MINISTRY OF AGRICULTURE, FISHERIES AND FOOD

CSG 15

Research and Development

Final Project Report

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Project title	To develop hop breeding material resistant to pests and pathogens
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Executive summary (maximum 2 sides A4)

The UK hop industry has declined steadily in area and production over the past decade against a situation of world over-supply and low prices for hops used for their bitterness, and an increase in prevalence of disease problems in the traditional varieties used for their aroma properties in beer. The UK hop industry needs to become more competitive in cost, quality and supply. Opportunities for the UK producer to increase export potential and provide import substitution depend on developing new varieties with lower costs of production, improved pest and disease resistance, and increased quality and consistency. This project aimed to develop breeding lines suitable for use in a commercial breeding project to achieve these objectives, concentrating on markedly improved resistance to hop wilt (*Verticillium albo-atrum*), downy mildew (*Pseudoperonospora humuli*) and damson-hop aphid (*Phorodon humuli*) in combination with desirable agronomic and brewing characteristics. In particular, pest and disease resistance was sought in dwarf breeding lines

A review of results from field tests of resistance to hop wilt over the period 1993-96 revealed 36 genotypes with markedly less infection than shown by the most resistant commercial varieties 'Wye Target' and 'Phoenix'. The most promising twelve genotypes were selected for this trial, comprising both dwarf and conventional types, and progeny were generated for assessment of their resistance to wilt. Resistance to hop wilt was tested a replicated field trial in an isolated site where the soil was infested with inoculum from virulent strains of *V. albo-atrum*. Infection of perennial rootstocks was determined through observation of internal and external symptoms, and by a re-isolation of the pathogen from tissues at the base of the bine prior to senescence in the autumn. In a separate field trial, cone samples were harvested from random seedlings within each progeny family and the resin quality determined by HPLC analysis of the dried cones. Three breeding lines were identified which conferred strong resistance to wilt whilst maintaining good brewing and agronomic quality attributes in their progeny. These lines have now contributed seedlings to a separate commercial hop breeding programme.

To improve the efficiency and speed of identification of suitable wilt-resistant parents for a crossing programme, work was conducted to develop a glasshouse progeny test of resistance to wilt. Basic procedures were outlined in replicated randomised experiments with seedlings raised in a frost-free glasshouse and inoculated when approx. 10cm tall with *V. albo-atrum* by dipping their roots in conidial suspension. The incidence and severity of wilt symptoms were recorded approx. 50 days after inoculation and scored visually. Results were compared with those from concurrent field tests of perennial material of the same progenies. The glasshouse tests produced high levels of symptom expression and there was a very good agreement between ranking of family results for field and glasshouse for symptom expression. There was less agreement between the glasshouse results and the incidence of infection in the field. It was concluded that glasshouse progeny tests can give a reliable indication of the relative resistance of the parental genotypes, providing a convenient screen to eliminate the more susceptible parents from further evaluation.

Ten dwarf female breeding lines, selected from segregating progeny of 'Wye Challenger', the most downy-resistant commercial UK hop variety, were planted in a randomised replicated field trial. Interspersed infected plants of 'Wye Target' provided a high and uniform inoculum of downy mildew and symptoms of the disease were recorded on the test lines early season, midseason and late season for two growing seasons. Large differences, consistent between blocks, were noted between genotypes in the amount of disease evident although the relative ranking of genotypes varied throughout the seasons. It was possible to identify lines which were more resistant than reference varieties 'Herald' and 'First Gold'. Also, detached leaf tests of the field-grown material were made under controlled environment conditions. There were marked varietal differences in sporulation on detached leaves but, because there were inconsistencies in the results for some genotypes, it was concluded that measurement of sporulation on detached leaves was not an accurate indication of field performance.

From the downy mildew field trial, five breeding lines were selected to represent the range of resistance expressed, and these were crossed to a common male parent. The resultant seedlings were inoculated in the glasshouse under high humidity with an aqueous suspension of *P. humuli* when at the two-leaf pair stage. Progeny were assessed after 3 weeks for those showing systemic infection. The level of resistance shown by the progeny corresponded well with the early season field susceptibility of the mother and confirmed that lines more resistant than 'First Gold' and 'Herald' can transmit good resistance to downy mildew to their progeny.

To identify genotypes suitable for future studies on the use of IPM crop management strategies in UK hop production, 34 selections, both dwarf and conventional genotypes, were identified from previous trials as resistant to damson-hop aphid and these were tested for their resistance to *Verticillium* wilt in a field trial. Wilt-resistant genotypes were established in unreplicated 20-plant field plots. From the mature plots, harvest yield data were obtained and random samples from the produce were analysed for brewing quality by HPLC. In addition, progeny from controlled crosses between these genotypes and a common male parent, were tested in a glasshouse screen for resistance to downy mildew. A dwarf genotype resistant to wilt, downy mildew and aphids, and with a yield potential close to commercially acceptable levels was identified and supplied to two farm trial sites.

During the period of this project, strategic research on the resistance of the hop to pests and diseases and the incorporation of these resistances into commercial varieties has been described in a total of 20 publications and 82 presentations. Publications have been in the popular, trade and scientific press. Presentations have included talks to hop growers, brewers, and students, and interviews on local and national radio and television, as well as poster presentations to scientific conferences.

Scientific report (maximum 20 sides A4)

1. INTRODUCTION

Hop (*Humulus lupulus*) production in the UK is divided approximately equally between the South East (Kent, Sussex, Hants) and the West Midlands (Hereford and Worcester) with approx. 40% of the area growing hops used for imparting bitterness to beer and the remainder growing hops used for their aroma qualities. The market value for bitter hops is subject to major fluctuations caused by year to year variation in crop yields and by marked cyclical changes in world market prices. After several years of over-supply on the world market, prices for bitter hops have been at, or below, the costs of production and the UK area of bitter hops has declined to 837ha in 2001 from 1242ha in 1997. Almost 60% of UK aroma hops are traditional varieties which are susceptible to all the main fungal diseases, and the area of aroma hops has declined steadily over the last decade to just 1118ha in 2001 as the incidence of hop wilt has increased.

To safeguard the domestic market, the UK hop industry needs to become more competitive in cost, quality and supply. Opportunities for the UK producer to increase export potential and provide import substitution depend on developing new varieties with lower costs of production, improved pest and disease resistance, and increased quality and consistency. The industry recognises that the development of dwarf hop varieties and low-trellis husbandry techniques provide a means to achieve these objectives.

This project aims to provide strategic underpinning for a separately-funded breeding programme to develop commercial hop varieties, by identifying elite hop breeding lines conferring to their progeny high levels of resistance to pests and pathogens and showing good general combining ability for cone and habit traits, in particular dwarf habit characteristics, and brewing quality. It concentrates on hop wilt (*Verticillium albo-atrum*), downy mildew (*Pseudoperonospora humuli*) and damson-hop aphid (*Phorodon humuli*). Plant breeding offers the only reliable approach to the control of hop wilt because there are no pesticides available for use against this disease. The evolution of more virulent strains threaten the UK hop industry and it is essential that all new varieties have resistance to hop wilt of a level to match the increased virulence of the pathogen. Increased susceptibility to downy mildew as a result of dwarf hop production techniques has been noted during the commercialisation of the dwarf varieties released by HRI to UK producers in 1996. Continued expansion of dwarf production in the UK requires that future varieties are considerably more resistant to downy mildew than those releases. To reduce the current complete dependence on pesticides for aphid control, an enhanced level of host plant resistance to aphid population growth is required. IPM strategies to provide consistent and effective control of the hop aphid are being developed. Unfortunately, aphid-resistant genotypes developed to date in other projects are uncommercial in many attributes. To evaluate the commercial costs and benefits from IPM strategies, this material needs to be developed further.

2. OBJECTIVES OF THE PROJECT

a) Hop wilt

To identify elite hop breeding lines of both conventional and dwarf genotypes able to confer to their progeny levels of field resistance to *V.albo-atrum* at least 20% greater than presently available in the most resistant commercial variety, 'Phoenix'.

b) Downy mildew

To identify dwarf genotypes able to confer to their progeny levels of field resistance to *P. humuli* at least 25% greater than presently available in dwarf varieties 'First Gold' or 'Herald'.

c) Damson-hop aphid

To evaluate the yield potential and field resistance to wilt and downy mildew of advanced selections also resistant to damson-hop aphid, thus providing genotypes with yields within 85% of commercial values for use in complementary projects on IPM in hops.

3. IDENTIFICATION OF BREEDING LINES FIELD-RESISTANT TO HOP WILT (objective a)

3.1 Combination of characteristics

Breeding lines combining strong resistance to wilt with good agronomic and brewing quality characteristics were sought for use in a programme of varietal improvement.

3.1.1 Experimental procedures

A review of results from field tests of resistance to hop wilt over the period 1993-96 revealed 36 genotypes with <10% infection; markedly less infection than shown by the most resistant commercial varieties 'Wye Target' and 'Phoenix' in these tests over this same period. Observation notes from breeding and selection plots were examined to assess the commercial suitability of these genotypes and twelve genotypes were selected for this trial, comprising both dwarf and conventional types. Seven genotypes were female and five were male. Controlled crosses were made to a common parents contributing aroma and bitterness. Female genotypes were crossed with male 43/72/2 to give an aroma background, and 28/77/145 to give a bitterness background. Male genotypes were crossed with 'WGV' for aroma and 'Admiral' for bitterness. Seed was obtained from all 24 crosses. Additionally, crosses were made with 'Phoenix' to provide reference progenies.

Seedlings were raised under glasshouse conditions. They were inoculated with an aqueous suspension of sporangia of *Pseudoperonospora humuli* at a concentration of 1.5×10^4 sporangia/ml when at the two true-leaf stage and, subsequently, with conidia of *Sphaerotheca humuli* when approximately 20 cm tall. From each family, at least 45 individuals showing greatest resistance to both pathogens were selected for transplanting to the field where they were established on a 'Worcester' system: plants 1m apart in rows 2.3m apart with two support strings per plant.

Softwood cuttings of a random 30 individuals of each family were taken immediately prior to transplantation and rooted under mist. They were planted through a paper mulch in an irrigated field and grown for a season producing dormant perennial rootstocks for field tests of resistance to hop wilt.

The following season, these rootstocks were planted in a replicated field trial in soil infested with inoculum from virulent strains of *V. albo-atrum*. Infection was determined through observation of internal and external symptoms, and by a re-isolation of the pathogen from tissues at the base of the bine prior to senescence in the autumn

In a separate field trial, cone samples of at least 250g fresh weight were harvested from a random ten seedlings within each progeny family. Resin quality was assessed by HPLC analysis of the cone samples dried to 10% moisture.

On the basis of the results from the field trials for resistance to wilt and the quality assessments, the "best" male and female parents were identified and used in a further crossing programme to complement their characteristics. Thus, conventional height parents were crossed with dwarf genotypes; parents susceptible to powdery mildew were crossed with resistant genotypes, etc. All the complementary parents were known to be resistant to wilt from previous trials, and several standard, well-tested genotypes were selected for this crossing programme. A total of six crosses were made and seed collected.

Following vernalisation for 6 weeks at 2^oC, seeds were planted individually in compost cells and resulting seedlings inoculated under high humidity with a 9×10^3 sporangia/ml aqueous suspension of *P. humuli* when at the two-leaf pair stage. Progeny were assessed after 3 weeks and those showing systemic infection discarded. From each family, 30 individuals were transplanted to the field and established on a 'Worcester' system to provide seedlings for assessment in the commercial breeding programme.

3.1.2 Results and discussion

In the replicated field trial for resistance to wilt disease, environmental conditions were conducive to infection and high levels of infection were recorded in reference varieties with 50% and 30% infection in plots of 'Wye Target' and 'Phoenix' respectively, the most resistant varieties in commerce.

Genotype	Family identity		Wilt infection (%)	Alpha -acid (%)	Beta -acid (%)	Co-humulone (%)
	Bitterness background	Aroma background				
24/84/27	18/97		59	8.5	3.4	36
"		13/97	92	6.3	3.1	43
44/84/20	19/97		56	10.5	3.8	35
"		14/97	- *	8.5	3.3	36
28/86/73	20/97		41	8.5	3.5	36
"		15/97	40	6.8	2.9	35
28/89/26	21/97		79	10.5	4.8	40
"		16/97	92	6.0	2.8	43
20/91/11	22/97		61	9.7	4.8	39
"		17/97	73	6.1	2.8	45
34/80/56	26/97		50	8.0	4.7	36
"		25/97	46	6.5	3.6	38
15/85/16	28/97		- *	8.1	3.2	35
"		27/97	- *	8.4	4.4	39
12/88/20	30/97		79	7.6	3.2	47
"		29/97	57	4.9	2.8	44
16/88/20	32/97		81	5.3	2.5	34
"		31/97	- *	4.6	3.0	41
17/88/60	34/97		63	6.0	3.4	40
"		33/97	47	5.2	4.2	49
18/88/57	36/97		79	6.1	2.7	37
"		35/97	60	5.3	3.7	41
36/91/54	38/97		80	5.9	2.8	34
"		37/97	38	5.9	3.8	37
Phoenix	24/97		78	5.8	3.4	37
"		23 /97	42	5.6	3.4	42

Table 1. Progeny tests for field resistance to wilt and quality attributes

* indicates that the progeny were not tested due to failures in propagation

Test progenies showed a wide range of susceptibilities (Table 1) with family means from 38% to 92% infection, despite all originating from parents classed as highly resistant. Thus, it was possible to distinguish the ability to transmit resistance to wilt between parents which had been recorded at similar levels of resistance to wilt. There was some interaction between the level of wilt infection and the genetic background of the cross but several genotypes were consistent in the transmission of resistance to their progeny, notably 28/86/73, 34/80/56 and 17/88/60. These breeding lines all showed at least 20% greater resistance to wilt in their progeny compared to those of 'Phoenix'.

Quality analysis of the same progenies showed some individuals to have alpha-acid contents up to 12.6% and cohumulone contents as low as 25%. The family means for quality attributes are shown in Table 1. Thus, it was possible to distinguish the parents on their ability to transmit desirable quality attributes to their progeny. Furthermore, despite being selected at random, eleven individual seedlings were considered to show sufficient yield potential to be selected for further evaluation as potential new hop varieties.

On the basis of these progeny test results, three lines were selected for use in the commercial breeding programme. Male line 44/84/20 produced the seedlings with the highest alpha-acid contents, yet with desirable low cohumulone, and with strong resistance to wilt in the cross with 'Admiral'. Male line 28/86/73, as well as consistent wilt resistance, gave progeny with moderately high alpha-acid contents and low cohumulone contents. Dwarf female line 17/88/60, also identified in the wilt tests, imparted good aroma characteristics to its progeny. Female line 34/80/56 was not selected for further use despite good resistance to wilt in its progeny because the progeny also displayed very poor agronomic features, notably the plant habits.

3.1.3 Conclusions

This project aimed to identify hop breeding lines conferring high levels of resistance to wilt disease to their progeny. From the most resistant lines in the HRI hop breeding programme, three breeding lines have been identified which achieve this objective whilst maintaining good brewing and agronomic quality attributes in the progeny. These lines have now contributed seedlings to a separate commercial hop breeding programme.

3.2 Seedling progeny tests

To improve the efficiency and speed of identification of suitable wilt-resistant parents for a crossing programme, work was conducted to develop a glasshouse progeny test of resistance to wilt.

3.2.1 Experimental procedures

To establish basic procedures for screening hop seedlings in a glasshouse for resistance to wilt, three progenies were generated in controlled crosses. Parents had been selected to produce similar levels of resistance in their progeny so that. non-genetic variation could be distinguished. Following vernalisation for 6 weeks at 2^{o}C, seeds were planted individually in compost cells and resulting seedlings inoculated when approx. 10cm tall with *V. albo-atrum* by dipping their roots in conidial suspension (1×10^4 or 2×10^3 or 4×10^2 conidia/ml). Seedlings were raised in a frost-free glasshouse and the experiment was laid out in a replicated randomised block design of each family at each inoculum concentration with four replicates, a replicate comprising 15 seedlings. The incidence and severity of wilt symptoms were recorded 53 days after inoculation and scored visually on a 0-4 scale from no symptoms (0) to killed by the pathogen (4).

To explore the potential of this system to distinguish between progenies, six controlled crosses were made between two females with differing levels of polygenic resistance and three males with differing levels of resistance assumed to be attributable to major gene resistance. Using the procedures already described, the resulting progenies were challenged by a root dip into a conidial suspension of *V. albo-atrum* (1×10^4 conidia/ml). A four-replicate randomised block design was again used with each replicate comprising 15 seedlings. Incidence and severity of wilt symptoms were recorded after 52 days.

To distinguish amongst progenies of resistant parents, nine controlled crosses were generated. The parents were all classed as “resistant” or “highly resistant” from previous field tests. Using the same procedures as previously, the progenies were challenged with a root dip into a conidial suspension of *V. albo-atrum* (1×10^4 conidia/ml). Incidence and severity of wilt symptoms were recorded after 50 days in the glasshouse and the results compared with those from concurrent field tests of perennial material of the same progenies planted in soil artificially infested with virulent strains of *V. albo-atrum*. These same progenies were again tested for a second season but were challenged with two concentrations of spore suspensions; 1×10^4 conidia/ml, as previously, and 5×10^4 conidia/ml.

In the final year of the project, the glasshouse tests were employed to assess progenies from previously untested parents where there was no expectation of the level of resistance to wilt in the progenies. Five progenies were tested using the glasshouse procedures developed in this project with an inoculum concentration of 5×10^4 conidia/ml. Concurrently, perennial material of the same progenies were assessed in field tests where each progeny was represented by 15 individuals, with five plants of each individual.

3.2.2 Results and discussion

The incidence and severity of wilt were similar in all three initial families when inoculated at the highest conidial dose (Table 2). Most plants were either symptomless or severely affected and %incidence gave the best measure of differences between families. Lower inoculum doses resulted in less wilt and inconsistent differences between families. Statistical analysis confirmed inoculum concentration as the main source of variation. It also indicated that replicate size and number could not be reduced without introducing too much error into the analysis of the results. Thus, 1×10^4 conidia/ml was chosen as the working inoculum concentration for the subsequent glasshouse tests, with all other experimental protocols remaining unaltered.

Inoculum dose	Family A	Family B	Family C
4×10^2 conidia/ml	45.0	18.3	30.8
2×10^3 conidia/ml	66.7	46.7	34.6
1×10^4 conidia/ml	85.0	88.3	88.9

Table 2 Incidence (%) of wilt symptoms in progenies in glasshouse tests

Results from tests to distinguish between progenies expected to differ in resistance to wilt indicated significant differences between both male and female parents with rankings of progeny exactly reflecting the known level of resistance of the parents. This indicated that both polygenic and major gene mediated resistance could be assessed in seedlings in the glasshouse. In the most susceptible combination, 65% of seedlings developed wilt symptoms while in the most resistant combination, only 18% were affected. This amount of variation allowed resistant parents to be distinguished from susceptible parents with confidence.

However, results from families derived only from known resistant parents showed a poor agreement between field and glasshouse progeny tests. Levels of wilt symptoms in the glasshouse were low with <45% incidence in the most susceptible family and it was not possible to rank, with confidence, the parents according to their ability to transmit resistance to wilt disease. This glasshouse test did not provide a sufficient challenge to distinguish amongst these resistant parents, although the procedures had previously been useful in distinguishing resistant from susceptible parents.

In contrast, during the repeat of this validation of seedling results with field results in a second season, the glasshouse tests produced high levels of symptom expression, even at the lower concentration, and there was a very good agreement between ranking of family results for field and glasshouse for symptom expression, particularly at the higher inoculum concentration (Table 3). The families showing lowest and highest incidences of symptoms in the field corresponded with those in the glasshouse and where field results were close, so were the results from the glasshouse.

Family identity	Glasshouse incidence of wilt symptoms (%) 5×10^4 conidia/ml	Field incidence of wilt symptoms (%) -second season	Field incidence of wilt infection (%) -second season	Field incidence of wilt infection (%) -first season
22/98	33	15	62	61
18/98	53	20	33	59
20/98	56	21	39	41
30/98	46	31	76	79
34/98	76	41	78	63

Table 3 Comparison of glasshouse and field progeny tests for resistance to wilt

The failure of the glasshouse tests in the first season indicated that the inoculum concentration of 1×10^4 conidia/ml, although satisfactory in most years, was sometimes too low to compensate for the variability of the glasshouse environment and the higher concentration of 5×10^4 conidia/ml was adopted for future glasshouse tests.

There was less agreement between the incidence of wilt in the glasshouse and the incidence of infection in the field. Many plants in the field show no symptoms but re-isolation of the pathogen from the basal bine tissues at the end of the season shows these plants to have been infected. Experience has shown that the incidence of infection is a more useful measure of the resistance of a genotype rather than symptom expression alone. The agreement between the field results between the two seasons of this test indicates that the field test procedures were satisfactory. The variation between seasons in some families can be readily explained by the type of resistance, polygenic or major gene, in each family and the variation caused by environmental effects in the field affecting the expression of polygenic resistance more than major gene resistance.

Symptom expression has, historically, been used to screen progenies in the field test to distinguish the susceptible genotypes, with a subsequent more detailed infection test to determine the resistance of the genotype. Thus, the glasshouse test appears to give a satisfactory replacement for the field progeny screen but does not give sufficient information to allow the resistance of the parental genotype to be ascertained with confidence.

To test this idea, for the final season of this work the glasshouse progeny test was compared directly with the field progeny screen rather than with the field resistance tests based on infection. The results (Table 4) indicate a good general agreement between the tests. Progenies 73/99 and 74/99 identified in the glasshouse test as susceptible were confirmed as susceptible in the field and, more importantly, progeny 75/99 was identified by the glasshouse procedures as resistant and this was validated by the field screen. The families with intermediate glasshouse scores gave intermediate or susceptible reactions in the field. These families were selected from the HRI hop breeding programme with no prior knowledge of their reaction to wilt disease. This is typical of the situation within the programme and this test has indicated that useful information can be obtained from glasshouse progeny tests.

3.2.3 Conclusions

The experiments described here have allowed procedures to be refined to provide a glasshouse progeny test for resistance to wilt. The results from these glasshouse experiments indicate that such progeny tests can give

a reliable indication of the relative resistance of the parental genotypes, providing a convenient screen to eliminate the more susceptible parents from further evaluation.

Family identity	Glasshouse score *	Field score **
73/99	2.68	4.7
74/99	2.82	3.9
75/99	0.71	1.8
76/99	1.42	2.7
77/99	1.22	4.2

Table 4 Comparison of glasshouse and field screens for resistance to wilt

* Mean of individual scores on 0-4 scale
** Mean of individual scores on 0-5 scale

4. IDENTIFICATION OF DWARF HOP LINES CONFERRING RESISTANCE TO DOWNY MILDEW (objective b)

4.1 Experimental procedures

Ten dwarf female breeding lines, selected from segregating progeny of a cross between 'Wye Challenger', the most downy-resistant commercial UK hop variety, and a dwarf male, were propagated by soft-wood cuttings to produce 24 dormant perennial rootstocks of each for testing for resistance to downy mildew in a field trial. To provide reference material, rootstocks of dwarf varieties 'Herald' and 'First Gold' were similarly produced. To provide field inoculum, 300 rootstocks of 'Wye Target', a highly susceptible commercial variety, were propagated and inoculated with an aqueous conidial suspension of downy mildew washed from sporulating leaf lesions. To provide guard material to surround the field plot, rootstocks of 'Wye Challenger' were also propagated.

All test rootstocks, as plots of six plants, were planted in field positions in a randomised replicated trial comprising four blocks. Six infected plants of 'Wye Target' were planted between each test plot to ensure that inoculum levels were high and uniform, and the entire trial was surrounded by plants of 'Wye Challenger' to minimise entry of extraneous inoculum into the trial area

Growing shoots were trained on to netting on a low trellis husbandry system. No fungicides for downy mildew control were applied and symptoms of the disease were recorded early season, midseason and late season for two growing seasons.

On the basis of results over the two seasons of the trial, five breeding lines were selected to represent the range of resistance expressed. These, together with 'First Gold' and 'Herald', were crossed to a common male parent and the resultant seeds collected for subsequent glasshouse progeny tests.

Following vernalisation for 6 weeks at $2^{o}C$, seeds were planted individually in compost cells and resulting seedlings inoculated under high humidity with a 9×10^{3} sporangia/ml aqueous suspension of *P. humuli* when at the two-leaf pair stage. Progeny were assessed after 3 weeks for those showing systemic infection. Results were compared with reference families derived from 'Wye Challenger' and 'Northern Brewer', and known to indicate resistance and susceptibility, respectively, in the parents.

To complement the progeny tests, detached leaf tests of the field-grown material were made under controlled environment conditions. For each of the dwarf breeding lines, six fully expanded young leaves were removed to humid chambers and inoculated at six random sites on each leaf with 70µm droplets of 1 x 10^4 sporangia/ml aqueous suspension. Sporangia production was measured after 7 days incubation at 13.5^{o}C with 19 hr daylength. This test was performed on two dates; at the end of May and one month later.

4.2 Results and discussion

Symptoms of the disease were noted on cones in all 'Wye Target' plots at the end of the first field season, indicating that the pathogen had established evenly throughout the trial.

Large differences, consistent between blocks, were noted between genotypes in the amount of disease evident throughout the seasons (Table 5). The relative ranking of genotypes varied with some more resistant to cone infection than early season shoot deformation, and *vice versa*. At each part of the growing season, genotypes were identified which were more resistant than reference varieties 'Herald' and 'First Gold'. In particular, breeding line 16/93/9 showed levels of cone infection equivalent to that seen in the adjacent guard rows of 'Wye Challenger', the most resistant commercial variety. Growers' reported experience of early season susceptibility of the two registered dwarf varieties, particularly 'First Gold', and the later cone resistance of 'Herald' were substantiated in this trial allowing confidence in the results for the test breeding lines.

Variety	Total no. deformed shoots in early season	Total no. deformed lateral shoots in midseason	Average incidence of cone infection in late season (%)	Comments
First Gold	15	76	37.5	very susceptible early season
Herald	6	76	22.5	cone resistance
16/93/9	2	5	10.5	highly resistant throughout
17/93/3	3	79	50.8	fully susc. in mid and late season
17/93/24	2	6	29.5	good resistance throughout
17/93/25	4	10	19.5	resistance in mid and late season
17/93/51	3	6	42.5	cone susceptibility
18/93/35	2	8	29.8	good resistance throughout
18/93/85	1	66	50.3	fully susc. in mid and late season
18/93/101	0	67	26.8	midseason susc.
19/93/8	4	34	30.3	moderate resistance throughout
19/93/25	5	103	52.0	fully susceptible

Table 5 Expression of downy mildew in field trial of dwarf breeding lines

There were marked varietal differences in sporulation on detached leaves incubated in a controlled environment, with results generally reflecting the severity of symptom expression in the field (Tables 5 and 6). As seen in the field symptom expression, the relative ranking of resistance, as expressed by sporangia production, changed between breeding lines in the later test. However, there were some inconsistencies

between the field observations and the detached leaves tests. In particular, line 17/93/24 was quite resistant throughout the field trial but detached leaves were highly susceptible. These results indicate that measurement of sporulation on detached leaves was not an accurate indication of field performance. The interaction of genotype and resistance to downy mildew infection with time and environment is clearly very complex.

Glasshouse tests for resistance to downy mildew amongst progeny of dwarf breeding lines selected for a range of symptom expression in the field showed the test lines to cover fully the range from resistance to susceptibility defined by the reference progenies (Table 7). The progeny of 16/93/9, in particular, showed greater resistance than the reference progeny derived from 'Wye Challenger' and nearly 40% better than the progeny of 'Herald'. The level of resistance shown by the progeny corresponded well with the early season field susceptibility of the mother. Both measures of resistance reflect systemic infection of the plant by the pathogen whereas cone infection in the field results from local, topical infection. Thus, seedling progeny tests have been shown to be a useful indicator of some aspects of field resistance. In commercial production, the amount of inoculum of *P.humuli* is the main determinant of the problem faced by the grower and most control measures aim to reduce inoculum levels which subsequently reduces the probability of cone infection. Varieties where the systemic over-wintering of the pathogen in the rootstocks is reduced will be considered the most resistant. This is more important in the cultivation of dwarf varieties where the higher planting density compared to traditional plantations provides a more humid environment conducive to the development of downy mildew. Thus, early season resistance is more important in dwarf varieties and the seedling progeny tests have been shown to give a good indication of this resistance.

Variety	Sporulation intensity 26 May (sporangia/ml)	Sporulation intensity 29 June (sporangia/ml)
First Gold	3573	1404
Herald	5244	444
16/93/9	1190	338
17/93/3	800	3095
17/93/24	4498	2328
17/93/25	530	284
17/93/51	2700	1706
18/93/35	356	348
18/93/85	3235	1315
18/93/101	6008	978
19/93/8	729	356
19/93/25	4800	1102

Table 6 Sporulation of *P.humuli* on detached leaves in controlled conditions

4.3 Conclusions

This project aimed to identify dwarf breeding lines able to confer on their progeny resistance to downy mildew greater than that currently available in the registered dwarf varieties 'First Gold' and 'Herald'. It has identified several lines as more resistant than these varieties and has confirmed through progeny tests that at least one of these, 16/93/9, transmits its good resistance to its progeny.

Female parent	Family no.	Field observations of parent	Severe symptoms (%)
First Gold	5/2000	early season susceptibility	56.3
Herald	4/2000	cone resistance	34.6
16/93/9	6/2000	highly resistant	21.3
17/93/25	7/2000	resistance later in season	61.3
18/93/85	8/2000	susceptibility later in season	35.3
18/93/101	9/2000	susceptibility midseason	76.3
19/93/25	10/2000	fully susceptible	69.3
Resistant reference	1/2000		29.9
Susceptible reference	2/2000		72.0

Table 7 Development of symptoms of downy mildew in progeny of dwarf selections

5. EVALUATION OF GENOTYPES SUITABLE FOR USE IN I.P.M. PROJECTS (objective c)

5.1 Experimental procedures

From previous trials where field plots were untreated with aphicides and natural infestations of damson-hop aphid were allowed to develop, 34 selections, both dwarf and conventional genotypes, were identified as resistant to damson-hop aphid. These were propagated by soft-wood cuttings to produce perennial dormant rootstocks which were tested for their resistance to *Verticillium* wilt in a field trial. For each selection, five plants were assessed for the development of external symptoms following planting of the rootstocks in soil infested with virulent strains of the pathogen. Results were related to the incidence of symptoms on reference varieties.

Those genotypes identified as resistant to wilt were further propagated to produce dormant rootstocks which were planted and established in unreplicated 20-plant field plots. These were grown and harvested under commercial husbandry procedures. From the mature plots, harvest yield data were obtained and random samples from the produce were analysed for brewing quality by HPLC. Comparative data were obtained from adjacent reference plots of 'Yeoman' and 'Wye Target'.

The resistance of these genotypes to downy mildew was assessed. Seed was collected from controlled crosses using a common male parent and the resulting progeny tested in a glasshouse screen the following season. Seedlings were inoculated at the two true-leaf stage with an aqueous suspension of sporangia (1×10^4 sporangia/ml) and recorded after three weeks. Results were compared with reference families derived from 'Wye Challenger' and 'Northern Brewer' and known to indicate resistance and susceptibility, respectively, in the parents.

A genotype resistant to wilt, downy mildew and aphids, and with a yield potential close to commercially acceptable levels was identified and propagated to provide dormant perennial rootstocks as planting material for complementary studies on the commercial exploitation of IPM in hops. It was supplied to two farm trial sites, one in the South-East and one in the West Midlands, to plant 0.4ha plots.

5.2 Results and discussion

In the field test for resistance to wilt, three of the aphid-resistant selections showed no symptoms and only one plant developed symptoms with a further five selections. This level of resistance was similar to that shown by reference varieties 'Yeoman' and 'Wye Target', both of which are considered to be resistant to the disease in commerce. Twenty four of the selections tested were tall, conventional habits and ten selections were dwarf plants. However, only one of those showing resistance was dwarf; selection 17/92/54. The remaining twenty eight susceptible selections were rejected from further trials. These results are consistent with the independent segregation of the traits for resistance to aphids and resistance to hop wilt. However, the shortage of selections which combine these resistances with dwarfness may indicate an adverse genetic association with plant habit.

The yield and analytical data (Table 8) obtained in 2001 from mature plots of the eight genotypes resistant to wilt showed four selections to produce unacceptably low yields (<30 Zn/ha). The yield of the dwarf selection was acceptable commercially and three others yielded comparably with the reference commercial varieties indicating no genetic barriers to combination of resistance to hop aphids with good yield potential. Indeed, the yield of one of the selections surpassed that of 'Wye Target' although its alpha-acid content was too low for it to progress to further commercial trials. Analytical data showed a wide range of values for the main quality parameters. None of the eight selections had an alpha-acid content or xanthohumol content above the reference varieties but several had low cohumulone contents, similar or less than that of 'Yeoman', which is considered to be a good quality attribute. In contrast, two selections had a cohumulone content >40% which is outside the commercially acceptable range. The genotypes were selected only for their resistance to aphids and these results indicate that no bias with regard to agronomic or quality traits is introduced by such selection.

Variety	Yield (Zn/ha)	Alpha-acid content (%)	Cohumulone content (%)	Beta-acid content (%)	Xanthohumol content (%)
14/92/8	21.6	6.7	33	2.5	0.34
14/92/41	19.8	4.1	45	1.8	0.20
20/92/56	50.2	8.6	33	2.9	0.50
17/92/24	23.5	5.3	30	2.7	0.24
17/92/54	32.1	9.5	26	4.0	0.38
21/92/42	21.3	7.3	43	2.8	0.25
27/92/31	35.5	6.5	33	2.6	0.36
27/92/33	34.1	9.5	28	3.4	0.50
Yeoman	36.1	10.2	30	4.8	0.62
Wye Target	45.6	10.8	37	4.5	0.67

Table 8 Harvest data from 2001 season

Glasshouse tests for resistance to downy mildew indicated that there was strong resistance in the progeny from two selections (Table 9) with the progeny showing less infection than in the resistant reference progeny. Similarly, two selections gave progeny with poor levels of resistance comparable to the susceptible reference. The downy-resistant selections were both low yielding and one of the downy-susceptible selections was one of the better yielding. This slight indication of an adverse association of yield and resistance to downy mildew

within aphid-resistant hop material requires further study to enable an efficient selection strategy to be developed.

From these data on the resistances of these aphid-resistant selections to wilt and downy mildew, together with the agronomic and quality data, genotype 17/92/54 was chosen as the most suitable for use in IPM studies. This genotype, established on farm trial sites, will be the basis of another project.

5.3 Conclusions

This project aimed to identify genotypes suitable for future studies on the use of IPM crop management strategies in UK hop production and, from the work presented here, it has been possible to select such a genotype. Although the crosses were not designed to examine the genetic interactions of the various traits, the indications from this work are that an appropriate hop breeding strategy should be able to produce an aphid-resistant variety of acceptable commercial quality.

Variety	Severe symptoms (%)
14/92/8	48.2
14/92/41	16.3
20/92/56	25.2
17/92/24	25.7
17/92/54	32.0
21/92/42	12.5
27/92/31	46.0
27/92/33	27.0
Resistant reference	21.6
Susceptible reference	51.6

Table 9 Development of symptoms of downy mildew in progeny of aphid-resistant parents

6. KNOWLEDGE TRANSFER

During the period of this project, strategic research on the resistance of the hop to pests and diseases and the incorporation of these resistances into commercial varieties has been described in a total of 20 publications and 82 presentations. Publications have been in the popular, trade and scientific press. The principal publications include :

Darby, P. (1997). ‘A century of hop breeding’. In: Brewing Room Book 1998-2000, pp 57-61, Pauls Malt, Suffolk

Proudlove, M., Darby, P., Baxter, D. & Young, G. (1998). Trial brewing with aphid resistant hops. Confidential report in *BRI Quarterly*, October 1998, 8-10, Nutfield.

Locke, T., Worsley, K. & Darby P. (1999). MAFF Horticulture information sheet '*Verticillium* wilt of hops', MAFF.

Darby, P. (1999). New selection criteria in hop breeding. Proceedings of the Scientific Commission of the International Hop Growers Convention, Pulawy, Poland, 1999, Ed. E. Seigner, 3-6.

Darby, P. (1999). Genetic improvement research leading to the breeding, development and commercialisation of varieties of dwarf hops. Preisverleihung der Rudolf Hermanns Stiftung 1999, 20-24, Geisenheim, Germany.

Darby, P. (2001). Single gene traits in hop breeding. Proceedings of the Scientific Commission of the International Hop Growers Convention, Canterbury, UK., 2001. Ed. E.Seigner. pp 76-80.

Darby, P. & Walker, C. (2002). Where next for the hop industry? *Brewers Guardian*, February 2002, 22-25.

Presentations have included talks to hop growers, brewers, and students, and interviews on local and national radio and television, as well as poster presentations to scientific conferences. A selection of the presentations specifically describing the work in this project include :

'Overview of the research programme' to Wye Hop Industry Board, Brewers' Open Day at Wye, 20 August 1997

'The future of hop growing in England' to the National Hop Association at Dormington, Hereford, 4 September 1997

'Developing a method for assessing wilt resistance in hop seedlings'. Poster presented to the 7th International *Verticillium* Symposium, Cape Sounion, Athens, Greece, 6-8 October 1997

'New Developments in Hop Growing' to the Institute of Brewing, Yorkshire and North Eastern Section at Tadcaster, Yorkshire, 6 November 1997

'Description of symptoms of hop root, leaf and cone pests and pathogens' to Willmot-Pertwee Ltd at HRI-East Malling, 2 December 1997

'Hop production in UK agriculture' to third year students of Agriculture and Agricultural Business Management at Wye College, University of London, 11 December 1997

'Hop breeding as a component of biological crop protection' to MSc students of Biological Crop Protection at Wye College, University of London, 16 March 1998

'Highlights of the HRI-Wye Hop Research 1998 season'. Talk to the NFU East Sussex and Kent Area Hops Forum, held at Bradbourne House, East Malling, 10 November 1998.

'Varietal resistance: past, present and future'. Presentations to Hop Growers Workshops on *Verticillium* wilt sponsored by MAFF Horticulture Division and organised through ADAS in Hereford on 10 March and at Bradbourne House, East Malling, 18 March 1999.

"Economic yield potential of dwarf hop varieties". Talk to an International Symposium on "New techniques in hop cultivation" held at Hopfenforschungsinstitut, Hüll, Germany on 17 May 1999.

"Origins of varietal resistance to wilt disease". Talk to MAFF Plant Health hop inspectors at a meeting in Wye, 27 May 1999.

"Current research and developments in hop breeding". Talk to the Institute of Brewing 2000 Brewers' Hop Day held at the offices of English Hop Products, Paddock Wood, Kent, 27 January 2000.

Interview with Meridian TV as part of Meridian News programmes on 'Organic hops and green beer', 22 September 2000.

'New hop varieties: what's new'. Presentation to Brewing Research International Hop Seminar, held at Redhill, 1 November 2000.

'Hop trials 2000'. Talk to the English Hops Ltd, Hop Seminar held at Scottish-Courage, Berkshire Brewery, Reading, 7 December 2000.

"Organic hops – nearly a reality". Presentation to Brewing Research International Organic Beer Seminar, Redhill, 31 January 2001.

"Aphid-resistant dwarf hops". Interview with BBC Radio 4 Farming Today, Hereford, 4 September 2001.

"The NHA Hop Supper". Interview with BBC Radio 4 Food Programme, London, 6 November 2001.

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