FINAL REPORT (September 1996)

Project Leader: Mrs. V.H. Knight
Horticulture Research International
East Malling
West Malling
Kent ME19 6BJ

Project Co-ordinator: Mr. D. Heaton
Boundary Farm
Matthews Lane
Hadlow
Nr. Tonbridge
Kent TN11 0JH

Location of project: HRI East Malling

Date project commenced: April 1994

Date project completed: September 1996

Key words: raspberry
breeding
trials
selections

Project No: **SF8a**
Title: **Breeding late summer and primocane raspberries**

Report of sandwich student, L. Jewsbury,
April - September 1996

CONTENTS

1. INTRODUCTION

1.1 HRI-EAST MALLING

In the early part of the century the benefits to agriculture from the Rothamsted Experimental Station were acknowledged by growers in the South East. The necessity for a local research station was discussed and in 1913 the Wye College Fruit Experiment Station was established at East Malling. Over the years the site has expanded greatly, from the original 23 acres to 240 hectares at present. With this has been a corresponding increase in the range of crops studied and scientific techniques involved.

Horticultural research and development has seen much restructuring in the past 20 years, not least with the formation of Horticulture Research International (HRI). In 1991 East Malling Research Station was integrated with six other research stations in the country to form HRI, with the headquarters based at Wellsbourne. Today HRI consists of six of the original seven research centres and is renowned world wide for achievements in many areas of research. The management structure of HRI is shown in fig. 1.

HRI's aim is to make advances in plant, animal and microbial science of relevance to horticulture and provide the U.K. horticultural industry with the benefits via technology transfer.

For this to be achieved, research projects require flexibility to adapt to changes in priorities of its customers whilst a link is maintained between all sectors of R and D to ensure rapid transfer of new advances.

HRI East Malling covers several areas of research, mainly top fruit (apples, pears and cherries), soft fruit (strawberries and raspberries), farm woodlands, woody ornamentals and hops.

Funding for research projects comes primarily from the Ministry of Agriculture, Fisheries and Food (MAFF), the Biotechnology and Biological Sciences Research Council (BBSRC) and other research and development councils. The horticultural industry, both U.K. and abroad, also contributes to the funding of research projects to varying degrees. In particular the raspberry breeding programme receives funding from MAFF, the East Malling Trustees and the Horticultural Development Council (HDC).

The Plant Breeding and Biotechnology Dept. at E. Malling runs several breeding programmes, including the raspberry programme. The HDC provide funding for a sandwich student to work on the raspberry breeding programme, under the supervision of Mrs. V H Knight, the project leader. All aspects of the breeding programme are undertaken by the sandwich student, including laboratory techniques and field work.

1.2 THE RASPBERRY

The genus *Rubus* is one of the most diverse genera of the plant kingdom. Raspberries, subgenus *Idaeobatus*, are distinguished from other species by the ability of the mature fruit to separate from the receptacle. The fruit is an aggregate of individual drupelets rather than a true berry, with each drupelet containing one seed. The most widely grown *Rubus* species is *R.idaeus.*

There are two fruiting types of *R.idaeus*, summer fruiting and primocane fruiting. The summer fruiting varieties produce only vegetative canes in their first year. The canes remain dormant throughout winter, flowering and bearing fruit in their second year. New vegetative canes appear during the cropping season and persist over winter as the old cane dies. The cycle continues as the new canes bear fruit the following year.

Primocane fruiting varieties produce new canes each year with the ability to fruit in their first season. The canes can be cut down at the end of the fruiting season, reducing maintenance costs for the growers during the winter season.

The cropping seasons of the two varieties vary. The season for summer fruiting types is between June and August. The primocane types fruit between August and October. By combining the two types in one plantation, high fruit yields can be obtained throughout the season.

1.3 THE RASPBERRY BREEDING PROGRAMME

Breeding objectives:

- Improved yield
- High quality fruit
- Improved plant habit
- Extension of season
- Resistance to pests and diseases

HORTICULTURAL RESEARCH INTERNATIONAL - Managerial Departments

Board of Directors
Chairman: Mr. G.T. Pryce

HRI Head Office, Wellesbourne:
Chief Executive: Prof. C.C. Payne
Company Secretary, Director of Finance: Mr. T.G. Heller
Director of Research Strategy: Prof. H.G. Jones
Director of External Affairs: Dr. J.J.M. Flegg

The HRI-sites with its Site Directors:

Wellesbourne	East-Malling Wye	Efford Kirton Stockbridge
Dr. I.R. Crute	Dr. A.R. Thompson	Dr. M.R. Shipway

HRI-East Malling:

Non-Science				Science			East-Malling
Administration	Commercial & Information Serv.	Horticultural Services	Facilities	Plant Breeding & Biotechnology	Entomology & Plant Pathology	Crop Science	Trust for Research
Mrs. A.M. Raffan	Mr. R. Newham	Mr. N.J. Osborne	Mr. B.M. Roberts	Dr. D.W. Simpson	Dr. M.F. Clark	Dr. B.H. Howard	Mr. A.F. Todd
Personell Accounts Canteen Cleaners	Liaison Photography Graphics Computing Library	Field- , Nursery- and Glasshouse services	Technical serv. (electricity and buildings)	Plant Breeding Biotechnology Hop (Wye)	Entomology Plant Pathology	Post Harvest Propagation Crop Physiology	Owners of the site

2. GERMINATION

2.1 INTRODUCTION

An investigation into the effect of two seed treatments on germination was included in the 1996 programme for all seed produced from the previous year's controlled crosses. The 1996 results and results from previous years will determine the future use of seed treatments. Included in the germination records were the numbers of spineless seedlings, which can be identified at the cotyledonary stage. As spinelessness is a characteristic desired by growers, seedlings without spines were potted in preference to those with spines. If germination was good enough, the spiny seedlings were removed from the seed tray with forceps and only spineless seedlings were allowed to grow.

2.2 METHOD

The seeds produced from the 1995 crosses received two different seed treatments per family (see Appendix 1), with twenty five families produced in total. Each family was allocated two sets of seed trays, labelled with different coloured tape to identify the seed treatment, and the seeds sown in the appropriate trays during January 1996. The seed trays were placed in the cold store, maintained at 4°C, for approx. two months.

The number of seedlings germinated per family were recorded for both seed treatments, with the number of spiny and spineless seedlings recorded in segregating progenies. Seedlings were counted at 3-4 day intervals, beginning 10 days after the

seed trays were moved from the cold store to the glasshouse. Accumulative counts were made for each seed tray. Records up to 38 days after transferal were made for the first batch of families and up to 28 days for the second batch.

Spinelessness was determined by the absence of glands on the cotyledons. The numbers of both spiny and spineless individuals were recorded each time for 7 out of the 16 families that were segregating, as the spiny seedlings were removed. In the other 9 segregating families germination was insufficient to discard the spiny individuals and only the total number germinated was recorded.

2.3 RESULTS

TABLE 2.a. Number of Germinated Seeds per Seed Treatment

Number of seeds per treatment	19395
Number of seeds germinated from bleach treatment	1938
Number of seeds germinated from acid treatment	3038

The germination results are based on records taken 28 days after the seed trays were removed from the cold store and transferred to the glasshouse.
Although the level of germination was higher from the acid treatment in most progenies, there were 2 progenies where the higher germination was from the bleach treatment and 6 progenies where the germination rates were very similar from both treatments.

2.4 DISCUSSION

The results show a higher germination from the acid treatment in 15 out of the 23 progenies, suggesting this to be a preferable treatment. Each year the progenies are genetically different and it is difficult to make universal recommendations for seed treatment in the future. However the results in 1996 are in agreement with the previous 3 years, and treating raspberry seed with acid has improved the germination rate in the majority of progenies in all 4 years.

Seven families produced sufficient numbers of spineless individuals for them to be potted in preference to those with spines, though at this stage all families contained some spiny seedlings.

3. SCREENING FOR GENETIC RESISTANCE TO *AMPHOROPHORA IDAEI*

3.1 INTRODUCTION

The large raspberry aphid, *Amphorophora idaei*, is commonly found in raspberry plantations in Britain. The aphid itself causes little or no damage to the plant but acts as a vector for four viruses;

- Black raspberry necrosis virus (BRNV)
- Raspberry leaf spot virus (RLSV)
- Raspberry leaf mottle virus (RLMV)
- Rubus yellow net virus (RYNV)

Direct virus control by chemical means is not possible, therefore alternatives must be sought. Three possible strategies are:
i)chemical control of the vector ii)breeding for resistance to all four viruses iii)breeding for resistance to the vector. Chemical control of the aphid is difficult as aphid infestation coincides with the flowering/fruiting period of the raspberry. Therefore, as four viruses are transmitted by one organism, the most appropriate approach is breeding for resistance to the aphid.

In early spring the aphid eggs hatch as the new leaves begin appearing on the raspberry canes. During spring and summer reproduction is asexual and live young are produced, mainly of wingless form. Development from 1st instar to adult is usually complete in 8-16 days. Colonies can easily be found on susceptible plants, usually on the stem and underside of new leaves. With reduced day length in autumn, winged males and sexual females are produced for sexual reproduction. In October the females deposit their eggs on primocanes to overwinter.

There are four known strains of *A.idaei* with strain 1 the most widespread and strain 4 quite rare. Genes for resistance to the aphid were identified over 30 years ago at East Malling and have proved invaluable to breeding programmes since. Some resistant genes provide resistance to specific strains whereas others, specifically A_{L518}, A_{10}, A_{K4a} and A_{cor1}, provide resistance to all known strains and are the most useful.

TABLE. 3a. Plant Resistance Genes in Relation to Strains of *Amphorophora idaei*

Genes	Origin	strain 1	strain 2	strain 3	strain 4
A_1	"BAUMFORTH A" (R.idaeus)	R	S	R	S
A_2	"CHIEF"(R.idaeus)	S	R	R	S
A_1 A_3	"CHIEF"(R.idaeus)	R	R	R	S
A_3 A_4	"CHIEF"(R.idaeus)	S	R	S	S
A_5	"CHIEF"(R.idaeus)	R	S	S	S
A_6	"CHIEF"(R.idaeus)	R	S	S	S
A_7	"CHIEF"(R.idaeus)	R	S	S	S
A_{L518}	L518 R.idaeus strigosus	R	R	R	R
A_{10}	"CUMBERLAND" (R.occidentalis)	R	R	R	R
A_{K4a}	"KLON 4a"(R.idaeus)	R	R	R	R
A_{cor1}	R.coreanus	R	R	R	R
A_{cor2}	R.coreanus	?	R	S	?

3.2 METHOD

Part of the protocol for the HRI-EM raspberry breeding programme is to screen all progeny from controlled crosses for aphid resistance. All susceptible seedlings are excluded and only seedlings classified as resistant will continue in the programme.

The parents used for the 1995 raspberry crosses were mainly heterozygous for A_{10} and/or A_{L518}.

In March the first eleven families were transferred to a glasshouse for germination at 22-24°C. Germination of the remaining families was deferred for one month in order to prevent the seedlings from growing too tall before inoculation. This measure was in response to the high number of seedlings lost in 1995 due to stem breakage during handling.

On reaching sufficient size, the seedlings were individually potted in 3" diameter pots, selecting for spinelessness where possible. It was intended to pot 150-200 per family but a few families had lower numbers, due to poor seed production and/or poor germination. In early April, a small number of *Amphorophora idaei* raised on Malling Landmark were received from the Scottish Crop Research Institute (SCRI), Dundee. M.Landmark pot plants, also maintained in the glasshouse, were used to culture the aphids whilst the seedlings were growing. As M.Landmark has the gene A_1 it is resistant to strains 1 and 3, and any aphids which thrive on it are strain 2 or 4, most likely to be strain 2. Aphid screening was undertaken using strain 2 in order to distinguish between seedlings carrying A_1 (susceptible to strain 2) and those carrying A_{10}, A_{L518} or A_{K4a} (resistant to strain 2).

Inoculation of the seedlings with *A. idaei* began in late April, with the tallest seedlings being inoculated first. A metal scoop was used to place 3 aphids on the youngest, fully expanded leaves of each plant, ensuring that only adult aphids were used. Adults were distinguished from the 4th instars by the presence of a reproductive spur on the abdomen. If used, immature aphids may not have reached the reproductive stage before the plants were recorded, possibly giving a false result.

The plants were recorded for resistance 3-4 days after inoculation. The presence of ≤ 2 aphids on a seedling, adults or nymphs, indicated resistance, whilst ≥ 8 aphids indicated susceptibility. Seedlings with 2-8 aphids were classed as intermediate and inoculated with one more aphid. Intermediates received an extra aphid up to two more times before a decision was made on their resistance status.

Susceptible seedlings were used as aphid stock plants during the inoculation period. On completion of inoculation all susceptible seedlings were disposed of, with only the Malling Landmark plants being retained. Resistant seedlings were maintained in the glasshouse until raspberry bushy dwarf virus testing was completed prior to being transferred to a roofless polytunnel, in trays labelled with the family number. The seedlings remained in the polytunnel for one-two months for hardening off before being planted in the field.

3.3 RESULTS

TABLE 3.b Numbers of Resistant and Susceptible Seedlings in each Family.

Family	Possible Resistant Genes of Parents	Number Potted	Number Res.	Number Sus.
6581	.x A_{10}	168	73	92
6582	.x A_{10}	91	10	88
6583	A_{10} A_{L518} .x A_{10} A_{L518}	200	68	126
6584	A_{10} A_{L518} .x A_{10} A_{L518}	200	87	114
6586	A_{10} .x A_{10} A_{L518}	150	69	82
6587	A_{10} A_{L518} .x A_{10}	150	107	42
6588	A_{10} .x A_{10}	150	82	60
6589	A_{10} .x A_{10}	150	86	60
6590	A_{10} .x A_{10} A_{L518}	200	155	38
6591	A_{10} A_{L518} .x A_{10}	200	156	33
6592	A_{10} .x A_{10}	200	98	88
6593	A_{10} .x A_{10}	150	121	19
6594	A_{10} .x A_{10}	150	111	38
6595	A_{10} .x A_{10}	150	126	14
6596	A_1 .x A_{10}	188	83	105
6597	A_1 or A_{10} .x A_{10}	150	108	37
6598	A_{10} .x A_{10}	150	132	16
6598/99	A_{10} .x A_{10}	100	73	17
6599	A_{10} .x A_{10}	74	69	2
6600	A_{10} .x A_{10}	150	119	7
6601	A_{10} .x A_{10}	51	50	1
6602	A_{10} .x A_{10}	141	102	23
6603	.x A_{10}	58	2	56
6604	.x A_{10}	73	48	25
6605	.x A_{10}	200	159	36

Out of a total of 3613 seedlings potted, 2294 were resistant to strain 2 of *A.idaei* and were retained to be planted for further selection, while 1224 were susceptible and discarded.

Fig. 3 Colony of *A.idaei* on a Susceptible Seedling

Fig. 3.a PIE CHART SUMMARY OF THE 1996 SEEDLING RESISTANCE TO A.idaei

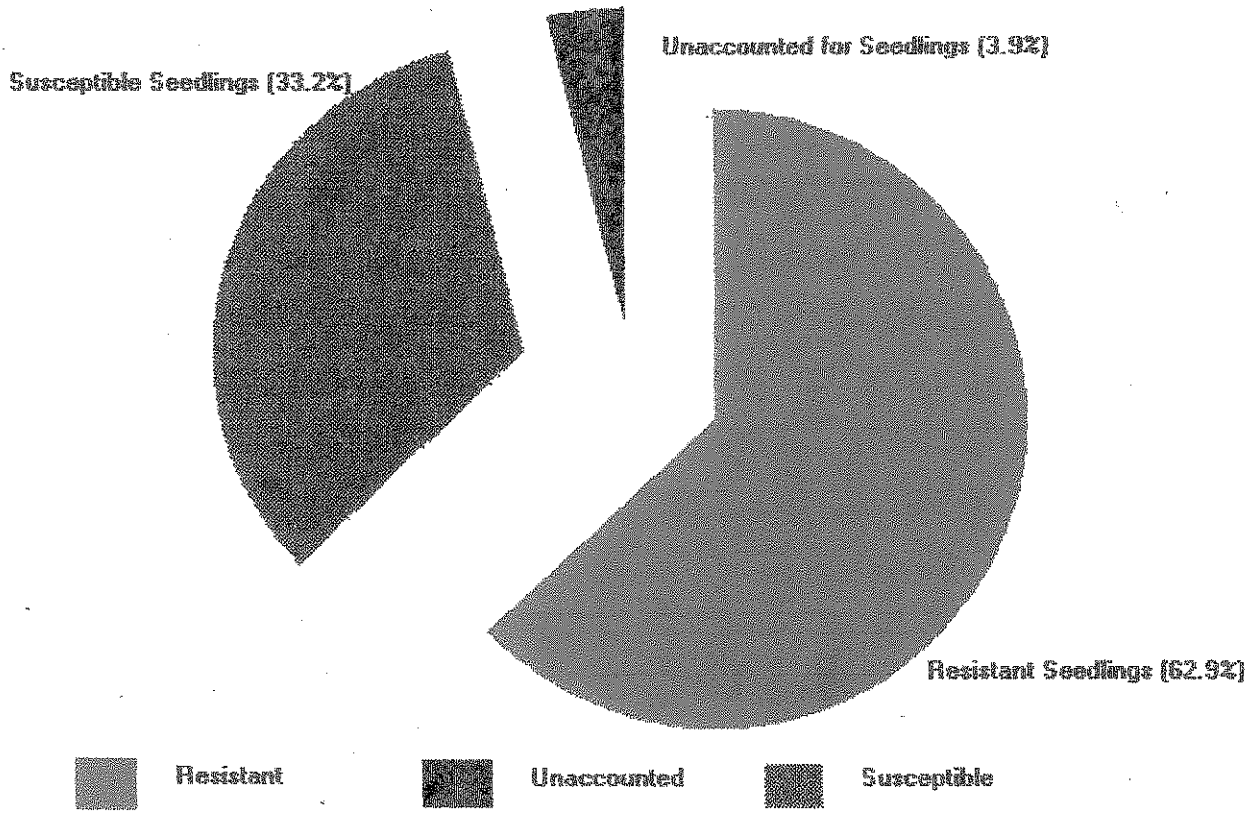
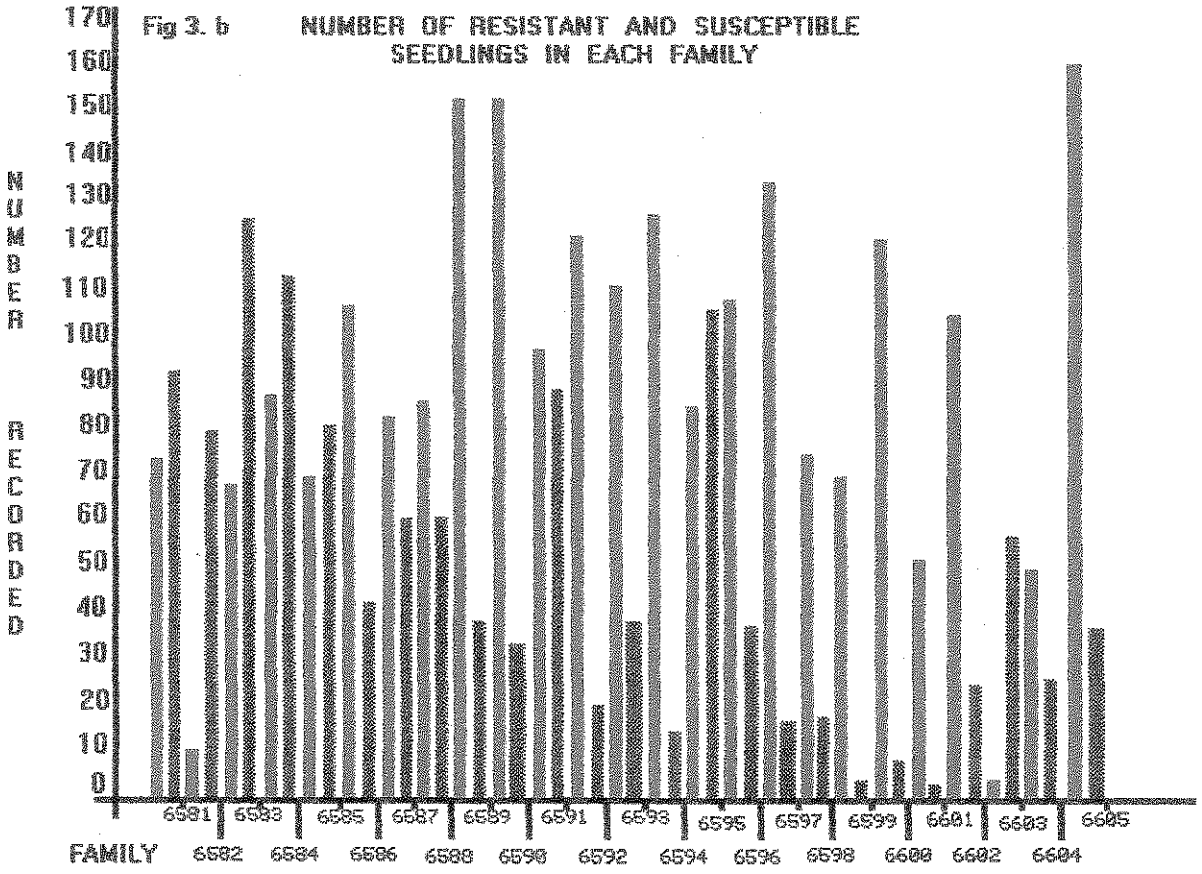


Fig. 3. b NUMBER OF RESISTANT AND SUSCEPTIBLE SEEDLINGS IN EACH FAMILY



3.4 DISCUSSION

As resistance to *A.idaei* is a dominant characteristic, parents that are both heterozygous for resistance would give rise to progeny segregating 3:1 resistant to susceptible. Likewise, progeny segregating 1:1 resistant to susceptible would be produced where only one parent carried a resistant gene.

The results showed 14 families, 6587, 6590 - 6595, 6597 - 6602 and 6605, segregating with a ratio of 3:1 or higher, suggesting both male and female parentage was heterozygous for resistance. The resistant progeny of these families can be classified as heterozygous for A_{10} and heterozygous for $A_{10} \pm A_{L518}$ in most cases, with the possibility of a proportion of seedlings being homozygous where both parents carried the same resistant gene.

Eight families segregated with a ratio of 1:1 suggesting heterozygosity of one parent and resistant seedlings to be heterozygous for resistance.

Three families, 6582, 6583 and 6603, had an unexpectedly high proportion of susceptible seedlings. This may be due to the misclassification of the supposedly resistant parents (possibly having A_1 instead of A_{10}), misclassification of the seedlings, lower germination or survival among aphid resistant seedlings, or a possible breakdown of resistance for reasons unknown.

The number of seedlings unaccounted for in 1996 was much lower than in 1995 (141 and 901, respectively). The majority of these were lost due to stem breakage during handling, but the number lost in this manner was greatly reduced by staggering germination. Several seedlings died due to insufficient watering, but it is

extremely difficult to check each pot and it may not be possible to find a workable solution to this problem.

Difficulty in classification of resistant and susceptible seedlings was experienced with the first batch to germinate as many appeared intermediate continuously. Few of those classified as susceptible had good colonies of aphids and, on the whole, the aphids were not thriving. The second batch to germinate exhibited strong susceptibility/resistance making classification much easier. One possibility for the difference was the inclusion of SusCon green, an insecticide routinely used to control vine weevil which was mistakenly added to the potting compost of the first batch, which could have adversely affected the aphids. However, the majority of families in the second batch were maintained in different glasshouses and though conditions were similar they were not necessarily the same, which may also have affected the aphids. Similarly the first batch were potted in mid April and inoculated between 25 April and 10 June, while the second batch were potted during the second week of May and inoculated between 7 June and 2 July, and again there may have been subtle differences in conditions which influenced aphid development. SusCon green is reported not to have any affect on aphids and the insecticide cannot be blamed implicitly, but ensuring SusCon green is not added to the potting compost in future may assist aphid screening.

4. RASPBERRY BUSHY DWARF VIRUS

4.1 INTRODUCTION

There are at least two isolates of the Raspberry Bushy Dwarf Virus (RBDV), the Scottish isolate (RBDV-S) and resistance-breaking (RBDV-RB). A single gene, *Bu*, confers resistance to RBDV-S and a number of important cultivars carry this gene. However, the gene does not provide resistance to RBDV-RB and an increasing number of raspberry canes in England have become infected with the virus.

The pollen-borne virus has the potential to rapidly spread through a plantation during the flowering season. Not only will the pollinated plant become infected, but also a proportion of the fertilized seeds. Symptoms of the virus may manifest as vivid leaf chlorosis; reduced fruit yield from drupelet abortion (crumbly fruit); loss of vigour including cane height and diameter, fruit size and number of emerging primocanes and floricanes. The lower fruit yield together with a high percentage of unmarketable crumbly fruit is commercially very damaging.

However, the symptoms are not reliable indicators of RBDV as they can be caused by other factors i.e. chlorosis caused by herbicide damage. Also, many infected raspberry canes express no obvious symptoms, making visual detection of the virus difficult.

Control of a pollen-borne virus is almost impossible in the field. It would require restriction of bee movements - the main pollinator, and/or prevention of flowering. Either method inhibits fruit production. Therefore breeding for resistance is a preferable approach. Unfortunately past investigations in this area have shown

differences between immunity to graft-inoculation and immunity to natural pollen-infection. Screening for resistance via pollen-infection would be more relevant but to undertake such a project would be extremely difficult without putting all the breeding material at risk. Therefore, the present raspberry breeding programme relies on early detection and destruction of infected canes.

For many years, RBDV at East Malling has been detected using the Enzyme Linked Immunosorbent Assay (ELISA). ELISA is favoured for being a generally reliable technique which can process several hundred samples in the course of one week. The technique is based on serology - a rabbit inoculated with proteinaceous receptors, in this case the plant virus, will form specific antibodies and an anti-serum can be produced. A sample of plant sap added to the anti-serum will result in precipitation if the virus is present. The principles of the ELISA technique are outlined in Fig. 4.b.

Fig. 4.a Leaf Chlorosis of Infected Raspberry Cane

4.2c The ELISA Test

Up to 45 samples were included, in duplicate, on one microwell plate, with 2 positive controls and 1 negative, again in duplicate.

See Appendix 2 for solutions used during the ELISA test.

100µl of a coating solution was added to each well of the microwell plate. This contained an agent, F(ab)2, which enabled the virus to adhere to the plate. The plate was placed in an incubator, at 30° C, for 4-5 hours.

7.5ml of grinding buffer was added to labelled tubes, corresponding to the sample number. Pestle and mortars were used to grind 0.15g ± 0.02g of leaf tissue per sample to a paste with a little grinding buffer. The remaining buffer was added to the mortar, mixed with the paste and returned to the appropriate tube.

After the incubation period the solution in the microwell plate was expelled and the plate washed 3 times with PBSTween, for 3 minutes each time. 100µl of sample was added to the corresponding wells of the plate and the plate stored in the fridge overnight.

The following morning the sample solutions were expelled and the plate again washed with PBSTween. 100µl of a solution containing Immunoglobulin (IgG), an RBDV-specific antibody, was added to each well and the plate incubated for 3-4 hours at 30° C. The IgG would attach to the virus if it was present in the well. The plate was again washed after the incubation period.

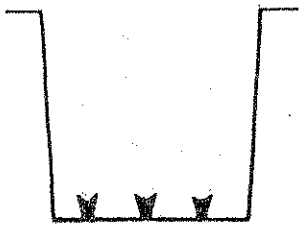
100µl of Protein A solution was added to each well. The solution contained Protein A-horse radish peroxidase (HRP) conjugate, an enzyme that recognises IgG and will adhere to it if present. The plate was incubated for 3-4 hours at 30°C.

After the incubation period the plate was washed and 100µl of a solution containing tetramethylbenzadine (TMB) substrate added. The TMB substrate reacts with the HRP conjugate producing a blue solution. The plate was left for 20 minutes to allow time for the TMB to react fully. Wells containing a blue solution were recorded as RBDV +ve. After 20 minutes the reaction was stopped with 25µl of 15% acid solution and quantitative readings taken of the reaction using a Bio-Rad Microplate Reader.

All plants that tested positive were destroyed and in selections where the majority of plants were positive, all plants from the selection were destroyed.

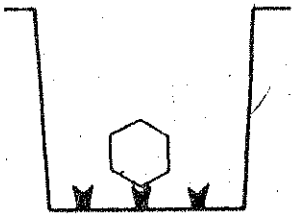
Principle of the ELISA technique

1. Specific antibody adsorbed to plate



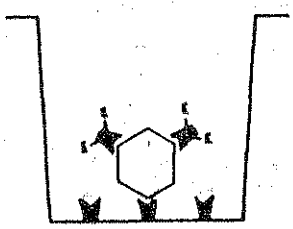
Wash

2. Add test sample containing virus



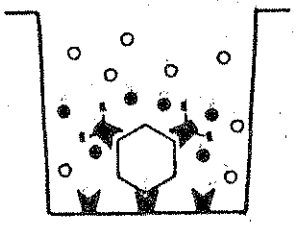
Wash

3. Add enzyme-labelled specific antibody



Wash

4. Add enzyme substrate



Colour intensity $\propto$ virus concentration

4.3 RESULTS

TABLE 4.a Number of Plants Which Tested RBDV Positive from Each Group in 1996

Group	Number Tested	Number Positive	% of Positives
Selections propagated 1994/95	507	387	76
Possible parents	375	55	15
HDC trial plants	917	113	12
Aphid resistant plants	910	13	1
Selections propagated 1995/96	790	81	10
Seedlings for propagation 1996/97	179	10	6

4.4 DISCUSSION

A very high percentage of the 1994/95 Primocane fruiting propagated selections tested positive for RBDV. This was probably due to the plants having been in the field for the whole of one flowering season and, in some cases, part of a second flowering season before being tested. On propagation the number of RBDV +ve plants would have been much lower, but as they remained in the field for over a year, the virus had time to spread to susceptible neighbours. It also suggests that, although the ELISA test failed in the hot summer of 1995, virus transmission had been extremely rapid.

The number of RBDV +ve plants in this group was much higher than in the 1995/96 propagated selections. This shows the importance of testing the plants in their first growing season and destroying all positives to reduce the spread of RBDV.

Testing plants to be used as parents is obviously important and it can be seen that, with 15% testing positive, avoiding using these plants prevents direct contribution to the spread of RBDV. However, a very small number of aphid resistant seedlings from the 1995 controlled crosses tested positive. This confirms that parents can pass the virus to their offspring and that ELISA testing cannot explicitly state that a plant is virus free. The test can only show that at the time of testing, the actual leaves sampled either did not contain RBDV or the concentration was too low to be detected.

5. THE CROSSING PROGRAMME

5.1 INTRODUCTION

Commercial raspberry growers require ever improving qualities of new cultivars, both in performance of the plant and in the marketability of the fruit. Performance of the plant includes resistance to pests and diseases, good cane health and strong laterals, spinelessness and well presented, easily picked fruit. Fruit qualities include size, colour, flavour, texture and shelf life performance. An extension of the traditional cropping season, by production of earlier and later fruiting varieties that can be grown in sequence with mid-season varieties, is also highly desired.

The raspberry breeding programme at East Malling is based on recurrent selection. Cultivars with favourable characteristics are intercrossed with the aim of producing hybrids which express a combination of the characteristics. In order to select the most suitable parents, knowledge of the selections and their characteristics is obviously required. Each year plants are assessed in the field and fruit from some plants is assessed in the laboratory. Plants in the field are assessed for yield, season, number of canes, strength of laterals, ease of plugging and fruit quality. Harvested fruit is assessed for fruit size, colour, taste, shelf life performance etc.

Selections do not necessarily have both types of assessment each year, but records are used to decide on the best parents for particular qualities.

5.2 METHOD

In 1996 33 controlled crosses of summer fruiting selections were carried out. The reasons behind each cross are outlined in Table 5.a.

TABLE 5.a The Main Objectives of the 1996 Crosses

Families	**Objectives**
6611 - 6620	To improve fruit quality in early ripening types
6621 - 6625	To improve plant habit and fruit texture for mechanical harvesting
6626 - 6629	To improve yield and fruit quality of mid/late season types
6630 - 6634	To increase fruit size of mid/late season types
6635 - 6636	To extend season for extra late ripening
6638 - 6639	To increase crop potential by encouraging floriferous laterals
6640 - 6643	Intercrossing selections with promising response to RBDV for possible resistance

Emasculation of the Female Parent

All crosses must be controlled, therefore emasculation of the female parent was essential to prevent self-pollination. Flowering laterals were chosen which were well attached to the cane and that looked strong enough to withstand the effects of wind and rain. Approx. 30 mature, unopened buds were required for each cross, with 5-10 buds on 3-5 laterals used per cross. All leaves, immature buds and open flowers were removed from the selected lateral. A circular incision round the base of the bud, just above the receptacle, was made using a scalpel sterilized in Industrial Methylated Spirit (IMS). This exposed the immature stigmas whilst removing the sepals, petals and stamens. The emasculated lateral was labelled with a tag bearing the date of emasculation, the identity of the female parent and the number of buds emasculated. A cloth pollinating bag was placed over the lateral and secured with wire. Laterals were left for 3-4 days to allow the stigmas to mature and become receptive to pollen.

Preparation of the Male Parent

The main method used for obtaining pollen was to select 4-5 laterals on the male plant which had several unopened, but near to flowering, buds. A lateral with 1-2 open flowers was a good indicator that the other buds were near to flowering. All open flowers and leaves were removed and the lateral covered by a thin perforated polythene bag, to prevent bees from contaminating the buds once open.
The laterals were left for 3-4 days, allowing the flowers to open and anthers to dehisce. After this period the flowers were removed, placed in labelled tubes and taken to the female plant for pollination.

The other method for obtaining pollen was used when the flowering of the male and female plants did not coincide. Mature, unopen buds were collected from the male plant and taken back to the laboratory. Fine forceps, sterilized in IMS, were used to remove the sepals and petals and collect the undehisced anthers into a petri dish. The anthers were left overnight at room temperature to dehisce, then stored in a desiccator in the fridge until needed.

Pollination

To pollinate the female plant, either the anthers of the male flowers were brushed against the receptive stigmas, or the stigmas dipped into the petri dish of lightly shaken, dehisced anthers. The process was repeated 2-3 times at an interval of 3-4 days. The procedure had to be carried out fairly quickly and the bag replaced on the lateral to prevent contamination by bees, which occur in large numbers in raspberry plantations during the main flowering period. After the first pollination the identity of the male parent was added to the tag on the female lateral.

Collection of the Ripe Fruit

The fruit was ready for collection 4-6 weeks from the final pollination. The pollinating bags were carefully removed and the fruit collected into a labelled container. At this stage the family number was used rather than the identity of both parents. Records were made of the number of well set fruit, poorly set fruit and number of flowers that failed to set for each cross.

Seed Extraction

Great care was taken throughout this procedure to ensure fruit or seed from different crosses did not become mixed. The fruit was placed in a blender with some water, agitated for 10 seconds and allowed to settle. The process was repeated, agitating for 5 seconds. The viable seeds sank to the bottom of the flask and the unfertilised seed and pulp floated, forming a distinct layer. The top layer was decanted off, leaving the viable seed in the flask. These were transferred to a container with a 10% bleach solution, left for 2 minutes then thoroughly rinsed in running water. The seeds were placed on a sheet of filter paper, labelled with the family number, and left to dry overnight. The following morning the seeds were placed in a labelled, waxed paper packet and transferred to the fridge.

The seeds were weighed at a later date, with the weight of 100 seeds and weight of the total amount of seed recorded. The estimated number of seed obtained from each cross was calculated from these figures.

The seeds will remain in the fridge until January 1997, when they will be treated before sowing in seed trays and transferred to the cold store.

5.3 RESULTS.

Seed was obtained from all 33 of the 1996 crosses.

TABLE 5.b Pollination Records from a Sample of Seven of the 1996 Controlled crosses

Family	Date Emas.	No. Flowers Emas.	No. Fruit Set Well	No. Fruit Set <10 Drupelets	No. Flowers Failed to Set	Est. No. Seed
6613	31/5	35	35			2180
6614	3/6	27	8	2	17	256*
6620	3/6	29	29			1669
6624	10/6	31	8	5	18	343*
6627	17/6	32	17	6	9	879
6637	20/6	34	33		1	3897
6642	10/6	24	22	1	1	971

* Actual seed number

Table 5.b shows the results obtained for a sample of seven families in the 1996 crossing programme. The different levels of success of the crosses can be seen in the Table. Families 6613 and 6620 were completely successful, whilst families 6614 and 6624 had poor results. Overall, in 5 out of 33 families $\leq$ 55% of the number of flowers emasculated set well, producing less than 1000 seed (estimated figures). The remaining 28 families were, reasonably successful, with an estimated seed number between 1000 and 3897.

5.4 DISCUSSION

The main reason for lost fruit/ failure of flowers to set was broken laterals or dead canes. In family 6640 one pollinating bag was found to be infested with grubs, which may have affected the seed count from the bag. Family 6642 had a high percentage of flowers set, but produced a fairly low number of seed (est. 971). This family had the lowest number of flowers emasculated, due to difficulty in finding suitable laterals, which probably contributed to the low seed count. However, the fruit produced may have been small with a low number of drupelets per fruit and, hence, a low seed number per fruit. Family 6627 had only 55% of flowers set, with no record of dead laterals. Reasons for this may have been poor pollinating techniques, low number of viable pollen grains or receptive stigmas or inherent incompatibility of the parents.

In general the 1996 crossing programme was successful, producing good seed numbers for most families with no crosses failing completely. Success in terms of germination will have to be seen in 1997 and success in terms of fruit quality will not be seen until 1999, emphasising the need for a long-term outlook when designing a crossing programme.

6. STAGE 0 TRIALS

6.1 INTRODUCTION

Selections that have exhibited particularly good characteristics during field assessments, in terms of yield, fruit quality and plant habit, are chosen for inclusion in the stage 0 trials. Separate trials are carried out each year for summer fruiting and primocane fruiting varieties, with 25-50 selections included in each. The trials record yield, fruiting period and fruit quality characteristics. Shelf life tests are also carried out.

Promising selections are included for assessment in stage 0 trials at least twice over a three year period. After analysis of the data, the best selections may be submitted for inclusion in the stage 1 trials, undertaken on commercial plantations at other locations in the country. Of the 80 or so selections assessed during the 3 year period, less than 10 may be selected for the stage 1 trials, ensuring only the most promising selections go forward.

6.2 METHOD

For each selection 1.5 - 2.5m of row was measured and marked with tape, from which the fruit was to be harvested. The majority of selections are present in the field as single plots, approx. 5.0m, but fruit picking is very time consuming and it is necessary to reduce the harvesting area to a more manageable size. Fruit was harvested twice weekly, from the first ripe fruit until there were less than 10 fruit per selection. Fruit was divided into marketable and unmarketable as it was picked and the weight recorded for each. The weight of 50 marketable fruit was also recorded. The unmarketable fruit was discarded, whilst the marketable was assessed for 9 quality characteristics, on a scale of 1-5 with 5 being the most desirable. The quality characteristics are shown in Table 6.a. Quality assessment was abandoned for a selection if there were less than 20 marketable fruit.

Two types of shelf life assessment were carried out on marketable fruit each week. The first type of assessment involved fruit individually laid out on a tray in rows of 10 or 20, with 3 replicates for each selection. The trays were incubated at 18°C and 90% relative humidity for 72 hours. After the incubation period, the fruit was examined for fungal fruit rots (*Botrytis cinerea, Cladosporium, Alternaria, Mucor/Rhizopus* and *Penicillium*) and the numbers of fruit infected with each type recorded per selection.

The second shelf life test involved punnets of fruit. When selections provided enough marketable fruit, three x 3/4 full punnets were placed on separate trays and again incubated at 18°C and 90% relative humidity. The whole punnets were graded on a 1-5 scale for rots and texture after 24, 48 and 96 hours.

TABLE 6.a Fruit Quality Grades on a 1-5 Scale

Character-istics	1	2	3	4	5
Redness	Very Dark	Dark	Medium	Pale	Fairly Pale
Brightness	Very Dull	Dull	Medium	Bright	Very Bright
Shape	Round	Roundish	Blunt Conical	Conical	Long Conical
Outline	Very Irregular	Irregular	Medium	Even	Very Even
Uniformity of Size	Very Variable	Variable	Medium	Uniform	Very Uniform
Texture	Very Soft	Soft	Medium	Firm	Very Firm
Cohesion	Very Crumbly	Crumbly	Slightly Crumbly	Mostly Whole	All Whole
Skin Strength	Weak		Medium		Strong
Flavour	Very Poor, Very Acid, No Raspberry Flavour, Foreign	Poor, Acid, Weak	Moderate, Bland	Good	Very Good, Aromatic, Strong Raspberry Flavour

6.3 RESULTS

The 1996 summer fruiting stage 0 trials included 48 genotypes, including 40 East Malling selections, 4 new cultivars or selections from overseas and 4 UK cultivars as controls (Gaia,Glen Ample, Julia and Leo). Summer fruiting selections were picked 16 times between 4 July and 27 August. All assessments were carried out for the summer fruiting selections and the data entered into the computer for analysis.

The stage 0 records will provide data on yield, mean fruit weight throughout the harvesting period, mean quality scores and dates at which 5, 50 and 95% of the fruit was harvested for each selection. Unfortunately this analysis will not be completed until October and thus cannot be included in my placement report.

The harvesting season for the summer fruiting selections in 1996 was slightly later than average and went on longer than expected. This delayed harvesting of the stage 0 primocane selections and some aspects of the primocane fruiting trial had to be adjusted. As the beginning of the crop had been missed the yield data would have been skewed in favour of late ripening primocane fruiting selections, so collecting yield data was abandoned. Twenty three East Malling selections, Polana and Kiwigold were picked twice a week from 22 August until 16 September. The standard UK cultivar Autumn Bliss was not available as a control because all plants had become infected with RBDV and removed. Instead of picking marketable and unmarketable fruit from a section of the plot, only marketable fruit was picked. The fruit quality assessments were carried out as usual, but the punnet shelf life tests were abandoned because of insufficient fruit and only the tests on individual fruit were carried out.

6.4 DISCUSSION

Complete stage 0 trials provide comprehensive data on yield, season, fruit quality and shelf life performance. Comparison of data from a three year period enables selections with the best overall performance to be forwarded for further trials.

Without the computer analysis of the 1996 data it is difficult to comment on the performance of particular selections. However, it was noticed that certain qualities could be affected, week to week, by environmental factors. During weeks of heavy rain, texture, skin strength and cohesion were noticeably affected, lowering the score for many selections. Brightness was difficult to record if the fruit was wet as the hairs on the surface of the fruit, which dull the appearance, were disturbed making the fruit appear brighter than usual. Also, the taste of many selections seemed to improve throughout the season, though this was probably due to an adjustment of the tasters palate rather than from any other factor.

7. SUMMARY

A total of 2198 seedlings from the 1995 controlled crosses were planted in the field in July 1996. All had been screened for aphid resistance and RBDV. Seven of the 25 families planted contained good numbers of spineless seedlings, from which cultivars suitable for commercial release may eventually be produced.

The ELISA test was unaffected by the weather during the 1996 summer, allowing a high number of plants to be tested for RBDV and the positives destroyed. This should have greatly reduced the spread of RBDV in the East Malling plantation during the 1996 season.

The crossing programme was completely successful in terms of pollination and seed production. However, due to the long term nature of the crossing programme, overall success of the crosses made may not be seen in the immediate future. New, improved cultivars may not be produced directly from the progeny but they could eventually lead to the release of good commercial cultivars by inclusion in future crossing programmes.

8. ACKNOWLEDGEMENTS

I would like to thank my supervisor, Mrs V H Knight, for her encouragement and support during my work placement. I would also like to thank the members of the Virology department who assisted with the ELISA testing and all members of the Plant Breeding and Biotechnology department for making my stay so enjoyable. Finally I would like to thank the Horticultural Development Council for funding my placement at HRI.

9. REFERENCES

1. Alston F.H, Knight V.H & Simpson D.W. 1988. The Contribution and Value of Resistant Cultivars to Disease Control in Fruit. pp 67-76. Control of Plant Disease: Costs and Benefits. *Clifford* B.C & Lester E. (Eds.)

2. Anon. 1989. AFRC Institute of Horticultural Research, East Malling 1913-1988.

3. Anon. 1995. HRI Annual Report 1993-1994.

4. Anon. 1992. HRI Corporate Plan 1994-1999.

5. Clark M.F & Adams A.N. 1976. Characteristics of the Microplate Method of Enzyme-Linked Immunosorbant Assay for the Detection of Plant Viruses. pp 475-483. Journal of General Virology 34.

6. Converse R.H, Stace-Smith R. & Jones A.T. 1987. Aphid Borne Diseases - Virus Diseases of Small Fruit. pp171, 194-196. Agriculture Handbook No.631.

7. Fisher G.C. 1991. Raspberry Aphids. pp 71. Compendium of Raspberry and Blackberry Diseases. American Phytopathological Society.

8. Jennings D.L. 1988. Raspberries and Blackberries: Their Breeding and Growth. Academic Press.

9. Jennings D.L, Knight V.H & McNicol R.J. 1989. Progress in the UK Raspberry Breeding Programme. pp 93-104. ACTA Horticulturae 262.

10. Keep E. 1989. Breeding Red Raspberry for Resistance to Diseases and Pests. pp 245-321. Plant Breeding Reviews 6.

11. Knight V.H. 1993. New Raspberries From Horticulture Research International, East Malling. pp 114-118. The Fruit Grower.

12. Knight V.H & Barbara D.J. 1995. Raspberry Bushy Dwarf Virus at HRI-East Malling. pp 105-110. New Developments in the Soft Fruit Industry. Proceedings of the ADAS/HRI Soft Fruit Conference.

10. APPENDIX 1

Treatments used on seed produced from the 1995 controlled crosses.

Treatment 1. Bleach.

Soak seeds for 24 hours in 2.5% NaOCl. Wash off under running water for 10 minutes. Put in distilled water in vials in the fridge. Change distilled water on days 2, 3, 5, 19, 22, 23 and 24.

Treatment 2. Sulphuric Acid.

Just cover seeds with concentrated H_2SO_4 for 90 minutes in an ice bath. Wash off acid with iced water, then wash under running water for 10 minutes. Put in distilled water in vials in fridge. Change distilled water on days 2, 3, 5, 18, 19, 22 and 23.

11. APPENDIX 2

Solutions used during ELISA testing.

Volumes used for two microwell plates, except PBS Tween.

PBS Tween (stock solution).

Phosphate buffered saline (PBS) + Tween 20:

500ml 10 x PBS concentrate

25ml 10% Tween 20

make up to 5L with distilled water.

Coating solution.

40µl $F(ab)_2$

20ml carbonate coating buffer pH 9.6:

1.59g Na_2CO_3

2.93g $NaHCO_3$

1000ml distilled water

PBS TOP (grinding buffer).

Phosphate buffered saline + Tween 20 + ovalbumin

+ PVP (polyvinylpyrrolidone Mr = 44 000):

20g PVP

2g albumin

make up to 1 litre with PBS Tween.

IgG solution.

40µl Immunoglobulin

20ml PBS Top

Protein A (HRP) solution.

For 1:2000 :

10µl Protein A-horse radish peroxidase conjugate

20ml PBS Top

TMB substrate solution.

2000µl 10 x tetramethylbenzadine (TMB) buffer

200µl TMB substrate

20µl H_2O_2

18ml distilled water

4.2 METHOD

As RBDV is a pollen-borne virus it is important to test as many plants as possible before they flower. However, the time between emergence of the new leaves in spring and the development of the flowers can be fairly short, especially with some cultivars. Therefore, it is impossible to test all plants at the Research Station in the time available and a decision must be made as to the most important selections for testing.

4.2a Selections chosen for ELISA testing in 1996:

Selections Propagated in Winter 1994/95. These selections should have been tested in 1995 but the failure of the ELISA test due to hot weather prevented this. It was important not to leave them for another flowering period as any RBDV positive canes could transmit the virus to previously uninfected canes.

Possible Parents for Controlled Crosses. Selections to be used as parents for the 1996 crosses required testing as the virus could be transferred between parents or passed on to the progeny.

HDC Trial Plants. A number of plants were due to be sent for trialling at other locations in the country. It was important to ensure the virus would not be transferred with the plants.

A.idaei Resistant Seedlings. Some seedlings from the 1995 crosses classified as resistant to *A.idaei* were tested for RBDV, depending on their parentage. Families

where both parents came from selections with completely RBDV-free histories were not tested at this stage. Families where one parent came from a selection with a minimal record of RBDV were initially classed as "test 25 seedlings". If any of the 25 tested positive, all seedlings in that family would be tested. Families where one or both parents came from selections with a history of RBDV were classed as "test all seedlings".

Selections Propagated in Winter 1995/96. Each year the plants propagated from the seedling plot the previous winter are tested to ensure RBDV +ve plants have not been introduced to the most recently planted field.

Seedlings to be Propagated Winter 1996/97. Seedlings to be propagated were included in the testing in a bid to reduce the number of plants that would require testing in 1997. It will also save on field space by excluding RBDV +ve plants from the next field planting.

4.2b Sample Collection

The youngest, fully expanded leaves were chosen for testing as these should contain a high concentration of the virus. Between 3 and 5 leaves from separate canes were included in one sample and many selections were bulk-tested. Bulk samples include up to 5 plants from the same selection, with 1-2 leaves from each plant. Plants in bulk samples that tested positive for RBDV were retested individually in order to identify RBDV-ve plants if possible.