Breeding of woolly aphid resistant dwarfing apple rootstocks

Roy Menzies
NSW Department of Primary Industries

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BREEDING OF WOOLLY APHID RESISTANT DWARFING APPLE ROOTSTOCKS

Roy Menzies and Lester Snare
N.S.W. Department of Primary Industries

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The purpose of this project is to develop a woolly aphid resistant dwarfing apple rootstock. This report presents the current status of the progeny bred to date.

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Media Summary

The woolly apple aphid (WAA) breeding project commenced in 1996 with the specific aim to develop WAA resistant dwarfing apple rootstocks for Australian conditions. Seedlings produced in the initial hybridization program are now under field evaluation by NSW Department of Primary Industries at the Orange Agricultural Institute. This is part of a long-term research project tackling one of the apple industry’s most serious pests.

About 500 rootstock selections from an initial 10,000 seedlings are 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. 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.

Currently available dwarfing rootstocks for apples are highly susceptible to WAA. Late autumn sees the aphids migrate to the roots which act as a source of infestation for other parts of the tree. If a dwarfing WAA resistant rootstock can be bred then the most important part of the life cycle can be broken.

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.

The 515 rootstock selections that survived the screening and propagation phase have been grafted to the vigorous scion variety, Jonagold. All of these rootstocks have been selected for desirable nursery ‘friendly ‘characteristics as well as freedom from pest and disease. Selection for vigour control, early fruiting (precocity), tree form and fruit production commenced in 2004.

The project is at an exciting stage with a wide diversity of tree shapes and sizes. It takes time to adequately evaluate and then commercially develop the elite stock of 20 to 30 selections. This time lag is difficult for both research staff and growers alike but it is an inevitable part of the process. The industry has shown commitment to continue the field evaluations and incorporate the elite rootstocks into the APFIP variety program. Links with overseas breeding programs are providing future testing for diseases such as fire blight and further cooperative sharing of progeny. This will result in apple trees that fulfil the initial aims of the project that encompass the following attributes.

- easy to propagate
- resistant to WAA and diseases
- suited to Australian conditions
- grower friendly in growth and production
**Technical Summary**

The Australian apple industry is continuing to adopt higher density, closer planted orchard systems. The economics of these pedestrian orchards with the associated reduced picking costs and increased production and fruit quality are arguably the only future for Australian apple production. Unfortunately the dwarfing rootstocks available for these orchard systems, in particular M26 and M9, are susceptible to attack by woolly apple aphid (WAA). 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.

Chemicals offer short term control of WAA but insecticide resistance, ecological disturbance and marketing restrictions make long term dependence on chemicals undesirable. Biological control with *Aphelinus mali* can assist in WAA control on the aerial parts of the tree when populations are low but with higher populations control is limited.

Rootstock breeding overseas, particularly at Geneva USA and in New Zealand, are providing some new rootstock progeny but already these are proving to be site specific and are often not able to resist higher WAA populations. The challenge was to breed WAA resistant rootstocks that could cope with Australian conditions and higher WAA populations and incorporate a range of dwarfing characteristics.

In 2000, the report on Project AP96019 outlined the parental selection and breeding strategies to incorporate WAA resistance with dwarf growth habit. The seedlings were screened for WAA in a glasshouse and susceptible seedlings were culled. 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.

Three rooted shoots of each selection were grafted with the variety Jonagold. These were then planted in the evaluation block. This process was carried out for three years to complete the preliminary evaluations from the initial three years of hybridisation. The evaluation block is randomised and includes 62 pollinators as well as industry standard rootstocks MM106, M26 and M9 for comparison. The high density planting aids in economy of space but also simulates the actual commercial situation. A combination of field recording for butts and yields and ratings for tree habit together with observations on pests, diseases and anchorage will provide a matrix of characteristics to select the elite stock for commercial testing. As with any breeding project, the selection for field evaluation is the beginning of an extensive process. However, the initial selection process over the last three years has produced approximately 515 candidates from the initial 10,000 seedlings. This is very encouraging as there is a wide range of tree vigour and tree forms with WAA resistance from which to select.

Recommendations from the project so far can be summarised in three points. Firstly, the industry has committed funds for the breeding project since 1996 and it is crucial that the support continues so that the significant potential of the breeding selections can be brought to commercial fruition. Secondly, while there will be efforts to reduce the long lag time from hybridisation to commercial sale and use, there must be strong caution against an early release of less than satisfactory rootstocks. Thirdly, the continued fostering of links with overseas breeders will help in reducing the length of evaluation time, open opportunities for acceptance
overseas, enable cooperative screening for other diseases such as fire blight and create opportunities for ‘swapping’ elite stock.

At this juncture of the project the technology 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 the crux of fruit tree breeding.
Introduction

The trend in apple growing in Australia is towards closer planting, using smaller compact trees to improve production, efficiencies, yields and returns. Whilst a number of the more vigorous rootstocks are resistant to woolly apple aphid (WAA) there are few options when dwarfing stocks are required. The objective of this breeding program is to develop a dwarfing apple rootstock with WAA resistance. The use of resistant rootstocks is one of the most effective methods of controlling this pest.

WAA is a major pest of the apple industry worldwide causing economic losses by reducing fruit quality and tree productivity. 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. 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. 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 (Nicholas 1997).

In terms of nursery production the presence of WAA in the stool bed is highly problematic and cannot be controlled without chemicals. (Malone and White 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.

Current options for growers wishing to use dwarfing rootstocks generally include M9, M26 or Mark and these stocks are not resistant to woolly aphid. Developing a host with resistance is the basis of this project. The Malling-Merton (MM) series of rootstocks derived from Northern Spy were selected partly for resistance to WAA (Lyth and Watkins 1981). However, they are not immune, and local infestations have been reported from as far back as 1927 (Le Pelley 1927).

Some of these infestations have been attributed to mutant aphid strains in other parts of the world. However, Knight and Alston (1972) confirm that Northern Spy (parentage unknown) and its MM derivatives remain of great value as sources of resistance. This resistance has been included in this breeding program but 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 the CG series which have *M. robusta* as one parent.

The Australian apple industry has acknowledged the significance of woolly aphid as a key pest as evidenced by initial funding for the project. The program now commences the all important identification of suitable stocks with potential material now available for early assessment. The implications for the commercial apple industry, the tree nursery industry and other breeders is that a more sustainable and profitable rootstock will be available in Australia that meets Australian conditions.
Materials & Methods

This four year funding period has two distinct parts. Firstly the completion of the propagation and nursery selection phases and, secondly the establishment of an evaluation block for orchard assessment of progeny. Table 1 shows the time scale and events for the breeding program since its commencement.

Table 1. The breeding program time line up to the present

<table>
<thead>
<tr>
<th>Time</th>
<th>What happened</th>
</tr>
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<tr>
<td>1996 and 1997</td>
<td>Initial cross pollinations</td>
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<td>1998 and 1999</td>
<td>Second cross pollinations</td>
</tr>
<tr>
<td>1998 to 2001</td>
<td>Seedlings “challenged” with woolly aphid in glasshouse</td>
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<td>1999 to 2002</td>
<td>Selected seedlings planted in stool beds for nursery evaluation</td>
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<tr>
<td>2000 to 2003</td>
<td>Selected seedlings from stool beds grafted with Jonagold</td>
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<tr>
<td>2001 to 2004</td>
<td>Grafted Jonagold trees planted in test orchard</td>
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</tbody>
</table>

Breeding strategies and pollination

The initial phase of the breeding program was parental selection. The critical characteristics sought in the parents were resistance to woolly aphid and a dwarf growth habit (Table 2). Disease resistance and ease of propagation are also important and formed part of the selection criteria but are less critical. Controlled crosses were carried out over a three year period from 1996 to 1999 using pollen from both local and overseas sources. This resulted in approximately 10,000 seedlings. Ideally, the plan was for 30,000 seedlings but resources were not available.

Table 2: Parentage groups that were used to provide sources of WAA resistance and genetic dwarfing

<table>
<thead>
<tr>
<th>Sources of woolly aphid resistance</th>
<th>Sources of dwarfing</th>
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<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 sieboldii – crab apple NZ (Snowbright A126)</td>
<td>Budagovsky 9* (Russia)</td>
</tr>
</tbody>
</table>

*Phytophthora resistant
+ Fireblight resistant (MM.106 is tolerant)

Culling woolly susceptible progeny

The primary and initial selection task was to cull or remove any seedling that showed susceptibility to WAA. Young seedlings were exposed to high numbers of healthy non-parasitised aphids in the glasshouse. 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 test orchard is adjacent to commercial plantings so that the selections will be potentially exposed to woolly during their evaluation. This will ensure that WAA resistance is not compromised.

**Selecting nursery friendly stock**

After eliminating WAA infested seedlings, the remainder were planted in a nursery. 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.

Those selections that met the 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. These graftlings were potted, held under shade house conditions and planted out the following winter.

**Field testing in the orchard**

The vigorous non-precocious Jonagold has been used as the test variety. This provides the most rigorous test for the rootstock candidates. Over 500 seedling rootstock selections have been selected and grafted during the 3 seasons from 2000 to 2003. There are three trees of each and they are being compared with MM106, M9 and M26.

**Evaluation orchard soil type**

The orchard evaluation block at the Orange Agricultural Institute (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 a red light clay of pH 6. Both A and B horizons are well structured. The site has been in fallow for over ten years and the soil is typical of that found on most local district orchards that are of reasonable fertility and vigour.

**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 1 m 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 will allow 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 provides not only adequate replication but a margin of safety when the elite crosses are propagated in situ. It is anticipated that elite selections will be converted to stools to facilitate propagation.

A simple two wired trellis has been erected to provide some support for the shallower root systems and the early crops.

*Figure 1: Spreading sawdust on the stool beds in summer 2002 to encourage rooting of the rootstock selections.*

*Figure 2: Removing rootstock selection from the stool bed nursery assessment block. The two selections on the right have excellent root and sucker production. The one on the left is a typical vigorous seedling type with poor sucker production.*
Results

Approximately 1700 trees have been 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. At this stage it is the 2001 and 2002 plantings which have provided the most data in terms of the rootstock performance in the field. The selections with the highest number of crosses are represented by CG5179 × Budagovsky 9, followed by Ottawa3 × MM110. These types have high numbers of plants with suitable nursery characteristics and are showing woolly aphid resistance. Orchard evaluation is required to assess dwarfing capability but some preliminary observations were made under stool bed conditions on their likeness to M9 and M26 rootstocks. Figure 2 shows the total number of selected progeny from each parental cross.

Table 3 lists the parentage and number of crosses planted over consecutive years including the bench mark industry standards. 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). To create greater diversity amongst rootstock candidates, parents used in the earlier crosses were re-allocated and additional crosses produced. This also allowed for the increasing quantity of flowers on more recently planted mother trees. A total of 1697 trees using 515 selected progeny with three replicates make up the planting. The remaining 152 trees are either the benchmark standards (M9, M26, and MM106) or pollinators.
Table 3: The progeny parents, the number of crosses selected and time of planting.

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<td>221</td>
<td>764</td>
<td>515</td>
<td>1697</td>
</tr>
</tbody>
</table>

At this early stage in the field evaluation process butt circumferences are the main numerical data available to analyse. The scatter plots in Figure 4 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. On the other hand the younger 2002 planting shows a more random growth response after two years’ growth.
Figures 3: Scatter diagrams for all progeny in each of the 2001 and 2002 plantings. The diamonds represent the standard benchmark rootstocks.

Figures 4 and 5 show the combined mean butts for each of the parental combinations for the 2001 and 2002 plantings. The standard errors are strongly influenced by the number of seedlings in each parental group. Where numbers are small variations are greater.

**Trunk circumference (mm):  trees planted in 2001**

*Figure 4: Tree growth of the older 2001 planted trees showing the 3 standard stocks in comparison with the mean value for all progeny from each of the 5 parental combinations*
Trunk circumference (mm): trees planted in 2002

Figure 5: Tree growth of the younger 2002 planted trees showing the three standard stocks in comparison with the mean value for all progeny from each of the 5 parental combinations.

Figure 6: Lester Snare, Technical Officer at OAI, examining the 2002 planting in the evaluation block.
Discussion

The project has successfully entered stage 3 as described by Campbell in 2000 with the establishment of all selected stocks in the evaluation test orchard. This has been achieved via the stooling of stocks and the selection of suitable candidates in the stock beds. All stocks now growing in the orchard evaluation block have been selected for desirable commercial nursery characteristics. Although not all of the juvenile characteristics of the seedlings indicate ideal dwarfing traits, a successful rootstock must be productive and friendly in the nursery for commercial acceptance. These characteristics include:

- 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
- Lack of burr knots
- Reliable and consistent rooting ability. This is one of the key commercial requirements alongside woolly resistance and dwarfing characteristics
- 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.

The early growth data collected from the evaluation orchard is already revealing some interesting facts about the selected seedlings, their parents and also the types of responses that indicate future dwarfing potential. Early growth of both dwarf and vigorous stocks has been similar. This is important in establishing a sound orchard tree that has a good root system and an optimal fruit bearing frame. Now after three years, the expected growth reduction in both the standards and the dwarf seedlings is beginning to show. This delayed growth reduction is the type of dwarfing that is ideal for compact orchards. There were, however, some very small trees that showed strong dwarfing in years 1 and 2. These trees will never develop into commercial bearing trees. The second important result from the preliminary growth data is the variation in growth and tree habit. The scatter diagrams in figures 5 and 6 shows an excellent spread of growth for the seedlings. Not only are there ideal growth and development patterns in a significant number of seedling stocks but also the variation will provide good options for further selection.

Rootstocks affect many aspects of an apple tree’s life from flowering, tree form and nutrition through to fruit colour and size and finally fruit quality and storage (Cummins and Aldwickle 1983). As such, the evaluation orchard is critical. The initial screening, selection and multiplication have been relatively demanding on resources. The evaluation phase will be particularly demanding on staff time to ensure that the elite stock finally presented for industry development are the best available. With both dwarfing characteristics and WAA resistance demonstrated on many of the selections, the other qualities will become more important in fine tuning the final product.
Technology Transfer

With the project in the early stages of development technology transfer has been limited to industry awareness. In 2003 the first significant field day was held at the Orange Agricultural Institute in June in conjunction with a couple of other research projects. Bayer, who produce the most effective chemical treatment currently available for WAA control, were also featured on the program. It was encouraging to receive very positive support from the technical staff of Bayer for the breeding project. They recognised that this approach provides the only long term answer to the WAA problem.

The next part of technology transfer involves the participation of overseas as well as local breeding and nursery groups. Developing the linkages necessary for the successful adoption of the final rootstock candidates has been facilitated by discussions with Terrence Robinson, University of New York, Cornell, USA. This has led to the offer of assistance for the screening of fire blight resistance and for possible inclusion of material into the NC140 evaluation program. Commercial development in Australia will be linked with the Australian Pome Fruit Improvement Program and other commercial nursery groups including ANFIC and Flemings & Associates. Both these companies have the distribution rights for CG rootstocks from Cornell that will be used in further evaluations at Orange.

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. Their comments were helpful and they were impressed by the range of growth habits and tree vigour. The Industry Development Manager (Val Hilton) from Apple & Pear Australia Ltd also inspected the site in 2003. Media releases resulting from the field days included the following.

- ‘WAA resistant rootstock breeding program’. Orchard walk and brochure, Orange Agricultural Institute, 28 May 2003
- ‘Keeping apple bug at bay’. NSW Agriculture Today, 29 May 2003
- ‘Benefits of WAA resistant rootstocks’. ABC Rural Radio (Central West), 5 June 2003
- ‘Woolly aphid still too wily’. Western Magazine, 5 June 2003
- ‘Beating woolly apple aphid – breeding rootstocks for the 21st century’ poster for 2nd national Conference, APAL, August 2004

Future activities
The first seven years of the project focussed on production and selection of the core rootstocks. The future will now centre on the selection process which will narrow those rootstocks to the final 20 or 30 elite stock. The next four to five years of field measurements and selection will provide a much greater source of interest and information for growers. Regular updates during this period will be important for growers to understand the progress and process on selection. There are and will be opportunities to incorporate other aspects of breeding, assessment criteria, dwarfing mechanisms and plant/disease resistance. Other examples include

- commercial WAA resistance testing for newly imported stocks (some CG stocks are not WAA resistant)
- the use of interstocks
- the mechanism of dwarfing
- the part for molecular biology
Recommendations

1. Industry recognition since the project’s conception in 1996 has been a great support. This investment must continue to ensure that the promise from the last 8 years is justified and fulfilled.

2. Commercial nurserymen and orchardists have given advice and direction during the development phase. This input should not only continue but it should also increase and be placed on a more structured framework.

3. The elite rootstock selections will be critically evaluated for commercialisation in the APFIP scheme. Variety compatibility will also be determined during this time.

4. International cooperative partnerships, in particular with plant breeders such as Terrence Robinson at Geneva, New York, have been commenced. These collaborative ventures need to be continued, expanded and formalised to enable shared testing of selections from both programs. Disease challenge with fire blight will also be part of the arrangement.

5. CG rootstocks from Geneva have recently been released from quarantine. These rootstocks should be included in the evaluation block. Arrangements have been made for this to happen. This evaluation will also provide advanced data on the performance of these rootstocks under Australian conditions.

6. Although the evaluation process is only in its infancy, we recommend caution in the future commercialisation of the elite stock. It is imperative that inferior material is not released to the industry. Despite the extra one or two years in testing, the integrity of the breeding program and the apple industry will be preserved.

7. Recently imported apple rootstocks appear to have varying degrees of WAA resistance. With the expertise in assessing WAA susceptibility we are in a position to offer a small testing service to the nursery industry. A similar situation could also exist for Phytophthora.
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Bibliography


