CONTENTS

PAGE

INTRODUCTION 1

FRUIT BREEDING AT EAST MALLING 2

BREEDING FOR PEST AND DISEASE RESISTANCE 3

Section 1: PHYTOPHTHORA EXPERIMENT

(1A) Introduction 4
(1B) Materials and Methods 5
(1C) Results 8
(1D) Conclusions and Improvements 13

Section 2: AMPHOROPHORA SCREENING

(2A) Introduction 14
(2B) Materials and Methods 17
(2C) Results and Discussion 17

Section 3: RASPBERRY BUSHY DWARF VIRUS

(3A) Introduction 20
(3B) ELISA Testing 21
(3C) Graft Inoculation in 1989 22
(3D) Resuts 23

Section 4: THE 1989 RUBUS CROSSING PROGRAMME

(4A) Introduction 25
(4B) Emasculation 26
(4C) Pollen Collection 26
(4D) Pollination 27
(4E) Seed Extraction 27
(4F) Seed Sowing 28

CONCLUSIONS 29

ACKNOWLEDGEMENTS 29

REFERENCES 30

APPENDICIES 32

INTRODUCTION

The first of my industrial placements was spent at the Institute of Horticultural Research, East Malling (IHR-EM), working under the supervision of V.H. Knight in the Breeding and Genetics Department. My work involved many aspects of raspberry breeding including field, laboratory and glasshouse studies.

INSTITUTE OF HORTICULTURAL RESEARCH - EAST MALLING

IHR-East Malling was founded in 1913 under the title "Wye College Fruit Experiment Station". The station became independent in 1920 and was known for the next 65 years as East Malling Research Station. Initially the station was partially funded by fruit growers but in 1946 the Ministry of Agriculture undertook full financial responsibility. Since 1980 however, there has been an increase in non-government funding. In 1987 East Malling joined with Littlehampton, Wellesbourne and the Hop Research Department at Wye College to make the I.H.R.

Today East Malling consists of about 237 hectares of land. The land consists of well drained sandy and loam soils of which 2/3 is used for experimental work at any one time. Throughout it's history the aims of East Malling have remained basically the same ;"to discover and apply scientific knowledge towards improvements in fruit production, storage and propagation and in the control of pests and diseases". Since 1980 East Malling has done research on woody ornamental species and in 1988 it embarked on a major new programme on farm woodlands.

FRUIT BREEDING AT EAST MALLING

The work of the Breeding and Genetics Department ranges from fundamental research through to production of finished varieties of apple, pear, plum, cherry, strawberry and raspberry. The breeding programme encompasses field vegetables, top and soft fruit and woody ornamentals. Breeding for pest and disease resistance is a major goal of many programmes within the Department. The raspberry breeding programme has produced Malling Autumn Bliss, the first primocane (autumn-fruiting) raspberry suitable for United Kingdom conditions, plus summer varieties Malling Admiral, Augusta, Joy and Leo, bred for improved cane and fruit characteristics and to extend the summer-fruiting season.

The raspberry breeding programme has several long term objectives which are:

(a) To extend the fruiting season,

(b) To improve fruit quality and yield,

(c) To increase mechanical harvesting potential,

(d) To incorporate resistance to pests and diseases.

Extending the season is done by crossing and breeding types which ripen either earlier or later than any existing cultivars and by producing early primocane types. Standard early ripenening cultivars such as Malling Promise and Glen Clova and the late-ripening cultivars Leo and Auguata are used as standards. The objective is to produce a long continuous season which is especially desirable for supplying supermarkets and the "Pick-Your-Own" market.

Fruit quality consists of many different factors including; good colour, firmness, shape and flavour. Fruit should also detatch easily from the receptacle which prevents damage during harvesting. Yield covers fruit size and quantity,

increased size aids harvesting. Fruit number can be increased by selecting for increased numbers of flowers per lateral,shorter nodes, or by breeding for the production of two laterals per node.

BREEDING FOR PEST AND DISEASE RESISTANCE

The main pests and disease of Rubus idaeus include; Amphorophora idaei (large raspberry aphid), Byturus tomentosus (raspberry beetle), Didymella applanata (spur blight), Elsinoe veneta (cane spot), Phytophthora species (root rot), raspberry bushy dwarf virus (RBDV) and other viruses, Sphaerotheca macularis (powdery mildew) and various fruit rots caused by Botrytis cinerea and Mucorales species.

The adult B. tomentosus causes damage to fruit buds and lays its eggs in fruit thus reducing yield and making raspberies unmarketable. D. applanata prevents formation of fruiting laterals by infecting buds. E. veneta causes spotting and pitting of canes and may also attack fruit.

S. macularis causes the production of mildewed, unmarketabe fruit and powdery leaves. Cane Botrytis acts in much the same way as D. applanata whereas fruit Botrytis produces grey mould on fruit. Breeding and selecting for resistance to A. idaei and Phytophthora are described in more detail in later sections.

With these objectives in mind comprehensive breeding programmes are formulated, the seedlings from which are both glasshouse and field tested before a variety can be produced. On average it takes 10-12 years to produce a new raspberry variety.

Section 1: PHYTOPHTHORA EXPERIMENT

(1A) INTRODUCTION

Root rot in raspberries can be caused by water-logged soils or by Phytophthora species and is characterised by necrotic roots and dying tops. Converse and Schwartze (1968) originally thought that Phytophthora erythroseptica was responsible for root rot in raspberries in North America, while pathologists in Europe thought that other species of Phytophthora, including P. megasperma var megasperma, were responsible (Duncan and Kennedy, 1986). Pathologists here and in North America now think root rot in raspberry may be caused by a species of Phytophthora specific to raspberry; tentatively named P. rubi (Duncan, unpub.).

The cultures used in this experiment were recieved from Dr. Duncan at the Scottish Crops Research Institue (SCRI) as Phytophthora megasperma var megasperma but in this report the causal organism is not defined to the species level.

The selections used in this experiment stem from back crosses from Rubus spectabilis (PURSH). R. spectabilis and F_1 hybrids between R. idaeus and R. spectabilis show some resistance to Phytophthora species. This experiment was designed to assess resistance in 2nd and 3rd backcross derivatives of R. spectabilis. Table 1 shows the selections tested, the number of generations from R. spectabilis and previous SCRI results, if any.

TABLE 1

Raspberry selections screened for resistance to *Phytophthora* at East Malling in 1989

SELECTION	GENERATION Ex. *R. spectabilis*	SCRI RESULTS
PRIMOCANE FRUITING		
5602/13	- ~~BC2~~	NT
5605/10	BC2	IT
5605/12	BC2	NT
5961/1	BC3	NT
5961/24	BC3	NT
5963/15	BC2	NT
5965/68	BC2	NT
5967/57	BC2	NT
5963/4	BC2	IT
5962/15	BC3	IT
5962/35	BC3	IT
SUMMER FRUITING		
5586/16	BC2	NT
5586/50	BC2	IT
5948/12	BC2	NT
5948/21	BC2	SU

NT=NOT PREVIOUSLY TESTED IT=INTERMEDIATE
SU=SUSCEPTIBLE

(1B) MATERIALS AND METHODS

From each clone, ten plants of similar size were selected. Five to be inoculated and five to act as controls. These were labelled and laid out in five blocks. Each block containing one inoculated and one non-inoculated plant per clone. The plants were then randomised within each block and given individual irrigation lines to prevent cross contamination. The initial height was recorded in cm.

One hundred ml of *Phytophthora* culture at $1x10^3$ zoospores per ml was added to each inoculated pot and 100ml of the culture medium was added to each of the control pots. Care was taken to agitate the inoculum to ensure an even distribution

of zoospores. Too violent agitation would have resulted in encystment and was therefore avoided.

In addition to the selections shown in Table 1, three cultivars were included; Latham the resistant standard; Glen Moy the susceptible standard and Meeker representing intermediate resistance. The leaf and stem symptoms were recorded once every seven days for a period of three weeks. The final records involved measuring stem lesions, counting the number of affected and healthy leaves and recording root rot grades. Leaves were judged as being "affected" if they showed any signs of yellowing, wilting, spotting or were dead. Root rot grades were measured on a scale of 1-5 (Fig. 1) and were assessed by comparing the inoculated plant with the non-inoculated plant within each clone/block combination.

Figure 1

Root rot grades 1-5

(1C) RESULTS

Percentage increase in height, percentage affected leaves, stem lesion length and root rot grades are shown in Table 2. As was expected Glen Moy proved to be extremely susceptible, with an average root rot grade of 5 (Figs. 2a and b). Latham and Meeker also behaved as expectd with the former showing considerable resistance (an average of less than 20% of the original root rotted). Meeker was intermediate between Latham and Moy.

The non-inoculated controls showed no symptoms, all having root rot grades of less than 20% and no stem lesions. The percentage of affected leaves in the controls ranged from 9-41. This was due to the method of recording which could not distinguish between those leaves affected by Phytophthora and those affected by lack of nutrients or normal senescence.

The inoculated 5965/68 plants showed an average height increase of 209%; 19% higher than the equivalent non-inoculated plants. Non-inoculated controls of all other selections showed a greater percentage height increase than their inoculated equivalents.

These initial results were very encouraging for future breeding, with only one selection (-5961/24) being more susceptible than Glen Moy with respect to stem lesions. Selection 5961/1 showed equivalent results to Meeker; with no stem lesions, low percentage affected leaves (15%) and a large percentage height increase (139%). Only the root rot grade of 2 showed a greater degree of susceptibility than Meeker. 5605/12 (Figs. 3a and b) had an average stem lesion lenght of 7mm which compares to an average of 0mm in both Meeker and Latham. All other results from 5605/12 show a greater or equal degree of resistance to Meeker and Latham.

5965/68 and 5602/13 had higher percentage height increases and lower percentage affected leaves than Latham and Meeker. Both these selections had no stem lesions and average root rot grades of 1. Thus 5605/12 and 5961/1 show resistance between that of Latham and Meeker. 5965/68 and 5602/13 show resistance greater than that of Latham. These 4 selections are all Primocame fruiting BC2 derivatives of *R. spectabilis*. In general the BC2 derivatives appeared more resistant than those of BC3, which was to be expected as the proportion of *R. spectabilis* in the genome is halved with each backcross generation.

TABLE 2

Phytophthora symptoms in inoculated and non-inoculated plants after 3 weeks, average over blocks

SELECTION	INCREASE IN HEIGHT (%)	AFFECTED LEAVES (%)	LESION LENGTH (mm)	ROOT ROT GRADE
		INOCULATED		
Latham	286	20	0	1
Meeker	154	39	0	1
Glen Moy	42	80	55	5
5602/13	427	11	0	1
5605/10	71	67	26	5
5605/12	210	25	7	1
5961/1	139	15	0	2
5961/24	67	63	67	5
5963/15	46	58	37	5
5965/68	209	16	0	1
5967/57	84	67	34	4
5963/4	78	53	15	5
5962/15	102	36	13	4
5962/35	78	50	39	5
5586/16	192	22	9	4
5586/50	128	52	15	4
5948/12	71	57	30	5
5948/21	110	70	25	5
		NON-INOCULATED		
Latham	355	41	0	1
Meeker	387	11	0	1
Glen Moy	207	14	0	1
5602/13	450	9	0	1
5605/10	174	14	0	1
5605/12	216	11	0	1
5961/1	178	14	0	1
5961/24	127	12	0	1
5963/15	92	15	0	1
5965/68	190	11	0	1
5967/57	193	19	0	1
5963/4	198	13	0	1
5962/15	135	15	0	1
5962/35	160	15	0	1
5586/16	280	12	0	1
5586/50	199	11	0	1
5948/12	197	18	0	1
5948/21	143	21	0	1

ROOT ROT GRADE: 1=Less than 20% of original root rotted
2=Between 21 and 40% " " "
3= " 41 and 60% " " "
4= " 61 and 80% " " "
5=Over 80% of original root rotted.

Figure 2

(a) Glen Moy non-inoculated control on left, inoculated plant 3 weeks after inoculation on right.Showing reduced height, stem lesions and affected leaves.

(b) Root system of non-inoculated control (left) and inoculated (right) of Glen Moy

Figure 3

(a) 5605/12 non-inoculated control on left, inoculated plant on right showing no decrease in height, healthy leaves and no stem lesions.

(b) Root systems of non-inoculated (left) and inocluated (right) of 5605/12.

(1D) CONCLUSIONS AND IMPROVEMENTS

Since this was the first *Phytophthora* experiment at East Malling and almost nothing is known about the genetics of *Phytophthora* resistance these results must be tentative. However, they appear to indicate 3 things;

(i) Large differences in response can be detected in pot plants inoculated with suspensions of zoospores,

(ii) *R. spectabilis* is a promising donor of reistance to *Phytophthora*,

(iii) that PF trial selections 5605/12, 5602/13, 5965/68 and 5961/1 show considerable resistance in material with good agronomic qualities.

Considerable difficulty arose from the need to remove compost from the roots in order to record root rot. To reduce time spent on this rather laborious and inefficient process,less peat could be used in the potting compost. The volume of compost could be reduced by decreasing the diameter of the plant pots but this could produce different results due to reduced growth. The recording of percentage root rot could be improved by increasing the number of grades. All other aspects of the experiment proved to be satisfactory.

Temperatures under glass during May and June in 1989 were exceptionally high, but the response of the standard cultivars was broadly similar to previous experiments done at lower temperatures at SCRI.

(2A) INTRODUCTION

The main aphid affecting raspberry production in the United Kingdom is *Amphorophora idaei* (Fig. 4). The adult *A. idaei* is about 3mm long with a pointed abdomen and long legs. The adults feed and produce nymphs on any susceptible plant and these nymphs undergo 4 instar (juvenile) phases before maturing into adults. Winged adults are produced in unfavourable conditions.

Although these aphids by themselves cause little damage to the raspberry crop, they are the vectors of 4 major *Rubus* viruses (Jones.,1976):

Black raspberry Necrosis Virus (BRNV),

Raspbery Leaf Mottle Virus (RLMV),

Raspberry Leaf Spot Virus (RLSV),

Rubus Yellow Net Virus (RYNV),

Resistance to these commercially damaging viruses is polygenic and combining such resistances would be very difficult. In contrast resistance to *A. idaei* is controlled by dominant major genes, $\underline{A}_1$-$\underline{A}_{10}$, and breeding for vector resistance is quicker and more effective. There are 4 European strains of *A. idaei* of which stains 1 and 3 have a high fertility but cannot colonise plants carrying gene $\underline{A}_1$. Strains 2 and 4 colonise plants carrying $\underline{A}_1$ but not plants carrying $\underline{A}_8$-$\underline{A}_{10}$ and $\underline{A}_{k4a}$. Strain 4 however is extremely rare in the field and biologically unfit. Therefore to identify seedlings carrying $\underline{A}_{10}$ inoculation was carried out with strain 2.

The genes and the strains to which they confer resistance are shown in Table 3, along with original source of the resistance gene (Knight,Keep and Briggs:1959; 1960; Keep,1977).

Figure 4

Amphorophora idaei showing both adult and nymphs feeding.

TABLE 3

The strain response of *Amphorophora idaei* resistance genes and their origin

GENE	ORIGIN	STRAIN OF *A. IDAEI* 1	2	3	4
A_1	Baumforth A (*R. idaeus*)	R	S	R	S
A_2	Chief (*R. strigosus*)	S	R	S	S
A_3	"	R	R	R	S
A_4	"	S	R	S	S
A_5	"	R	S	S	S
A_6	"	R	S	S	S
A_7	"	R	S	S	S
A_8	L518 *R. strigosus*	R	R	R	R
A_9	"	R	R	R	R
A_{10}	Cumberland (*R. occidentalis*)	R	R	R	R
A_{k4a}	*R. idaeus*	R	R	R	R

R = RESISTANT
S = SUSCEPTIBLE

The peak of aphid population coincides with the main summer harvests so spraying is both ecologically and economically unacceptable. As virus transmission is so rapid it is extremely difficult to prevent virus by using aphidicides so genetic resistance to the virus vector is an effective method of maintaining virus free crops. To this end, all young seedlings undergo glasshouse screening for *A. idaei* resistance. Those seedlings tested in 1989 were from the 1988 crossing programme all of which had at least one parent with genetic resistance to *A. idaei*.

(2C) MATERIALS AND METHODS

The seedlings were inoculated on the youngest leaf with 3 adult aphids (using a metal label as a scoop). After 3-5 days the seedlings were examined and susceptible seedlings destroyed. A seedling was judged to be susceptible if the adult aphids had thrived and produced nymphs. A total absence of aphids showed clear resistance. Any indeterminate seedlings were re-inoculated and the aphids given a further 3 days to settle and reproduce.

(2C) RESULTS AND DISCUSSION

Table 4 lists the 1989 families, the parental resistance genes and the numbers of susceptible and resistant seedlings per family. Although the presence of A_{10} in the parental genetic makeup is known it is unknown whether these parents are heterozygous or homozygous for this gene. If both parents were heterozygous for A_{10} it would be expected that only one in 4 of their prodgeny would be susceptible. If one parent were hererozygous and one homozygous for A_{10} or both were homozygous, one would expect no susceptibles.

In general the results are as expected, with progenies derived from parents who both posess A_{10} showing a high proportion of resistance seedlings. There is one notable exception however; family 6395 in which only 14 out of 65 plants were resistant, despite both parents posessing A_{10}. This decreased resistance may be due to severe infestation with *Tetranychus urticae* (red spider mite) or to incorrect parental genotyping. Approximately 3500 seedlings found to be resistant to *A. idaei* were planted out for assessment of their agronomic properties in future years.

TABLE 4

The results of screening the 1989 families with *Amphorophora idaei*

FAMILY	GENES FOR RESISTANCE	RESULTS	
		RES	SUS
PRIMOCANE FRUITING			
6361	$\underline{A}_{10}$ $^*\underline{A}_{L518}$ x	147	102
6362	$\underline{A}_{10}$ $^*\underline{A}_{L518}$ x $\underline{A}_{10}$	104	46
6363	$\underline{A}_{10}$ $^*\underline{A}_{L518}$ x $^*\underline{A}_{10}$ $^*\underline{A}_{L518}$	4	0
6364	$\underline{A}_{10}$ $^*\underline{A}_{L518}$ x $^*\underline{A}_{10}$ $^*\underline{A}_{L518}$	29	41
6365	$\underline{A}_{10}$ $^*\underline{A}_{L518}$ x $^*\underline{A}_{10}$ $^*\underline{A}_{L518}$	71	26
6366	$^*\underline{A}_{10}$ $^*\underline{A}_{L518}$ x $^*\underline{A}_{10}$ $^*\underline{A}_{L518}$	33	7
6367	$\underline{A}_{10}$ x $^*\underline{A}_{10}$ $^*\underline{A}_{L518}$	7	0
6368	$^*\underline{A}_{10}$ $^*\underline{A}_{L518}$ x $^*\underline{A}_{10}$ $^*\underline{A}_{L518}$	138	75
6369	$^*\underline{A}_{10}$ $^*\underline{A}_{L518}$ x $^*\underline{A}_{10}$ $^*\underline{A}_{L518}$	135	105
6370	$\underline{A}_{10}$ x $\underline{A}_{10}$	54	26
6371	$\underline{A}_{10}$ x $^*\underline{A}_{10}$ $^*\underline{A}_{L518}$	62	38
6372	$^*\underline{A}_{10}$ $^*\underline{A}_{L518}$ $^*\underline{A}_{1}$ $^*\underline{A}_{2}$ x $^*\underline{A}_{10}$ $^*\underline{A}_{L518}$	13	19
6373	$^*\underline{A}_{10}$ $^*\underline{A}_{L518}$ $^*\underline{A}_{1}$ $^*\underline{A}_{2}$ x $^*\underline{A}_{10}$ $^*\underline{A}_{L518}$	211	39
6374	x $\underline{A}_{10}$	59	6
6375	x $\underline{A}_{10}$	122	23
6376	x $\underline{A}_{10}$	135	65
6377	x $\underline{A}_{10}$	17	5
6378	x $\underline{A}_{10}$	63	34

TALBE 4 (Cont.)

FAMILY	GENES FOR RESISTANCE	RESULTS	
		RES	SUS
SUMMER FRUITING			
6379	$\underline{A}_{10}$ *$\underline{A}_1$ *$\underline{A}_2$ x $\underline{A}_{10}$ *$\underline{A}_1$	162	88
6380	$\underline{A}_{10}$ x $\underline{A}_{10}$ *$\underline{A}_1$ *$\underline{A}_{12}$	147	95
6381	$\underline{A}_{10}$ *$\underline{A}_1$ x $\underline{A}_{10}$ *$\underline{A}_1$	90	79
6382	$\underline{A}_{10}$ *$\underline{A}_1$ x *$\underline{A}_{10}$ *$\underline{A}_{L518}$	76	79
6383	$\underline{A}_{10}$ *$\underline{A}_1$ x $\underline{A}_{10}$ *$\underline{A}_1$	180	40
6384	$\underline{A}_{10}$ *$\underline{A}_1$ x $\underline{A}_{10}$ *$\underline{A}_1$	130	117
6385	$\underline{A}_{10}$ *$\underline{A}_1$ x $\underline{A}_{10}$ *$\underline{A}_1$	120	90
6386	$\underline{A}_{10}$ x $\underline{A}_{10}$ *$\underline{A}_1$	163	178
6387	$\underline{A}_{10}$ *$\underline{A}_1$ x $\underline{A}_{10}$ *$\underline{A}_1$	52	64
6388	*$\underline{A}_{10}$ *$\underline{A}_1$ *$\underline{A}_{L518}$ x $\underline{A}_{10}$ *$\underline{A}_{L518}$	93	132
6389	$\underline{A}_{10}$ *$\underline{A}_1$ x $\underline{A}_{10}$	124	75
6390	$\underline{A}_{10}$ x $\underline{A}_1$	102	132
6391	$\underline{A}_{10}$ x $\underline{A}_{10}$ *$\underline{A}_1$	107	93
6392	$\underline{A}_{L518}$ *$\underline{A}_1$ x *$\underline{A}_1$	179	21
6393	$\underline{A}_{10}$ x $\underline{A}_{10}$	148	46
6394	$\underline{A}_{L518}$ *$\underline{A}_1$ x $\underline{A}_{10}$ *$\underline{A}_1$	113	137
6395	$\underline{A}_{10}$ x $\underline{A}_{10}$	14	41
6396	$\underline{A}_{10}$ x *$\underline{A}_{10}$ *$\underline{A}_1$ *$\underline{A}_{L518}$	161	33
6397	$\underline{A}_{10}$ x $\underline{A}_{10}$	55	60
6398	$\underline{A}_{10}$ *$\underline{A}_1$ x $\underline{A}_{10}$ *$\underline{A}_1$	54	35
6399	$\underline{A}_{10}$ *$\underline{A}_1$ x *$\underline{A}_1$ *$\underline{A}_{L518}$	135	65
6276	$\underline{A}_{10}$ $\underline{A}_1$ x $\underline{A}_1$	83	119

* = May or may not contain two or more resistant genes.

(3A) INTRODUCTION

The only pollen borne raspberry virus in the United Kingdom is Raspberry Bushy Dwarf Virus (RBDV). Alone RBDV can cause "Raspbery Yellows" disease which is most obvious in late spring/early summer. Symptoms include chlorotic line patterns on leaves and yellow veins (Knight, Manwell and Barbara, 1978). Fruit can become crumbly and unmarketable due to the failure of druplets to set. These symptoms are weather dependent and increased light intensities and temperatures can reduce visable symptoms. Also leaf symptoms are sometimes totally absent and green-leaved plants can index RBDV positive.

RBDV can act with Black Raspberry Necrosis Virus to cause "Raspberry Bushy Dwarf" Disease. RBDV itself consists of quasi-isometric particles about 33nm in diameter (Jones, 1986). There are at least 3 known strains; serologically indistinguishable but differing in their *Rubus* host range (Barbara *et al*, 1984). Antisera can be raised to R.B.D.V. and so an ennzyme linked immunosorbent assay or ELISA,can be used providing a relatively simple and quick method of virus detection.

Raspberry parents, selections and seedlings in the breeding programme are tested for RBDV as it is seed borne, as well as pollen borne, and is therefore highly infectious. Any plants giving a positive result are grubbed to prevent spread of the virus.

(3B) ELISA TESTING

Leaves from plants to be tested were collected into labelled polythene bags. The ELISA plates were labelled and coated with a specific antibody $F(ab')_2$. The $F(ab')_2$ is diluted 1:2000 in coating buffer (Appendix 1(a)) and 0.1ml was then added to each well. The plate is then covered and incubated at 30°C for approximately 6 hours. This allows the $F(ab')_2$ to bind to the plate.

Whilst the plate is incubating 0.15g of leaf was ground in a pestle and mortar then diluted in 7.5ml of buffer. The buffer used was phosphate buffered saline and Tween (Appendix 1(b)). The Tween was added to prevent any non-specific binding as this would produce anomalous results (Clark and Adams, 1977). After coating, the plates were washed with PBS-Tween for three minutes. This was repeated 2 more times, then the plate was dried.

The test samples were pipetted into the wells in duplicate, 0.1ml per well. Positive and negative controls from known infected and healthy plants were added to each plate. The plates were then left to incubate at 6°C overnight. This allows any RBDV antigen present in any sample to bind to the antibody present on the plates.

After incubation the plates were washed in PBS-Tween as before and the second antibody (Immunoglobulin G) was added. This IgG was diluted 1:1500 in the PBS-TOP (PBS-Tween,Ovalbumin and Polyvinylpyrrolidine) buffer (Appendix 1(c)). 0.1ml was added to each well. The plates were then covered and incubated at 30°C for 3-4 hours.

The plates were washed and dried as before and 0.1ml of diluted conjugate was added to each well. The conjugate, which consists of Protein A and Horse Radish Peroxidase, was diluted

1:3000 in PBS-TOP. The plate was once more covered and incubated at 30°C for 2-3 hours to allow the Protein A to bind to the IgG. IgG is used as the Protein A cannot bind directly to the antigen.

Finally the substrate was added. The substrate is hydrogen peroxide diluted 1:1000 in water with TMB (3,3',5,5'-Tetramethyl Benzidine) diluted 1:100 and substrate buffer 1:10 (Appendix 1(d)). The reaction between substrate and Horse Radish Peroxidase produces a blue colour. Thus if any RBDV was present in the test sample then the IgG and HRP-Protein A conjugate would have bound and the HRP would catalyse the reaction. Thus any blue colour was taken as a positive indication of RBDV.

(3C) GRAFT INOCULATION IN 1989

Seven out of 8 Primocane trial selections have only indexed RBDV-negative in the field to date. To test if this is due to inate resistance or lack of exposure to the virus these selections were inoculated with the virus by "in-arch bottle grafting". 5605/10 has been found to be infected with RBDV in the field, however following inoculation in 1987 (grafted plants tested in 1988,89) 5605/10 failed to become infected with the D200 strain and further plants of 5605/10 were inoculated in 1989. The other 7 PF selections were grafted with both the D200 and the RB strain in 1989. These plants will be tested for RBDV for the first time in spring 1990.

Actively growing, young cane tips (approx. 10cm) of plants infected with either the D200 or the RB strain of RBDV were used as the inoculum source. Equal numbers of young pot plants of each selection were inoculated with RBDV D200 and RB. The shoot tip from the inoculum source was stripped of it lower leaves and tesselated as closely as possible to the stem of the

selection to be graft-inoculated.

Using a scalpel a vertical incision was made upwards on the inoculum and downwards on the selection. These slits should be of equal length, about 1-2 cm long, and should be below at least one actively transpiring leaf. The inoculum was then slotted in the selection and the two cut surfaces were held tightly together by "Stericrepe" tape.

The graft-inoculum was kept alive by placing its cut base in a glass tube full of water wired onto a bamboo cane. This ensures the continued transpiration of the detached inoculum shoot. Virus particles move across the juncture from inoculum to selection. After 6 weeks the stericrepe was removed and the sucess or failure of each graft recorded.

The primocane selections inoculated in 1989 were;5602/13, 5605/12, 5961/1, 5961/24, 5963/15, 5965/68, 5967/57 and 5605/10.

(3D) RESULTS

One hundred out of 101 plants grafted with RBDV in 1987 and negative in 1988 were still negative in 1989. Plants from 12 out of 14 selections grafted with RBDV-D200 all indexed negative and probably are resistant to D200. Of the two selections susceptible to D200 by grafting, only one has become infected naturally. Three out of eight selections grafted with RBDDV-RB also indexed negative, however numbers of plants/selections were too small for these results to be definitive.

Seven out of 50 possible parents were found to be RBDV-infected in the field. Healthy substitutes were found for five out of seven. Healthy plants within the block were found of the other two parents and these plants used for crossing. Twelve percent of seedlings or selections propagated from the breeding

plots during winter 1988/89 were found to be infected in summer 1989. The majority were only partially infected; the RBDV-positive plants were grubbed while the RBDV-negative plants were retained. Where selections were infected throughout, the whole block was grubbed.

Three progenies derived from an RBDV-susceptible parent were tested following aphid screening in 1989 but all 164 seedlings were negative. Five progenies introduced as seed from Minnesota and mostly derived from the RBDV-susceptible cultivar Redwing were also tested after aphid screening. Four out of 395 seedlings were possibly RBDV-infected and discarded prior to planting.

Section 4: THE 1989 RUBUS CROSSING PROGRAMME

(4A) INTRODUCTION

Each year clones which have previously shown one or more outstanding quality are selected as parents. The 1989 breeding objectives are as follows:

Primocane fruiting raspberries

Increased yield

Improved fruit quality

Increased earliness and condensed ripening of fruit

Improved shelf-life

Beetle resistance

Summer fruiting raspberries

Increased yield

Improved fruit quality

Increased earliness

Increased lateness

Improved plant habit

Decreased lateral length

Condensed ripening of fruit

Mechanical harvesting potential

The crossing programme had 5 stages; emasculation, pollen collection, pollination, seed extraction and seed sowing. Those clones selected as parents were RBDV tested using the ELISA technique previously described. Only those found to be virus free were used as parents.

(4B) EMASCULATION

The female parent was prepared for pollination by emasculation. A strong, firmly attached lateral with between 4 and 8 advanced buds and at least one open flower is ideal for this process. All leaves, any open flowers and very immature buds were removed. The sepals, petals and stamens of the remaining buds were then removed by making a single, circular incision around the base of the bud, thus leaving the immature receptacles.

To prevent cross pollination by insects or wind the whole lateral is enclosed within a paper bag. This had been waxed to ensure it is waterproof and pierced to allow circulation of air. Between 20 and 30 buds per cross were emasculated depending on availability. The emasculated flowers continued to develop within the bags and the stigmas were recptive 4-5 days after emascultion.

(4C) POLLEN COLLECTION

Two methods of obtaining uncontaminated pollen samples were used. How closely the parental flowering seasons coincided determined which method was used. If, at the time of female emasculation, several suitable laterals could be found on the male parent then the preferred method was "bagging". A suitable male lateral also has at least one open flower and several advanced buds. All the leaves and any open flowers were removed and the buds enclosed within an aerated waxed paper bag. Thus by the time the stigmas on the female were receptive the male bags contained open flowers with dehisced anthers.

If the female parent flowered much later than the male, pollen was collected, extracted and stored. This involved the collection of many advanced buds. The immature anthers from

these buds were removed using forceps and left at ambient temperature to mature and dehisce overnight in a petri dish.

(4D) POLLINATION

The extracted pollen was transferred to the receptive flowers using a paint brush. The pollen from bagged flowers was transferred by gently brushing whole flowers with the dehisced anthers over the stigmas.

Two or three pollinations per cross were performed over 6-7 days. To ensure a continuous supply of pollen further laterals on the male parent were "bagged". After the final pollination the female bags are sealed and secured firmly and left to ripen. Each lateral on the female parent was carefully labelled, giving both parents, the date and number of flowers emasculated. Between every process from emasculation to pollination all equipment used, including hands was washed in alcohol to prevent cross contamination.

(4E) SEED EXTRACTION

Once ripe the fruit was picked and the percentage set determined the fruit from each cross was given its new family number and the seed was extracted. During extraction care was taken to ensure each seed lot was kept separate.

The fruit was liquidised with a small amount of water for about 30 seconds. Water was added to allow the pulp to be decanted off and the seeds to settle out. Dead seeds floated and were removed with the pulp.

Once all the pulp was removed, the clean seeds were sterilized using a dilute "Milton" solution. They were then allowed to dry overnight on filter paper labelled with the family

number. Numbers of seeds were estimated by weighing a sample of 100 seeds/cross plus the total weight of seeds per cross.

(4F) SEED SOWING

Due to the low primocane germination rate, large numbers of seeds are needed to produce the desired amount of seedlings per family. When a cross produced low seed yield a percentage (usually 50%) was kept for scarification with concentrated sulphuric acid before sowing. This breaks down the seed coat and thus should increase the germination rate.

Seed not for scarification was sown immediately. Seed trays were filled three quarters full with ordinary compost and surfaced with seed compost, a fungicide (6g Thiram/gallon of water) was watered-on to prevent "damping off". The seed trays were then stacked and covered in black polythene sheets at ambient temperature to ripen for 8-12 weeks. They will then be transferred to $3^{o}C$ for at least 12 weeks stratification.

CONLUSIONS

This placement has provided me with invaluable experience in field, glasshouse and laboratory research. Involvement in every aspect of the breeding programme has increased my awareness of the importance of plant breeding especially in those aspects related to pest and disease resistance. In these times of increased ecological awareness it seems very unfortunate that breeding programmes such as the IHR-EM raspberry programme should be in jeopardy due to lack of government funding.

ACKNOWLEDGEMENTS

I would like to thank Mrs V.H. Knight and all the staff of the Breeding and Genetics Department for a rewarding and enjoyable placement at East Malling and the HDC who funded my placement. Also Anne Morton of the Virology Department who helped me during my work on RBDV.

REFERENCES

Barbara, D.J., Jones,A.T., Henderson,S.J.,Wilson,S.C., Knight V.H..,1984, "Isolates of Rasperry Bushy Dwarf Virus-differing in Rubus host range", Ann. Appl. Biol.(1984), 105,pp 49-54.

Clark, M.T. and Adams, A.N., 1977, "Characteristics of the microplate method of ELISA for the dection of plant viruses", J. Genet. Virol., 34 pp 475-483.

Converse.R.H.and Schwartze,C.D., 1968. "A root rot of red raspberry caused by Phytophthora erythroseptica". Phytopathology 58. pp 56-59.

Duncan, J.H. and Kennedy, D.M. (1986) "Root rot and Die-back of raspberry".Scottish Crop Research Institute Report for 1985,p90.

Jones, A.T.,1976, "Effects of Resistance to A. rubi in Raspberry (Rubus idaeus) on the spread of aphid-borne viruses", Ann. Appl. Biol.,82,pp130-131.

Jones, A.T., 1986, "Advances in The Study, Detection And control of viruses of Rubus with particular reference to the United Kingdom", Crop Res.(Hort. Res.), Vol. 26, pp27-171.

Keep, E., 1977, "Breeding for resistance to aphids in Rubus and Ribes", SROP/WPRS Bulletin 3, pp79-84.

Knight, R.L., Keep, E.and Briggs, J.B., 1959, "Genetics

of resistance to Amphorophora rubi(Kalt) in the raspberry,I. The gene $\underline{A}_1$ from Baumforth A.", J. Genet (56), pp261-280.

Knight, R.L., Keep, E. and Briggs, J.B., 1960, "Genetics of resistance to Amphorophora rubi(Kalt) in the raspberry. II .The genes $\underline{A}_2$-$\underline{A}_7$ from the American variety, Chief" Genet. Res., (1), pp319-331.

Knight, V.H., Manwell, W.E.,Barbara,D.J,,1978, "Raspberry Virus Investigation" , EMRS Report For 1977, pp130-131.

APPENDIX I

(a) Phosphate Buffered Saline and Tween 20 (PBS-Tween) pH 7.4

8.0g NaCl,

0.2g $KH_2 PO_4$,

2.9g $Na_2H PO_4.12H_2O$,

0.2g KCl,

0.2g $Na N_3$

0.5ml Tween 20

Make up to 1 litre in water.

(b) PBS-TOP

2.0% Polyvinylpyrrolidine

0.2% Ovalbumin

Make up to 1 litre in PBS

(c) COATING BUFFER pH 9.6

1.59g $Na_2 CO_3$

2.93g $Na HCO_3$

0.2g $Na N_3$

Make up to 1 litre with water

(d) SUBSTRATE BUFFER

97ml Diethanoldiamine

800ml H_2O

0,2g NaN_3

add HCl to give pH 9.8.