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

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.

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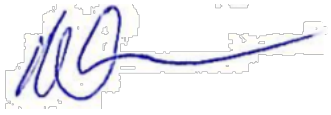
Signature  Date: 29 April 2019

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GROWER SUMMARY

Headline

NIAB EMR continues to breed and select improved rootstocks for apple and pear. Trials with our own selections as well as material from other breeding programmes are also ongoing. This project (2015-2020) encompasses the work covered in former project TF182 (breeding) and TF172a&b (trialling).

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 are also important in the nursery.

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

For UK growers, the AHDB involvement in the development of new rootstocks from NIAB EMR's programme will ensure material will be available to UK levy payers. The AHDB helps to 'steer' breeding objectives to meet the specific requirements of UK growers and ensures that appropriate newly selected rootstocks are trialled further before release to the UK industry.

INN has members in the USA, Chile, South Africa, Australia, New Zealand and throughout 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.

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

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

Summary of progress

Preliminary trials:

- Two new trials, one for apple and one for pear were planned for the 2018-19 winter
- Harvest of existing preliminary pear trial was once again not possible, due to lack of set in this plot which appears to be particularly prone to spring frost
- Vigour records were taken and only a couple of rootstocks appear to be in an interesting vigour range but this could be due to the lack of production in the trees, at least in part

Second stage trials:

- The 2014 trial evaluating Canadian apple rootstocks started to show interesting differences on both vigour and productivity traits. Several of this rootstock showed interesting vigour control and good yield efficiency in the reporting season. This trial is likely to be completed in 2019 or 2020. Verification of pest and disease resistance reports for this genotypes is also recommended
- The new EUFRIN apple rootstock trial was recorded for the first time although production on this first season was low in general
- In addition to the usual winter records, LiDAR was used for the first time in an effort to make tree vigour recording more accurate and cost-effective

Crossing programme

- More than 5,500 flowers were hand pollinated in spring 2018 of which approximately half corresponded to crosses of interest for genetic studies as well as breeding
- Nearly 3,000 apple and 2,670 pear seeds were sown to raise the 2019 seedlings populations

Selection

- Field records (vigour, crop load and suckering) were taken on existing apple and pear populations and a number of ‘fast-tracked’ preliminary selections were made in to speed up the pest and disease resistance evaluation of the apple pipeline

Pest and disease screening

- Preliminary results fire blight screening of pear selections were received from INRA. PQ34-3 appeared to be fairly resistant
- *P. cactorum* screening of hardwood cuttings was inconclusive but some progress is being made in the optimisation of a cut-shoot test. From 2019, at least part of the apple seedlings will be screened prior to potting with a zoospore spray

- Woolly apple aphid (WAA) populations thrived in the GH screen and some resistance breaking strains were detected but did not survive the winter

Financial Benefits

Although this is a very long-term project, there are major financial advantages to the development and selection of rootstocks with improved agronomic performance including reduced pruning and picking costs and the ability to grow material with reduced pest and disease susceptibility and AHDB support to this projects ensures that UK growers will have access to new UK-bred rootstocks.

Action Points

None at this point.

SCIENCE SECTION

Introduction

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 are also important in the nursery. Whilst there are few international breeding programmes generating tree-fruit rootstocks, NIAB EMR involvement in rootstock development dates back to its foundation with the subsequent release of the world-famous series of apple rootstocks; M. (Malling) and M.M. (Malling-Merton in collaboration with the, as was, John Innes Horticultural Institution).

In 2008, EMR (now NIAB EMR), the AHDB tree fruit panel 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. The programme has been renewed several times since and current funding incorporating breeding, preliminary trialling and UK second stage trials runs until 2020.

For UK growers, the AHDB also acts as the UK licensee for the EMRC with the intention of making new rootstocks released from EMR's programme, widely available to UK levy payers. AHDB also helps to 'steer' breeding objectives to meet the specific requirements of the UK growers and ensures that newly selected rootstocks are trialled further before release to the UK industry.

INN has members in the USA, Chile, South Africa, Australia, New Zealand and in the EU (represented by EVI). 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 is not unusual for new rootstock to take 30-35 years. Selection of parental material, crossing, seedling selection and first stage trialling, which are carried out at NIAB EMR, takes around 10 years. Promising material is then propagated and released for HDC-funded trials in the UK and INN-funded trials at appropriate sites around the rest of the world. As trial results accumulate, validating which selections are most promising, these rootstocks are then propagated to build up sufficient material for distribution before it is possible to co-ordinate effective 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 to identify a range of apple, pear and quince rootstocks with desirable size control, precocity and productivity, with resistance to diseases and pests where applicable.

Aims and objectives

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

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

INN partners in 2018 further clarified the importance of focusing on combined pest and disease resistance. They ranked the importance resistances as 1) fire blight (FB); 2) woolly apple aphid (WAA); 3) collar/crown rot (Pc) and replant disease (ARD); 4) European apple canker and stated that they would not consider the commercial release of any genotype without significant tolerance or resistance to FB and WAA; whilst these are not as critical in the UK as in other regions the TF panel understands the risk of severity for both to be increasing and the importance of international commercial value in new rootstocks. Based on these priorities, a number of selections in the pipeline will be deselected in 2018-19. Similarly, some families in the programme will be discarded and others re-classified as germplasm for pre-breeding (e.g. pyramiding of FB resistances or introducing new sources of resistance).

Materials and methods

The breeding programme is an ongoing effort of which different steps are briefly described below. More detailed methodology is included in the relevant part of the yearly update if necessary.

Preliminary trials

After one or two years of growth in pots, selections are grafted with a common scion (currently ‘Gala’ for apples and ‘Conference’ for pears) and established in replicated trials that include standard commercial rootstocks for control purposes.

In these trials tree vigour is assessed by the measurement of tree volume in the form of the height and spread of the tree crown and by the recording of trunk girth at 15 cm above ground level; where appropriate, fresh weights at the time of grubbing are 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 (kg per cm^2 of cross section) are calculated. Records are taken on tree health, graft compatibility and anchorage.

Traditionally, rootstock trials at NIAB EMR have not been pruned other than to remove suckers after recording. However, this has not led to the best agronomical evaluation of the new selections. After discussions with the EMRC executive management committee and the HDC tree fruit panel, it was decided to correctively prune ongoing trials in February 2015 and to follow a conservative pruning strategy more in line with commercial orchard practice thereafter. This year, pruning weights are being recorded in the beginning of April, 2019.

The best selections after this preliminary evaluation are subsequently propagated to enter the second stage trials funded by AHDB Horticulture under this same project (TF224) in the UK and by INN overseas.

Second stage trials (previously under AHDB Horticulture project TF172a&b)

The second stage trials are performed as described for preliminary trials but usually with greater level of replication as more material is available per genotype and, in the case of apple, can involve more than one scion cultivar. During the reporting year the apple trial SP250, with Canadian rootstocks, and a multisite (EUFRIN-coordinated) trial with 18 rootstock genotypes were evaluated.

The tree vigour of the two second stage trials was assessed both manually, as described above for the preliminary trials, but also by the means of imaging by an unmanned aerial vehicle (UAV) and by LiDAR. LiDAR is a technology that uses pulsed laser light to measure the distance between a target and a sensor to make image representations of an object. The second stage trials were imaged by LiDAR and UAV imaging in September 2018. The UAV imaging was conducted by the company Outfield Technologies. A comparison of estimated tree vigour from manual assessments and LiDAR imaging will be presented in this report.

Image analysis of trial data

Raw input image files from the software 'LA Recorder' were pre-processed in the software 'LA Decision Support' to separate trees from different rows by colour. The tree crown area of each tree was thereafter determined in the software ImageJ (ImageJ 1.x, available at https://imagej.net). Trees in the row closest to the LiDAR were selected based on a colour threshold. Each tree was thereafter subsequently marked and the area of selected colour calculated through the function 'Analyze Particles'. The support stakes in the field were used as a reference size in the pictures.

Statistical analysis of trial data

All statistical analyses were conducted in the software R (https://www.R-project.org).

The differences between rootstocks for traits measured in 2018 were analysed with a linear mixed-effect model approach.

Differences between rootstocks in the trial 'SP250' were analysed using a mixed linear model. The analysis was conducted using the 'lmer' function in the R package 'lme4'. In the mixed-effect model, rootstock and scion were included as fixed effects in the analysis of SP250, whereas block were included as random effects. The interaction term between rootstock and scion was excluded based on model selection. In the EUFRIN trial, rootstock and ARD-treatment were included as fixed effects, whereas block was included as a random effect. The best linear unbiased estimate of all traits were obtained from the fixed effect of rootstock from the same model. The effect of rootstock in the trial RF187 was analysed using anova (function:aov), with rootstock and block as factors.

The normality of the residuals from the model were checked through a Shapiro-Wilk normality test (function: 'shapiro.test') and Q-Q-plots. Traits which had non-normally distributed residuals were either log or square root-transformed for numeric and integer traits, respectively. A few traits did not have normally distributed residuals after transformation and differences between rootstocks were therefore analysed with a Kruskal-Wallis test (function: 'kruskal.test').

Breeding activities:

Crossing

Parental genotypes that carry one or more phenotypic traits of interest are selected and a crossing programme is designed aiming to combine those desirable characteristic 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 the 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 for until the following January.

Raising seedling populations

Seeds are stratified in the cold-store (between 2 and 4 °C) in trays of moist compost and perlite mix for 16 weeks. After this period, seed trays clearly labelled with progeny numbers are placed in a glasshouse (at ~ 18°C) for germination. Individual seedlings are potted and labelled as they become large enough to handle safely and grown on for around two 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 standards rootstocks are interspersed in the seedling population as controls. Rootstocks ‘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. Seedlings are budded with a common scion 12-13 months after planting and the controls are bench grafted the winter after that and planted in the field during the second summer in the field of the seedling population.

Records on bud take and production of suckers are taken in the first two years of the population and, thereafter, for the three to four years, seedlings are evaluated with regards to vigour and suckering. As the common scion comes into fruit, crop load and fruit size are recorded and any other differences attributable to the rootstocks (e.g. incidence of bitter-pit) are noted if significant as is pest and disease incidence (in the suckers or crown) and any other detrimental characteristic observed (e.g. burr-knots, brittle wood, poor anchorage, etc.). The most promising seedlings on each population are selected for propagation usually five or six years after planting.

Propagation

Interesting seedlings are selected and marked out with tape in the field during 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 earthed-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 and unhealthy selections can be discarded (e.g. severe mildew infection or infestation by woolly apple aphid on families segregating for resistance)

Hardwood cuttings (ideally ~ 30 cm in length) are taken of these selections at the beginning of December and dipped in 0.5% (Indole-3-butryic acid) IBA solution for 5 s prior to insertion into a heated cutting bin to a depth of 6 to 8 cm. The cutting bin consists of 30 cm layer of a 1:1 mixture of peat and fine bark over a 5 cm layer of coarse sand. A soil warming cable maintains bed temperature at 25ºC. Air temperature is cooled via ventilation to outside. Cuttings are left until rooted and then potted into 2 L pots, in late January or early February and grown on in unheated glasshouse. Ease of propagation is also a key selection criterion and recalcitrant selections are discarded.

Pest and disease resistance screening

Fire-blight (FB)

Usually, fire-blight inoculations were carried out for apple genotypes in Agroscope (CH). This service was discontinued in 2017 so not testing could be carried out in the reporting year. Seven pear genotypes (5 EM selections, 'Old Home' and 'BP1') were included free of charge in the routine screening at INRA Angers (France). The full protocol for this testing was not provided to us and the results we present are only tentative.

Woolly apple aphid (WAA)

Colonies of *Eriosoma lanigenum* (WAA) collected from the field/glasshouse or from plant material maintained over winter in the cold store at East Malling in Kent are used to challenge rooted cuttings in the glasshouse. Aphids are added to each tree 2-3 times between June and September, depending on the season. Scoring is carried out at the end of the growing season. Individuals will be considered resistant if WAA failed to establish colonies and susceptible if they have succeeded. Genotypes considered resistant will usually be re-tested in a second season for confirmation as will any selection presenting conflicting results amongst replicates.

Phytophthora cactorum:

Development of a glass house pot-based test for determining susceptibility to *P. cactorum* continued in 2018, using the same protocol described in the 2016/2017 annual report. The experiment used hardwood cuttings from a number of rootstock genotypes rooted in early 2018. These were randomised and potted up in extra-large root trainers in July and moved into a glasshouse compartment with temperature control. Inoculation was performed in August using three different isolates of *P. cactorum* (mixed and separate) previously grown and re-isolated from apple. Each hardwood cutting was inoculated with 15 ml of inoculum consisting of $2x10^{-4}$ zoospores suspended in diluted compost extract. In the case of testing a mixture of *P. cactorum* isolates, three or four replicates per genotype were inoculated with a mixture of zoospores from three *P. cactorum* isolates (P295, 62471, and R36/14; ratio 1:1:1). For each genotype, one replicate mock-inoculated with diluted compost extract was used as a control. Where enough hardwood cuttings were available for a genotype, additional replicates were inoculated with inoculum containing zoospores from only one of the three isolates. Disease symptoms were recorded in October. This involved scoring of symptoms in the stem area of inoculation (measuring the length of any visible lesions) as well as recording root colour and production of new roots.

In addition, we have initiated the development and validation of a cut-shoot inoculation protocol in collaboration with Dr Charlotte Nellist and Mr Matteo Luberti (PhD student at NIAB EMR). In brief, the protocol involves the following: 1) cut shoots (~22cm long) from

different genotypes are surface-sterilised using a bleach solution, rinsed and the cut ends sealed with wax. 2) Shoots are then randomised, wounded and inoculated with agar discs containing mycelia of an individual *P. cactorum* isolate. At least three replicates per each genotype-isolate combination are inoculated. 3) Controls consist of cut shoots mock-inoculated with agar discs that contain no mycelia. 4) Following inoculation, cut shoots are suspended horizontally in a tray containing moist paper. Trays are then sealed with plastic and placed in a controlled environment growth room at 20°C in 18hr/6hr light/dark cycles. 5) Disease symptoms are recorded after 4 weeks, which involves measuring any visible lesions. The cut shoot protocol has been tested in a limited number of genotypes thus far (on dormant shoot) and it will be tested on both dormant and active shoots of a wider range of genotypes in 2019. If it proves sufficiently discriminating and reproducible, this protocol would allow us to test a large number of genotypes rapidly.

Results

Preliminary Trials

Pear trial, 'Conference' (RF187)

The pear trial was planted in plot RF187 in 2014 and contains 10 selections of pear rootstocks, all planted with 'Conference' as a common scion. 'Quince A' is included as a standard in the trial. Although the trees in this trial flowered during spring 2018, they did not produce any crop, which was likely due to a late frost. The correlation between Trunk Cross-Sectional Area (TCA) and tree volume was lower for this pear trial than for the apple trials (R^2=0.62), TCA also had a wider distribution compared to the distribution in the apple trials.

Quince A was the least vigorous rootstock of all rootstock genotypes in the trial, both in terms of TCA and tree volume. PQ37-2 was slightly more vigorous than Quince A.

Table 1. Mean (±standard error) for number of suckers, TCA and tree volume for the pear rootstocks planted in RF187

Rootstock	Number of suckers	Trunk cross sectional area (TCA, cm^2)	Tree volume (m^3)
PQ37-2	0.8 (±0.8)	11.2 (±6.5)	3.55 (±2.5)
PQ37-3	2 (±0.7)	12.0 (±2.5)	5.31 (±0.5)
PQ37-5	0.3 ±(0.3)	27.5 (±10)	6.52 (±3.5)
PQ37-7	0.5 (±0.5)	16.8 (±2.9)	3.16 (±0.9)
PQ37-8	0 (±0)	16.4 (±3.9)	5.10 (±2.2)
PQ38-2	0.3 (±0.3)	18.4 (±3.4)	6.46 (±3.1)
PQ39-1	0.5 (±0.5)	21.1 (±7.3)	6.16 (±3.14)
PQ39-3	1 (±0.5)	17.9 (±7.8)	4.51 (±2.9)
PQ39-4	0 (±0)	18.4(±7.7)	4.20 (±1.7)
PQ39-5	0 (±0)	21.6 (±6.0)	4.36 (±1.5)
Quince A	0 (±0)	8.05 (±5.9)	1.34 (±1.3)
P-value for effect of rootstock (d.f. 10)	0.09[1]	0.9	1.0

[1]Trait analysed with Kruskal-Wallis test due to non-normal distribution of residuals. Degrees of freedom for rootstock are 10 Chi-square=16.2

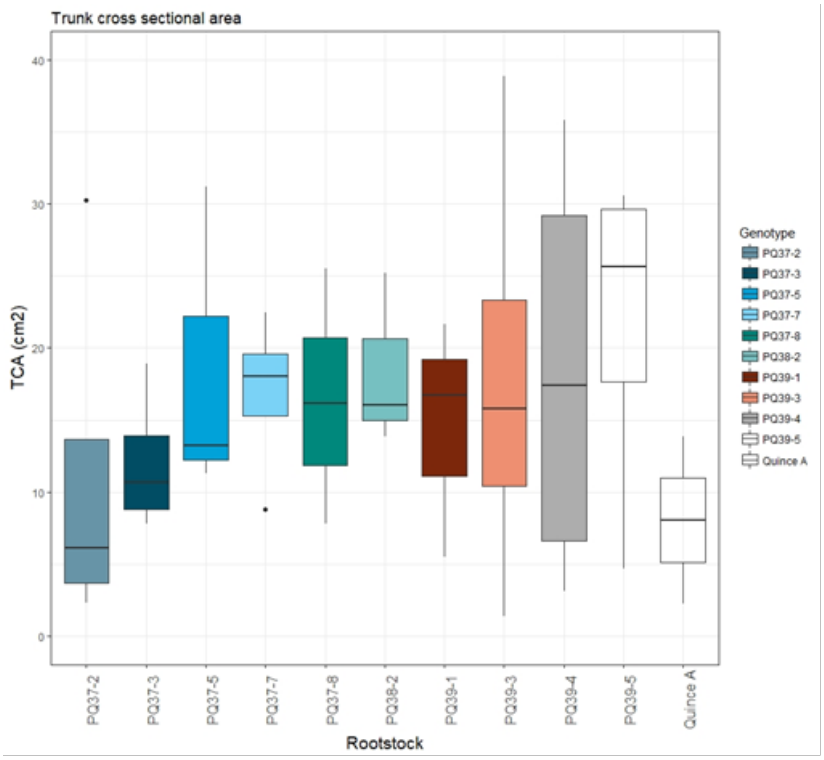

Figure 1. Boxplot of trunk cross-sectional area (TCA) for pear rootstocks in the trial RF187

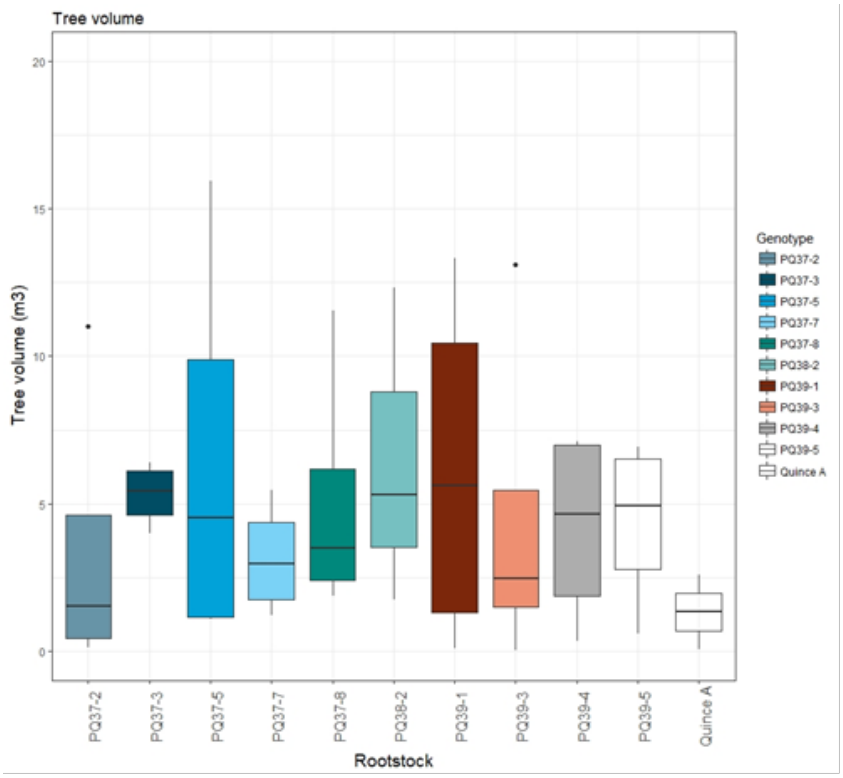

Figure 2. Boxplot of tree volume for pear rootstocks in the trial RF187

Pear trial, 'Conference' (SP257)

For all genotypes selected for inclusion in the preliminary pear rootstock trial, roots were collected from rootstocks with successful grafts for DNA extraction and trueness-to-type (TTT) testing. Grafted rootstocks that were determined to be healthy and true-to-type were planted in December 2018 (Table 2).

Table 2. Replicates confirmed as true-to-type (TTT) for inclusion in the preliminary pear rootstock trial planted in 2018

Genotype / Selection	Inclusion in trial	Number of TTT trees included in trial
PQ34-3	Trial	5
PQ35-2	Trial	5
PQ35-3	Trial	3
PQ37-1	Trial	5
PQ34-1	Trial	5
PQ39-2	Trial	5
PQ39-6	Trial	5
PQ39-7	Trial	5
PQ41-9	Trial	5
PQ42-33	Trial	5
PQ42-47	Trial	5
PQ43-34	Trial	4
PQ44-11	Trial	5
PQ44-26	Trial	5
QC*	Trial (control)	5 (not genotyped)
Q-ELINE*	Use as guard in trial	2 (not genotyped)
QA*	Use as guard in trial	2 (not genotyped)

*As provided by commercial nursery; presumed to be true to type

Apple trial, 'Gala' (plot code to be assigned post-planting)

For all genotypes selected for inclusion in the preliminary apple rootstock trial, roots were collected from rootstocks with successful grafts for DNA extraction and TTT testing. A number of rootstock genotypes were excluded as trial entries due to pest and or disease resistance susceptibility (Table 3); others due to lack of sufficient healthy grafted trees. Several genotypes will be kept under observation as guard for potential use as parental material. The trials will be comprised of the remaining healthy, TTT successful grafts (Table 3) and will be planted in early spring 2019.

Table 3. Replicates confirmed as true-to-type (TTT) for inclusion in the preliminary apple rootstock trial to be planted in 2019.

Genotype /Selection	Inclusion in trial	Number of live trees confirmed as TTT
M.116	Trial (control)	8
M.27	Trial (control)	3
M.9	Trial (control)	2
M432-203	Use as guard in trial (no mayor FB[1] resistance, WAA[2] resistant)	5
M432-217	Discard (no mayor FB resistance, WAA response inconclusive)t	-
M480-3	Use as guard in trial (no mayor FB resistance, WAA resistant)	5
M482-11	Discard (no mayor FB resistance, WAA response inconclusive)	-
M482-13	Use as guard in trial (no mayor FB resistance, WAA resistant (TBC[3]))	5
M482-158	Discard (no mayor FB resistance, WAA susceptible)	-
M508-1	Trial entry (confirm resistances through inoculation)	8
M508-49	Trial entry (confirm resistances through inoculation)	7
M509-22	Use as guard in trial (FB unknown, WAA response inconclusive)	5
M546-22	Re-propagate (FB resistance TBC; WAA resistant; no live grafted trees)	-
M546-110	Use as guard (FB resistance TBC; WAA resistant; insufficient trees).	2
M547-1	Re-propagate? (FB resistance TBC; WAA susceptible)	-
M547-8	Trial entry (confirm resistances through inoculation)	4
M547-41	Trial entry (confirm resistances through inoculation)	7
M547-72	Discard (FB resistance unlikely, WAA susceptible)	-
M549-83	Trial entry (confirm resistances through inoculation)	8

[1] FB; Fire blight
[2] WAA; woolly apple aphid
[3] TBC; to be confirmed

Second Stage Trials

Canadian rootstock selections (SP250)

The trial SP250 was planted in 2014 and contains six Canadian rootstock genotypes and the four standard varieties ‘M27’, ‘M9’, ‘M26’ and ‘MM106’. The trial is under conventional management with ‘Gala’ and ‘Braeburn’ as scions. Due to errors during propagation, the different rootstocks are planted in uneven sample sizes. The trial is therefore statistically analysed with a mixed linear model.

The fruit was harvested, graded and weighed in September (Gala) and October (Braeburn) 2018 and the breakdown of fruit yield per fruit size averaged over the two scions ‘Braeburn’ and ‘Gala’ is presented in Figure 3.

The girth and volume of the trees were measured in December 2018. The average vigour effect of the M9 rootstocks in this trial is slightly higher than M26, which should be taken into account when M9 is compared to the other rootstocks in this trial.

SJM15

SJM15 was only grown with ‘Gala’ as a scion, therefore all estimate means are based on the rootstock’s performance with this particular scion. This rootstock has a similar tree volume and TCA to M27 (Table 5), but had a higher estimated yield and yield efficiency than M27. SJM15 also had the highest estimated mean yield efficiency of all the rootstocks in this trial both when measured as a function of tree volume and TCA. However, the rootstock produced a high proportion (35%) of apples below 65 mm (Table 4). The tendency of SJM15 to produce small fruit was also noted in the harvest of 2017.

SJM127

SJM127 was only grown with ‘Braeburn’ as a scion, therefore all estimate means are based on the rootstock’s performance with this particular scion. The rootstock has a similar effect on vigour to M26 both measured as tree volume and as TCA. SJM127 yielded almost twice as much as M26 (15 and 27 kg/tree for M26 and SJM127 , respectively), and therefore had a better yield efficiency both when expressed as kg yield/tree volume and kg yield/TCA. SJM127 also had a better yield efficiency than M9 and MM106, but it was lower than for M27.

SJM167

The SJM167 rootstocks produced trees with a higher estimated mean tree volume and TCA than M26. The tree volume was 1.7m^3 larger than for M26 and the TCA 1.2 cm^2 larger. SJM167 also yielded more than M26, and had higher yield efficiency (as per tree volume and TCA). SJM167 had a high proportion (44%) of fruit that was graded as above 75mm in diameter, however M9 had a similar proportion of large fruit in this trial (46%).

SJM188

The trees with SJM188 as a rootstock were slightly less vigourous than M26. The yield efficiency of SJM188 was higher than for M9, M26 and MM106 (Table 4).

SJP84-5162

SJP84-5162 was only grown with 'Braeburn' as a scion, therefore all estimate means are based on the rootstock's performance with this particular scion. Trees on the SJP84-5162 rootstock had a smaller estimated mean tree volume than M27, but a larger TCA (Table 5). In the tree volume measurements from 2017, SJP84-5162 was larger than M27 and only had a slightly smaller tree volume than M26 (6.9, 3.9 and 7.2m^3 . The rootstock might therefore have been pruned harder than the others during winter 2017-18. SJP84-5162 had an estimated mean yield of 17.5 kg/tree and a higher yield efficiency than an estimated was higher than M9, M26 and MM106. This rootstock had a higher mean incidence of bitter pit than any of the other rootstocks, with an average score of 2 on a scale from 1-5 where 2 indicates that less than 5% of all fruit have symptoms of bitter pit. However, there was no statistically significant effect of rootstock on the incidence of bitter pit and SJP84-5162 did not produce fruit with higher bitter pit incidence in the previous year.

SJP84-5174

The trees planted with the rootstock SJP84-5174 had a higher tree estimated mean volume than M26 (2.7m^3 larger), but were very similar in TCA (Table 5). The yield efficiency of SJP84-5174 was 0.3 kg/m^3 higher than for M26 but had the same yield efficiency when measured as kg/cm^2

SJP84-5217

SJP84-5217 is very similar to SJP84-5174 in tree volume and trunk size (Table 5). It did, however, have an estimated mean yield of 1.9 kg more fruit per tree than SJP84-5174 (Table4). It therefore had a higher yield efficiency (as per tree volume and TCA) than the standard rootstocks M9, M26 and MM106.

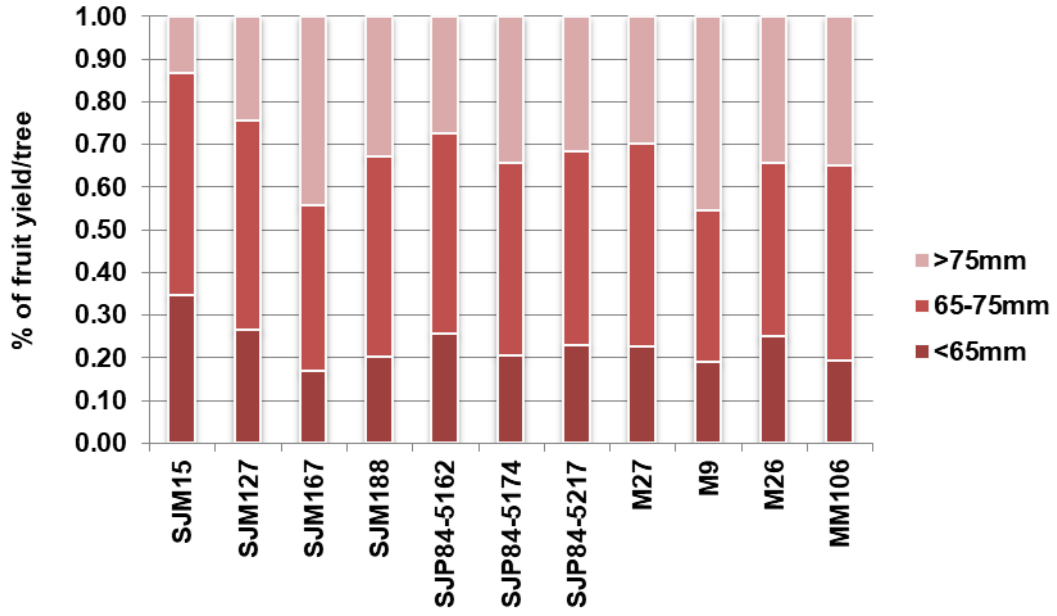

Figure 3. Percentage of fruit yield by fruit size averaged over the two scions 'Braeburn' and 'Gala' except for rootstock SJM127 (planted with 'Braeburn') and SJM15 (only with 'Gala')

Table 4. Estimated means of fruit yield and number of fruit per tree by fruit size class. The means are estimated from the linear mixed model and averaged over the level of scion

Rootstock	Fruit yield by size (kg/tree)			Number of fruit by size (No./tree)		
	<65 mm	65-75 mm	>75 mm	<65 mm	65-75 mm	>75 mm
SJM15	5.8	13.1	0.9	88	95	4
SJM127	4.8	16.4	4.0	60	115	22
SJM167	1.5	8.0	10.4	25	52	51
SJM188	2.0	10.6	5.2	30	70	25
SJP84-5162	2.7	9.2	3.1	37	61	15
SJP84-5174	2.1	10.4	5.9	31	68	28
SJP84-5217	2.7	10.4	5.0	35	69	24
M27	1.7	7.2	2.9	23	48	14
M9	1.7	5.8	9.7	30	37	47
M26	2.3	6.1	4.3	38	40	22
MM106	2.1	11.7	6.8	28	78	34
P-value for effect of rootstock[1]	0.08	0.17	0.07	0.09	0.11	0.07
Chi-square$_{(6,16:10)}$[4]	16.8	14.2	17.0	16.3	15.6	17.3
P-value for effect of scion[1]	0.34	0.94	0.61	0.04 *	0.63	0.94
Chi-square$_{(15,16:1)}$[2]	0.9	0.0061	0.26	4.10	0.23	0.0058
P-value for effect of scion:rootstock[1]	1	0.34	0.14	1	0.35	0.22
Chi-square$_{(14,15:1)}$[2]	0	0.89	2.14	0	0.87	1.50

[1] *, ** and *** indicate the significance level at 5, 1 and 0.1% respectively.
[2] Numbers within brackets indicate the following degrees of freedom; (null model, alternative model: difference between models)

Table 5. Estimated means for six Canadian rootstock selections as well as four standard rootstock varieties. The table also shows the p-values for the effect of rootstock, scion and rootstock:scion interaction from a linear mixed model. For bitter pit and number of suckers, the arithmetic mean is shown

Rootstock	Yield (kg/tree)	Number of fruit per tree	Bitter pit (1-5)	Number of suckers per tree	Tree volume (m^3)	TCA[1] (cm^2)	Yield efficiency	
							As per TCA (kg/cm^2)	As per tree volume (kg/ m^3)
SJM15	18.9	179.8	1.8	0.0	6.9	11.5	2.0	3.3
SJM127	27.0	211.1	1.8	0.0	11.1	17.5	1.6	2.5
SJM167	21.6	143.6	1.8	0.0	12.1	18.8	1.2	2.0
SJM188	19.3	137.9	1.7	0.0	9.9	15.1	1.3	2.1
SJP84-5162	17.5	131.2	2.1	0.0	6.7	13.0	1.3	2.6
SJP84-5174	20.3	147.8	1.6	0.0	13.1	17.6	1.2	1.7
SJP84-5217	22.2	152.7	1.8	0.0	13.7	17.3	1.4	1.8
M27	14.0	97.3	1.4	0.2	7.5	10.1	1.7	2.8
M9	19.5	130.9	1.7	0.1	14.1	17.9	1.1	1.5
M26	15.0	115.6	1.7	0.2	10.4	17.6	0.9	1.7
MM106	22.7	154.5	1.7	0.0	19.2	26.9	0.9	1.1
P-value for effect of rootstock[2]	0.04 *	0.046 *	0.45 (KW)[3]	0.42 (KW)[3]	3.51e-06 ***	1.19e-06 ***	0.004 **	0.0001 ***
Chi-square$_{(6,16:10)}$[4]	19.4	18.6	9.96	10.2	43.8	46.5	25.7	35.3
P-value for effect of scion	0.99	0.94	0.03 * (KW)[3]	0.93 (KW)[3]	0.04 *	0.0004 ***	0.02 *	0.06
Chi-square$_{(15,16:1)}$[4]	0	0.005	4.6	0.006	4.26	12.4	5.18	3.37
P-value for effect of scion:rootstock	1	1	0.2 (KW)[3]	0.13 (KW)[3]	1	1	0.82	1
Chi-square$_{(14,15:1)}$[4]	0	0	22.6	24.5	0	0	0.04	0

[1] Trunk cross sectional area; [2] *, ** and *** indicate the significance level at 5, 1 and 0.1% respectively; [3] Difference in trait analysed with Kruskal-Wallis test due to non-normal distribution of residuals. Degrees of freedom for rootstock, scion and rootstock:scion interaction are 10, 1 and 18 respectively; [4] Numbers within brackets indicate the following degrees of freedom; (null model, alternative model: difference between models)

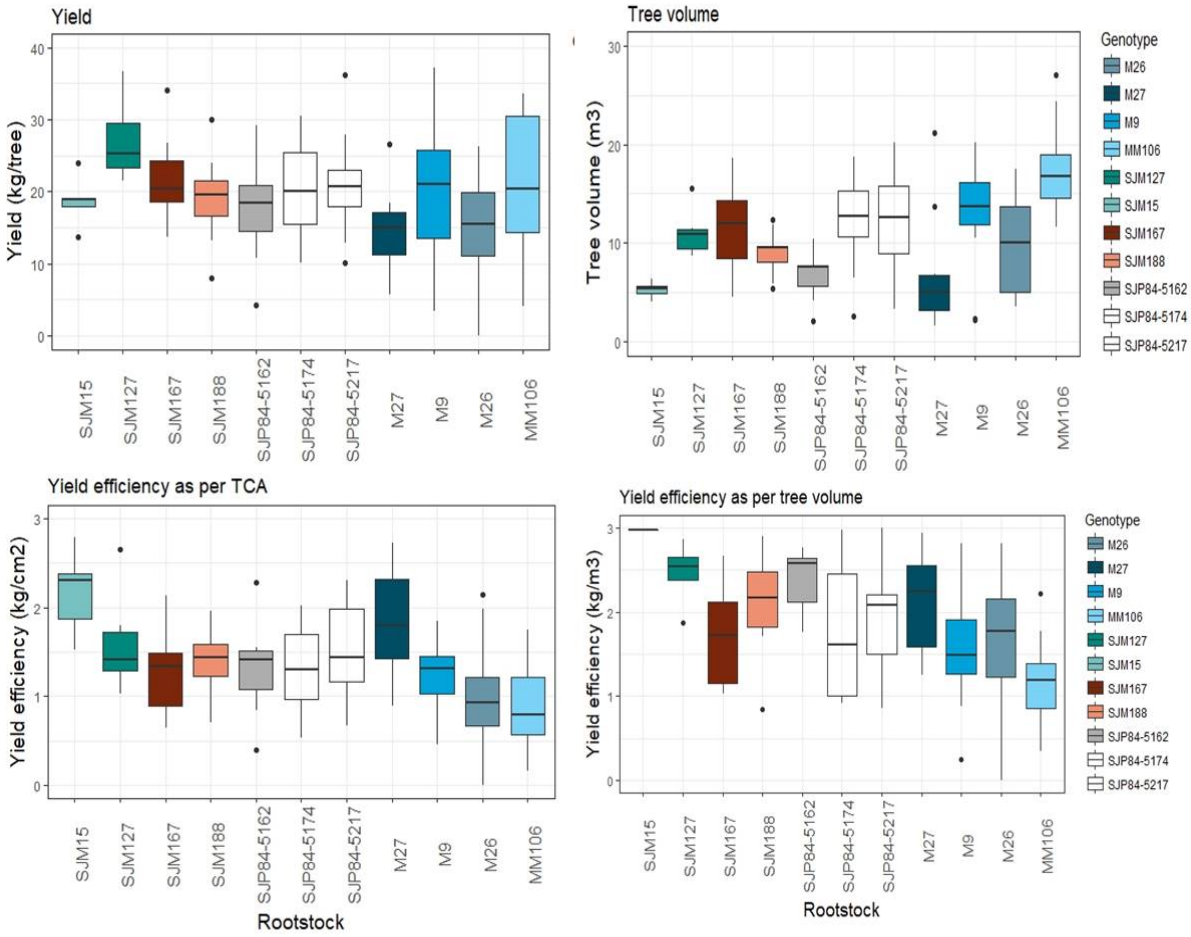

Figure 4. Boxplots of yield, tree volume and yield efficiency as a function of Trunk Cross-sectional Area (TCA) and tree volume across the two scions 'Braeburn' and 'Gala' except for rootstock SJM127 (planted with 'Braeburn') and SJM15 (only with 'Gala')

EUFRIN multisite apple rootstock trial

In 2017 NIAB EMR became one of the sites for the new international rootstock trial coordinated by the European Fruit Research Institutes Network (EUFRIN) which will evaluate rootstocks in 19 sites from Norway to Spain. Six of the 18 apple rootstocks planted in these trials in 2017 are from the EMRC (Table 6).

The trial was designed to compare a subset of the genotypes under apple replant conditions. To this aim, the whole trial was planted in the plot of a recently grubbed apple orchard. The non-apple replant condition blocks were treated with granular Basamid® (soil sterilant containing 97% dazomet) as per common practise in many areas of continental Europe. The trial is divided in two sub-trials: one for genotypes of a comparable vigour to M.9 or smaller (plot CW152) and another for genotypes of similar or more vigorous than M.26 (plot CW153). All rootstocks within this trial have the 'Gala' mutation 'Galaval' as a scion. G.11 was included in both trials.

The fruit was harvested, graded and weighed in September. The girth and volume of the trees were measured in December 2018.

Table 6. Number of trees of each genotype planted in the EUFRIN trial at NIAB EMR in 2017 in each of the two field plots (CW152 and CW153) according to expected vigour.

Genotype	CW152 (~ M.9 size)		CW153 (> M.26 size)	
	Treated	ARD[1]	Treated	ARD[1]
AR10 3 9			12	
AR295-6	12	12		
AR486-1	12			
AR680 2	12			
AR835-11	12			
B10/MITCH N°62396	12	12		
G11	12	12	12	
G202			12	
G41	12	12		
G935			12	12
N°3038	12			
P67	12	12		
M.9 (Pajam2)	12	12		

[1] ARD = Apple Replant Disease (= not treated with Basimid®)

A multisite trial for pear and quince rootstocks for pear is planned to be planted in 2019. The trial will include 4 rootstocks, of which two are quince selections from the NIAB EMR breeding programme; QR196-9 and QR530-11. The trial will be planted in six countries in addition to East Malling.

EUFRIN: M9 sized rootstocks

Six of the rootstocks that were included in this subset of the trial were planted in both apple replant plots and in plots treated with a soil sterilant. The statistical significances of the effect of replant conditions on yield and vigour traits are shown in Table 7. ARD

condition had a statistically significant ($p<0.01$) effect on yield, with the apple replant conditions having a 0.9 kg/tree lower estimated yield than treated plots. Apple replant also had a significant effect on the number of suckers produced. However, the estimated number of suckers in ARD plots was only 1 more than for treated plots. The estimated yield efficiency as per TCA was lower for the untreated ARD plots, with trees that were 0.19 kg/cm^2 less productive than the treated plots. There was no statistically significant interaction between ARD condition and rootstock for any of the analysed traits. Thus, all rootstocks were similarly affected by apple replant conditions during this initial stage and none of them showed any particular tolerance or susceptibility to such conditions thus far.

N°3038

N°3038 had the smallest estimated tree volume of all the rootstock in the trial (Table 7). The estimated yield was 4.1 kg of fruit per tree, and N°3038 had the highest yield efficiency of all rootstocks. The majority of the rootstocks produced apples that had developed >95% over-colour. However, as seen in Figure 5, N°3038 had occurrences of lower percentages of over-colour.

AR295-6

Bred at East Malling from a cross between Ottawa 3 x *M. robusta* 5. Susceptible to WAA and fairly tolerant to fire blight. AR295-6 was one of the rootstocks with the smallest estimated tree volume and TCA. In comparisons to the other rootstocks it had a good yield efficiency, both in the terms of yield/volume and yield/TCA. AR295-6 had a lower median percentage of over colour than any of the other rootstocks (Figure 5).

AR486-1

Bred at East Malling from a cross between Ottawa 3 x M7. Susceptible to fire blight and woolly apple aphid.

AR680-2

Bred at East Malling from a cross between M26 x M7. The rootstock is moderately susceptible to fire blight and susceptible to woolly apple aphid.

AR835-11

Bred at East Malling from a cross between MI793 x M9. Low susceptibility to fire blight but susceptible to WAA. AR835-11 was one of the most vigorous rootstocks among the ones in trial, but had the lowest yield of all of them (2.1 kg/tree) and therefore had a poor yield efficiency of 0.3.

B10

Budagovsky 10 is a release from the breeding programme at Michurinsk University of Agriculture (Russia), originating from a cross between M27 x *M. robusta* 5.

Geneva 11 (G11)

Bred by the Geneva rootstock programme and patented in the US in 1997. This rootstock originates from a cross between M26 x *M. robusta* 5 and is described by the breeder as resistant to fire blight and partially tolerant to replant disease.

Geneva 41 (G41)

G41 is a release from the Geneva rootstock breeding programme. The rootstock is from a cross between M27 x *M. robusta* 5 and is described by the breeder as resistant to fire blight and tolerant to woolly apple aphid, replant disease and *Phytophthora* crown rot. At 95.5 g, G41 had the highest estimated average fruit weight of all rootstocks in this trial.

P67

Bred at the Institute of Pomology and Floriculture in Skierniewice from a cross between a Swedish and a Polish rootstock (Alnarp 2 x Budagovsky 9; A2 x B9). P67 was low yielding in our trial with yield efficiencies of 0.7 and 0.5 for kg/m^3 and kg/m^2 respectively only.

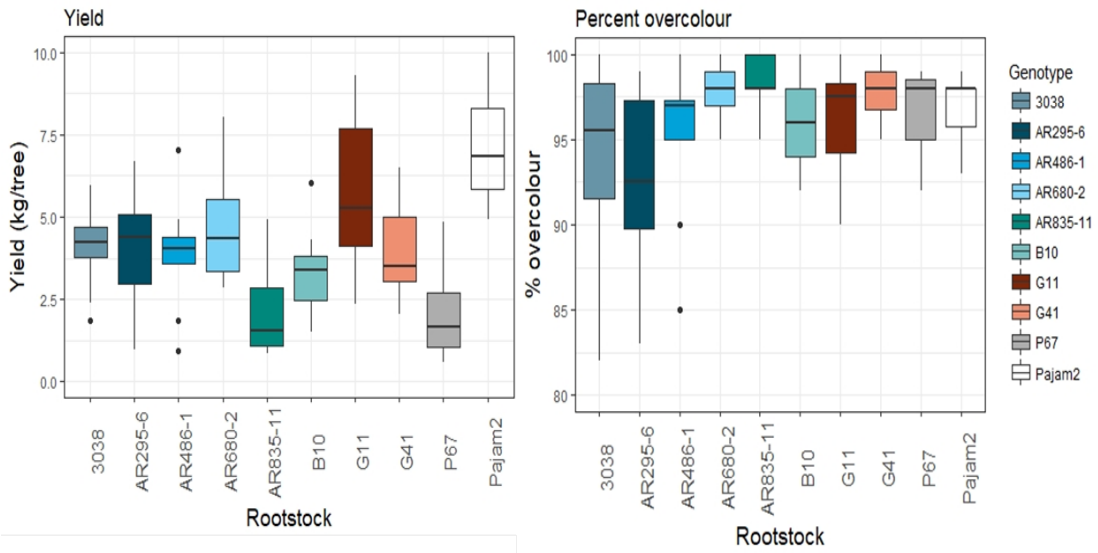

Figure 5. Boxplots for yield and percent over colour of fruit for the soil sterilant treated plots in the EUFRIN trial. The figure shows the values for rootstocks with a similar vigour to M9.

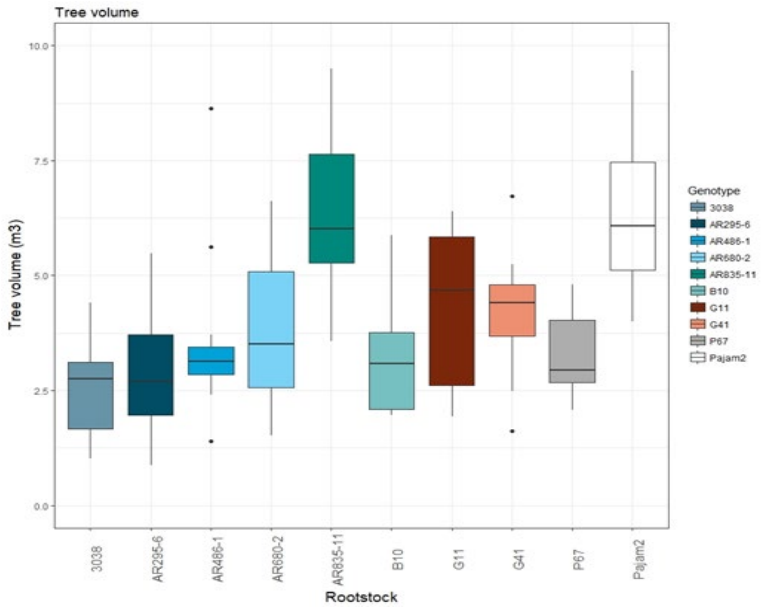

Figure 6. Boxplots of the tree volume for soil sterilant treated plots in the EUFRIN trial with M9-sized rootstocks

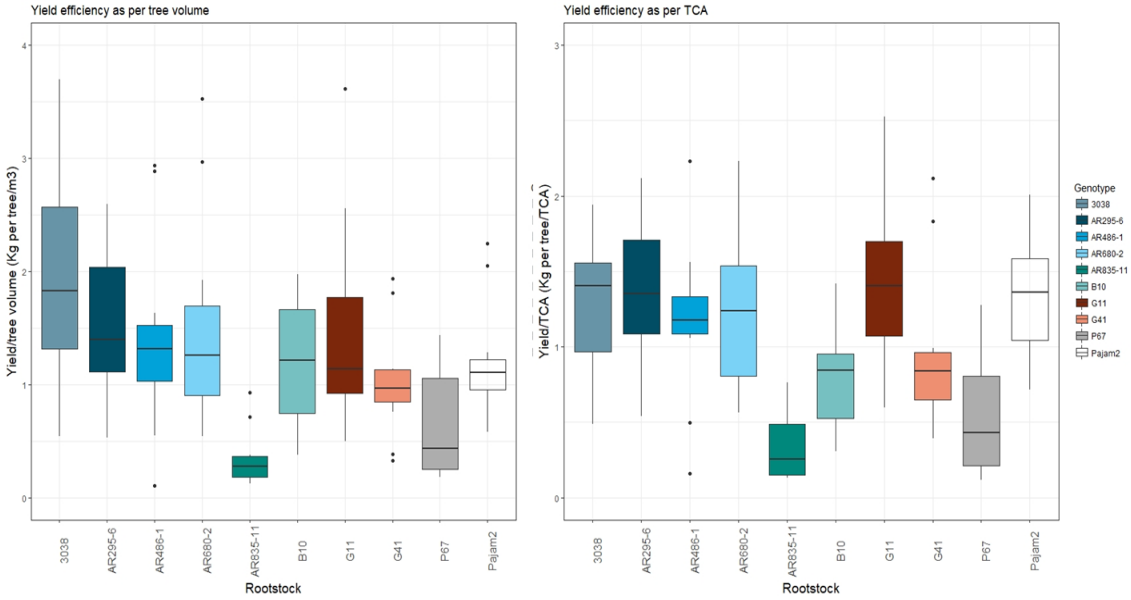

Figure 7. Boxplots of yield efficiency for the soil sterilant treated plots in the EUFRIN trial with M9-sized rootstocks. Yield efficiency is presented both as a function of tree volume and of Trunk Cross-sectional Area (TCA)

Table 7. Best linear unbiased estimates (BLUE) for the 10 rootstock genotypes that are included in the EUFRIN trial of M9-sized rootstocks. The table also shows the p-values for the effect of rootstock, Apple Replant Disease (ARD) treatment and rootstock:ARD interaction from a linear mixed model. The arithmetic mean is shown for the trait bitter pit due to a non-normality of residuals

Rootstock	Yield (kg/tree)	Number of fruit per tree	Average fruit weight (g)	Bitter pit (1-5)	Number of suckers per tree	Tree volume (m^3)	TCA[1] (cm^2)	Yield efficiency	
								As per TCA (kg/cm^2)	As per tree volume (kg/ m^3)
N°3038	4.1	67.6	72.7	1.2	0.2	2.6	3.4	1.3	1.9
AR295-6	4.1	54.5	76.9	1.1	0.3	2.9	2.7	1.4	1.5
AR486-1	3.9	71.4	61.8	1	0.2	3.6	3.6	1.2	1.4
AR680-2	4.7	68.2	72.3	1.1	0.6	3.8	4.0	1.2	1.5
AR835-11	2.1	26.5	78.6	1	0.8	6.4	6.5	0.3	0.3
B10	3.5	47.5	79.0	1	0.1	3.1	4.3	0.9	1.3
G11	5.0	67.4	86.8	1	0.0	4.3	4.1	1.2	1.3
G41	4.1	47.7	95.5	1	0.1	4.1	4.4	1.0	1.0
P67	2.2	26.8	84.0	1	0.3	3.5	4.3	0.5	0.7
M9 (Pajam 2)	6.5	89.8	73.0	1.1	0.2	6.3	5.7	1.2	1.1
P-value for effect of rootstock[2]	0.0001 ***	9.8e-05 ***	0.10	0.3426[4]	0.22	8.6e-07 ***	5.6e-09 ***	0.0002 ***	0.0003 ***
Chi-square$_{(5,14:9)}$[3]	33.7	33.8	14.6	10.1[4]	11.8	45.1	56.8	31.3	31.7
P-value for effect of ARD	0.005 **	0.0506	0.61	0.93[4]	3.2e-06 ***	0.5	0.83	0.037 *	0.1
Chi-square$_{(13,14:1)}$[3]	7.72	3.82	0.26	0.007[4] **	21.7	0.39	0.04	2.57	4.36
P-value for effect of rootstock:ARD	1	1	1	-	1	1	1	1	1
Chi-square$_{(13,14:1)}$[3]	0	0	0	-	0	0	0	0	0

[1] Trunk cross sectional area

[2] *, ** and *** indicate the significance level at 5, 1 and 0.1% respectively.

[3] Numbers within brackets indicate the following degrees of freedom; (null model, alternative model: difference between models)

[4] Difference in trait analysed with Kruskal-Wallis test due to non-normal distribution of residuals. Degrees of freedom for rootstock and treatment are 9 and 1, respectively.

EUFRIN: M26-sized rootstocks

Four rootstocks with a similar effect on vigour to M26 are included in this subset of the EUFRIN trial. The rootstock G935 was planted in both ARD conditions and in plots treated with soil sterilant. The remaining rootstocks were only planted in treated plots. Yield data for 2018 is only available for replicate 1 and 2 (each consisting of a plot of 3 trees) due to missing records. The yield and yield components of this trial is therefore not analysed statistically, but the data will be included in the analysis in subsequent years.

Apple replant condition had a significant effect on the number of suckers produced, with a higher production of suckers in untreated plots compared to plots treated with basimid. There was no significant effect of ARD on any of the other measured traits. No interaction term was estimated as only one rootstock was grown in apple replant plots. Results are presented in Tables 8 and 9 and Figures 8 and 9.

AR10-3-9

AR 10-3-9 is a selection from a cross between MM106 x M27. AR 10-3-9 is susceptible to WAA and fire blight. AR10-3-9 had a similar yield and yield efficiency to G202.

Geneva 11 (G11)

Geneva 11 is a rootstock from the Geneva breeding programme. According to the breeder this variety is resistant to fire blight, partially resistant to apple replant disease and susceptible to woolly apple aphid. G11 was the smallest and had the highest mean yield of the four rootstocks in this part of the trial producing 5.5 kg yield per tree. This rootstock also showed good yield efficiency (2 kg/m^3 and 2.5 kg/cm^2) and average fruit size (94.5 g).

Geneva 202 (G202)

Geneva 202 is resistant to fire blight and WAA, but not apple replant disease, according to the breeder.

Geneva 935 (G935)

Geneva 935 is a rootstock from the Cornell University and USDA-ARS joint Geneva rootstock breeding programme that was patented in 2005. The rootstock is described by the breeder as a dwarfing rootstock resistant to fire blight, *Phytophthora* crown rot and with a high tolerance to ARD.

Table 8. Best linear unbiased estimates for the 4 rootstock genotypes that are included in the EUFRIN trial of M26-sized rootstocks. The table also shows the p-values for the effect of rootstock and Apple Replant Disease (ARD) treatment from a linear mixed model. The arithmetic mean is shown for the trait bitter pit and number of suckers due to a non-normality of residuals

Rootstock	Bitter pit (1-5)	Number of suckers per tree	Tree volume (m^3)	TCA[1] (cm^2)	% Over colour
AR10-3-9	1	0	4.5	6.0	97.4
G11	1	0	3.5	3.7	98.5
G202	1	3	4.3	5.4	97.0
G935	1	1	4.0	4.6	94.2
P-value for effect of rootstock	0.73	0.30	0.31	0.0037 **	0.022 *
Chi-square$_{(7,4:3)}$[4]	1.3	3.64	3.54	13.5	9.58
P-value for effect of ARD	0.54	0.0097 **	0.3802	0.67	0.097
Chi-square$_{(6,7:1)}$[4]	0.37	6.67	0.77	0.19	2.75

[1] Trunk cross sectional area
[2] *, ** and *** indicate the significance level at 5, 1 and 0.1% respectively.
[3]Numbers within brackets indicate the following degrees of freedom; (null model, alternative model: difference between models)
[4]Difference in trait analysed with Kruskal-Wallis test due to non-normal distribution of residuals. Degrees of freedom for rootstock and treatment are 3 and 1, respectively.

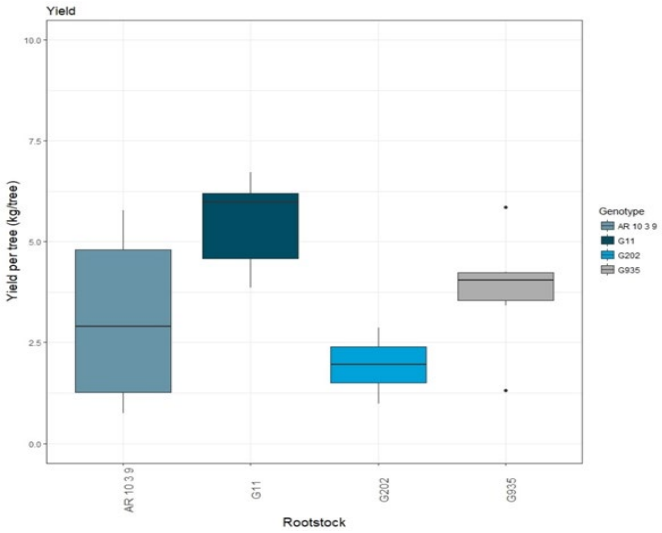

Figure 8. Boxplot of yield for four rootstocks in plots treated with a soil sterilant. The yield data is based on only two replicates

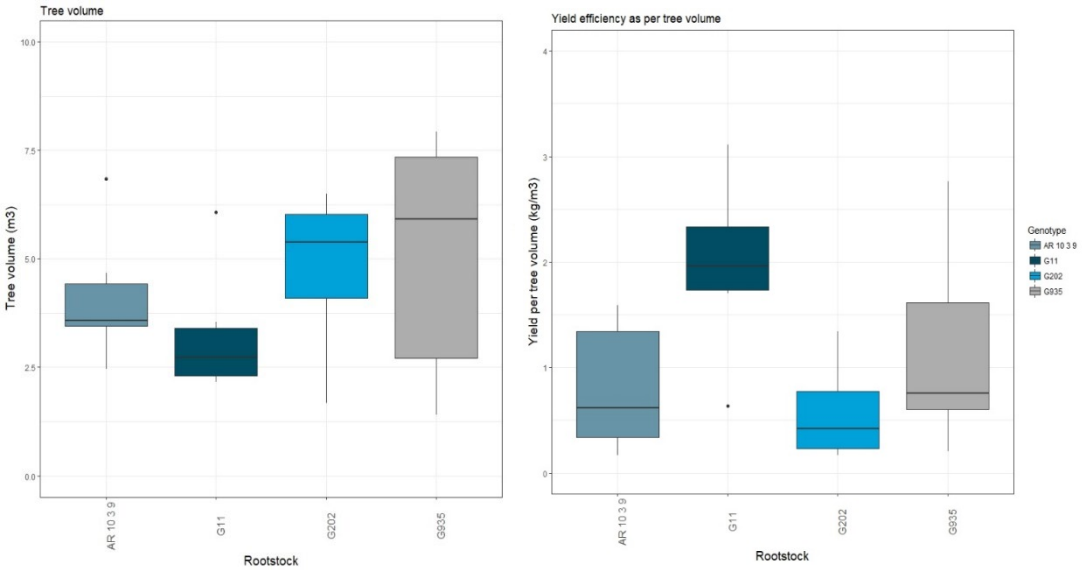

Figure 9. Boxplot of tree volume and yield efficiency for four rootstocks in plots treated with soil sterilant

Table 9. Arithmetic mean (±standard error) for yield components for the four rootstock genotypes that are included in the EUFRIN trial of M26-sized rootstocks. All means are for trees planted in plots treated with soil sterilant

Rootstock	Yield (kg/tree)	Crop load (No. fruit/tree)	Average fruit weight (g)	Yield efficiency (kg/m^3)	Yield efficiency (kg/cm^2)	Fruit yield by size (kg/tree)			Number of fruit by size (No./tree)		
						<65mm	65-75mm	>75mm	<65mm	65-75mm	>75mm
AR10-3-9	3.1 (±0.9)	35(± 8)	82.0 (±10)	0.8 (±0.2)	0.6 (±0.2)	1.5 (±0.4)	1.6 (±0.7)	0.0 (±0)	23 (±6)	12 (±5)	0 (±0)
G11	5.5 (±0.5)	62 (±10)	94.6 (±8)	2.0 (±0.3)	2.5 (±1.0)	2.1 (±0.6)	3.3 (±0.2)	0.1 (±0.08)	37 (±10)	24 (±2)	0.8 (±0.5)
G202	2.7 (±0.8)	33 (±12)	83.9 (±5)	0.8 (±0.3)	0.6 (±0.3)	1.2 (±0.4)	1.5 (±0.5)	0.0 (±0.03)	22 (±8)	12 (±4)	0.2 (±0.2)
G935	3.8 (±0.6)	48 (±8)	81.4 (±9)	1.2 (±0.4)	0.9 (±0.2)	2.1 (±0.4)	1.7 (±0.6)	0.0 (±0)	35 (±9)	13 (±4)	0 (±0)

Automated phenotyping of tree vigour

Measuring tree size in an objective and time-efficient manner is key to increase the scale of replicated evaluation in the breeding programme. In 2018 we attempted to UAV imaging and (tractor based) LiDAR technology to automate this process.

The drone approach will need further testing before we could deploy into the programme but the results from the LiDAR tests were very encouraging as with some further development it will allow us to not only accurately determine tree size but also record tree architecture (Figure 10).

Figure 10. Screen capture from LiDAR equipment output showing a range of tree sizes and architectures in a young trial planting

The tree canopy area of the trees in the trials SP250 and EUFRIN was estimated using LiDAR imaging in September 2018. These trials were also manually assessed for tree volume in December 2018. These two trials incorporated both fully grown trees (SP250) and younger trees (EUFRIN). As seen in Figure 11, there was a good correlation between the manual assessment of tree volume and the tree canopy area as estimated by LiDAR (R2 = 0.66). The correlation of tree canopy area to TCA was lower, with R^2 = 0.60. TCA was more highly correlated with tree volume than tree crown area R^2 = 0.73.

Some of the limitations for this system were the presence of tall weeds in the rows at the time of recording and low branches in some of the trees. The accuracy of this recording system could be improved addressing both of those issues in 2019.

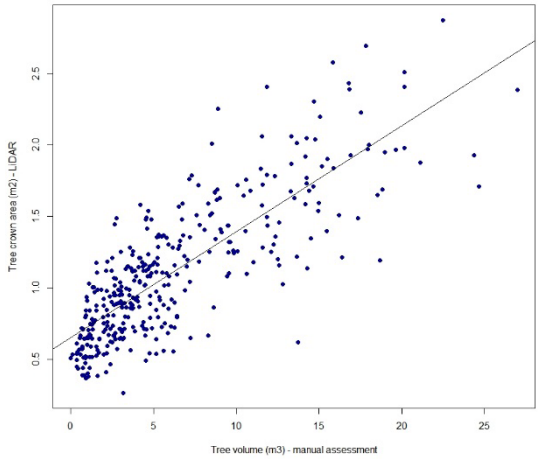

Figure 11. Correlation between tree crown volume as estimated from manual measurements and the tree crown area calculated from Lidar imaging

Breeding activities

New seedling populations

More than 2,800 seeds from six apple crosses were sown in winter 2017-18 to raise over 1,000 seedlings (Table 10); seedling number were negatively affected by high temperatures in the period following germination. Climatic conditions in early summer favoured high incidence of mildew and *Aphis pomi* infestations that were difficult to control without causing damage to such young plants.

These crosses were design to introduce pest and disease resistance in dwarfing and semi-dwarfing genetic background but none of them combined both fire blight (FB) and woolly apple aphid (WAA) resistance. Following feedback on breeding priorities from the EMRC policy group in 2018 which will require all new material under consideration for commercial release to be resistant to both, these families have now been re-classified as part of the pre-breeding section of the programme. Therefore, the seedlings were not planted in the field but potted and retained in a poly-tunnel over the winter to be screened through GH inoculation with WAA (M613, M616 and M617); FB-linked DNA markers (M612, M614, M615) and dwarfing linked markers (all) in 2019 prior to propagation and further evaluation. Additional inoculation test will be carried out to determine resistance to *Phytophthora cactorum* (Pc) and apple canker in due course as appropriate. Seedling from this families could be used for genetic studies and those carrying suitable combination of alleles will be grafted on to precocious rootstocks to use them in crossing as soon as possible.

No pear seedling were due to be raised in 2018 and seeds from the 2017 crosses were kept in store for germination in 2018-19.

Crossing and germination

The crossing programme was carried out between the 16th of April and the 5th of May; wide temperature fluctuations during flower development and in the pollination period negatively affected fruit set and seed viability; pear crossing was particularly challenging. In total, 5,544 apple flowers (Table 11) were hand pollinated including 2,232 for genetic studies; these seedlings will be evaluated for disease resistance by PhD student(s) in projects linked to the breeding programme and selected individuals used in breeding programme. For pear, only 512 could be pollinated in the six crosses planned for 2018 (Table 12). This was partly due to state of the trees in the old pear gene bank plot which is due to be grubbed in 2019-20 and the fact that the trees in the new gene bank were too young to flower in 2018. Trees in the new gene bank are due for verification in 2019 and could be used for crossing from 2020 onwards.

Nearly 3,000 apple and 2,670 pear seeds were sown in early January 2019 from the 2018 crosses as well as stored seed (Table 13) and will be germinated in spring 2019 following stratification.

Table 10. Apple rootstock seedlings germinated in 2018

Family	Parentage	Sown	Potted	Objective of the cross	Woolly Apple Aphid		Fireblight		Other testing		
					Segregation	Test	Segregation	Test	Dw[1]	P. cactorum	Canker
M612	G11 x AR295-6	53	10	High productivity in dwarfing types with improved P&D[2] resistance	All susceptible	N/A[3]	~ 50% FB_R5[4]	SSR markers 2019	SSR markers 2019-20	Cut shoot 2020-21 TBC[8]	TBD
M613	M116 x AR295-6	198	85	Introducing WAA and P cactorum resistance from 'Northern Spy' into dwarfing types	Segregate for Er1 (~50:50)	GH inoc[6]. 2019	Only partial tolerance at best	GH inoc. 2020/21 (Er1 only)	SSR markers 2019-20	Cut shoot 2020-21 TBC	TBD
M614	G30 x AR440-1	134	60	High productivity in semi-dwarfing types with improved P&D resistance	All susceptible	N/A	~ 50% expected to carry FB_R5	SSR markers 2019	SSR markers 2019-20	Cut shoot 2020-21 TBC	TBD
M615	Evereste x G30	1,690	625	FB resistance pyramiding on semi-vigorous background	All susceptible	N/A	~25% expected to carry FB_R5 & FB_E[5]	SSR markers 2019	SSR markers 2019-20	Cut shoot 2020-21 TBC	TBD
M616	Novole x M9	325	147	Introducing new sources of FB and P cactorum resistance into a dwarfing background	Unknown	GH inoc. 2019	Expected segregation - No markers	TBD[7]	SSR markers 2019-20	Cut shoot 2020-21 TBC	TBD
M617	M. koreana x M9	409	150	Introducing new sources of canker resistance into a dwarfing background	Unknown	GH inoc. 2019	Unknown	TBD	SSR markers 2019-20	TBD	Cut shoot 2020-21 TBC

[1] Dw = Dwarfing alleles
[2] P&D = pest and disease resistance
[3] N/A = Not applicable
[4] FB_R5 = fire blight resistance from *M. robusta* 5; [5]FB_E = fire blight resistance from 'Evereste'
[6] Glasshouse inoculation
[7] TBD = To be decided
[8] TDC = To be confirmed

Table 11. Apple rootstock crosses made in 2018 indicating numbers of flowers pollinated, fruit set, viable seeds collected and the objectives of the cross

Cross	Flowers pollinated	Fruit set	Viable seeds	Objective(s)
AR295-6 x G202	457	62	145	Breeding population
M116 x G935	233	111	337	Breeding population
M116 x G11	361	51	161	Breeding population
G11 x AR295-6	466	45	12	Pre-breeding population (no WAA_R[1])
M.116 x AR295-6	339	47	21	Pre-breeding population (no FB_R[2])
N. Spy x Evereste	317	163	704	Resistance combination/Mapping
M116 x M. robusta 5	402	53	154	Resistance combination & pyramiding
G202 x M547-41	402	123	159	Resistance pyramiding/Breeding
M. robusta 5 x G30	149	0	0	Breeding population/Mapping
M. robusta 5 x AR295-6	186	15	188	Breeding population/Mapping
M. zumi callocarpa x MM106	228	62	0	European apple canker studies
M. hartwegii x MM106	280	187	17	European apple canker studies
M. floribunda x MM106	256	10	6	European apple canker studies
Novole x MM106	252	210	131	P. cactorum mapping/Breeding
Red Melba x MM106	250	12	37	P. cactorum mapping/Breeding
Torstein x MM106	239	46	143	P. cactorum mapping/Breeding
Novole x Cox	312	306	196	P. cactorum resistance studies
Red Melba x Cox	190	50	200	P. cactorum resistance studies
Torstein x Cox	225	34	77	P. cactorum resistance studies

[1] WAA = segregation for woolly apple aphid resistance
[2] FB = segregation for fire blight resistance

Table 12. Pear rootstock crosses made in 2018 indicating numbers of flowers pollinated, fruit set, viable seeds collected and the objectives of the cross

Cross	Flowers pollinated	Fruit set	Viable seeds	Objective(s)
P525-3 x BP1	144	22	2	Repeat of promising breeding population
Old Home x BP3	274	87	592	FB[1] resistance
P298-18 x BP2	63	14	74	FB resistance, good rooting ability
P298-18 x PQ34-6	75	13	77	FB resistance, vigour control
OHF333 x BP1	41	6	29	FB and pear decline tolerance, dwarfing, precocity
OHF333 x B627	35	12	82	FB and pear decline tolerance, dwarfing, precocity

[1] FB = fire blight

Table 13. Apple and pear families sown in 2018; genus, parentage and the year the seed was produced are indicated as well as the number of seed sown per family and the type/purpose of cross

Genus	Year	Family	Cross	Seed sown	Comments
Malus	2018	**M618**	AR295-6 x G202	145	Breeding family
Malus	2017	**M619**	AR86-1-20 x G11	198	Breeding family
Malus	2016	**M620**	Bud 9 x Evereste	10	Pre-breeding family (no WAA_R[1])
Malus	2018	**M621**	G11 x AR295-6	12	Pre-breeding family (no WAA_R)
Malus	2018	**M622**	G202 x M547-41	159	Breeding family
Malus	2017	**M623**	G202 x Evereste	22	Breeding family
Malus	2018	**M624**	M. floribunda x MM106	6	Canker studies/Breeding family
Malus	2018	**M625**	M. hartwegii x MM106	17	Canker studies
Malus	2018	**M626**	M. robusta 5 x AR295-6	188	Breeding population/Mapping
Malus	2018	**M627**	M116 x G935	337	Breeding family
Malus	2018	**M628**	M116 x G11	161	Breeding family
Malus	2018	**M629**	M116 x AR295-6	21	Pre-breeding family (no FB_R[2])
Malus	2018	**M630**	M116 x M. robusta 5	154	Breeding family
Malus	2018	**M631**	N. Spy x Evereste	704	Breeding family/Mapping
Malus	2018	**M632**	Novole x MM106	131	P. cactorum studies/Breeding
Malus	2018	**M633**	Novole x Cox	196	P. cactorum studies
Malus	2014	**M634**	Novole x M116	58	P. cactorum studies/Breeding
Malus	2018	**M635**	Red Melba x MM106	37	P. cactorum studies/Breeding
Malus	2018	**M636**	Red Melba x Cox	200	P. cactorum studies
Malus	2018	**M637**	Torstein x MM106	143	P. cactorum studies/Breeding
Malus	2018	**M638**	Torstein x Cox	77	P. cactorum studies
Pyrus	2016	**PRP63**	Old Home x B602	58	Breeding family
Hybrid	2017	**PRP64**	OHF51 x Pyronia (2x)	65	Breeding family
Pyrus	2017	**PRP65**	Old Home x BP3	270	Breeding family
Pyrus	2018	**PRP66**	Old Home x BP3	592	Breeding family
Pyrus	2018	**PRP67**	P298-18 x BP2	74	Breeding family
Pyrus	2018	**PRP68**	P298-18 x PQ34-6	77	Breeding family
Pyrus	2018	**PRP69**	OHF333 x BP1	29	Breeding family
Pyrus	2018	**PRP70**	OHF333 x B627	82	Breeding family
Pyrus	2009	**PRP71**	BP3 op	109	Breeding family
Pyrus	2009	**PRP72**	OHF34 op	403	Breeding family
Pyrus	2009	**PRP73**	OHF51 op	331	Breeding family
Pyrus	2009	**PRP74**	OHF87 op	326	Breeding family
Pyrus	2010	**PRP75**	BP1 x P. betulifolia	254	Breeding family

[1] WAA = segregation for woolly apple aphid resistance
[2] FB = segregation for fire blight resistance

Evaluation of existing seedling populations

Apple

Twenty-nine apple families were assessed by breeders in September and October 2018 (Table 14) Records on vigour, crop load, suckering and fruit size were taken as appropriate and, in certain genotypes, other traits such as poor anchorage or the incidence of bitter-pit or burr-knots were also recorded. Woolly apple aphid colonization was also noted and susceptible seedlings from segregating families deselected.

Table 14. Apple rootstock seedlings evaluated in autumn 2018; year of planting, plot and parentage are indicated as well as number of selections made where appropriate. Cells shaded in green, purple and orange indicate breeding, pre-breeding and discarded families, respectively

Year planted	Plot	Family	Parentage	Number of seedlings	Number of pre-selection
2012	SP241	**M555a**	G30 op	123	9
2012	SP241	**M556a**	Ottawa 3 op	85	9
2012	SP241	**M559a**	Bud 9 x M9	56	0
2012	SP241	**M560a**	AR86-1-20 x G11	184	16
2012	SP241	**M561**	M27 x G30	6	0
2012	SP241	**M562a**	MM106 x G202	212	17
2012	SP241	**M563a**	MM106 x Bud 9	83	4
2012	SP241	**M564**	G202 x AR295-6	10	1
2012	SP241	**M565**	Bud 9 x M116	8	4
2013	SP246	**M566**	Bud 9 x Evereste	20	3
2013	SP246	**M567**	M27 x G11	11	2
2013	SP246	**M568**	Torstein x M27	4	0
2013	SP246	**M569**	Torstein x M9	11	0
2013	SP246	**M570**	G202 op	86	9
2013	SP246	**M571**	G11 op	76	5
2014	SC204	**M573**	Bud 9 x Evereste	6	0
2014	SC204	**M574**	Evereste x M9	303	17
2014	SC204	**M575**	M9a x Evereste	5	0
2014	SC204	**M576**	A469-4 x MH101	26	0
2014	SC204	**M577**	Evereste x G30	5	0
2014	SC204	**M578**	G11 x AR295-6	52	3
2014	SC204	**M579**	G30 x M27	15	0
2014	SC204	**M580**	G30 x AR295-6	148	5
2014	SC204	**M581**	M27 x G11	41	4
2014	SC204	**M582**	MM106 x G30	35	4
2014	SC204	**M583**	Torstein x M27	210	None yet
2014	SC204	**M584**	Torstein x M9	124	None yet
2014	SC204	**M585**	M9 x Sally	9	4
2014	SC204	**M586**	M26 x AR633-1	16	0

Additionally and in accordance with the new guidelines for selection, 10 families were fully deselected and 12 were re-classed as for pre-breeding only. Although only families planted in 2012 were due for pre-selection in 2018, the most promising individual from the 2013 and 2014 populations were also chosen and cut down to initiate propagation in an effort to eliminate susceptible germplasm and speed up the identification of new parental material. As soon as these selections produce new growth leaf tissue will be collected for DNA genotyping for FB resistance markers which will inform further

propagation efforts. Families M584 and M585 were generated to introduce a new source of Pc resistance in the programme but they were not sufficiently precocious to give any indication of yield potential in 2018 so will continue their field evaluation in 2019.

All families planted between 2015 and 2017 (Table 15) were also re-classed according to new priorities; six families will be grubbed in 2019 and ten considered for pre-breeding only. Records were taken for families planted in 2015 and 2016 (M587–M602) and leaf samples were collected from the 2017 families that will be retained for marker screening in 2019.

Table 15. Apple rootstock seedlings yet to be evaluated; year of planting, plot and parentage are indicated as well as the next steps of the selection process in each case. Cells shaded in green, purple and orange indicate breeding, pre-breeding and discarded families, respectively

Year planted	Plot	Family	Parentage	Number of seedlings	Next step of selection
2015	SC205	M587	G202 x AR295-6	54	Complete shortened field evaluation
2015	SC205	M588	AR295-6 x G202	57	Complete shortened field evaluation
2015	SC205	M589	Evereste x G30	613	Complete shortened field evaluation
2015	SC205	M590	M13 F x M116	14	Complete shortened field evaluation
2015	SC205	M591	MM106 x G30	91	Complete shortened field evaluation
2015	SC205	M592	G30 x M27	205	Complete shortened field evaluation
2015	SC205	M593	Bud 9 x Evereste	65	Complete shortened field evaluation
2015	SC205	M594	Novole x M116	45	Complete shortened field evaluation
2016	SC207	M595	A469-4 x MH123	80	Discard
2016	SC207	M596	M13 F x Bud9	280	Discard
—[1]	SC207	M597	Evereste x G202	6	Propagate for crossing and other testing
2016	SC207	M598	Evereste x AR295-6	346	Complete shortened field evaluation
2016	SC207	M599	Novole x AR295-6	119	Complete shortened field evaluation
2016	SC207	M600	Bud 9 x Evereste	287	Discard
2016	SC207	M601	M116 x AR295-6	198	Complete shortened field evaluation
2016	SC207	M602	M13 x G11	202	Complete shortened field evaluation
2017	SC208	M603	AR86-1-20 x G11	1	Screen with FB and Dw markers
2017	SC208	M605	Novole x M116	14	Screen with Dw markers
—[1]	SC208	M606	Evereste x G202	12	Screen with FB and Dw markers
2017	SC208	M606	Evereste x G202	34	Screen with FB and Dw markers
2017	SC208	M607	Evereste x G11	172	Screen with FB and Dw markers
2017	SC208	M608	Evereste x AR295-6	157	Screen with FB and Dw markers
2017	SC208	M608	Evereste x AR295-6	347	Screen with FB and Dw markers
2017	SC208	M609	M13 x AR295-6	349	Discard
2017	SC208	M610	Bud 9 x Evereste	537	Discard
2017	SC208	M611	AR295-6 x G30	231	Discard

[1] screened with fire blight (FB) resistance linked markers following germination; screened for woolly apple aphid (WAA) resistance by direct insect inoculation in the glasshouse. Individuals retained are classed as resistant to both WAA and FB.

Pear

Records were taken on vigour, suckering and crop load and, if present, fruit size for families planted in 2013 and on survival and suckering only for those planted in 2015. Families planted in 2017 were budded with ‘Concorde’ in August 2018. (Table 16).

No selections were made on pear families in 2018.

Table 16. Pear rootstock seedlings under filed evaluation in 2018; year of planting, plot, parentage and number of seedlings per family are indicated

Year planted	Plot	Family	Parentage	Number of seedlings
2013	SP247	**PRP49a**	PB11-30 x OHF333	69
2013	SP247	**PRP50a**	OHF87 x BP1	132
2013	SP247	**PRP51**	OHF87 x P525-3	4
2013	SP257	**PRP52**	BP1 x P525-3	351
2015	SC206	**PRP51a**	OHF87 x P525-3	102
2015	SC206	**PRP53**	OHF333 x BP1	78
2015	SC206	**PRP54**	OHF333 x Pyronia (2x)[1]	32
2015	SC206	**PRP55**	Old Home x BP3	15
2015	SC206	**PRP56**	P298-18 x *P. serotina* 'Kumloi'	173
2017	SC207	**PRP57**	BP1 x *P. betulifolia*	135
2017	SC207	**PRP58**	OHF333 x Junsko Zlato	9
2017	SC207	**PRP59**	OHF51x Pyronia (2x)	56
2017	SC207	**PRP60**	Old Home x BP3	183
2017	SC207	**PRP61**	OHF69 x BP2	46
2017	SC207	**PRP52**	OHF333 x Farmingdale	119

[1] Diploid interspecific hybrid between pear (*Pyrus*) and Quince (*Cydonia*)

Pest and disease resistance screening

Fire blight (FB)

Apple:

No fire blight inoculations were carried out for apple in 2018 but an in house screening will be set up from 2019.

Pear:

Six genotypes of pear were introduced in the INRA susceptibility screening during the reporting year; four advanced selections and two parental genotypes (Old Home and BP1) and their response in order of tolerance is presented in Table 17. It is not clear why the genotype susceptibility index (GSI) for well-known resistant cultivars like 'Old Home' and 'Harrow Sweet' in this particular experiment placed them in Category C and the pathology team at INRA provided no further explanation so it is difficult to draw firm conclusions from these results. However, it is clear that BP1 (a commonly used parent in the breeding programme) is extremely susceptible and should only be crossed with highly resistant parents. PQ34-3 was the most tolerant of the selections tested. PQ34-1, PQ35-2 and PQ35-3 appeared in this study to be susceptible but with considerable lower (GSI) scores than 'Comice'. We plan to replicate this testing at NIAB EMR in 2020.

Table 17. Summary of results for fire blight inoculation screening carried out by the pathology team at INRA Angers). Genotypes are ranked in order of tolerance as indicated by the genotype susceptibility index (GSI)

Genotype[1]	Shoots inoculated	Frequency Index	Severity Index	GSI	Susceptibility Class[2]	Reported Susceptibility[3]
Old Home	9	0.89	15	14	C	Very Resistant
Harrow Sweet	24	0.75	30	25	C	Very Resistant
PQ34-3	13	0.6	45	32	C	-
PQ35-3	15	0.9	43	41	D	-
PQ34-1	15	0.87	58	51	D	-
PQ35-2	10	0.60	80	52	D	-
Comice	21	1.0	81	81	E	Very Susceptible
BP1	12	1.00	90	90	E	-

[1] Genotypes underlined were provided by INRA as susceptible and resistance controls
[2] Qualitative scale from A (Fully Immune) to E (Very Susceptible); intermediate classes designations not provided by INRA
[3] As reported in literature

Phytophthora cactorum (Pc)

Propagation of hardwood cuttings was successful in 2017/2018, which allowed inclusion of 20 genotypes in the *Phytophthora cactorum* (Pc) resistance screen. 20 genotypes were screened with a mixture of Pc isolates P295, 62471, and R36/14; 6 genotypes were screened with isolate P295; 11 genotypes were screened with isolate 62471; and 11 genotypes were screened with isolate R36/14 (Table 18) For all genotypes tested, no clear root or shoot symptoms were observed in any inoculated replicates, whether genotypes were inoculated with the mixture of isolates or with individual isolates (Table 18 and Figure 12). As a result, the experiment will be repeated in 2019 using Pc isolates that are identified as pathogenic in cut shoot experiments (see below).

As a proof-of-concept experiment to test the efficacy of the cut shoot inoculation protocol and the pathogenicity of various Pc isolates in apple, dormant cut shoots of 'Gala' and 'Cox' were inoculated with different individual isolates (Figure 13 and Table 19). Isolates had previously been obtained from either strawberry or apple. For each isolate, five replicates of 'Gala' and five replicates of 'Cox' were inoculated. In addition, replicates of each genotype were included as un-inoculated controls. The cut shoot inoculation protocol proved promising, with distinct disease symptoms observed for some isolates in both 'Cox' and 'Gala' (Table 19 and Figure 14). Two strawberry isolates (11-40 and 17-21) and two apple isolates (R36/14 and P295) were pathogenic in apple, with disease symptoms generally more severe for inoculation with isolate R36/14. In each case, 'Cox' appeared to be more susceptible than 'Gala'. Following the promising results from this initial cut shoot inoculation test, the protocol will be used to screen a larger number of genotypes using isolates R36/14 and P295, in both dormant and active cut shoots. Updates on these experiments will be provided during the next reporting period.

Table 18. Summary of *Phytophthora cactorum* resistance screening for apple rootstock genotypes following inoculation with zoospores from isolates P295, 62471, and R36/14, either in a mixture for 3 isolates or for each isolate individually. Many genotypes were not tested with the individual isolates (NT), and were only inoculated with the three-isolate mixture

Genotype	Number of replicates for each isolate-genotype combination				Results
	Mixture	P295	62471	R36/14	
AR10-3-9	4	2	3	3	No clear symptoms
AR295-6	3	NT	NT	NT	No clear symptoms
AR440-1	4	2	2	2	No clear symptoms
AR680-2	4	NT	2	2	No clear symptoms
AR682-6	4	3	2	3	No clear symptoms
M306-20	4	NT	NT	NT	No clear symptoms
M430-249	4	NT	NT	NT	No clear symptoms
M432-203	4	NT	3	3	No clear symptoms
M432-250	4	NT	NT	NT	No clear symptoms
M480-3	4	NT	NT	NT	No clear symptoms
M482-13	4	NT	NT	NT	No clear symptoms
M482-158	4	NT	3	3	No clear symptoms
M546-110	4	3	3	3	No clear symptoms
M547-1	4	NT	NT	NT	No clear symptoms
M547-41	4	3	3	3	No clear symptoms
M547-72	4	NT	2	2	No clear symptoms
M551-50	3	2	3	3	No clear symptoms
M552-108	4	NT	NT	NT	No clear symptoms
M552-92	3	NT	NT	NT	No clear symptoms
M9 (Pajam 2)	4	NT	1	1	No clear symptoms

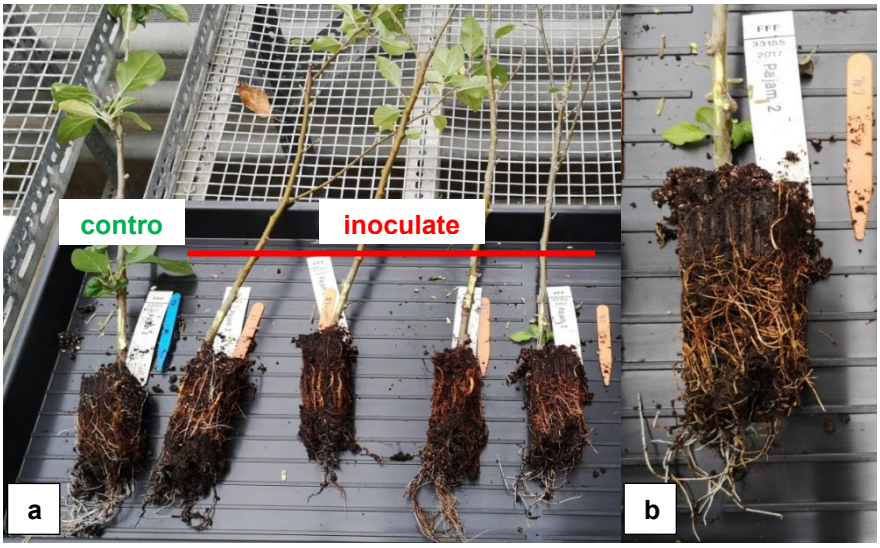

Figure 12. Hardwood cuttings of M9 (Pajam 2) screened for resistance to *Phytophthora cactorum*. **a)** Absence of clear disease symptoms in the four replicates inoculated with a mixture of *P. cactorum* isolates, compared to the un-inoculated control. **b)** Close-up of healthy stem and roots in an M9 (Pajam 2) hardwood cutting inoculated with a mixture of *P. cactorum* isolates.

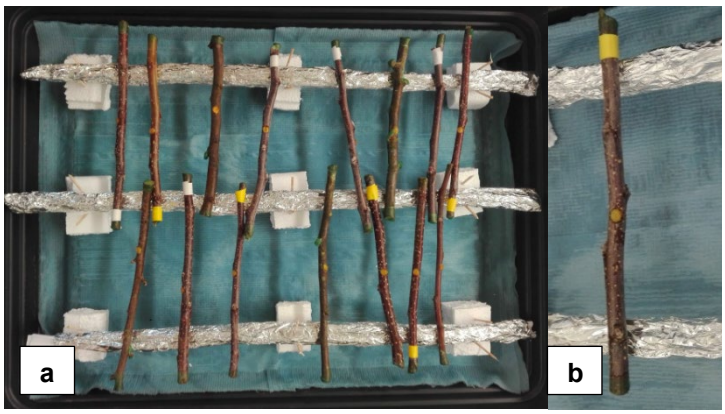

Figure 13. Experimental setup for the cut shoot inoculation test. **a)** Cut shoots suspended horizontally in trays containing moist paper towel. **b)** Close-up of a cut shoot that has been wounded and inoculated with an agar disc containing mycelia. (Photographs courtesy of C. Nellist and M. Luberti).

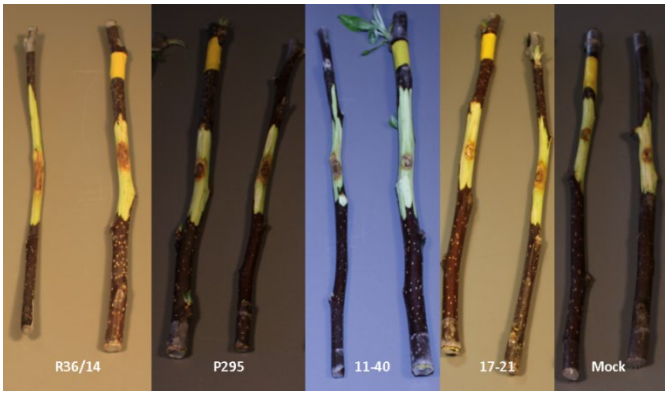

Figure 14. Stem lesions in cut shoots of 'Cox' and 'Gala' (labelled with yellow tape) 4 weeks after inoculation with different individual isolates of *P. cactorum*. (Photographs courtesy of C. Nellist and M. Luberti).

Table 19. Summary of *Phytophtora* resistance screening in cut shoots of apple using 12 different individual isolates. Four isolates (highlighted in orange) produced clear disease symptoms 4 weeks after inoculation

Isolate	Source of isolate	Pathogenicity observed
12-420	Strawberry	-
P414	Strawberry	-
P421	Strawberry	-
P404	Strawberry	-
11-40	Strawberry	+
10300	Strawberry	-
17-21	Strawberry	+
R36/14	Apple	++
62471	Apple	-
P295	Apple	+
SCRP370	(*P. infestans*)	-
SCRP376	(*P. infestans*)	-

Woolly apple aphid (WAA)

In 2018, 22 selections from this programme were included alongside advanced selections and cultivars (Table 20) in a glasshouse (GH) randomised screening for WAA susceptibility. Four replicates of each genotype were inoculated with aphids in June (re-inoculated at least once if colonies did not appear) and one replicate was left un-inoculated. All plants were scored at the end of the summer on a 0 to 3 scale ('0' = no colonies present to '3' = large colonies) and a final resistance score given based on the average score for the inoculated replicates. It is worth noting that populations in the GH thrived and the non-inoculated replicates of several genotypes were colonised by the aphids.

Unexpectedly, aphid colonies were found in plants of plant carrying on genotypes known to be resistant, namely AR86-1-20 (Er1; Northern Spy resistance) and G202 and G41 (Er2; *M.robusta* 5 resistance). The plans used in the screening were later confirmed as TTT leading to the conclusion that resistance breaking strains were represent in the GH. Unfortunately, these colonies were short-lived and could not be maintained for further studies.

This calls into question whether some of the genotypes classed as susceptible could carry a resistance gene but were colonised by resistance breaking strains. In future, we would need to consider the cost-benefit implications of genotyping all plants to be entered in the resistance screening and the need to more carefully monitor the emergence of possible resistance-breaking biotypes.

Table 20. Summary of the results for woolly apple aphid screening at NIAB EMR in 2018

Genotype	Replicate					Average (1,3-5)	Final score
	1	2[1]	3	4	5		
AR10-2-5	2	2	2	2	3	**2.25**	Susceptible
AR10-3-9	3	0	3	3	3	**3**	Susceptible
AR295-6	3	3	3	3	3	**3**	Susceptible
AR440-1	1	0	2	2	2	**1.75**	Mod susceptible
AR486-1	2	0	3	3	3	**2.75**	Susceptible
AR628-2	3	2	3	3	3	**3**	Susceptible
AR680-2	2	0	3	3	3	**2.75**	Susceptible
AR682-6	2	0	2	1	2	**1.75**	Mod susceptible
AR837-9	3	0	3	3	3	**3**	Susceptible
AR839-9	1	0	1	1	1	**1**	Fairly resistant
AR86-1-20	0	0	2	3	3	**2**	Susceptible[2]
G11	3	1	3	3	3	**3**	Susceptible
G202	0	0	3	1	1	**1.25**	Susceptible[2]
G41	2	0	0	2	1	**1.25**	Susceptible[2]
G935	3	3	3	3	3	**3**	Susceptible
GB VF80-2	3	2	3	3	3	**3**	Susceptible
GB-VF80-1	3	3	3	3	3	**3**	Susceptible
GH HDC80	2	0	0	3	3	**2**	Unclear
M116	1	1	1	1	1	**1**	Fairly resistant
M306-20	3	1	2	3	3	**2.75**	Susceptible
M432-203	2	0	2	1	0	**1.25**	Unclear
M480-3	2	0	1	0	2	**1.25**	Unclear
M482-13	3	3	3	3	3	**3**	Susceptible
M482-158	3	2	3	3	3	**3**	Susceptible
M508-1	2	0	1	1	0	**1**	Unclear
M546-110	0	0	2	1	0	**0.75**	Unclear
M547-1	3	0	3	3	2	**2.75**	Susceptible
M547-41	0	0	1	1	1	**0.75**	Fairly resistant
M547-72	2	0	3	1	1	**1.75**	Unclear
M552-108	3	3	2	3	3	**2.75**	Susceptible
M552-89	3	2	3	3	3	**3**	Susceptible
M553-112	3	0	3	1	3	**2.5**	Unclear
M553-117	1	1	2	2	3	**2**	Unclear
M553-124	3	0	3	3	3	**3**	Susceptible
M553-127	1	0	0	1	1	**0.75**	Fairly resistant
M553-2	3	0	0	1	2	**1.5**	Unclear
M553-64	2	0	1	0	1	**1**	Unclear
M554-135	2	0	0	0	2	**1**	Unclear
M554-17	2	0	1	2	0	**1.25**	Unclear
M554-40	3	3	3	3	2	**2.75**	Susceptible
M554-95	3	2	0	0	1	**1**	Unclear
M555-185	3	1	3	0	2	**2**	Unclear
M9 (Pajam2)	2	3	3	3	3	**2.75**	Susceptible
MM106	2	0	1	1	1	**1.25**	Fairly resistant
R104	2	0	3	3	3	**2.75**	Susceptible
R59	3	2	3	3	3	**3**	Susceptible
Voinesti	3	3	3	3	3	**3**	Susceptible

[1] Not inoculated control

[2] Genotypes are reported as resistant to WAA and the plants used were verified as true-to-type

Genotyping

Preliminary work was done to verify the usefulness of SSR (microsatellite) markers in the regions where WAA resistance genes have been previously mapped for the use in marker-assisted selection in the apple rootstock program. A small number of SCAR (Sequence Characterised Amplified Region) markers possibly linked to WAA resistance in 'Northern Spy' (*Er1*) have been published in literature. However, analysis of SCAR markers is cumbersome, and preliminary screening with a small number of genotypes showed that these markers are not particularly informative. As a result, SSR markers that could potentially be used as an alternative were identified using various available apple linkage maps (Figure 15). In total, 13 SSR markers were tested on a small number of genotypes (Table 21). A marker was considered potentially informative if alleles were present only in 'Northern Spy' and its resistant progeny, and not in susceptible genotypes or in genotypes that carry other sources of WAA resistance. Of the 13 SSR markers test, seven markers are potentially informative, while the remaining six SSR markers were either not informative or unclear. These seven potentially informative markers are a starting point, but more work needs to be done, including screening of segregating populations. Identification of SSR (and SNP) markers linked to WAA resistance will be continued, particularly as part of a (CTP) PhD research project that will commence in 2019.

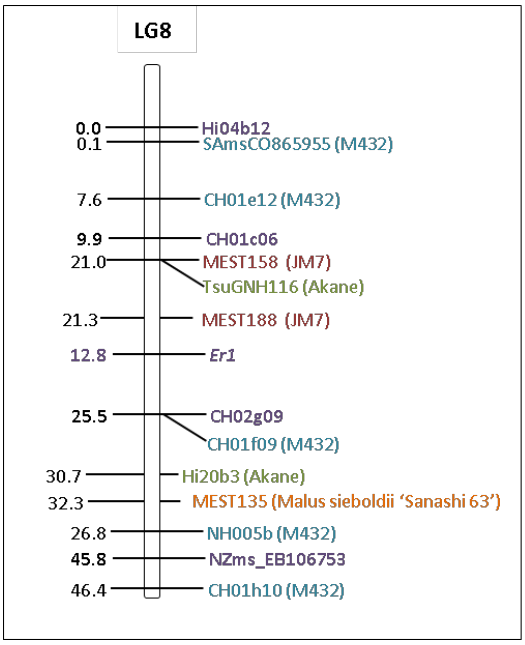

Figure 15. Representation (not to scale) of potential SSR markers for identification of *Er1* from 'Northern Spy'. Markers and distances (in cM) in bold are as published in Bus et al 2008. Other markers have been added through comparison to linkage maps from "M432", "Northern Spy", "Akane", "JM7", "Sanashi" and the Apple Integrated Map (distances in cM are as published in the individual linkage maps)

Table 21. Results obtained in small number of genotypes using 17 SSR markers with potential for use in screening for WAA resistance from 'Northern Spy'. Alleles present only in 'Northern Spy' and its resistant progeny are shown in bold font. Potentially informative markers are highlighted in green. Non-informative and unclear markers are highlighted in grey and yellow, respectively

SSR marker	*M. robusta* 5	Northern Spy	Malus floribunda	M116	MM106	M9	G41	G202
CH01c06	147/160	160/**170**	156/160	160/**170**	160/**170**	160/187	147/160	147/160/178
CH01e12	233/247	250/**258**	242/248	248/**258**	250/**258**	250/276	233/247	233/247
CH01f09	104	**125**/139	111	**125**/139	**125**/131	129/131	104/139	104/131
MEST135	149/154	139	139	139/141	139	139/143	139/154	139/149
MEST188	267	271	267/271	271	271	271	267/271	267/271
Hi04B12	136	**145**	133/142	134/**145**	134/**145**	130/140	136	136
NH005b	334/362	**337**/355	376	**337**/340	**337**/376	376	341/362	334/376
CH02g09	116	110/**137**	102/134	110/**137**	134/**137**	134/152	110/116	116/134
Hi20b03	203/206	215/**231**	223	**231**/234	223/**231**	215/223	234	223
NZms_EB106753	164/170	164/170	161/164	164/173	164	164/170	164/173	164/170
CH01h10	89/111	91/99	90/104/112	99/116	99	99/116	91/112	89/99
TsuGNH116	244	**246/249**	246	**246**	**246/249**	244	244/**246**	244
MEST158	223/254	250	223/236	236/250	236/250	236/250	236/250	236/245

Knowledge and Technology Transfer

INN visit to NIAB EMR – East Malling, July 2018

EMRC management committee meeting – East Malling, November 2018

EMRC management committee meeting – East Malling, February 2019