulus L.) grown in Quebec

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Abstract:
Hop (Humulus lupulus L.) is a dioecious plant cultivated in most temperate areas of the world, mainly for its inflorescences known as hop cones. Hop cones contain hop bitter acids (α- and β-acids), and polyphenols such as prenylated chalcones and proanthocyanidins (Kavalier et al. 2011). Hop leaves may also contain polyphenols (Ceh et al. 2007). The present work was conducted to determine the yield and the content of α- and β-acids, desmethylxanthohumol, xanthohumol and proanthocyanidins in cones and leaves of 5 cultivars (Cascade, Galena, Nugget, Willamette, Brewers gold) grown in Houblonnière Gosselin (Quebec, Canada). Cones, leaves and stems of each plant were separated to measure fresh and dry weights of each part. α- and β-acids, desmethylxanthohumol and xanthohumol were determined by HPLC coupled to UV detection while proanthocyanidins were determined by HPLC coupled to fluorescence detection. Total polyphenols were determined by Folin-Ciocalteu assay. Our data showed that Nugget had the lowest cone yield (113.5 g of dry cones / plant) and Galena had the highest cone yield (603.5 g of dry cones / plant). Cones of Nugget had the highest concentration of α-acids (10.8 % DM), proanthocyanidins (1.7 % DM) and total polyphenols (4.6 % DM) whereas cones of Galena had the highest content of β-acids (6.7 % DM) and cones of Brewers Gold had the highest concentration of xanthohumol (0.6 % DM). Leaves of Nugget had the highest concentration of α-acids (0.09 % DM), while leaves of Galena had the highest content of proanthocyanidins (1.1 % DM) and total polyphenols (3.18 % DM) while leaves of Brewers Gold had the highest concentration of xanthohumol (0.014 % DM). No correlation was found between yield and cones and leaves polyphenol content. Moreover, hop leaves contained small amounts of xanthohumol, α- and β-acids but higher amounts of proanthocyanidins and total polyphenols.

Keywords: Hop, xanthohumol, α-acids, β-acids, proanthocyanidins, total polyphenols

References:
Ceh B, Kac M, Kosir IJ, Abram V (2007) Relationships between xanthohumol and polyphenol content in hop leaves and hop cones with regard to water supply and cultivar. International Journal of Molecular Sciences 8: 989-1000
Kavalier AR, Litt A, Ma C, Pitra NJ, Coles MC, Kennelly EJ, Matthews PD (2011) Phytochemical and morphological characterization of hop (Humulus lupulus L.) cones over five developmental stages using High Performance Liquid Chromatography coupled to Time-of-Flight mass spectrometry, Ultrahigh Performance Liquid Chromatography photodiode array detection, and light microscopy techniques. Journal of Agricultural and Food Chemistry 59: 4783-4793

Contribution to etiology of occurrence of monecious hop plants

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Abstract:
In this work we monitor influence of external conditions during hop cultivar Magnum cultivation on occurrence of abnormities in a stand in extreme year 2009. Heterozygous origin of hop probably enables also occurrence of anther flowers in cones and changes in inflorescence structure. Cause of changes is regarded in extreme weather conditions, which modified year ontogenesis, photosynthesis, metabolism and morphogenic processes by changes of character of endogenous substances. Every reproductive meristeme in leaves axil responded to degree of external conditions changes. We suppose different activity of not only root system, but of total hop metabolism, which evinced changes under stress conditions i.e. in synthesis of gibereline or cytokinine substances. Degree of changes conditioned by changes of metabolism proved by modification of heterozygous character of pistil plants of cultivar Magnum.

Keywords: heterozygous hop cultivar, modification of genetical base

References:

Changes in the rate of photosynthesis, transpiration and plant pigments content (chlorophylmetric units) during the vegetation of hop

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Abstract:
The paper evaluated changes in the rate of photosynthesis, transpiration and plant pigments content (chlorophylmetric units) during the vegetation in the hop plant. The device LC pro + (portable infrared gas analyzer) and Chlorophylmeter SPAD – 502 was used for the measurement of physiological processes. The generic model of the photosynthetic rate was defined in relation to the growth phase of the hop during the vegetation of hop plants. The results of measuring photosynthetic rate (2007 – 2009) showed that photosynthesis gradual increase during the extensive growth (BBCH 32 - 37) and photosynthetic activity increased in the flowering stage of hop plants (BBCH 61 - 65). On the contrary, hop plants reduce assimilation towards maturity of hops (BBCH 69 - 81). A similar trend was found for measurement of pigments (chlorophylmetric units) in the leaves of hop. Transpiration rate decreases with the course of hop plants ontogeny.
The average rate of photosynthesis during the vegetation in the years (2007 - 2009) ranged from 5.61 to 7.82 mmolCO2.m-2.s-1. The average transpiration rate ranged from 1.02 to 1.21 mmolH2O.m-2.s-1. The average values of pigment content (chlorophylmetric units) ranged from 36.78 to 44.07.

Keywords: hop, photosynthesis, transpiration, chlorophyl

References:

Section 4

Hop Cultivation and Management

Lectures

The Influence of Hop Root Age on the Quality of Hop

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Abstract:
Hop (*Humulus lupulus*), one of the important ingredients in brewing, provides bitterness, aroma and fullness to beer. Therefore, it is difficult to brew beer in equal quality using the hops with fluctuated quality, as the hop quality may vary due to climate, cultivation method, root condition, etc. The object of this study is to elucidate the influence of hop root age on the contents and the quality of the components in the dried hop cones including α-acid and oil, and then the beer quality. The length of the vine, leaf size, and stem diameter were also monitored throughout the cultivation season. In 2010 and 2011 crop seasons, Czech Saazer from 8 hop gardens, whose root age are different to each other were selected for this study. Of the younger root aged hops, leaf size and stem diameter were larger and flowering was late. Content of α-acid was higher in the younger root aged hops. Although the total amount of oil was almost the same between 8 lots, the balance of terpene components were characteristic to the root age of the hops. It was also confirmed that the hop aroma in beers was different in the younger root aged hops. From these results, it was suggested that vegetative growth of young root is strong, and this affects the secondary metabolism in hop itself, thus to the beer aroma quality. Therefore, elucidation of the root age of hop may enable us to select proper hops to brew beers consumers really expect. Detail will be discussed in the presentation.

Keywords: Hop root age, Czech Saazer, α-acids, terpene contents

References:

Transpiration – an important contribution to overall water balance in hop plantation.

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Water is often a limiting factor of hop (*Humulus lupulus* L.) growth. Plantations are usually grown at the relatively arid sites and they often require irrigation. Proper knowledge of the partitioning of aboveground water loss among hop and understorey weeds transpiration and soil evaporation may lead to management tools to increase water availability for the hop plants. Evapotranspiration of the Czech hop plantation (variety Preminat) was investigated during the growing season 2011. We measured two components of the water balance: total evapotranspiration (using the Bowen ratio energy balance method) and transpiration of the bines (using the stem heat balance sensors type EMS SF 62). In addition, we calculated the potential evapotranspiration (PET). At the beginning of growing season, evaporation from the bare soil (with dry surface) made up usually no more than 50 % of PET. Exceptional were days after the rain events when evaporation reached almost 100 % of PET. When the crop was fully developed, measured evapotranspiration reached up to 90 % of the PET. Even at the end of the growing season only one half from the total amount of vaporized water was transpired by the hop bines while second half came from the soil surface or understory weeds. This ineffective portioning of water balance may lead in dry years to the stress conditions and induce necessity of the irrigation. This work was supported by the Ministry of Agriculture as part of the project QH81049 and by OPVK reg. No. CZ.1.07/2.3.00/30.0017.

Keywords: transpiration, evapotranspiration, water use efficiency, Bowen ratio, trunk heat balance method.

References:

Lindroth, A., Cermak, J., Kucera, J., Cienciala, E., and Eckersten, H. (1995). Sap flow by the heat-balance method applied to small-size salix trees in a short-rotation forest. *Biomass and Bioenergy,* **8**, 7-15.

Cryopreservation and Maintance of Hop Material in USDA Germplasm Collection

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Abstract:
The US Department of Agriculture, Agricultural Research Service, National Clonal Germplasm Repository (NCGR) has the responsibility for conservation of the hop (Humulus L.) genetic resources. The collection includes about 530 accessions representing 7 taxa (species and subspecies) from 21 countries. This germplasm includes wild collected seeds, virus and viroid tested plants growing in greenhouses or screenhouses, in vitro cultures, and cryogenically preserved pollen and meristems. The tissue culture collection is primarily composed of pathogen-tested cultivars and wild collected species. The cryopreserved clonal collection was established from the tissue cultured plants and includes 73 accessions stored using the encapsulation-dehydration technique. These cryopreserved samples are safeguarded at the USDA ARS National Center for Genetic Resources Preservation in Ft. Collins, Colorado. Hop stunt viroid recently appeared in the main hop production areas of the Pacific Northwestern United States. The plants NCGR distributes have tested negative for this viroid. Hop germplasm from the NCGR is distributed to researchers internationally in accordance with country, regional and state phytosanitary regulations.

Keywords: germplasm, cryopreservation, In vitro culture, virus, viroid, *Humulus*

References:

Hop cultivation trials in some Italian regions: alfa- and beta-acid content of hop cones

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Abstract:
In Italy where the hop cultivation is still at pioneering level some Europeans and Americans varieties have been cultivated in four Italian regions: Sardinia island, Piemonte (north west), Emilia-Romagna (north central) and Friuli Venezia Giulia (north East). The aim of this study was to verify the attitude of Italian climate to hop cultivation and the quality of the hop cones. Since the hop plants were only 2-3 years old it was not still possible to evaluate exactly the yield, nevertheless the first results were very encouraging. Different Hop trellis have been tested (4-6 meters high) and the results obtained in terms of quality were very interesting. Hop cones were analyzed and alfa and beta acid content have been evaluated. The five analogue of alfa-acid have been characterised, i.e. humulone, cohumulone, adhumulone, prehumulone and posthumulones according to EBC methods. As known the relative amount of the adhumulones witin the alfa-acids is fairly constant between varieties (ca. 15%) while the relative amounts of humulone and cohumulone are rather variety dependent (ca. 20-50%) while pre- and posthumulone are present in very low concentrations. Cohumulone is often associated with a low hop quality, the belief is that hops with high levels of cohumulone produce a harsh and unpleasant bitterness with a negative impact on head retention. Although this is not finally proven, hop varieties with relatively low cohumulone content are still largely preferred. All hop cones obtained in Italy presented an alfa-acid level compared to the same variety cultivated in Hallertau (Germany), Saaz (Czech Republic) and Yakima valley (USA). The levels of cohumulone were similar or slightly lower than the average content.

Keywords: hop trellis, alfa and beta acid content

References:
1) Verzele M., De Keukeleire D. Chemistry and analysis of Hop and Beer Bitter Acids. Elsevier, 1991
2) Analytica-EBC Methods, Fachverlag Hans Carl, Nurnberg, Germany, 2005
3) Hops and Hop products, Manual of Good Practice. EBC Technology and Engineering Forum, Fachverlag Hans Carl, Nurnberg, Germany, 1997

Trial growing low trellis hops in Czech Republic in 2009-2011

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Abstract:
Lack of labour for spring works, which escalated in the Czech Republic in 2007, brought a renewal of the idea in the cultivation of hops in low trellis. Low trellis was built on the hop garden of Hop Research Institute. The plants were planted in the autumn in 2008. The yield and chemical composition of the hop cones were found out on experimental variants in the years 2009-2011, which mainly represented the variety Saaz, Premiant and Sládek. Variety First Gold represented the control variant. The three-year production results showed that only variety Sládek can achieve profitability in this type of cultivation. The paper in conclusion is occupied with the use of Czech mobile hop picking machine HUN-30 in the Czech hop growing areas.

Keywords: Hops, Humulus lupulus L., low trellis, yield, chemical analysis, mobile hop picking machine

References:
JEŽEK, J.: KŘIVÁNEK, J.: CINIBURK, V.: KOŘEN, J. Development of low trellis in Czech Republic. In SEIGNER, E. (ed.). Proceedings of the Scientific Commission of I.H.G.C., Poland, Lublin, 19 - 23 June 2011. Hüll: Hop Research Center (Scientific Commission of International Hop Growers´Convention), 2011, p. 119. ISSN 1814–2206.

Energy consumption and quality control during hop kilning - latest results of an ongoing research project

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Abstract:
After harvest green hops show water contents of 70 to 80 % which must be reduced to approximately 10 %. During the hop kilning process fresh air is heated to approximately 65 °C. With the help of the company WOLF Anlagen-Technik GmbH & Co. KG a pilot heat exchange system was integrated into an industrial size hop kiln of the Hallertau region in Germany. Goal of the project was to decrease the consumption of fossil resources for heating and to recover volatile hop components from the exhaust air of the hop kiln.
The collected materials were analysed using methyl-tert-butyl-ether (MTBE) extraction and subsequent gas chromatography. For quantification of the results an isotope dilution array was used. The heat exchanging process generated condensate which was analysed in the same way. Additionally green and dried hops were analysed on their humulone and lupulone concentrations (HPLC, acc. to EBC 7.7) as well as hop oils (GC-MS: ether extraction). Eight substances (myrcene, linalool, terpineol, nerol, farnesol, geraniol, α-humulene, β-caryophyllene) were quantified and summed up to at least 0.25 mg hop oils per hour and m3 exhaust air.
The exhaust air had a mean temperature of 31.3 °C. Post heat exchange the air left the HE-unit with a mean temperature of 23.2 °C. In the mean, fresh air was heated from 15.0 °C to 24.4 °C. The heat recovery resulted in an efficiency of approx. 50 %. These figures led to a calculated 38 kW/h energy recovery or a reduced energy consumption of 20.8 %. According to the current price level (0.8 € per litre fuel oil) a theoretical saving of approx. 200 € per day the kiln is running resulted. Depending on the harvested hop varieties and the region hops are kilned over a period of up to 30 days per year.

Keywords: hop kilning, hop oils, humulones, lupulones, energy recovery

References:

Section 4

Hop Cultivation and Management

Posters

The main physiological disorders of roots covered seedling in hop

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Abstract:
The results of many experiments and researches in hop, but also for example in forestery practice, confirm the fact, that the roots covered seedling has higher biological value in comparison with roots non-covered seedling. Although its production is considerably expensive, unlike roots non-covered seedling it has higher rooting, stands which come from it have higher productivity, longevity, and thus also economic prosperity. Advantages of this seedling lie in qualitatively higher physiological parameters, which together with genetical quality and morphological parameters decide about its biological value and they are significantly influenced by abiotic factors. Presented work analyzes abiotic disorders of roots covered seedling and their symptoms, on of which thorough knowledge we can elaborate and realize adequate proposal of preventive and revitalization measures, and thus ensure its production in high quality.

Keywords: hop, roots covered seedling of hop, physiological disorders, abiotic stresses, unsuitable nutrition

References:

Analysis of activity of inclined belt conveyors with different belt structure when separating impurities from hops

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Abstract:
An important role within a picking line is played by inclined belt conveyors, whose function is significantly related to removing any undesirable impurities as well as to quality of the final hop product.
The measurement task was to analyze quality of work performed by two inclined conveyors set with belts of various surface structure. At the same time the costs of inclined conveyors production was assessed.
The belt which is nowadays used with inclined conveyors has lugs on its surface which take a form of truncated cones. The designed belt has its lugs formed by longer segments in skew lines. For both of the belts the two research teams designed and made laboratory inclined conveyors whose incline and circumferential belt speed can be changed. In between the two inclined conveyors was inserted a belt conveyor which enabled an even dosage of hops. Comparison of the inclined conveyors quality of work was based on determination of percentage amount of the material separated from the hop cones taken at the outlet of the hop picker, and further from the waste after the set of hop picker inclined conveyors. The inclined conveyors perimeter speed was set at 0.55 m.s-1 and the dosing conveyor perimeter speed was set at the value of 0.24 m.s-1. The calculation determined the timing of the input material in a way to reach a passableness of 0.1 kg.s-1. The measurement was carried out at three inclines of the conveyors.
The detailed analysis and statistical assessment of measured values prove that both of the belts separate impurities approximately the same way. Simultaneously were compared the costs of production and life of the designed inclined conveyor. Life of the fully utilized belt is relatively short – about 3 years. This is influenced by the belt material (rubber) and an uneven load together with tension in the belt caused by the central guide ridge. A disadvantage of the separating belts central guide is a frequent breaking of the drive and driven drums due to a combined bending load of the shaft.
Life of the designed belt is guaranteed by the producer for the period up to 15 or 20 years. A longer life relates to the material used (PVC), which does not tend to ageing as it is with rubber, and newly designed guide wedges, which do not cause undesirable tension inside the belt as they are placed on the belt edge, having a much smaller cross-section then with the current belt. Specific weight of the designed belt is about 25% smaller compared to the current one, which is why the belt structure is also less strained when in operation. From the point of the economical assessment, usage of the designed inclined conveyors is definitely favourable, as the return on the costs of exchange of 6 inclined conveyors in the picking line makes only 3.13 years at a comparable separation quality. Based on the presented analysis, a comparison of a set of six former and six new inclined conveyors will be carried out in regular operation of the picking line this years harvest season.
Supported by the Ministry of Agriculture of the Czech Republic, Project No. QI101B071.

Keywords: inclined belt conveyor, separation, hop cones

References:

JECH, J. et al. (2011): Machinery for plant production 3 - Machines and equipment for post-harvest and storage technologies. Profi Press s.r.o., Prague, 368. (In Czech and Slovak)

PODSEDNÍK, J., RYBKA, A., HEŘMÁNEK, P., HONZÍK, I. (2011): Evaluation of the quality of the separation of admixtuters from the hop product grown on high and low trellis constructions. Mechanizace zemědělství, roč. 61, č. Zvláštní vydání, Prague, 193-200. (In Czech)

RYBÁČEK, V. et al. (1980): Hop Production. SZN, Prague, 426. (In Czech)

RYBKA, A., HEŘMÁNEK, P., HONZÍK, I., JOŠT, B. (2011): Analysis elimination of the impurities in harvesting and processing of hops. Mechanizace zemědělství, roč. 61, č. Zvláštní vydání, Prague, 9-15. (In Czech)

SRIVASTAVA, A.K. et al. (1993): Engineering Principles of Agricultural Machines. ASAE, Michigan, USA, 601.

Cover Cropping Systems for Organic Hop Production in the Yakima Valley, USA

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Abstract:
Organic hops are currently not required for beer to be certified organic in the United States. However, a 2010 ruling by the National Organic Standards Board dictates that certified organic beer will have to be brewed with certified organic hops beginning in 2013. US organic hop production has been increasing in recent years and is expected to continue to increase (Turner et al., 2011). Weeds are traditionally controlled through repeated tillage and mowing inputs in organic hopyards, but attaining adequate weed management remains problem. Cover crops are often used in organic hopyards to increase soil organic matter, improve soil quality, and create a more manageable groundcover in the inter-row. This experiment addresses this issue by analyzing the impact of different cover crops on weed populations in an organic hopyard. The experiment was conducted in a certified organic hopyard near Yakima, Washington. Eight treatments with two controls and six different cover crops were established and their effect on weed populations was observed. Treatments consisted of several grasses and leguminous combinations that were chosen for their ability to produce copious aboveground biomass, survive compaction, and grow vigorously. The hop plants and cover crops were first established in the summer of 2010. Cover crops were established again in April 2011 and April 2012 using a split-plot design with three different hop varieties and three replicates. Data were collected for percent groundcover, plant count, and biomass by species for the cover crops and weeds. Hop yield and relative acid content were also evaluated. Preliminary data suggest a significant reduction in weed biomass and groundcover under cover crops when compared to controls.

Keywords: Hops, Organic, Cover Crops, Weeds

References:
Turner S. F., C. A. Benedict, H. Darby, L. A. Hoagland, P. Simonson, J. R. Sirrine and K. M. Murphy. 2011. Challenges and opportunities for organic hop production in the United States. Agronomy

Organic hop growing in Czech Republic

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Abstract:
In the Czech Republic the organic growing of hops began regularly in 2009. Hop Research Institute Ltd. motivated the first three growers from Saaz and Tirschitz hop growing region to enter the growing organic hops. Experiments with alternative protection of hops were implemented in 80s of the last century in the Hop Research Institute. In 2012, organic acreage reached a total of 10.7 hectares of hops. The variety Saaz and Premiant are cultivated. Chmelařství, cooperative Žatec processes hops for granules type 90. Beer tasting are used to evaluate the attributes of conventional and organic hops.

Keywords: Hops, Humulus lupulus L., organic hops, growing, protection, organic beer

References:

Production and propagation of virus free hops in the Czech Republic

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Abstract:
Hop as a perennial and vegetatively propagated crop is endangered by virus diseases. They cause economic losses showing in lower yield and lower contents of alpha bitter acids, which are very important for beer production. To solve this problem an improvement programme has been started in the Czech Republic. Production of virus free plant material was started in 1980s and propagation began in 1991. The obtaining virus free plant material by heat therapy and meristem culture, as well as the propagation of plant material and certification procedure are described. Health control of important viruses i.e. ApMV, HLV and HMV is perform by ELISA. Dot-blot hybridisation is used for HLVd determination. Health status is assessed at all level of propagation process: *in vitro* cultures, technical and space isolation, high quality mother plants and propagated material in glasshouses, hop gardens and nurseries.
The results were obtained with the help of financial support from the project of MSM 1486434701 granted by Ministry of Education, Youth and Sport Czech Republic.

Keywords: Hops, Humulus lupulus L., virus free rootstocks

References:

Section 5

Hop, Indispensable Raw Material for Brewing

Lectures

The origin of staling aldehydes: hop versus malt

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Abstract:
Notwithstanding the consensus that has been reached in brewing industry about the key role aldehydes play in the sensory perception of beer ageing[1], the possible mechanisms for their formation are still under discussion. Aldehydes in aged beer might result from iso-α-acids degradation[2] and Strecker degradation of amino acids[3], among several other reaction pathways according to the literature, but apparently also from interactions between both cited pathways, deduced from our study. Based on model reactions, it was observed that degradation of iso-α-acids, concomitant with beer flavour instability, is generating Strecker aldehydes in the presence of corresponding amino acids. With the aim to elucidate the relative significance of the pathways leading to these ageing flavours, this was further explored in brewing practice, focussing on the marked instability of *trans*- vs. *cis*-iso-α-acids during storage.
The relationship between the storage-induced degradation of (reduced) iso-α-acids and the increase in staling aldehydes was studied in authentic beer samples, exclusively bittered with commercial isomerised hop extract, *cis*-iso-α-acids, *trans*-iso-α-acids, dihydroiso-α-acids and tetrahydroiso-α-acids, respectively. All beers were derived from unhopped basic 2-hL brews, prepared under identical conditions. Unhopped and conventionally bittered beers (pellets, CO_2-extract) served as references.
The results of this study revealed that the contribution of iso-α-acids to the development of aldehydes in aged beer through degradation and subsequent reaction with amino acids, as was illustrated in model reactions, is only a minor pathway in the beer matrix. In this study, the decisive factor with a large impact on the aldehyde content of both fresh and aged beers, was the type of malt used for brewing. Based on the present findings it is concluded that critical factors related to the brewing process and the beer matrix itself, such as malt and wort quality, are more important regarding the flavour stability of beer, irrespective of the mode of bittering.

Keywords: aldehydes, beer, iso-α-acids

References:
1. De Clippeleer et al. EBC 2011.
2. Hashimoto and Kuroiwa 1975; Hashimoto and Eshima 1977, 1979; Kaneda et al. 1989.
3. Soares da Costa et al. 2004; Vanderhaegen et al. 2006; Guedes de Pinho and Silva Ferreira 2006; Suda et al. 2007; Saison et al. 2009.

Towards understanding the origin of American hop aroma in beer

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Abstract:
Notable differences exist between American and European hops in terms of the types of flavor they contribute to beer. Brewers tend to describe the former as contributing citrusy, fruity and in some instances floral aromas to beer, while the latter are often described as contributing herbal, tobacco, woody, and spicy notes. Single-hop brewing trials were carried out with Cascade, Chinook, Centennial, Citra, Simcoe, East Kent Goldings, Hallertau Mittelfrüh and Saaz to identify hop-derived volatiles that contribute to "American" hop aroma in beer. The eight resultant beers were evaluated using both sensory and instrumental analyses. The sensory analysis identified Centennial as having the highest piney and green hop aromas while Citra and Simcoe were characterized as being very fruity, citrusy, and tropical (especially Citra). The Hallertau Mittelfrüh was similar to the East Kent Goldings and these two were more floral and rose like than the Saaz sample and more melon and DMS than the American varieties. Volatile analysis of the beer samples was performed using a stir bar sorptive extraction (SBSE) of the beer samples followed by separation on a gas chromatograph with FID and mass selective detectors. The SBSE procedure resulted in over 300 peaks being separated by the GC. Principal components analysis of the GC data yielded distinct separations between the American varieties Centennial, Chinook, Citra, and the European varieties such as Hallertau Mittelfrüh thereby identifying a clear separation between the citrusy hops and the European (non-citrusy) hop. Mapping the sensory data to the instrumental data via Generalized Procrustean Analysis revealed interrelationships between the aromatic descriptors and the individual volatile compounds that were separated by the GC.

Keywords: Hop aroma, beer flavor, American hops

References:

A sensory method developed for screening of dried hop cones for specific aroma traits

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Abstract:
Aroma-type hops are essential components of the brewing process imparting many of the specific flavours and aromas that define the style of beer. Hop breeding research aims to develop selections that would confer new, unique sensory characteristics to beer.

The identification of promising seedling plants relies primarily on chemical analysis of secondary plant metabolites, because collecting sensory data on breeding populations of several hundreds of individual plants is a significant challenge. This challenge was addressed by developing a sensory methodology to rapidly screen a large number of dried hop samples for a specific aroma trait. In Year 1, hop selections (n210) were assessed for the perceived intensity of citrus aroma by a 6-member trained sensory panel. Reference standards were used, and control hop samples were chosen for exhibiting weak, moderate and strong intensities of citrus aroma, respectively. The reliability of the screening method was established in Year 2, where 52 of the hop selections evaluated in Year 1 were re-screened. The developed sensory method was found to provide robust sensory data pertaining to intensity of citrus aroma exhibited by dried hop cones.

In the second step of the research, a smaller number of hop selections (n41) were screened for the presence/absence of 9 aroma traits (grapefruit, passionfruit, feijoa, banana, hay, ginger, onion/garlic, sweaty, and piney). The purpose was to identify hop selections with salient, specific aroma nuances that could be of interest to the brewing industry. Differences were established between the hop selections, especially in terms of frequency of citation of passionfruit, onion/garlic and ginger aroma notes. The presentation concludes with a discussion of the need for and challenges of developing a simple beer model system to assess the impact of a particular hop selection on beer flavour.

Keywords: hop aroma, sensory evaluation, trained panel

References:

Elucidation and evaluation of hop polyphenol influence on lager beer flavor and flavor stability.

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Abstract:
RESEARCH INTENT:
Hops are a relatively rich source of antioxidants such as the flavanoid polyphenols. Although the use of whole hops and whole hop pellets seems to be in decline, and thus a total contribution of polyphenols to beer is in decline, evidence exists to suggest that whole hop or hop solid material (cellulosic fraction) affects beer flavor and flavor stability. The goal of this research is to provide a better understanding of how, and more explicitly - which - hop polyphenols affect beer flavor and flavor stability.
METHODOLOGY:
Polyphenols were extracted from hop solids and examined for polyphenol (flavonoid) content and antioxidant potential. Sucessive fractionation by solid phase extraction was performed in order to elucidate the most potent antioxidant species. Beers were dosed with the polyphenol rich extract and cold or force-aged for several weeks. Fresh and aged beers were assessed by chemical and sensory analysis to determine the hop extract effect on lager beer flavor and flavor stability. Chemical analyses performed on the extracts and/or beers to identify antioxidants and antioxidant character include: Phloroglucinolysis, RP-HPLC-MS-ESI, FRAP, DPPH•, ESR, ICP-AES.
RESULTS:
Despite being 99% phenolic in nature, phloroglucinolysis indicated that (+)-catechin and and other flavan-3-ols represented only 2% of the hop extract material by weight (w/w%), with a procyanidin mDP of 2.72. The extract contained caffeic acid, methylated xanthohumols, flavan-3-ol-glycosides, flavanonol glycosides, flavonol glycosides and other unknown compounds. Fractionation of the extract allowed for identification of several classes of prenylflavanoids that could be further correlated with varying levels of antioxidant character via DPPH• antiradical capacity. Chemical analysis of the fresh and aged beers confirmed an anti-staling effect of the hop extract. Sensorially, beers treated with polyphenols were statistically different from beers that did not receive polyphenols.

Keywords: hops: polyphenols: flavan-3-ols: proanthocyanidins: flavonols: beer flavor: antioxidants

References:
1. Ting, P.L. et al. 2004.U.S. Patent: Method and composition for improving the flavor stability of malt beverages.
2. Irwin et al. 1991. The role of copper, oxygen and polyphenols in beer flavor instability. J. Am. Soc. Brew. Chem 49(3): p. 140-149.
3. Walters et al. 1997. Comparison of (+)-catechin and ferulic acid as natural antioxidants and their impact on beer flavor stability. 1. Force aging. J. Am. Soc. Brew. Chem. 55(2): p.83-89.
4. Forster, A. 2007. Influence of hop polyphenols on beer flavor, in Hop Flavor and Aroma in Beer: Int. Brew. Symp. Corvallis, OR.

5. McLaughlin et al. 2008. Bitterness modifying properties of hop polyphenols extracted from spent hop material. J. Am. Soc. Brew. Chem. 66(3): p. 174-183
6. Callemien and Collin. 2008. Use of RP-HPLC-ESI(-)-MS/MS to differentiate various proanthocyandin isomers in lager beer extracts. J Am. Soc. Brew. Chem. 66(2): p. 109-115

Beer's Bitter Structural Chirality (Solved)

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Abstract:
The longstanding question regarding the absolute stereochemistry of the cis and trans iso alpha-acids (1) derived from the precursor alpha-acids (2) during the beer brewing process has been resolved via X-ray crystallography. This result not only elucidates in detail the molecular structure of humulone and several of its derivatives, but resolves confusion over the hops chemical isomerization mechanism which has lasted for greater than three decades. Future work into the structure-function relationship can now build on these results associating health benefit claims for mild to moderate consumption of beer with specific structural details of these bitter tasting molecules.

Keywords: Stereochemistry, Isohumulone, Tetrahydroisohumulone, Chirality, Crystalography, Isomerization

References:
(1) Keukeleire, D. De: Verzele, M. Tetrahedron 1971, 27, 4939-4945.
(2) Keukeleire, D. De: Verzele, M. Tetrahedron 1970, 26, 385-393.

Section 5

Hop, Indispensable Raw Material for Brewing

Posters

The use of hop-derived polyphenolic extracts to improve beer flavour and flavour stability

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Abstract:
The use of whole hops as a raw material in brewing suffers from a number of drawbacks. The paramount problem is that the amount of aromatic and flavouring constituents in hops varies considerably from batch to batch according to the climatic and soil conditions prevailing during hop cultivation, the harvest time, the time elapsed between harvesting and drying, as well as the drying and storage conditions. Therefore, the use of whole hops during brewing is inappropriate for delivering a final product with consistent sensory qualities. In modern brewing technology advanced hop products, derived from pure resin extract, are used increasingly in order to achieve a more consistent hoppy character in existing brands and to develop new beers with innovative flavour profiles. However, advanced hop products do not contain hop polyphenols that are considered as potent antioxidants. This is important in view of the major challenge of achieving flavour stable beers. The present study describes a method for brewing beer comprising the addition of polyphenol-rich extracts prepared from hops at specific steps during or after the brewing process. The proposed method enhances the mouthfeel, the reducing power and the stability of beer. Furthermore, the addition of hop oil essences to beer in combination with hop polyphenolic extracts were assessed by sensory evaluation. Fully advanced hopped beer, made with a combination of hop polyphenol extract, isomerised hop alpha acid extract and hop aromatic oil, was preferred over a conventionally hopped beer. This finding shows the potential of the novel hopping technology to improve beer flavour.

Keywords: Beer flavour, hop polyphenols, hop essential oils, sensory evaluation, flavour stability

References:

Stability of hop beta acids and their decomposition products during natural ageing

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Instability and inclination to oxidative changes are determining properties of hop beta acids in dependence on storage conditions. This property has an impact on both hop storage and beer brewing as well. In the course of one month storage about 50% of pure beta acids decompose under aerobic conditions at ambient temperature. After six months degree of degradation is more than 90%. Rate of degradation is significantly slower in leaf hops, especially during the first 6 months of storage. Protective effect of lupulin glands biomembranes substantially reduce rate of raw hops deterioration. High sensitivity of beta acids to oxygen has proven after their application on the surface of solid carriers (cellulose powder, silica sand). Rate of oxidation depended on storage temperature. More than 80 % of beta acids were lost after 24 hours at a room temperature. Analysis of mass spectra obtained by DART-orbitrap MS fingerptinting shows that big proportion of degradation products form compounds that arise from cyclisation of side isopentenyl chains of beta acids (tricyclolupulones, hydroxytricyclolupulones). The beers hopped by partially degraded beta acids at a dose of 20 g/hl showed distinctive sensorial bitterness. Bitterness was not in any way an unpleasant and lingering. Structural changes of beta acids in the course of ageing explain one of the reasons why bittering potential of older hops do not decline proportionally to alpha acid content decrease. Comparison of mass spectra of beta acids decomposition products in a natural hop ageing and laboratory wort boiling test shows that some unpaired fragments are found besides of many identical fragments. It indicates that beta acids decomposition products resulting from natural hop ageing and wort boiling are not fully identical.

Keywords: beer, beta acids, bitter taste, hop, HPLC analysis, lupulones, mass spectrometry, oxidation reactions, tricyclolupulones

References:

Haseleu, G., Intelmann, D., Hofmann, T. (2009): Structure determination and sensory evaluation of novel bitter compounds formed from beta acids of hop (*Humulus lupulus* L.) upon wort boiling. *Food Chem*. 116, 71-81.

Section 6

Hop, Beer and Health

Lectures

Screening of a commercial hop extract for substances active in neural stem cell differentiation

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Abstract:
Hops, the medicinal plant 2007 (Germany), comprises a special class of flavonoids, the prenylflavonoids. These flavonoids in common have a prenyl, geranyl, farnesyl group or a pyrano- or furano-ring and show multiple medicinal effects. Xanthohumol, the most concentrated Prenylflavonoid, is well known in cancer prevention because of inhibitory effects in three steps of carcinogenesis. But also minor, lower concentrated flavonoids show interesting activities. 8-Prenylnaringenin for example shows remarkably high growth inhibition in leukemic cells with multi-drug resistance [1]. Furthermore, this flavanone is the most potent phytoestrogen known so far. 8-prenylnaringenin imitates activities of estradiol and is therefore discussed for hormone replacement therapy [2]. The risk of cerebral infarctions raises after menopause in women. Estradiol seems to prevent brain damage in neurodegenerative processes [3]. A relevant effect of estradiol is to increase the number of newly differented neurons in brain [4]. By differentiation of adult stem cells, which are found also in human brain, the number of neurons is increased. This study addressed the question, if rare prenylflavonoids from hops can promote neuronal differentiation in stem cells, since hop flavonoids may cross the blood-brain barrier [5].
In a commercial hop extract rich in prenylflavonoids, the xanthohumol content was reduced by some recrystallization steps. Following an activity-guided fractionation, a chalcone was identified as a potent inducer of neuronal differentiation. Differentiating activity thereby was quantified by a luciferase-assay based on doublecortin, a marker for young neurons. Remarkably, the activity of this prenylchalcone was significantly higher than the activity of the well-known differentiation factor retinoic acid.

Keywords: estradiol, neurodegenerative diseases, xanthohumol, 8-prenylnaringenin

References:
[1] Diller RA, Riepl HM, Rose O, Frias C, Henze G and Prokop A. Planta Med 2007: 73: 755-761.
[2] Heyerick A, Vervarcke S, Depypere H, Bracke M and De Keukeleire D. Maturitas 2006: 54: 164-175.
[3] Alonso de Lecinana M and Egido JA. Cerebrovasc Dis 2006: 21 Suppl 2: 48-53.
[4] Tanapat P, Hastings NB, Reeves AJ and Gould E. J Neurosci 1999: 19: 5792-5801.
[5] Rad M, Humpel M, Schaefer O, Schoemaker RC, Schleuning WD, Cohen AF and Burggraaf J. Brit J Clin Pharmaco 2006: 62: 288-296.

Effect of xanthohumol on brewing yeast cells

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Abstract:
Xanthohumol (XN), a prenylated chalcone from hops and beer, is a phenolic compound that has received considerable attention in recent years. This compound has a range of interesting biological properties that may have therapeutic utility: anti-inflammatory, antioxidant, antilipoperoxidative activities as well as antiangiogenic, antiproliferative and apoptotic effects, mainly assessed in vitro studies that reasonably suggest a potential chemopreventive activity.
In order to understand how xanthohumol affects a brewing yeast's metabolism, yeast viability and vitality were studied during the production of a xanthohumol enriched beer (10 mg/L xanthohumol) on a 50 L pilot plant scale. The results showed that yeast viability was slightly decreased by xanthohumol, but on the other hand yeast vitality in the xanthohumol enriched brewing trials was slightly better. The content of higher alcohols, esters and organic acids was similar to the control in all the xanthohumol enriched brewing trials, however the content of sulphur dioxide, acetaldehyde and saturated fatty acids was lower in the xanthohumol enriched brewing trials.
Due to the fact that yeast vitality was slightly decreased by xanthohumol, experiment with zymolyase was done. Zymolyase is known to exhibit beta-1,3-glucanase and a residual protease activity and thus affects yeast cell wall integrity.
We used treatments with zymolyase to find out if xanthohumol addition can increase or decrease cell wall damage. Two strains (brewing and wild type) were used for comparison. Yeast cells treated by xanthohumol are significantly more sensitive to zymolyase treatment. As xanthohumol increased the sensitivity for zymolyase treatment, this fact should be used for increase of therapeutic effect for supresion of yeast infection.

Keywords: Xanthohumol, yeast, yeast vitality, yeast viability, cell wall

References:
Magalhães P.J., Carvalho A.B., Gonçalves L.M., Pacheco J.G., Guido L.F., Brányik T., Rodrigues P.G., Kuncová G., Dostálek P., Barros A.A.: The Impact of Xanthohumol on a Brewing Yeasts Viability, Vitality and Metabolite Formation. J.Inst.Brew. 117(3) 368-376 (2011).

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