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New Trend in Hop Breeding at the Hop Research Center Huell

Hop cultivars have been developed at the Hop Research Center Huell since 1926. Until recently, all breeding programmes pursued the objectives to develop aroma varieties of the classical European noble aroma type and high yielding, high-alpha varieties. Both groups fully satisfied the demands of growers and brewers worldwide. However, initiated by the US craft brewers and also taken up by other creative brewers worldwide novel hop-derived aroma and flavour notes in beer attracted much attention. Therefore, new breeding efforts started in 2006 which already brought forth several experimental lines which impart multifaceted floral, fruity and citrusy aroma impressions to beer. Two hop varieties introducing mandarin-orange or fruity-piney notes to beer have already been submitted for registration for Plant Variety Rights and others will follow.

Descriptors: hop breeding, hop aroma, flavour, fruity and citrusy notes, floral nuances

1 Introduction

Breeding research at the Hop Research Center (HRC) Huell has a long tradition. Due to severe infections of downy mildew causing dramatic losses of yield and quality in German hop production, the researchers at Huell started their first breeding programme in 1926. Improved disease resistance while retaining the noble aroma and flavour of the old land races like in Hallertauer Mittelfrueher (Mfr.) or in the Saazer "Formenkreis" (group of varieties including Saazer, Spalter and Tettnanger) were the major objectives for Huell cultivars. Pursuing these aims all hop breeding programmes at Huell and also worldwide were focused to develop hops which showed the typical aroma impressions of the old land races including Fuggle and Golding in representing the fine, classical hoppy aroma and flavour of European style, in combination with improved agronomic performance and multiple disease resistances. This trend in the aroma sector was continued until into the 1980s and early 1990s [1, 2, 3, 4, 5], since brewers seemed to be quite reluctant to accept new aroma types and notes in their beers [6].

In the early 1970s breeding objectives at the HRC Huell were changed to satisfy the new demands of brewers who wanted varieties with increased alpha-acid contents for a more economical way of bittering beers. Based on the English bitter hops Bullion and Brewers Gold, US breeders developed so-called high-alpha cultivars providing alpha-acid contents of more than 12 % and higher yields. In 1975 Comet was the first, but less successful cultivar, followed by Galena, Eroica and Nugget [6] which paved the way in this high-alpha sector. At Huell Herbert Ehrmaier developed the first German high-alpha cultivars with Hallertauer Magnum [4] in 1993 which was an exceptional success in finally representing the hop cultivar with the largest acreage worldwide. Hallertauer Taurus followed in 1995 and finally Herkules was released in 2006, the latest Huell high yielding, high-alpha cultivar.

Today, a clear distinction in aroma versus bitter hop varieties based on the aroma features and alpha-acid content is getting more and more complicated. Dual purpose hops suitable for bittering as well as for bringing special aroma impressions to beer have filled in the gap between both categories.

For a long time aroma quality of hop was only of concern for a specific part of brewers who considered hop-derived beer flavour as their marketing strategy and thus used higher hopping rates and did not buy hops on the basis of alpha-acid contents. Only this group of creative brewers thoroughly selected hop varieties based on origin, harvest time and growing region [7]. Especially, the craft brewers` scene, which started in the 1980s, driven by their special aroma and beer philosophy revolutionized the use of hop. They realized their ideas on the one hand in beers which were hoppier, more bitter and with higher alcohol contents such as the IPA (India Pale Ale) category. On the other hand, they were producing lower alcohol beers with substantial, unique flavour [8]. Today, this trend, which originally started in the USA, more and more inspires creative brewers worldwide who respond to special consumers` wishes for beers with greater character by utilizing different hop cultivars imparting special aroma, flavour and bitter qualities.

Already over the last decade aroma types with clearly distinctive, more fruity, flowery and spicy, but still typically hoppy aroma notes have been developed at the HRC Huell such as in the cultivars Saphir, Opal and Smaragd which significantly increased the portfolio of new aroma and flavour impressions for brewers.

Complying with the demands of brewers to create beers with unique, clearly distinguishing aroma characters a new breeding programme has been started at the Hop Research Center Huell in 2006 to develop hop varieties with floral, fruity, tropical and exotic

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flavours, irrespective of higher or lower alpha-acid contents. While this programme started in Germany only few years ago, commercial hop cultivars matching these novel aroma and flavour impressions are already available in the USA as well as in Australia and New Zealand. Already in 1972 derived from the U.S.D.A. breeding programme Cascade was released with its characteristic citrus and grapefruit-like notes. Certainly this variety was selected in those days for its agricultural performance and as alternative for Fuggle, but soon it turned out that Cascade contained unique aroma components and thus was already used in the early 1990s in a specialty niche [7] in the brewing sector and is still the most widely used hop cultivar in this craft brewers` scene. Furthermore, Amarillo, Centennial, Citra, Simcoe and various other hops from private US breeding programmes are in demand with craft brewers in these days for their unique, clearly distinctive aroma profile. Also the Australian aroma breeding programme, which started in the 1980s [5], brought out hop varieties named as Topaz, Stella, Galaxy and Summer which impart fruity flavours to beer comparable to grapefruit, passion fruit, apricot, melon, black currant and blackberry and even from flowers [9]. Moreover, HortResearch Riwaka Hop Research Centre (now Plant & Food Research) in New Zealand had by 2000 developed a triploid hop variety named Nelson Sauvin reminding in its aroma and flavour contribution to the grape variety Sauvignon Blanc which brings the typical white wine fruitiness [10].

Although, some of these hop varieties were already in use for decades, now in the hand of craft brewers and other creative brewers completely new flavour perceptions could be obtained with these hops through their ample use of hops combined with late addition in the whirlpool or even through dry-hopping as well as by the utilization of green, un-dried hops.

The key word “flavour hops” created by Charles N. Papazian, president of the American Association of Brewers, almost 20 years ago significantly influenced the current trends on the beer market and also inspired the breeding programme at the Hop Research Center Huell. The progress of this new trend in the Huell breeding work will be presented and discussed in the following.

2 Materials and Methods

2.1 Plant material

All specific crosses were conducted with hop cultivars and female breeding lines, respectively, in the hop breeding yard of the Hop Research Center Huell, Bavarian State Research Center for Agriculture, Huell-Wolnzach, Germany. Pollen was taken from selected males derived from own breeding programmes which were grown in a hop breeding yard with exclusively male genotypes in Freising, Germany. Four to six weeks after germination all seedlings from these crosses were screened for disease resistance or tolerance after artificial infection with powdery mildew (caused by *Podosphaera macularis* ssp. *humuli* Braun & Tak.) and subsequently with downy mildew (*Pseudoperonospora humuli* Miyabe. & Takah.) sporangia in greenhouse chambers. Seedlings which survived these infection tests were grown in a so-called “growth hall” from May onwards to estimate their vigour, cone set and their resistance/tolerance towards aphids and powdery mildew under natural infection conditions. Moreover, their flowers allowed the breeder to distinguish their sex. Plants with poor vigour, revealing growth abnormalities or high disease and pest susceptibility were culled. In late fall or in the next early spring, female seedlings were planted in the Huell breeding yard to assess their agronomic performance, resistance, aroma expression and also their chemical composition in the course of their three-year-testing. Individuals showing male flowers were transplanted to the male breeding yard in Freising. Satisfying vigour and growth as well as tolerance to Verticillium wilt, downy and powdery mildew under field conditions were further basic selection criteria. Advanced selections were grown in the Huell hop yard providing heavy clay soil and also in Rohrbach with sandy soil conditions in two replications with six plants each. Based on the organoleptic evaluation of ripe cones by the breeder hop lines with interesting aroma impressions in the field were harvested and chemically analysed. Some experimental lines presented here only exist as small plots at the hop breeding yard Huell due to their very early developmental stage, but their exceptional aroma justified their harvest and chemical analysis.

2.2 Analysis of cone qualities

The evaluation of specific aroma characteristics of the presented Huell breeding lines and of the reference cultivars Hallertauer Mfr. and Cascade was done organoleptically. The dried cones with a water content of approx. 10 % were assessed by the breeder and later on by a panel of hop experts consisting of brewers, traders and growers. In addition, the dried cones from the reference material and from the most promising Huell experimental lines were chemically analysed. The analysis of essential oils as well as of bitter compounds was conducted at the HRC Huell.

The alpha- and beta-acids were determined by the HPLC (high performance liquid chromatography) method according to ANALYTICA-EBC 7.7 [11]. The data presented here are based on the analysis of several samples from different locations and years. Details are given in table 1. The total oil content was measured by steam distillation following the methods ANALYTICA-EBC 7.10 [11], however, the value given in table 2 is only based on a single measurement of a composite sample representing a mixture of 2–3 harvest samples from the year 2011.

Table 1 Number and origin of hop samples analysed by HPLC

experim. lines/cv.	samples	location	year of harvest
2007/018/013	14	5	2008–2011
2007/019/008	8	4	2009–2011
2008/020/004	8	3	2009–2011
2009/001/718	5	2	2010, 2011
2009/002/706	4	1	2010, 2011
2000/109/728	73	13	2007–2011
2006/078/009	5	2	2009, 2011
2008/059/003	7	2	2009–2011
Cascade	4	1	2008–2011
Hallertauer Mfr.	10	2	2008–2011

Table 2 Bitter acids and total oil content

	EBC 7.7			EBC 7.10
experim. lines/cv.	alpha-acids (%)[1]	beta-acids (%)[1]	cohumulone (%)[2]	total oil [3,4]
2007/018/013	7.0–10.0	5.0–6.5	28–35	2.1
2007/019/008	9.0–12.0	4.5–5.5	23–26	1.5
2008/020/004	6.5–10.0	4.0–6.5	35–40	2.0
2009/001/718	7.4–8.5	3.1–3.4	27–28	1.6
2009/002/706	6.9–7.5	7.3–7.9	27–28	0.8
2000/109/728	18.0–23.0	4.5–6.0	22–30	4.4
2006/078/009	16.0–18.0	5.5–6.5	21–24	2.5
2008/059/003	17.0–19.0	5.0–6.0	22–27	3.8
Cascade	4.5–7.0	4.5–7.0	30–38	1.8
Hallertauer Mfr.	3.0–5.0	3.5–5.5	19–23	0.95

[1](%) w/w; [2]relative % of a-acids; [3]ml/100 g dried hop; [4]single measurement of a composite sample

The headspace gas chromatography (GC) was used to determine the essential oil compounds. One gram of dried hop cones was ground, 1 µl of enanthic acid ethyl ester was added as an internal standard, before the vial was sealed and was ready for the GC analysis. Headspace gas chromatography was carried out using a Dani 8500 gas chromatograph + FID (flame ionization detector) equipped with a Dani headspace sampler HSS 86.50. The polyethylene glycol fused silica cross bonded capillary column Permabond from Macherey-Nagel with 50 m x 0.25 mm ID and 0.23 µm of film thickness was used. The temperature programme started at 60 °C for 10 min, increased to 170 °C at 2 °C/min. and then to 200 °C at 6 °C/min., held for 20 min. The sample vial was kept at a temperature of 110 °C for 80 min. and then 5 ml of the headspace was injected into the column. The injector and detector temperatures were 200 °C and 210 °C, respectively. The carrier gas was helium, at a flow rate of 1.2 ml/min and the split ratio was 1:25. The various essential oil compounds were identified via mass spectrometry in cooperation with the Technical University Munich, Chair of Chemical Technical Analysis [12]. The majority of the hereby identified compounds was confirmed by using commercially available standards, while 2-methyl butyl isobutyrate and 2-methyl butyl 2-methyl butyrate were synthesized in the own laboratory for that purpose.

Table 3 Number and origin of hop samples analysed by head space GC

experim. lines/cv.	samples	location	year of harvest
2007/018/013	5	3	2008, 2010, 2011
2007/019/008	3	3	2010, 2011
2008/020/004	3	2	2010, 2011
2009/001/718	3	2	2010, 2011
2009/002/706	2	1	2010, 2011
2000/109/728	6	4	2007, 2008, 2010, 2011
2006/078/009	1	1	2011
2008/059/003	2	2	2011
Cascade	4	1	2008 - 2011
Hallertauer Mfr.	10	2	2008 - 2011

Based on headspace GC the area of each chromatographic peak assigned to a specific essential oil compound is reported. The mean values for the respective peak areas presented here are based on the analysis of two up to ten hop samples obtained from various locations and years, with the exception of line 2006/078/009 were data are only available for a single hop sample. Details are listed in table 3. Moreover, to provide better insight into the special aroma notes of each experimental line in comparison to Cascade and Hallertauer Mfr. the data set was shown as the relative aroma concentrations of each variety to one another, where essential oil substances within the specific aroma category citrusy, fruity, floral, herbal, spicy and woody notes were summed up.

Assignment of single essential oil compounds to specific aroma impressions was done based on the associations given by *Steinhaus and Schieberle* [13], *Kishimoto* [14], *Whittock and Koutoulis* [5], *Schönberger and Kostelecky* [15], *Perflavory, USA* [16] or on the authors` own perceptions.

3 Results and Discussion

3.1 Breeding history – Genetic background of the various crosses

For decades in the Huell breeding history experimental lines with fruity, wild American aroma notes which did not match with the selection criteria for typical European noble hop aroma characteristics were discarded, only some of these aroma types were retained when providing a source of increased resistance to powdery mildew and Verticillium or due to their potential for higher contents of alpha-acids. But in the meantime, the attitude towards hop aroma had completely changed and thus, starting in the summer of 2006 the first crosses were conducted with hops revealing the potential to transmit citrusy, fruity and floral aroma notes to the progeny. Until the season of 2011, thirty-three crosses were conducted following this breeding objective.

In 13 of these crosses the US cultivar (cv.) Cascade was taken as mother. Cascade originated from an open pollinated Fuggle seedling which itself brought traits from the English noble hop Fuggle and the Russian cv. Serebrianker. Thus, it was expected that this old US cultivar developed in 1955 and released in 1972 [17] had the potential to transmit the classical English aroma style combined with wild American hop characteristics from its unknown father. Cascade´s contribution to beer is described as pleasantly, medium in strength, well balanced in its bittering potential, and another crucial feature for being selected as mother in this breeding programme is its unique floral, spicy and citrus aroma and flavour when used in brewing beer. Among all flavour hops, which inspire creative brewers and especially the US craft brewers` scene, Cascade is at current the most widely used hop cultivar. Considering the father side, Huell breeding lines were selected which had the potential to transmit basic resistance or tolerance to powdery mildew and downy mildew as well as good agronomic performance. Considering specific aroma characters, the selection of suitable males was more challenging and less predictable, since aroma assessment and analytical data could only be compiled from sisters of these males and in only one case this father was

already used in crosses so that information was available from its progeny. Nevertheless, for some male pollinators, which derived from crosses of the Huell aroma cultivar Opal with attributed fruity aroma, it was expected that in the respective progeny a spectrum of fruity aroma nuances of European and American style contributed by both parents would be realized.

New differentiating aroma impressions were also tried to be produced in 15 crosses with Huell hop breeding material on both parent sides. Since all father plants – with only one exception – descended from Cascade crosses, in hybridizing them with Huell aroma cultivars or Huell aroma breeding lines also in these cases the intention was to combine traditional European hoppy aroma with so far not so traditional hop aroma notes conveying the impression of black currant and other fruits.

Furthermore, crosses which aimed to produce high yielding high-alpha varieties with broader disease resistance generated seedlings which finally turned out to match this breeding objective for fruity-citrusy and piney notes. In these crosses Huell breeding lines were used on the mother and/or the father side which possessed already introgressed wild North American germplasm which held promise for higher alpha-acid potentials and for typical American hop aroma characteristics. Moreover, it was expected that these parents would transmit their higher level of disease tolerance, in particular to Verticillium wilt which caused problems in the direct offspring of Cascade.

3.2 Selection of seedlings matching this breeding objective

All seedlings produced in the various crosses mentioned before were pre-selected for disease resistance or tolerance to powdery and downy mildew in the greenhouse and had to show good performance with regard to growth, vigour, cone set and cone shape in the growth hall.

Starting in spring 2008 up to now 2,200 pre-selected female individuals from all 33 crosses which were directed to create “flavour hops” were grown in the Huell breeding yard, while 251 male individuals were grown in the Freising male breeding yard.

Especially, in the progeny of the 13 Cascade crosses some seedlings were prone to show wilting symptoms due to Verticillium during their growth in the Huell hop yard which is highly infected with mild pathotypes of *Verticillium albo-atrum*. In addition, a num-ber of seedlings showed a mixture of female and male flowers with varying proportions. These hermaphrodite phenotypes occur often in the offspring of crosses between North American and European germplasm. Presumably, they are the result of incompatibilities between the different types of sex chromosomes and of autosomal sex determining factors [6, 18] found or presumed in the different *Humulus lupulus* subspecies. Already during their growth in the growth hall some seedlings from these “wide” crosses between Cascade and Huell males had shown growth abnormalities with extreme branching, drastically reduced length growth and failed flowering which might also be the result of genetic incompatibilities. All seedlings revealing developmental disorders and abnor-mal sex were discarded. Out of 1,361 female seedlings from these Cascade crosses, which have been grown in the Huell breeding yard since 2008, so far 77 experimental lines which showed broader disease tolerance, satisfying agronomic performance and in particular, promising organoleptic aroma evaluations were harvested starting in 2008 till 2011 and their cones were chemically analysed.

Fourteen breeding lines from seven different Cascade crosses which satisfied the breeder`s expectations during their two-year-growth as single plants were grown in replications in Huell and at another hop breeding yard in Rohrbach with different soil conditions for another two years.

2000/109/728, 2006/078/009 and 2008/059/003 derived from traditional high-alpha breeding programmes. Proving superior alpha-acid contents in combination with high yield they were already at an advanced stage of development when they attracted attention due to their distinctive aroma characters.

Pedigree of the eight promising experimental lines with special aroma features:

- 2007/018/013: Cascade x Huell male (Opal x wild PM resistant male)
- 2007/019/008: Cascade x Huell male (94/045/001 x wild PM resistant male)
- 2008/020/004: Cascade x Huell male (Perle x 2002/026/504)
- 2009/001/718: Cascade x Huell male (Opal x wild PM resistant male)
- 2009/002/706: Cascade x Huell male (Opal x wild PM resistant male)
- 2000/109/728: 94/075/758 x 97/060/720
- 2006/078/009: 94/075/758 x 2002/061/731
- 2008/059/003: 2000/118/716 x 2002/061/731

3.3 Aroma evaluation and chemical data

All these hops mentioned before had shown exceptional fruity, citrusy and floral notes along with herbal, spicy and woody aroma impressions when organoleptically evaluated by the breeder in the field and therefore they were harvested and chemically analysed. All these experimental lines from the different crosses reached their specific ripeness late in the season and were harvested around the 20th of September in each year using a picking machine. The cones of each hop sample were dried and stored at 4 °C. Short after harvest the aroma of the dried hop samples was evaluated by the breeder, while two to six weeks after har-vest the samples of the various new breeding lines and also from Hallertauer Mfr. and Cascade as reference material were assessed by a panel of hop experts bringing together the Huell breeder, hop traders and brewers including craft brewers from the USA.

The following aroma descriptions were given:

- Hallertauer Mfr. (reference for typical European style noble aroma): mild, piney, woody, earthy, slightly citrusy

- Cascade (reference for a typical "flavour hop" aroma): medium, floral, citrusy
- 2007/018/013: fruity aroma revealing a strong tangerine note with slightly sweet aroma impressions
- 2007/019/008: intensive, long lasting flowery aroma including various fruity nuances including passion fruit, grapefruit, gooseberry and pineapple; comparable to the bouquet of a fine white vine
- 2008/020/004: with various fruity aroma notes including citrus, melon, mint, banana and strawberry impressions
- 2009/001/718: strong, pleasantly fruity aroma revealing citrus-, water melon-, grapefruit-like notes plus a refreshing aroma note and the impression of honey
- 2009/002/706: fruity, slightly sweet aroma revealing honeydew melon and strawberry notes
- 2000/109/728: strong, pleasant, fruity, spicy, fresh notes
- 2006/078/009: with intensive fruity aroma including citrus and mint impressions and an especially strong banana note
- 2008/059/003: various fruity notes with a clearly perceptible pineapple note, but also floral impressions similar to lavender and slightly peppery

These aroma perceptions of the various experimental lines were supplemented with the respective chemical data, where Hallertauer Mfr. and Cascade were used as references with classical European aroma style and fruity-citrusy aroma impressions, respectively.

The HPLC data on bitter acids compiled in table 2 confirmed the breeder`s intentions to produce "flavour hops" with lower and higher alpha-acid contents as well. While the breeding lines 2007/018/013, 2007/019/008, 2008/020/004, 2009/001/718 and 2009/002/706 had alpha-acid contents varying from 6.5 to 12.0 %, 2006/078/009 and 2008/059/003 showed humulone contents of more than 16.0 % and 2000/109/728 up to 23 %. Beta-acid values in all breeding lines were lower, around 4.5–6.5 %.

The cohumulone proportion of the alpha-acid analogs ranged from 22–40 % in those hops which derived directly from Cascade crosses and was lower (21–30 %) in the Huell germplasm based hops (Table 2). Despite great efforts so far, the role of high cohumulone contents above 30 % on poor bitterness quality and inferior brewing value could neither be fully confirmed nor invalidated [19].

Concerning their yield only a rough estimation of their expected potential could be obtained, since almost all experimental lines were at an early stage of development. They are quite promising with yields ranging from 1,800 to 2,200 kg /ha for those lines with lower alpha-acids and reaching 2,500 kg/ha in the high-alpha lines. Line 2009/001/718 and 2009/002/706 were harvested for the very first time and thus, their yields were not determined.

All lines being preselected for resistance or tolerance towards powdery and downy mildew also showed these behaviours under field conditions. In addition, all advanced selections proved tolerance towards mild Verticillium pathotypes.

Today hop addition during the brewing process is often conducted based on the oil content in ml/L wort as the aroma relevant measurable parameter [20]. Therefore, in preparation for the brewing trials the oil content was determined for all these hops (Table 2). In almost all experimental lines clearly higher oil contents were detected in comparison to Hallertauer Mfr., but similar levels to Cascade. Quite astonishing was the high oil content of 2008/059/003 and in particular of 2000/109/728 with 3.8 and 4.4 ml/100 g dried hop, respectively.

Table 4 shows the compiled data of the various essential oil compounds based on headspace GC which is the standard technique in the laboratory of the HRC Huell for the rapid assessment of seedlings from all breeding programmes. The GC headspace data set of these lines represents a compilation of samples produced at different locations and from different years (Table 3).

As expected, great variability of these aroma compounds was observed reflecting different growing conditions, seasonal effects and in addition, a wider range of variation in the analysis of the essential oils using GC (data not shown). Before discussing the results, it should be mentioned that when using headspace GC certainly only the volatile compounds could be determined, while less volatile or nonvolatile components of the hop matrix were missing.

Out of 76 peaks of the GC chromatograms obtained from the eight most promising experimental lines 39 identified essential oil compounds could be assigned to specific odour and in few cases also to defined flavour impressions comprising fruity, citrusy, floral, spicy, woody and herbal aroma notes. The allocation of the various oil compounds to these specific aroma and flavour perceptions was done based on the assignments given by Steinhaus and Schieberle [13], Kishimoto [14], Whittock and Koutoulis [5], Schönberger and Kostelecky [15], Perflavory, USA [16] and on own perceptions of the respective reference substances. When analysing these experimental lines two more prominent peaks occurred on the GC chromatogram which only appeared as background peaks in former breeding lines and Huell cultivars following the classical aroma breeding goals. One of these new peaks in the GC chromatogram of 2007/018/013 appearing directly after the beta- and alpha-selinene peaks amounted to 5.2 % of the total oil content and thus had the same expansion as the humulene peak. In 2000/109/728 this unknown oil compound was 0.22 %. Another peak occurred in the GC run of four here described breeding lines (2008/059/003, 2000/109/728, 2009/001/718 and 2006/078/009) after the cadinene peak representing 0.2–1.4 % of the total oils. This oil peak was already detected in the Huell cultivars Opal and Smaragd with attributed special fruity notes, but was missing in Cascade and Hallertauer Mfr.. These so far unknown peaks and other more prominent peaks (S10, S13, S14b, S15, S15b see Table 4) which already occurred in Hallertauer Mfr., but with minor expansion, should be investigated as soon as possible using MS-GC (mass spectrometry in combination with GC).

Despite the relatively small number of substances identified so far, and being fully aware of the remarkable range of variation in the analysis of the essential oils in hop samples [20] this series of data compiled in table 4 clearly demonstrate the exceptionally distinctive aroma of all experimental lines in comparison to Cas-

Table 4 **Essential oil compounds of eight Huell experimental lines depicted as area of GC peaks (mean value x 10^3) in comparison to Cascade and Hallertauer Mfr. based on headspace GC: 6 citrusy, 7 fruity, 4 floral, 9 herbal, 3 spicy/terpenic, 10 woody compounds including 9 with no odour /flavour assignment, 1 off-flavour (off-f.) and 9 non-identified substances**

		experimental lines								cultivars	
essential oil compounds		2007/018/013	2007/019/008	2008/020/004	2009/001/718	2009/002/706	2000/109/728	2006/078/009	2008/059/003	Cascade	Hallertau. Mfr.
α-terpinene	citrusy	54	81	52	72	60	193	92	110	34	32
limonene	citrusy	477	747	298	613	716	912	930	338	378	129
β-citronellol	citrusy	10	0	0	25	0	37	32	51	12	64
nerol	citrusy	45	52	48	50	42	90	49	116	72	24
β-cubebene	citrusy	36	41	45	42	29	31	45	51	60	36
2-decanone	citrusy	219	59	128	140	156	146	78	113	48	78
2-methyl butyl isobutyrate	fruity	2914	2099	1867	1795	4166	3895	3804	1615	2163	1264
2-methyl butyl 2-methylbutyrate	fruity	128	94	57	24	142	98	224	52	52	39
methyl nonanoate	fruity	78	39	12	61	57	48	44	23	47	47
2-undecanone	fruity	146	136	201	151	166	170	220	139	105	181
isoamyl acetate	fruity	383	179	226	240	355	277	237	236	239	278
4-methyl-2-pentanone	fruity	740	655	413	836	542	424	581	764	522	645
isobutyl isobutyrate	fruity	2097	988	1460	941	2560	2405	1813	1001	1258	897
ocimene	floral	557	501	551	412	410	908	575	1033	590	345
pentadecanone	floral	22	0	5	53	9	24	31	0	39	97
linalool	floral	202	202	337	255	94	151	524	325	197	426
geraniol	floral	45	24	31	12	20	24	0	26	17	11
α-pinene	herbal	763	665	643	455	479	1083	835	1416	777	431
2-methyl-3-buten-2-ol	herbal	13620	8985	9849	7999	15327	5995	7752	6330	11798	14569
α-cubebene	herbal	5	0	17	34	20	9	23	0	0	14
α-selinene	herbal	645	1560	901	671	1230	87	1145	952	131	82
β-selinene	herbal	675	1355	859	679	1167	123	1087	899	156	131
farnesene	herbal	40	0	150	614	195	0	0	13	167	0
2-nonanone	herbal	503	107	221	178	259	1576	661	472	98	255
E-β-ocimene	herbal	138	81	40	258	118	2200	274	1452	82	38
β-bourbonene	herbal	0	0	90	0	3	0	0	0	5	5
tri-decanone	spicy	0	0	43	26	37	25	39	46	35	43
myrcene	spicy	35228	41317	52314	42636	19525	152750	68616	183305	36603	13425
β-pinene	spicy	2275	2065	2349	1855	1727	3485	2487	4523	2607	1595
sabinene	woody	255	524	145	1982	302	1766	837	3001	199	28
γ-terpinene	woody	19	19	18	26	19	48	44	120	17	20
perrilene	woody	136	111	175	145	100	173	159	234	192	120
α-copaene	woody	139	47	80	195	126	190	271	402	132	193
aromadendrene	woody	123	34	437	76	53	74	48	135	77	0
humulene	woody	2170	227	311	4892	312	6665	4733	1241	1997	7457
γ-muurolene	woody	96	31	94	180	75	214	214	348	95	205
cadinene	woody	246	52	103	389	126	500	494	784	204	488
caryophyllene oxid	woody	17	7	16	26	9	19	21	0	20	36
β-caryophyllene	woody	792	181	502	1914	359	3330	2692	1872	853	2349
enanthic acid methyl ester	odourless or unknown	710	311	703	387	466	712	679	412	256	576
2-octen-4-one	odourless or unknown	119	184	61	152	148	202	204	96	80	60
methyl 6-methyl heptanoate	odourless or unknown	493	303	317	389	298	521	341	474	220	238
α-ylangene	odourless or unknown	74	44	46	27	21	35	22	80	23	54
calarene	odourless or unknown	132	4	68	98	38	224	92	158	253	229
4-decenoic acid methyl ester	odourless or unknown	414	108	78	258	176	353	203	155	106	210
viridiflorene	odourless or unknown	71	22	216	33	22	12	29	29	6	6
4,8-decadienic acid methyl ester	odourless or unknown	162	150	106	310	177	295	365	166	97	180
selinadiene	odourless or unknown	516	0	645	21	332	0	0	586	8	0
dimethyl sulfide	off-f.	74	67	78	107	77	185	109	181	91	43
S10	not identified	438	739	613	537	522	1023	1591	1494	669	307
S13	not identified	35	16	16	11	37	115	44	46	5	1
S14b	not identified	115	539	349	456	64	531	89	583	215	41
S15	not identified	138	81	40	258	118	2200	274	1452	82	38
S15b	not identified	85	111	106	88	64	83	109	88	54	35
S16	not identified	61	89	27	57	32	42	17	142	76	65
S16b	not identified	51	40	67	36	43	37	58	62	61	43
S19	not identified	0	5	0	4	5	0	24	19	0	0
S20	not identified	211	241	228	140	128	173	365	186	83	266
S24	not identified	64	40	55	56	35	61	55	55	75	54

cade as typical “flavour hop” and in particular to Hallertauer Mfr. as reference for the classical European aroma type.

Myrcene was found as the main oil compound in the new Huell breeding lines varying between 36 and 83 % (percentage of all essential oil compounds identified not shown). A high myrcene level is typical for Cascade and several other US hop varieties which is in contrast to that found in the noble European hop Hallertauer Mfr.. With the exception of line 2009/002/706, they all showed myrcene contents as high as Cascade or even significantly higher as found in the high-alpha breeds 2000/109/728 and 2008/059/003. On the other side, it is well-known that the myrcene content varies significantly depending on location, seasonal effects, harvest time and storage conditions. Moreover, beta-pinene as the second highest compound further contributed to the spicy, green character. In brewing, myrcene is considered as the crucial feature of the green hop aroma and is often found in dry-hopped beers, but at very low levels in beers with standard hop addition (kettle-hopped beer) which also applies for pinene in beer [20].

Despite the high contents of humulene and beta-caryophyllene in the lines 2000/109/728 and 2009/001/718 which are comparable to those in Hallertauer Mfr., these sesquiterpenes will according to recent findings based on threshold analysis in beer [14] not contribute to the beer aroma. Which specific essential oil compounds really contribute to woody, piney, earthy notes in beer is still under investigation [21]. Figure 1 provides insight into the relative aroma concentrations of each variety to one another, where normalized values of each category herbal, spicy and woody odour and flavour are summarised. Based on these results, the new Huell selections 2009/001/718, 2000/109/728 and 2006/078/009 may have similar potential as Hallertauer Mfr. for imparting herbal, spicy or woody nuances to beer. In particular, 2000/109/728 already attracted attention during the organoleptic evaluation due to its piney-fresh aroma nuances.

Already during the evaluating of the aroma quality of these experimental lines their fruity and citrusy odour was outstanding. Definitely, exceptionally high contents of citrusy and fruity essential oil compounds were found in all experimental lines in the chemical analysis when compared to Cascade and Hallertauer Mfr. (Fig. 2).

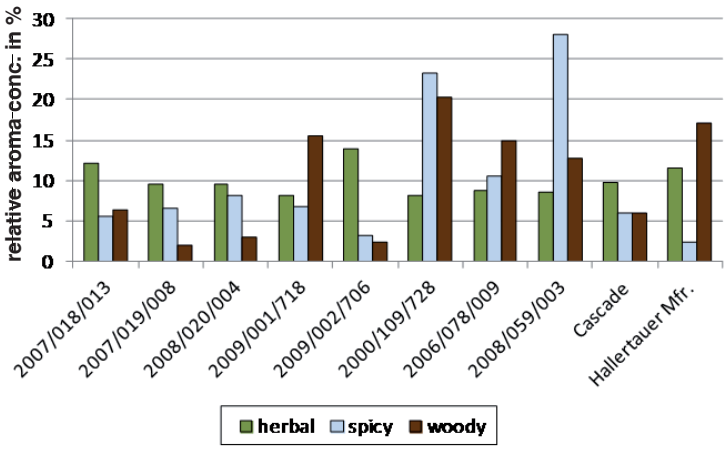

Fig. 1 Relative aroma concentrations of each variety to one another with focus to herbal, spicy and woody notes based on headspace GC

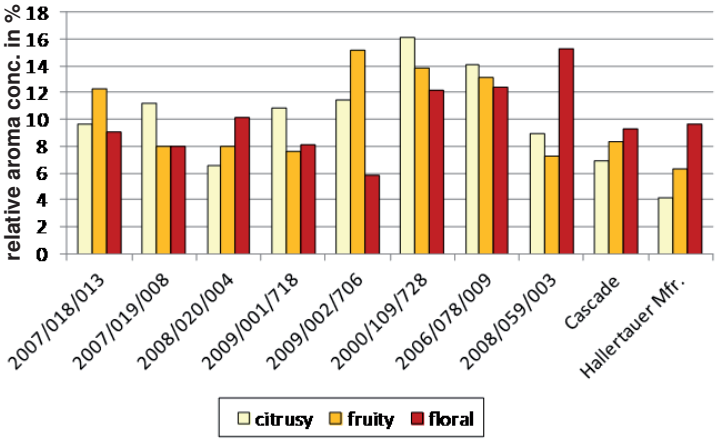

Fig. 2 Relative aroma concentrations of each variety to one another with focus to citrusy, fruity and floral notes based on headspace GC

Here in particular, the similar or even significantly higher contents of limonene, nerol and 2-decanone as citrusy substances along with 2-methyl butyl isobutyrate (2MIB) and isobutyl isobutyrate (IBIB) as fruity compounds should be mentioned. Chemists and brewers are still trying to elucidate which of these hop-derived compounds with citrusy or fruity odour and flavour nuances (Table 4) really contribute to these aroma impressions in beer. Only recently, 2MIB and IBIB were found along with isoamyl isobutyrate in the New Zealand cv. Nelson Sauvin. In this context two volatile thiols, 3-mercapto-4-methylpentan-1-ol and 3-mercapto-4-methylpentyl acetate, in synergy with 2MIB [22] could be identified as the crucial components being responsible for the Sauvignon Blanc aroma in beer, which also gave rise to the name of this hop cultivar.

The high linalool contents (Table 4) of 2008/020/004, 2006/078/009 and of 2008/059/003 and the significantly enhanced geraniol level in 2007/018/013 when compared to that in Hallertauer Mfr. may contribute to floral-citrusy aroma impressions in beer [23]. Especially, when taking into account that geraniol, linalool and also nerol can contribute to an increased citrusy aroma perception via specific synergistic transitions during the fermentation of beer [24].

Certainly, as already mentioned before the composition and amount of the essential oil compounds of the hops presented here varied significantly depending on the microclimate and the soil conditions of the respective growing location. Moreover, clear seasonal effects showed in the data sets of the various hop samples harvested in different years (data not shown), while the time of harvest for each hop was vital. These factors which influence the content of alpha-acids and the content and the composition of essential oils were already addressed in recent investigations by *Bailey* in examining the sensory properties of dry-hopped lager beer produced with hop samples harvested at different times and crop locations [25].

The authors are fully aware of the difficulties in assigning aroma information obtained from dried cones of a specific hop variety which was based on organoleptic assessment or on chemical analysis of the volatile essential oil compounds to its flavour in beer. In particular, since the complex processes involved in the development of aroma and bitterness quality during the brewing of beer are still far from being fully understood. Starting already in

the 19th century researchers tried to elucidate the secrets of hop aroma. Today more than 300 oil compounds have been identified [26], but their impact on hop aroma in beer is only partly explored [15]. So far a few hop substances have been identified as indicator substances for perceived aroma and flavour in beer. Beside linalool, other compounds such as geraniol, beta-citronellol, myrcene or ethyl 4-methylpentanoate and 4-mercapto-4-methylpentan-2-one (4MMP) have been identified as so-called "character impact compounds" which really contribute to perceived aroma impressions in beer due to their low sensory threshold [14, 15, 20, 23].

The already remarkable complex interaction of aroma compounds which contribute to beer aroma and flavour is getting more and more unpredictable when including the influence of additive, synergistic or masking interactions [23] and of different brewing processes and hopping regimes [21].

In addition to the essential oils, the composition of the bitter fraction as well as polyphenols, they all contribute and influence the aroma and flavour perceptions in beer [19]. Therefore, brewing trials are urgently needed to get at least a first impression about the flavour potential of these new Huell breeding lines.

3.4 Brewing trials by others

First brewing trials with eight of these new Huell breeding lines were performed by various brewers and thus, a rough idea about their potential to impart distinctive aroma notes to beers could be gained.

For example, in single-hopped beer brewed with the Huell experimental line 2007/018/013 aroma notes reminding to tangerine, orange, lime and pear as well as to floral nuances could be perceived [27]. These brewing trials also revealed the Sauvignon Blanc aroma of line 2007/019/008 and the multifaceted fruity notes of 2008/059/003 with pineapple, banana, citrus, strawberry and melon impressions [27]. Another brewer used the line 2007/018/013 as exclusive hop addition in significant quantities (250 g/hl) to produce a "Bavarian" American Pale Ale (Toft, pers. comm.), whereby in particular a pronounced mandarin orange aroma and flavour could be perceived in this beer. Moreover, recent trial brews proved the fruity-piney aroma impressions of 2000/109/728 (Schönberger and Gahr, pers. comm.) and its extraordinary aroma and flavour characters.

At present, more brewing trials are being conducted with all these breeding lines presented here using various brewing procedures including traditional hopping as well as dry-hopping where completely different aroma and flavour characteristics might be produced.

Based on these brewing trials, the breeding lines 2007/018/013 and 2000/109/728 have been submitted for registration for Plant Variety Rights to the European Plant Variety Office at Angers, France. Line 2007/019/008 and 2009/002/706 will follow.

4 Conclusion and Summary

For the first time the German breeding work brought forth hops with fruity, citrusy and floral aroma notes which are urgently sought by brewers worldwide who want to create clearly distinctive beers in the premium sector. With these first Huell breeding lines, which can be included in the group of "flavour hops", the defined goal was achieved to combine traditional European aroma style elements mainly based on essential oil components with spicy, herbal, woody notes with more unusual – non-hoppy compounds reminding to various fruits and flower fragrance notes. Moreover, these breeds were especially selected to allow growers an environmentally sound and economically advantageous production of these hop varieties due to increased disease resistance and enhanced agronomic effectiveness. Several hop varieties with convincing brewing trials by imparting fruity, citrusy and floral aroma and flavour notes to beer were or will be made available for brewers after harvest 2012.

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