Shelagh Marshall

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## Introduction

Horticulture Research International was officially formed in 1990, combining the expertise and experience of seven long established research centres.

The international research reputations of Wellesbourne, formerly the National Vegetable Research Station, in field vegetables; East Malling formerly East Malling Research Station, in fruit and hardy nursery stock; Littlehampton, formerly the Glasshouse Crops Research Institute, in glasshouse crops and mushrooms; and the Hops Research Department based at Wye College have been augmented by the addition of the former ADAS Experimental Horticulture Stations. Situated at Efford, Kirton and Stockbridge House, these have close links with the horticultural industry the future success of which depends so heavily on research and development input from HRI.

East Malling is HRI's centre for research on perennial crops, being founded in 1913 by fruit growers alert to the benefits that research offer their industry. The Station's primary concerns are tree fruits (principally apples and pears but also plums and cherries); soft fruits (strawberries and raspberries); hops: woody ornamentals; and, as a recent addition, broadleaved farm woodland tree species. In addition to crop orientated research, are underpinning basic science research programmes in plant physiology, genetics, molecular biology, immunodiagnostics and analytical chemistry. East Malling maintains close associations with comparable research stations world-wide, and has strong academic links with universities, particularly in the training of postgraduate students from the United Kingdom and from overseas.

East Malling is set in the heart of Kent, the Garden of England and the major area of fruit and hop growing. The Station covers 220 hectares, which besides experimental plots and orchards includes laboratories, controlled environment facilities and glasshouses. A new post-harvest storage research facility was opened by Agriculture Minister John Gummer in April 1992.

Much of the work at East Malling is carried out under contract to the Ministry of Agriculture, Fisheries and Food and Department of Education and Science, but an increasing proportion is funded by agencies such as the Overseas Development Administration, the Horticultural Development Council, the Apple and Pear Research Council, the Commission of the European Communities and other industrial partners from the UK and abroad. Because they must be ever ready to adapt to changing customer preferences, to give the highest attention to food quality, and to minimise pesticide use by adopting resistant varieties and biological pest control strategies developed to benefit both the environment and the consumer, fruit growers continue to contribute to research costs through the East Malling Research Association and the Nuclear Stock Association.

Whilst at East Malling, I was involved in the Raspberry Breeding Programme, working with Mrs. V.H. Knight as part of the Breeding and Genetics Department.

## REVIEW

### Enhancement of *Rubus* Seed germination

### Introduction

Difficulty in obtaining high germination of limited seed numbers is a common problem faced by *Rubus* breeders. Blackberry seed is more difficult to germinate than raspberry seed, and low germination levels are often obtained. It appears that the seeds are "doubly dormant", germination being restricted by both the hard seed coverings and a dormant embryo (Galletta et al., 1989). Various attempts have been made to overcome this dormancy, to promote early spring germination following autumn sowing, so that a high proportion of seeds germinate in the first year. In breeding programmes, seeds which do not germinate in this first year are usually discarded, yet ironically these "difficult" seeds might be especially valuable genetically (Heit and Slate, 1950).

Stratification (moist chilling) is the most common method used to break dormancy. Many pretreatments have been tested either prior to, or without stratification, including chemical and manual scarification of the endocarp, exposure to various temperature and light regimes and application of growth hormones. In nature, dormancy is broken by the exposure of moist seed to low winter temperatures, often after it has passed through the digestive tract of a bird (Jennings, 1988).

The study of *Rubus seed* germination and dormancy is also of interest to scientists trying to devise an effective weed control programme for *Rubus cuneifolius* (American bramble). *R. cuneifolius* escaped from cultivation after being introduced into South Africa in the 1980s, and has become one of the most serious and costly weeds in Natal owing to its vigorous vegetative growth and copious seed production.

## Seed structure and development

*Rubus* fruits are aggregates of drupelets formed by the ripening together of a number of ovaries all from the same flower, adhering to a common receptacle. In the centre of each drupelet is a pyrene with a hard endocarp enclosing one or occasionally two seeds. The endocarp consists of two layers of elongated, parallel scleroid cells at right angles to each other and each several cells thick, the outer one forming the characteristic ridged pattern of the pyrene (Jennings, 1988). Although the endocarp is entirely maternal in origin, its size and shape is considerably influenced by interactions between the pollen parent and maternal tissues. The endosperm pellicle and the seed coat are known to be impermeable to gases. Histochemical tests have shown that hydrocyanic acid is present (Warr et al., 1979).

Seeds from full-sized green fruits germinate as readily as those from ripe fruits after scarification and stratification (Galletta et al., 1989), however seeds from fruits which have not started to swell do not germinate (Dale and Jarvis, 1983), suggesting that the embryo probably reaches full size when the fruit is nearly ripe.

The number of defective (collapsed) seeds appears to be higher for blackberry varieties than raspberry varieties (Kerr, 1954), but there does not seem to be any correlation between the germination of seeds and the percentage of aborted seeds (scott and Ink, 1957).

## Manual and Chemical Scarification of Seeds

Scarification involves breaking down of the seed coat and is achieved either manually with a scalpel or with the use of chemicals.

### Manual

Manual scarification of seeds is tedious, yet exceptionally high germination levels have been achieved by this method. Kerr (1954) obtained 88% and 90% germination of a blackberry selection by removing endocarps manually prior to stratification for 5 months, and Nesme (1985) obtained 100% germination in less than 15 days, at 20°C, with naked embryos or if the three seed coats were nicked, for five varieties of raspberry. Seeds with nicked endocarps did not germinate until the testa and endosperm were injured, and intact nutlets never germinated. Mechanical removal of the endocarp was found to be essential for any germination of cloudberry (Rantala, 1976; Warr et al., 1979).

Kex et al. (1985) studied in vitro germination and growth of *Rubus* seeds and embryos, obtaining the best germination (81%) and subsequent survival to soil (47%) with halved seeds, without the need for stratification (see Table 1). The lowest germination resulted from the halved-embryo treatment. It appeared that the physical action of cutting the seed in half did not impede development, however, the significantly reduced seed development percentages

of the embryo and halved-embryo treatments suggested that some of the embryos were damaged during seed coat removal. Germination levels may have been improved by discarding empty seeds, as seeds were extracted by crushing single berries between layers of paper towel, whereas usually fruit is macerated in a blender and floating empty seeds are decanted off.

Table 1

The effect of various seed treatments on the germination of *Rubus* seeds and embryos in vitro and survival in soil (Ke et al., 1985)

| Treatment | Attrition Sources (avg. % of original n) | | | | |
|---|---|---|---|---|---|
| | n | % germination (avg[z]) | seeds that germinated but did not develop | seedlings that died in soil | efficiency[y] |
| Seed (control) | 710 | 62.8 | 13.0 | 17.8 | 0.32 |
| Embryo | 316 | 69.6 | 18.0 | 19.8 | 0.32 |
| Halved seed | 602 | 81.1 | 13.5 | 20.8 | 0.47 |
| Halved embryo | 320 | 56.8 | 20.0 | 17.3 | 0.20 |
| LSD | | 6.5 | 2.6 | NS | 0.08 |

[z] Each value represents the average of 10 different hybrid seed lots.
[y] Efficiency = percentage of seeds that germinated and survived in soil.

Chemical

As early as 1919 Rose suggested that dormancy in *Rubus idaeus* seed was probably due to the high breaking strength of the endocarp and suggested treatment with concentrated sulphuric acid ($H_2SO_4$). Acid scarification causes carbonization of the endocarp, removing the need for manual scarification.

Heit and Slate (1950) found that 45 to 60 minutes of treatment at room temperature gave a good stand of seedlings the first year. They noted that the acid temperature sometimes reached 50°C during treatment, which for any longer period is likely to result in embryo injury. Moore et al. (1974) improved this treatment by using an ice bath to control the acid temperature. This prevents heating but also reduces acid activity, so that longer treatment periods are required for satisfactory scarification. They found that treatment for 3 hours in an ice bath prior to stratification resulted in increased blackberry seed germination and earlier seedling emergence. Before treatment the seed coat must be absolutely dry, or the heat generated by the treatment will kill the embryo (Ourecky, 1975).

The effectiveness of $H_2SO_4$ pretreatment is possibly due to its eroding or softening of the endocarp, facilitating gaseous exchange, and partly to its action as an oxidizing agent promoting chemical changes within the seed (Jennings and Tulloch, 1965). Campbell et al. (1988) found that scarification in $H_2SO_4$ was successful only if the carbonized embryo was removed. Lasheen and Blackhurst (1956) studied the biochemical changes associated with dormancy and stratification of blackberry seed and observed that during stratification starch decreases as sucrose and reducing sugars increase in the seed. The initial rate of starch breakdown was accelerated by $H_2SO_4$ treatment for 45 minutes followed by stratification, providing evidence that the seed coat does act as a barrier to germination during the removal of dormancy.

Pretreatment with hypochlorite solutions also increases germination. Scott and Ink (1957) found that soaking *Rubus* seeds in a 1% sodium hypochlorite (NaOCl) solution for one or two weeks was beneficial, but longer treatments had a detrimental effect. Jennings and Tulloch (1965) found that

the optimum concentration of NaOCl varied for different seed samples and that the most suitable concentration was 0.5% NaOCl, inducing more seeds to germinate than $H_2SO_4$, following six weeks of stratification. At certain strengths calcium hypochlorite (CaOCl) proves to be a better agent for pretreatment than NaOCl, and addition of a small (saturating) quantity of calcium hydroxide ($Ca(OH)_2$) to pretreatment solutions is beneficial and induces earlier germination. Treatment with higher concentrations of hypochlorite solutions leads either to a delay in germination or to a complete failure to germinate. $Ca(OH)_2$ reduces this tendency for germination to be inhibited and permits higher concentrations of the pretreatment solutions to be used advantageously. This led Jennings and Tulloch to hypothesise that the $Ca(OH)_2$ absorbs carbon dioxide or neutralises acids, either of which may be released during oxidizing processes involving the pretreatment chemicals. Pretreatment for 20 minutes with concentrated $H_2SO_4$ followed by six days with 1% CaOCl and $Ca(OH)_2$ was found to be beneficial for all of the seed samples. Interestingly soaking *R. cuneifolius* seeds in a 15% NaOCl solution without $Ca(OH)_2$ for 18 hours induced 85% germination when followed by 20 weeks stratification (Campbell et al., 1988). A direct relationship was found between the degree of endocarp scarification and germination, using electron microscopy.

Promising results were also achieved in a preliminary experiment when seeds were treated with 1.0% thiourea (Jennings and Tulloch, 1965). With another batch of seed however, less success was achieved by pre treatment with a range of thiourea concentrations.

Small seeds may require shorter chemical scarification treatment times to prevent the chemicals penetrating and killing the embryos (Moore et al.,

1974), as was the case with "Ark 578", a very small seeded diploid blackberry selection.

## Stratification

Most reports seem to agree that stratification is the most useful procedure used in efforts to overcome the presence of physiological embryo dormancy. Lasheen and Blackhurst (1956) studied the physiological changes which occur during stratification of "Lawton" blackberry seeds. They found that an acidic growth inhibitor gradually disappeared during moist chilling, and there was evidence of a growth promoter after four months (the time of optimum germination) but not after seven months.

Seeds which have been scarified prior to stratification appear to require shorter periods at low temperature. The optimum length of stratification in Jennings and Tullochs' experiments (1965) with pre treated raspberry seed was six weeks. Seeds which were treated with a hypochlorite solution with calcium hydroxide added germinated more rapidly when they had received six weeks of stratification, and although the total germination was not very different for those given six and four weeks of stratification, the more rapid germination of the former more than compensated for the extra chilling time provided.

Seeds of *R. cuneifolius* require at least one month of stratification to break dormancy of approximately 60% of the seeds (Van Staden and Campbell, 1984).

Scott and Ink investigated the effect of a second stratification period on germination of "Merton Thornless" blackberry seed. Germination of seeds was increased in most of the seed lots, and in a few cases was more than doubled following the second stratification period, indicating that the seeds had not been stratified sufficiently by the first cold treatment to break their dormancy. Another blackberry selection (US-1410) held continuously moist in a greenhouse for 12 months and then stratified once, germinated as well, or better than seeds stratified twice (see Table 2).

Jennings and Tulloch (1965) found that when their pretreatment conditions were optimal, some seed samples did not require a stratification period, yet for others this was still essential. They concluded that this difference between seed samples was due to the variation in extent of dormancy and sensitivity to chemical and environmental factors. Raspberry seeds can germinate without stratification provided that they do not undergo a prolonged period of drying before being sown (Dale and Jarvis, 1983). In Jennings and Tullochs' experiments the seeds were dried for several weeks in a chamber over calcium chloride. Dale and Jarvis demonstrated that seeds which are air dried overnight and sown immediately after pre-treatment give a similar mean germinability to seeds which are also chilled. Seeds which are air dried for several weeks, treated and then sown had a lower mean germinability than either of the other two treatments (see Table 3). It appears that when seed is stored dry, sufficient inhibitors move from the testa and endosperm into the embryo to induce embryo dormancy, which can only be broken by low temperatures. Care must be taken during stratification, to maintain an adequately moist condition to prevent secondary dormancy (Ourecky, 1975).

Attempts to induce germination without stratification and thereby accelerate the reproductive cycle of *Rubus* species have had limited success. Lundergan and Carlisi (1984) treated two blackberry cultivars with oxygenated distilled water for 30 minutes after acid scarification. The seeds were however air dried for 3 days prior to the acid treatment. Seed germination varied greatly between the two cultivars, the percentage of "Comanche" germination comparing favourably with previously reported germination rates of *Rubus* seeds that have undergone stratification. Kex et al. (1985) used in vitro techniques to germinate *Rubus* seeds and embryos, and obtained 57% to 81% germination within 8 to 12 days. However this procedure is both expensive and labour intensive.

Table 2

Germination of US-1410[a] blackberry seeds given no pretreatments before one or two stratification periods (adapted from Scott and Ink, 1957).

| Stratification periods | Percentage of seeds germinated by indicated date | | | |
|---|---|---|---|---|
| | Feb. 4, 1952 | Apr. 10, 1952 | July 30, 1952 | Aug. 31, 1953 |
| 5 months (1951-1952)<br>3 months (1952-1953) | 16.8 | 24.0 | 24.8 | 46.5 |
| 3 months (1952-1953) | 0 | 0 | 0 | 51.8 |

[a] US-1410 is an $F_1$ seedling selection of Brainerd X Merton Thornless

Table 3

Germinability of seeds from four raspberry seed samples given three drying and chilling regimes before sowing (from Dale and Jarvis, 1983)

| Cultivar | Drying and chilling regime | | |
|---|---|---|---|
| | Overnight drying, not chilled | Overnight drying, chilled | 7 weeks drying, not chilled |
| Malling Jewel | 70 | 55 | 43 |
| Glen Clova | 79 | 87 | 44 |
| Glen Isla | 67 | 56 | 22 |
| M31 | 82 | 80 | 27 |
| Mean | 75 | 69 | 34 |

(Seeds were pre-treated after drying by soaking in concentrated sulphuric acid for 20 minutes followed by immersion for seven days in a solution of calcium hypochlorite with excess calcium hydroxide.)

## Effects of environmental factors on seed germination

### Light

Jennings and Tulloch (1965) noted that deep sowing gave significantly better results when light was provided, but that there was no significant difference between planting depths when it was not. In a later experiment (Jennings, 1971) the difference between two replicates of potted seeds was shown to have a large effect, one set being placed on a table receiving more direct sunlight than the other set placed on the floor. Average seedling emergence in the "floor" series occurred approximately two weeks later than in the "table" series. Van Staden and Campbell (1984) found that one month of stratification and the presence of light was necessary to break dormancy of approximately 60% of *R. cuneifolius* seeds. The seeds kept in the dark had

approximately 20% germination. This light effect was most pronounced in seeds subjected to an alternating incubation temperature regime.

Temperature

The growing temperature to which a plant is exposed during seed development affects the embryo size of seeds and their time of germination (Dale and Jarvis, 1983). Seeds from plants grown at 20°C germinated slightly later, and had on average larger embryos, than those plants grown at 16°C. This also affects the ratio of homozygous and heterozygous seedlings (Jennings, 1971).

Scott and Ink (1957) found that germination was favoured if moist seeds were kept at greenhouse temperatures for a period before stratification. Experiments with "Norfolk Giant" seeds (Jennings and Tulloch, 1965) showed that the total germination was much higher for seeds which had received longer periods of stratification, although germination in the first four weeks after sowing was mostly confined to seeds which had first received 4,6 or 8 weeks of high temperature and light treatment. It would therefore appear that the seeds given the early high temperature and light treatment had acquired a stimulus for growth, but that in the absence of adequate stratification inhibitors of germination had not been removed sufficiently, limiting the extent of germination. Seeds which had received adequate stratification periods were capable of germination, though this was delayed, probably whilst the necessary stimulus for growth was acquired. Van Staden and Cambell (1984) found that when seeds of *R. cuneifolius* were incubated at 20°C for 2 months, then scarified prior to stratification, their overall germination was reduced severely. The best germination was achieved after acid scarification prior to

one month of stratification and subsequent incubation at an alternating temperature of 10/20°C in the presence of light. Further work with *R.cuneifolius* (Campbell et al., 1988) demonstrated that alternating temperatures of 10/20°C and 15/30°C give greatest germination. Seeds scarified with 15% NaOCl for 18 hours prior to incubation for 8 weeks at an alternating temperature of 15/30°C resulted in 85% germination. Unfortunately very little work with *Rubus* species to study the effects of fluctuating temperatures on germination have been carried out. Work with other species has demonstrated its germination enhancement effect. The germination of pin cherry (*Prunus pensylvanica* L.) is greatly improved by drastic temperature fluctuation treatment. The optimum treatment regimen is a 24 hour soak in 0.5M hydroxylammonium chloride followed by 30 days stratification at 5°C, prior to a 10 day germination period with 12 hours at 5°C alternating with 12 hours at 30°C. This treatment results in over 75% germination (Laidlaw, 1987).

Genetic factors affecting seed germination

Inherent factors varying among genetic clones are operative in seed germinability. It is often found that some clones fail to germinate well under any treatment. Scott and Ink (1957) found that seeds of blackberry selections differed greatly in their germination (from 29% to 73%) when compared directly. Seeds of the blackberry cultivar "Comanche" appear to have high germination levels when compared to other blackberry clones (Moore et al., 1974; Lundergan et al., 1984).

It appears that some genotypes are capable of germinating but fail to develop further (Kex et al., 1985). Differences among blackberry selections in the percentage of aborted seeds in normal appearing nutlets have been

observed, but germination of the seeds was not correlated with the percentage of aborted seeds (Scott and Ink, 1957).

Nesme (1985) found that virtually inbred seeds of raspberry had the highest percentage of ungerminated immature embryos. In Jenning's study (1971) of genetic factors affecting seedling emergence in raspberries, inbreeding effects on seedling emergence were associated with reductions in embryo size, whilst maternal effects on emergence were negatively correlated with maternal effects on embryo size. A large maternal influence on dormancy means that the time when germination begins is predetermined by the seed parents. The adverse effects of inbreeding only seem to be revealed when conditions are such that maternally induced dormancy is removed, giving the larger cross bred embryos which emerge first a selective advantage over later emerging inbred embryos. In nature, this results in heterozygotes being selected, thereby minimizing the consequences of inbreeding and maintaining heterozygous but stable populations.

<u>Application of growth hormones</u>

Heit and Slate (1950) found that dusting blackberry seeds with "Graino", a hormone powder, before germination gave a negative response. Seed numbers and size were not increased by application of gibberellin to young fruit (Topham, 1971). Application of gibberellic acid to raspberry seeds which have not received stratification, appears to have no significant effect, but it considerably increases the germination capacity of seeds which have been stratified (Jennings and Tulloch, 1965). Addition of $10^{-5}$M gibberellic acid or $10^{-7}$M benzylamine purine to oxygenating solutions used to treat scarified blackberry seeds did not improve germination (Lundergan and Carlisi, 1984).

However, Warr et al. (1979) found that application of gibberellic acid at concentrations of 5.8 X $10^{-6}$M and 5.8 X $10^{-5}$M to manually scarified bakeapple seeds (*Rubus chamaemorus* L.) in petri dishes incubated at 18°C, produced significantly higher germination percentages over controls.

## Dormancy mechanism of *Rubus* seeds

It is known that many fleshy fruits contain inhibiting substances that prevent germination of the seed while in contact with the juice or when still inside the fruit (Evenari, 1949). The mechanism of seed dormancy in *Rubus* after extraction from fruit has been linked to a number of factors. Ourecky (1975) stated that "dormancy is due to three conditions: impermeability of the seed coat to air and water, mechanical resistance of the seed coat to swelling of the embryo, and the requirement of the embryo for certain physiological changes which occur only under proper conditions of air, moisture, and temperature". However, these conditions are barriers to germination once dormancy has been induced, and dormancy is a result of the effects of growth inhibiting and promoting substances found within seed, whose levels are affected by temperature and moisture (Jennings and Tulloch, 1965). The germination inhibitors are initially present in the testa and endosperm tissues, but when seed is stored dry, sufficient inhibitors move into the embryo to induce embryo dormancy, which can only be broken by low temperatures (Lasheen and Blackhurst, 1956). Scarification of the endocarp before drying facilitates the removal of the inhibitors before they are translocated to the embryo (Jennings and Tulloch, 1965) and removes the mechanical resistance to germination. Chemical treatments may also increase germination by increasing the permeability of the endocarp to gases and water.

The failure of seeds of *R. cuneifolius* to germinate without a period of stratification (Van Staden and Campbell, 1984) was probably the result of growth inhibitors moving into the embryos whilst they were dried. Nesme (1985) found no evidence of embryonic dormancy with nearly 100% of naked embryos germinating in less than 15 days, without any after ripening treatments. However, as these seeds did not experience any drying treatments, this is not evidence that embryonic dormancy does not exist, simply that in this case it was avoided.

Once embryonic dormancy has developed, stratification appears to be the most effective method for removal of inhibitors. As inhibiting substances disappear during stratification, growth promoting substances are formed. This suggests that post sowing factors are effective when the inhibitor - promoter balance of the seed has been modified by such treatments, possibly by reducing the inhibitor concentration below a threshold level (Jennings and Tulloch, 1965). Growth promoting substances appear also to be acquired when adequate light is provided after sowing.

Seeds appear capable of re-entering a state of dormancy unless planted immediately after completion of their stratification period. Jennings and Tulloch suggest that the processes that lead to dormancy are reversible, and that factors which determine the direction of the processes are sensitive to environmental variation.

Discussion

*Rubus* seeds appear to rapidly enter a state of embryonic dormancy after they have been harvested, especially if they are dried. In breeding programmes, storage of dried seed is convenient, allowing all fruit to be harvested before treatment begins. Seeds are also far easier to handle when they are dry. Although many *Rubus* species achieve high levels of germination if seed is chemically scarified immediately after seed removal, this is not a convenient time for planting of seedlings and also levels of survival in soil if seedlings are planted outside the same year are poor. Many breeding programmes involve screening of seedlings for resistance to pests, which could not be accommodated in this time scale. Therefore effective methods of overcoming embryonic dormancy, resulting in high levels of early spring germination are of most use to *Rubus* breeders.

Techniques which eliminate the need for a stratification period, and allow seedlings to be sown the same year as seed production may be of use in accelerating genetic advance in specific areas of breeding programmes.

In spite of the value of the various techniques used to enhance seed germination, erratic seedling emergence is still recognized as a major problem for *Rubus* breeders. The main cause is the considerable difference in response to treatments found between genetic clones, especially as breeding for high levels of seed germination is an objective fairly low on a breeder's list of priorities.

Jennings and Tulloch's (1965) pretreatment for 20 minutes with concentrated $H_2SO_4$ followed by six days with 1% CaOCl and $Ca(OH)_2$ prior to stratification is probably the chemical treatment which will induce greatest germination from most seed lots, although optimum treatment times may need to be adjusted for seed other than raspberry seed. The application of growth hormones to seeds may be of use where particular genetic clones are favoured, or when levels of inbreeding are high.

Sowing seeds in late summer and storing them at greenhouse temperatures for a year, then stratification the following year may result in greatly improved germination levels, although this would add an extra year to evaluation time. It would be interesting to determine if seedlings which emerge after a second period of stratification are of a higher value than those which emerge after only one period of stratification, within a breeding programme.

The prospects of establishing an effective weed control programme for *R. cuneifolius* are unlikely at present. Its extremely hard seed coat probably allows germination only after prolonged periods of stratification combined with degradation of the seed coat. Seed reserves could pose a long term weed problem.

Even though the mechanism of *Rubus* seed germination is not clear, dormancy is best viewed as a reversible system (Jennings and Tulloch, 1965). Those attempting to increase germination should aim to control the rates of forward and reverse chemical processes, and ensure that the progress of the reverse process towards secondary dormancy is minimal.

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## Raspberry Production in the UK

Raspberry production in the UK averages 22,500 tonnes each year (Knight et al., 1989). Production greatly increased after 1970 in England and Wales, due to the development of PYO outlets. Over 90% of the Scottish crop goes to processing outlets, but the demand from processors has dropped as they can now import cheaper raspberry pulp from Europe.

An increased market, along with a need to raise and maintain plantations that are virus and disease-free, has resulted in a continued demand from growers for new, improved raspberry varieties. Raspberry breeding programmes have been successful in providing varieties which are meeting more of the growers' needs. This is reflected in the change in varieties grown on large scale over recent years. The UK raspberry breeding programme is divided between East Malling and SCRI (Scottish Crop Research Institute), Dundee.

### Raspberry breeding at East Malling

The raspberry breeding programme at East Malling has been running since 1922, and has produced many highly successful varieties such as Malling Jewel, Malling Exploit, Malling Promise, Malling Leo and Malling Autumn Bliss. English growers are particularly interested in late-ripening raspberries and the East Malling programme concentrates on late summer fruiting raspberries (traditional biennial cropping raspberries which crop from mid-July onwards) and primocane raspberries (annual cropping raspberries which crop on the young cane from early August onwards). The main objectives are outlined below.

Extending the season

Recently bred red raspberry cultivars have greatly increased the season of raspberry production from the traditional 4-6 weeks in mid-summer, to a continuous production from the beginning of July to late October. This has largely been achieved by breeding earlier and better primocane fruiting cultivars, whose season of production now overlaps that of late summer fruiting cultivars. Fig. 1 shows how the seasons of Malling Leo, Malling Augusta and Malling Autumn Bliss overlap to produce a continuous supply of raspberries throughout the summer. Augusta is the first cultivar arising from a backcrossing programme using *Rubus cockburnianus* as donor of many flowered laterals and late season. Autumn Bliss with *R. arcticus*, *R. occidentalis* and *R. idaeus strigosus* in its ancestry, has been widely planted in England (Knight et al., 1987). It is only since the release of Autumn Bliss that primocane raspberries have become a commercial crop in England.

High yield

Higher yields can be achieved by increasing fruit size and number of flowers per lateral and decreasing internode length in summer fruiting raspberries, and increasing fruit size and the bearing surface along with earlier season in primocane fruiting raspberries.

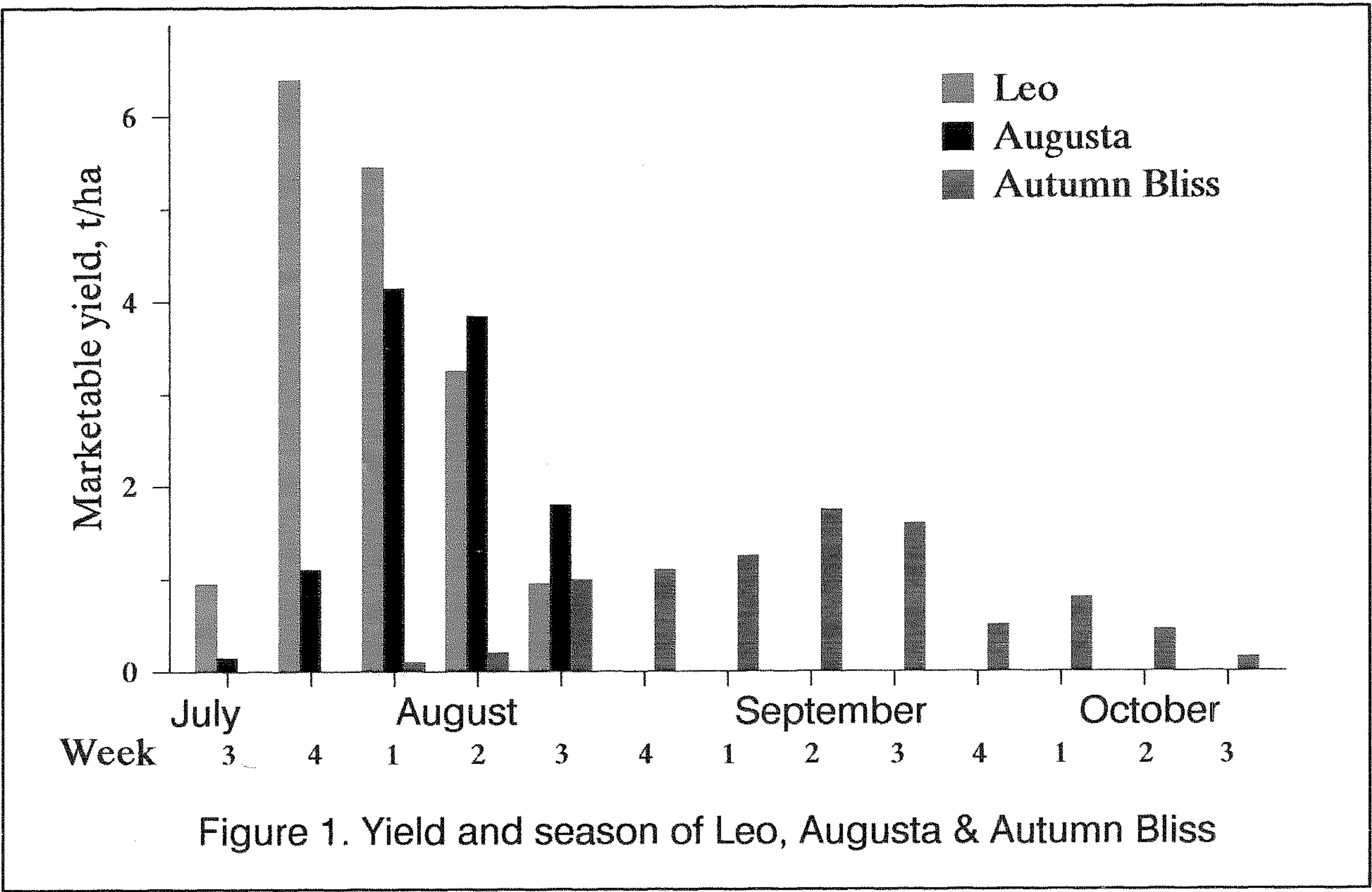

Figure 1. Yield and season of Leo, Augusta & Autumn Bliss

## Good quality fruit

The main aspects of fruit quality are size, shape, colour, texture and flavour. Breeding for other characteristics such as pest and disease resistance can have a detrimental effect on fruit colour as some resistance genes are obtained from black-fruited raspberry species such as *R. occidentalis*.

## Good plant habit

Good plant habit is important for cane management and affects the amount of crop picked. Canes should be sturdy and upright, with a good growth that is not too vigorous. Fruit should be well displayed on strongly attached laterals.

## Pest and disease resistance

Breeding for resistance to pests, diseases and viruses has become an important objective in raspberry breeding in the last couple of decades, due to the widespread occurrences of outbreaks of *Phytophthora*, Raspberry bushy dwarf virus etc. Some pests and diseases can be controlled by chemical means but the general trend is to minimise pesticide use , and host plant resistance has an important part to play in reducing dependency on chemicals.

Spinelessness

Spineless plants are welcomed by growers and fruit pickers. All manual operations are easier with spineless plants, which saves time and money. Spines are also a source of mechanical injury to the fruit, especially in high winds. Spinelessness is controlled by a single recessive gene which originated in a progeny of an old Scottish cultivar, Burnetholm. The procedure used to select for spinelessness is very simple because spineless and spiny seedlings can be distinguished immediately after germination. Spiny seedlings have glandular hairs around the edge of the cotyledons, whereas spineless seedlings do not. Much of the breeding material is heterozygous for spinelessness and if possible only spineless seedlings are planted for evaluation. The first spine-free cultivars were Glen Moy and Glen Prosen. Autumn Cascade is the first spine-free primocane cultivar.

A number of other objectives are also taken into consideration, such as shelf-life and the production of a raspberry suitable for mechanical harvesting.

References

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## Aphid Resistance

### Introduction

The large raspberry aphid *Amphorophora idaei*, occurs on wild and cultivated red raspberries throughout Europe. Although it does not usually cause much direct physical damage to host plants, it is a vector of four major raspberry viruses (Raspberry leaf spot virus (RLSV), Raspberry leaf mottle virus (RLMV), Black raspberry necrosis virus (BRNV) and Rubus yellow net virus (RYNV)). These viruses can result in serious decreases in plant vigour, fruit yield and quality. RYNV and RLMV are acquired after a feeding time of 1 hour or less (Keep, 1989).

Eggs of *A. idaei* hatch in early March and aphids feed on young buds, later living on the undersides of the new terminal growth of leaves. Populations of aphids build up asexually during spring and early summer by producing live parthenocarpic nymphs (Fig. 2). Development from nymphs through the four instar phases to adults takes approximately 4-8 days. The populations reach a peak during July and August on the primocanes, but a rapid decline occurs during September and sexual forms occur by the end of this month (Jennings, 1988). Eggs are laid usually on the lower parts of canes, from October to December.

Due to the rapid transmission of the aphid-borne viruses and the fact that infestations occur throughout the harvesting period, insecticide spraying is not effective or desirable. For these reasons, the benefits of breeding raspberries for strong host resistance has been recognized for a long time, and is a routine part of most raspberry breeding programmes.

Figure 2

Adults and nymphs of *Amphorophora idaei*

Resistance to *A. idaei* is controlled by major genes which vary in their ability to control the four strains of *A. idaei* that have been identified. Some of these genes are able to give resistance to all four strains whilst others have a complementary action.

Table 1 shows the origin of the major aphid resistance genes, that have been identified to date, and their resistance (R) and susceptibility (S) to each of the four strains of *A. idaei*. The genes $A_{10}$, $A_{K4a}$, $A_{L518}$ and $A_{cor1}$ are especially desirable as these genes confer resistance to all known strains.

Table 1

Resistance to the four strains of *Amphorophora idaei* conferred by different *Rubus* resistance genes

(adapted from Keep, 1989)

| Genes | Origin | Strain of *A. idaei* 1 | 2 | 3 | 4 |
|---|---|---|---|---|---|
| $A_1$ | "Baumforth A" (*R. idaeus*) | R | S | R | S |
| $A_2$ | "Chief" (*R. idaeus*) | S | R | S | S |
| $A_1A_3$ | "Chief" (*R. idaeus*) | R | R | R | S |
| $A_3A_4$ | "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 | ? |

Materials and Method

The seedlings of each progeny were laid out in the glasshouse on capillary matting. Strain 2 aphids were collected from Malling Delight and Malling Orion plants growing in the field. As these varieties have the gene $A_1$, and are therefore resistant to strains 1 and 3, strain 2 is the only strain that colonises them (strain 4 is extremely rare). Malling Landmark pot plants were used as stock plants initially, whilst the aphid population built up in size. Rows of 10 seedlings were inoculated at any one time by placing

these adult aphids onto the leaves of each plant, using a metal scoop. After 3-4 days these plants were recorded as resistant (no aphids present) or susceptible (adults and nymphs present on the underside of the leaves). Susceptible plants were discarded or used as further stock plants to help to increase the aphid numbers.

## Results

Table 2 shows the results of the aphid testing for the seedlings raised in 1992 from the 1991 crossing programme, along with the known or possible resistance genes carried by their parents.

Unfortunately very few of the seedlings were actually tested for aphid resistance in 1992 as the aphids died a few weeks after the testing started, due to vaporization and drift of Metasystox used to treat some nearby plum trees. We were however able to obtain an insight into the degree of susceptibility of some of the progenies. While searching for aphid stock, many seedlings had become infested on standing and these were recorded and removed. However this does mean that regrettably many of the seedlings planted this year will probably be grubbed due to their lack of aphid resistance and consequent infection with aphid borne viruses.

Table 2

Results of *Amphorophora idaei* screening of the 1992 progenies

| Family | Parents | | Genes for resistance | | Results Res | Sus |
|---|---|---|---|---|---|---|
| Primocane Fruiting | | | | | | |
| 6471 | Ruby | X 280/115 | | X $*A_{10}*A_{L518}$ | 31 | 12 |
| 6472 | 5961/24 | X 6321/12 | $*A_{10}*A_{L518}$ | X $A_{10}$ | - | - |
| 6473 | 6280/115 | X 5961/24 | $*A_{10}*A_{L518}$ | X $*A_{10}*A_{L518}$ | - | - |
| 6474 | 6280/115 | X 6291/41 | $*A_{10}*A_{L518}$ | X $A_{10}$ | - | - |
| 6475 | 6280/126 | X 6328/15 | $*A_{10}*A_{L518}$ | X $A_{10}*A_{L518}$ | - | - |
| 6476 | 6328/189 | X 6321/12 | $A_{10}*A_{L518}$ | X $A_{10}$ | - | - |
| 6477 | 6215/30 | X 6328/155 | $*A_{10}*A_{L518}$ | X $A_{10}*A_{L518}$ | - | - |
| 6478 | 6300/47 | X 6321/3 | $A_{10}$ | X $A_{10}$ | - | - |
| 6479 | Ruby | X 6280/126 | | X $*A_{10}*A_{L518}$ | 24 | 15 |
| 6480 | 6280/126 | X 5605/12 | $*A_{10}*A_{L518}$ | X $A_{10}$ | - | - |
| 6481 | 6328/44 | X 6300/47 | $A_{10}*A_{L518}$ | X $A_{10}$ | - | - |
| 6482 | 6328/44 | X 6321/3 | $A_{10}*A_{L518}$ | X $A_{10}$ | - | - |
| 6483 | A.Bliss | X Ruby | $A_{10}$ | X | - | - |
| Summer fruiting | | | | | | |
| 6484 | 7815A12 | X 6166/98 | $A_{10}$ | X $*A_{10}*A_{L518}$ | 8 | 9 |
| 6485 | 5795/93 | X 5796/23 | $A_{10}$ | X $A_{10}$ | - | - |
| 6486 | 5796/23 | X 7815A12 | $A_{10}$ | X $A_{10}$ | - | - |
| 6487 | 5928/22 | X 7815B8 | $A_{10}*A_{K4a}$ | X | 45 | 17 |
| 6488 | 5928/22 | X 6166/98 | $A_{10}*A_{K4a}$ | X $*A_{10}*A_{L518}$ | 9 | 1 |
| 6489 | 6166/98 | X 6230/22 | $*A_{10}*A_{L518}$ | X $*A_1*A_{10}*A_{L518}$ | 8 | - |
| 6490 | 6166/98 | X 6312/5 | $*A_{10}*A_{L518}$ | X $*A_{10}*A_{K4a}$ | 19 | 4 |
| 6491 | 6156/84 | X 6230/22 | $A_{10}*A_1*A_2$ | X $*A_1*A_{10}*A_{L518}$ | - | - |
| 6492 | 6288/20 | X 6355/48 | $*A_1*A_{10}$ | X $A_{10}$ | - | - |
| 6493 | 5833/9 | X 16A10 | $A_{10}$ | X | 20 | 17 |
| 6494 | 6399/21 | X 16A10 | $A_{10}*A_1*A_2*A_{K4a}$ | X | - | 3 |
| 6495 | 6399/21 | X 6230/22 | $A_{10}*A_1*A_2*A_{K4a}$ | X $*A_1*A_{10}*A_{L518}$ | - | - |
| 6497 | 5833/29 | X 3650/3 | $A_{10}$ | X $A_{10}*A_1$ | - | - |
| 6498 | 6156/84 | X 3650/3 | $A_{10}*A_1*A_2$ | X $A_{10}*A_1$ | - | - |

(* indicates where a gene may be present)

## Conclusions

Screening seedlings for *A. idaei* resistance pre-planting is an effective and economical part of the breeding programme. The use of major resistance genes in breeding for host resistance has been very effective. Aphid testing also allows a further insight into parental genes, by consideration of the ratios of resistant to susceptible offspring. Unfortunately with the limited amount of data resulting from this years testing it is not possible to do this.

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## Raspberry Bushy Dwarf Virus

### Introduction

Raspberry Bushy Dwarf Virus (RBDV) is a pollen and seed-borne virus, and therefore represents a large problem once plants have become infected in the field. No means of removing the virus from plants has been found, the only methods of control being the use of resistant plants and the removal of infected material before flowering occurs. Raspberry bushy dwarf disease as described by Cadman (1961) was probably induced by infection with Black Raspberry Necrosis Virus (BRNV) and RBDV, possibly augmented by infection with additional viruses (Jones, 1979). The symptoms of RBDV are often not apparent initially, and are not clear in latter stages as symptom expression is considered to be dependant on both genetic resistance to symptom expression and to environmental factors. Infection can lead to a great loss in cane height and diameter and yield in some plants (hence its name). Crumbly fruit has also been linked to RBDV (Murant et al., 1974), although this can occur for a number of unrelated reasons, along with decreased mean berry weight (Jones, 1979) and premature defoliation of fruiting canes (Wilson et al., 1983). Jones et al. (1981) suggest that RBDV is the casual agent of yellows disease. In an infected plant, yellowing of leaves in May/June and crumbly fruit often indicates a high probability of an RBDV infected plant.

There are two types of RBDV isolates which have been identified. The type previously studied in the UK such as D200, and a RBDV-resistance breaking form (RBDV-RB). D200 isolate is controlled by a single dominant gene *Bu* (Barbara et al., 1984). Inheritance and resistance to other isolates is

however more complex, giving a range of susceptibility/resistance. This makes screening for resistance to isolates other than D200 difficult.

RBDV is particularly prevalent in the raspberry plots at East Malling, having been introduced inadvertently via some infected seed from the USSR. A great deal of time is spent each year testing field material for the virus using the ELISA technique. It is particularly important to ensure that all plants used as parents in the crossing programme are virus-free.

ELISA

Enzyme-linked immunosorbant assay (ELISA) as described by Voller et al. (1976) is an effective method of detecting the presence of plant viruses in crude leaf samples. Barbara and Clark (1982) developed a simple indirect ELISA technique using $F(ab')_2$ fragments of immunoglobulin, which is the most efficient and effective procedure for detecting the presence of RBDV in field material.

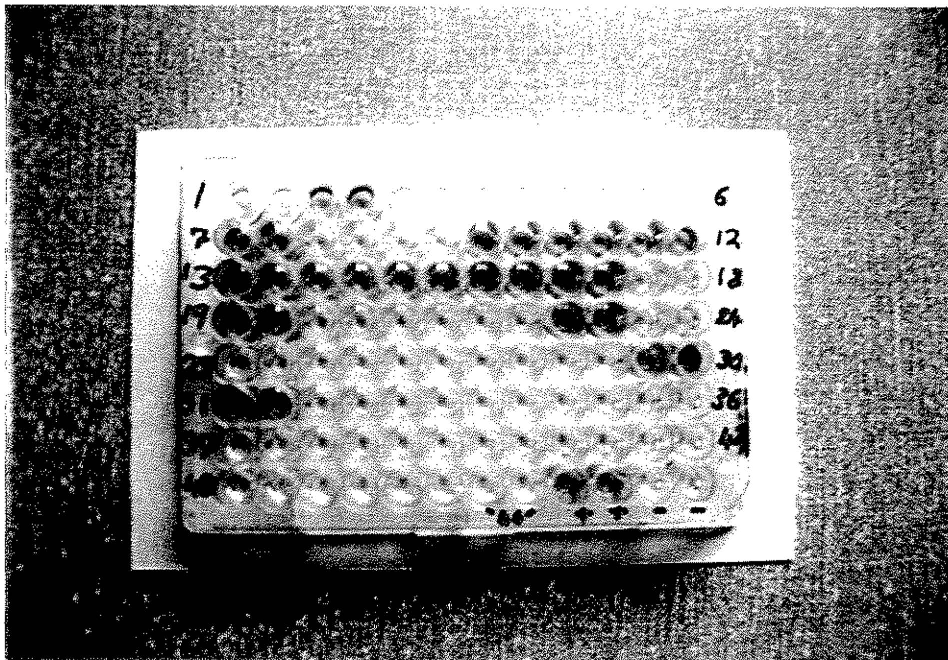
Materials and Method

Each test was performed on 92 samples, using two microwell plates. These samples either consisted of leaves from an individual plant, or were "bulk samples", containing leaves from 2-5 plants.

The two microwell plates were initially coated with $F(ab')_2$ diluted in coating buffer (1:500) (see appendix) and incubated at 30°C for 4-5 hours. Leaf samples were ground up in a phosphate buffered saline grinding buffer (see appendix), at a concentration of approx. 0.02g/ml. After washing the

plates with PBSTween (see appendix), 100$\mu$l of each sample was loaded in duplicate along with a positive and negative control for each plate. The plates were stored in the fridge overnight. The next day, the samples were washed out and IgG diluted in PBSTOP (1:2000) loaded into each well, followed by incubation of the plates at 30°C for 3 hours. Protein A-HRP conjugate diluted in PBSTOP (1:1000) was then loaded after washing the plates with PBSTween and the plates were incubated once more at 30°C for 3 hours. After a final wash, 100$\mu$l of substrate (see appendix) was loaded into each well and the plates left to develop for 20 minutes before being read. A pair of blue wells indicates that virus is present in a sample (see Fig. 3), provided that the positive and negative controls are blue and clear respectively. This procedure is outlined diagrammatically in Fig. 4.

Figure 3

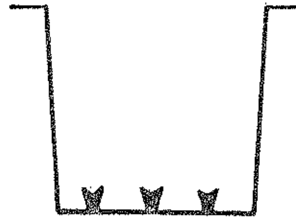



An ELISA plate with 14 out of 46 positive samples, plus positive and negative controls in the bottom right hand corner.

Figure 4

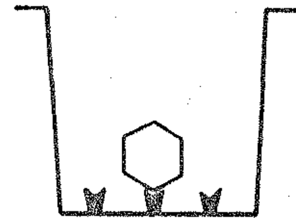
## Principle of the ELISA technique

1. Specific antibody adsorbed to plate



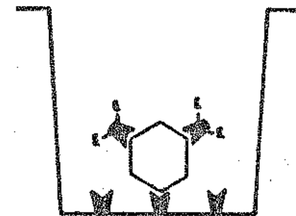
Wash

2. Add test sample containing virus



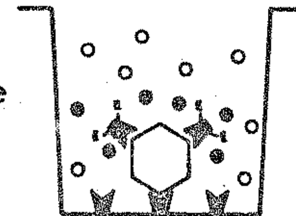
Wash

3. Add enzyme-labelled specific antibody



Wash

4. Add enzyme substrate



Colour intensity $\propto$ virus concentration

## Results

A wide range of plants were tested for RBDV in a purely qualitative manner, including parent plants for the crossing programme, samples of the 1992 seedlings and a large amount of field material. When a bulk sample tested positive, the individual plants within the sample were then tested separately. All plants that gave a positive result were cut down, although some had started to flower and may have added to the spread of RBDV in the field at East Malling. These plants will be totally grubbed (ie. have their roots removed) during winter 1992, to ensure that they do not grow back and flower next year. Flowering had begun due to time lost because of some difficulties experienced with the antibodies and a failure of the positive controls to show the presence of virus, due to a decrease in virus levels caused by the hot weather in June. In July additional positive controls were collected from the field. In early July the field positives had more virus than the glasshouse positives, but by the end of the month the virus content under glass had increased to normal levels.

## Conclusions

The ELISA technique devised by Barbara and Clark (1982) is a very effective way of testing raspberry plants for the presence of RBDV. It is unfortunate that the time most suitable for performing these tests is the short period of time after the first leaves appear in spring, and before flowering occurs, which is a busy period because of the necessity to start the crossing programme.

It was interesting to discover by default that a large variance occurs in virus concentration in field material between the young and old leaves. Young leaves appear to have a much higher virus concentration than older leaves. The youngest leaves are routinely sampled, and this years experience emphasises the importance of always using the youngest leaves available, to ensure that virus is not missed. Older leaves can have too low a virus concentration to test positive and an infected plant could be wrongly recorded as healthy.

In an effort to safeguard against future failure of the positive controls due to hot weather, the possibility of a freeze-dried positive sample is being investigated.

It appears that the only real hope of totally eradicating RBDV from the East Malling raspberry breeding plots is a thorough and concentrated testing programme at the critical time after leaves first appear and before flowering occurs, followed by immediate removal of positive material. Unfortunately this would require more hands, the funding for which is not available. At the moment promising lines which become infected with RBDV are grubbed and lost as parents or potential varieties, which is inefficient. Of even greater concern is the spread of RBDV in commercial plantings, some of which is due to the resistance-breaking strain.

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## Appendix

PBSTween

Phosphate buffered saline

5ml/l 10% Tween 20

PBSTOP (grinding buffer)

Phosphate buffered saline

5ml/l 10% Tween 20

2g/l ovalbumin

20g/l PVP (polyvinylpyrrolidone)

Coating Buffer (pH 9.6)

1.59g/l $Na_2CO_3$

2.93g/l $NaHCO_3$

diluted in distilled water

Substrate Buffer

2ml 10xTMB (Trimethylbenzadine) buffer

0.2ml TMB substrate

0.02ml $H_2O_2$

18ml distilled water

## *Phytophthora* Root Rot

### Introduction

Raspberry root rot is now the most serious threat to raspberry production in Europe (Duncan and Kennedy, 1991). During the 1980's many severe outbreaks of *Phytophthora* species caused widespread and often devastating damage both in Europe and North America, commonly in areas that were previously free from disease (Jennings, 1991). Duncan et al. (1987) identified 28 isolates of *Phytophthora* in 19 red raspberry stocks from widely separated parts of the British Isles. *Phytophthora* infection causes dieback due to necrosis of older roots, less production of feeder roots and stem lesions, resulting in a reduction in growth and eventual death.

After some uncertainty (Duncan et al., 1987), the most important cause of root rot in the Northern Hemisphere (isolated from more than 80% of all outbreaks in the UK), has been identified as *P. fragariae* var. *rubi* (Duncan and Kennedy, 1991). In the past this pathogen has been referred to as *P. erythroseptica*, *P. megasperma* and *P. fragariae*. *P. fragariae* var. *rubi* is closely related to the fungus which causes red core disease of strawberry, formerly referred to as *P. fragariae* and now renamed *P. fragariae* var. *fragariae*.

Tests for resistance have given some promising results. Strong resistance has been discovered in several sources independently derived from *R. ideaus strigosus* eg. Latham. Early backcross derivatives of *R. coreanus*, *R. pileatus*, *R. phoenicolasius* and *R. spectabilis*, have been identified as resistant during screening at SCRI and EM (Knight et al., 1989). Also the

hybrid berry Tayberry has been shown to be resistant to *Phytophthora* spp..

However, most resistant lines identified lack commercial quality and require further backcrossing to produce a resistant cultivar (Knight et al., 1989). To ensure that resistance is not lost, this backcrossing must be carefully monitored using screening techniques.

## Materials and Method

Motile zoospores of four strains of *P. fragariae* var. *rubi* were used in all inoculations, diluted in compost leachate. To ensure that a suitable concentration was used, zoospores were counted in a sample of the solution, and diluted to give 1000 zoospores/ml. The concentration of zoospores after inoculation was also determined, to check that the inoculum had remained effective. The temperature and humidity in the glasshouse was recorded throughout the testing period.

Two separate experiments were carried out, to asses the levels of resistance to *P. fragariae* var. *rubi* in the 1992 seedling progenies and also promising selections that have been propagated, but have not been previously tested.

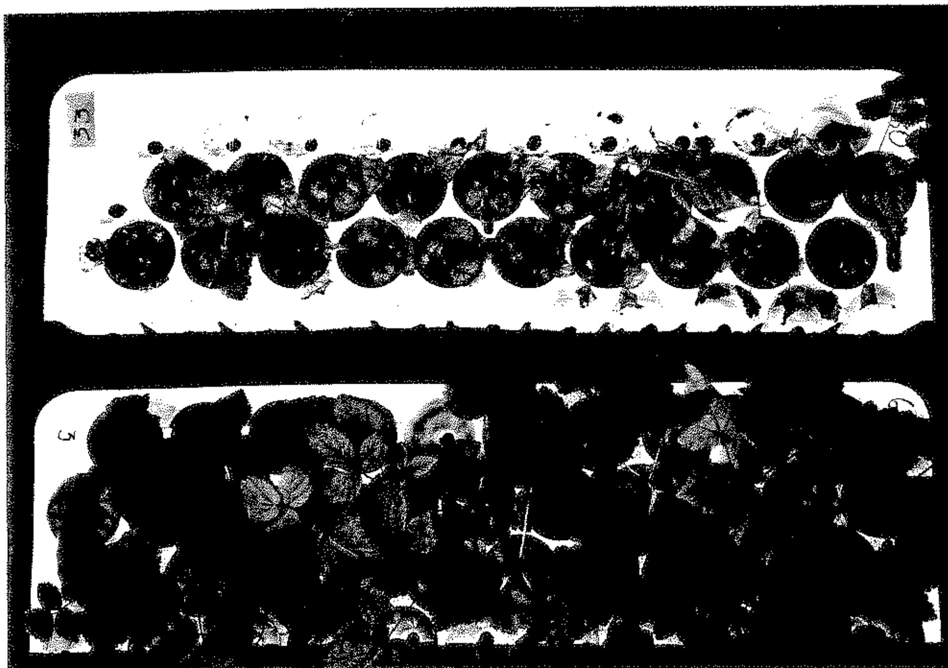
### Experiment A

27 seedling progenies were tested. Seedlings were pricked out from seedling trays and sown in cellular trays, holding 50 seedlings each. These

were transferred to the glasshouse, where they were allowed to acclimatise for 2-3 weeks prior to inoculation

Each individual seedling was inoculated with 5ml of the zoospore solution. Between 89 and 200 seedlings were inoculated per progeny (see Table 4). All the seedlings were recorded as healthy, diseased or dead, 2, 3 and 4 weeks after inoculation (see Fig. 5). Diseased individuals were identified by the presence of blackened stems, usually accompanied by wilting of the leaves.

Figure 5



Raspberry seedlings 3 weeks after innoculation with *Phytophthora*. The top tray were a susceptible progeny innoculated with 1000 zoospores/ml. The bottom tray were controls innoculated with compost leachate.

Experiment B

Pot plants raised from root cuttings of 22 promising selections were tested, with three controls (Latham, Meeker and Glen Moy).

The plants were clearly numbered and their heights measured before being arranged in 5 randomised blocks, each containing one plant to be inoculated, and one control plant (of similar height) for each selection. Irrigation lines were set up for each plant, to ensure that no cross contamination occurred during watering. 100ml of zoospore solution was used to inoculate each test plant and 100ml of compost leachate containing no zoospores used to inoculate each control plant.

These plants were recorded three weeks after inoculation. The height of each plant, and the length of any stem lesion was measured, and the root rot grade determined (see Table 3 and Fig. 6) by comparison of each test plant with its corresponding control plant.

Table 3

Root rot grades

| Root rot grade | % of root rotted |
|---|---|
| 1 | <20 |
| 2 | 21-40 |
| 3 | 41-60 |
| 4 | 61-80 |
| 5 | >80 |

Figure 6

Root rot grades 1-5 from left to right.

Results

The health status of the seedling progenies 4 weeks after inoculation are shown in Table 4. The number of healthy seedlings for many of the progenies is high, and although the % of healthy seedlings ranged from 40-99%, we suspect that the very high temperatures experienced during the testing period (regularly >35°C) are likely to have adversely affected the zoospores. Previous studies have shown that temperatures higher than 30°C stop growth of *P. fragariae* var. *rubi* colonies (Duncan et al., 1987). Screening for *Phytophthora* resistance in controlled environment cabinets has demonstrated that resistance appears to be greatly increased at higher temperatures

(Knight, unpublished data), indicating that the motile zoospores are deactivated at warmer temperatures, and fail to initiate infection.

If resistance levels at lower temperatures are relative to those at higher temperatures, progenies 6476, 6485 and 6502 have noticeably higher resistance than the other progenies. Progeny 6502 was derived from a *Phytophthora*-resistant *R. idaeus strigosus* Canadian selection. The very high level of resistance in 6476 and 6485 was unexpected and suggests that one or both of the parents in each progeny is resistant.

Table 5 shows the results of testing promising selections. Once again, the effect of the high temperatures experienced in the glasshouse during testing must be taken into account when analyzing this data.

Latham and Meeker (the two resistant controls) both have characteristic low root rot grades for their inoculated plants and show little difference between mean % increase in height for both inoculated and non-inoculated plants. Glen Moy (the susceptible control) has a mean root rot grade of 4.3, a high mean stem lesion length for inoculated plants and a noticeable difference in mean % increase in height for inoculated and non-inoculated plants. Selection 6220\26 shows very promising results, having a mean root rot grade very similar to Latham, virtually no stem lesions and almost identical mean % increase in height for inoculated and non-inoculated plants. Other selections ie. 5790\36, 5928\63, 5930\12 and 5948\12 also gave promising results.

Conclusion

The most resistant selection, 6220/26 is a primocane fruiting selection with commercial potential. The performance of 6220/26 in 1992 agrees with earlier work which indicated that primocane types tend to have more resistance than summer fruiting types. The other four selections which did well (5790/36, 5928/63, 5930/12 and 5948/12) are all summer fruiting and these results are encouraging. 5948/12 is a very early ripening derivative of *R. spectabilis* and 5790/36 and 5930/12 are high yielding types with commercial potential.

It is important to remember that conditions in screening tests should be kept as similar as possible to field conditions. Because the temperature under glass in May/June 1992 was very high, the conditions experienced by the pathogen may have been less conductive to disease than those normally experienced in the field. At high temperatures plants may be wrongly classified as resistant and the screening method unreliable. From 1993 onwards the screening will be done in a new glasshouse with cooling facilities and the maximum temperature kept to 22°C.

Combined efforts between breeders, pathologists and chemists will be needed to overcome this major problem. Sites for raspberry production should be carefully selected and heavy, poorly drained soils avoided, as preliminary work has shown that waterlogging can result in more severe root rotting (Duncan et al., 1987). Integrated management techniques including planting on ridges to improve drainage from canes and carefully monitored use of fungicides are likely to prove most beneficial. Planting material should always come from certified cane nurseries, to ensure that plants are not infected. If these precautions are taken, varieties with moderate resistance to *Phytophthora* will perform well and the efforts of raspberry breeders will

be of most value.

Table 4
Health status of seedlings 4 weeks after inoculation with *Phytophthora* zoospores

| Progeny | No. of seedlings | % of seedlings healthy | diseased | dead |
|---|---|---|---|---|
| 6471 | 100 | 79 | 12 | 9 |
| 6472 | 83 | 74 | 12 | 14 |
| 6474 | 97 | 88 | 10 | 2 |
| 6476 | 100 | 99 | 0 | 1 |
| 6477 | 99 | 78 | 17 | 5 |
| 6479 | 195 | 88 | 8 | 4 |
| 6481 | 100 | 83 | 4 | 13 |
| 6482 | 99 | 91 | 1 | 8 |
| 6483 | 200 | 87 | 2 | 11 |
| 6484 | 100 | 47 | 29 | 24 |
| 6485 | 99 | 97 | 1 | 2 |
| 6486 | 99 | 58 | 7 | 35 |
| 6487 | 99 | 70 | 7 | 23 |
| 6488 | 100 | 40 | 2 | 58 |
| 6489 | 98 | 66 | 6 | 28 |
| 6490 | 100 | 47 | 39 | 14 |
| 6491 | 100 | 62 | 17 | 21 |
| 6492 | 98 | 63 | 14 | 22 |
| 6493 | 95 | 64 | 19 | 17 |
| 6494 | 98 | 86 | 4 | 10 |
| 6495 | 99 | 65 | 20 | 15 |
| 6496 | 98 | 89 | 7 | 4 |
| 6497 | 100 | 70 | 16 | 14 |
| 6498 | 100 | 45 | 10 | 45 |
| 6500 | 174 | 74 | 8 | 18 |
| 6501 | 192 | 89 | 6 | 5 |
| 6502 | 152 | 91 | 3 | 6 |

Table 5

Results of *Phytophthora* testing of promising selections and control varieties

| Selection | Mean % increase in height | | Mean stem lesion length (mm) | | Mean root rot grade | |
|---|---|---|---|---|---|---|
| | inoc | con | inoc | con | inoc | con |
| Latham | 117 | 148 | 0 | 0 | 1.3 | 1 |
| Meeker | 124 | 149 | 4 | 0 | 2.2 | 1 |
| Glen Moy | 60 | 135 | 61 | 0 | 4.3 | 1 |
| 5312/5 | 207 | 415 | 48 | 0 | 4.4 | 1 |
| 5790/36 | 126 | 176 | 0 | 0 | 2.4 | 1 |
| 5795/93 | 143 | 214 | 34 | 0 | 4 | 1 |
| 5795/107 | 105 | 205 | 40 | 0 | 2.8 | 1 |
| 5928/63 | 193 | 271 | 2 | 0 | 2.2 | 1 |
| 5928/114 | 90 | 119 | 10 | 0 | 3 | 1 |
| 5930/12 | 174 | 238 | 9 | 0 | 2 | 1 |
| 5930/46 | 135 | 204 | 47 | 0 | 4.2 | 1 |
| 5948/12 | 142 | 161 | 9 | 0 | 2 | 1 |
| 5951/6 | 150 | 347 | 49 | 0 | 4.6 | 1 |
| 5969/63 | 157 | 198 | 61 | 0 | 3.4 | 1 |
| 5969/99 | 178 | 206 | 11 | 0 | 2.8 | 1 |
| 6156/84 | 186 | 227 | 88 | 0 | 3 | 1 |
| 6166/79 | 228 | 295 | 40 | 0 | 3.4 | 1 |
| 6166/98 | 109 | 132 | 33 | 0 | 3.4 | 1 |
| 6166/99 | 84 | 148 | 24 | 0 | 3.6 | 1 |
| 6215/30 | 169 | 379 | 47 | 0 | 3.8 | 1 |
| 6215/76 | 215 | 243 | 9 | 0 | 3 | 1 |
| 6220/26 | 96 | 93 | 4 | 0 | 1.4 | 1 |
| 6225/11 | 158 | 423 | 42 | 0 | 2.6 | 1 |
| 6230/22 | 171 | 238 | 78 | 0 | 3.7 | 1 |
| 6251/39 | 99 | 155 | 15 | 0 | 2.4 | 1 |

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## Stage 0 Selection Trial

### Introduction

Promising selections that have been propagated and assessed for 2/3 cropping seasons, are further assessed for yield and fruit quality in the breeders "Stage 0 Trials", before being submitted for "Stage 1 Trials" at the National Fruit Trials, Brogdale. This ensures that only those selections which produce a high yield of good quality, marketable fruit are entered for trial. Two separate Stage 0 Trials are carried out each year for summer fruiting and primocane fruiting raspberries. A couple of named varieties were picked as standards. The results of the summer fruiting Stage 0 Trials are presented below. Primocane fruit is still being picked and assessed.

### Materials and Method

Only a small length is recorded (between 1.5 and 2.5m) as picking is both time consuming and labour intensive. The portion of canes to be picked are clearly marked using yellow tape and the length measured and recorded. Ripe fruit is picked twice a week, throughout the harvesting period, for each stage 0 selection. Fruit is separated as it is picked into marketable and unmarketable fruit, and each is weighed and recorded. 50 marketable fruit for each selection are counted and weighed. The marketable fruit is also assessed for quality, by grading each selection on a 1-5 scale for various quality characters (see Table 7). By observing the range and mean of these grades, an accurate assessment of the fruits' characters can be obtained. Also, any changes in fruit quality within a season can be identified eg. darkening of colour.

Table 6

Fruit quality assessment scale

| Character | 1 | 2 | 3 | 4 | 5 |
|---|---|---|---|---|---|
| Colour redness | Pale | Fairly pale | Medium | Dark | Very dark |
| brightness | Very bright | Bright | Medium | Dull | Very dull |
| Shape | Long conical | Conical | Blunt conical | Roundish | Round |
| Outline | Very even | Even | Medium | Irregular | Very irregular |
| Uniformity of size | Very uniform | Uniform | Medium | Variable | Very variable |
| Flavour | Very good (aromatic) | Good | Medium | Poor | Very poor (acid) |

Results

The data was entered into a computer and then processed by the computing department. The actual lengths of rows picked / selection were entered and all selections converted to 10m, in order that direct comparisons could be made. Yields of marketable fruit, unmarketable fruit and total fruit yields were calculated / 10m of canes, along with mean fruit weights (Table 7). Mean gradings for the fruit quality characters were also obtained (Table 8).

Selections 6225/11, 6226/37, 6225/53 and 6166/98 performed particularly well in this trial, all producing a high yield of good quality, marketable fruit. 6225/11 has an "apricot" coloured fruit, which may require further work to improve shelf life, as these fruits tend to deteriorate rapidly once picked. However, its very high yield and late season are very promising. 6166/98 has a particularly good plant habit, making cane management and

picking easier. 6226/37 and 6225/53 both produce brightly coloured, even, uniform, roundish fruit with a good flavour. 7815B8 is a Scottish selection which has performed well at the National Fruit Trials, and was picked for comparison; it had by far the largest fruits.

Table 7

Marketable and unmarketable yield (kg/10m row) and mean fruit weight for the 1992 Stage 0 summer fruiting trial

| Variety /selection | Marketable fruit (kg) | Unmarketable fruit (kg) | Total yield (kg) | Mean fruit weight (g) |
|---|---|---|---|---|
| 6166/23 | 56.18 | 8.69 | 64.87 | 3.5 |
| 6225/11 | 51.33 | 5.74 | 57.06 | 2.6 |
| 6228/20 | 48.89 | 5.93 | 54.81 | 3.5 |
| 6226/37 | 48.12 | 3.47 | 51.59 | 2.7 |
| 6235/49 | 43.32 | 9.39 | 52.71 | 3.9 |
| 7815B8 | 43.02 | 6.81 | 49.83 | 5.3 |
| 6225/53 | 42.73 | 6.07 | 48.80 | 2.7 |
| 6160/24 | 40.96 | 5.24 | 46.20 | 2.8 |
| 6166/98 | 40.91 | 9.37 | 50.28 | 2.6 |
| 6230/22 | 40.84 | 3.33 | 44.17 | 2.8 |
| 6156/41 | 37.63 | 4.96 | 42.59 | 3.2 |
| Tulameen | 37.20 | 3.26 | 40.46 | 3.6 |
| 6166/99 | 34.78 | 5.06 | 39.84 | 2.8 |
| 6235/68 | 32.49 | 7.07 | 39.56 | 3.9 |
| M. Delight | 31.89 | 21.31 | 53.19 | 3.6 |
| 5928/63 | 31.24 | 9.92 | 41.16 | 3.2 |
| 5588/81 | 30.18 | 8.17 | 38.35 | 2.8 |
| 5928/127 | 28.94 | 3.06 | 32.00 | 2.6 |
| 5958/41 | 28.73 | 4.64 | 33.37 | 2.2 |
| 5959/68 | 28.41 | 7.55 | 35.96 | 3.0 |
| M. Leo | 27.21 | 5.19 | 32.40 | 2.6 |
| 6235/8 | 25.08 | 3.24 | 28.32 | 4.0 |
| 6235/1 | 24.55 | 7.29 | 31.84 | 3.9 |

Table 8

Fruit quality assessment of the 1992 Stage 0 summer fruiting trial

| Variety /selection | Redness | Bright | Shape | Outline | Uniform (size) | Flavour |
|---|---|---|---|---|---|---|
| 6166/23 | 3.6 | 2.6 | 2.2 | 2.1 | 3.5 | 3.4 |
| 6225/11 | yellow | 1.6 | 2.9 | 3.1 | 2.9 | 1.9 |
| 6228/20 | 2.1 | 2.6 | 2.6 | 2.6 | 3.3 | 3.4 |
| 6226/37 | 2.7 | 2.0 | 3.6 | 1.8 | 1.9 | 2.7 |
| 6235/49 | 3.4 | 2.7 | 1.6 | 3.2 | 3.2 | 3.1 |
| 7815B8 | 2.9 | 2.1 | 3.4 | 1.7 | 2.1 | 1.2 |
| 6225/53 | 3.0 | 2.1 | 3.7 | 2.0 | 2.4 | 2.5 |
| 6160/24 | 3.9 | 1.6 | 3.9 | 2.3 | 2.4 | 2.7 |
| 6166/98 | 3.2 | 2.7 | 2.0 | 1.4 | 3.2 | 2.8 |
| 6230/22 | 2.7 | 1.0 | 2.0 | 1.4 | 3.3 | 3.0 |
| 6156/41 | 3.0 | 2.7 | 3.8 | 2.4 | 2.8 | 3.5 |
| Tulameen | 2.9 | 1.7 | 2.1 | 2.1 | 3.7 | 1.8 |
| 6166/99 | 3.1 | 2.8 | 2.9 | 2.1 | 3.3 | 3.3 |
| 6235/68 | 2.7 | 1.8 | 2.6 | 3.0 | 2.9 | 2.2 |
| M. Delight | 1.3 | 2.1 | 2.1 | 2.4 | 3.4 | 3.6 |
| 5928/63 | 3.8 | 2.9 | 2.4 | 4.3 | 3.5 | 2.6 |
| 5588/81 | yellow | 2.7 | 3.2 | 3.0 | 2.7 | 2.2 |
| 5928/127 | 2.9 | 2.9 | 4.4 | 2.6 | 2.3 | 3.0 |
| 5958/41 | 3.8 | 4.0 | 3.8 | 2.0 | 2.0 | 4.5 |
| 5959/68 | 4.0 | 2.3 | 2.5 | 3.6 | 2.9 | 3.1 |
| M. Leo | 3.1 | 2.7 | 4.3 | 1.8 | 2.0 | 2.2 |
| 6235/8 | 1.6 | 2.0 | 2.6 | 2.2 | 2.8 | 1.9 |
| 6235/1 | 3.4 | 3.7 | 2.1 | 3.0 | 3.8 | 3.0 |

## Conclusions

The Stage 0 trial is an effective way of gaining information on the most promising selections, prior to deciding which to submit for further trials at Brogdale. As the fruit quality assessment is performed by students, it gives the breeder a second opinion the market acceptability of the fruit. The quality assessment also identifies any aspects of fruit quality that may limit its commercial potential. Individuals which are good in nine out of ten attributes may be useful parents.

## The Crossing Programme

### Introduction

New progenies are produced by crossing two superior and hopefully complementary parents. It is in the choice of parents that the breeders skill and knowledge of the crop are all important. The programme is devised during the winter, and initiated once flowers start to open in late May.

### Materials and Method

If the flowering seasons of the parents do not coincide, pollen must be collected from the earlier parent and stored. This is done by removing anthers from buds, allowing them to dehisce at room temperature over night, and storage in a desiccator at 4°C.

Preparation of the female parent involves selection of well secured laterals with a high number of large buds. All leaves, open flowers and immature buds are removed from the lateral and the remaining buds emasculated. This involves removal of sepals, petals and stamens by a scalpel cut, ringing the base of the bud, leaving only the immature stigmas. The scalpel and fingertips are rinsed in alcohol before emasculating another clone. The lateral is then covered by a waxed paper bag to prevent cross pollination, and labelled. Between 25 and 30 flowers are emasculated for each cross.

Laterals of the male parent are covered with a waxed bag, first removing all leaves, open flowers and developing fruit. When the female flowers are receptive, fresh flowers containing large quantities of pollen are removed from the bagged male parent and placed in a labelled tube. The bags are replaced for further pollen collection at a later date.

The first pollination is carried out 3/4 days after emasculation, and one or two further pollinations carried out at 2/3 day intervals. The flowers collected from the male parent are used as "brushes" to apply the pollen to the receptive stigmas (see Fig. 7). If pollen has been stored in a desiccator, the petri dish is taken out to the field, and pollen is applied using a fine haired brush. After pollination, the male parent is written on the label and the bag is replaced.

Mature fruits are collected and placed in labelled containers. Seeds are extracted by macerating fruits in a blender with water, and decanting off the pulp and unfertilized floating seeds. The clean seeds are soaked in a diluted solution of "Milton" sterilizing fluid for one minute, and then rinsed thoroughly. The seeds are dried on filter paper overnight and then stored at 4°C until sowing. Estimates of seed numbers are made by weighing 100 seeds and the total seed for each cross. Some seed lots with low numbers are treated with concentrated sulphuric acid, prior to sowing, to increase their germination percentage (see Review).

The seeds are sown in seed trays three quarter filled with ordinary compost, followed by a thin layer of seed compost. Seeds are scattered evenly on the surface and a further thin layer of seed compost sieved over them. They are then watered with a fungicide (6g of thiram/gallon of water) to prevent

"damping off". The seed trays are after-ripened in the glasshouse at ambient temperature, under black polythene for 3 months, and then transferred to the cold store for at least 12 weeks. In spring the seed trays are returned to the glasshouse for germination and early growth.

Figure 7

Pollination of an emasculated flower.

Results

The number of emasculated flowers, fruit set and approximate number of seeds are given for 4 out of 45 of the 1992 crosses in Table 9. The first two crosses (3655/48 x Glen Moy and 3655/48 x Latham) demonstrate the effect that a poor pollen sample can have on fruit set. Glen Moy pollen was collected in the field at East Malling and produced a 97% fruit set and a large amount of seed. Due to a lack of flowering Latham plants at East Malling, the Latham pollen was sent from Canada. This pollen sample was fairly small and appears to have been largely infertile by the time it reached its female partner, resulting in only 27% fruit set and a small amount of seed. The second two crosses (6235/1 x Tulameen and BC82-21-18 x 5928/22) demonstrate the effect of fruit size on amount of seed produced. 6235/1 and Tulameen both produce large fruit, and although fruit set was only 65%, the amount of seed produced was high. BC82-21-18 is a small fruited, *Rubus idaeus strigosus* selection from Canada, and although fruit set was 92%, the amount of seed set was relatively low.

Table 9

Examples of fruit set and seed numbers obtained from some of the 1992 crosses

| Cross female | | male | Number of emasculated flowers | Number of fruit set | Approximate number of seeds obtained |
|---|---|---|---|---|---|
| 3655/48 | x | Glen Moy | 29 | 28 | 3118 |
| 3655/48 | x | Latham | 30 | 8 | 378 |
| 6235/1 | x | Tulameen | 26 | 17 | 2155 |
| BC82-21-18 | x | 5928/22 | 24 | 22 | 1281 |

Conclusions

It is important for breeders to plan their crossing programmes carefully, in order to ensure maximum fruit set. This involves careful and frequent records of season and the number of flowers available, plus knowledge of the typical fruit set of each parent. Where seed numbers obtained are low, the use of seed germination enhancement techniques may be very beneficial, and sufficiently large seedling progenies can still be obtained.

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## Conclusion

Working at East Malling has given me a real insight into fruit breeding, and the fruit industry at large. I am sure that some of the knowledge I have gained will be relevant to courses that I have chosen for my final year, and will therefore help me with my future studies. I also feel that the contrast between the two work placements I have undertaken will help me with future career decisions. My time away from college has also helped with my ability to settle into new environments, and has been most enjoyable.