Final report

to Trustees of the Brewers' Research and Education Fund

Project from July 2017 to July 2020

British Hops: Genetic Marker Population Material - Maintenance of Plant Material.

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

Executive Project Summary

To safeguard the British hop industry, new hop varieties suitable for British conditions need to be developed more efficiently and much more quickly than in the past. The British Hop Association believes that molecular marker-assisted selection (MAS) techniques offer this potential but a prerequisite for their development is access to marker populations; large plant populations satisfying very specific criteria. This project aimed to maintain and characterise four hop marker populations in the field, thereby making available suitable materials for a separate project to develop MAS, particularly SNP (single nucleotide polymorphisms) markers. SNP markers are a molecular technique which utilises specific DNA sequences and has proved very powerful in other crops but with only limited application yet to hops. Furthermore, by facilitating a separate project, this BREF-funded project has allowed additional funding to be gained for the appointment and training of a successor to Dr Peter Darby who retired at the end of March 2020.

For each of four crosses between unrelated complementary parents, about 300 unselected individual hop seedling plants were established in the field at Wye Hops Ltd. These populations were assessed for a range of characteristics of value to the hop breeding programme with traits recorded over at least two seasons. For all traits, the heritability patterns observed agreed closely with expectations from the published mode of inheritance. These populations can be used confidently to provide materials to determine putative SNP molecular markers. Cones and cuttings have already been taken from them for such purposes.

The E H Wade Fund, a charitable trust set up by the daughter of one of the co-founders of the Wolverhampton and Dudley Breweries, has provided funds to develop SNP markers in hops for a PhD studentship jointly provided through NIAB-East Malling Research and the University of Reading. Klara Hajdu was appointed to the postion, starting in January 2019. In addition to her studies, she has also been training as a part-time hop breeding assistant at Wye Hops Ltd and has taken on leadership of the programme since April 2020.

Summary of objectives and what has been achieved

The application gave the objective for the project as:-

> "This project seeks to maintain a resource for the development of marker-assisted selection techniques taking British hop breeding forward into the molecular age whilst a complementary project to develop the genetic markers is initiated."

The full proposal expanded this objective into five main aims. The background and progress achieved for each of these is summarised.

- To safeguard the future of the British hop industry by facilitating adoption of molecular selection techniques to breed new British hop varieties

Due to its high sensitivity to environmental influences, particularly daylength and the presence of diseases, hops only grow optimally in the region in which they were selected. The British hop industry urgently requires development of its own new hop varieties to meet the rapidly changing international market for hops and to address the unpredictable consequences of environmental change. New molecular selection techniques offer the potential to increase greatly the efficiency of the hop breeding programme and dramatically shorten the lead time with comprehensive selection possible within weeks of a seedling starting to grow. The BHA wishes to embrace this new technology. However, a pre-requisite for the development of suitable molecular selection is access to appropriate marker populations.
This project has allowed four putative marker populations to be established and maintained in the field at Wye Hops Ltd and, from their heritability patterns, shown that these families are suitable to use as marker populations. With such materials made available by this project, applications have been possible for separate funding to allow the first steps to be made towards the adoption of molecular selection. It has enabled the BHA to gain access to the required expertise and laboratory facilities, both of which it lacked and could not have resourced without the existence of the marker populations.
To date, whole genome DNA sequences for the parents of the 'Pilgrim' marker population have been determined and leaf samples taken of all their progeny for genotyping.

- To provide a permanent resource for the development of marker-assisted selection based on molecular techniques such as SNPs

The four marker populations derived from parents 'Cascade', 'Boadicea', 'Fuggle' and 'Pilgrim' have been established in a plot occupying 0.41 ha adjacent to the permanent germplasm collection at Wye Hops Ltd. This plot is not part of the turnover of materials associated with the selection cycle of the main breeding programme and will, like the germplasm collection, act as a permanent resource. To maintain this plot, the plants have required all the horticultural field-work effort for the specialised husbandry operations associated with commercial hop growing; stringing, training, routine protective spraying, fertilizer application, bine management and cutting, irrigation, harvest operations, weed control, and wirework maintenance.
The crosses to provide marker families were not from the main breeding programme but were devised to meet very specific criteria. Parents were chosen to be complementary for as many

traits as possible and unrelated by pedigree, Progenies were entirely unselected during seedling raising before planting in the field plot so as not to introduce any bias. Families were of over 200 individuals, sufficient size to enable robust statistical analysis of the phenotypic data to provide publishable results. From the characterisation done in this project, simple traits in all the populations have been shown to have independent segregation of alleles confirming that Mendelian segregation is occurring without hinderance. Thus, all four populations are entirely suitable for use to determine molecular markers such as SNP (single-nucleotide polymorphism) markers. SNPs are DNA sequences which vary by just a single nucleotide, occurring throughout the genome at relatively high frequency. It will be possible to associate statistically a trait of interest with particular SNPs which act as indicators for the trait without any need to determine any causal relationship.
The associated separate project facilitated by this project has started evaluating Repeat Amplification Sequencing as a technique to find SNP markers. This innovative new technique uses transposon sequence derived markers and, if successful, offers low-cost, high-volume DNA sequencing suitable for application in a practical hop breeding programme. Initial tests have been very encouraging revealing a high abundance of genetic polymorphisms present in the hop genome. However, their equitable distribution across the hop genome has yet to be determined.

- To characterise four suitable marker populations.

During the project, the expression of six traits was recorded segregating amongst four families. These traits were selected as being under simple genetic control from the action of few genes. They were resistances to powdery mildew provided by R2, R4 and Rsov sources, resistance to aphids, sex type and dwarfness. For each trait, one parent in a cross expressed the trait whilst the other complementary parent did not. Wherever possible, a trait was included in more than one family and these were recorded in more than one year to minimise seasonal environmental variation. Also, the total number of seedling plants recorded for each trait in each family always well exceeded 200 to provide for statistical robustness. In all instances, the observed segregation patterns were in close statistical agreement with those expected theoretically from published sources confirming unbiased Mendelian segregation. Variation attributable between families and between seasons was mostly insignificant. Thus, the heritability patterns within the four families developed during this project have been shown to meet all the criteria for use of the families as marker populations for future development of molecular selection markers. The characterisation recorded for the six traits here will allow rapid association with molecular markers for each trait when each seedling has been genotyped. Furthermore, having shown the populations to be suitable for these simple traits, the populations will also be suitable to extend the use to determine molecular markers for genetically more complex traits such as downy mildew or wilt resistance, or flavour components.

- To supply materials for separate tests conducted elsewhere to assess resistance to wilt disease, and for assessment of the intensity and quality of the aroma.

Resistance to hop wilt disease is seen as the priority for molecular marker determination in the British hop breeding programme, offering the greatest benefit in saving time, cost and resources. To achieve this, the segregation of resistance to wilt disease in a suitable marker

population needs to be related through advanced statistical association analysis (Quantitative Trait Linkage) to the genome sequences from each seedling plant in the population and their two parents. As part of the studentship and in anticipation of this work, Klara Hajdu attended a workshop in Slovenia in early May 2019 where the methodology of the pathogenicity assay was demonstrated. In late May 2019, cuttings were taken immediately prior to hop training from 260 individuals from the 'Pilgrim' population at Wye Hops Ltd and rooted in glasshouses at NIAB-East Malling Research. A total of over 4000 pots of cuttings were obtained, hoping to generate at least twelve small perennial rootstocks from each individual seedling plant. Following overwintering as bare roots, 182 genotypes and 6 reference varieties, including the two parental lines, were potted up in replicated large scale *Verticillium wilt* pathogenicity tests which started in a class 2 licensed growing compartment at NIAB-EMR in July 2020 and will be completed by November 2020.
Similarly as part of the studentship, cones were sampled at maturity from every female plant in the 'Cascade' marker population. This consisted of 218 female plants confirmed over two seasons, and 13 where there was some ambiguity between observations due to rogues and tangled adjacent positions in the field. Laterals were selected from the upper and lower parts of each plant on both sides of the bine to give cone samples of approx. 50g fresh weight. Fresh samples were vacuum packed and stored frozen at NIAB-East Malling Research at -24º to await analyses of the resins and oils at Reading University. Furthermore, hop cones from seven individuals from this 'Cascade' population have been harvested for brewing trials and genotyping of these individuals has commenced.

- To appoint and train a successor to Dr Darby.

Through Wye Hops Ltd, funding was secured in April 2018 from the E H Wade Fund to provide for a PhD studentship jointly provided through NIAB-East Malling Research and the University of Reading. Ms Klara Hajdu was appointed to this position, starting in January 2019. Although the studentship includes an element of training in classical hop breeding to provide a successor following the retirement of Dr Peter Darby, Ms Hajdu has also been employed as a part-time hop breeding assistant at Wye Hops Ltd in addition to her studies for the PhD. During 2019 under the guidance of Dr Darby, she actively participated in all the main operations of the current hop breeding programme. As well as work at the main breeding hop garden at China Farm, Canterbury, she visited and recorded observations at the dwarf trials site in Herefordshire, at the conventional plot trials site near Faversham, Kent, and at the elite stocks facility at Stockbridge Technology Centre, Yorks. Additionally, she visited all the farm trial sites for several of the current projects including projects continuing IBD-funded projects. Dr Darby retired at the end of March 2020 and Ms Hajdu has taken on leadership of the projects. However, Dr Darby will continue as a supervisor for the studentship keeping close contact on progress with, at least, quarterly scheduled meetings for the next two years. The BHA have agreed with him that Ms Hajdu can call on him for guidance as and when she feels it is necessary.

Main Report

Introduction

British hops have an estimated value to the brewing industry of over £10 billion. The hop area in the UK at harvest 2019 was 958 ha, localised in areas of Kent and Sussex, Hereford and Worcester, with a production of 33,918 Zn (1,695.9 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 traditional aroma varieties, mainly *cv.* Goldings and *cv.* Fuggles which are both heritage varieties selected by the named hop growers in 1790 and 1875 respectively. British hops represent 1.5% of world hop production and provide the brewer with a secure supply of a diverse range of aroma and flavour hops. The challenge for the British hop industry, represented by the British Hop Association (BHA), is to increase export sales in the next five years beyond the current estimate of 40% of domestic production, utilising the hop varieties already in production and bringing forward several new high-impact selections currently in farm trials. However, shortly beyond this immediate horizon, the BHA needs to provide the market with new varieties meeting the increasing interest and demand for hop-forward varieties which can be grown against an increasingly variable and extreme environment. Therefore, UK hop breeding now needs to be able to respond much more quickly than in the past.

Since its foundation in 1906, selection in the British hop breeding programme has been based on classical techniques observing the expression of characteristics. Such phenotypic selection in hops is greatly influenced by seasonal, maturity and environmental factors which results in a long lead time with at least 10 years between making a cross and planting selections from it on farms. Furthermore, phenotypic selection is very inefficient in use of resources. Experience has shown that about only one selection from 100,000 seeds sown advances to farm trials. Selection based on the genetic component alone, with confounding environmental and maturity influences removed, so-called molecular selection techniques, has the potential to speed up selection greatly allowing identification of promising individuals in progeny as soon as there is sufficient seedling growth to allow DNA extraction.

Molecular selection uses properties of DNA to provide markers, or indicators, of the presence of genes controlling the traits of interest. The greater the number of markers, the more likely that the markers will be physically close to the gene and the more effective the selection technique. With older molecular techniques, less than 1000 markers have been determined in hops but in recent years new techniques based on SNP markers have found more than 17,000 markers in hops (Matthews *et al* 2013, Falconer 2016). Marker Assisted Selection techniques (MAS) have been successfully applied in other crop breeding (Collard and Mackill 2008) and, with this number of SNP markers in hops, they could realise the potential for molecular selection in hops, possibly reducing the number of years requiring in-field observation to little more than 5 years.

To retain its importance in the international hop market and to safeguard the future of the hop industry in the UK, the BHA created a wholly-owned subsidiary company, Wye Hops Ltd., in 2007. As well as maintaining the National Hop Collection, the basis of all British hop breeding since 1906, this company is continuing to deliver a hop breeding programme for the

UK following the closure of the programme at Imperial College (formerly Wye College), Wye. The BHA raises a voluntary levy from all hop growers in the UK to provide the core funding for Wye Hops Ltd which receives no public funds. To address the needs for faster variety development, the BHA feel that this is an appropriate time for Wye Hops Ltd to embrace modern breeding techniques such as molecular marker assisted selection based on SNP markers. However, a prerequisite for such work is the availability of suitable marker populations.

Marker populations are the unselected, unbiased progeny from a cross between unrelated parents complementary for traits which segregate independently. Through advanced statistical techniques of association analysis such as quantitative trait linkage (QTL), SNPs are sought which are associated with desirable characteristics shown by individuals in the progeny. The level of probability needed for such association is around 10^{-9} (Matthews 2013) and, therefore, large populations are required to provide sufficient statistical robustness. To be publishable in a reputable journal, a population size of more than 200 is required. For hops, this is a major investment in land and resources and many studies have only had access to smaller populations, often less than 100 (Henning *et al*, 2017).

Objectives

The main objective of this project was to facilitate adoption by the BHA hop breeding programme of molecular marker-assisted selection techniques to speed up breeding of new British hop varieties. The aim was to provide suitable hop populations for the development of SNP markers, maintaining this as a permanent resource whilst a complementary project to develop these genetic markers was initiated.

This project aimed to establish and maintain four defined progenies in the field at Wye Hops Ltd as putative marker populations, to characterise these populations for several traits where the inheritance patterns were known so as to confirm their suitability as marker populations, and to supply materials from these populations for separate tests conducted elsewhere as part of complementary projects.

The existence of suitable marker populations is a prerequisite for attracting funding for complementary projects. By providing these populations and thereby securing additional funding, this BREF project aimed to enable the appointment and training of a successor to Dr Peter Darby who has led the BHA programme since 1981.

Procedures

Four controlled crosses were made in 2015 using, as mothers, *cvs.* ‘Boadicea’, ‘Cascade’, ‘Fuggle’ and ‘Pilgrim’. For each cross, the male parent was chosen to be complementary for as many traits as possible and of unrelated pedigree to the mother. However, where possible, some communality in pedigree between crosses was sought to link the four populations, minimising any effects arising from non-segregation of translocations within the genome, a problem later highlighted for hops by Zhang *et al* (2017). The common pedigrees also reflected the interconnected nature of the pedigrees of the main commercial breeding programme at Wye Hops Ltd. The resulting 1200+ seedlings were raised in 2016 in a glasshouse and subjected to an epidemic of powdery mildew using a defined strain of the disease. The virulence of the isolate (vBv1v3) allowed expression of seedling resistance to

this disease from R2, R4 and Rsov. Seedlings 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 of 2016-17.

During the 2017 establishment year, the seedlings from two of the families where resistance to aphids was expected to be expressed were left unsprayed with insecticides to allow preliminary observation of their infestation by hop aphid, and preliminary records made of the sex of each seedling from all four crosses.

During 2018 the seedlings were in their first season as mature fully-developed hop plants allowing the segregation of dwarfness to be recorded and the sex of each seedling confirmed. Conditions allowed records to be taken of resistance to hop aphids in unsprayed plots of one family to confirm preliminary observations.

During 2019, to confirm preliminary observations, the resistance to aphids was recorded for the second family initially recorded in 2017. Also, the expression of dwarf traits was confirmed in the three families where segregation was expected. Cuttings were taken in the spring for a separate project from the plants in the 'Pilgrim' family to generate perennial rootstocks for testing against Verticillium wilt disease to determine the segregation of resistance in this family. Cones from every individual female from the 'Cascade' family were sampled at harvest to provide materials for a separate project for volatiles profiling and preliminary flavour studies.

During 2020, fresh hop aroma was assessed in all females in the 'Cascade' population and seven individuals harvested to provide sufficient for brewing trials to be carried out in the new research brewery at NIAB-EMR. Genotyping the DNA sequences for these individuals has also started as part of the separate project.

The expression of many characteristics in hops is influenced strongly by environmental factors and, therefore, the appearance of every individual in the mapping populations required assessment over more than a single season to ensure that only DNA from individuals with consistent results is used in development of SNP molecular markers, avoiding erroneous associations and linkages.

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. It was contracted to grow the hops in this project to such an industry standard. All husbandry operations associated with commercial hop growing were required including stringing, training, routine protective spraying, fertilizer application, bine management and cutting, irrigation, weed control, and wirework maintenance. 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. Data was stored on an MS Access database, backed-up frequently on several media including the MS OneDrive cloud for security.

Results and Discussion

a) Crosses 2015 and seedling raising

The choice of parents is critical to the successful development of suitable marker populations, laying the foundations for all subsequent work. The area of plot available at China Farm to accommodate marker populations was sufficient for 1350 mature hop plants. With an objective of about 300 plants per family, four populations were sought and *cvs.* 'Boadicea', 'Cascade', 'Fuggle' and 'Pilgrim' were selected as mothers to reflect the breadth of the breeding programme at Wye Hops Ltd whilst providing a range of all the known single gene traits actively selected in the current programme yet also encompassing more complex traits. The crosses were as follows.

'Cascade' cross designated 20/2015. 'Cascade' is derived from 'Fuggle' combined with wild USA germplasm. It is a conventional height variety providing, in itself and in many progeny varieties worldwide, the new high impact flavours associated with craft brewing. It is susceptible to v2 and v4 powdery mildew strains, and aphids. It has a relatively high cohumulone content. To complement 'Cascade', male 28/07/79 was selected. This male is derived from 'Boadicea' and 'Russian' pedigree. It is a dwarf variety with R2 and R4 resistance to powdery mildew. It is resistant to aphids and selected from a family in which all sibling females show exceptionally low cohumulone content.

'Boadicea' cross designated 31/2015. 'Boadicea' is derived from wild Japanese germplasm. It is a dwarf variety selected as the first commercial variety with resistance to aphids. It is resistant to all powdery mildew strains except v2 and this has been shown to be due to R2 homozygosity. To complement 'Boadicea', male 50/95/24 was selected. This male is derived from selection 37/91/22 which was highlighted as the main source of a dominant gene for susceptibility to powdery mildew designated Rsov (Darby 2013). The action of Rsov can only be detected in crosses with a parent homozygous for powdery mildew resistance. It is a tall variety with susceptibility to aphids.

'Fuggle' cross designated 40/2015. 'Fuggle' is a conventional height variety derived from old English germplasm. It is a susceptible to all pests and diseases and is well regarded for its traditional aroma. To complement 'Fuggle', male 15/01/11 was selected. This male is derived from the same 'Russian' germplasm as 28/07/79. It is a semi-dwarf variety with R4 resistance to powdery mildew and resistance to wilt disease.

'Pilgrim' cross designated 50/2015. 'Pilgrim' was selected from a complex pedigree within the main UK hop breeding programme including common ancestry with 'Boadicea'. It carries R2 resistance to powdery mildew as well as strong resistances to wilt disease and downy mildew. To complement 'Pilgrim', male 316/01/10 was selected. This male is derived from Alsation 'Strisselspalt' germplasm. It has been found to be susceptible to UK strains of wilt disease and initial tests indicate that it is also relatively susceptible to downy mildew.

Thus, these crosses meet the criteria for marker populations of parents being unrelated and complementary within a cross with most traits present in more than one cross yet providing some communality of pedigree between crosses.

To ensure that the populations meet the criterion for sufficient numbers, excess seed were sown and excess seedlings potted on to anticipate losses due to failures in germination and

deaths and runts after potting on from seed trays into 1 litre pots (Table 1). Between potting on and planting out in the field, the seedlings were screened against powdery mildew and care was taken to draw the field population equally from the resistant and susceptible members of each family to ensure that no selection bias was introduced.

Table 1. Seedling raising of putative marker populations.

Family	No. sown	% germination	No. potted	Runts	No. planted
20/2015	1180	63.5	425	1	301
31/2015	1180	59.5	432	21	283
40/2015	944	89.6	432	17	311
50/2015	708	46.9	310	5	305

b) Powdery mildew R2 resistance.

The observed segregation of resistance provided by the R2 gene was tested against a Chi-square distribution to detect if there was any significant deviation from expectation. As detailed by Darby (2001), the mode of action of this gene is postulated to be a single dominant major gene giving a 1:1 segregation between resistance and susceptibility.

Table 2. Segregation for R2 resistance to powdery mildew.

Family	Rest.	Susc.	Chi Sq	Prob
20/2015	105	112	0.23	0.63
50/2015	135	145	0.36	0.55

For both families carrying R2, Chi-square values were obtained with probability greater than 0.05 (Table 2) indicating insignificant deviation of observed numbers from expectation.

c) Powdery mildew R4 resistance

As for the R2 resistance, the observed segregation of resistance provided by the R4 gene in two families was tested against a Chi-square distribution with expectation of a 1:1 ratio of resistance to susceptibility (Darby 2001).

Table 3. Segregation for R4 resistance to powdery mildew.

Family	Rest.	Susc.	Chi Sq	Prob
20/2015	207	217	0.24	0.63
40/2015	183	232	5.79	0.02

Both Chi-square values (Table 3) confirm that observations were consistent with expectations for the action of a single major gene for resistance at >1% probability. The largest contribution to the Chi-square value was from family 40/2015 which has ‘Fuggle’ as the mother and showed slight skew towards susceptibility. This very probably reflects the very high susceptibility of ‘Fuggle’ to powdery mildew.

d) Powdery mildew susceptibility gene (Rsov)

Family 31/2015 is derived from 'Boadicea' which is homozygous (true-breeding) for R2 powdery mildew resistance. 'Boadicea' has been used in 40 other crosses in past years at Wye Hops Ltd and all its seedlings have shown resistance to vBv1v3 virulence. However, Darby (2013) postulated the presence of a single dominant gene for susceptibility (Rsov) in some specific breeding lines at Wye Hops Ltd and it was believed that the male parent in this cross carried this gene. Therefore, a 1:1 ratio of resistance to susceptibility would be expected in this family despite the true-breeding nature of 'Boadicea'.

Table 4. Segregation for Rsov resistance to powdery mildew.

Family	Rest.	Susc.	Chi Sq	Prob
31/2015	221	190	2.34	0.13

The Chi-square value obtained for the segregation of resistance in this family (Table 4) has a probability greater than 0.05 confirming that observations are consistent with expectations for the action of a single major gene for susceptibility. Also, this confirms that the gene is present in the male parent.

e) Resistance to Damson-hop Aphid

The presence of damson-hop aphids on each individual in each of two families was scored in early July, approx. 7 weeks after the first aphid migrants were detected in the hops growing at Wye Hops Ltd. Plants were scored as infested if three or more aphids were found on any leaf. This is the same criterion as used for an IBD-funded project 2007-2009 (Darby, 2009).

Table 5. Colonisation by hop aphids

Family	Year of assessment	Free of aphids	Aphids present	Chi Sq	Prob 1:1
20/2015	2017	157	128	2.95	0.86
	2018	155	135	1.38	0.24
31/2015	2017	135	127	0.24	0.62
	2019	138	140	0.01	0.91
	Totals:	585	530	4.58	
Analysis	Deviation (1 df)			2.71	0.10
	Heterogeneity (3 df)			1.87	0.60

Hop aphids are known to be killed by air temperatures above 30° and such temperatures occurred for several days in early July 2019 immediately prior to assessment. Very few aphids were found on the main parts of the leaves but healthy aphids were found close to the veins on leaves in these progeny where it is presumed the micro-climate was cooler and more humid.

Darby (2009) proposed the action of a single dominant gene for resistance to aphids in 'Boadicea'. Therefore, the ratio of infested to clean plants in this family was tested by a Chi-square Test against the expectation of a 1:1 ratio. All calculated values gave a probability >0.05 (Table 5) indicating no significant departure of observations from expectations. Furthermore, no significant heterogeneity was detected between seasons or between families. On an individual basis, there was very good agreement between seasons. In family 20/2015, only 22 individuals which were clean of aphids in 2017 supported aphid colonisation in 2018. Similarly in family 31/2015, 25 individuals with no aphid colonisation in 2017 were recorded as susceptible to infestation in 2019. The numbers free of aphids in both years of assessment for each family are shown in Table 6.

Table 6. Individual plant infestation by hop aphids

Family	Free of aphids in both years	Aphids present	Chi Sq	Prob 1:1 with 82% expression
20/2015	135	157	3.31	0.07
31/2015	110	152	0.11	0.75

In the IBD-funded project 2007-2009, it was found that only 82% of resistant plants were detected by defining resistance as severely as less than three aphids on a leaf (Darby 2009). Testing these results here against 82% expression of resistance gave Chi-square values which indicate no significant deviation from expectation. These results are, therefore, entirely consistent with previous work and indicate consistent control by action of a single gene in both families.

f) Sex of seedlings

Hops have an XY chromosome system for sex expression (Neve 1991) which acts in exactly the same manner as for humans. However in hops, a 1:1 ratio of males and females is rarely observed due to the effects of pollen age, pollen competition in the hop flower and maternal influences. In most hop families, a ratio of either about 2:1 or 4:1, females to males, is observed in the absence of any selection (Neve 1991).

Table 7. Observed numbers of females and males in each marker family

Family	Female	Male	Ratio
20/2015	218	79	2.76
31/2015	229	50	4.58
40/2015	220	89	2.47
50/2015	215	85	2.53

As seen in Table 7, all putative marker families in this project show a preponderance of females in ratios in agreement with Neve (1991). Of the total of 1185 seedlings where the sex could be determined, only 17 (1.4%) showed an inconsistency between years and these individuals will be excluded from association studies for SNP marker detection. A single

individual in family 50/2015 was a true hermaphrodite. From these results, it would seem that the behaviour of chromosomal systems in these populations are functioning fully as expected.

g) Dwarfness

Identification of dwarf plants and their distinction from simply weak plants, especially for males, was difficult. In particular, growing conditions during 2019 caused many problems for identification of dwarf hop plants. Early regrowth necessitated early training which led to vigorous growth. Thus, many of the female dwarfs grew over the top wire at 4.6m high and could only be identified as dwarf by their shorter internode length. A period of drought and excessively high temperatures during early July that year co-incided with the onset of flowering in male plants which truncated vegetative growth prematurely. Thus, many males were of short stature although not true dwarfs. There was, therefore, some degree of misclassification.

Table 8. Segregation for dwarfness in each marker family

Family	Year of assessment	Dwarf	Tall	Chi Sq (3:5)	Prob
20/2015	2018	94	203	4.34	0.04
	2019	94	201	4.00	0.05
31/2015	2018	117	162	2.34	0.13
	2019	118	163	2.42	0.12
40/2015	2018	95	214	6.02	0.01
	2019	91	206	5.96	0.01
	Totals: (6 df)	609	1149	25.08	
Analysis	Deviation (1 df)			6.13	0.01
	Heterogeneity (5 df)			18.95	<0.01

The ratios of dwarf to tall plants in these families were tested by a Chi-square Test against the expectation of a 3:5 ratio. Such a ratio would be obtained if the expression of dwarfness was controlled by a 2-gene epistatic mode of inheritance as has been postulated (Darby 1994, Henning *et al* 2017). Despite inevitable misclassification, Chi-square values calculated for all families (Table 8) confirm that observations are consistent with expectations of a 3:5 ratio at 1% probability or more. However, there was significant heterogeneity. Observations were highly stable between seasons despite the difficult weather in 2019 and this heterogeneity was attributable to differences between families, notably the ‘Fuggle’ family (40/2015) where comparatively few dwarfs were observed. The father of this family, however, was a semi-dwarf and the genetics controlling partial expression of dwarfness are not yet elucidated although experience suggests that greater numbers of taller plants would be expected than from a true dwarf parent.

The characterisation of the populations carried out in this project has examined single gene traits, two-gene epistatic traits and chromosomally-determined traits. With the exception of the Rsov resistance to powdery mildew, all traits have been tested across at least two families

eliminating error from specific genetic interactions. In all instances, the genetic systems have been shown to function in agreement with expectation from previous published works. Thus, independent segregation of alleles in Mendelian manner is occurring in all four families. All these families have been shown to meet the criteria for use as genetic marker populations.

Succession planning

By facilitating a separate project, this BREF-funded project has allowed additional funding to be gained for the appointment and training of a successor to Dr Peter Darby who retired at the end of March 2020. The funding was secured in April 2018 from the E H Wade Fund to provide for a PhD studentship, jointly provided through NIAB-East Malling Research and the University of Reading, to develop SNP markers for traits important to the breeding programme carried out by Wye Hops Ltd. The E. H. Wade Fund is a charitable trust set up by the daughter of one of the co-founders of the Wolverhampton and Dudley Breweries. The post was advertised in May 2018 and interviews held in June. Although an appointment was made, the initial candidate proved unsatisfactory and left soon after starting. The position was re-interviewed and Ms Klara Hajdu appointed, starting in January 2019. Her studentship includes an element of training in classical hop breeding and she has also been employed as a part-time hop breeding assistant at Wye Hops Ltd in addition to her studies for the PhD. She has been gaining experience in all aspects of the current breeding programme including seed sowing, seedling glasshouse disease screening, assessment of males, virus testing, recording aphid infestation, selection of females, harvest, and sample analysis. As well as at the main breeding hop garden at China Farm, Canterbury, this has involved visiting and recording at the dwarf trials site in Herefordshire, at the conventional plot trials site near Faversham, Kent, and at the elite stocks facility at Stockbridge Technology Centre, Yorks. During 2019 under the guidance of Dr Darby, Ms Hajdu visited all the farm trial sites for several of the current projects including the development of varieties with stronger flavour and the development of a wilt-resistant substitute for ‘Fuggle’; both continuations of IBD-funded projects.

The BHA consider that this experience will provide a successor following the retirement of Dr Peter Darby and have indicated to Ms Hajdu that she will be offered the permanent full-time position of hop breeder at Wye Hops Ltd at the completion of her studentship. Dr Darby retired at the end of March 2020 and Ms Hajdu has taken on leadership of the projects. However, Dr Darby will continue as a supervisor for the studentship keeping close contact on progress with, at least, quarterly scheduled meetings for the next two years. The BHA have agreed with him that Ms Hajdu can call on him for guidance as and when she feels it is necessary.

Outputs

The principal aim of this BREF-funded project was to develop and maintain a large field collection of hop plant populations, fulfilling the criteria for their use as marker populations which are a prerequisite for separate projects to develop SNP molecular markers. Thus, the principal output from this project would be access to suitable material to use in complementary molecular projects. The work done in this project has shown that the four populations being maintained at Wye Hops Ltd are entirely suitable to use for this purpose.

As described, materials derived from these populations are currently being used in separate projects to this end.

The existence of these populations has also allowed additional funding to be obtained. A component of this funding has been to provide suitable training for a successor hop breeder at Wye Hops Ltd. Thus, an important output from this BREF funding has been for the BHA to be able to appoint a trainee hop breeder.

Information about this project has been disseminated through reports and meetings including 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. However, restrictions on meetings and travel as a result of Covid-19 have greatly limited dissemination during 2020.

Meeting with Mr David Thompson, Wade charities trustee, at Stocks Farm, Worcs., 20 January 2017.

Report to R&D Committee of the British Hop Association, at spring meeting held at Thame, 3 May 2017.

Meeting with Mr David Thompson, trustee of Helen Wade Fund to discuss future projects and view seedling disease screening procedures at Nursery, 25 May 2017.

Scientific Commission meeting of the IHGC at St Stefan am Walde, Austria, 25-29 June 2017.

Report to R&D Committee of the British Hop Association, held at China Farm, Canterbury, 18 August 2017.

Visit and tour of breeding plots at China Farm for NorthEast Plants, New Brunswick, Canada on 14 September 2017.

Visit and tour of breeding plots at China Farm for Dr Tetsu Sugimura, Kirin Company Ltd., R&D Division on 22 September 2017.

Annual Newsletter distributed to all growers via Producer Groups and to brewers as link to BHA website in IBD Newsletter on 25 September 2017.

Report to R&D Committee of the British Hop Association, held at James Figg Inn, Thame, 22 November 2017.

Report to Hop Industry Committee of the Institute of Brewing and Distilling, held at Curlew Street, London, 8 December 2017.

Seminar to University of Nottingham MSc Brewing Science, “Current Trends in hop usage for flavour” presented with Prof Ray Marriott of University of Bangor, held at Sutton Bonington Campus, Nottingham, 12 January 2018.

Report to R&D Committee of the British Hop Association, at spring meeting held at Thame, 17 April 2018.

Press pack distributed for Wye Hops Open Day, 23 July 2018.

Report to R&D Committee of the British Hop Association, held at China Farm, Canterbury, 23 August 2018.

Open Day for BHA growers held at China Farm, Canterbury, 23 August 2018.

Conducted tour of breeding plots at China Farm, Kent for Mike Unsworth, Operations Manager for Shepherd-Neame Ltd, 13 September 2018.

Submitted first interim report on maintenance of mapping populations to BREF, 24 September 2018.

Report to Hop Industry Committee of the Institute of Brewing and Distilling, held at Curlew Street, London, 23 November 2018.

Report to R&D Committee of the British Hop Association, held at James Figg Inn, Thame, 27 November 2018.

Hajdu, K., Cockerton, H.M., He, J.Q., Darby, P., Harrison, R.J. and Armitage, A.D. (2019) Cost effective genotyping for 21st century hop breeding. Proceedings of the Scientific Commission of the International Hop Growers Convention, Bischoffsheim, France (ISSN 2512-3785). Ed. F. Weihrauch. pp 6 – 9.

Seminar to University of Nottingham MSc Brewing Science, "Current Trends in hop usage for flavour" presented with Prof Ray Marriott of University of Bangor, held at Sutton Bonington Campus, Nottingham, 11 January 2019.

Lecture given to University of Cambridge MPhil Biotechnology students at Jesus College, Cambridge, 4 February 2019.

PhD Project introduced to NIAB-EMR Board, February 2019.

Visit from David Thompson, Trustee of Helen Wade Fund and E J Thompson Fund, 6 March 2019.

Reports to R&D Committee of the British Hop Association, at spring meeting held at Whitchurch-on-Thames, 16 April 2019.

Attendance at Hop Verticillium wilt workshop at Slovenian Institute of Hop Research and Brewing, Zalec, Slovenia, May 2019.

Visit to breeding plots from Cameron and Laura Ealam, New Zealand hop growers, accompanied by Guy Perry of Hopsteiner, 23 May 2019.

Presented keynote opening address to 2019 ASBC conference held in New Orleans, Louisiana, USA describing history and objectives for British hop breeding, 24 June 2019.

Visit to breeding plots from Drew Gaskell and Dean Monshing, Yakima Chief Hops, USA, 13 August 2019.

Report to R&D Committee of the British Hop Association, held at Parsonage and China Farms, Canterbury, 20 August 2019.

Visit and tour of breeding plots at China Farm for Karl Vanevenhoven, COO Yakima Chief Hops, Belgium, accompanied by Chris Daws, English Hops Ltd., 19 September 2019.

Visit and tour of breeding plots at China Farm for Dr Peter Kopp, Associate Professor, Department of History, University of Colorado, Denver, USA. 23 September 2019.

Submitted second interim report on maintenance of mapping populations to BREF, 16 October 2019.

Presented PhD in hop breeding at various student outreach events:
Reading University Undergraduate students' day, NIAB-EMR, October 2019.
Nottingham University DTP-PhD students' day, NIAB-EMR, October 2019.
Apprenticeship Fair 2020 - Hosted by four Kent MPs, Maidstone, February 2020.

Visit from Brett Porter and three colleagues, Director of Brewing and Innovation, Anheuser-Busch Craft breweries, USA., 8 November 2019.

Presentation "Molecular markers in hop breeding" at BHA Hop Technical Day held at NIAB-EMR, East Malling, Kent, 18 November 2019.

Report to R&D Committee of the British Hop Association, held at Queens Head, East Malling, 19 November 2019.

Report to Hop Industry Committee of the Institute of Brewing and Distilling, held at Curlew Street, London, 10 January 2020.

Seminar to University of Nottingham MSc Brewing Science, "Current Trends in hop usage for flavour" presented with Prof Ray Marriott of University of Bangor, held at Sutton Bonington Campus, Nottingham, 17 January 2020.

BSPP grant application; £3800 won for 2020 summer undergraduate student working on Hop PhD project: "Phenotyping Verticillium wilt resistance in Hop to novel, hyper-virulent forms of Verticillium non-alfalfa through large scale pathogenicity assays"

Draft paper; "Characterisation of novel sources of *Verticillium wilt* resistance in hop through QTL mapping" (planned submission date 2021; Journal: Theoretical and Applied Genetics).

Possible further work

Planned observations of the mapping populations *in situ* at Wye Hops Ltd have been completed. Individual plants have been characterised for their sex, dwarf habit, resistance to aphids, and resistances to powdery mildew. All field observations have been confirmed over more than one growing season and each trait in more than one family. This data is stored in a secured database and can now be used in statistical analyses in complementary projects to determine if any associations can be identified with SNP molecular markers.

Ambiguous and contradictory results have been highlighted and these individuals will be excluded from linkage analyses to avoid erroneous conclusions. In the complementary project, DNA profiles from all the parents of these mapping populations are being obtained and will be used to verify the parentage of each individual plant in the population so that, again, erroneous data will not be included and used in the development of SNP molecular markers.

Rootstocks derived from family 50/2015 are being screened in licensed growing facilities at NIAB-East Malling Research to observe the segregation of resistance to Verticillium wilt disease in this family and, hence, determine any association with specific SNP markers. The mapping populations were designed such that resistance to Verticillium wilt disease is also expected to segregate in the 'Fuggle' family, 40/2015. The field materials of family 40/2015 growing at Wye Hops Ltd can be used in the future to validate any putative molecular markers for wilt resistance.

Volatiles profiling and preliminary flavour studies using GC-MS and olfactometry facilities at the University of Reading will be used to analyse the samples harvested from the 'Cascade' population, 20/2015. The presence of specific compounds, notably thiols and their precursors, within the mapping populations can be used to investigate the inheritance of flavours and, hopefully, identify SNP markers for these flavours. A new Research Brewery and Vinery has been set up at NIAB-EMR and it is planned to conduct experimental brews with individuals from the 'Cascade' mapping population to take this work forward into the hop flavours imparted to beers.

The mapping populations being maintained at Wye Hops Ltd were designed to provide materials segregating for several other characteristics. These include resistance to downy mildew and the traditional flavours associated with 'Fuggle'. Maintaining these populations in the field will enable such future work to be carried out. The DNA of individuals in these

populations, when sequenced, will allow identification of further useful SNP markers for several other traits. Thus, the populations developed and maintained in this BREF project will allow hop breeding at Wye Hops Ltd to respond rapidly to new challenges in the future.

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