SCIENTIFIC COMMISSION OF THE INTERNATIONAL HOP GROWERS´ CONVENTION

COMMISSION SCIENTIFIQUE DU COMITE INTERNATIONAL DE LA CULTURE DU HOUBLON

WISSENSCHAFTLICHE KOMMISSION DES INTERNATIONALEN HOPFENBAUBÜROS

Report on the meeting of the Scientific Commission of the I.H.G.C. from 19 – 23 June 2011 in Lublin, Poland

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With this meeting of the Scientific Commission (SC) in Poland we have continued the tradition to bring together hop scientists from all around the globe in the name of the International Hop Growers´ Convention to discuss their work. Following the invitation of Prof. Dr Ewa Solarska from the University of Life Sciences in Lublin, as host and Dr. Elisabeth Seigner from the Bavarian State Research Center for Agriculture, as chairperson of the SC, 52 participants from 13 hop growing nations joined this meeting.

In 22 papers and 22 posters the hop scientists presented their research on the following topics:

- Classical hop breeding
- biotechnological work
- molecular investigations on hop
- chemical analysis of hop compounds
- management of hop diseases and pests
- physiology of hop
- improvements in hop production

Our special thanks go to Prof. Dr Ewa Solarska for her excellent job in organizing this meeting. We say thank you to her team from the university and all persons who undertook various tasks and thus helped to make this meeting a memorable event for us. Certainly we are also grateful to the various sponsors who supported the mission of the SC by their financial backing. We highly appreciate the various contributions of our hop scientists and experts in providing their knowledge and ideas in papers, posters and discussions which made this meeting a big success for the sake of the hop and brewing industry.

All contributions are compiled in the Proceedings which are available online on our website http://www.lfl.bayern.de/ipz/hopfen/10585/index.php.

Summary

Session I: Hop Breeding

Excellent brewing quality, increased levels of resistance to diseases and pests, enhanced stress tolerance and high yield are the main objectives in all hop breeding programs. This was also shown by breeders from Australia, Poland, Germany, and the Czech Republic in their contributions. From the economical point of view high alpha varieties are imperative, although aroma varieties with special aroma qualities are increasingly in demand by brewers. A relatively new trend is the development of aroma varieties with unique, clearly distinctive aromas and flavors, as presented by Australian and German scientists. In particular hops with fruity, citrous and flowery aroma compounds are of special interest. Based on GC-MS (gas chromatography-mass spectroscopy) the Australian researchers already provided a comprehensive description of the odor active essential oil compounds characteristic of their new varieties e.g. Southern Saaz, Summer, Stella, Topas, and Galaxy.

At current, also hop varieties with higher contents of special compounds such as beta acids, xanthohumol and other biologically active polyphenols are being developed. These compounds would open new options for the application of hops in pharmaceutical-medicinal fields. In this context the new Czech variety Vital may arouse interest, because Vital shows the double content of desmethylxanthohumol in comparison to other varieties.

Moreover, breeding of dwarf hops especially adapted to the growth on low trellis systems is pursued. They are the basic prerequisites so that growers can exploit the economic and ecological advantages of this innovative production system.

Breeding for powdery mildew (PM) resistance is still a major objective as contributions from Germany and Poland showed. During selection for resistance in the greenhouse and in hop fields under natural infection conditions promising PM resistant wild hops could be detected, as presented by the Polish breeder. At the Hop Research Center Huell wild hops were screened for novel resistance mechanisms at cell level by using tissue specific staining techniques and fluorescence microscopy. In future, various resistance mechanisms should be combined via cross-breeding in order to achieve long-term resistance towards powdery mildew.

At the Czech hop research institute currently wild hops from North Ossetia are being characterized. Also in this case, wild hops are considered as the essential genetic resources in providing new sources for resistance to diseases, pests, and climatic stress.

Session II: Biotechnology

The contributions from Slovakia and the Ukraine demonstrated that meristem culture and *in vitro* propagation were standard procedures used in breeding and in the production of virus-free planting material. *In vitro* storage is getting more and more important for the preservation of healthy base material in breeding or of special genetic resources. Furthermore, suspension cultures are being examined in how far they can be used in biofermenters for the synthesis of secondary plant metabolites such as polyphenols and flavonoids. In addition,

more efficient, optimized protocols for the regeneration of complete plants from any explantat are being worked out to create the basis for successful genetic engineering. Another application of *in vitro* techniques is the use of colchicin in doubling the chromosomes of *in vitro* shoots as crucial step for the production of tetraploid mother plants in breeding for triploids. The chromosomal background of monoecious hop plants showing predominantly male or female flowers with few flowers of the opposite sex has been examined by Slovenian colleagues. Using the flow cytometer they detected in the progenies of various diploid parents that monoecious plant with male phenotype and few male flowers had a triploid set of chromosomes, while female hops with a few male flowers were diploid. The sex expression in hops is still full of secrets and studies with triploids, tetraploids and aneuploids are necessary to provide more details.

Session III: Molecular investigations on hop

The molecular characterization of the hop genome is in rapid progress. As a new marker technology the diversity arrays technology (DArT) has been presented by Australian researchers. On the basis of a genetic diversity study using 92 hop genotypes and 730 DArT markers the precision, high through-put and efficiency of this new marker technology has been proven. Furthermore, the genome analytical work is focused on the identification and mapping of QTLs (quantitative trait loci) for complex traits such as the synthesis of xanthohumol and desmethylxanthohumol as presented by a Czech scientist. Applying the QTL analysis a Slovenian group had identified loci (regions on the genome) which were associated with the synthesis of alpha acid, yield formation, and with the resistance to *Verticillium* wilt. Slovenian researchers also conducted comprehensive studies to identify genes directly involved in the resistance to *Verticillium* wilt. Thereby, they found astonishing sequence homologies to two already known and well-characterized resistance genes Ve1 and 2 in tomato. Now after these first steps of successful identification and cloning of these genes they are aiming to develop markers for selection and to conduct functional tests of the isolated genes in model systems.

Comprehensive knowledge on the various regulator elements to control key genes of the synthesis of biologically active flavonoids pave the way to create hop varieties especially tailored for the requirement of the pharmaceutical and medicinal sector. Thus, a research team from the Stuttgart-Hohenheim University achieved the metabolic engineering of key genes involved in the biosynthesis of flavonoids by in the insertion of a regulator gene from Arabidopsis into the landrace Tettnanger. The transgenic Tettnanger plants formed reddish-pink cones in which higher levels of anthocyanins, rutin, and isoquercetin could be detected. Following the same objective, Czech scientists studied various lupulin gland specific transcription factors of hop which regulate the expression of different genes of the flavonoid pathway. Functional analysis of hop transcription factors was performed in Arabidopsis, petunia and tabacco. In addition, it was found that various hop transcription factors of the lupulin gland biosynthesis could be disturbed by hop stunt viroid infections. These findings may explain the losses of alpha acid content observed in several hop cultivars which were described by Japanese and US American researchers.

Session IV: Chemical analysis of hop compounds

Several researchers are concerned with polyphenols which have aroused interest since the 1990th due their newly discovered health-promoting effects. Also in brewing beer polyphenols have gained in importance in contributing to stabilize the beers due to their antioxidative potential. Thus, studies have been conducted to find out the various factors influencing polyphenol or flavonoid biosynthesis in various systems: e.g. in leaves of field grown hops as well as in *in vitro* shoot and callus cultures. Currently, the polyphenol composition of 160 hop cultivars from the Huell world hop collection is being analyzed using the UHPLC technique which allows a rapid and improved chemical separation of the hop compounds. The objective is to clarify whether based on quercetin and kaempherol glycosides a clear characterization and differentiation of the various aroma and bitter varities can be achieved.

Moreover, the impact of the isomerization of the alpha acids on the composition of hop resins and essential oils has been presented in a poster by a Czech colleague.

Session V: Management of hop diseases and pests

Despite all efforts in breeding for resistance plant protection remains a crucial factor to prevent significant loss of yield and quality caused by diseases and pests. Especially, in organic hop production alternative measures to control pests and disease are urgently needed. Here probiotic microorganisms so-called effective microorganisms (EM) may provide a solution. As reported by a Polish scientist, EMs with fermented plant extracts showed the same efficacy to control downy and powdery mildew as well as two spotted spider mite and aphis as already established means such as Myco-Sin as plant strengtheners, whey and quassia, respectively. Generally, copper-based fungicides play the decisive role in controlling fungal diseases, in particular to control downy mildew in organic hop production. However, since copper compounds have been classified as very hazardous by European environmental protection agencies, efforts are being made to reduce the amount of copper applied. Investigations in Germany revealed the possibility that by using two new copper hydroxide formulations the amount of copper to effectively control downy mildew could be reduced by 50 % - in particular when applied in combination with plant strengtheners such as Frutogard. The effect of copper on physiological processes like the photosynthesis and transpiration has been investigated in the Czech Republic. Both procedures showed increased levels after the application of copper fungicides, but returned to the initial levels 10 -14 days later. Also the accumulation of the heavy metal in leaves and cones had been analyzed after repeated copper fungicide treatments.

In field trials in Poland the full efficacy of Movento with the active compound spirotetramat to control aphids has been confirmed. In this way besides Confidor and Teppeki also Movento increases the currently available spectrum of effective means to control aphids in Polish hop yards as part of the anti-resistance strategy. Since Hexythiazox has been used as miticide in the Czech Republic since 1996, its biological efficacy was checked. In these tests

spider mite populations collected from different Czech hop growing regions still showed high susceptibility towards the active compound thiazolidine. This product together with a newly registered miticide out of the group of the bifenazates contribute to the successful realization of an integrated plant protection strategy. In parallel to the testing of the efficacy of various pesticides also an extensive screening for residues of pesticides was conducted for hop and hop products in the Czech Republic (CZ). In all hop samples the detected residues were always well below the fixed maximum residue levels confirming the quality standards of hop produced in the CZ.

Favored by climatic and agrotechnical changes as well the hop snout weevil *Neoplinthus* turned out to cause more serious problems in Slovenian hop gardens. Since there are no effective insecticides available to control this pest, up to 70 % of damage has been observed. Various alternative control measures are being tested, also entomo-pathogenic nematodes, although their practical use seems to be difficult. Also in Czech hop yards different species of hop flee beetles had been found, but only *Psylliodes attenuatus* caused damage. Another pest in the Czech Republic (CZ) was the alfalfa snout beetle which could be controlled most efficiently by applying insecticides in spring, when the young adult pests emerged to the surface during a phase of soil warming.

In Germany over the last years increased symptoms of wilt have been observed in hops, even hop cultivars assessed as being *Verticillium* tolerant showed wilting leaves and bines. To clarify whether specific cultivation measures or new, more aggressive pathotypes of *Verticillium* were the cause for this increased wilting, from different regions of the Hallertau the *Verticillium* fungus has been isolated from hop bines with wilting symptoms. Subsequently, the fungal isolates were examined using a molecular APLF based approach and artificial inoculation tests in the growth chambers which were conducted by Slovenian researchers. In these tests for the very first time mild as well as lethal *Verticillium* pathotypes could be confirmed in the Hallertau. First of all, a molecular test for mild and lethal *Verticillium* types directly from bines should be elaborated. Furthermore, phytosanitary measures are being taken to “cure” wilt infected hop yards and to prevent further spread of *Verticillium*. In addition, bioantagonists such as specific bacterial or fungal strains should be identified to ward off infections with *Verticillium* in hops.

In the USA an efficient strategy for the management of powdery mildew on hops is being elaborated. Besides other approaches eight effective synthetic fungicides with five different modes of action have been registered. However, successful control very much depends on the timing of the application of a specific active agent and its mode of action to the various growth stages of leaves and cones. Comprehensive studies in 2007 and 2008 showed that the cones were highly susceptible to PM infections in late July to early August. Especially during this critical period highly effective fungicides had to be applied to ensure best cone quality at harvest. In Slovenia since 2005 the damage on hop leaves caused by *Cercospora* and *Phoma* has increased dramatically. Before an effective disease management could be started, crucial knowledge on pathogenicity, aggressiveness of the fungal pathogens and epidemiology had been gained in comprehensive artificial infection tests and by monitoring using a Burkard spore

trap. At the same time the resistance of different hop cultivars could be assessed and *in vitro* tests gave information on the efficacy of different fungicides. The analysis of meteorological data from 2000 - 2009 revealed that severe outbreaks of *Phoma* and *Cercospora* infections could be correlated with intensive and frequent rain falls.

Virus infections can lead to significant losses of yield and quality under conducive weather and stress situations depending on the susceptibility of the hop variety and the virus species. At the Czech hop research institute 43 hop varieties were tested in 2005 and 2006 for hop mosaic (HMV) and apple mosaic virus (ApMV) using the DAS-ELISA (double antibody sandwich enzyme-linked immuno sorbent assay). A very high rate of infection with ApMV became obvious in 54 % of tested hops, 39 % were infected by HMV and 28 % showed both viruses. This finding showed that as part of the quality management high priority should be given to virus-free gene resources and virus-free planting material.

Session VI: Physiology of hop

From 2007 – 2009 the rate of photosynthesis and transpiration had been measured using Czech hop varieties and breeding lines at different developmental stages revealing a significant effect of the year and genotype. In warm and sunny years the new breeding lines showed higher rates of photosynthesis than the older varieties. Moreover, the highest rate of both physiological processes was detected at stage BBCH 32 (= code for the phenological development of the plant) with rapid elongation growth and at the transition from the vegetative to the generative stage (BBCH 65). When reaching the technical ripeness both rates declined.

Session VII: Improvements in hop production

The production of hops on low trellises in comparison to high trellis systems shows significant economic and ecological benefits. However, for this new production system special machines and devices are necessary which have to be adapted to the use in low trellis systems. In a project Czech experts developed for this purpose e.g. special cutting and pruning devices, sprayers and tilling machines. In Germany a mechatronic device for fully automatic stringing was constructed and tested in practice. A sensor controlled device for single plant treatment during the watering procedure was developed which made the work with hop much easier and in particular decisively improved the protection of the user while watering with pesticides. By utilizing optical sensors in combination with special nozzles the amount of pesticides could be reduced drastically, since the nozzles only opened when the bine or wire were recognized

Excursion and information about hop growing in Poland

On the second last day the participants had the opportunity to visit five hop gardens in the Lublin region. In this area approximately 80 % of Polish hops are produced (total acreage 1,700 ha). By acreage Marynka is the most important Polish cultivar. Showing a content of 8-9 % alpha acids and pleasant aroma it is assigned as dual purpose hop. The hop stands were promising in producing good yield, however, the Polish hop growers are bothered by a very bad contract situation.
Besides conventionally produced hop yards an organic hop garden could be visited. The grower reported about the successful application of effective microorganisms (EM) to control

diseases and pests. He applied the EM in combination with the extract of stinging nettle, dandelion or tansy (*Tanacetum vulgare*).
On the last stage of this excursion at the farm of this organic hop farmer detailed information on the various fields of EM applications could be obtained.

I hope that all delegates got a lot of useful information in the lecture part as well as in the excursion. So that an abundance of information and new ideas can be taken home together with possibly already existing concepts for joint projects with partners from the hop and brewing industry.

The best of luck and success in all fields of hop research.

Dr. Elisabeth Seigner

Scientific Commission, IHGC July 2011

Papers and Posters presented:

I. Session: Hop Breeding

New hop (Humulus lupulus L.) aroma varieties from Australia
Whittock, S., Koutoulis, A.

Trends in hop breeding – new aroma and bitter qualities at the Hop Research Center Huell
Lutz, A., Kneidl, J., Seefelder, S., Kammhuber, K., and Seigner, E.

New knowledge in Czech hop breeding
Nesvadba, V., Krofta, K., Poloncikova, Z.

Vital – new Czech hop variety
Krofta, K., Nesvadba, V., Patzak, J.

Variability of wild hops (Humulus lupulus L.)
Nesvadba, V., Krofta, K., Marzoev, A., Pšenáková, I., Faragó, J., Mursaliev, M., Kyrdaliev, K., Polončiková, Z., Henychová, A.

Resistance mechanisms of different hop genotypes to hop powdery mildew
Oberhollenzer, K., Seigner, E., Lutz, A., Eichmann, R., Hückelhoven, R.

Breeding for resistance to hop powdery mildew in Poland
Skomra, U.

II. Session: Biotechnology

New biotechnological approaches of growing hop in the Ukraine
Melnychuk, M., Boiko, A., Kukovenko, V., Kliuvadenko, A., Likhanov, A., Dubrovin, V., Overchenko, V., Drozd, P., Shulga, V.

Use of tissue culture techniques to hop improvement
Faragó, J., Pšenáková, I., Faragová, N.

Development of triploid plants of hop (Humulus lupulus L.)
Trojak-Goluch, A., Skomra, U., Agacka, M.

Ploidy and sex expression in hop plants
Škof S., Čerenak A., Jakše J., Bohanec B., Javornik B.

III. Session: Molecular Investigations on Hops

A diversity arrays technology (DArT) platform for high-throughput genotyping of hop (Humulus lupulus L.)

Howard, E., Whittock, S., Jakše, J., Carling, J., Matthews, P. D., Probasco, G., Henning, J. A., Darby, P., Cerenak, A., Javornik, B., Kilian, A., Koutoulis, A.

Molecular mapping of QTLs for xanthohumol and DMX contents in hop
Patzak, J.

QTL mapping of Verticillium resistance and yield traits in hop
Jakše, J., Cerenak, A., Radisek, S., Satovic, Z., Luthar, Z. and Javornik, B.

Isolation and characterization of Verticillium resistance gene homologs in hop
Majer, A., Javornik, B., Jakše, J.

Transcription factor pap1/AtMYB75 regulates flavonoid production in transgenic hop (Humulus lupulus L.)
Gatica, A., Farag, M., Stanke, M., Born, U., Alheit, K., Matoušek, J., Wessjohann, L., Weber, G.

Functional analyses of lupulin gland-specific regulatory factors from WD40, bHLH and Myb families of hop (Humulus lupulus L.) show formation of crucial complexes activating chs_H1 genes.
Matoušek, J., Patzak, J., Kocábek, T., Fussy, Z., Stehlík, J., Orctová, L. and Durajsamy, G.

Complementation analysis of hop transcription factors using Arabidopsis thaliana genes in transient system and in transgenotes
Kocábek, T., Matoušek, J.

Hop stunt viroid (HSVd) disease causes alteration of expression of hop transcription factors from MYB, bHLH and WRKY families
Füssy, Z., Stehlík, J. , Patzak, J., Matoušek, J.

IV. Session: Chemical Analysis of Hop Compounds

Differentiation of the World Hop Collection by means of the low molecular polyphenols
Kammhuber, K.

Study of the production of secondary metabolites in shoot and callus cultures and field grown plants of hop
Ürgeová, E., Polívka, L., Faragó, J., Vaverková, S.

Effect of genotype and growing period on antioxidant activities of hop leaf extracts
Faragó, J., Pšenáková, I., Kraic, J.

Bioactive Compounds in Hop Cultivars Growing in Poland
Sosnowska, B., Solarska E.

Anticholinesterase activity of hops
Szwajgier, D., Borowiec, K., Solarska, E.

Influence of isomerisation on the composition of hop resins and essential oils.
Stasiak, M.

V. Session: Management of Hop Diseases and Pests

Probiotic microorganisms with fermented plant extracts in protection of organic hops
Solarska, E.

Downy mildew control in organic hops: How much copper is actually needed?
Weihrauch, F., Schwarz, J., Sterler, A.

The effect of application of copper fungicides on photosynthesis parameters and level of elementary copper in hops
Krofta, K., Ježek, J., Pokorný, J., Pulkrábek, J.

Movento - new insecticide for aphids control on hops
Korzeniowski, M., Solarska, E.

Hexythiazox, the miticide for spider mite (Tetranychus urticae Koch) control in Czech hops
Vostřel, J.

Pesticide screening programme of hops by V.F. HUMULUS
Vojtěchová, D., Kroupa, F.

Hop snout weevil (Neoplinthus tigratus porcatus Panzer) is the important insect pest of hop (Humulus lupulus L.) in Slovenia
Rak Cizej, M., Radišek, S.

Hop protection against alfalfa snout beetle (Otiorhynchus ligustici L.) with the help of meteorological data in Bohemian and Moravian hop gardens
Vostřel, J., Klapal, I., Kudrna, T.

Species of hop flea beetles (Chrysomelidae, Alticinae) attacking hop plants in Czech hop gardens
Vostrel, J., Klapal, I.

Studies of Verticillium wilt in hops
Seefelder, S. ,Drofenigg, K., Seigner, E., Niedermeier, E., Berg, G., Javornik, B., Radišek, S.

Development of a rapid molecular in-planta test for the detection of Verticillium pathotypes in hops and strategies for prevention of wilt
Drofenigg, K., Zachow, C., Berg, G., Radišek, S., Seigner, E., Seefelder,S.

Strategies for management of powdery mildew on hop cones
Nelson, M.E., Gent, D.H., Grove, G.G.

Management of Cercospora and Phoma leaf spot on hops in Slovenia
Radišek, S., Leskovšek, L., Javornik, B.

Evaluation of health status in hop varieties
Svoboda, P., Nesvadba, V.

VI. Session: Physiology of Hop

The physiological parameters of hop plant (Humulus lupulus L.)
Pokorný, J., Pulkrábek, J., Nesvadba, V.

VII. Session: Hop Production

Development of low trellis in Czech Republic
Ježek, J., Křivánek, J., Ciniburk, V., Kořen, J.

Development of a mechatronic device for fully automatic wire stringing in hops
Portner, J., Gobor, Z., Fröhlich, G., Kammerloher, T.

Sensor controlled single plant treatment in the pesticide application
Portner, J., Fuß, S.

Pesticide reduction through sensor implementation
Portner, J., Fuß, S.