Project Title:	Raspberry: evaluation of advanced selections from the HRI breeding programme
Project Number:	SF 8c
Project Leader:	Victoria H. Knight HRI – East Malling West Malling Kent ME19 6BJ
Report:	Annual report, March 2003
Key Worker:	James Williams, Manchester Metropolitan University, HRI, East Malling, sandwich student, April-Sept. 2002
Location of Project:	Horticulture Research International East Malling
Project Co-ordinator:	Harriet Duncalfe Well End Farm Wisbech
Date Project Commenced:	1 April 2002
Date Completion due:	31 March 2003
Keywords:	raspberry, breeding, evaluation, trials

The results and conclusions in this report are based on an investigation conducted over one year. The conditions under which the experiment was carried out and the results obtained have been reported with detail and accuracy. However, because of the biological nature of the work, it must be borne in mind that different circumstances and conditions could produce different results. Therefore, care must be taken with interpretation of the results especially if they are used as the basis for commercial products recommendations.

CONTENTS

Page

Fact Sheet 06/03 Raspberry Project SF 8c

Raspberry trials - summer fruiting and primocane fruiting

Fact Sheet 06/03

Raspberry

Project SF 8c

Horticultural Development Council

Bradbourne House
East Malling
Kent ME19 6DZ
T: 01732 848383
F: 01732 848498
E: hdc@hdc.org.uk

Raspberry trials - summer fruiting and primocane fruiting

by Vicky Knight, HRI East Malling

This report covers the Stage 0 summer and primocane fruiting trials at East Malling in 2002. The summer fruiting (SF) selections were picked from 14 June - 15 August while the primocane fruiting (PF) selections were picked from 22 July - 4 October. The trials were only picked twice a week so the proportion of unmarketable fruit is higher than it would be on a commercial farm. The shelf life evaluations of the PF selections were curtailed on 11 September because of technical problems.

Summer fruiting (SF) trials

25 SF selections were picked, 16 of which had been picked previously, and compared with Glen Ample and Tulameen. 13 and 21 selections produced a higher yield than Glen Ample and Tulameen, respectively. However only 6549/31 had larger fruit than Glen Ample (overall mean 4.97g compared to 4.73g). 6545/26, 6544/80, 6551/38 and 6551/40 were significantly earlier than Glen Ample and had more condensed ripening seasons. Glen Ample is considered to have a good shelf life, particularly texture. 11 selections had less post-harvest fruit rots than Glen Ample, while 14 were rated as having a better texture after 72 hours at 18°C and 90% relative humidity.

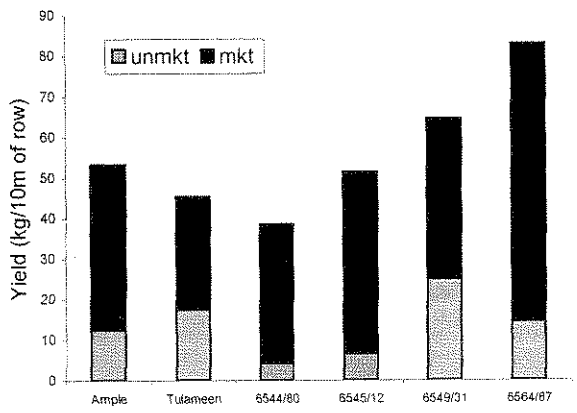
4 selections have been chosen to go forward to further trials:

6544/80 Ripened very early, with a condensed season in 2000-2002. Produced a lower yield than Glen Ample but had a very high percentage of marketable fruit each year. The fruit is attractive with excellent colour and shape, good flavour and is well presented to the pickers. Shelf life was fairly good with low incidence of rots and moderate texture in the punnet.
Planted as a main entry in HDC SF 41b in Oxfordshire in July 2002.

6545/12 Ripened earlier and had a higher yield of marketable fruit than Glen Ample in 2001 and 2002, plus a very high percentage of marketable fruit. Fruit is firm, very cohesive and regular in outline. Shelf life was moderate, low incidence of rots and reasonable texture but the fruit went rather dark and dull after 72h.
Planted as a main entry in HDC SF 41b in Oxfordshire in July 2002.

6549/31 Ripens at much the same season as Glen Ample and produced slightly higher yield. Had the largest fruit in the EM trials in 2001 and 2002 (overall mean fruit weight was 4.49g and 4.97g in 2001 and 2002, respectively) and fruit size held up well during the season. Fruit is long, conical in shape and was highly rated for flavour, texture and cohesion. Shelf life was moderate with good punnet texture and low rots but the fruit went dark and uneven in colour after 72h.
To be propagated from roots in spring 2003 for inclusion in grower trials.

6564/87 Ripens 4-5 days later than Glen Ample. Similar yield to Glen Ample in 2001, but much higher yield in 2002. Plant has good habit with long, ascending laterals. Fruit quality was good, particularly flavour and colour. Shelf life was fairly good, low rots, fair texture but slightly dark and uneven colour.
To be propagated from roots in spring 2003 for inclusion in grower trials.



Primocane fruiting (PF) trials

13 PF selections, plus Autumn Bliss, were harvested from two older plots (RF157 and RF161), most of which had been recorded previously. Another 23 genotypes were picked from a young plot (RF167), including 17 selections which had not been harvested before. This plot had been planted very late in spring 2001 and had not established very well. The yield figures in 2002 are unreliable because of the differences between plots, but the fruit quality and shelf life records are useful.

a) Selections from RF157 and RF161
12 out of 13 selections produced a higher yield than Bliss, but it was a poor plot of Bliss. Fruit size was disappointing. On different picks 5 selections had larger fruit than Bliss but the overall mean fruit weight of Bliss was higher than any of the selections. Bliss was also rated as having the best flavour. Several selections had similar ripening seasons to Bliss. Three were earlier (6523/8, 6529/85 and 5690/74) and had reached 95% pick by the end of August. Three selections were 1-2 weeks later than Bliss.

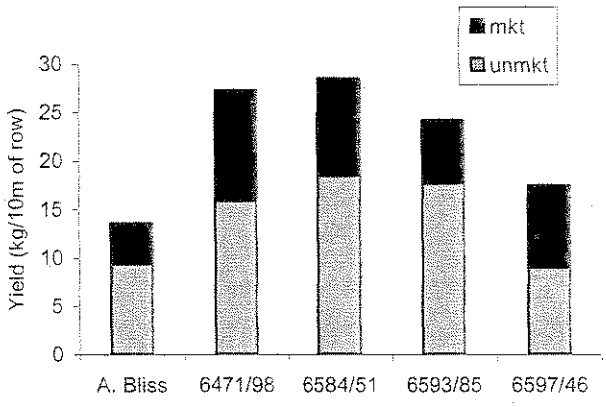
b) New selections from RF167
Bliss in the young plot started cropping 10 days later than it did in the older plot so the season and yield data are probably atypical in 2002. 10 selections were rated as having a better flavour than Bliss, 15 had firmer fruit and almost all selections were lighter red and had a more regular outline.

6471/98, 6523/8 and 6529/85 were selected for Meiosis trials in 1999 or 2000. Results from East Malling in the interim suggest that they have some good attributes but that the fruit size is probably too small. Another three selections (6584/51, 6593/85 and 6597/49) have been selected for grower trials. They were propagated in spring 2002 for Meiosis and were picked again at East Malling in 2002:

6584/51 Produced a fairly high yield and cropping season was similar to Bliss. Fruit was firmer, brighter and more regular in outline than Bliss but smaller. Shelf life was moderate, low rots but moderate texture.

6593/85 Produced a fairly high yield but had a low percentage of marketable fruit in 2002. Season was slightly earlier than Bliss. Fruit quality was average on most counts but flavour was good and it was the sweetest of the PF selections. Shelf life was moderate to poor.

6597/46 Yield was only moderate but it had reasonably large fruit until September. Fruit had good colour, fair flavour and was fairly firm. Season was a few days later than Bliss. Shelf life was fair, generally low incidence of rots and moderate texture.



Further trialling

6 main entries and Octavia from East Malling were planted in HDC SF Trial 2 (SF 41b), including 6544/80 and 6545/12. The other main entries were identified as promising from earlier Stage 0 trials. The plants in the HDC trial were cut down after planting to aid establishment and the trial will crop for the first time in 2004. The 6 PF selections are due to be planted as unreplicated observation plots on four grower sites in 2003. These grower trials are run by Meiosis Ltd.

Varieties within HDC SF 41b will be recorded by Janet Allen, National Cane Fruit Specialist, who then makes recommendations for commercial release.

FUNDING

Funding for raspberry breeding at East Malling is provided by DEFRA, East Malling Trust for Horticultural Research and the HDC.

DEFRA
Department for
Environment,
Food & Rural Affairs

East Malling Trust for Horticultural Research

HDC

Further information may be obtained from the HDC Project Report SF 8c Raspberry trials at HRI East Malling.

1. INTRODUCTION

1.1 Horticulture Research International (HRI)

Horticulture Research International (HRI) is the single largest team of horticultural research and development scientists in the world. Its customers include UK Research Councils, Government Departments, the EC, Overseas agencies, growers, grower-funded levy bodies and the commercial industry. HRI's mission is "to innovate and communicate for the benefit of producers and consumers of horticulture and other plant based products". The company is a non-Departmental Public Body (NDBP) responsible to the UK's Department for Environment, Food & Rural Affairs (DEFRA, formerly MAFF).

HRI manages world class facilities on five sites in the UK, and is being increasingly contracted to work internationally on a range of horticultural crops. HRI East Malling is the centre for research on perennial crops.

The research at HRI-EM focuses primarily on-

- tree fruits (principally apples and pears but also plums and cherries)
- soft fruits (strawberries, raspberries and black currants)
- hops
- hardy nursery stock
- farm woodland species

Particular research strengths at East Malling are the programmes, funded by the DEFRA and industry, on crop physiology, genetics, molecular biology, immunodiagnostics and analytical chemistry. East Malling maintains close associations with comparable research stations worldwide, and has strong academic links with universities. The East Malling site and buildings are leased to HRI from the East Malling Trust for Horticultural Research whose offices are nearby in Bradbourne House. The Trust supports a substantial number of research projects and contributes in other ways to the underpinning of relevant sectors of the UK horticultural industry.

Initiated by reduced funding from both DEFRA and the public sector in recent years HRI has embarked on a major cost saving and restructuring exercise. This began in 1999 and focused on cost minimisation throughout the entire structure of HRI while scoping new funding opportunities and internal communication. This resulted in the old structure of sites and departments being replaced with 'themes' and 'teams' in order to make the science base at HRI more robust, integrated and organised. Three overarching themes were introduced from April 1st 2002.

The Plant Breeding and Biotechnology department has become the Perennial Breeding and Genetics team and is part of the Crop Improvement and Biotechnology theme. The overall remit of the Perennial Breeding and Genetics team is the improvement of perennial crops such as top fruit (apples and pears), stone fruit (cherries and plums) and soft fruit (raspberries and strawberries), as well as hops, hardy ornamentals, timber trees and nuts. The research encompasses genetic investigations on key traits such as resistance to pests and diseases, flowering, fruit quality and plant architecture. The strategy is an integrated approach involving

conventional breeding, genome analysis and genetic modification. Extensive genetic resources are available and these are exploited throughout the research programme leading to the development of new cultivars of fruit crops, hops, hardy ornamentals and timber trees.

1.2 The Raspberry Breeding Programme at HRI-EM

Mrs Vicky Knight manages the Raspberry breeding programme at HRI-EM, assisted each year by a sandwich student from April to October, plus one or two summer casuals. The main objective of the programme is to produce new superior varieties of raspberry for the UK and overseas markets. New varieties created by the breeding programme should have desirable characteristics such as possessing pest resistance, producing high yields, with attractive, flavoursome fruit with a good shelf life.

Two types of raspberry are included in the breeding programme at HRI-EM. Summer fruiting (SF) and primocane fruiting (PF) selections are bred in alternate years to produce seedling populations in the following April. Seedling progenies are selected for absence of spines and resistance to the large raspberry aphid. Aphid resistant seedlings are then tested for Raspberry Bushy Dwarf Virus (RBDV) to prevent seed transmission of the virus and the introduction of the virus into the field when the seedlings are planted.

Seedling plantations are monitored and particularly good seedlings are chosen for further evaluation. These seedlings are lifted from the field and replanted as 10-plant plots within propagation fields, where further monitoring is carried out. The most promising propagated selections are chosen for inclusion in the Stage 0 trials each summer. Selections that perform particularly well enter further trials at other sites and might be named as new varieties to be commercially grown in the UK or abroad. A new late ripening summer fruiting variety, Octavia, was named in 2002.

2. GERMINATION OF 2002 RASPBERRY SEEDLINGS

2.1 Introduction

In 2001 twenty-four controlled crosses were made between primocane fruiting selections. Seeds were extracted from the resulting fruit, which were stored at 2-4°C in a coldstore until January 2002. The seed lots from each particular cross were given family numbers (between 6763 and 6786) which were a continuation of the family numbers from the previous years.

The period of dormancy within cold storage mimicked the winter period experienced in nature. Several conditions are required for raspberry seeds to remain in a state of dormancy. The seeds must be dehydrated and possess a coat that is completely impermeable to air and water. This deprives the seeds metabolic pathways and prevents them of speeding up to a pace that would cause germination. Secondly the seed coat needs to be firm enough to withstand the dilation of the embryo within. In nature dormancy is also controlled by a certain chemical(s) found within the seeds which act as acidic, ether-soluble, growth-inhibiting substances. Such substances are found in different concentrations throughout the seed, highest in the endosperm where it is likely that they are produced. From there they probably diffuse to other tissues within the seed where their concentrations are considerably lower. Such chemicals control the metabolic pathways which initiate germination.

The breaking of dormancy of the seeds is achieved by altering the external conditions and physiology of the seeds themselves. Such changes allow air and water to diffuse into the seeds providing them with the desired internal environment for the embryo to metabolise and start to divide. This is due to the increase in the activity of metabolic enzymes and an increase in internal sugars. Environmental changes also cause the breakdown of the acidic ether-soluble growth-inhibiting substances (which also have an influence), probably accompanied by the formation of growth promoting chemicals.

In nature germination of raspberry seeds is generally in the region of 5 and 10%. For this reason, the seed produced from the crosses were treated so as to increase the germination percentage in order to obtain sufficient numbers of germinating seeds from each cross. The treatment used to break seed dormancy and condition them for germination involved soaking the seeds in concentrated sulphuric acid (H_2SO_4) for 90 minutes in an ice bath. The sulphuric acid causes scarification of the seeds coats and the ice bath helps to reduce the heat generated from the process. Once the seed coat has been broken down air and water can diffuse into the seed feeding the metabolic pathways which cause germination. Following this treatment, germination percentage is further increased by soaking the seeds in distilled water, which encourages the growth inhibiting substances to leach out.

The percentage of seeds that eventually germinate and the date at which they do so is effected by a number of different conditions. From the time of pollination up until germination the seeds are prone to damage which could prevent growth from occurring. Mechanical damage during seed extraction could harm the seeds. This might prevent the seeds successfully entering a phase of dormancy when relocated to the cold-store. Therefore, the low temperatures experienced within the cold-store

would kill the embryo. Another factor to consider which also affects both germination percentage and the time of germination is the maternal parent of the particular seed lots. The maternal parent greatly influences the endosperm size. Seeds with small endosperms tend to emerge earlier due to the smaller quantities of dormancy-inducing agents within them. Consequently larger seeds with larger endosperms contain higher quantities of inducing factors resulting in their belated germination.

The presence of spines was investigated once the seed lots had began to germinate. Spiny stems, laterals and petioles are undesirable and farmers prefer to grow *Rubus* cultivars which are spineless.

There are several known genes, which determine the degree of spininess shown in a plants phenotype. A very common gene used in breeding is the recessive gene *s* discovered by Lewis (1939). This gene is very suitable for breeding spineless cultivars as it causes the cotyledons to be glandless and therefore have an even outline when observed under a lens. Seedlings possessing the spine-producing gene, *S*, can therefore be identified as soon as they emerge from the soil due to the glandular hairs protruding from the edge of the cotyledons. The dominant allele of the gene *S*, instigates spiny stems, laterals and petioles. The crossing programme aims to reduce the incidence of spiny seedlings by using homozygous spineless or heterozygous spiny selections as parents. The aim of the seed treatment is to produce sufficient number of seedlings per family so that the spiny seedlings can be discarded in segregating progenies and still achieving the required number of spineless seedlings for the next stage of the programme.

2.2 Method

The seed lots were removed from winter dormancy within the cold-store in January 2002. The seeds from each particular seedlot were then treated with sulphuric acid (H_2SO_4) for 90 minutes in an ice-bath. The acid was then washed off using iced water and returned to the cold-store in vials containing distilled water. After two, four and seven days the seeds were washed and the distilled water within the vials was changed. Following the seventh wash the seeds were dried overnight to facilitate sowing. The ideal number of seeds to be sown into each tray is around 250, making best use of the space within a tray and preventing overcrowding of seedlings once they emerge. For this reason each family was split up into between three and ten sample (all of equal weight) depending on the number of seeds available. One sample of each family was counted and it was assumed that the other samples contained the same number of seeds, there may have been some slight variation. The number of seeds per sample for each family was recorded for latter reference allowing germination percentages to be calculated once germination was complete. Each sample of seeds was then evenly sown into trays of damp compost, watered and moved into the glass-house cold-store where the temperature was maintained at 2°C. At this temperature the seeds absorbed water from the compost, preparing them for germination. Every tray was labelled with its new family number and its individual tray number.

Due to the limited bench space and also to spread the workload, the seedlots were removed from the glass-house cold-store and allowed to germinate in two separate batches on different dates. Batch 1 consisted of families 6763 to 6774 and batch 2

consisted of the remainder of the families, 6775 to 6786. Moving the seeds from the cold store into a glasshouse maintained at 20-22°C results in fairly rapid and synchronised germination.

Batch 1 was removed from the glasshouse cold-store on 1st March 2002 and the first seedlings emerged 11 days later. Germination was recorded by counting the number of seedlings which had emerged within a particular tray twice a week, until the seedlings were large enough to prick out into pots. Once the number of germinated seedlings within a tray had reached about fifty the presence or absence of spines was recorded. This entailed examining the cotyledons of each seedling through a 10X lens. The presence of hairs projecting from the edge of the cotyledons indicated the presence of the gene *S* and that the plant would be spiny. A number of families either had all spiny or all spineless seedlings and therefore no selecting was required for such families. If the progeny was segregating for spiny/spineless seedlings and, if the germination rate was high enough, then the spiny seedlings could be discarded. The accumulative number of seeds germinated, including any discarded spiny seedlings, was calculated after each session.

Batch 2 was moved from the cold-store on 2nd April 2002 and was the first seedlings emerged after 7 days in the glasshouse. Germination and spininess was recorded twice a week as before.

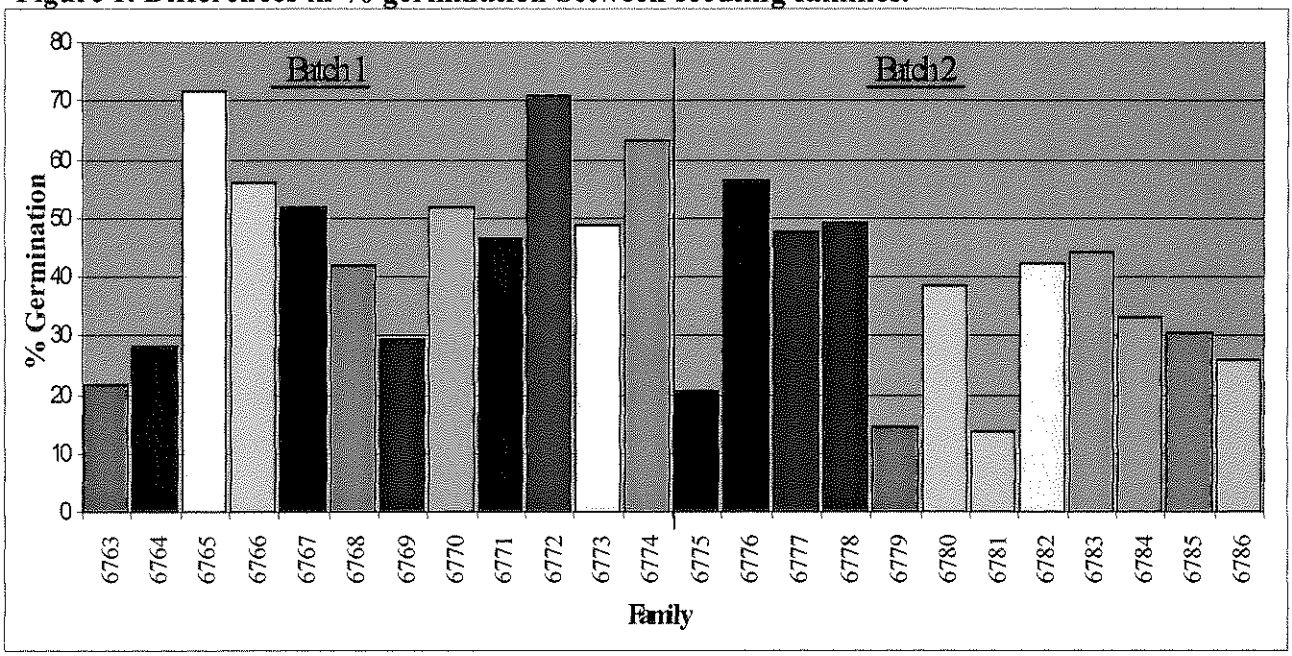
Recording of batch 1 continued until 2nd April 2002, thirty-two days after being removed from the cold-store. Recording of batch 2 continued until 30th April 2002, twenty-eight days after leaving the cold-store. Germination actually continued after these dates but due to the seedling having reached optimum size they were pricked out and potted up. The required number of seedlings from each family was potted up into 7.5cm pots. For batch 1 potting up began on 3rd April followed by batch 2 on the 1st May. If numbers were plentiful only the most suitable seedlings were potted up, all others being discarded. Therefore it was impossible to continue monitoring germination once potting up had begun. If the required number of potted seedlings was met all but one tray of that family was discarded. The tray retained was as a precaution in case any seedlings died from that family. If the required number of seedlings was not met all the trays of that family were retained so that germination could continue until either the required number was met or germination had ceased.

2.3 Results

Table 1. Percentage germination for families 6763-6786 in 2002.

Family	Total number of seeds sown	Total number of seeds germinated	Percentage germination
Batch 1			
6763	2192	473	21.6
6764	2640	743	28.1
6765	2930	2094	71.5
6766	1440	805	55.9
6767	1872	971	51.9
6768	792	332	41.9
6769	855	252	29.5
6770	2088	1079	51.7
6771	1920	896	46.7
6772	1869	1324	70.8
6773	624	304	48.7
6774	1452	918	63.2
Batch 2			
6775	1701	353	20.8
6776	1872	1058	56.5
6777	645	308	47.8
6778	1477	724	49.0
6779	1920	275	14.3
6780	852	329	38.6
6781	1440	198	13.8
6782	2080	881	42.4
6783	1398	620	44.4
6784	2248	745	33.1
6785	1463	444	30.4
6786	2032	528	26.0
Total	**39802**	**16654**	
Mean % germination			**41.6**
Standard deviation			**16.4**

Figure 1. Differences in % germination between seedling families.



2.4 Discussion

Figure 1 clearly illustrates the magnitude of variation between germination percentages for the different seedling families. There is a 57% difference between the poorest germinating family (family 6781 at 13.75%) and the most successful germinating family (family 6765 at 71.47%). There were considerable differences in the time at which it took for the emergence of the first seedling within a tray after leaving the cold-store. In family 6776 germination was rapid in all eight trays whereas in family 6775 germination was much slower.

There are several incidents which may have occurred at numerous different times from pollination up until removal from the cold-store, which may explain both, the wide range of percentages of seeds germinating, and differences in the dates of initial germinating. Bad frost or very wet weather succeeding pollination could cause weaker seeds to become unviable. Mechanical damage during seed extraction could also have injured the seeds from one seed lot more than that of another. This maybe due to inconsistencies in the precision of the extraction technique used for different seed lots. It may also be due to differences in the mechanical tolerances of the seeds from different families, causing some seedlots to become more damaged than others. The mentioned reasons could also prevent the seeds successfully entering a phase of dormancy when relocated to the cold-store. The low temperatures experienced therefore within could kill the embryos of damaged seeds. The vast change in environment and adjustment required by the seeds when moved into or out of the cold-store maybe more easily achieved, and with less damaging effects by certain families. Such families may experience increased percentages of germination due to such qualities. All of the above may also effect the time taken to germinate once out of the cold-store. Self repair of damaged seeds of some weaker seed lots may have been required before true germination could take place. Another determinant of the germination date is the size of the endosperm within the seeds. Seeds with small endosperms tend to emerge earlier due to the smaller quantities of dormancy-inducing

agents within them. The maternal parent of a cross has an influence on the endosperm size of its seeds. Therefore, one possible explanation for certain families with early germination may be that they had a maternal parent that produced seeds with smaller endosperms. If so, the seeds would contain smaller quantities of dormancy-inducing agents and so germinate earlier.

Figure 1 illustrates that, in general, batch one experienced more successful germination than batch two. The overall mean percentage of seeds germinated per tray for batch one was 49% compared to 34% for batch two. The reason for these differences maybe due to the prolonged period of dormancy experienced by batch two, due to their latter removal from glasshouse cold storage. However this is unlikely as in the wild, under certain conditions, raspberry seeds can remain in a dormant state for years and still be able to germinate successfully once conditions change. It is more likely that the parents of the batch two seed lots played a more significant role in their reduced percentage of germination. In particular the maternal parent for reasons already discussed.

The selecting for spinelessness was carried out with a high proportion of success. The predicted spine determining genes for each seedlot was confirmed by the state of the cotyledons of germinated seeds (using the technique outlined in the method). Families 6763, 6764, 6765, 6767, 6769 and 6780 segregated and at the calculated ratios. All but families 6763, 6779 and 6780 produced the required number of spineless seedlings to be potted up. These three families had too low percentages of germinating seeds in order to be able to discard the spiny seedlings and only keep the spineless ones. For this reason families 6779 and 6780 were never selected for spinelessness. Selection in family 6773 stopped after twenty-five days when it was recognised that too few seeds were germinating. This resulted in both spineless and spiny seedling being potted up for these three families once germination had stopped.

As a result of seed treatment and selection for spinelessness virtually all the required numbers of seedling for potting up was met allowing the next stage of the project to be started.

3. SCREENING FOR APHID RESISTANCE

3.1 Introduction

Two species of aphids live on raspberries, the large raspberry aphid, *Amphorophora idaei*, and the small raspberry aphid, *Aphis idaei*. Five strains of *Amphorophora idaei* are recognised (strains 1-4 and X) and the HRI breeding program produces seedling progenies which are segregating for resistance to *A. idaei*.

Through extensive research into resistance of certain raspberry varieties it has been established that there are a number of genes which determine resistance to *A. idaei*. Some resistance genes confer resistance to two or three strains (eg A_1 and A_2) while some confer resistance to strains 1-4 (eg A_{10}, A_{L518} and A_{K4a}). Much of the breeding material has A_{10} and, in order to identify seedlings with A_{10}, we screen the plants with strain 2. At the moment strain X is relatively rare but can overcome A_{10} under certain environmental conditions. We have no gene that confers strong resistance to strain X.

Resistant plants have biochemical components in the cuticular waxes on the leaves which the aphids do not like. The aphids are repelled before they have a chance to transmit any viruses they may be holding in their gut. When an aphid has identified a particular plant as being resistant it will simply walk (or fly if it has the ability) onto adjacent material until it finds a host that is susceptible and suitable for colonisation.

Primary colonisation of a previously uninhibited crop is accomplished through winged, adult aphids, which by chance, come to land on the crop. This is mainly as a result of their turbulent, only slightly controlled flight. The colony then reproduces, increasing their numbers, and spreads throughout the crop by the young, wingless aphids simply walking in search of nutritionally richer material.

Direct feeding of the aphids causes relatively little damage to raspberry plants. *Amphorophora idaei* poses a serious problem in commercial raspberry growing because it is a vector of four significant raspberry viruses. These are; Black Raspberry Necrosis Virus (BRNV), Raspberry Leaf Mottle Virus (RLMV), Raspberry Leaf Spot Virus (RLSV), and Rubus Yellow Net Virus (RYNV).

The main effect of these viruses is the formation of diseases known as mosaics. Transmission of the mosaic-causing viruses is attained through a semi-persistent manner. That is, in order to infect the crop on which the aphid is feeding, it previously has to have fed on an infected crop for up to one hour. Usually symptoms only arise due to co-infection of two or more of the four viruses. Visible indications of infection, seen in the leaves, vary depending on which two viruses are concurrently introduced. Typical symptoms however are various forms of chlorosis and vein banding, sometimes accompanied by downward curling of the leaves. The biggest concern of infection for commercial growers is the increasing reduction in yield which occurs with time. The only solution, other than extensive spraying of insecticides (which is difficult close to harvest), is to grow cultivars which are resistant to the aphid vector. Varieties with aphid resistance produce high yields for many years and result in considerable improvements in the economy of commercial raspberry growing.

3.2 Method

Once the seedlings in each new family had been potted up they were returned to the glass-house and placed on the benches. The number of seedlings per family to be screened for aphid resistance was determined by the aphid resistance status of the parents and the expected segregation ratio. The ideal number of seedlings per family to be planted in the field is around 100. Where 1:1 resistance to susceptibility was expected, 250 seedlings were potted. Where 3:1 was expected, 150or 200 seedlings were potted, depending on other attributes of the parents. Each family was divided up into blocks of fifty seedlings, arranged in five rows of ten. Plastic labels were placed into the four corner pots of each block stating the family number. A narrow gap was left between adjacent blocks of the same family allowing room for watering. Wider gaps were left between blocks of different families to help keep them separate.

On 16th April a small colony of *Amphorophora idaei* strain 2 arrived by post from the Scottish Crop Research Institute, Dundee. These aphids were transferred onto the young expanding leaves of two plants of Malling Landmark. M. Landmark has the gene A_1 and is resistant to strains 1 and 3. Aphids which established, thrived and reproduced on M. Landmark had to be strain 2 or 4. These two plants were the initial aphid stock plants. The aphids prefer the underside of the young leaves, so after being carefully transferred by hand, they would walk under the leaves and begin to feed. After ten days on the Landmark plants the aphids on the stock plants had multiplied sufficiently for screening to begin.

The method used to screen for *Amphorophora idaei* resistance was inoculation, followed by, a period for establishment, followed by, visual classification of resistance/susceptibility. Inoculation involved the transfer of three adult aphids from a stock plant onto the youngest leaves of the chosen seedling. Identification of an adult aphid was made through the presence of a distinct tailpiece protruding from the abdomen. It was important not to transfer 4th instar nymphs by mistake because they would not produce nymphs immediately. The largest seedlings were inoculated first and formed new rows of tens, at the front of which was a label stating the family number, the number of aphids put onto each plant (3), and the date the plants were inoculated.

A period of three or four days was usually enough for the aphids to establish a small colony on any susceptible plants. Therefore recording was done three or four days after inoculation. Recording entailed visually checking stems and the undersides of all the leaves of the inoculated plants allowing classification into one of three categories:-

1. RESISTANT-completely free of aphids, no adults and no nymphs
2. SUSCEPTIBLE-one or more adults and several nymphs
3. INTERMEDIATE-either 1 or 2 adults with no nymphs, or a few nymphs but no adults

Resistant seedlings were recorded and moved into a labelled red tray. Susceptible seedlings were recorded and then were either discarded or kept as additional stock plants. Intermediate seedlings were inoculated with a fourth aphid and placed in rows of tens, separate to the other seedlings. These plants were left for three or four more days, after which classification would be more accurate.

Full trays of resistant seedlings were moved outside to a partially sheltered poly-tunnel to harden off prior to planting.

All recording of inoculated seedlings was completed by 28th June. In certain families resistant seedlings waited in the glasshouse to be tested for Raspberry Bushy Dwarf Virus (RBDV) prior to planting.

3.3 Results

The aphid resistance genes of the 2001 parents and the aphid screening results can be seen in Table 2.

Table 2. Aphid resistance genes of the parents of the 2002 families, the number of seedlings potted per family and the number of individuals recorded as resistant, susceptible or broken/dead.

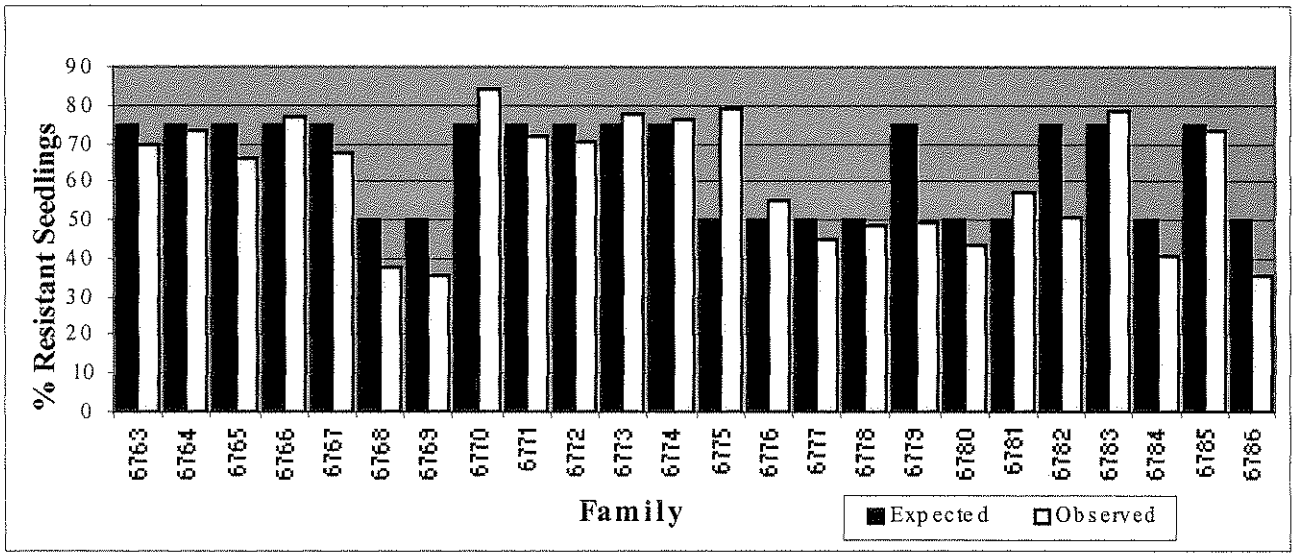
Family	Parents		*Amph.* Resistance genes		Number			
	Female	Male	Female	Male	Potted	Resistant	Susceptible	Broken/ dead
6763	6523/8	6529/85	A_{10}	A_{10}	158	110	43	5
6764	6523/8	6590/113	A_{10}	A_{10}+A_{L518}	200	146	40	14
6765	6529/85	6597/46	A_{10}	A_{10}+A_1	200	132	52	16
6766	6531/79	6590/113	A_{10}	A_{10}+A_{L518}	200	154	40	6
6767	6592/11	6529/85	A_{10}	A_{10}	200	135	62	3
6768	J. Squire	6531/62	sus	A_{10}	250	95	146	9
6769	Polana	6531/62	sus	A_{10}	218	77	111	30
6770	6584/51	6592/11	A_{10}+A_{L518}	A_{10}	200	168	24	8
6771	6590/149	6593/39	A_{10}+A_{L518}	A_{10}	150	108	40	2
6772	6593/39	6583/44	A_{10}	A_{10}+A_{L518}	150	106	39	5
6773	6593/39	6590/113	A_{10}	A_{10}+A_{L518}	150	116	25	9
6774	6593/39	6597/46	A_{10}	A_{10}+A_1	150	114	29	7
6775	Polana	6531/76	sus	A_{10}+A_{L518}	250	198	15	37
6776	6583/44	J. Squire	A_{10}+A_{L518}	sus	250	137	112	1
6777	6583/44	Polana	A_{10}+A_{L518}	sus	250	113	127	10
6778	6583/44	6471/98	A_{10}+A_{L518}	sus	250	122	107	21
6779	6590/149	6523/8	A_{10}+A_{L518}	A_{10}	200	99	81	20
6780	J. Squire	6592/11	sus	A_{10}	250	109	134	7
6781	Polana	6953/85	sus	A_{10}	250	143	100	7
6782	6593/85	6592/11	A_{10}	A_{10}	200	101	50	49
6783	6597/46	6593/85	A_{10}+A_1	A_{10}	200	157	33	10
6784	6471/98	6531/79	sus	A_{10}	250	101	148	1
6785	6590/113	6597/46	A_{10}+A_{L518}	A_{10}+A_1	200	146	38	16
6786	6590/149	6471/98	A_{10}+A_{L518}	sus	200	71	128	1
				Total	4976	2958	1724	294
				Percentages		59	35	6

As a result of knowing the resistant genes of the parents it was possible to predict the ratio of resistant:susceptible seedlings in the progenies. These predicted ratios are shown in table 3. In some cases these predicted ratios might be slightly incorrect due to changes in susceptibility under certain conditions. Sometimes, as a result of shading, lower leaves do not receive sufficient amounts of light. This causes premature senescence which could instigate changes in the makeup of the cuticular waxes of leaves. As a result, aphids that find themselves on senescent leaves are able to feed and reproduce. Therefore, some seedlings might be classified wrongly as susceptible seedlings, slightly altering the true expected ratio.

Table 3. Percentages of resistant, susceptible and broken/dead seedlings for each family and a comparison between the expected and actual resistance: susceptibility ratios for each family.

Family	Resistant plants (%)	Susceptible plants (%)	Broken/ dead plants (%)	Ratio Resistant : Susceptible	
				Expected	Actual
6763	70	27	3	3:1	7:3
6764	73	20	7	3:1	7:2
6765	66	26	8	3:1	7:3
6766	77	20	3	3:1	4:1
6767	68	31	1	3:1	7:3
6768	38	58	4	1:1	2:3
6769	35	51	14	1:1	4:5
6770	84	12	4	3:1	8:1
6771	72	27	1	3:1	7:3
6772	71	26	3	3:1	7:3
6773	77	17	6	3:1	4:1
6774	76	19	5	3:1	4:1
6775	79	6	15	1:1	8:1
6776	55	45	0	1:1	5:4
6777	45	51	4	1:1	1:1
6778	49	43	8	1:1	5:4
6779	50	41	10	3:1	5:4
6780	44	54	3	1:1	4:5
6781	57	40	3	1:1	3:2
6782	51	25	25	3:1	5:3
6783	79	17	5	3:1	4:1
6784	40	59	0	1:1	2:3
6785	73	19	8	3:1	7:2
6786	36	64	1	1:1	2:3
Mean	61	33	6		

Figure 2. Comparison of expected and observed percentages of aphid resistant seedlings for each family.



3.4 Discussion

The mean percentage of resistant seedlings (61%), shown in Table 3, highlights that the Raspberry crossing programme at East Malling in 2001 achieved the aim of producing mainly aphid resistant seedlings. However, Table 3 also shows that there is a considerable amount of deviation from this mean, giving a substantial range between the families with the highest and lowest percentages of resistant seedlings, 84% for 6770 (expected 75%), compared to 35% for 6769 (expected 50%). This greater than anticipated range is due to the observed resistant:susceptible segregation ratios being rather different to the expected ratios for certain families. The extent of these differences is demonstrated in Figure 2. There are several acceptable explanations for such irregular segregation patterns.

One explanation is that results may have been skewed due to there being too few seedlings in certain progenies, more being required to show a true ratio of segregation. This could result in the observed percentage of resistant seedlings either falling short or exceeding the expected percentage. A shortfall in the number of resistant seedlings was most likely to be caused by competition for light and subsequent shading as discussed previously. Another possible explanation for the excess of susceptibles may be due to the plant in question being inoculated with one or more aphids that were just about to give birth. If this was the case, the pregnant aphids might have given birth to a number of nymphs then identified the plant as being resistant and so walked off in search of a susceptible plant. Within the three or four days following inoculation the newly born nymphs would not have matured enough to enable them to move to a susceptible host. They would therefore still be present on the resistant plant when recording was carried out and as a result the plant could have been wrongly classified as susceptible.

However, Figure 2 actually shows that, for the majority of families, the expected and observed percentages of resistant seedlings were very similar. This highlights that the genes for resistance, inherited by the seedling populations, were accurately predicted.

The families whose observed segregation ratios differed from their expected ratios the most were 6775, 6779 and 6782 with differences of plus 29%, minus 25% and minus 24% respectively. Families 6779 and 6782's much lower percentage of resistant seedlings might be due to their incorrect classification for the reasons given above. Family 6775's much higher percentage of resistant seedlings might be due to the majority of its dead or broken plants being susceptible. As a result, such plants would not have been recorded as being susceptible, therefore skewing the results in favour of a higher percentage of resistant plants. Alternatively the resistance genes in the parents of these three families may have been incorrectly documented.

In family 6775 the very low percentage of susceptibles could indicate that Polana had a resistance gene, however the ratios in other families derived from Polana (6769, 6777 and 6781) were fairly close to 1:1 indicating that Polana was fully susceptible. Another possible explanation is that the other parent of 6775, 6531/76, was homozygous for A_{10}.

As a result of screening the 2002 seedling population for *Amphorophora idaei,* approximately 2,900 resistant seedlings were planted in the field for further evaluation. They will be immune to colonisation, leaving only a slim possibility of them contracting any of the four viruses for which the aphid is a vector. Any new varieties that may eventually arise out of this seedling population will therefore be resistant to strains 1-4 of the aphid and suitable for commercial growing.

4. SCREENING FOR RASPBERRY BUSHY DWARF VIRUS

4.1 Introduction

Raspberry Bushy Dwarf Virus (RBDV) is an isometric RNA virus with a diameter of about 33nm which has been identified within raspberry crops world-wide for some time. Despite the name, bushiness and dwarfing is only seen when a plant is co-infected with RBDV and another virus (usually one of the four viruses previously mentioned as being transmitted by *A. idaei*) Another common symptom of the virus is the yellowing of leaves in spring and early summer. Pale yellowing of some finer veins gradually develops into full-blown yellow leaves throughout the plant, caused by inter-veinal chlorosis. The presence of yellowing symptoms in May/June is often indicative of RBDV infection and is one reason why certain selections are tested at HRI. However the display of leaf yellows is inconsistent between genotypes and so is not diagnostic. Later in the season yellowing of the foliage can have other causes.

The most devastating effect of the virus to the raspberry industry is the development of crumbly, unmarketable fruit. This is a result of up to 43% of the fruit's drupelets falling to set, as well as uneven development of the ones that do set. Such raspberries have irregular outlines along with poor cohesion, resulting in them breaking up into individual drupelets when picked. RBDV infection could result in an economically crippling 30% plus reduction in saleable fruit, varieties with resistance to RBDV are therefore very desirable.

Resistance to the some isolates of the virus in some UK cultivars is due to the presence of a single dominant gene *Bu*. However, seedlings and selections are not routinely tested for the presence of *Bu* because testing entails grafting, which is very labour intensive and it can take up to two years to get the results. Also there are resistance breaking isolates of the virus at East Malling which can overcome *Bu*. The current strategy at HRI is to limit the amount of RBDV in the breeding material by testing for the virus and to select for resistance to field infection.

RBDV is impossible to control because of its method of transmission. RBDV is transmitted via infected pollen and to seedlings via infected seeds. The virus can therefore rapidly spread throughout crops during the pollinating season by bees and other pollinating insects. It has been suggested that infection can be amplified by cross contamination within hives of bees collecting from plants of variable RBDV incidence.

From 29th April to 25th June 2002 a range of different material was tested for the presence of RBDV using Enzyme linked Immunosorbent Assay (ELISA). A number of factors played a part in determining which material required testing which are mentioned below. It is too time consuming to test all the breeding material each year but key categories are tested including parents, some of the young seedlings prior to planting and any material going from HRI out to the industry.

At the beginning of each season it is important to test all reagents used in the ELISA test to ensure adequate discrimination between positive and negative samples.

4.2 Method

Initial testing of the potential parents for the 2002 crossing program, some of the 2002 seedlings and the selections chosen for trial were tested as bulk samples with leaves from five plants together. This cut down the workload in that not every single plant had to be individually tested. If a bulk sample was RBDV positive, then the five plants making up that sample were tested again as individual samples. This identified which particular plants were positive and which were negative for presence of RBDV. All microwell plates were read using a measurement filter of 450nm and a reference filter of 620nm.

RBDV Test plates

In order to obtain clear results from ELISA it was necessary to carry out initial calibrations on antisera and protein reagents in order to determine the optimum concentrations. Raspberry samples of known RBDV incidence were used for the test plates and ideally the difference in absorbance values of the positive and negative samples should be in the order of 10-fold.

Five test plates were run following the protocol but with various stocks of IgG, $F(ab)_2$ and protein A in varied dilutions. All test plates used several leaves of four controls of known RBDV incidence. The controls used were Lloyd George, Barnaulskaya and 4427, all known to be infected and kept within a containment glasshouse, and a selection of Loganberry known to be RBDV-negative and kept within a glasshouse free of RBDV.

The first two plates tested the reliability of stocks of IgG and $F(ab)_2$ received from HRI-Wellsborne on 26th July 2001 and an older sample of IgG stored in a fridge from 29th August 2000. Both plates used an old sample of protein A at a concentration of 1 in 2000. Two further plates using the same controls were carried out to test various dilutions of new preparations of $F(ab)_2$ and IgG antisera received from HRI-W on 23rd April 2002. Also a stock of protein A received from Sigma in March 2001 was compared with the old protein A used for the previous two plates.

One final test plate was carried out to test the reliability of a new stock of protein A received from Sigma on 26th April 2002 and to compare it with the old protein A. No further testing of $F(ab)_2$ and IgG was required as plates 3 and 4 had determined that the 2002 stocks of both worked well and at what concentration they were most effective.

Potential summer fruiting parents for the 2002 crossing programme

Due to RBDV being readily transmitted via pollen and seeds it was necessary to test all potential parents of the 2002 crossing programme. This year 33 selections had been identified as potential parents because of their desirable attributes. Most parents were growing in the field as a single plot made up of 10 plants or stools. Material from every stool of the 33 potential parents required testing for presence of RBDV before they could be used as parents. If RBDV-positive plants were identified they were grubbed to prevent further spread. The selection was then either, dismissed as a suitable parent, or accepted if there were a number of RBDV negative stools

remaining and the risk of RBDV within these stools was low due to a number of aspects.

2002 Seedlings

Twelve out of 24 of the 2002 seedling progenies required RBDV testing due to the potential risk of having inherited the virus from their parents. These twelve families were identified as being possible carriers due to some recorded incidence of the virus within one or both of their parents. If one of the parents had tested bulk positive for RBDV in 2001 but a number of stools had been identified as being negative and used for the cross, all of the resulting 2002 seedlings required RBDV testing. This was due to only a limited sample of the negatively identified stools being tested. There is a chance that other material from such stools could be infected and therefore, so could their offspring. If a seedling progeny had a parent that tested completely negative for RBDV in 2001 but that was known to be susceptible by its history, a sample of 25 seedlings were tested. If none of the 25 tested positive all of the remaining seedlings were assumed to be negative and so were planted in the field. It is not definite that the untested seedlings were negative, it is only assumed on the basis that their parents had limited history of RBDV and that the 25 tested were negative. If one or more of the 25 seedlings tested positive for RBDV all of the family would be tested.

HDC and Meiosis trials

It is very important that no RBDV-positive material is passed on from HRI to trials or raspberry farms. In 2002 root cuttings were taken from a number of selections which had been chosen for further trialling by the HDC and Meiosis. Approximately 550 individual plants from 21 different selections were to be tested before going on to HDC SF Trial 2. In addition, 261 plants from 7 different selections destined for Meiosis trials were also tested before being handed over to Meiosis Ltd. All plants were held in a glasshouse while testing was carried out and no plants flowered before testing was completed.

4.3 Results

RBDV Test plates

Results of the first two plates showed that the 2001 IgG failed at concentrations of 1 in 500 and 1 in 1000 as all samples came up negative. Better results were seen for the older IgG especially when used at a concentration of 1 in 500 combined with the 2001 $F(ab)_2$ at a concentration of 1 in 1000. However, the three positive controls only showed up as being slightly positive giving only minimum resolution between them and the negative control. Further testing was therefore required in order to achieve an acceptable difference between the positive and negative samples.

Results of the third and fourth test plates indicated that the two new samples of $F(ab)_2$ and IgG worked better than the older samples used for the first two plates. Absorbance values were higher for the older protein A suggesting that the March 2001 stock is not as good. The ideal concentration at which to use the older protein A was shown to be 1 in 1000. Little difference could be seen between samples where the only difference was the concentration of $F(ab)_2$ suggesting that 1 in 1000 is an

acceptable dilution. There were however significant differences between samples where the only alteration was the concentration of IgG. This indicated that the best concentration of IgG to use would be 1 in 500.

Results of the final test plate showed that the new (2002) protein A gave considerably higher absorbance values than the older protein A. At a concentration of 1 in 2000 the new protein A gave absorbance differences of nearly X10 between the positive and negative controls. However, absorbance values for the negative controls were slightly high but not high enough to indicate the presence of RBDV.

As a result of the five test plates the stocks of $F(ab)_2$, IgG and protein A chosen to be used for all further RBDV testing were as follows:-

2002 stock of $F(ab)_2$ at a concentration of 1 in 500,
2002 stock of IgG at a concentration of 1 in 1000,
2002 stock of protein A at a concentration of 1 in 2000

The positive control, Barnaulskaya, consistently gave high absorbance values for all test plates and was therefore suitable to be the glass-house positive control for all further tests. A selection of Autumn Bliss in the field was identified as clearly being infected with RBDV by the extent of leaf yellows it possessed and was therefore chosen to be the field positive control for all further tests. The negative control, Logan, consistently gave low absorbance values for all test plates and was therefore suitable to be the glass-house negative control for all further tests.

Potential summer fruiting parents for 2002 crossing program

Of the 33 potential parents for the 2002 crossing program six selections had bulk samples which tested positive for RBDV. These selections, 6512/50, 6564/87, 6507/56, 6639/26, 6495/53 and 6507/35, consisted of between 5 and 14 plants and were therefore individually tested for RBDV to identify which particular plants were harbouring the virus.

Selections 6564/87, 6495/53 and 6507/35 were found to be only partly positive, each having only one positive plant out of ten or eleven. The plants identified as being RBDV positive were grubbed as soon as possible after recognition to prevent spread of the virus to the remaining RBDV negative plants. More plants of 6512/50 were infected but negative stools were identified and because of the desirable attributes possessed by selections 6512/50 and 6564/87, both were accepted as parents to be used in the 2002 crossing program. The remaining RBDV-negative stools provided sufficient numbers of flowers for their intended crosses and they were used as females to limit spread. All seedlings derived from either of these two selections will require to be tested for RBDV in 2003 before being planted in the field. Selections 6495/53 and 6507/35 possessed less desirable attributes and had insufficient numbers of flowers, so both were dismissed as parents for the 2002 crossing programme.

Selection 6507/56 was identified as having four positive and six negative plants and selection 6639/26 was identified as having one positive and three negative plants. These ratios of positive to negative plants were too high to ensure that the negative plants were completely free of the virus. It only ensures that the leaves tested were

free of RBDV. As a result both selections were dismissed as parents for the 2002-crossing programme.

The remaining 27 selections potential parents were completely free of RBDV and were all used in the 2002-crossing programme.

2002 Seedlings

Of the 12 families tested for presence of RBDV only one seedling in family 6772 was identified as being RBDV positive (Table 4). One bulk sample in family 6772 was the only positive sample out of all the seedlings tested. The seedlings from this batch were therefore tested individually for RBDV. Results showed that seedling number 69 of this family was the only positive plant and it was discarded.

Only one RBDV-positive seedling out of 661 tested highlights the effort put into testing the parents prior to crossing in 2001 and shows that it is an effective strategy for minimising RBDV in the breeding material at HRI-EM.

Table 4. Results of testing the aphid resistant seedlings in 12 families for RBDV.

Family	Number of seedlings tested	Number of RBDV negative seedlings	Number of RBDV positive seedlings
6763	25	25	0
6764	25	25	0
6771	109	109	0
6772	110	109	1
6773	121	121	0
6774	121	121	0
6778	25	25	0
6781	25	25	0
6782	25	25	0
6783	25	25	0
6784	25	25	0
6786	25	25	0
Total	**661**	**660**	**1**

HDC and Meiosis trials

Results of testing the selections chosen to go forward for HDC trials showed that only one plant out of 546 has RBDV (Table 5). One bulk sample from the selection 6507/35, containing plants numbered 11-15, was the only positive batch out of all the selections tested. The plants from this batch were therefore tested individually and number 11, which was the only positive plant was destroyed.

Table 5. Results of testing HDC Summer Fruiting Trial 2 plants for RBDV.

Selection	Number of plants raised in 2001	Number of RBDV negative plants	Number of RBDV positive plants
Main entries (60 plants required of each)			
6385/1	61	61	0
6390/47	34	34	0
6428/1	64	64	0
6506/37	54	54	0
6512/50	65	65	0
6544/80	55	55	0
6545/12	51	51	0
Guards from HRI-EM (15 plants required of each)			
5928/114	20	20	0
6166/98	17	17	0
6413/59	15	15	0
6487/74	17	17	0
6507/35	17	16	1
Guards from overseas (15 plants required of each)			
Kitsilano (BC85-18-16)	11	11	0
Cowichan (BC87-14-20)	4	4	0
BC89-2-89	4	4	0
BC89-33-84	16	16	0
BC89-34-41	11	11	0
BC90-8-11	4	4	0
BC90-8-20	5	5	0
Rubaca	4	4	0
Wei-Rula	17	17	0
Total	**546**	**545**	**1**

All of the plants selected to go forward for Meiosis trials were identified as being RBDV negative. This emphasises the scrutiny placed on sourcing healthy material of selections chosen for further trialling. The RBDV-positive plant of 6507/35 from the HDC trial was discarded ensuring that all plants passed on were RBDV-negative.

4.4 Discussion

The results of this seasons ELISA testing to screen for presence of RBDV within raspberry material at HRI are very encouraging. Only a tiny proportion of the material tested was found to be RBDV positive. This highlights how important controlling the prevalence of the virus is considered at HRI-EM and the strict precautions in place to limit transfer of the virus once identified.

Initial ELISA test plates established the most effective stocks and concentrations of IgG, $F(ab)_2$ and protein A to use when testing the actual samples. This ensured that RBDV-negative and positive samples could be accurately distinguished.

The elimination of four potential parents from the 2002 crossing programme left 29 parents suitable for the program, 27 of which showed no RBDV at all, and 2 which showed a limited presence of the virus. Negative stools were deemed suitable parents on the basis of the limited incidence of RBDV and their desirable attributes. These 29 parents hopefully will not pass on RBDV to their seedlings. However, it is not guaranteed that all the resulting seedlings will be RBDV negative because only a limited sample of the potential parents was tested. Some plants may have shown no presence of RBDV in ELISA results but other untested regions of the plant may have harboured the virus. For this reason, all seedlings derived from 6512/50 and 6564/87 will be tested in 2003.

Table 4 emphasises the precautions taken when choosing the parents for the 2001 crossing program (the same as used for the 2002 crossing program mentioned above). The one RBDV-positive seedling was found to be from family 6772, one of the four progenies identified as most likely to possess the virus. This indicates that one of the parents of the seedling, either 6593/39 or 6583/44, is susceptible to RBDV and that there was some incidence of the disease at some point. The suspicious parent was 6593/39 due to having shown a limited presence of RBDV within one of its plants in 2001. This plant was grubbed but the virus may have been transferred by bees prior to grubbing and been absent within a sample tested from such an infected stool.

Table 5 shows only one plant from selection 6507/35 tested positive for RBDV and was discarded. The remaining HDC selections, along with all the Meiosis selections, tested negative for RBDV and were forwarded for their advanced trials. These plants pose no threat to the raspberry industry.

5. 2002 CROSSING PROGRAMME

5.1 Introduction

In 2002, 30 summer fruiting crosses were made using 29 summer fruiting parents in different combinations. The 29 parents were narrowed down from 33 potential parents as a result of screening as previously described. Summer fruiting and primocane fruiting crossing programmes are carried out in alternate years at HRI-EM in order to make the workload each summer manageable and limit field and glasshouse charges. In 2002 the crossing programme was between summer fruiting selections.

The parents were selected for their desirable attributes such as good habit, resistance to pests and diseases, fruit quality, harvesting season or yield. The aim is to cross two parents with different advantageous attributes to identify individual seedlings with all the good characters combined. For example, cross a very early ripening parent with moderate shelf life with an individual with good shelf life but late ripening and select for seedlings that are very early ripening as well as having good shelf life. Alternatively the cross may result in further improvement of a single recognised characteristic such as producing seedlings that are extra late fruiting when both parents were moderately late fruiting.

The objective of the 2002 crosses can be seen in Table 6. Desirable improvements in summer fruiting types are to produce selections that fruit very early in the season and produce others that fruit very late, as well as having high yields of good quality fruit. The aim being to produce new varieties that have overlapping harvest periods, integrating the summer and primocane fruiting harvesting seasons so that commercial growers have high yields of quality fruit throughout a prolonged raspberry season.

There is no guarantee that all the seeds produced from each cross will germinate into plants that express the superior characteristics that the cross is designed to improve. This is due to each seed of the seed lots being genetically distinct as a result of independent assortment of chromosomes and crossing over of chromatids during the metaphase of meiosis which produced the ovules and pollen within the parent flowers. Further variation among seedlings is because of the random pairing of pollen and ovules at pollination. For these reasons, only one seedling out of a population of 100 might express superiority in the characteristics or combination of characteristics that the cross was designed to improve. Therefore, as many as possible of the seedlings produced from each cross are to planted into seedling plots in 2003, following pre-field selection for spinelessness, aphid resistance and freedom from RBDV.

Table 6. The major objectives of each cross of the 2002 crossing programme and the new family numbers given to the resulting seed lots.

Family	Parents		Major objectives of each cross
	Female	Male	
6787	Malahat	6390/47	Increased earliness by intercrossing very early and early selections
6788	6544/80	6390/47	
6789	6544/80	6545/26	
6790	6545/26	6390/47	
6791	6545/26	6413/59	
6792	Malahat	6592/48	Increased earliness by crossing a very early primocane fruiting selection with early summer fruiting selections
6793	6390/47	6592/48	
6794	6414/14	6592/48	
6795	6511/58	6390/47	Early ripening, high yield and good fruit quality
6796	6511/58	6399/84	
6797	6545/12	6399/84	
6798	6545/12	6511/58	
6799	6545/12	6544/80	
6800	Malahat	6569/49	Early ripening, high yield incorporating double laterals ex *R. coreanus*
6801	6548/10	6569/49	
6802	Octavia	6549/31	Extra late crosses
6803	Octavia	6564/87	
6804	Tulameen	Octavia	
6805	6385/1	Octavia	
6806	6553/25	6385/1	
6807	6558/29	Glen Ample	
6808	6558/78	6495/58	Extra late ripening and good shelf life
6809	6558/83	6549/31	
6810	6554/64	Glen Ample	Late ripening and good quality of fruit
6811	6556/44	6385/1	
6812	6564/87	Glen Ample	
6813	6564/87	Tulameen	
6814	6564/87	6495/58	
6815	Tulameen	6570/13	Late ripening, increased numbers of flowers per lateral ex *R. coreanus*
6816	6559/95	6570/31	

5.2 Method

Thirty-three potential parents were selected and tested for presence of RBDV (as described in previous section) which resulted in twenty-nine being deemed suitable for crossing. Approximately 30 well set fruit would produce the required numbers of seeds per cross so 30 buds were needed on the female parent. The female flowers were selected while still unopened buds, ensuring that no bee pollination had already occurred. The best female buds to use are ones that are just about to open with well developed stigmas, which following fertilisation, would produce a large number of viable seeds. Predicting which buds would mature into workable flowers was tricky and meant that the 30 female flowers for a particular cross would be selected from a number of different laterals on different stools of the selection based on the stage of

development of the buds. Between four and six laterals were chosen for each female parent, incorporating from three to ten buds on each so that there were around 30 usable flowers for the cross. Fruit resulting from each cross will does not ripen until six weeks after initial pollination. Therefore the flowers selected must be on strong laterals with limited risk of breaking during this fruit maturation period.

Small, immature buds and any open flowers were removed from the chosen laterals. A sharp, sterile scalpel was used to emasculate the remaining large buds by making a shallow incision around the widest part of the bud, thus removing all petals, sepals and anther bearing stamens, and leaving the stigmas unharmed. The scalpel and the fingertips were dipped in 100% ethanol after pollination to prevent pollen contamination of the next female parent. Emasculated laterals were enclosed in windowed, white female pollinating bags to prevent insect pollination. The bags were closed around the lateral, which had been pruned to remove obstructing foliage, by winding one end of a long piece of wire around the base of the bag, the other end of which was then attached to a nearby lateral, cane or wire for support. If a particular selection was to be used as a female parent more than once, the female bags from different crosses were colour coded with tape. An aluminium label was placed around the base of the lateral, stating the female parent, the male parent, the number of buds emasculated and the date of emasculation. The first female parents to have flowers selected and emasculated were 6390/47, 6414/14 and 6545/26 on 7th May. These parents were emasculated at such an early date because of their early flowering time. Emasculation of the other female parents occurred as and when they, and the male parents with which they were to be crossed, were at the correct stages of flowering.

Flowers of the male parent were also chosen at the bud stage so as to ensure that no pollen contamination had occurred by bees feeding on a number of different selections. Thirty or so male flowers selected for a particular cross would be located on several laterals on different stools. These chosen laterals were bagged in clear perforated thin plastic bags, closed around the lateral using a small piece of wire without an attached support lateral as they were only required for a period of around two weeks while pollination was being carried out. If a particular selection was to be used as a male parent more than once, more laterals were bagged as required.

Three to five days after bagging, the male flowers should have opened and their anthers dehisced (identified by yellow, dusty anthers), and the female stigmas should have fanned out to a suitable state for the pollination. Pollen was indirectly collected from the male parent by detaching a number of opened flowers and collecting them into a labelled tube. The pollen from 12-20 male flowers was sufficient for pollinating about 30 emasculated female flowers. The technique used for pollination was holding a male flower with tweezers by its peduncle and brushing its anthers against the fanned out stigmas of the female flowers. Alternatively, if the male parent flowered considerably earlier than its female counterpart, anthers were extracted from large flower buds into a petri dish, and allowed to dehisce at room temperature, and then stored in a desiccator. Stored pollen was applied to stigmas by lightly dipping the stigmas into the dish of pollen. After pollination had been carried out the female bags were replaced and left to allow fertilisation to occur. Fertilisation only occurred if enough viable pollen had been applied to the stigmas and consequently travelled to the ovules. For this reason, the pollination procedure was repeated after three and six days, if necessary, to give the best chance of successful fertilisation and production of

fruit containing viable seeds. Once the stigmas had turned brown they were no longer receptive.

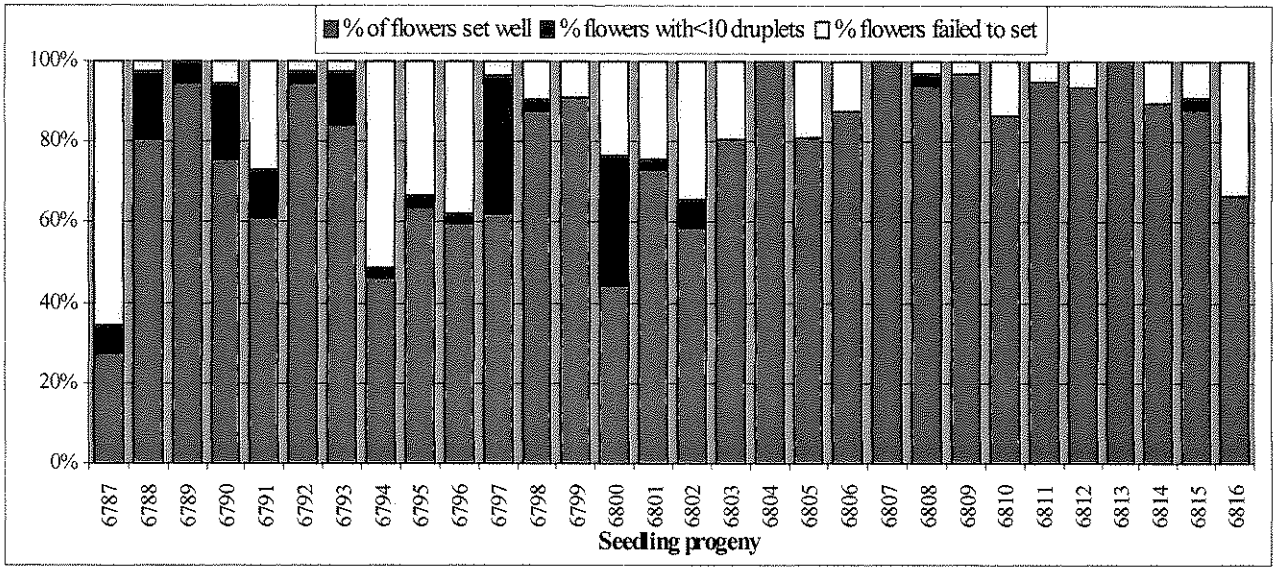
After pollination had been carried out two or three times the female flowers were left for around five weeks to allow them to develop into fruit. Fruit that set well, along with the drupelets of fruit that set badly, were collected and numbers of fruit to have set well, set with less than ten drupelets, and failed to set, along with number (if any) of broken laterals was recorded. Fruit from particular crosses matured at different times depending on which date pollination was successful, so sometimes it took up to two weeks to collect all the fruit from one cross.

When all the fruit from a particular cross was collected, the seeds were extracted using a blender. The seeds were left to dry overnight and bagged up into labelled bags and stored in the cold-store. The seed number per cross was estimated by weighing the seed lot and weighing three samples of 100 seeds.

5.3 Results

Between 29 and 41 flowers were emasculated per cross and Figure 3 shows the percentages of flowers which set well, set badly and failed to set within each cross. The results of the controlled crossing programme in 2002 are shown in Table 7.

Figure 3. A comparison of the outcome of the 30 pollinations shown as percentages of total flowers pollinated per cross.



5.4 Discussion

Figure 3 shows that there is substantial variation in the outcome of flowers pollinated for each cross. Very pleasing results can be seen for crosses to produce the new seedling progenies: 6804, 6807 and 6813, in that all flowers emasculated for these

Table 7. The number of flowers emasculated for each cross, the pollination results and the estimated number of seeds extracted.

Family	No. flowers emasculated	No. fruit			No. lost laterals	Estimated no. of seeds
		Set well	<10 drupelets	failed to set		
6787	29	8	1	19	1	522
6788	41	33	7	1	0	1752
6789	35	33	2	0	0	2652
6790	37	28	7	2	0	1980
6791	41	25	5	11	1	1538
6792	37	35	1	1	0	1939
6793	38	32	5	1	0	2390
6794	41	19	1	21	2	1842
6795	33	21	1	11	1	1337
6796	37	22	1	14	1	1135
6797	29	18	10	1	0	1038
6798	32	28	1	3	0	2043
6799	33	30	0	3	0	2530
6800	34	15	11	8	1	441
6801	37	27	1	9	0	1267
6802	29	17	2	10	1	1049
6803	31	25	0	6	0	1692
6804	33	33	0	0	0	2844
6805	32	26	0	6	1	1914
6806	32	28	0	4	0	3042
6807	32	32	0	0	0	2921
6808	33	31	1	1	0	1463
6809	35	34	0	1	0	4356
6810	30	26	0	4	0	3059
6811	39	37	0	2	0	2232
6812	31	29	0	2	0	4344
6813	32	32	0	0	0	3900
6814	29	26	0	3	0	3941
6815	34	30	1	3	0	2083
6816	39	26	0	13	1	2452
Total	**1025**	**806**	**58**	**160**	**10**	**65698**
Mean	**34.2**	**26.9**	**1.9**	**5.3**	**0.3**	**2190**
% of Total		**78.6**	**5.7**	**15.6**		

three crosses set well into good fruit. This 100% success might be because the female flowers were at an ideal state and size on the date at which they were pollinated, with mature fanned stigmas allowing successful intake of pollen and fertilisation. The pollen used on the cross might have been at the perfect stage, anthers having formed correctly due to good weather then de-hissing immediately prior to pollination,

releasing viable pollen for the cross. Following pollination conditions, such as the weather, might have been particularly suitable for pollen tube growth, fertilisation and production of well-set fruit. For 100% of the fruit to have set well probably required a combination all three events.

The least successful crosses were progenies 6787 and 6794, with 66 and 51% of flowers failing to set, respectively. However, the reason for these high percentages of failed fruits is different for each cross. Family 6787 had one broken lateral which resulted in 6 flowers failing to set. This leaves 13 flowers that failed to set because of other influences, such as both parents flowers not being at the correct stage when the cross was made or bad weather (cold and wet) prior to and succeeding pollination. Family 6794 suffered two broken laterals which accounts for approximately 14 of the 21 flowers which failed to set. As the majority of the flowers on the unbroken laterals set well (only one fruit with less than ten drupelets), sufficient seed was produced in seed lot 6794.

The number of badly setting fruit was high for families 6797 and 6800, as indicated by the middle bands for their corresponding bars on Figure 3. This partial setting of fruit might be due to the parents possessing genes which adversely influence fertilisation and result in only partial setting of some fruit. Uneven pollinating of a flower's stigmas due to inexperience in the pollinating technique could also result in badly set fruit. Also, the pollen collected from the male parent may have been in a limited supply, resulting in it being applied too thinly. Weather conditions prior to and succeeding pollination could have resulted in damage to a number of stigmas preventing fertilisation of every ovule of some flowers. All four suggested reasons would have resulted in a reduced number of drupelets forming.

Overall, the mean percentage of fruit to have set well was high at 79%. This resulted in a mean of 2190 seeds being extracted for each new seedling progeny. Table 8 shows that there is a considerable range between the estimated number of seeds for different families. The highest number of seeds was extracted from the fruit of families 6809 and 6812, estimated at 4356 and 4344 respectively (both had less than 100% of fruit setting well). The reason for such big seed lots was probably due to both families having large fruit consisting of greater numbers of drupelets than other families; neither family had any fruit that set with less than ten drupelets. An abundance of drupelets for the two families therefore released vast numbers of seeds when the extraction procedure was carried out. The crosses creating families 6787 and 6800 produced the smallest seed lots of 522 and 441 estimated number of seeds, respectively. Family 6787 has already been mentioned in having one broken lateral and several flowers which failed to set. Family 6800 has previously been mentioned as having a large number of fruit with less than ten drupelets. This, combined with eight fruit being lost on a broken lateral, resulted in a low number of drupelets from which seeds could be extracted.

An ideal number of seeds per family is around 1000 and only two families (6787 and 6800) have less than 1000 seeds. All seed lots have the potential to produce 250 seedlings per family in 2003 and in all but families 6787 and 6800 the required numbers for spineless selection and aphid screening should germinate.

6. STAGE 0 FRUITING TRIALS

6.1 Introduction

Twenty-five summer-fruiting selections were chosen for inclusion in the stage 0 trials in 2002 on the basis that they might have commercial potential. These were selected a week prior to the first picking date of 15th June. At this time the presence and abundance of green immature fruit was a good indicator of which selections were going to be high yielding. Selections that had been propagated from seedling plots in winter 2000/01 were too weak to be included in the stage 0 trials in 2002. Other selections were rejected because they performed badly in the 2001 stage 0 trials. In addition to the 25 selections, two industry standards, Tulameen and Glen Ample, were included for comparison. In past years up to 50 summer-fruiting selections have been chosen for stage 0 trial but this year, due to many selections being rejected because of factors mentioned above, only twenty-five were chosen. Thirty-five primocane fruiting selections were also chosen as stage 0 trials but they will continue to fruit for some weeks after this report is finished and therefore results and summary data will not be included in the main body of the report. More primocane fruiting selections were chosen than summer-fruiting selections simply because there were more available that were suitable for stage 0 trialing in 2002.

Stage 0 trials entail picking all the marketable and unmarketable fruit from the chosen selections throughout their harvesting period. Marketable and unmarketable fruit is weighed at each pick and the marketable fruit is evaluated for nine fruit quality attributes. Once a week shelf life tests are set up and the fruit recorded after 48 and 72 hours. Totals yields (expressed in kilograms per 10 meters of row) and mean fruit attribute scores are calculated for the whole season after fruiting has ceased.

It is important that the fruit is picked at regular intervals so that fresh fruit does not become rotten while on the plants while waiting to be picked. The stage 0 trials were picked twice a week (Mondays and Thursdays) but commercially raspberries for the fresh market are picked at least every two days.

The eventual outcome of stage 0 selections will be determined by the mean scores that selections receive for each of the nine fruit quality attributes and the five shelf life attributes examined. The mean scores of the nine initial attributes inspected were compared to those of Tulameen and Glen Ample and if a selection's scores were equal to or better than the standards for every characteristic they are considered promising. The mean scores for flavour, brightness and redness are particularly important when determining the outcome of selections. Good flavoured, bright fruit, which is not too dark, is very desirable. Seven of the nine attributes examined have threshold values which must be met if a selection is to go on to further trials. If a selection produces fruit that has exceptionally good flavour, that is very bright and pale-red in colour, but one other attribute, such as cohesion, falls below the threshold minimum, the selection will be dismissed as a potential commercial variety. Shape and uniformity of size are the two exceptions, where mean scores do not play much, if any, part in a selections future. There is no particular preferred shape of raspberry fruit that is desired by commercial growers. Uniformity of fruit size is not essential either but similar sized fruit looks better in the punnet. Large fruit is desirable because it is quicker and hence cheaper to pick.

When evaluating shelf life, selections were compared to Glen Ample only as it has particularly good shelf life, whereas Tulameen only has moderate shelf life. Low rots and good overall punnet texture are particularly desirable for the fresh market. Selections identified as having poor fruit, bad shelf life or low total yields would be discarded as potential varieties but might be kept at HRI if they possessed other desirable attributes, such as pest or virus resistance, or exceptional early or late fruiting.

6.2 Method

On 12th June twenty-five summer fruiting selections were chosen to be included in 2002 stage 0 trials. A small length of row (between 1.6 and 3.3m) of each selection was chosen and each end marked with yellow tape. The length of row of each selection to be picked was measured and recorded so that total yields could be standardised into comparable units, kilograms per 10 metres of row. Each selection was allocated a tray into which punnets of picked fruit would be transported from the field to the laboratory. The trays were labelled with their selection and row number. The first pick for the summer-fruiting selections was Friday 14th June. The labelled trays, along with empty punnets, were placed at the first yellow tape and the pickers asked to pick all the ripe and overripe fruit between the two tapes. Any under-ripe fruit was left to mature. The picked fruit was taken to a fruit laboratory to be assessed.

Fruit assessment involved weighing the marketable and unmarketable fruit from each selection. Fifty marketable fruit, was counted into an empty punnet and weighed in order to calculate the mean weight of individual fruit for each selection at each pick. If fifty fruit were not present the weight of fifty fruit was estimated by dividing the marketable fruit weight by the number of fruit present then multiplying by fifty. If ten or more marketable fruit had been picked it was evaluated for nine different attributes. The attributes assessed and the guide-lines followed when awarding scores can be seen within Table 1 of Appendix A. If less than ten marketable fruit had been picked no assessment was made as it was considered too small a sample.

Picking continued until all the fruit was ripe and no yellow/green immature fruit could be seen within the marked length of row. The final pick for the summer-fruiting selections was 15th August, when only selections 6558/78 and 6558/83 were picked.

Shelf life tests were set up on a Monday and ideally two punnets per selection, each three-quarters full, were stored at 18°C and 90% relative humidity in a controlled environment cabinet. Commercially raspberries are stored and transported at 3-4°C so this was a harsh test. After 48 and 72 hours the fruit assessment was evaluated for incidence of rots, texture and appearance following the guide-lines seen within Table 2 of Appendix A.

The data recorded for the 27 summer fruiting genotypes was entered into two excel files, one for yield and fruit quality attributes and one for shelf life. This data was then summarised. The 37 primocane fruiting selections were evaluated in the same way and picking, which commenced on 22nd July, will continue until all the fruit has ripened.

6.3 Results

Considerably different mean weights of individual fruit were obtained for different selections (Table 8). Selection 5649/31 had the highest mean fruit weight at 4.97g, compared to 2.93g, the lowest mean fruit weight, collected from selection 6558/78. The mean fruit weight from all selections was 3.71g, noticeably lower than both Tulameen (4.20g) and Glen Ample (4.73g). There were also large differences in the marketable yields obtained from the twenty-seven genotypes (Table 8). Selection 5644/80 had the highest percentage of marketable fruit (89%) compared to selection 6558/64, which had only 42% marketable. The mean marketable yield was calculated to be 65% which was lower than would be acceptable commercially but this was a reflection on the relatively long intervals between picks.

Table 8. Yields and overall mean fruit weight of the stage 0 genotypes in 2002, expressed in kg/10m of row and sorted by total yield, descending from the greatest to lowest yields.

Selection	Yields (kg/10m row)				
	Total	Marketable	Unmarketable	Marketable as % of total	Mean weight (g) of one fruit
6564/87	82.93	68.41	14.52	82	3.84
6558/78	73.56	44.71	28.84	61	2.93
6550/9	66.43	52.42	14.01	79	3.81
6549/31	64.54	39.51	25.03	61	4.97
6558/83	61.22	34.67	26.55	57	3.12
6548/13	59.00	42.92	16.08	73	4.02
6559/95	58.48	40.50	17.99	69	4.29
6556/29	58.13	37.01	21.12	64	4.11
6558/64	58.01	24.26	33.75	42	3.41
6556/44	55.67	30.66	25.01	55	4.18
6558/56	55.63	26.22	29.41	47	2.97
6564/63	55.63	41.79	13.84	75	3.57
6554/64	54.22	25.59	28.64	47	4.18
Glen Ample	53.36	40.91	12.45	77	4.73
6551/38	53.04	39.65	13.39	75	4.34
6545/12	51.43	44.82	6.61	87	3.59
6549/99	50.84	28.50	22.34	56	3.19
6549/24	50.60	25.34	25.26	50	3.76
6551/40	48.06	24.13	23.92	50	3.81
6558/29	47.64	23.54	24.11	49	3.69
6548/24	46.40	34.26	12.14	74	3.54
6545/26	46.10	35.54	10.55	77	2.98
Tulameen	45.47	27.93	17.54	61	4.20
6548/51	42.71	34.02	8.70	80	3.36
6564/30	42.51	23.95	18.56	56	3.14
6553/25	38.55	26.06	12.49	68	2.97
6544/80	38.46	34.28	4.18	89	3.45
Total	**1458.59**	**951.57**	**507.02**		
Mean	**54.02**	**35.24**	**18.78**	**65**	**3.71**

Of the nine different fruit quality attributes; redness, brightness and flavour are most significant when deciding the outcome of stage 0 selections. The other attributes, apart from shape and uniformity of fruit size, are less important but have threshold scores that must be met if a selection is to progress to further trials. If mean attribute scores are equal to, or more than those obtained by Tulameen and Glen Ample, they are considered acceptable. Thirteen of the twenty-five selections were darker than Glen Ample, which had a mean score of 3.2 (Table 9). Selection 6558/56 had the palest coloured fruit, with a mean score of 4.1, and selections 6558/83 and 6564/30 had the darkest, both with means scores of 2.2. Tulameen was considered brighter than Glen Ample, with a mean score of 3.2 (compared to 2.5) but there were four selections that were brighter than Tulameen. Selections 6551/38 and 6551/40 had the brightest fruit with a score of 3.6 and 6558/29 had the dullest at 1.7. The selection with the highest mean score for flavour was 6549/31 with a mean score of 3.9 and there were twenty-two other selections that had higher mean flavour scores than Tulameen which had a score of only 2.5. Glen Ample had considerably better flavour (3.2), which was bettered by six selections. The selection with the worst flavoured fruit was 6551/38, which had a mean score of 2.0. Cohesion was consistently high for every selection. Skin strength was generally high and only 6549/24 scored less than 3.0.

Table 9. Mean scores given to nine fruit quality attributes of marketable fruit collected from each selection over the harvesting period.

Selection	Quality parameters								
	Redness	Brightness	Shape	Outline	Uniformity of size	Texture	Cohesion	Skin strength	Flavour
G. Ample	3.2	2.5	2.5	2.5	3.7	2.5	4.2	4.2	3.2
Tulameen	3.1	3.2	3.7	2.7	2.8	2.4	4.0	4.6	2.5
6544/80	3.3	3.0	3.6	3.1	2.6	2.8	4.7	3.8	3.3
6545/12	2.7	2.5	3.5	3.6	2.6	3.3	5.0	3.5	3.2
6545/26	3.1	2.6	3.7	3.4	2.9	3.4	4.8	3.5	2.6
6548/13	4.0	2.6	2.9	1.9	2.4	2.8	3.6	4.4	2.9
6548/24	3.7	3.0	2.1	2.8	2.8	3.7	4.2	4.7	3.0
6548/51	3.2	2.0	1.7	3.7	3.8	3.9	4.8	4.2	2.6
6549/24	4.0	2.9	3.1	2.6	2.5	2.6	5.0	2.9	3.2
6549/31	2.9	3.0	4.3	1.8	2.2	3.3	4.8	3.7	3.9
6549/99	2.8	2.4	2.2	2.1	3.4	2.8	4.7	4.0	3.4
6550/9	3.4	3.1	3.3	2.6	2.8	2.5	4.3	4.3	2.9
6551/38	3.0	3.6	3.9	2.7	3.2	2.7	4.9	4.4	2.0
6551/40	2.3	3.6	3.8	2.0	2.8	2.3	4.8	4.3	2.2
6553/25	3.3	3.2	2.7	2.3	2.9	1.9	4.7	4.1	2.9
6554/64	3.0	2.9	3.2	1.5	2.9	1.8	4.5	3.7	3.4
6556/29	3.9	2.9	2.5	3.0	2.6	2.9	4.9	3.9	2.8
6556/44	3.1	2.5	2.2	3.4	2.6	3.7	4.9	4.2	3.1
6558/29	3.5	1.7	3.1	2.2	2.2	4.1	5.0	4.5	3.2
6558/56	4.1	2.7	1.7	2.3	3.0	3.4	4.7	4.1	2.7
6558/64	3.1	2.6	3.2	2.1	2.5	3.5	4.4	4.6	2.6
6558/78	2.9	2.7	1.7	2.8	2.9	3.9	4.0	3.6	3.2
6558/83	2.2	2.3	3.1	2.3	2.1	3.3	4.8	4.7	3.3
6559/95	3.3	1.8	3.7	2.8	1.9	3.1	4.8	4.6	3.1
6564/30	2.2	3.0	2.5	3.1	3.1	2.9	4.6	4.7	2.7
6564/63	2.7	3.3	3.1	2.8	1.8	2.3	4.6	4.2	2.9
6564/87	3.2	3.3	3.3	2.8	2.1	2.7	4.6	4.1	3.5
Mean	**3.2**	**2.8**	**3.0**	**2.6**	**2.7**	**3.0**	**4.6**	**4.1**	**3.0**

Pick 1 was on 14th June and pick 19 was on 15th August in 2002. The earliest ripening selections were 6545/26, 6544/80, 6551/38 and 6551/40 while the latest selections were 6558/29, 6558/78 and 6558/83 (Table 10). The length of the picking season ranged from 21 days in selection 6551/40 to 36 days in selection 6558/78. Tulameen and Glen Ample were picked for 29 and 32 days, respectively.

Table 10. Dates at which 5%, 50% and 95% of total yields were picked for 2002 SF selections, sorted descending by 5% pick dates, earliest at the top.

Selection	5% Pick date	50% Pick date	95% Pick date
6545/26	15-Jun	27-Jun	11-Jul
6544/80	16-Jun	25-Jun	8-Jul
6551/38	17-Jun	28-Jun	10-Jul
6551/40	18-Jun	30-Jun	9-Jul
6564/30	19-Jun	3-Jul	17-Jul
6545/12	20-Jun	5-Jul	22-Jul
Glen Ample	21-Jun	6-Jul	23-Jul
6548/51	22-Jun	6-Jul	21-Jul
6549/31	22-Jun	4-Jul	21-Jul
Tulameen	23-Jun	5-Jul	22-Jul
6548/13	23-Jun	6-Jul	24-Jul
6558/64	23-Jun	8-Jul	25-Jul
6564/63	23-Jun	6-Jul	23-Jul
6559/95	24-Jun	7-Jul	28-Jul
6549/24	26-Jun	5-Jul	21-Jul
6548/24	27-Jun	11-Jul	27-Jul
6550/9	27-Jun	10-Jul	25-Jul
6553/25	27-Jun	9-Jul	24-Jul
6556/29	27-Jun	13-Jul	31-Jul
6556/44	27-Jun	11-Jul	1-Aug
6564/87	27-Jun	10-Jul	25-Jul
6554/64	30-Jun	11-Jul	27-Jul
6558/56	2-Jul	15-Jul	30-Jul
6549/99	3-Jul	13-Jul	26-Jul
6558/29	4-Jul	19-Jul	5-Aug
6558/78	5-Jul	24-Jul	10-Aug
6558/83	6-Jul	22-Jul	7-Aug

In the shelf life tests five selections had better punnet texture after 48 hours than Glen Ample, which had a mean score of 3.9, the best of which were selections 6558/83 and 6548/24, both with means scores of 4.2 (Table 11). The fruit for these five selections was considered almost as good as when just picked after 48 hours at 18°C (this is very good). However, nine selections had better punnet texture after 72 hours than Glen Ample did, which had a reduced mean score of 2.9, selection 6558/83 remaining on top with a score of 3.6 (Table 12). The selection with the worst punnet texture after both 48 and 72 hours was 6551/40, which had mean scores of 2.5 and 2.0, respectively. After 48 hours all selections appeared to have limited numbers of fruit with rots, only selection 6545/26 had more than five rotten fruit on average, with a mean score of 3.7 (Table 11). However, after 72 hours there were greater differences in rot scores between different selections. Selection 6558/83 had the best mean score for rots at 4.2 (on average less than five fruit per punnet had rots) after 72 hours

(Table 12). Selection 65551/40 had the worst score for rots at 2.0 (on average 41-80% of the fruit in each punnet had rots) after 72 hours. Fourteen of the selections had higher means scores for rots than Glen Ample, which had a score of 3.2 after 72 hours. All of the selections that had better scores for punnet texture than Glen Ample also had better rot scores suggesting that the two attributes are complimentary to one another. Tulameen had poorer scores than Glen Ample for all attributes after 48 and 72 hour except for brightness.

Table 11. Mean scores given to stage 0 selections after 48-hour shelf life, sorted in descending order of punnet texture.

Selection	Punnet texture	Redness	Brightness	Colour	Rots
6558/83	4.2	2.0	1.8	3.4	4.3
6548/24	4.2	3.2	3.0	2.5	4.4
6548/13	4.1	3.7	2.2	2.7	4.5
6558/78	4.1	2.8	2.7	3.3	4.7
6548/51	3.9	3.2	2.4	2.9	4.7
len Ample	3.9	3.0	2.9	2.9	4.4
6549/31	3.8	2.5	2.9	2.4	4.4
6558/64	3.7	3.0	2.7	3.2	4.6
6559/95	3.7	3.1	1.9	2.7	4.6
6550/9	3.6	3.1	2.6	2.8	4.3
6558/29	3.6	3.7	1.8	2.5	4.4
Tulameen	3.5	2.7	3.5	2.7	4.7
6545/12	3.5	2.2	2.0	2.4	4.3
6545/26	3.5	2.0	2.0	2.5	3.7
6558/56	3.5	4.5	2.2	3.0	4.9
6564/63	3.5	2.2	2.6	2.2	4.5
6553/25	3.4	3.3	2.9	2.3	4.5
6556/44	3.3	3.0	2.4	2.5	4.3
6549/24	3.2	4.1	3.1	2.6	4.1
6549/99	3.2	2.2	2.4	2.5	4.4
6554/64	3.2	2.8	2.3	2.0	4.5
6544/80	3.2	3.0	3.2	2.7	4.5
6551/38	3.2	2.2	3.3	2.3	4.2
6556/29	3.2	3.7	2.0	2.5	4.5
6564/30	3.1	1.3	3.1	2.2	4.4
6564/87	3.1	2.5	2.6	2.4	4.5
6551/40	2.5	2.0	3.5	2.0	4.0
Mean	**3.5**	**2.9**	**2.6**	**2.6**	**4.4**

Table 12. Mean scores given to stage 0 selections after 72-hour shelf life, sorted in descending order of punnet texture.

Selection	Punnet texture	Redness	Brightness	Colour	Rots	Fruit Texture
6558/83	3.6	1.0	1.4	3.0	4.2	2.9
6548/13	3.5	3.5	1.2	2.4	3.8	3.0
6558/78	3.2	2.3	2.2	2.4	3.8	3.2
6548/24	3.2	3.2	2.3	2.2	3.7	3.0
6558/29	3.2	3.5	1.5	2.1	3.7	3.6
6556/44	3.1	2.6	2.0	2.0	3.4	3.2
6558/64	3.1	2.3	2.2	3.0	3.6	3.1
6559/95	3.0	3.1	1.6	2.1	3.7	2.7
6548/51	2.9	3.1	1.9	2.5	4.0	3.6
Glen Ample	2.9	2.9	2.0	2.4	3.2	2.6
6549/31	2.8	2.0	2.3	1.8	3.1	2.8
6550/9	2.8	2.8	2.1	2.4	3.5	2.3
6553/25	2.8	3.2	2.0	1.6	2.9	2.0
6564/87	2.8	2.4	2.2	2.1	3.4	2.6
6549/99	2.7	1.8	2.0	2.0	3.2	3.0
6558/56	2.7	4.4	1.9	2.1	3.9	2.7
6545/12	2.7	2.4	1.3	2.2	2.9	2.6
6564/30	2.6	1.0	2.5	1.9	3.2	2.5
Tulameen	2.5	2.7	2.8	2.3	3.0	2.3
6551/38	2.5	1.7	3.2	2.0	2.7	2.5
6564/63	2.5	2.0	2.4	2.0	3.7	2.5
6549/24	2.4	3.6	2.2	2.1	3.0	2.4
6545/26	2.3	1.7	1.5	2.2	2.2	2.7
6556/29	2.3	3.4	1.9	2.2	3.0	2.0
6554/64	2.2	2.3	2.0	1.6	2.9	2.4
6544/80	2.0	3.0	1.8	2.3	3.5	2.3
6551/40	2.0	1.5	2.5	1.8	2.0	2.0
Mean	**2.8**	**2.6**	**2.0**	**2.2**	**3.3**	**2.7**

6.4 Discussion

The Raspberry breeder at HRI-EM, will study the raw and summarised Stage 0 data and decide what will be the next stage for each of the 25 selections picked in 2002. All aspects mentioned in this section plus the field records taken over the years will be taken into consideration in order to determine whether a selection is seen as a potential new variety. If a selection does go on to advanced trials at HRI or elsewhere, it is likely that it will have been chosen due to it having consistently moderate to good scores for every attribute as opposed to having outstanding scores for one or two attributes plus low scores for other attributes. Selections that do have good scores for all nine fruit characteristics examined, also have to have good shelf life and high yields of marketable fruit.

Selections which fail to meet all the criteria of a new variety may be used as parents in the future. Some selections picked in 2002 will be picked again in 2003 to confirm their potential.

The Stage 0 trials at HRI are not replicated and they are recorded by inexperienced students each year. Mean fruit weights and yield figures are reliable but any subjective scoring is less reliable. Flavour, texture and skin strength are particularly difficult to score and all the Stage 0 data is used in conjunction with the breeders records. Further replicated trials are needed of the most promising selections, preferably at more than one site, before the final decision can be made to name as a new variety.

ACKNOWLEDGEMENTS

I have received continual support throughout this six-month placement as Assistant Raspberry Breeder and would like to show gratitude to Vicky Knight for providing the opportunity to participate within the raspberry breeding programme at HRI-EM. The first hand experience I have gained under the supervision of Mrs Knight has been extremely beneficial to my University studies and will definitely aid me in obtaining success in future projects and career avenues. I am extremely grateful for the help received from many other members of staff at HRI-EM, Jesus Garcia-Alonso and Catherine Herrod in particular.

I would also like to acknowledge the Horticultural Development Council (HDC) for funding the placement which has enabled me to increase my knowledge in a number of areas of scientific research and has enabled me to improve my communication skills both within the work place and when writing scientific reports.

Appendix – Stage 0 fruit analysis guidelines

Table 1. Scoring guidelines for assessing nine attributes of fruit quality.

ATRIBUTE	SCALE					
1. Redness	Yellow/ Apricot 6	Pale 5	Fairly pale 4	Medium 3	Dark 2	Very dark 1
2. Brightness	Very bright 5	Bright 4	Medium 3	Dull 2	Very dull 1	
3. Shape	Long conical 5	Conical 4	Blunt conical 3	Roundish 2	Round 1	
4. Outline	Very even 5	Even 4	Medium 3	Irregular 2	Very irregular 1	
5. Uniformity of size	Very uniform 5	Uniform 4	Medium 3	Variable 2	Very variable 1	
6. Texture	Very firm 5	Firm 4	Medium 3	Soft 2	Very soft 1	
7. Cohesion	All whole 5	Mostly whole 4	Slightly crumbly 3	Crumbly 2	Very crumbly 1	
8. Skin strength	None broken. Strong 5	1 or 2/10 broken 4	3-5/10 broken. Moderate 3	6 or 7/10 broken 2	8-10/10 broken. Weak 1	
9. Flavour	Very good, aromatic, strong raspberry flavour 5	Good 4	Slightly acid, moderate, bland 3	Poor, acid, weak 2	Very poor, very acid, no raspberry flavour, foreign 1	

Note

Texture was assessed by holding a raspberry between the index finger and thumb and lightly squeezing, repeating with about five fruit to get a true assessment.

Skin strength was assessed by lightly rubbing the surface of a raspberry with the thumb five or six times, also repeating with about five fruit to get a true assessment.

Table 2. Scoring guidelines for assessing 48 and 72 hour shelf-life.

ATRIBUTE	SCALE					
1. Redness	Yellow/ apricot 6	Pale 5	Fairly pale 4	Medium 3	Dark 2	Very dark 1
2. Brightness	Very bright 5	Bright 4	Medium 3	Dull 2	Very dull 1	
3. Uniformity of colour within each fruit	Very uniform colour 5	Uniform colour 4	Slightly uneven colour 3	Uneven colour, slightly blotchy 2	Very uneven colour, very blotchy 1	
4. Texture of the whole punnet	As picked 5	Almost as good as just picked 4	Slightly collapsed, a few squashed fruit or a very slight amount of free juice 3	Moderately collapsed, several squashed fruit, sunk in punnet, slight amount of free juice 2	Very collapsed, sunk in punnet, lots of free juice 1	
5. Rots	No rots 0% 5	1-5 rots 2-10% 4	Several rots 11-40% 3	Many rots 41-80% 2	Almost whole punnet rotten 81-100% 1	
6. Texture of individual fruits	Very firm 5	Firm 4	Medium 3	Soft 2	Very soft 1	