

Project title: Membership of the East Malling
Rootstock Club

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AUTHENTICATION

We declare that this work was done under our supervision according to the procedures described herein and that the report represents a true and accurate record of the results obtained.

Ms Felicidad Fernández
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CONTENTS

Grower Summary	1
Headline	1
Background and expected deliverables	1
Summary of the project and main conclusions	3
Action point for growers	3
Science Section	4
Introduction	4
Method	5
Summary of the project and progress made	7
Crossing programme planned for 2010	18
Update on other science projects	20

GROWER SUMMARY

Headline

A new funding arrangement has been established to develop improved rootstocks for apple and pear at East Malling Research (EMR).

Background and expected deliverables

Improved rootstocks are essential for profitable and sustainable production in tree-fruit crops. Factors important to growers include dwarfing (to reduce the cost of pruning and picking), induction of precocious and reliable cropping, freedom from suckers, good anchorage and resistance to pests and diseases. Ease of propagation and good scion-stock compatibility is also important at the nursery stage. There are few breeding programmes generating tree-fruit rootstocks. East Malling Research (EMR) involvement in rootstock development dates back to its foundation with the subsequent release of the world-famous series of M. (Malling) and M.M. (Malling-Merton in collaboration with the John Innes Institute) apple rootstocks. As a consequence of the decline in government support for 'near-market' research in the 1990's, industry support for the programme was sought and between 1992 and 2007, breeding apple and pear rootstocks formed one of the objectives of the East Malling Apple and Pear Breeding Club (APBC). The Apple and Pear Research Council and more recently the Horticultural Development Company (HDC) were the UK Licensees for the material developed as part of the APBC, which included two new rootstock releases, M.116 for apple and EMH, a quince rootstock for pear.

In 2008, EMR, the HDC and the International New Varieties Network (INN) launched a Rootstock Club (EMRC) to breed, develop, distribute and commercialise new rootstock breeding material from EMR, world-wide.

EMR has a wealth of breeding lines, derived from, UK, USA, Canadian and Japanese material, encompassing diverse agronomic variation and a wide range of resistance to pests and diseases. Defra has funded and is continuing to fund underpinning research at EMR on genetic mapping of rootstocks and the development of molecular markers for pre-selection of key rootstock characters. The programme is strengthened by EMR's wide range of international contacts and collaborations.

For UK growers, the HDC also acts as the UK licensee for the East Malling Rootstock Club

(EMRC) and will make new rootstocks released from EMR's programme, widely available to UK levy payers. The HDC helps to 'steer' breeding objectives to meet the specific requirements of the UK growers and will ensure that newly selected rootstocks are trialled further before release to the industry.

The INN has members in the USA, Chile, South Africa, Australia, New Zealand and across Europe. In each country, members can produce virus-free (VF) certified rootstocks and premium quality VF certified finished trees. INN members will arrange, evaluate and select from their own trials to identify those rootstocks best suited to each country's specific growing conditions.

It can take over 30 years to develop a new rootstock. Selection of parental material, crossing, seedling selection and first stage trialling which is carried out at EMR, takes around 10 years. Promising material is then propagated and released for HDC-funded trials in the UK and INN-funded trials at sites around the rest of the world. As trial results accumulate, indicating which selections are most promising, these stocks must be propagated to build up enough material for distribution before it is possible to co-ordinate a world-wide release.

The EMRC will complete the evaluation of apple, pear and quince rootstock material developed by the former APBC currently in the pipeline, with the aim of producing a range of apple, pear and quince rootstocks with desirable size control, precocity and productivity, with resistance to diseases and pests where applicable. New breeding material will also be produced taking account of potential climate change scenarios, using a new streamlined system previously developed in an associated project funded by the Department for the Environment, Food and Rural Affairs (Defra).

The club aims to develop a range of apple, pear and quince rootstocks to suit different growing conditions. Breeding objectives include:

- dwarfing and semi-dwarfing stocks for apple and pear
- improved scion-graft compatibility, in particular for pear
- precocity and productivity
- fire-blight and/or woolly apple aphid resistance
- tolerance to replant disease

Summary of the project and main conclusions

Currently, there are 40 selections at different stages of evaluation for pear and nine apple selections from the EMR programme have been included in the latest HDC-funded trial planted in February 2010. A total of 11 apple and 5 pear progenies are currently at different stages in the selection pipeline. Since the club was created, good progress has been made:

- 4 apple and 8 pear selections have entered propagation for preliminary trials.
- 14 apple and 11 pear seedlings were identified as interesting and were cut back for propagation in 2010-11.
- 7 new apple progenies have been raised (three planted in 2009 and four to be planted in 2010).
- 13 new crosses (7 for apple and 6 for pear) were carried out this spring (2010).

Financial benefits

No financial benefits will arise from this project until new rootstocks are released to the industry.

Action points for growers

As promising material is identified by the breeding team at EMR, selections will be entered into HDC-funded trials (TF 172).

SCIENCE SECTION

Background

Improved rootstocks are essential for profitable and sustainable production in tree-fruit crops. Factors important to growers include dwarfing (to reduce the cost of pruning and picking), induction of precocious and reliable cropping, freedom from suckers, good anchorage and resistance to pests and diseases. Ease of propagation and good scion-stock compatibility is also important at the nursery stage. There are few breeding programmes generating tree-fruit rootstocks. East Malling Research (EMR) involvement in rootstock development dates back to its foundation with the subsequent release of the world-famous series of M. (Malling) and M.M. (Malling-Merton in collaboration with the John Innes Institute) apple rootstocks.

In 2008, EMR, the HDC and the International New Varieties Network (INN) launched a Rootstock Club (EMRC) to breed, develop, distribute and commercialise new rootstock breeding material from EMR, world-wide.

For UK growers, the HDC also acts as the UK licensee for the East Malling Rootstock Club (EMRC) and will make new rootstocks released from EMR's programme, widely available to UK levy payers. The HDC helps to 'steer' breeding objectives to meet the specific requirements of the UK growers and will ensure that newly selected rootstocks are trialled further before release to the industry.

The INN has members in the USA, Chile, South Africa, Australia, New Zealand and across Europe. In each country, members can produce virus-free (VF) certified rootstocks and premium quality VF certified finished trees. INN members will arrange, evaluate and select from their own trials to identify those rootstocks best suited to each country's specific growing conditions.

It can take over 30 years to develop a new rootstock. Selection of parental material, crossing, seedling selection and first stage trialling which is carried out at EMR, takes around 10 years. Promising material is then propagated and released for HDC-funded trials in the UK and INN-funded trials at sites around the rest of the world. As trial results accumulate, indicating which selections are most promising, these stocks must be propagated to build up enough material for distribution before it is possible to co-ordinate a world-wide release.

The EMRC will complete the evaluation of apple, pear and quince rootstock material developed by the former APBC currently in the pipeline, with the aim of producing a range of apple, pear and quince rootstocks with desirable size control, precocity and productivity, with resistance to

diseases and pests where applicable. New breeding material will also be produced taking account of potential climate change scenarios, using a new streamlined system previously developed in an associated project funded by the Department for the Environment, Food and Rural Affairs (Defra).

Aims and objectives

The club aims to develop a range of apple, pear and quince rootstocks to suit different growing conditions. Breeding objectives include:

- dwarfing and semi-dwarfing stocks for apple and pear
- improved scion-graft compatibility, in particular for pear
- precocity and productivity
- fire-blight and/or woolly apple aphid resistance
- tolerance to replant disease

Method

The breeding programme is an ongoing effort of which different steps are briefly described below:

Crossing

Parental genotypes that carry one or more traits of interest are selected and a crossing programme is designed aiming to combine those desirable characteristics into the resulting seedlings. Controlled crosses are carried out in Spring. First, the anthers of the intended male parent are extracted from unopened blossoms to avoid cross contamination and placed in Petri dishes until they dehisce, releasing their pollen. Pollen is stored in a desiccator at 3 °C, remaining viable for up to 4 years. Secondly, petals are removed from the flowers of the intended female (balloon stage) and pollen of the chosen male placed on the receptive stigmas. Fruits are then left to develop and ripen naturally and seeds are carefully extracted after harvest.

Fresh seeds are washed and soaked in water for 2 - 3 days with daily rinses to remove germination-inhibiting compounds. They are then air-dried and stored at 3 °C until the following January.

Raising seedling populations

Seeds are stratified in the cold-store (> 4 °C) in trays of moist compost and Perlite mix for 16 weeks. After this period seed trays are placed in a glasshouse (at ~ 18°C) for germination.

Seedlings are potted individually as they become large enough to handle safely and are grown on for a couple of months. In their first summer seedlings are planted out in the field and left to establish for a whole growing season.

Field evaluation of rootstock seedlings

In the first winter, 1-year-old bare-rooted plants of commercial rootstocks are interspersed in the seedling population as controls. 'M.27', 'M.9', 'M.26' and 'M.M.106' are used for apple populations and quince rootstock 'EMA' and 'EMC' are used in the pear populations. Both seedlings and controls are budded with the same scion the following summer and left to grow.

For the three to four years of establishment of each population, records are taken for each seedling of vigour, production of suckers and pest and disease incidence in those suckers. As the common scion comes into fruit differences attributable to the rootstocks, such as fruit size and crop load, are also recorded for two seasons and the most promising seedlings are selected for propagation.

Propagation

Each selection is marked out in the field in the summer and cut back below the budding union the following autumn. To encourage growth of shoots from the rootstock and their subsequent rooting, stumps are covered up with compost in the spring and again during the summer. Leaf samples of each selection are taken at this stage to allow future DNA identification. Pest and disease incidence of the stocks is recorded during the summer so that unhealthy selections can be discarded.

Hardwood cuttings (ideally ~ 30 cm in length) are taken of these selections at the beginning of December. The cuttings are dipped in 0.5% (indole-3-butyric acid) IBA solution for 5 seconds prior to insertion into a heated cutting bin to a depth of 6 to 8cm. The cutting bin consists of a 30 cm layer of a 1:1 mixture of peat and fine bark over a 5cm layer of coarse sand through which runs a soil warming cable which maintains the bed temperature at 25°C. Air temperature is cooled with ventilation to the outside. Cuttings are left until rooted and then potted into 2 litre pots, typically in late January or early February, and grown on under unheated glass. Ease of propagation is also an important selection criterion and reticent selections will also be discarded.

Preliminary trials

After a year or two of growth in pots, selections are grafted with a common scion ('Cox' or 'Fiesta' for apples and 'Conference' for pears) and established in replicated trials that include commercial rootstocks for control purposes.

In these trials tree vigour is assessed by the measurement of tree volume (either in the form of the number and length of shoots for trees up to 3 years old or by the measurement of the height and spread of the tree crown in older trees) and by the recording of trunk girth at 15 cm above ground level. Where appropriate, tree fresh weight at the time of grubbing is also recorded as a measure of relative vigour.

Total yields and yields of class one fruit (>65 mm and 55-65 mm) are measured for each tree and cumulative yields and yield efficiencies are calculated.

In all trials notes on tree health, graft compatibility and anchorage are made as required.

The best selections after this preliminary evaluation are then propagated to enter further trials funded by HDC (TF172) in the UK and by INN overseas.

Summary of the project and progress made

Currently, there are 40 selections at different stages of evaluation for pear and nine apple selections from the EMR programme have been included in the latest HDC-funded trial planted in February 2010. A total of 11 apple and 5 pear progenies are currently at different stages in the selection pipeline. Since the club was created, good progress has been made.

1.1. New seedling populations

1.1.1 Apple

Seeds from the 2009 crosses were extracted and two open pollinated seed-lots were also collected in order to ensure that a sufficiently large population could be raised in 2010. Seed trays went into cold storage on 19th January and, after 12 weeks of chilling they are currently being germinated for a summer planting of the following families:

- M553 (AR86-1-20 x Geneva 202): 164 seeds
- M554 (M.M.106 x Geneva 30): 414 seeds
- M555 (Geneva 30 (= Robusta 5 x M.9) o.p.): 400 seeds
- M556 (Ottawa 3 (= M.9 x Robin Crab) o.p.): 400 seeds

All seedlings arising from the controlled crosses will be planted and numbers will be made up to ~ 1000 with individuals from the open pollinated families. A total of 348 new seedlings from three different progenies (Table 1) were planted in 2009.

Table 1. New progenies planted in 2009

Progeny number	♀		♂		Planted
	Rootstock	Characteristics	Rootstock	Characteristics	
M550	AR86-1-20	semi-invigorating (95% of M.M.106), waa ¹ resistance, good yields	M.9 EMLA	dwarfing, waa ¹ susceptible, low chilling requirement	83
M551	M.16	invigorating (parent of M26), late leafing	M.9a	dwarfing, waa ¹ susceptible, low chilling requirement	118
M552	White Angel	source of resistance to powdery mildew	M.9 EMLA	dwarfing, waa ¹ susceptible, low chilling requirement	147

¹ waa woolly apple aphid

The small size of the 2009 population was due to poor germination and low survival rate, which are not uncommon in progenies segregating for low vigour.

1.1.2. Pear

The pear pipeline is well stocked and recently planted trials are currently being evaluated and consequently, as this crop is of lower priority to the club, no new progenies were raised in 2009. Similarly, at the EMRBC technical meeting held in January 2010 it was decided that no pear progenies would be raised in 2010 and seed produced from 2009 crosses will be stored for future germination.

At the next technical meeting to be held in September 2010, the club needs to decide what proportion of the total breeding effort they wish to see dedicated to pear, possibly somewhere in the region of 25 – 30%. It is expected in the future that progenies for pear rootstocks will be raised every two or three years to facilitate selection as well as the establishment and management of subsequent trials.

1.2. Seedling populations in the pipeline:

In 2009, the evaluation of apple and pear progenies for the EMRSC was carried out with the assistance and advice of Dr Kate Evans (Washington State University) working as a consultant for EMR.

1.2.1. Apple

All seedlings in the pipeline have been budded with the columnar scion SA544-28 for evaluation of vigour, yield, etc. Suckering is also recorded. Progenies M545, M546, M547, M548 and M549, all budded in 2008, were not ready for evaluation and continued to establish in 2009. Seedlings from the following older progenies were evaluated and de-suckered in July and August 2009:

- M432 (M.27 x M.116): 79 individuals
- M430 (M.I.S x M.27): 83 individuals
- M480 (M.9 x M.116): 20 individuals
- M481 (M.9 x Geneva 202): 18 individuals
- M482 (mixture between M480 and M481 seed): 205 individuals
- M508 (M.13 x JM7): 61 individuals

1.2.1. Pear

Seedlings from three progenies planted in 2008, plus quince controls EMA and EMC, were budded with Concorde in August 2009:

- PQ42 (OHxF51 x *P. amygdaliformis*): 160 individuals
- PQ43 (OHxF69 x *P. amygdaliformis*): 98 individuals
- PQ44 (OHxF333 x *P. betulifolia*): 27 individuals

Seedlings from progenies planted in 2005 (budded 2006) were evaluated and de-suckered in July – August 2009:

- PQ40 (OHxF51 x Kumloi): 9 individuals
- PQ41(OHxF34 x Kumloi): 66 individuals

1.3. Selection

1.3.1. Apple

Three seedlings from progeny M345 (M.M.106 x Totem) and 11 from M360 (AR86-1-20 x M.9), first identified as promising in 2008, were selected in August 2009 and cut back in December 2009 for propagation in 2010-11.

1.3.2. Pear

Three seedlings from progeny PQ38 (*Pyrus* QR708-36 op) and 8 from PQ39 (*Pyrus* QR517-9 op) were selected in August and cut back in December 2009 for propagation in 2010-11.

1.4. Propagation

Seedlings selected in 2008 were cut back and covered with compost in 2009 to encourage the production of shoots. This will be repeated in spring 2010 to increase the number of replicates per selection, except where seedlings have been subsequently de-selected or died.

1.4.1. Apple

In 2008, 10 seedlings from progeny M306 (AR86-1-20 x M.20) were pre-selected for propagation. Of those, three have now been deselected due to severe infestation of woolly apple aphid or severe mildew symptoms. One failed to produce any shoots and was also discarded whilst two more produced only very few shoots, so they were cut back again to attempt propagation in 2010-11. Cuttings from the remaining seedlings were taken on 8th Dec 2009 for propagation (Table 2).

Table 2. Results of propagation¹ of apple rootstock selections in 2009

Selection	Cuttings taken	Rooting quality ²				Total
		Very good	Good	Fair	Poor	
M306-6	24	8	6	3	1	18
M306-20	30	9	5	4	2	20
M306-79	32	2	5	4	2	13
M306-189	23	12	5	2	2	21

¹ cuttings were treated with IBA and placed in heated bins for rooting and potted up after 7 weeks.

² based on amount and strength of roots produced

1.4.2. Pear

Of the 10 seedlings from PQ37 progeny (OHxF87 x B627 (= *P. betulifolia* x *P. calleryana*) that were pre-selected in 2008, three died after being cut back and cuttings were taken from the remaining eight seedlings on 8th Dec 2009 for propagation (Table 3).

Table 3. Results of propagation¹ of pear rootstock selections in 2009

Selection	Cuttings taken	Rooting quality ²				Total
		Very good	Good	Fair	Poor	
PQ37-1	14	0	1	0	1	2
PQ37-2	14	0	1	1	8	10
PQ37-3	14	0	0	3	1	4
PQ37-4	14	0	0	0	0	0
PQ37-5	28	0	0	1	4	5
PQ37-6	10	0	0	2	1	3
PQ37-7	14	2	1	3	3	9
PQ37-8	10	0	0	0	4	4

¹ cuttings were treated with IBA and placed in heated bins for rooting and potted up after 7 weeks.

² based on amount and strength of roots produced

1.5. Preliminary trials

1.5.1. Apple

The most recent apple trial (DM169) was grubbed in winter 2008-09 and results were reported to the club last year. Four stocks from this trial were identified for further evaluation in the UK and have been included in the latest HDC-funded trials, planted in March 2010 at EMR. B24, R59 and R104 will be compared to AR852-3, AR839-9, M.9, M.26 and M.27 in a conventionally managed orchard with 'Royal Gala' and 'Braeburn' as scions. R80 has been included in the organic trial alongside AR10-3-9, AR809-3, AR835-11, M.M.106 and M.116, all worked with 'Red Falstaff'.

1.5.2. Pear

Three trials of rootstocks for pear were evaluated in 2009; PR180 (planted in 1998), DM177 and DF178 (both planted in 2006). In all three trials, quince rootstocks EMA and EMC were included as controls and in all cases the comparison of vigour between both controls was unexpected. In PR180, the vigour of both controls was not statistically different. It was originally suggested that the lack of difference may have related to the different origins of the rootstocks – EMA stocks were produced at EMR and EMC in a commercial nursery. In the case of DM177 and 178, all EMA and part of the EMC controls were obtained from 'Blackmoor' nursery whereas the rest of the EMC controls came from 'Keepers' nursery. It was expected that the EMC rootstocks obtained from two different nurseries would perform similarly. However, the girths of trees on EMC rootstocks from 'Keepers' nursery are similar to those of trees on EMA rootstocks and their tree volume is intermediate between that of those on EMA and EMC from

'Blackmoor'. This might be due solely to the different origin of the stocks but it seems more likely that some of the controls are not true-to-type. Whilst DNA analysis in apple and pear is performed routinely at EMR (see update on other research later on), DNA fingerprinting for quince is more difficult and has not yet been fully optimised. Tests carried out 2009 using some samples from this plots and EMR own stocks were inconclusive and seemed to suggest possible mixtures. Work will continue in 2010 to optimise a fingerprinting protocol for quince and samples of EMA and EMC from several nurseries will be included.

The oldest trial, in PR180, has now been grubbed and final records including grubbing weight have been taken. A preliminary analysis of the results is summarised in Table 4. So far we have only analysed data for selections where at least five of the six original trees remained but a second round of analysis for selections of lower survival rate is currently underway. Please note that, as mentioned before, the vigour of both controls in this trial was not statistically significant.

Table 4. The effects of *Pyrus* and *Quince* (including EMA and EMC) rootstocks on the growth and cropping of 'Conference' pear trees in plot PR180.

Rootstock	2009 Data						Cumulative Data (2001-2009)		
	Girth (cm)	Tree Volume (m ³)	Grubbing Weight (kg)	Total Yield (kg)	Class 1 fruit >65mm (kg/tree)	Class 1 fruit 55-65mm (kg/tree)	Total Yield (kg/tree)	Class 1 fruit >65mm (kg/tree)	Yield efficiency (kg/cm ²)
QR193/13	25.7	14.6	16.9	25.0	5.4	13.6	91.8	32.9	1.74
QR517/9	31.3	19.2	23.9	37.3	5.4	20.8	129.7	26.6	1.60
QR530/18	22.2	10.3	10.2	13.3	0.2	3.7	69.5	10.0	1.73
QR708/36	30.4	17.7	22.8	32.4	3.8	18.4	143.4	24.3	1.96
QR719/3	29.2	17.3	20.5	30.5	8.4	15.9	126.0	49.0	1.85
EMA	23.8	13.5	14.3	22.2	0.9	11.6	83.2	19.4	1.87
EMC	22.9	11.8	12.8	23.5	1.5	11.0	103.7	25.2	2.54
SED (24 d.f.)	1.35	2.17	2.40	2.73	2.06	2.27	10.36	6.24	0.223
Significance ¹	***	**	***	***	**	***	***	***	**
LSD p=0.05	2.77	4.44	4.91	5.64	4.24	4.69	21.39	12.89	0.461

¹ *, ** and *** indicate rootstock effect significant at the 5, 1 and 0.1% level respectively.

Comments:

- None of the *Pyrus* rootstocks provided similar or lower vigour than the controls and none of them was considered sufficiently dwarfing to meet the current needs of the UK industry.
- QR517/9 (*Pyrus*) was significantly more vigorous and higher yielding than the controls but produced only similar yields of Class 1 fruit (55-65 mm and >65mm).
- QR708/36 (*Pyrus*) was more vigorous than EMA or EMC. It produced higher total yields than the controls but similar Class 1 fruit yield over the duration of the trial.
- QR719/3 (*Pyrus*) was more vigorous than EMA and EMC and had a higher cumulative yield but it was less yield efficient (YE) than EMC.

- QR193/13 (quince) was statistically of similar vigour to the controls and had a similar yield (in 2009 and cumulatively). It produced a higher Class1 >65 mm yield than EMA but the same as EMC.
- QR 530/18 (quince) had similar vigour to the controls but cumulative yields were the same or smaller than those of the controls. It performed well in 2008 (see EMRBC annual report 2008-09) with higher total yield and yield of Class 1 fruit (55-65mm and >65mm) than EMC but it did not stand out in 2009.
- None of the selections analysed so far was more YE than the controls.

The two younger trials planted in 2006, DM177 (*Pyrus* stocks) and DM178 (quince stocks) were evaluated for the third time in 2009 (Table 5 and 6) but it is still early to reach conclusions. Additionally the number of tree replicates available for each stock varies greatly which will complicate the analysis.

Table 5. The effects of *Pyrus* (and quince controls EMA and EMC) rootstocks on the growth and cropping of Conference pear trees in plot DM177.

Rootstock	2009 data					Cumulative data (2007-2009)		
	Girth (cm)	Tree Volume (m ³)	Total Yield (kg)	Class 1 fruit >65mm (kg/tree)	Class 1 fruit 55-65mm (kg/tree)	Total Yield (kg/tree)	Class 1 fruit >65mm (kg/tree)	Yield efficiency (kg/cm ²)
PQ34-1	10.1	3.4	3.0	1.5	0.8	3.0	1.5	0.38
PQ34-2	8.4	1.6	1.1	0.1	0.6	1.1	0.1	0.16
PQ34-3	12.3	4.0	6.5	1.7	2.7	7.1	2.0	0.59
PQ34-4	8.2	1.6	0.4	0.0	0.3	0.4	0.0	0.09
PQ34-5	9.6	3.9	2.5	0.3	0.5	2.6	0.3	0.33
PQ34-6	13.3	4.6	5.3	0.0	0.7	7.1	1.1	0.50
PQ35-1	8.8	1.8	1.9	0.0	0.7	2.2	0.2	0.35
PQ35-2	11.1	2.1	10.6	1.4	2.7	10.8	1.6	1.10
PQ35-3	3.5	0.0	0.9	0.4	0.0	0.9	0.4	0.92
EMA	12.5	5.3	4.0	1.2	1.6	5.2	2.1	0.43
EMC ¹	9.8	2.4	2.2	0.0	0.9	4.5	1.2	0.57
EMC ²	12.1	3.9	3.2	0.9	1.2	5.2	1.5	0.46
SED (38)	1.53	1.58	1.72	0.70	1.07	2.11	1.08	0.201
Significance ³	***	***	***	***	ns	***	**	***
LSD	3.10	3.20	3.48	1.41	2.17	4.26	2.18	0.408

¹ from Blackmoor nursery

² from Keepers nursery

³ *, ** and *** indicate rootstock effect significant at the 5, 1 and 0.1% level respectively, ns indicates no significant effect.

Table 6. The effects of Quince (including EMA and EMC) rootstocks on the growth and cropping of Conference pear trees in plot DM178.

Rootstock	2009 data					Cumulative data (2007-2009)		
	Girth (cm)	Tree Volume (m ³)	Total Yield (kg)	Class 1 fruit >65mm (kg/tree)	Class 1 fruit 55-65mm (kg/tree)	Total Yield (kg/tree)	Class 1 fruit >65mm (kg/tree)	Yield efficiency (kg/cm ²)
PQ-1	11.0	2.2	3.7	0.6	2.0	5.4	0.9	0.61
PQ-2	12.1	2.8	4.6	0.1	3.0	8.0	1.3	0.62
PQ-3	9.7	2.2	4.0	0.5	1.8	5.9	1.2	0.64
PQ-6	13.4	5.6	7.7	1.0	4.0	9.6	2.0	0.68
PQ-7	9.0	1.0	4.8	1.4	2.2	4.8	1.4	0.75
PQ-8	11.9	5.9	6.8	1.3	4.0	12.6	2.0	1.12
PQ-9	11.0	3.0	2.0	0.9	0.4	2.4	1.1	0.24
PQ-10	10.8	2.5	1.5	0.5	0.5	2.2	1.0	0.25
PQ-11	8.5	1.4	1.6	0.0	0.8	1.6	0.0	0.22
PQ-12	8.7	1.7	3.6	1.3	1.5	3.9	1.4	0.63
PQ-13	9.4	2.3	4.7	0.3	1.9	7.1	1.3	1.00
PQ-16	13.5	5.7	6.8	1.6	3.3	8.1	2.5	0.56
PQ-18	12.5	3.3	5.7	1.4	3.0	8.6	2.9	0.74
PQ-19	8.8	1.9	1.9	0.3	1.1	3.7	0.7	0.52
PQ-20	11.9	4.0	4.3	1.5	0.9	5.7	2.1	0.48
PQ-21	11.6	3.7	5.1	0.0	2.3	6.0	0.3	0.56
PQ-22	14.0	4.2	7.3	1.8	2.5	8.9	3.1	0.57
EMA	12.9	4.9	6.0	1.6	2.5	7.9	2.7	0.60
EMC ¹	10.1	3.3	3.8	1.1	1.4	6.4	2.6	0.72
EMC ²	12.7	2.9	5.1	0.7	2.0	9.5	3.0	0.71
SED (48)	1.63	1.51	2.27	0.97	1.64	3.48	1.57	0.268
Significance ³	***	**	*	ns	ns	*	ns	ns
LSD	3.27	3.03	4.56	1.94	3.30	7.00	3.15	0.539

¹ from Blackmoor nursery

² from Keepers nursery

³ *, ** and *** indicate rootstock effect significant at the 5, 1 and 0.1% level respectively, ns indicates no significant effect.

Comments on DM177

- PQ35-3 appears to be the least vigorous in the trial including all controls but only one tree is available.
- PQ34-2, PQ34-4 and PQ35-1 are less vigorous than the EMA controls having shown both smaller girths and tree volumes.
- PQ34-6 is the only selection statistically more vigorous than all the controls.
- So far, PQ35-2 seems to have a higher yield and yield efficiency than the controls but only one tree is available.

Comments on DM178

- PQ-7, PQ-11 and PQ-12 are less vigorous than the EMA controls having shown both smaller girths and tree volumes. PQ-13 and PQ-19 have also shown smaller girths than

EMA but the tree volumes are not significantly different.

- When compared to EMC from 'Keepers' nursery, PQ-7, PQ-11, PQ-12 and P-19 have produced trees with smaller girth but no significant differences have been seen in tree volume. None of the stocks was less vigorous than EMC from 'Blackmoor' nursery for either girth or tree volume.
- None of the selections showed statistically larger girths than EMA, whereas PQ-6, PQ-16 and PQ-22 had larger girths than EMC from 'Blackmoor'.
- There was no significant difference between the yields of the rootstock selections and any of the control rootstocks in 2009. Generally, this is the first proper set of cumulative data for this plot and shows no significant differences in the cropping performance of any of the selections compared to any of the standards for either yield or yield efficiency. However, PQ-9, PQ-10 and PQ-11 show smaller cumulative total yields compared to EMC from 'Keepers' nursery.

1.6. Dr Evans consultancy:

Dr Evans was contracted as a consultant to EMR in 2009 in order to facilitate the transition in the management of the programme to the current breeder. Her work concentrated in three areas:

1.6.1. Germplasm and crossing

Lists of rootstock germplasm were updated and annotated; allowing easy reference to a set of documents compiled from the Apple and Pear Breeding Club files. She also advised on the design of the 2009 crossing programme.

1.6.2. Selection

Advice and training in seedling selection criteria was given and, with EMR's breeder, progenies were scored and selections made as appropriate.

1.6.3. Literature review on marker assisted selection (MAS) in rootstocks

A literature survey was produced covering current opportunities for MAS in apple and pear rootstocks. This report is available in the EMRSC web page and summarized in section 1.7. of this report.

1.7. Use of DNA markers in the East Malling Rootstock Club.

Accurate pre-selection of new apple and pear rootstocks in breeding programmes is difficult and time consuming as there are few useful traits, such as disease and pest resistance, that can be tested relatively easily, whilst identifying differences in vigour and other important traits requires long-term trials. Breeders are constantly striving to identify faster and more efficient pre-selection techniques; molecular markers would appear to be the obvious tool, however markers linked to key rootstock traits have only recently become a focus of attention (primarily by the groups at East Malling Research, UK, Plant & Food Research, New Zealand and USDA, Geneva, New York). Currently markers have been identified for the following traits of interest:

- **Vigour:** Dwarfing in apple rootstocks appears to be polygenically controlled. However the first molecular markers linked to a major quantitative trait locus (QTL) influencing dwarfing in apple rootstocks have been identified in a progeny of 'M.9' x 'Robusta 5'. The dwarfing gene, *Dw1*, was mapped at the top of linkage group (LG) 5 but it did not account for the full variation of this trait, the markers were not tightly linked and one of them is not easily transferable to other populations. It should be possible to identify markers linked to the more complete dwarfing, such as that displayed by 'M.27', from the work currently ongoing at EMR. Other studies have concentrated on scion vigour but none of the markers identified are particularly useful for MAS at this time.
- **Root architectural traits:** Mapping of the prominent fine root formation typical of high performance apple rootstocks is ongoing and could be linked to tolerance to apple replant disease. Several significant loci modulating root architecture have been identified, in particular, a large QTL on LG 11 that explained approximately 40% of the genetic variation in the 'Ottawa 3' x 'Robusta 5' progeny.
- **Resistance to woolly apple aphid (waa):** Markers have been identified for several of the genes that confer resistance to waa, namely *Er1* (from 'Northern Spy'), *Er2* (from 'Robusta 5') and *Er3* (from *Malus sieboldii* 'Aotea 1'). As biotypes of aphids have been found that are able to overcome each of these resistances separately, the identification of these markers will enable the identification of selections with more than one gene (pyramided resistance) thus increasing the durability of the resistance.
- **Resistance to fire blight in apple:** A major QTL associated with fire blight resistance derived from the variety 'Fiesta' has been identified in LG7, it appears to be stable in different genetic backgrounds and explains ~ 38% of the phenotypic variation. A fire blight resistance QTL from 'Robusta 5' is located in LG 3 and accounts for 80% of the phenotypic variation in the progeny observed. Resistance QTLs have also been identified from *M. floribunda* 821 and the ornamental cultivar 'Evereste' on LG 12, explaining between 40% and 50-70% of the phenotypic variation.

- **Resistance to fire blight in pear:** Four putative QTLs from 'Harrowsweet' have been identified but the polygenic nature of this resistance is unclear as, together, they can only explain up to 50% of the variance.

More markers are available for traits mostly of interest to scions, such as resistance to mildew, scab and phytophagous pests such as aphids. These traits can also be important for rootstocks in the nursery rather than the orchard, particularly if production is organic. Several of these markers could be readily applied to a rootstock breeding programme and may prove useful for pre-selection; however, their use will depend greatly on the genetic background of the crosses to be tested and how closely linked each particular marker is. New gene-specific markers are highly likely in the near future not only from the mapping of segregating progenies but also from the application of the new apple genome sequence. Nonetheless, the costs involved in using linked markers for progeny pre-selection, at this stage, are not justified by the expected benefits. On the other hand, the use of DNA markers for fingerprinting could prove extremely useful for the management of any breeding programme.

An essential requirement for breeding programmes in the early stages of selection and multiplication for trialling is ensuring trueness-to-type of the germplasm. To do so on morphological traits alone is a major challenge as novel material has usually not been fully characterised at this stage and, in many cases, related germplasm (harder to distinguish) undergoes propagation concurrently. In recent years, Simple Sequence Repeats (SSR) markers, also known as microsatellites, have become the marker of choice for fingerprinting in plants. Many such markers are available for apple and pear and different research groups have used different marker sets. The EMR fingerprinting protocol for apple and pear follows the recommendations arising from the 2006 ECPGR (European Collaborative Programme for Crop Genetic Resources) workshop on fingerprinting, where international experts decided to nominate standard SSR sets to fingerprint, amongst other crops, pear and apple. Following discussions at the EMRSC technical meeting held at EMR in January 2010, it was decided that all rootstock selections entering propagation for preliminary trials will be fingerprinted. This will allow us to ensure that all rooted cuttings to be used as trial material or to establish stoolbeds are true-to-type. Controls used for comparison in the trials will also be fingerprinted and compared to EMR's database. Hopefully this will avoid future problems in data analysis.

1.8. EMRSC web page

The EMRBC internet site (www.emrootstockclub.com) is now live and new contents will be added regularly. In the future we hope to include results from INN trials on this page so all members can easily access this information as it is generated. Club members' suggestions on

further contents are welcome. This site 'hangs' from the EMR main page and contains a restricted area, for club members only, where contracts, reports and other relevant information can be found. To access this area, please contact the breeder (felicidad.fernandez@emr.ac.uk) for user name and password details.

2. Crossing programme planned for 2010:

2.1. Apple

The main aim of the apple programme is to introduce pest and disease resistance into the 'Malling breeding lines' with particular emphasis on resistance to fire blight and woolly apple aphid (waa) in order to produce resistant, dwarfing and/or semi-dwarfing rootstocks. We also aim to introduce heat tolerance and water use efficiency in combination with suitable nursery characteristics and appropriate vigour. In the next month we expect to carry out between four and seven controlled crosses using some of the following potential parents:

- **M.9:** dwarfing, fire-blight and woolly apple aphid (waa) susceptible, low chilling requirement
- **M.27** (M.13 x M.9): very dwarfing, waa susceptible, poor anchorage
- **M.M.106** (Northern Spy x M.1): semi-vigorous (~60-70% of seedling stock), precocious, heavy cropping, reasonable anchorage, good water use efficiency (wue), waa resistant, susceptible to mildew and collar rot.
- **M.116** (M.M.106 x M.27): semi-vigorous (~95% of M.M.106), very resistant to crown and collar rots, waa resistant, fairly good wue, low suckering
- **AR86-1-20** (M.M.106 x M.27): sibling of M.116, moderately vigorous, waa resistant, fire-blight susceptible
- **Budagovsky 9** (M.8 x Krasny Shtandard): selected in Poland, dwarfing (~M.9), precocious, winter hardy, fairly fire-blight resistant (according to recent research), collar-rot resistant, moderate resistance to mildew and scab in the nursery
- **Geneva 11** (M.26 x *Malus robusta* 5): dwarfing (~M.9), very precocious, good yield efficiency, adequate rooting, low suckering, no burr-knots, fairly resistant to fire-blight, moderately waa resistant.
- **Geneva 30** (*Malus robusta* 5 x M.9): semi-dwarfing (~M.7, ~55-65% of seedling stock), precocious, high yield efficiency, reasonable anchorage, winter hardy, collar rot and fire-blight resistant.
- **Geneva 202** (M.27 x *Malus robusta* 5): semi-dwarfing (~M.26, ~45-55% of seedling stock), high yield efficiency, waa resistant; crown rot and fire-blight resistant.
- **Hashabi (MH) 10.1:** selected in what is now Israel from the 1920s to 1940s, very good

heat tolerance, vigorous but the least invigorating of the Hashabi series (as classified by Prof. Raphael Assaf in 1980s), productive, some susceptibility to nematodes, fairly susceptible to mildew.

The planned crossing programme is as follows but it will be modified depending on the flowering times and blossom production of the different parents:

- M.116 (or AR86-1-20) x M.9
- Geneva 30 x M.116 (or AR86-1-20)
- M.M. 106 x Budagovsky 9
- Budagovsky 9 x M9
- M.M.106 x Geneva 202
- M.27 x MH10.1
- Geneva 11 x MH10.1

2.2. Pear

The main aim of the pear programme is to produce improved *Pyrus* stocks with a range of vigour to avoid incompatibility problems currently experienced between some pear cultivars and quince stocks. We also aim to move away from Asian pear germplasm which is very susceptible to pear decline whilst incorporating good disease resistance and other desirable characteristic (ease of propagation, precocity etc). In the next month we expect to carry out between two and four controlled crosses using some of the following potential parents:

- **PB 11-30**: very early, precocious heavy cropper
- **OHxF 87**: semi-dwarfing, precocious, promotes early spurring, slightly more dwarf than OHxF 97
- **OHxF 333**: more invigorating than BA29, resistant to fire blight and pear decline
- **'Junsko Zlato'**: very early ripening *P. communis* cultivar
- ***Pyrus betulifolia***: very vigorous, excellent rooting, winter hardy, resistant to fire-blight, leaf spot, and tolerant to pear decline and wet soils.
- **Bien Donne Pear 1 (BP1)**: selected in South Africa; dwarfing, heat tolerant, not very easy to root

The planned crossing programme is as follows but it will be modified depending on the flowering times and blossom production of the different parents:

- BP1 x *Pyrus betulifolia*
- 'Junsko Zlato' x OHxF 333
- PB 11-30 x OHxF 333
- OHxF 87 x BP1

3. Update on other science projects at EMR in 2009-10

EMR continues to carry out research commissioned by the UK government designed to benefit the fruit industry and intended to be disseminated as widely as possible. This section provides a short summary of two of those projects.

3.1. Defra project GC0140: Fingerprinting the UK National Apple & Pear Collections

This project 3-year project, completed in March 2010, aimed to optimise fingerprinting protocols for apple and pear and to provide Defra with information to facilitate the management of the UK National Fruit Collections (NFC). Defra holds these valuable genetic resources that comprise approximately 2200 apple and 560 pear accessions as well as other fruit crops.

In recent years, the use of DNA markers for characterisation of germplasm collections has become increasingly common. The markers most commonly used, known as Simple Sequence Repeats (SSRs), are areas of the genome of repetitive sequence (e.g. AT AT AT AT AT AT) and variable length (eg. 'AT AT AT' vs 'AT AT AT AT'). These variations in length can be detected in the laboratory and compiled to create a genetic 'fingerprint' of an individual tree. Such fingerprints are invaluable aids to the management of collections, e.g. when checking for trueness to type after propagation or for detecting likely duplicates. In addition, the determination of incompatibility (S) genotype by molecular methods is proving useful for fingerprinting Rosaceous tree fruits as well as providing agronomically useful data.

The main objective of this project was to use SSR markers to fingerprint the apple and pear accessions in the NFC thus enabling checking of identities and detection of synonyms. In total, 559 pear and 2,162 apple accessions were analysed with twelve SSRs chosen from the marker sets recommended by the ECPGR for each genus. A set of eight control genotypes for each crop were also included in the analysis to allow for the internal harmonization of data and to aid comparison of results with other studies. Analysis of the SSR data identified a total of 443 and 1,613 unique accessions of pear and apple respectively, with the remaining individuals having at least one other accession with identical SSR profile. A total of 43 and 193 groups of suspected duplicates were identified in pear and apple.

These fingerprints will prove to be an extremely valuable reference set for testing the trueness-to-type of the recently re-propagated pear collection and the soon to be re-propagated apple collection. The re-propagated accessions will be fingerprinted using the same methodology used in this study and the two data sets compared – thus avoiding in most cases the need for

laborious and time-consuming morphological comparison. Additionally, self(in)compatibility genotypes were determined for 1,696 apple accessions. A total of 48 pear and 304 apple accession have been confirmed as polyploids.

These same fingerprinting protocols are being applied by EMR in its commercial fingerprinting service and will be used to check trueness-to-type of the material produced and distributed by the EMRSC in future.

3.2. Defra project WU0115 ‘Genomics tools for preselection of water-use efficiency in top fruit rootstocks’

This four-year project started in 2008 and aims to identify molecular markers associated with water-use efficiency (WUE) in rootstocks to inform breeding and germplasm selection. The work revolves around an EMR rootstock mapping progeny that is expected to provide useful information in many traits of importance for commercial rootstock breeding such as dwarfing, rooting, anchorage in addition to WUE. The project is structured around five main objectives.

3.2.1.1. Determine the most suitable physiological measurements for screening WUE in the mapping progeny and develop a generic technique.

Seven commercial rootstocks representing a wide range of vigour have been screened over the last two years (both in pots and under field conditions) with a range of physiological measurements related to WUE potential. Biomass accumulated per litre of water transpired was identified as a particularly useful trait for screening rootstocks in 2008. Although there was some variation between experiments in carbon isotope composition, stomatal conductance, and midday water potential, certain rootstocks showed very consistent relative values for each of these traits, suggesting that they should also be included in a screening programme. Combining the results of both years, ‘M.M.106’ appears to be relatively water use efficient followed by ‘M.116’, while ‘M.M.111’ appears to be the least water use efficient rootstock studied.

3.2.1.2. Determine WUE of mapping progeny (‘M.27’ × ‘M.116’) seedlings with protocol optimised in Objective 1.

The seedlings from the mapping progeny are currently being propagated and screening will begin when sufficient numbers have been obtained to establish a replicated experiment.

3.2.1.3. To saturate the outline molecular map for the mapping progeny.

An outline map of this progeny, comprising 151 SSR markers, was produced as part of the previous Defra-funded project. Increasing marker density is essential for the identification of areas controlling genes of interest. In the last two years good progress has been made and the current map comprises 326 SSR markers. Additionally, more than 800 DaRT (Diversity Arrays Technology) markers have been screened in this progeny and are in the process of being incorporated into the map.

3.2.1.4. Identify markers linked to WUE.

This objective requires the joint analysis of phenotypic data from Objective 2 and molecular data from Objective 3 and consequently has not yet started.

3.2.1.5. Use WUE information on germplasm (Objective 1) to inform crossing programme for the EMRBC.

Crosses carried out for the EMRSC in 2009 and those planned for 2010 include M.M.106 and M.116 as parents, with the intention to develop breeding lines and selections of commercial interest and improved WUE.