Breeding woolly aphid resistant dwarfing apple rootstocks (continued AP96019 and AP00007)

Lester Snare
NSW Department of Industry and Investment

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BREEDING OF WOOLLY APHID RESISTANT DWARFING APPLE ROOTSTOCKS

Lester Snare
Industry and Investment N.S.W.
The purpose of this project is to develop a woolly aphid resistant dwarfing apple rootstock. This final report presents the current status of the progeny bred and recommendations for future evaluation.

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Media Summary

The woolly apple aphid (WAA) rootstock resistant breeding project commenced in 1996 with the specific aim to develop WAA resistant dwarfing apple rootstocks for Australian conditions. Seedlings produced in an initial hybridization program have been under field evaluation by Industry and Investment N.S.W. (I&I) at the Orange Agricultural Institute. This is part of a long-term research project tackling one of the apple industry’s most serious pests.

Initial seedlings were planted in a test orchard at the Institute in a bid to develop a dwarfing apple rootstock which is also resistant to the devastating woolly aphid.

WAA is a major pest of apple trees in Australia infesting both the roots and shoots. The roots are the main feeding and breeding site providing a springboard for woolly to feed and damage the above ground parts of the tree. The sap-sucking insect is one of the key pests affecting the Australian apple industry. The insect not only sucks the sap of the tree and reduces tree vigour but also causes marks and stickiness on the fruit, which can result in a loss of market premiums for growers. Infestations also make conditions difficult for pickers. Many of the currently grown dwarfing stocks such as M.9 and M.26 are very efficient at reducing growth and increasing productivity of apple trees but are also very sensitive to attack from woolly aphid.

While some breeding programs in New York and New Zealand are tackling this challenge the need for a local program was critical. The warmer Australian climate and higher pest pressure make woolly aphid control more difficult.

The options for apple growers to control WAA are limited. Chemicals are effective in the short term but resistance, cost, ecological disruption and market restrictions are major damaging side effects. Biological control is effective but it can be very inconsistent. Resistant rootstocks with dwarfing growth habits similar to the current commercially used rootstocks, M26 and M9, are the only long term solution for apple growers.

This project has produced a range of apple rootstocks with varying size controlling abilities suitable for high density production systems in Australia. This will result in apple trees that fulfil the initial aims of the project that encompass the following attributes.

- easy to propagate
- yield efficient
- dwarfing traits
- resistant to WAA
- suited to Australian conditions
- grower friendly in growth and production

The rootstocks bred in this program have remained free of woolly aphid infestation through out the screening process. These stocks provide growers with the foundation for an economic and sustainable orchard system.
This project aims to develop dwarfing, woolly aphid resistant apple rootstock material which allows for improved production efficiencies. Woolly apple aphid (*Eriosoma lanigerum* Hausm) is a major pest of most commercial apple varieties and expensive to control with current registered chemicals. The importance of rootstocks with genetic resistance to below ground infestation is highlighted when coupled with susceptible commercial varieties like Cripps Pink and Fuji strains. Biological control for the majority of growers has proven to be limited.

The Australian apple industry is continuing to adopt higher density, closer planted orchard systems. A recent extension program, “Future Orchards 2012” conducted over a number of years in Australia highlighted the economic benefits and management of smaller pedestrian orchards. Dwarfing rootstocks are the foundation for a successful high density planting system. The selection of rootstock combined with the scion variety provides the tree vigour and final tree size which in turn determines its planting density. In close planted systems the rootstock allows the tree to be maintained in its allotted space. The rootstock also has a dominating effect on tree precocity, the trees ability to crop in its early years.

**The Breeding Program**

The initial phase of the breeding program included parental selection. The critical characteristics sought in the parents were resistance to woolly aphid and a dwarf growth habit. Disease resistance and ease of propagation are also important and formed part of the selection criteria. It was important to incorporate a diversity of species of both WAA resistance and dwarfing into the program for a number of reasons. Firstly, resistance by pests such as WAA to chemicals has been common but, although biotype resistance by pests to resistant plants is rare, it can happen. The CG series and *Malus seibodii* provided the diversity needed to prevent biotype resistance. Secondly, the genetic diversity increases the overall chance of developing selections suitable to local Australian conditions.

Initial pollinations were completed over two years and in their first year young seedlings were subject to intense WAA pressure in a glasshouse. Healthy aphids were placed directly on the seedlings and those that succumbed to the pest were eliminated. The second evaluation stage and nursery propagation was carried out in stool beds. Seedlings were selected using a range of nursery friendly criteria: lack of spikes, lack of burr-knots, good root and sucker production, moderate vigour, absence of WAA and powdery mildew. Following stooling of promising selections rooted shoots of each selection was grafted with the variety Jonagold. These 1,697 grafted trees were then planted in the evaluation block for field evaluation. A combination of field recording of tree circumference, yields and ratings for desirable tree habit together with observations on pests, diseases and anchorage has provided a matrix of characteristics for selection of the elite stock. Trees of each selection were planted in a statistically designed experiment with three standard rootstocks as comparisons: MM106, M.9 and M.26.

The final phase of the programme included screening of elite stocks for *Phytophthora cactorum* susceptibility. Identification of resistant genotypes involved using an excised stem technique. This technique as used by Jeffers *et al* (1981), using cuttings
from rooted stools, facilitated a pre-screening programme that could handle a volume of material in a short period of time. The timing of this procedure ensured that stocks with suitable WAA resistance and dwarfing capacity were not eliminated at an earlier stage based purely on degrees of *Phytophthora* susceptibility.

**Research findings**

In the 12 years from commencement in 1996, this project has achieved not only 4 years of crosses, propagation of crosses/grafted trees for field evaluation but also a significant evaluation phase assessing fruiting capacity and dwarfing capability. Russo and Robinson (2007) point out that 5 years is too short a period to critically evaluate rootstock performance and that an eight year period is required for field evaluation alone. This allows accurate assessment of potential profitability, production efficiencies and assessment of impacts of year to year variation in weather.

Selections identified in this Australian breeding program are promising in terms of productivity, exceed the productivity of M9, fulfil a range of vigour requirements suitable for high density orchard plantations, and up to this point, remain free from woolly aphid.

It is recommended that in order for the program to provide maximum benefit to industry propagation of elite stools should continue to provide rooted stools for further bulking up and distribution for secondary orchard evaluation. Ideally, these stocks would potentially form part of a future evaluation program using different scion combinations which can be evaluated in second level orchard trials across a range of regions and in collaboration with the Australian Pome Fruit Improvement Program (APFIP)

Interest to screen for fire blight and further field evaluation has been received from Cornell University, Geneva in the USA. An offer to include Australian material in their program was received in 2010.
Introduction

Woolly apple aphid (WAA) is a major pest of the apple industry worldwide causing economic losses by reducing fruit quality and tree productivity. The WAA rootstock resistant breeding project commenced in 1996 with the specific aim to develop WAA resistant dwarfing apple rootstocks for Australian conditions. Both biological and chemical control measures have been used to control woolly aphid (*Eriosoma lanigerum*) however the long term solution is genetic. Chemicals provide a short term solution but the number and effectiveness of sprays is declining and consumer pressure demands a reduced level of pesticides for fruit production. Both long and short term measures are needed for effective control. This need is reflected in the number of projects currently investigating WAA management in other parts of the world. On the chemical side, ‘softer’ alternatives are being tested to control the attacks on the aerial parts of the tree, while soil treatments are also being refined. Predators such as the European earwig (*Forficula auricularia*) and the parasitoid wasp (*Aphelinus mali*) provide varying degrees of control but even with predators and chemical control programs, fruit and vegetative growth can be adversely effected. Nicholas (1997) points out that in the case of the WAA parasite its lifecycle can lag behind that of the woolly aphid life cycle and may not prevent woolly aphid from causing damage.

WAA is a native of North America but, in the early days of the developing apple industry, it quickly adapted to Australian conditions. While a range of chemicals was initially employed to control WAA, the major breakthrough was the release of a resistant rootstock, “Northern Spy”, in the early 1900s. Northern Spy was quickly adopted as it had a better root system than the locally selected “Winter Majetin”. Where Northern Spy was used WAA was now restricted to the aerial parts of the tree where it was easier to control with predators or chemicals.

Following the adoption of Northern Spy, all major rootstock breeding programs worldwide incorporated it to provide WAA resistance. Most of the UK selections, including the Merton series such as M.793 and the Merton-Malling series MM101 to MM115 were bred with Northern Spy as a parent. Although these new rootstocks were readily adopted in Europe, in Australia many of the older orchards continued to be propagated on seedling rootstocks and there was a slower acceptance of WAA resistant alternatives. However, by the 1960s WAA resistant rootstocks were becoming the standard for Australian apple orchards.

Orchards planted around this time were generally only low and medium density predominantly grown of MM106 or Northern Spy rootstocks. Gradually the benefits of higher density orchards were becoming apparent. The economics of these pedestrian orchards with the associated reduced picking costs and increased production and fruit quality became arguably the only future for Australian apple production. This point is supported by the development of the Future Orchards 2012 program which promoted the benefits of high density plantings to Australian apple producers.

The M9 rootstock and its various clones is the most planted rootstock in the world and is the basis for many intensive plantings in Australia. It produces a tree that is 30% the size of seedling, precocious, productive and produces large size fruit. Although M9 is planted widely around the world it does have high susceptibility to fire blight (*E. amylovora*) which Australia has thus far avoided. Robinson (2009) also points out that M9, in many parts of the world, is not well adapted to climates with high soil temperatures. This is also true of M9 planted in Australia which
experiences higher evaporation rates than other parts of the world. The warmer Australian climate and higher pest pressure potentially make woolly aphid control difficult.

Unfortunately the dwarfing rootstocks available in Australia for intensive orchard systems, in particular M26 and M9, are susceptible to attack by WAA. The attack of WAA on dwarfing rootstocks is accentuated because the tree is already less vigorous. Damage to the root system of young trees severely reduces the tree’s ability to uptake water and nutrients and produce new growth. In older and more vigorous trees, the effects are not as great. As this pest predominantly uses the root system for the critical times in its life cycle, genetic resistance by the rootstock is pivotal to the long term success of new apple orchards. In terms of nursery production the presence of WAA in the stool bed is highly problematic and cannot be controlled without chemicals. (Malone 1994). When stocks are earthed up aphids become buried and can remain active. In some cases when infested stock is planted as orchard trees, trees remain weak and unproductive.

The challenge

The challenge was to breed WAA resistant dwarfing rootstocks that could cope with Australian conditions and high WAA populations. Resistance from Northern Spy has been included in this breeding program but the program also includes resistance from Malus sieboldii and Malus robusta (originated from M. prunifolia × M. baccata, selected Ontario, Canada). In this breeding program, the Malus robusta component has been introduced through some of the Cornell-Geneva series which have M.robusta as one parent. Appendix one shows the parentage used in the hybridisation program. Recent genome mapping work reports molecular markers for genes conferring woolly aphid resistance. Bus et al (2008) report that the Er1 and Er2 genes derived from Northern Spy and Robusta 5, respectively are the major genes used by breeders to improve the resistance of apple rootstocks to WAA. This validates their use for resistance in the Australian breeding program.

There are frustrations for growers and breeders alike in facing long time frames from pollination to commercialisation of new stocks. While some areas of breeding are being accelerated, it is difficult to change the time taken for field evaluations. This applies particularly to rootstocks. An eight year period is required for field evaluation alone in most over seas breeding programs. This allows accurate assessment of potential profitability, production efficiencies and assessment of impacts of year to year variation in weather.

Some promising selections have being ear-marked to be part of the elite stock for incorporation in secondary orchard evaluation trials and the Australian Pome Fruit Improvement program. There are some risks in being premature, but by using a range of assessors including nurserymen, breeders and orchardists, there are reasonable possibilities of making some good decisions.

The implications of this breeding project and more widely, greater rootstock evaluation in Australia is the provision of better information to explore the relationship between rootstock and location and how tree performance is affected. Finding the exceptions and interactions with cultivars and rootstocks on which they are grafted is of considerable value for local producing regions. The NC-140 regional research project in the USA utilises this approach for rootstock evaluation.
The development of an Australian rootstock along with through evaluation under Australian conditions will better help producers to manage the extent and patterns of changing climatic effects on orchard-system performance.

Materials & Methods

Breeding strategies and pollination

The initial phase of the breeding program was parental selection. A collection of rootstocks already existing at the Orange Agricultural Institute was supplemented with further desirable parent material from a range of sources available in Australia. The critical characteristics sought in the parents were resistance to woolly aphid and a dwarf growth habit. Disease resistance and ease of propagation was also important and formed part of the selection criteria but was less critical. Parental selection was critical to the success of the program. Table one list the sources of WAA resistance and sources of dwarfing.

Table 1. The breeding parents

<table>
<thead>
<tr>
<th>Sources of woolly aphid resistance</th>
<th>Sources of dwarfing</th>
</tr>
</thead>
<tbody>
<tr>
<td>• MM Series (102, 105, 106, 108, 111*, 115)</td>
<td>• Malling series (M.9, M.26, M.27*)</td>
</tr>
<tr>
<td>• Northern Spy</td>
<td>• Ottawa 3*</td>
</tr>
<tr>
<td>• CG* series from Geneva, NY (CG 5179, CG 6210, CG 7707)</td>
<td>• P.2 (Poland)</td>
</tr>
<tr>
<td>• *Malus seiboldii – crab apple NZ (Snowbright A126)</td>
<td>• Budagovsky 9* (Russia)</td>
</tr>
<tr>
<td>*Phytophthora resistant</td>
<td>+ Fireblight resistant (MM.106 is tolerant)</td>
</tr>
</tbody>
</table>

Following the procurement and collection of pollen from both local and overseas sources controlled crosses were carried out over a three year period from 1996 to 1999. Rootstock parents were required to be planted at Orange Agricultural Institute and then produce enough flowers and pollen for hand crosses to be performed. Pollen supplies of resistant varieties were supplemented by QDPI at Stanthorpe and SARDI at Lenswood, South Australia. Two visits to Havelock North, New Zealand enabled crosses to be carried out with germplasm not available in Australia at the time. This resulted in approximately 10,000 seedlings. Ideally, the plan was for 30,000 seedlings but resources were not available. Figure one illustrates the breeding program process.
Figure 1. Breeding Program process and Timeframes

Parental selection

Seeds produced

Pre-screening for woolly aphid

Selected seedlings in stool beds

Seedlings removed

Hybridisation 1998 and 1999

Selected seedlings in stool beds.

Evaluation in test orchard – 3 reps

Trees planted (240 seedling selections) 2001/2002

Evaluation block completed July 2004


Final selection of elite rootstocks for commercial testing. (2010)

Grafted to Jonagold July 2001

Seedlings removed

Screening nursery characteristics

Grafted to Jonagold July 2003

947 trees planted (275 seedling selections) 2003/2004

Pre-screening
Culling woolly aphid

The primary and initial selection task was to cull or remove any seedling that showed susceptibility to woolly aphid. Young seedlings were exposed to high numbers of healthy aphids in the glasshouse when they were 100-150mm high. The percentage resistance varied from 24%-88% (Campbell 2000). Those that succumbed were eliminated. Very few susceptible seedlings escaped detection and the few that did have been rogued out from the stool beds in the nursery evaluation block. The survivors were planted in a nursery stool bed and stooled for rootstock production. This process was repeated over a three year period from 1998 to 2001.

Selecting nursery friendly stock

During the establishment of stools, seedlings were discarded if they failed to meet the following requirements.

- Freedom from burr knots (swelling formed on stems from root initials)
- Relative freedom from spikes
- Some tolerance to scab and mildew
- Easy to propagate with good root production in the stool.

Early determination of dwarfing capability in the stool bed is difficult however internode spacing and excessive vigour were observed in relation to other traits, this included relative freedom for spikes. Figure 2 illustrates the variability amongst stocks. Those selections that met these criteria were then ready for field testing. The candidates had now been reduced from the original 10,000 seeds to 515 seedlings that could be grafted and planted in the test orchard.

Figure 2. Variability of spike production

![Figure 2. Variability of spike production](image)

Stocks with excessive spike production were culled from the program (far right)

Field testing in the orchard
Over 800 rootstock types were selected and grafted during the 3 seasons from 2000 to 2003. Those selections-stools that met the nursery criteria were ready to be harvested for field testing. Three rooted stocks were harvested from each stool and whip and tongue grafted to the vigorous Jonagold variety. The graftlings were potted, held under shade house conditions and planted out the following winter. The vigorous non-precocious Jonagold has been used as the test variety. This provides the most rigorous test for the rootstock candidates. Selections were inter-planted with MM.106, M.9, M.26 and a suitable pollinator variety. Just as there was a wide and extremely interesting range of growth responses in the stool beds, there was also a wide range of tree vigour, feathering, bud break and precocity exhibited by the young orchard trees. Blossom time, yield figures and butt circumference measurements were taken annually to ascertain tree performance. Photo points were taken of each tree to provide a record of tree height. This provided many opportunities to select a small number of elite stock for eventual commercial testing.

**Evaluation orchard block design**

The evaluation block is approximately 140 × 60 m with a gentle slope to the east. Trees are planted in double rows to maximise land usage as well as simulating a high density orchard. The double rows are 1m apart, trees 1.5 m apart and the distance between double rows is 4.5 m. This gives a planting density of 2963 trees per hectare.

The layout has been designed to accommodate sequential plantings as planting material became available. The design is set up as a split block design but with some modifications. A blocking approach was used to allocate tree positions. Pollinators were allocated to the sets of double rows proportional to the number of trees in the row. This allowed for adequate fruit production on the scion material. The standards of M26, M9 and MM106 were positioned using a blocking approach across the planting. The rootstock material was allocated in a similar fashion to the standards taking into account the slope of the land. The crosses are replicated three times across the planting. This provided not only adequate replication but a margin of safety when the elite crosses are propagated in situ. Elite selections were converted to stools to facilitate propagation.

A simple two wired trellis was erected to provide some support for the shallower root systems and the early crops. The test orchard is adjacent to commercial plantings so that the selections were potentially exposed to woolly during their evaluation. Approximately 1700 trees were established in the evaluation orchard. The first group were planted in 2001 with subsequent plantings in 2002, 2003 and 2004. The largest planting was in June of 2004 where 700 trees were planted out. A small number of selections from one cross do not necessarily indicate that there was a heavy culling. Small parent mother trees, low flower numbers and unfavourable weather conditions at flowering time contributed to the varying numbers of seedlings originally produced during the earlier phase of the hybridisation program. (Campbell 2000).

**Evaluation orchard soil type**

The orchard evaluation block at the Orange Agricultural Institute, NSW (elevation 922 m), is typical of the Orange apple district growing conditions. The region has a winter spring dominant rainfall pattern with a mean annual rainfall of 949 mm.
The soil is volcanic in origin and is classed as a krasnozem type originating from a weathered basaltic rock. The 300 mm deep A horizon is a red brown clay loam, with a pH of 6.5. This overlies red light clay of pH 6. Both A and B horizons are well structured. The evaluation orchard had been in fallow for over ten years and the soil is typical of that found on most local district orchards that produce commercial crops. Soil sampling and leaf tissue analysis were used to confirm nutrient status.

The nature of fruit tree breeding and the future

Some aspects of fruit tree breeding have been accelerated but it is still a long process until a new creation is finally launched into the commercial orchard world. The wait is even longer when breeding rootstocks. (Atkinson et al 2001) The alternative is a rushed evaluation with a much higher risk of failure.

The past four seasons have provided field data to select the elite stock groups. These stocks could be incorporated into the APFIP evaluation sites and included for testing in the Cornell-Geneva program.

The technology utilised in this Australian program is typical of that used for fruit tree and rootstock breeding worldwide. In the future there is potential to investigate methods for shortening the time frame as well as assessing other horticultural characters that are linked to dwarfing. Molecular biology, gene markers and linked characters will all increase in importance but horticultural field testing remains crucial for success of the program.

Phytophthora Screening - Phytophthora cactorum.

Screening for P. cactorum. was also a part of the selection process for identification of elite stocks. P. cactorum is a fungal disease that effects both roots and above ground parts of apple trees. Disease of the roots is referred to as crown rot and infected above ground parts generally known as collar rot. Several factors favour disease development, including excessive soil moisture, moderate soil temperatures, wounds, time of the year and rootstock susceptibility.

An excised stem technique as described by Jeffers et al. (1981) and Brown (1993) was used to indicate susceptibility or resistance to Phytophthora cactorum using pathogenic isolates obtained from the plant pathology herbarium, Orange Agricultural Institute. Excised twigs were placed on inoculated agar in jars. These were sealed, incubated and then twigs examined for lesions. Although flooding of young seedlings is a standard technique for crown rot resistance determination Bus (2009) points out that the flooding method is unreliable when applied to mature trees. The technique was trialled initially with known susceptible and resistant apple rootstocks and photo points taken. This allowed for accurate determination of not only technique, but also interpretation of results of progeny from the breeding program.
Results

The results show that there are a number of progeny from this program that will fit into size categories that are comparable to those provided by the industry standard stocks of M9, M26 and MM106. The new rootstocks compare favourably with these stocks in terms of yield and tree size and remain free from woolly aphid. This has been the case through the duration from seedling to a five year old grafted orchard tree in the evaluation block.

Early measurements from the breeding program showed the diversity of growth rates in very young trees. Clustering around the diamonds was positive indicating that some of the new stocks were performing close to industry standards at an early stage.

Figure 3 Early growth responses of stocks planted in 2001 and 2002

![Scatter diagrams for all progeny in each of the 2001 and 2002 plantings. The diamonds represent the standard benchmark rootstocks.](image)

The scatter plots in Figure 3 show the range of growth responses of the selections in the 2001 and 2002 plantings. There was a significant variation in the progeny for the random effects: maternal (not paternal) parent, designation, and row and tree position. These variations justify the randomisation and statistical design of the evaluation block. The differences between 2001 and 2002 plantings show how the tree growth in the older planting is more clearly demonstrating the differences in tree vigour and dwarfing characteristics.

Performance of stocks under field conditions in the evaluation orchard

Data collected from the evaluation orchard revealed some interesting facts about the selected seedlings, their parents and also the types of responses that indicate dwarfing potential. Early growth of both dwarf and vigorous stocks had been similar. This is important in establishing a sound orchard tree that has a good root system and an optimal fruit bearing frame.

For the purposes of this project breeders classed elite productive stocks into three tree sizes.

- Semi- dwarf MM106 (Group 1)
- Dwarf M26-M9 (Group 2 Elite)
- Very dwarf< M9 (Group 3)
Yield efficient trees both above and below M26/ M9 categories were also identified. Although this was not a direct requirement of the project, it seemed prudent to identify and hold this material in view of the absence of woolly aphid and their potential suitability to different soil types climate and growing systems.

Group 1-Semi-dwarf

Figure 4 below represents crosses in this group. Yield efficiency and cumulative yield are presented MM106, M9 and M26 are charted on the right side.

Figure 4. Group 1 Semi-dwarf cumulative yield and yield efficiency

The two charts below show the 5 best crosses in this group
Table 2. Parentage of the five best crosses in Group One

<table>
<thead>
<tr>
<th>Cross Designation</th>
<th>Male Parent</th>
<th>Female Parent</th>
</tr>
</thead>
<tbody>
<tr>
<td>96-100-36</td>
<td>Ottawa 3</td>
<td>MM 110</td>
</tr>
<tr>
<td>97-110-119</td>
<td>CG5179</td>
<td>Bud 9</td>
</tr>
<tr>
<td>99-136-1</td>
<td>Ottawa 3</td>
<td>CG602</td>
</tr>
<tr>
<td>96-100-79</td>
<td>Ottawa 3</td>
<td>MM 110</td>
</tr>
<tr>
<td>96-100-28</td>
<td>Ottawa 3</td>
<td>MM 110</td>
</tr>
</tbody>
</table>

Group 2-Dwarf elite group

The cross selections indicated in group two, figure 5 are of most interest as they are close to M9 size. Cross numbers 91-110-40, 99-118-21 and 99-150-10 have yield efficiencies greater than some of the standard rootstocks. Table 3 shows the origin of these selections. It is interesting to note that 4 of these crosses have CG5179 from Geneva in their parentage.

Figure 5. Dwarf elite group cumulative yield and yield efficiency
The two charts below show the 4 best crosses in this group.

Table 3 Parentage of best crosses in group two

<table>
<thead>
<tr>
<th>Cross Designation</th>
<th>Male Parent</th>
<th>Female Parent</th>
</tr>
</thead>
<tbody>
<tr>
<td>97-110-40</td>
<td>CG5179</td>
<td>Bud 9</td>
</tr>
<tr>
<td>99-118-21</td>
<td>Ottawa 3</td>
<td>CG5179</td>
</tr>
<tr>
<td>99-150-10</td>
<td>M9</td>
<td>CG5179</td>
</tr>
<tr>
<td>99-168-5</td>
<td>Bud 491</td>
<td>CG5179</td>
</tr>
</tbody>
</table>

The images above show relative size of rootstocks compared with M9 (far right).
Group 3 Smaller than M9
These are trees that are suitable for very high densities approximately above 3000 trees/ha

Figure 6. Smaller than M9
Table 4 Parentage of the 5 best crosses in group 3

<table>
<thead>
<tr>
<th>Cross Designation</th>
<th>Male Parent</th>
<th>Female Parent</th>
</tr>
</thead>
<tbody>
<tr>
<td>96-100-24</td>
<td>Ottawa 3</td>
<td>MM 110</td>
</tr>
<tr>
<td>99-111-6</td>
<td>Bud 9</td>
<td>CG5179</td>
</tr>
<tr>
<td>99-144-10</td>
<td>M9</td>
<td>MM108</td>
</tr>
<tr>
<td>99-150-5</td>
<td>M9</td>
<td>CG5179</td>
</tr>
<tr>
<td>99-164-19</td>
<td>M27</td>
<td>CG 6210</td>
</tr>
</tbody>
</table>

**Phytophthora Screening**

Screening for *Phytophthora cactorum* was initially performed on a series of commonly known rootstocks, including MM106, MM104 and M27 with known levels of resistance or susceptibility. Lesion length was measured and scored to confirm technique accuracy and assist with interpretation of further testing results. The virulence of the stored isolate of *Phytophthora cactorum* (DAR 60701) obtained from the Australian Scientific Collections Unit was also determined. Excised stems showing necrosis were re-isolated to confirm the presence or absence of *P. cactorum*. The results below confirmed accuracy and provided confidence to perform screening on stock from the breeding program.

<table>
<thead>
<tr>
<th>Rootstock</th>
<th>Result following testing</th>
</tr>
</thead>
<tbody>
<tr>
<td>M26</td>
<td>Mildly susceptible</td>
</tr>
<tr>
<td>MM106</td>
<td>Susceptible</td>
</tr>
<tr>
<td>MM104</td>
<td>Susceptible</td>
</tr>
<tr>
<td>CG7707</td>
<td>Resistant</td>
</tr>
<tr>
<td>M793</td>
<td>Resistant</td>
</tr>
<tr>
<td>P1</td>
<td>Considerable resistance</td>
</tr>
<tr>
<td>M27</td>
<td>Resistant</td>
</tr>
</tbody>
</table>

Sixteen selections from the program were then screened following the process as outlined in materials and methods. The pathology report results were encouraging with two selections showing susceptibility using the outlined protocol. One selection showed an eye spot lesion that was not typical of a *Phytophthora* resulting in no re-isolation of the fungus. Selections from the elite crosses were not infected using the described protocol. The pathology report indicates that further testing is desirable.
Discussion

The project has now successfully completed the orchard evaluation phase and allowed for the identification of a number of selections that targeted the original objectives of producing a woolly aphid resistant dwarfing apple rootstock. The program has evolved through parental establishment and selection, woolly aphid resistance selection, propagation of suitable stocks, dwarfing trait selection, pre screening for Phytophthora, and finally, evaluation in a test orchard.

The data has shown that a number of selections have been bred in a range of sizes that show potential for high density plantings under Australian conditions. Under the protocols outlined the selections have remained free from woolly aphid. The selections identified in group two require bulking up for further investigation in field trials. Selections in group one and three also have desirable traits and material should be maintained for any further potential breeding. It is interesting to note that Cornell University at Geneva is currently focussing its commercialisation efforts on 4 apple rootstocks (Warner 2009). These include:

- Geneva 11 - a 1978 cross of M26×Robusta 5
- G.41 - a 1975 cross of M27×Robusta 5
- G. 935 - a 1976 cross of Ottawa 3× Robusta 5
- Geneva 202 - a 1975 cross of M27 ×Robusta 5

The dates of when these crosses were bred indicate the time frames required for commercialisation, but more importantly the need to be prudent when maintaining germplasm for future use or release (Robinson et al 2009). The JM series rootstock releases from Japan which were produced in the early 1970s also took well over a decade to be named and registered. (Soejima et al 1998)

The key objectives of the Australian program take into account both desirable nursery traits and performance of the rootstock in a test orchard in an environment typical for quality apple production. Selection has encompassed the following selection criteria

- Resistance to WAA
- Upright growth habit for ease of handling in the stock bed
- Highly productive stool beds. All of the stools harvested had a minimum of 4 suckers.
- Lack of spininess or minimum number of spines.
- Lack of brittle wood and Reduced numbers of burr knots
- Reliable and consistent rooting ability. This is one of the key commercial requirements alongside woolly resistance and dwarfing characteristics. i.e. a capacity to reproduce
- A likeness to other dwarfing rootstocks similar to the parentage. This task was subjective and largely based on the investigators knowledge of managing stool beds and a range of dwarfing stocks over many years.
- Profitable yield development over time
- Yield efficiency

Table 5 indicates that based on cumulative yield and varying tree numbers per hectare the elite selections in group two, grafted to Jonagold, are competitive with M9 and M26 which are industry standard rootstocks. Tree densities around the world, particularly Europe were increasing up until the mid-1990s’ but in more recent times there has been a trend towards more moderate planting densities ranging between 2,800 and 3,800 trees. The tree densities presented below are considered to be in the range suitable for Australian orchards.

Table 5. Yield based on different tree densities

<table>
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<tr>
<th>Cross ID.</th>
<th>Cumulative Yield per Tree</th>
<th>Yield (tonnes) at Trees per Hectare</th>
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* These selections show excellent tree traits and potential for improved yields

As indicated in materials and methods, Jonagold was used as the test variety. This vigorous variety was used so that an early expression of the dwarfing capacity could be identified given the overall time frame of the breeding program. Given that identified selections have a dwarfing habit these rootstocks offer potential to achieve consistent high quality fruit by allowing more uniform light to penetrate the canopy and avoid shading, a significant factor in the loss of productivity. The division of selections into 3 vigour groups provides flexibility for future testing and if necessary, the option for selecting slightly higher or lower vigour types to manage varying types of growing systems.

As the Australian industry moves from low to high density plantings, new rootstocks cannot be recommended unless there are sustained efforts to investigate soil and climatic adaptability, size control, root anchorage, precocity, pest resistance, productivity and propagation. Most evaluation programs recognise the importance of evaluating interaction between genotypes and the environment (Hardner et al 2008). In the same way that growth characteristics of rootstocks in Northern America are different to that of Northern Europe, where most high density systems were developed, so are they also different under Australian conditions.
Technology Transfer

This Australian breeding program has planting material that can now be incorporated into secondary orchard trials. This protocol is similar to that of the NC-140 Regional Research Project in the USA which seeks to enhance practices in temperate fruit production by focussing on rootstocks. Now that material is available there is also an opportunity to include material into this program.

Until this point the transfer of information has evolved around creating an awareness of the program and also more generally the importance of the rootstock and the role that it plays in high density plantations. To a large degree the Future Orchards 2012 program, which commenced in 2006, has fortuitously assisted with this process directly by promoting high density plantings and reinforcing the benefits to Australian growers. This breeding project sits well with the key objectives of the 2012 program by aiming to improve grower’s attitude towards intensification and providing a better understanding of yield efficiency.

Over the last funding period commencing 2005 the investigator and team have:

- Attended twice yearly all Future Orchards 2012 program and monitored developments and sentiment related to rootstocks and intensification.
- Participated as an evaluator in the 2012 program and attended discussion groups with facilitators.
- Met with two evaluation co-ordinators of APFIP (Australian Pome Fruit Improvement Program) and inspected the evaluation orchard at Orange.
- Reported annually to Apple & Pear Australia Ltd (APAL) on project progress for inclusion into APAL annual reports which is disseminated to all levy payers.
- Inspected evaluation block with Dr Terence Robinson, apple rootstock breeder, Dept. Hort. Sciences, Cornell University, Geneva and John Wilton, AgFirst, New Zealand. This provided useful insight and direction into progress of the breeding project.
- Maintained contact with commercial groups like Australian Nurserymen Fruit Improvement Company in relation to rootstock development and gauging interest in future evaluation strategies. A number of nursery operators have also visited the site to inspect progress of the planting. This has provided a commercial perspective in terms of rootstock requirements.
- Participated as an evaluator for APFIP Orange NSW evaluation block and as such gained insight into new apple varieties and rootstock interaction.
- Presented progress report at the 2008 APAL annual conference in Hobart.
- Maintained contact with apple rootstock breeder Dr Terrence Robinson, Cornell University, Geneva, New York and received offer for testing of Australian bred stocks in the USA. (June 2010). Dr Robinson inspected the evaluation orchard in 2009.

Publications include:


It is always difficult for breeding programmes to release information until they have suitable selections for release and secondary testing. Now that the primary evaluation period is complete information on the selected progeny can be disseminated. Discussions with the Technical editor Apple and Pear Australia Ltd is underway to release information and articles can now be prepared for future publishing in professional journals. As stated earlier, commercial development in Australia will be linked with the Australian Pome Fruit Improvement Program and other commercial nursery groups.

**Recommendations**

The key recommendations of this study are to:

1. Continue to propagate elite stocks so that material can be bulked up and tested in secondary orchard trials with commercial scion varieties.

2. Evaluate the elite rootstock selections independently for full commercialisation and in association with the Australian Pome Fruit Improvement Program. Rootstocks from the following programs could be included.
   - Cornel-Geneva CG series
   - AR-series from East Malling
   - Selections from the Aotea programme in New Zealand
   - JM series from Morioka Japan

3. Develop in conjunction with industry a national rootstock assessement program which includes key local and overseas material for field testing. This concept has the support, in principal of APFIP as their current program focuses largely on scion varieties only. Suggested sites could include Tasmania and NSW. Discussions with the APFIP evaluation coordinator, indicates a need for this in Australia. This may well form part of a collaborative industry program as is the case with the NC-140 Regional Research Project in the USA. This evaluation is necessary to reduce risk to growers as high density plantings, despite the well documented returns, may cost 10 to 20 times more to establish and thus enhance economic risk. Evaluation could form part of the PIPS program and include the
4. Submit planting material from this program to Cornell University, Geneva, USA for screening for fire blight susceptibility.

5. Determine molecular markers in the newly bred stocks for genes conferring resistance to WAA. This could be completed in conjunction with Cornell University or completed in Australia.

6. Maintain international cooperative partnerships, in particular with plant breeders such as Terrence Robinson at Geneva, New York and explore possibilities for Australia to collaborate in apple rootstock evaluation.

7. Offer a testing service for screening of woolly apple aphid and Phytophthora. Recently imported apple rootstocks appear to have varying degrees of WAA resistance. With the expertise in assessing WAA susceptibility Industry and Investment NSW is in a position to offer a small testing service to the nursery industry for independent testing of any new stocks.

8. Investigate the suitability of the Australian rootstocks for hard wood cutting propagation.

9. Investigate funding opportunities so that a small secondary orchard evaluation program can continue to gain maximum return from this program.

Acknowledgements

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This project has been supported in the field by Stephen Gottschall, Industry & Investment NSW. Remy Van de Ven, I&I NSW provided statistical advice, planning and analysis. Thanks are also extended to former and present staff of I&I NSW, Jill Campbell, Suzie Newman, Delia Dray and Roy Menzies who contributed to concept development and input in the early phases of the project.

Finally, I wish to thank Shane Hetherington and Deidre Gunning, Orange Agricultural Institute for plant pathology support.
Bibliography


## Appendix 1: Parentage used in the hybridisation program

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<th>Cross Designation</th>
<th>Male Parent</th>
<th>Female Parent</th>
<th>No. of Crosses reaching Evaluation Phase</th>
<th>No. of Crosses still under Evaluation in 2010</th>
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