Final report

Project from October 2014 to October 2016

The selection of stronger flavour within the UK hop breeding programme.

submitted by Dr Peter Darby on behalf of Wye Hops Limited, a subsidiary company of the British Hop Association.

Summary

It is anticipated that the demand for British hops will be greatly influenced by the craft and cask ale sectors who use flavour hops to produce a product characterised by more intense hop flavours. Currently, the demand for such hops is frequently being met in the UK by imports. British hop growers require suitable new flavour varieties incorporating the specific requirements for UK hop production. Since 2011, the development of flavour hops has been a primary objective of the Wye Hops programme and this project assessed the first progenies from this new initiative. The project aimed to identify potential new varieties and future parents, evaluating and comparing breeding techniques whilst looking for analytical indicators of strong flavour. The project assessed individual hop seedlings in the field derived from crosses made 2011-2013. Male progeny were assessed during July. Female progeny with an economic yield of cones in September were individually harvested to provide samples under code to a trade panel of merchants, brewers and suppliers who assessed the quality and intensity of the aroma of the dried cones. Those samples highlighted in the assessment were analysed for their composition. From records of agronomic and quality attributes, 32 individuals with more intense aroma, suitable to advance to yield plots or to use as future parents, were identified and propagated. A pedigree breeding strategy produced almost all the selections and inbreeding was not found to give any marked advantage. Breeding for specific oil composition, although successful, did not develop individuals with strong aroma. Relatively high total oil content and, in particular, high monoterpene content and high levels of thio-alcohols characterised several of the selections giving strong aroma.

Introduction

Traditionally, hops have been classified as either bitter or aroma. Bitter hops are high in alpha-acids and impart bitterness to beer during the boiling phases of the brewing process whilst aroma hops have a specific, desired essential oil composition which imparts aroma and flavour to beer when added later in the process. Recently, a new class of hop variety has been distinguished in which the alpha-acid content is well above that of a traditional aroma hop yet which is used primarily for imparting strong and distinctive flavour. The term “Flavour Hop” has been coined to describe these new varieties and examples include *cv.* Cascade from the USA, *cv.* Galaxy from Australia and *cv.* Nelson Sauvin from New Zealand.

Flavour hops have been championed by the craft brewing movement which started in the late 1970s in the USA. These small and micro-breweries produce a premium product characterised by more intense flavours and a higher alcohol content than mainstream commercial beers. According to the US Brewers Association, craft beers represented about 12% of US beer sales in 2015 with sales growing at 13% pa in an otherwise slightly declining (-0.2%) US beer market. The demand for hops from craft brewers is disproportionately high and some estimates are that nearly 43% of US hop production is to supply this small sector. In the UK, the craft sector is much smaller than in the USA with an estimated 2.5% of market share in 2015 but with a 23% pa growth rate. CAMRA’S *Good Beer Guide 2016* reported that 204 new breweries opened in the UK in 2015, taking the total to 1,424. It is also strongly influencing the cask ale sector which represents 17% of UK on-trade sales. Thus, the UK is reflecting the USA and it is anticipated that the demand for UK hops will be greatly influenced by the requirements of the craft and cask ale sectors in future years.

At present, the demand for flavour hop varieties by the UK craft and cask ale sectors is frequently being met by imports of hops. The flavour hop varieties sought are difficult to grow successfully in the UK, lacking adaptation to the UK latitudes which results in much later maturity than the commercial British crop. Furthermore, these ex-UK hop varieties often lack resistance to the spectrum of diseases present in Britain, notably to wilt disease. Many of the desired varieties are protected by Plant Variety Rights or patents which preclude them from being grown generally. To compete in this market and provide an alternative to imported hops, British hop growers require suitable new flavour varieties reflecting the latitude, light intensity, pest and disease spectrum, and agronomic requirements of the UK.

The hop area in the UK at harvest 2015 was 895 ha, localised in areas of Kent and Sussex, Hereford and Worcester, with a production of 27,130 Zn (1,356.5 tonnes). The industry has been stable with only small changes in area or production since 2005, reflecting a change in market away from the commodity alpha-acid market. More than 60% of the area is now occupied by aroma varieties, mainly *cv.* Goldings and *cv.* Fuggles which are both old varieties selected by the named hop growers in 1790 and 1875 respectively. However, few of the British varieties would be classed as flavour hops although some accessions in the National Hop Collection are currently being re-assessed for this purpose.

Perception of flavour and, in particular, flavour intensity is subjective and appropriate analytical parameters do not yet exist. It has been suggested that the compounds responsible for the distinct flavours are mainly thiols present in very low amounts in the essential oils. Although more than 40 thiols have been characterised in hops, detection of these is at the limits of current analytical technology and new thiol compounds continue to be identified. For example, in *cv.* Cascade methyl-sulfanyl-pentan-2-one (MSP) and S-3-(1-hydroxyhexyl) cysteine have been proposed as the determinants of the distinctive flavour whilst in *cv.*

Nelson Sauvin it is 3-sulfanyl-4-methylpentan-1-one. It is likely that most of the compounds responsible for distinctive flavour are still unknown.

To retain its importance in the international hop market and to safeguard the future of the hop industry in the UK, the British Hop Association (BHA) created a wholly-owned subsidiary company, Wye Hops Ltd., in 2007. As well as maintaining the National Hop Collection, this company is delivering a new hop breeding programme following the closure of the EMR programme at Imperial College, Wye. Since 2011, the development of flavour hops has been a primary objective to sustain future hop production in Britain, aiming to produce new hop varieties, both conventional and dwarf types, with more intense aromas combined with improved pest and disease resistance and superior agronomic traits. Experience has been that more intense aroma in the dried cones usually translates into stronger flavour in beer. This project assessed the first progenies arising from this new initiative.

<u>Objectives</u>

The project aimed to identify potential new varieties amongst the progeny derived from crosses made 2011-2013 growing as 3,006 individual plants in the field. Those individuals showing good agronomic adaptation as growing plants combined with high aroma intensity in their dried cones would be taken forward by propagation and establishment of multi-plant plots suitable for yield measurement and provision of sufficient quantities for pilot brewing trials.

The project also aimed to make selections from the progenies suitable to add to the germplasm collection as future parents for flavour in the on-going crossing programme. In particular, such parents would include males with improved pest and disease resistance.

The project used diverse approaches to increasing flavour in British hops and a further aim was to evaluate and compare these breeding techniques to allow the planning of efficient future crossing programmes. Pedigree breeding had been used with specific mother varieties, notably *cv.* Cascade, or from specific germplasm sources such as wild American. Although usually deleterious in hop breeding, inbreeding had been used to facilitate expression of unusual flavours. Such inbreeding had been used sucessfully elsewhere to develop the *cvs.* Cascade and Citra. By choice of parents, variation in expression of specific hop essential oils, particularly farnesene and selinene, had been sought.

With the aim of providing a more objective selection criterion for future progenies in the BHA programme, samples of dried hops from selections made in this project were subjected to detailed resin and oil analyses. In particular, the analysis of thiol compounds was explored to find simple, practical analytical indicators for distinctive flavours.

<u>Procedures</u>

The project assessed 3,006 individual hop seedlings in the field at Wye Hops Ltd. These seedlings were derived from crosses made 2011-2013 and all progenies included both conventional plant habits and dwarf types. The 28 crosses made in 2011 used *cvs.* Cascade, Keyworth's Early and Keyworth's Midseason as mother parents with several of the male parents chosen for their resistance to aphids. All mother parents in the 21 crosses made in 2012 had farnesene at elevated levels in their essential oils, and several of the male parents (families 22-32/12) had been chosen from their proven ability to give progeny with elevated farnesene levels. Some of these crosses also aimed to explore inbreeding within *cvs.* Cascade, Saaz and Fuggle (families 51-55/12). The 23 crosses made in 2013 used wild USA germplasm and aimed to combine this with resistance to disease carried by the complementary parent.

Each batch of seedlings was raised under glasshouse conditions and screened for resistance to downy mildew and powdery mildew diseases to allow selection of the most resistant genotypes. At least 30 resulting seedlings from each family were transplanted to field plots where every individual seedling was given a unique identifier and position. Seedlings were planted within families which were unreplicated. The plants were irrigated as necessary and left to establish perennial rootstocks, senescing naturally into the winter.

During the 2014 growing season prior to the start of this project, all progeny had been given appropriate husbandry and a full protective spray programme except for those progeny from the 2011 crosses where resistance to aphids was expected to segregate. For these, the spray programme excluded insecticides. Male progeny from the 2011 crosses had been assessed during June and July for their main characteristics including flowering period, pest and disease incidence, plant habit, and lupulin gland quantity. Female plants in the 2011 and 2012 progenies which produced an economic yield of cones at harvest in September 2014 had been individually harvested, with cone samples dried to 10% moisture in specialised kilns, pressed, wrapped and cold-stored. Assessments were made at harvest of the level of pest and disease, cone maturity, cone and picking characteristics and the plant habit and productivity for each selection. No assessments were made of the 2013 progenies which had been left to establish perennial rootstocks.

The project started in October 2014 with HPLC analysis of the content and composition of the resins of all samples from the 2014 harvest. The results, together with the observations made at harvest, were used to draw up a short-list of samples to provide to a trade panel of merchants, brewers and suppliers assembled and hosted by Charles Faram and Co. Ltd. The panel were provided with the samples under code numbers, including reference varieties and, therefore, assessed blind the quality and intensity of the aroma of the dried cones. Those samples highlighted in the assessment were further analysed for their oil composition by GC and for specific thiol compounds by GC-MS. Scrutiny of these records of agronomic and quality attributes allowed identification of individuals which might be suitable to advance to yield plots or to use as future parents in the commercial hop breeding programme of the BHA. Family records and sib data were used to inform the selection of male genotypes from the 2011 crosses.

During 2015, the main growing season of the project, progeny from all three cross-years were available for assessment in the field. They were grown using commercial husbandry and females were selected, harvested and assessed in the field as described previously for 2014. Dried samples of all selections, including reference varieties, were provided under code to a panel of ten growers of the BHA Next Generation Group comprising hop growers under the age of 40. The intensity of the aromas and flavours detected were used to draw up a short-list of samples which were forwarded to the trade panel who assessed blind the quality and intensity of the aroma, as described previously. As in the previous year, samples highlighted in this assessment were subjected to further detailed analysis of their oil composition and thiol content to determine any association between sensory and analytical parameters. Individuals which might be suitable to advance to yield plots or to use as future parents in the commercial hop breeding programme were identified from the results of the aroma panel and the analysis data. Also during 2015, males from the 2012 crosses were identified and selected for addition to the parental germplasm collection as described. All selections made following the 2014 harvest were propagated by softwood cuttings taken from regrowth in spring 2015 to produce perennial dormant rootstocks which were planted in winter 2015 to establish during the 2016 season.

During the final year of the project, the 2016 growing season, the progeny from the 2012 crosses were grubbed from the field to make space available for progeny from crosses made in 2015 for objectives separate from this project. However, selections from the 2012 progeny were transferred to propagation beds prior to grubbing of the remainder of the seedlings and cuttings taken in the spring for further trials. Progeny from the 2013 crosses were fully mature plants and males were assessed as described.

The project used land at China Farm, Canterbury, owned and managed by Elverton Farms Ltd., where a 7-acre hop garden solely for hop breeding work had been constructed in 2007 with a grant from the IBD. China Farm is an existing commercial hop farm producing high quality aroma varieties. Therefore, it had the equipment, facilities and staff with experience required for hop husbandry to a high standard. Ancillary facilities such as offices, storage (including cold storage) and glasshouses were available close to the breeding garden. The programme of work, the materials and the facilities for this project were entirely from resources managed by Wye Hops Ltd., and included specialised equipment such as miniature hop kilns and press for sample preparation. HPLC and GC analysis of cone samples were performed in the laboratories of Lupofresh Ltd., one of the UK hop merchant companies, working to industry quality standards. Analysis by GC-MS was undertaken through the University of Bangor, Wales, Tristaroma in Colmar, France and Nyseos in Montpellier, France. Data was stored on an Access database, remotely backed-up on a daily basis for security.

Results and Discussion

Samples collected at harvest 2014

A total of 76 duplicate samples of dried cones were prepared from selections made amongst the 2011 crosses (18 selections) and 2012 crosses (39 selections), including 19 samples from reference varieties. Approximately equal numbers of seedlings were available for selection in both year groups and this imbalance in selected numbers reflects the poor agronomic characteristics of many of the seedlings derived from *cv.* Cascade. This USA variety, selected in Oregon, is not well adapted to the higher latitudes of the UK and many of its seedlings showed poor adaptation; flowering and maturing late, and many having very small cones.

Samples of all selections were analysed by HPLC for resin contents and compositions. This data (part shown in Table 2) was considered together with notes made about the selections in the field at harvest. Selections were rejected on negative comments for the presence of powdery mildew disease, poor distribution of cones, poor plant habits and if cones were considered unsuitable in shape or density for machine harvest. As a result, samples from 39 field positions, plus four reference varieties, were selected to be assessed by the trade panel. In addition, a sample from GE77 was provided to the panel although there had been insufficient quantity of cones to provide a duplicate sample for analysis.

The samples were presented under code numbers which gave their field position. The reference varieties, *cv.* Endeavour, *cv.* Fuggle and two of *cv.* Cascade, could not be identified as known varieties by the panel. The panel scored each sample for the intensity of the aroma on a 1-10 point scale of increasing intensity and reached a consensus value following a discussion period at the end of the assessment session. The panel also recorded their impressions of the predominant flavour notes for each sample. The results, ordered by aroma intensity, are shown in Table 1.

The reference varieties, despite being anonymous, ordered in intensity as would be predicted with cv. Fuggle scoring 5 whilst *cv.* Cascade scored 7. These samples also solicited comments on their flavour typical of the varieties. Five selections scored equal to *cv.* Cascade and two, both seedlings of *cv.* Cascade, were considered to give greater aroma intensity. Thus, it has been possible to identify stronger flavour within the breeding materials of Wye Hops Ltd. Comments on flavour notes showed many diverse descriptors with individual differences even between seedlings in the same family.

Table 1. Trade panel assessment of the aroma of samples from 2014 harvest

Variety	**Sample No.**	**Intensity**	**Comments**
43/11/1	FI81	9	Banana and floral
41/11/24	FI37	8	Lychees
43/11/38	FJ28	7	Raspberry, orange
Cascade	FJ35	7	Floral, citrus, geranium
45/11/23	FK09	7	Tropical, fruity, sl ester
21/12/4	FZ01	7	Citrus, spicy mango
32/12/28	GC75	7	Earthy, sl sulphur
Cascade	GE57	7	Spicy American
52/12/18	GE77	7	Zesty fruit
31/11/22	FG72	6	Slightly musty
23/12/30	FY39	6	Rhubarb, mouldy

25/12/5	FZ09	6	Fresh citrus
26/12/24	FZ62	6	Phenolic
29/12/11	GB11	6	Sulphury, earthy
31/12/25	GC20	6	Pine polish
31/12/50	GC45	6	Sulphur and garlic
32/12/19	GC66	6	Slight sulphur
33/12/2	GC85	6	Lemon and lime
Endeavour	GD06	6	Apple, pine
51/12/18	GE18	6	Chemicals
52/12/11	GE70	6	Unpleasant
52/12/41	GE100	6	Rancid lemon
55/12/11	GF81	6	Grassy, earthy
55/12/32	GF102	6	Pine, peppery
Fuggle	ET42	5	Spicy and earthy
33/11/8	FH28	5	Smokey
53/11/15	FN34	5	Paraffin
21/12/44	FX61	5	Sickly
26/12/2	FZ40	5	Woody
26/12/40	FZ78	5	Cardboard
28/12/62	GA101	5	Soft fruits
30/12/6	GB53	5	Menthol
30/12/28	GB75	5	Bready
30/12/37	GB84	5	Unpleasant
30/12/41	GB88	5	Floral
31/12/17	GC12	5	Spicy, nice
34/12/10	GD17	5	Mushroom
34/12/24	GD31	5	Esters, pear drops
42/12/9	GD74	5	Slightly peppery
55/12/27	GF97	5	Slightly banana
26/12/21	FZ59	4	Sour
33/12/8	GC91	4	Spicy pine
55/12/2	GF72	4	Pine
55/12/7	GF77	4	Sweet silage

As seen in Table 2, there was no apparent association between the intensity of aroma and any of the measured resin parameters. Although *cv.* Cascade is notable for high cohumulone composition of its alpha-acid, the samples with higher perceived aroma intensity did not generally have high cohumulone. Indeed, sample FI81with the strongest intensity had a particularly low cohumulone content. Only one selection, FG72 exceeded the cohumulone content of *cv.* Cascade. Similarly, there were no apparent differences in aroma intensity, resin contents or compositions between samples from families 51-55/12 where inbreeding had been used compared with other progeny.

Table 2. HPLC analyses of samples presented to trade aroma panel, ordered by perceived intensity of the sample aroma.

Variety	**Sample No.**	**Intensity**	**LCV**	**HPLC alpha**	**Co-humulone**	**HPLC beta**	**Co-lupulone**	**Xantho -humol**	**DMX**
				%	% of α	%	% of β	%	1000x%
43/11/1	FI81	9	8.1	7.9	19.5	3.4	38.2	0.52	50
41/11/24	FI37	8	9.3	8.7	34.3	3.4	51.1	0.54	70
43/11/38	FJ28	7	9.7	9.4	24.5	4.1	41.3	0.58	30
Cascade	FJ35	7	5.5	5.3	40.2	4.9	53.2	0.36	85
45/11/23	FK09	7	8.9	8.5	31.8	6.1	47.6	0.60	80
21/12/4	FZ01	7	9.8	9.1	26.6	3.1	44.1	0.54	19
32/12/28	GC75	7	8.8	8.2	23.3	3.4	44.5	0.37	36
Cascade	GE57	7	4.1	3.6	40.7	4.6	52.1	0.35	64
52/12/18	GE77	7	-	-	-	-	-	-	-
31/11/22	FG72	6	4.9	4.5	43.6	2.5	62.1	0.27	15
23/12/30	FY39	6	7.6	7.3	34.3	7.5	52.4	0.56	123
25/12/5	FZ09	6	10.1	9.6	19.3	4.5	38.2	0.50	61
26/12/24	FZ62	6	6.9	6.4	25.2	3.7	41.8	0.35	62
29/12/11	GB11	6	7.5	6.7	22.9	4.6	42.3	0.39	39
31/12/25	GC20	6	4.4	4.2	27.4	7.6	49.4	0.42	127
31/12/50	GC45	6	5.5	5.1	26.6	7.6	49.4	0.47	81
32/12/19	GC66	6	8.0	7.3	24.1	4.9	42.7	0.43	59
33/12/2	GC85	6	8.8	8.3	23.8	3.4	44.2	0.48	51
Endeavour	GD06	6	10.8	10.5	29.6	5.1	50.3	0.52	57
51/12/18	GE18	6	4.8	4.3	34.6	3.2	47.2	0.29	33
52/12/11	GE70	6	5.0	4.7	37.9	2.7	53.1	0.31	24
52/12/41	GE100	6	6.5	6.1	35.1	4.2	46.8	0.56	51

55/12/11	GF81	6	8.6	8.0	26.4	4.5	47.7	0.49	47
55/12/32	GF102	6	8.2	7.3	28.4	3.3	48.1	0.42	55
Fuggle	ET42	5	6.2	5.9	30.5	3.7	50.2	0.34	34
33/11/8	FH28	5	9.7	9.2	28.9	5.9	44.5	0.65	85
53/11/15	FN34	5	7.6	7.1	26.9	3.4	44.6	0.39	46
21/12/44	FX61	5	5.9	5.4	34.8	3.7	51.8	0.29	56
26/12/2	FZ40	5	7.9	7.4	19.8	3.7	42.3	0.35	47
26/12/40	FZ78	5	6.8	6.2	25.3	3.9	47.5	0.53	47
28/12/62	GA101	5	10.6	9.9	25.9	3.6	45.0	0.84	51
30/12/6	GB53	5	7.5	6.9	34.9	6.4	54.0	0.50	88
30/12/28	GB75	5	7.3	6.7	34.3	3.6	52.8	0.42	64
30/12/37	GB84	5	9.5	8.7	31.9	3.5	55.0	0.64	53
30/12/41	GB88	5	5.5	5.3	26.6	8.6	46.5	0.55	96
31/12/17	GC12	5	8.3	7.6	31.1	5.8	53.5	0.48	114
34/12/10	GD17	5	10.5	10.3	25.4	5.9	45.3	0.43	50
34/12/24	GD31	5	14.4	13.6	26.0	4.8	48.2	1.11	72
42/12/9	GD74	5	9.2	8.5	38.4	3.8	59.0	0.53	50
55/12/27	GF97	5	9.4	8.7	26.1	3.6	43.0	0.48	58
26/12/21	FZ59	4	6.3	5.8	16.7	3.5	35.4	0.32	38
33/12/8	GC91	4	7.0	6.8	27.7	3.2	48.1	0.38	61
55/12/2	GF72	4	10.0	9.4	26.2	4.3	48.4	0.49	51
55/12/7	GF77	4	7.7	7.4	33.9	4.3	52.9	0.49	62
Challenger	FI57	NP	6.7	6.3	22.6	5.1	42.7	0.32	81
Admiral	FK41	NP	14.6	14.1	36.3	7.1	55.5	1.09	92
Target	FK44	NP	9.3	8.9	32.7	5.3	51.0	0.76	47
Herald	FK47	NP	10.4	9.9	29.0	4.1	51.0	0.57	65
Sovereign	FQ62	NP	4.3	3.8	24.7	2.2	39.7	0.24	20

LCV Lead Conductance Value
DMX Desmethylxanthohumol
NP Sample not presented to trade panel

There was also no apparent association between the intensity of the aroma and most components of the essential oils (Table 3). However, the total steam-distilled oil content of the samples with the highest aroma intensity (scores 7-9) was generally higher than the other samples and the myrcene content of these samples with intense aroma was also higher. The samples with the highest aroma intensity had oil contents and myrcene contents similar to that in *cv.* Cascade. The farnesene content of *cv.* Cascade is also a notable feature of the variety but there was no discernible association amongst these samples between farnesene content and aroma intensity. The analyses did confirm that all selections from families 22-42/12, where the crosses had been designed to give a farnesene content in the progeny, did contain farnesene well above trace levels.

Table 3. GC analyses of the essential oils of samples presented to trade aroma panel, ordered by perceived intensity of the sample aroma.

Variety	**Sample no.**	**Inten -sity**	**Total oil**	**Myr-cene**	**Cary-ophyllene**	**Farn-esene**	**Hum-ulene**	**Selin-ene**	**Ger-aniol**	**Lina-lool**
43/11/1	FI81	9	0.75	35.1	5.8	1.1	21.1	11.6	0.21	0.27
41/11/24	FI37	8	1.0	32.8	10.4	1.2	32.1	1.4	0.11	0.34
43/11/38	FJ28	7	0.7	28.6	15.5	0.3	4.8	20.8	0.14	0.49
Cascade	FJ35	7	0.9	38.3	7.8	8.2	22.2	4.2	0.37	0.42
45/11/23	FK09	7	1.2	27.3	14.5	2.1	24.9	2.6	0.21	0.74
21/12/4	FZ01	7	0.8	19.8	22.1	0.2	25.8	0.7	0.09	0.26
32/12/28	GC75	7	1.2	27.8	19.9	4.6	1.7	20.3	0.28	0.6
52/12/18	GE77	7	1.1	26.7	15.3	0.3	4.7	25.2	0.11	0.39
25/12/5	FZ09	6	0.3	26.4	8.7	4.9	25.8	7.1	0.15	0.34
29/12/11	GB11	6	0.5	26.3	7.9	4.7	21.8	8.7	0.09	0.31
31/12/50	GC45	6	0.6	29.5	1.2	3.3	4.5	33.4	0.11	0.5
33/12/2	GC85	6	0.3	20.3	10.2	7.8	29.8	7.3	0.15	0.09
Endeavour	GD6	6	1.1	23.8	3.0	6.5	5.3	33.6	0.52	0.23
55/12/11	GF81	6	0.2	10.0	14.0	0.4	49.5	2.5	0.21	0.42
55/12/32	GF102	6	0.4	18.7	8.2	0.2	26.8	15.5	0.05	0.16
Fuggle	ET42	5	0.9	28.3	9.2	6.7	30.3	1.7	0.13	0.64
28/12/62	GA101	5	0.3	18.4	7.4	6.4	20.6	10.0	0.15	0.27
30/12/6	GB53	5	0.5	24.2	5.9	2.1	6.3	34.1	0.12	0.22
31/12/17	GC12	5	0.3	6.8	10.5	6.7	38.9	2.5	0.21	0.43

The steam-distilled oils obtained for GC analysis were further used at the University of Bangor to analyse thiol content. Thio-esters (Table 4) were readily detected and showed considerable variation between samples, S-methyl hexanethioate in particular. For this compound there was a hint that it was at elevated levels in the samples with low aroma intensity but no general association could be detected between thio-ester content and aroma intensity which could be used to act as an indicator for selection for stronger intensity within the breeding programme.

Table 4. Thio-ester contents of samples presented to trade aroma panel, ordered by perceived intensity of the sample aroma.

Variety	Sample No.	Intensity	M6E	M5E	M7E
			ppm	ppm	ppm
43/11/1	FI81	9	448.6	49.0	4.4
41/11/24	FI37	8	73.7	81.8	0.4
43/11/38	FJ28	7	396.4	107.5	8.0
Cascade	FJ35	7	178.2	73.9	0.5
45/11/23	FK09	7	130.3	457.7	-
21/12/4	FZ01	7	421.8	63.1	37.7
32/12/28	GC75	7	515.8	545.1	97.2
Cascade	GE57	7	102.5	99.5	-
52/12/18	GE77	7	590.3	86.0	10.9
25/12/5	FZ09	6	331.6	630.1	7.6
29/12/11	GB11	6	950.8	215.0	158.6
31/12/50	GC45	6	1719.7	179.1	215.1
33/12/2	GC85	6	251.8	519.7	7.6
Endeavour	GD06	6	209.0	172.3	1.7
55/12/11	GF81	6	408.7	304.3	32.1
55/12/32	GF102	6	559.3	160.2	12.1
28/12/62	GA101	5	1886.5	763.9	243.1
30/12/6	GB53	5	238.5	105.6	-
31/12/17	GC12	5	2174.3	438.1	77.9

M6E S-methyl hexanethioate
M5E S-methyl-4-methyl pentanethioate
M7E S-methyl heptanethioate

Analysis failed to detect any of the thio-alcohols such as MSP even in *cv.* Cascade despite such thiols being known from published studies to be present. However, upto and including 2014, all published research on the thiol content of hops infer the content from analysis of beer made with the hops. None actually measured the content by direct extraction from cones. It is not practical to brew a beer for each putative selection from the breeding programme. Therefore, efforts were made to develop a methodology and further dried cone samples of *cv.* Cascade were supplied to University of Bangor who undertook experiments on extraction and detection. Despite considerable experience of the analysis of thiols in other projects, the thio-alcohols from hops proved to be too labile during extraction and provided materials at the limits of detection with the equipment available. Results lacked any consistency. The

University of Bangor declined to do further analysis of thiol content for the following 2015 season.

All seven genotypes which scored at least 7 from the trade aroma panel were selected for further evaluation in yield trials. Four of these were seedlings of *cv.* Cascade. In addition, pedigree information was used to select a further four seedlings from amongst those short-listed for the aroma panel to maintain a breadth of germplasm. Thus, a seedling of *cv.* Saaz (FZ9), *cv.* Fuggle (GA101), *cv.* Colgate (GB53) and *cv.* Tolhurst (GC12) were selected as potential parents. Finally, three seedlings of *cv.* Endeavour from the aroma assessment (GC85, GF81 and GF102) were selected for further evaluation because of their outstanding agronomic features, notably dwarf habit and yield. Although the primary selection criterion in this project was stronger aroma, this illustrates the multiplicity of secondary objectives sought during a practical breeding programme. All genotypes selected for further evaluation will be assessed in a subsequent, separate test of their resistance to wilt disease.

Amongst the 2011 crosses, 30 males were noted to be of interest during field assessment in the 2014 season. This included 11 genotypes displaying resistance to aphid colonisation in unsprayed plots. These notes, together with the analyses of their sisters, the performance of the families with the trade aroma panel, and from knowledge of which female selections had been made, were used to select 13 males to propagate and add to the germplasm collection.

Samples collected at harvest 2015

Progeny from all three cross-years, 2011-2013, were available for selection during 2015. Samples for aroma assessment were obtained from 79 selections; 22 from 2011 crosses, 39 from 2012 crosses and 18 from 2013 crosses. The lower number of selections from the 2013 crosses clearly reflected the poorer agronomic performance of progeny resulting from wild and USA origins. In addition, two reference samples of *cv.* Cascade were obtained. From the lack of any association between strength of aroma and resin characteristics found during 2014, it was decided not to take duplicate samples from most genotypes to have a separate sample for analysis. Only the 12 genotypes with the most promising agronomic features were harvested to give also a sample for HPLC analysis (data not presented).

Samples were assessed by the BHA Next Generation Group of young hop growers on a ten point scale of increasing intensity and only those samples scored as 8 or above by at least one of the Group were forwarded to the trade aroma panel. This resulted in 29 samples going forward for the trade assessment under code, including two samples of *cv.* Cascade. The results from the trade panel are shown in Table 5. The reference *cv.* Cascade again scored 7 on the ten-point scale. Although the second reference sample was only scored as a 6, the flavour was distinctly of citrus notes. Fifteen samples were judged at least equal in intensity to the reference and six of these samples exceeded the intensity of *cv.* Cascade. Several of the samples with the most intense aroma were from the 2013 crosses indicating that wild germplasm can provide a useful source for this trait. Although *cv.* Keyworth's Midseason was the mother of FG41 and *cv.* Bramling Cross the mother of FO31, all four other strong aromas from the 2011 crosses were seedlings of *cv.* Cascade. Furthermore, only three of the samples with the strongest aroma were derived from the 2012 crosses but these were also all seedlings of *cv.* Cascade. Thus, of the fifteen strong aromas, seven were seedlings of *cv.*

Cascade. Of the 3006 seedlings in the field for assessment during 2015, only 836 were derived from *cv.* Cascade. This indicates that *cv.* Cascade is very much more effective in transmitting strong aroma to its progeny than would be expected by chance. As with the 2014 harvest, the results from the trade aroma panel assessment of samples from the 2015 harvest indicated that stronger flavour can be obtained within the BHA breeding programme and that a range of unusual and distinctive flavours could be ascribed.

In contrast to 2014 when eleven selections were harvested from the seedlings resulting from inbreeding crosses, nine of which were forwarded to the trade aroma panel, from the 2015 harvest only four selections were made in this group and none of the samples from these progressed to the trade panel.

Table 5. Trade panel assessment of the aroma of samples from 2015 harvest

Variety	**Sample no.**	**Intensity**	**Comments**
47/13/6	DM32	8	Lemon, grapefruit
13/13/25	DW7	8	Sweet floral
15/13/4	DW55	8	Geraniums, berry fruits
18/13/15	DY52	8	Rose
30/11/17	FG41	8	Cherry, floral
43/11/35	FJ25	8	Esters
28/13/64	DI35	7	Lemon, leather
14/13/2	DW19	7	Fruit, spicy
43/11/12	FJ2	7	Tobacco, esters
51/11/26	FM45	7	Strawberry, cherry
Cascade	FM58	7	Cut grass, spicy
54/11/35	FO14	7	Fish, cheese, sulphur
60/11/10	FO31	7	Leather polish
21/12/28	FX45	7	Malty, paper
21/12/49	FX66	7	Orange, esters
23/12/17	FY26	7	Banana
27/13/11	ED33	6	Rich
Cascade	EE26	6	Citrus
50/11/22	FL86	6	Peach, grass
52/11/1	FM61	6	Burnt milk
52/11/34	FN1	6	Hay, dry wine
62/11/39	FP86	6	Oatmeal, black pepper

67/11/36	FS79	6	Sour milk, menthol
23/13/23	EB15	5	Stone fruits
60/11/21	FO42	5	Stale, lilies
62/11/35	FP82	5	Sulphur, rosemary
67/11/49	FS92	5	Sour milk
52/12/41	GE100	5	Fruity, spicy
61/11/6	FO86	4	Floral

With no duplicate samples being available for most of the selections, each sample from the aroma assessment had to be used again if required. Unfortunately, not all the samples were usable after the aroma assessment. Therefore, samples were not used for a routine GC analysis of the oils, as in the previous year, but supplied to specialist companies for more detailed and unusual analyses.

Samples were sent to Twistaroma, Colmar, France for oil analysis using SPME techniques. This protocol collects and analyses the volatiles in the air above the sample rather than collecting the oils by distillation. As such, it might be considered to be more relevant to the perceived aroma. However, because it uses a different collection method, the relative distribution of compounds is different from steam-distillation and the values for each component of the oils cannot be equated between the techniques. Despite this, the results (Table 6) again indicated that the samples perceived as having stronger intensity of aroma frequently had a higher myrcene content reflecting the total monoterpene content. However, there were several exceptions to this generalisation.

For each component, the technique also allowed the proportion in the aroma to be determined, splitting between the free compound available to the atmosphere and the proportion bound within the sample tissue. Several reports in the brewing literature cite geraniol and linalool as the main determinants of hop flavour in beer. Thus, SPME analysis was used for these compounds to determine if there was any association between the free compounds in the air above the samples and the perceived intensity of the aroma. Unfortunately, no such association could be found for either geraniol or linalool (Table 6).

Table 6. Detailed SPME analysis of the composition of essential oils of samples presented to trade aroma panel, ordered by perceived intensity of the sample aroma.

Sample no.	**Inten-sity**	**Free Geraniol**	**Free Linalool**	**Bound Geraniol**	**Bound Linalool**	**Mono-terpenes**	**Myrcene**	**Sesqui-terpenes**
		%	%	%	%	%	%	%
DW55	8	0.23	0.77	0.19	0.19	17.3	15.5	42.6
FG 41	8	0.29	1.27	0.08	0.09	3.8	3.3	83.7
FJ 25	8	0.30	0.50	0.13	0.00	13.0	11.1	35.4
DI 35	7	0.82	1.06	0.33	0.32	16.3	15.7	64.4
FX 66	7	0.69	1.51	0.52	0.22	16.3	14.9	48.7

FY 26	7	0.30	0.19	0.17	0.29	6.2	5.6	60.1
FO 14	7	0.30	0.77	0.05	0.03	6.6	6.4	71.8
FX 45	7	0.15	1.53	0.07	0.05	17.9	17.4	70.7
FM 45	7	1.11	1.24	1.22	0.68	25.2	24.2	44.1
FO 86	6	0.19	1.82	0.00	0.00	8.9	7.5	50.7
FO 42	5	0.03	2.15	0.00	0.00	7.1	6.5	51.4
FS 92	5	0.12	0.55	0.05	0.09	5.2	4.3	69.6

At the Trends in Brewing conference held in Ghent in April 2016 a presentation from Nyseos, Montpellier, France indicated that a method for direct measurement of thio-alcohol content of dried hop samples was available within the company's laboratories. Following discussions, four samples were provided under code by Wye Hops to test the potential for this expensive analysis. At fifteen times the cost of a GC analysis this could not be a routine testing system for selection within a breeding programme producing more than 75 samples each season. The four samples comprised *cv.* Cascade, known to contain the thiol 4MSP, *cv.* Sovereign, considered to be likely to be of very low thiol content if any, and two samples considered to have the strongest aroma from the trade assessment.

The results of the thiol analysis (Table 7) showed that thio-alcohols (4MSP and 3MH) could be detected at the level of parts per billion. However, these compounds have a very low flavour threshold and can be detected strongly in beer even at these low concentrations. It was also possible to detect precursors of the thiols such as C4MSP, the precursor of 4MSP. The detection of 4MSP and its precursor in *cv.* Cascade and the low levels of thiols in cv. Sovereign gave confidence to the results from this laboratory. The analysis indicated that the test samples with strong aroma intensity had relatively high levels of specific thiols, exceeding those in *cv.* Cascade. Although on too small a scale to draw any firm conclusions, these tests seem to confirm that thio-alcohols could act as useful indicators of stronger flavour.

Table 7. Thiol contents of selected samples (in µg/kg)

Variety	**4MSP**	**C4MSP**	**3MH**
Cascade	2.5	1.1	13.9
DM32	6.0	2.5	0.0
DY52	3.5	1.3	68.5
Sovereign	0.5	0.9	0.0

4MSP 4-methyl-4-sulfanylpentan-2-one
C4MSP Cysteine-4-methyl-4-sulfanylpentan-2-one
3MH 3-mercaptohexan-1-ol

All fifteen seedlings identified by the trade panel as having aroma intensity equal or greater than the reference sample of *cv.* Cascade were selected for propagation for further assessment. From records of their agronomic performance, seven of these were chosen for evaluation in yield trials (five conventional genotypes and two dwarf types) and eight for addition to the germplasm collection to use as future parents. Also from records of the agronomic performance of those seedlings selected for the trade panel, three other selections for further assessment were made. As stated previously, these seedlings with good field records were analysed by HPLC and all had cohumulone contents in excess of 40%.

Amongst the 2012 crosses, 42 male seedlings were noted to be of interest during field assessment in the 2015 season. These records, together with information and data from their siblings were used to select 12 males to propagate and add to the germplasm collection. During 2016, the males amongst the seedlings from the 2013 crosses were assessed in the field and 27 were highlighted. Selections will be made from these to progress to parental germplasm plots.

Outputs

The project has succeeded in its main objective to identify new seedlings in the British hop breeding programme with more intense aroma as an indicator of potential to provide stronger flavour in beer. Over the two years of the project, 32 female genotypes have been taken forward for further evaluation as a result of the selection protocols described here; ten in each season for assessment of their yield and brewing characteristics, and twelve as potential parents in the future crossing programme. In addition, at least twelve male seedlings have been selected from each cross-year also to add to the parental collection.

The project also aimed to compare different strategies to achieve stronger flavour hops. The choice of parents as used in a pedigree breeding strategy has been shown to be a successful approach and some specific parents, such as *cv.* Cascade, have been found to be particularly effective in transmitting intense aroma to their progeny. Similarly, wild USA germplasm has generated some progeny with particularly intense aroma. In this project, inbreeding has not produced any marked advantage with just one selection from an inbreeding cross going forward. Breeding for specific composition in the essential oil proved successful with attempts to incorporate farnesene. However, the presence of this oil did not associate with stronger aroma.

The project aimed to find simple, practical analytical indicators for distinctive strong flavours. Most parameters were found to show no association including all features of the resins and most of the oil traits. Although many of those samples with strong aroma had relatively high total oil content and, in particular, high monoterpene content, some of the samples with strong, intense aroma did not conform to this pattern. Only the presence of low flavour threshold thio-alcohols gave any indication of a marker for strong flavour but analysis of these compounds is still, at this stage, only available in specialist laboratories for a small number of samples.

Technology transfer

Information about this project has been disseminated through reports and meetings including trade journals, industry committees such as the Hop Industry Committee of the IBD, participation in hop producer-group meetings and seminars, as well as lectures to students and visits to universities and hop research centres throughout the world. During the period of this report, aspects of this work have been described as detailed below:-

Report to R&D Committee of the British Hop Association, held at Shepherd-Neame Brewery, Faversham, 18 November 2014.

Presentation "The Wye Hops programme - seven years on" to English Hops Ltd., Annual conference held at Marstons Brewery, Burton-on-Trent, 20 November 2014.

Meeting and visit to germplasm plots at China Farm with Mercy Morris, Conservation Manager for Plant Heritage, 4 December 2014.

Report to Hop Industry Committee of the Institute of Brewing and Distilling, held at Curlew Street, London, 18 December 2014.

Presentation "The future for the British hop breeding programme" to BHA Conference, Hampton Estate, Surrey, 5 March 2015.

Presentation "Activities at Wye Hops Ltd" to UKC Ale and Cider Society, University of Kent, Canterbury, 19 March 2015.

Attended IBD/Botanix tasting of experimental brews held at Botanix, Paddock Wood, Kent, 10 April 2015.

Report to R&D Committee of the British Hop Association, at spring meeting held at Thame, 22 April 2015.

Meeting with Prof. Jim Dunwell and colleagues at Dept. of Agriculture, University of Reading, 23 April 2015.

Visit and tour of breeding plots for James van der Watt, manager in Brewing Developments , and Xiang Yin, Director Brewing Raw Materials for SAB Miller, UK, 15 May 2015.

Visit and tour of germplasm plots for Mette Thomsen, Bioforsk, Kapp, Norway, 2 June 2015.

Visit and tour of breeding plots for Max Alexander, BBC, 4 June 2015.

Visit and tour of breeding plots for Lupulina Brewery, Girona, Spain accompanied by Julian Herrington, Brewery consultant, 18 June 2015.

Meeting with Prof Robbie Waugh, University of Dundee, and colleagues at James Hutton Institute, Dundee, 26 June 2015.

Presentation "Outline of hop variety development" to SAB Miller lunchtime seminar, Woking, 2 July 2015.

Visit and tour of breeding plots for Mike Benner, Managing Director SIBA, 14 August 2015.

Report to R&D Committee of the British Hop Association, held at China Farm, Canterbury, 19 August 2015.

Conducted tour of breeding plots at China Farm during an Open Day for 29 members of BHA, 19 August 2015.

Visit and tour of germplasm plots at China Farm for Paul Herbert, Kent Brewery, 11 September 2015.

Visit and tour of breeding plots at China Farm for Dr Matthew Moscou, Group Leader at the Sainsbury Laboratory, Norwich, 15 September 2015.

Annual Newsletter distributed to growers via Producer Groups and to brewers as an insert into September 2015 edition of Brewer and Distiller International. Also, posted on British Hops website.

Interview with Pete Brown, beer journalist and author, accompanied by John Humphreys, Shepherd-Neame, Faversham, 22 September 2015.

Attended public launch of Kent Green Beer Fortnight at Canterbury Food and Drink Festival, 25 September 2015.

Visits to Huell Hop Research centre, Germany, Slovenian Hop and Brewing Research Institute, and Hop Research Institute, Czech Republic, 28 September - 2 October 2015.

Presentation “Hop variety developments” to IBD ‘Business of Brewing’ Symposium, Harrogate, Yorks., 9 October 2015.

Interim report on IBD-funded project “The selection of stronger flavour within the UK hop breeding programme” sent to Brewers Research and Education Fund trustees, 13 October 2015.

Visit from Theo Freyne, Deya Brewing Co., Cirencester to discuss British hop varieties and see production facilities, 29 October 2015.

Visits to USDA-ARS, Corvallis, University of Oregon; Hop Breeding Company, Toppenish; Haas Innovations Centre, Yakima; Roy Farms, Moxee, 2 - 6 November 2015.

Report to R&D Committee of the British Hop Association, at autumn meeting held at Thame, 17 November 2015.

Presentation “Recent objectives for the Wye Hops breeding programme” at EHL Conference and AGM, St Austell Brewery, Cornwall, 27 November 2015.

Report to Hop Industry Committee of the Institute of Brewing and Distilling, held at BBPA, Brewers Hall, London, 3 December 2015.

Meeting with Dr Carol Wagstaff at Knowledge Transfer Partnership, Reading University, 7 December 2015.

Interview with Sophie Atherton, beer journalist, for article in CAMRA magazine, 9 December 2015.

Provided extensive notes describing concepts and recent progress with hop breeding programme to Jane Peyton, journalist, for article about innovation in hops for CAMRA magazine, 8 March 2016.

Presentations “Hop variety developments” to SIBA BeerX 2016 at Ice Sheffield Arena, 17 March 2016.

Visits to southern hemisphere hop research centres; Plant and Food Research, New Zealand meeting Dr Ron Beatson, 7-8 April 2016, Australia meeting Dr Simon Whittock, 14-15 April 2016, SAB Hop Farms, South Africa meeting Lauren Steytler, General Manager, 18-19 April, 2016.

Report to R&D Committee of the British Hop Association, at spring meeting held at Thame, 26 April 2016.

Visit and tour of breeding plots at China Farm for Dr Paul Matthews, Hopsteiner USA, 12 May 2016.

Telephone interview with Michael Jenkins of on-line magazine Hop and Barley, 24 June 2016.

Conducted tour of Stocks Farm, Worcs. for students from UC Davis, USA led by Prof Charlie Bamforth, 20 July 2016.

Conducted tour of breeding plots at China Farm for Dr Vladimir Nesvadba and two colleagues from Hop Research Institute, Czech Republic, 8 August 2016.

Report to R&D Committee of the British Hop Association, held at China Farm, Canterbury, 17 August 2016.

Visit and tour of germplasm plots at China Farm for microbrewers from Kent, 13 September 2016.

Hosted visit of IBD Southern Section to hop collection at Queen Court, Ospringe, Kent on 19 September 2016.

Presentation “Developments in hop research” to evening meeting of IBD Southern Section at Shepherd-Neame Brewery, Faversham, Kent on 19 September 2016.

Hosted visit of East Anglian Craft Brewers to hop collection at Queen Court, Ospringe, Kent on 20 September 2016.

Attended launch of Kent Green Beer Festival at Canterbury Food and Drink Festival, 23 September 2016.

Telephone interview with Richard Croasdale, Editor-in-Chief, Ferment magazine, 28 September 2016.

Annual Newsletter distributed to all growers via Producer Groups and to brewers as an insert into October 2016 edition of Brewer and Distiller International. Also posted on BHA website.