Final Report

Commercial development of subtropical mandarin hybrids

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CT09023

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Media Summary

Mandarin growers operating in subtropical Australia need better varieties if they are to remain competitive on both domestic and international markets. There is a significant gap between consumer expectations and the mandarin fruit they actually consume, and new improved varieties can close this gap. The problem is long-standing, and more than 20 years ago Queensland grower organisations approached their state Department of Agriculture and Fisheries to collaborate in a private mandarin breeding program to start tackling the problem; the Mandarin Hybridisation Project. More than 50,000 hybrids resulted and were carefully tested to identify a few that showed the most promise. This project aimed to progress this high quality material toward commercial production.

More than 16,000 original hybrids remained to be assessed and this was completed during the project, with the last progeny block bulldozed in May 2014. No new hybrids were produced, focussing instead on existing material and getting it ready for commercial production. Although close to 500 individual hybrids showed commercial potential when originally selected from the progeny blocks, an extensive period of stage-two testing using conventional rootstocks and wider spacing gradually identified faults with most of these and less than 20 were finally considered worthy of testing in commercial orchards. With such an exhaustive list of essential traits, it is hardly surprising that such a small fraction of the original hybrid population (<0.05%) had all the necessary characteristics in the one cultivar.

A major obstacle to commercial production of the selected cultivars was the presence of very high seed numbers (20-30 per fruit) and this was successfully solved by this project. Through the production and screening of large numbers of variants, we were able to obtain low-seeded selections of the best quality hybrids. These were then carefully compared as daughter trees to ensure undesirable changes had not occurred in other traits such as acidity and productivity, and to identify the best few to go into a final stage of commercial testing.

Commercial test blocks of low-seeded variants of three high quality hybrids were planted at the end of the project, and will provide fruit for test marketing and production testing ahead of full-scale commercialisation.

The project was successfully guided by a management committee comprising representatives of the contributing organisations. Important issues such as a commercialisation strategy module and an open tender process for commercial-scale testing were undertaken by this committee. Industry engagement activities occurred in every year of the project, providing opportunities for growers, marketers and contributing organisations to inspect and taste the new mandarin cultivars as they progressed toward commercialisation. Feedback from this engagement was critical to identifying which hybrids had the best commercial potential.

Improving the profitability of commercial mandarin growing in the subtropics, via the availability of better quality mandarin cultivars, was the key objective of this breeding project. With new high quality low-seeded mandarins now established in commercial quantities, and industry players eager to gain access, this goal is within reach.

Technical Summary

There has been a sharp decline in the profitability of citrus growing businesses in subtropical Australia over the last few decades, and significant improvements in technology are required to boost efficiency and ensure sustainability in an increasingly competitive world citrus market. Better mandarin cultivars are a critical component of the technology needed to improve profitability and growers recognised this fact many years ago when they collected voluntary levies and engaged with the Queensland Department of Agriculture & Fisheries to undertake a private breeding program. Such long-term vision and foresight provided a wealth of genetic material from which this project could re-select and quickly move the best selections toward commercial production.

Some 16,566 hybrids remained to be assessed from original progeny blocks, containing the offspring of 36 parents in more than 200 parental combinations. During the project, 38 new hybrids were selected and re-propagated from these progeny blocks and added to the 399 previous selections present as daughter trees. The last progeny block was culled in May 2014 when trees were almost 10 years old.

A second round of assessment and culling occurred, using daughter trees grown on conventional rootstocks at wider spacing. Once these trees commence fruiting they were described in detail at 3-4 times during the season, and this occurred for multiple seasons. Genotypes that performed poorly or had ‘fatal flaws’ could be quickly removed from the program but most required assessment for at least four seasons. Many genotypes performed well in one season but then performed poorly subsequently, and this in itself became a major reason for culling many of the original selections.

Five original selections had performed well as daughter trees and so were established in large plantings on commercial orchards in Mundubbera and Gayndah. Three of these have now entered the final stage of commercial testing with PBR applications lodged. Rootstock testing showed that a range of existing industry rootstocks would be adequate, and these field trials will enable any early signs of incompatibility to be identified prior to large-scale plantings.

Exports are critical to industry profitability, and have always been a feature of mandarin businesses in the subtropics. Postharvest testing of hybrids from this breeding program resolved concerns that high Brix fruit may be more prone to off-flavour development along the export supply chain. The problem of off-flavours was induced by the use of low permeability shellac-based fruit coatings (‘waxes’) which was further exacerbated at higher storage temperatures. It was not related to the Brix content of the fruit, and indeed high Brix fruit seemed less prone to the problem. The three hybrids included in the supply chain simulations performed at least as well as the three cultivars which are currently exported commercially. Although genetic aspects of differences in supply chain performance are poorly understood, further work in this area is low priority given that shellac-based coatings are the major reason for off-flavours and this problem can be easily fixed.

Consumers want less seeds in their fruit, and they want it more than every before. Hybrids from this program were extremely seedy (20-30 per fruit) and a major achievement of this project was to solve this problem. The best hybrids from the program are now available with greatly reduced seed numbers, thanks to the planting, screening and re-testing of large numbers of irradiated buds. More than 60 low-seeded variants of eight advanced hybrids were developed and tested during the project and the best of these have already been used to establish the final stage of testing in commercial-size orchard blocks. The discovery of problems such as low productivity, high acidity, and bitter flavours reinforced the need for careful testing of daughter trees from multiple low-seeded variants of high quality hybrids.

Clear guidance from a project Management Committee and regular industry engagements in every year of the project have helped to ensure this breeding program quickly progressed the best genetic material toward commercial production. This is now within reach, and the eagerness of industry stakeholder who have seen the material first-hand to start commercial production, suggests that the project is close to providing new mandarin cultivars that meet industry and consumer expectations.

Chapter 1: Progeny block assessment and Original Selection propagation

At the commencement of this project approximately one third of the original ~50,000 hybrids generated in the Mandarin Hybridisation Project remained to be assessed. The last of these hybrids was generated in the 2002 flowering season, with the resulting fruit harvested in May 2003 and the hybrid seedlings field-planted in February and March 2004. One of the key objectives of this project was to complete the first-stage selection of this primary material so that all progeny blocks could be removed. To do this, the 5 remaining progeny blocks at BRS were assessed on multiple occasions throughout each fruiting season to identify any hybrids of sufficient merit for selection. Budwood was then taken from these new selections at the end of each season, propagated onto rootstocks, and the resulting nursery trees planted into the high security compound. This chapter provides an outline of the progeny material that was selected, the source families that gave rise to it, and the process of quickly making selections so that progeny blocks could be bulldozed. The project was primarily focussed on commercial development of selections made years earlier, and there was little enthusiasm from either industry or funding bodies to continue the primary breeding work. However the breeding team were determined that the years of hard work and investment would not be undermined by the usual rush-to-release that plagues tree crop breeding internationally.

Five progeny blocks, G1, G2, H1, H2 and J, containing a total of 16,566 mandarin hybrids had been field planted at BRS between May 2001 and February 2004. Table 1.1 gives the details on these different plantings, including some of the parentage they contained.

Table 1.1: Planting dates, tree numbers, and family/parental composition of hybrid progeny blocks assessed during CT09023.

Block	Planted	No. of hybrids	No. of families	Main parents
G1	May 2001	1,635	44	Burndale, Daisy, Ellendale, Encore, Fortune, Fremont, Hickson, Imperial, Kinnow, Minneola, Murcott, Nova, Orlando, Ortanique, Page, Wekiwa.
G2	Jan. 2002	1,608	14	Ellendale, Encore, Fallglo, Fortune, Hickson, Kinnow, Monarch, Murcott, Ortanique, Weikiwa
H1	Jan. 2002	1,992	27	Afourer, Arrufatina, Chironja, Daisy, Ellendale, Encore, Fallglo, Fortune, Hickson, IM111, Kinnow, Murcott, Nova, Orlando, Ortanique, Topaz.
H2	Apr. 2003	5,960	85	Chironja, Bakers Sweet, Daisy, Ellendale, Ellenor, Encore, Fallglo, Fremont, Fortune, Hickson, Imperial, IM111, Kinnow, Mineola, Monarch, Murcott, Nova, Orlando, Ortanique, Page, Temple, Topaz, Umatilla, Wilking, 01C028, 01C035, 01C049, 01C050.
J	Mar. 2004	5,371	76	Afourer, Daisy, Ellendale, Ellenor, Encore, Fallglo, Fremont, Fortune, Hickson, Imperial, IM111, Minneola, Monarch, Nova, Ortanique, Temple, 00C018, 01C028, 01C049, 01C050, 02C065, 02C105, 02C110, 02C116.

It can be seen from Table 1.1 that a large and diverse population of hybrids was available for selection during the course of this project. An increasing usage of BRS-bred parents is also evident in the younger progeny blocks. The breeding program was initially plagued by an absence of good parents to use in the annual hybridisations, but as new material fruited and was selected, we were able to incorporate some of this back into the program. As a consequence the breeding program now has an abundance of good monoembryonic parents that are superior to the named commercial cultivars that were the only choice earlier in the breeding. Monarch, Ellenor and IM111 are QDPI-bred cultivars originating from efforts prior to 1970, whereas those with code numbers are selections that have been made since 2000. The increasing number of families and diversity of parents seen in these last 5 progeny blocks of the MHP was a consequence of deliberations by the Management Committee and new breeder who had taken over the program in 1998. At that time it was realised that the crossing program was too narrowly based and that a greater diversity was needed in order to identify other parental combinations that may give commercially useful progeny. Whilst this unavoidably increased the complexity of the program it had the distinct advantage of revealing the heritability of various traits and allowed the breeding team to estimate the value of individual parents. By allowing the assessment of these progeny blocks during this project we were able to capitalise on the decisions made by the Management Committee to expand the program. Consequently, there are many parents that we would never use again (such as Ortanique, Weikwa, Orlando, Minneola, Imperial) or would use with extreme caution knowing their serious limitations (such as Afourer, Hickson and Kinnow).

The assessment of progeny blocks occurred on multiple occasion during each season so that hybrids of different maturity times could be accurately assessed. It is virtually impossible to determine the optimum maturity time of a new mandarin hybrid simply by looking at the fruit, as some hybrids can colour very early in the season and yet be still highly acid until some months later. With young seedling trees at such high density it was often the case that only a single piece of fruit was available for assessment and the breeding team became proficient at determining whether fruit should be picked and assessed or left on the tree until the next round of assessments. For example, in the 2010 season the progeny blocks were assessed on the 15-17 March, 7-9 June, 2-4 August and 4-6 October with these multiple assessments increasing the likelihood of assessing new hybrids close to their optimum and also ensuring that genotypes with the potential for maturity at the extreme ends of the season were not missed. Where individual hybrid trees had multiple fruit, it was then possible to assess this at different dates. A system of coloured pin-markers and trunk sprays was used to identify hybrids that warranted re-assessment during the next round, for example if they were excessively acidic but otherwise suitable for selection.

Table 1.2 summarises the number and types of Original Selections made from the 5 progeny blocks in each year of the project.

Table 1.2: Number of Original Selections and parentage of genotypes discovered and re-propagated from different progeny blocks in each year of the project CT09023.

Year	Total selections	Progeny block					Parentage of selections
		G1	G2	H1	H2	J	
2010	4		2			2	Fortune, Encore, Monarch, Murcott, Hickson
2011	17	3		2	5	7	Daisy, Ellendale, Encore, Fremont, Hickson, Imperial, IM111, Monarch, Murcott, Nova, 00C018, 01C028, 01C049, 01C050, 02C065, 02C105, 02C110
2012	10					10	Daisy, Hickson, Imperial, IM111, Monarch, Murcott, 00C018, 01C028, 02C105, 02C110, 02C116
2013	7					7	Afourer, Daisy, Ellenor, Encore, Fallglo, Fortune, Monarch, 02C065,
Total	38	3	2	2	5	26	

The progeny blocks G1, G2, H1 and H2 were removed in November 2011 after a final round of assessments in late August of that year. Hybrid trees in these block ranged in age from 8 years 7 months to 10 years 6 months at the time of their removal. Selected rows in J Block were culled in August 2012 when they were 8 years 5 months old, because they had been identified as poorly performing families and their removal made additional space for nearby families to grow and flower, and also improved access for spraying. The remainder of J Block was removed in May 2014 when trees were 9 years 8 months old.

Figure 1.1: Removal of progeny blocks following final assessment. Selective removal of poor quality families in J Block in August 2012 prior to complete removal of this block in May 2014.

Experience from this project shows that conventional mandarin hybrid blocks need to be retained for 8 to 10 years in a breeding program, even allowing for the fact that many trees (perhaps >30%) have still not commenced fruiting after this time. Previous work at Merbein in southern Australia showed that some mandarin hybrids had still not commenced fruiting after more than 20 years. Retaining progeny blocks for more than 10 years is considered unwise because the high density plantings (10,000 trees/ha) used in the BRS program causes between-plant competition to increase exponentially as trees age which further reduces their likelihood of fruiting. But more importantly, the length of the juvenile period is a heritable trait and so hybrids that only commence fruiting at a very old age are likely to pass this trait to their progeny if they are used as a parent. Conversely, hybrids that fruit at a young age are likely to produce progeny that also tend to fruit while relatively young. Because the BRS program is designed as a multi-generational breeding populations we need to make selections that have merit as both commercial cultivars AND new breeding parents. Reducing the length of the juvenile period of mandarin hybrids is one of the corner-stones of the BRS program and so progeny block removal after 10 years is desirable, even if it means that some potential selections have been missed.

A third benefit of “early” progeny block removal is that it allows resources to be devoted to the next generation of hybrids, and for limited land resources to be replanted. For example, the removal of H1 Block in November 2011 was followed by a brief wet season fallow and then immediate replanting to new mandarin hybrids in April 2012. Similarly J Block, which was bulldozed in May 2014 was back under mandarin hybrids by April 2015. This again reinforces the commitment of the breeding team to driving genetic progress through multigenerational mandarin breeding.

Chapter 2: Re-assessment and culling of Stage 2 selections

At the commencement of this project more than 400 Original Selections had been made by the Mandarin Hybridisation Project. These had been propagated onto conventional nursery rootstocks and then field planted in the high security compound at BRS. An additional 38 selections were made from the remaining progeny blocks (G1, G2, H1, H2, J) during this project (see Chapter 1) and these were also re-propagated and planted into the high security compound. All of these Original Hybrids needed to be assessed in order to identify any that truly had sufficient merit to become commercial cultivars.

Two young nursery trees of each Original Hybrid were field planted the season following their selection from progeny blocks, such that the collection of germplasm to be assessed in the high security compound varied in age depending on when selections had originally been made. Trees generally took 2-3 seasons to recommence fruiting and assessment of such trees commenced immediately. Every fruit tree was assessed at multiple times throughout the fruiting season in order to capture hybrids when they were close to their optimum maturity. These assessment times were normally late-March, June, late-July and September.

For each assessment period, the breeding team worked collectively to assess and describe the characteristics of each tree. Assessed fruit quality characteristics included: size, shape, skin texture, peelability, skin thickness, rag, external colour, internal colour, Brix, acidity, firmness, seed number, albedo retention, crop size, rind oil offensiveness, skin blemish, taste, chewiness, granulation, navel and areola, alternaria presence, scab level and maturity time. Trees were also rated for crop load and health, and given an overall score which reflected the breeding teams opinion of the likely commercial potential of the hybrid (taking all factors into account). A series of 'fatal flaws' were identified and carefully watched out for during the assessment process. These were traits that we believed would always prevent a hybrid becoming commercially successful, regardless of how well the hybrid performed for other characteristics. One such example is an offensive rind oil smell that can sometimes be overlooked when making the original selections from the progeny blocks. It is a minty smell that is released as the rind is peeled and occurs in about 1 in 20 selected hybrids (and in the American cultivar Osceola). We refer to this smell as 'the skunk' and any hybrids with this trait are immediately removed from the program. Likewise, granulation is a 'fatal flaw' and any hybrids that show this flesh disorder are immediately removed. Granulation has caused significant problems for the Australian citrus industry over recent decades, and tends to be aggravated in warmer production areas. Work at BRS has shown that the trait is strongly inherited and so by immediately culling hybrids with this 'fatal flaw' we not only avoid future problems for the commercial industry but also help to remove the trait from our breeding populations.

Original Selections in the high security compound were not only assessed at multiple points during the season, but also for multiple seasons. Mandarins are notoriously difficult to phenotype and hybrids that produce outstanding fruit in one season can often produce very poor fruit quality the next. New hybrids do not get planted in the high security compound unless they showed outstanding fruit quality when selected from the original progeny blocks. And so it is one of the frustrations of mandarin breeding that genotypes can appear to be highly suitable when originally selected, but then have poor fruit quality attributes when examined in more detail. The reverse situation has never occurred! The stability of quality is perhaps the most important trait in any new mandarin, and it is also the most difficult to measure. By assessing all hybrids at multiple times of the season and for multiple seasons, it becomes possible to identify those few selections that consistently produce high quality fruit. The assessment process followed by the breeding team involves having on hand all previous data for individual trees at the same time that the new assessments are being made. In this way it is possible to refer back to past performance to identify if hybrids have had a consistent problem with a particular trait. This system has proven extremely useful in deciding whether individual trees should be culled or kept for further assessment.

A primary tenet of the breeding program at BRS, and this project in particular, is that the majority of available resources should be devoted to the most advanced material. For this to occur, poor hybrids must be identified and culled as quickly as possible so that they do not consume further resources. Major culls of Original Selections occurred in the high security compound throughout the period of this project, such that the planting is now a patch-work of trees, with many open spaces where poor performing hybrids have been removed. For example, in the 2011 season 81 trees were identified as consistently poor performers and were cut out in August of that year. While this has been important for creating space in which to plant new Original Selections from the progeny blocks, the great benefit of this extensive and continuous culling process has been to focus efforts on the better material. This is the material with the most potential value commercially and as future breeding parents.

Figure 2.1: Selective culling of poor performing Stage 2 genotypes from the high security compound has left a patchwork of trees, and allowed the breeding team to focus their efforts on the better quality hybrids.

Through a process of annual culling, and careful trait description across multiple seasons, this project has identified a population of about 50 high quality mandarin genotypes, of diverse parental background, now held in the high security compound at BRS. This was the venue for a fruit display when members of the Hort Innovation board visited BRS during the course of this project.

Figure 2.2: Members of the Hort Innovation Board and local industry leaders inspect trees and fruit samples within the high security compound at BRS, the site of all Stage 2 assessments of hybrids from the program.

Chapter 3: Propagation and semi-commercial evaluation of Advanced Selections

Selections from the breeding program that had consistently shown outstanding performance were planted in semi-commercial sized blocks on commercial orchards in the Central Burnett. Initially there were five of these Advanced Selections and more became available as the project progressed. Budwood from these Advanced Selections was irradiated prior to propagating the trees for the semi-commercial blocks to enable low-seeded mutations to be identified simultaneously with the commercial assessment of these Advanced Selections. All nursery operations were performed at BRS, to facilitate security and integrity of the planting material. The field plantings occurred at five different sites in the commercial production area, plus an additional top-worked site. These sites were carefully chosen so as not to compromise the security of the material, or to spread the material too thinly across sites and decrease operational efficiency through increased administration and operational costs. Any less sites and we would have exposed the program to potential failure should a site be poorly managed, change ownership or be destroyed by a natural event like hail. It could not have been foreseen at the commencement of this project that two significant flood events would occur during the life of the project, one of which was the largest on record. Flooding in December 2010 reached 7.92m on the Burnett River at Bundaberg and was significantly exceeded in January 2013 when it peaked at 9.53m. Prior to this the record had been 9.04m set in January 1890. Not surprisingly, major damage resulted to almost all trial sites and had to be rectified by the breeding team and collaborating growers.

Figure 3.1: A minor flood in 2010 followed by a major event in 2013 caused significant damage to project trial sites in Mundubbera and Gaydah. The breeding team assisting clean-up of trial sites and adjacent orchard area so that irrigation could be resumed.

Commercial sites were also chosen to provide an efficient and effective means of assessing Advanced Selections within a semi-commercial context. It was based on the trustworthiness of the grower (particularly in relation to maintaining confidentiality), high horticultural standards, grower interest, and the biophysical appropriateness of the site.

Figure 3.2: Planting four of the six semi-commercial trial sites established around Mundubbera/Gayndah during the project period.

Trees were propagated on two major rootstock (Troyer and Benton) to enable performance comparison with Original Selections in the high security compound at BRS. In addition, a large number of new experimental rootstocks (such as US812, Cox and Fraser) were also included on a limited scale to provide preliminary information on their potential usefulness in the production environment.

The objective was to establish a minimum of 50 replicates of each Advanced Selection on at least two sites, planted in such a way that individual selections could be culled as appropriate without compromising commercial growing practices. This was accomplished at the first two sites by alternate-planting the five different Advanced Selections down each row, creating an opportunity to cull poor selections while keeping rows intact. However in practice and as with almost all high density plantings, this selective culling did not take place even after two of the Advanced Selections were dropped from the program; it was simply easier to leave all trees in place.

Data was collected and collated by the breeding team from BRS. Most assessment were made in the field but on some occasions fruit and/or extracted juice samples needed to be processed in the laboratory in Bundaberg. Characteristics measured include those used on the Stage 2 selections (including size, shape, skin texture, peelability, skin thickness, rag, external colour, internal colour, Brix, firmness, seed number, albedo retention, crop size, rind oil offensiveness, skin blemish, taste, chewiness, granulation, navel and areola, alternaria presence, scab level and maturity time) as well as wind-rub, pack-out, on-tree-storage, and postharvest performance before and after supply-chain simulations. Taste panels were a critical part of the assessment process and used throughout the project, both formally and informally. Significant spatial and temporal effects on fruit quality mean that large scale consumer evaluation trials seldom represent an efficient use of resources in mandarin assessment; fruit quality simply varies so much over time and across production sites. Rather, the assessment philosophy of this project was that mandarin quality description is an ‘unending synthesis’ in which information from multiple years, maturity times, locations, consumer/grower contexts, and growing conditions is considered collectively to identify the most promising genotypes to progress through the breeding process. These semi-commercial plantings have not only determined which Advanced Selections warrant commercialisation but has also generated information to help support their future growth. For example, preliminary rootstock information has been collected from a small range of stocks, along with field-reaction observations on diseases like alternaria brown spot and mandarin scab. The fact that these sites were located on different orchards also helped to generate useful information to support future commercial uptake and production.

Many thousands of trees were budded at the BRS nursery in order to generate sufficient material for the semi-commercial sites. These sites were to serve multiple purposes, one of which was the identification of low-seeded mutations of the Advanced Selection, and so all buds had to be irradiated prior to their propagation. This resulted in large numbers of tree losses in the nursery, particularly when working with new cultivars with varying and unknown sensitivity to irradiation. Consequently, many rounds of budding were performed in order to generate sufficient trees (Figure 3.3).

Budwood of the required Advanced Selections was collected from the source tree in the high security compound at BRS then taken to Brisbane for irradiation treatment in the University of Queensland gamma cell. Upon return to Bundaberg, this irradiated budwood was quickly worked onto different rootstock trees. A number of experiments were conducted during this period to determine the effects of different dose rates and attenuation, and care was taken to ensure this information followed surviving trees when they were eventually planted in the field. Observations on leaf deformity of emerging buds was also recorded, in response to data presented at the 11th International Citrus Congress (Wuhan, China 2008) showing that these were the buds most likely to carry low-seeded mutations.

Figure 3.3: Newly budded trees in the nursery at BRS, January 2010, destined for semi-commercial trial sites at Mundubbera and Gayndah. Of the 1,741 trees budded on this occasion some 956 were re-budded in May 2010 on account of high bud losses caused by the treatment. These trees were eventually field planted at Sites C and F (see Table 3.1).

A total of six trial sites were established on commercial orchards during the life of the project, and served multiple purposes toward the overall objective of the commercial development of subtropical mandarin hybrids. Table 3.1 provides key information for these six trial sites:

Table 3.1: Key characteristics of the six semi-commercial evaluation sites established on commercial orchard during the life of this project.

Site Code	Location	Planting date	Tree spacing	No. of trees	Advanced Selections	Rootstocks	Selections made
A	Gayndah	26/3/2009	1.3 x 7	272	00C018, 01C011, 02C059, 02C061, 02C063	Benton, Cox, Fraser, Swingle, Troyer, US812	11C011, 11C012, 11C013, 11C017, 11C018, 11C019, 12C013, 12C014,
B	Mundubbera	23/4/2009	1.4 x 7	271	00C018, 01C011, 02C059, 02C061, 02C063	Benton, Cox, Fraser, Swingle, Troyer, US812	11C001, 11C002, 11C003, 11C004, 11C005, 11C006, 11C007, 11C008, 11C009, 11C010, 11C020, 11C021, 12C003, 12C004, 12C005, 12C006, 12C007, 12C008, 12C009, 12C010, 12C017, 12C020, 12C022, 12C031, 13C001, 13C002, 13C003, 13C004, 13C005, 13C006, 13C007
C	Mundubbera	16/3/2011	1.5 x 7.3	255	00C018, 00C029, 02C055, 02C061, 02C062, 02C063, 02C109, 08C004, 08C005, 09C018	Benton, C35, Swingle, Troyer, US812, 59-24-8, 63-199-31, 63-199-49, 3796, 3806, 3812, 3822, 3835	15C001, 15C002
D	Mundubbera	30/1/2012		126	11C001, 11C003, 11C004, 11C005, 11C006, 11C007, 11C008, 11C009, 11C010, 11C011, 11C012, 11C012a, 11C012b, 11C013, 11C017, 11C018, 11C019, 11C020, 11C021	Benton, Swingle, Troyer, 62-137-2	Daughter trees, no re-irradiation

E	Boyne	5/9/2012	3 x 6	65	07C001, 11C001, 11C003, 11C011, 11C012, 11C012a, 11C012b, 11C013, 11C017, 11C018, 11C019, 11C020, 11C021	Sweet Orange	Top-worked, no re-irradiation
F	Gayndah	13/12/2012		396	00C018, 00C029, 01C011, 02C059, 02C061, 02C062, 02C063, 06N006, 08C004, 08C005, 09C018, 11C001, 11C003, 11C004, 11C005, 11C006, 11C007, 11C008, 11C009, 11C010, 11C011, 11C012, 11C012a, 11C012b, 11C013, 11C017, 11C018, 11C019, 11C020, 11C021, 12C003, 12C005, 12C006	Benton, C32, H639, Swingle, Troyer, UCR-6A-38-8, US812, 59-24-8, 63-199-49, 3784, 3796, 3802, 3806, 3812, 3831, 3834, 4033	15C003, 15C004, 15C005, 16C001, 16C002, 16C003, 16C004, 16C005, 16C006, 16C007, 16C008, 16C009, 16C010, 16C011, 16C012, 16C013, 16C014, 16C015, 16C016, 16C017, 16C018, 16C019.

Chapter 4: Rootstock effects on Advanced Selections

The main driver of mandarin production and quality is the genetics of the scion cultivar. Rootstock genetics can none-the-less provide some opportunities for further improvements, and given that scion cultivars must be grown on a rootstock then it is worthwhile identifying some of the better choices before a new scion cultivar goes into commercial production. Issues of graft incompatibility also need to be considered because popular rootstocks are not always suitable for every scion cultivar. As an example, Troyer is the most important and widely used citrus rootstock in the world, and yet it is incompatible with Imperial mandarin.

Because this project aimed to establish plantings under commercial conditions with large numbers of trees of the Advanced Selections, the opportunity existed to simultaneously incorporate a diverse range of rootstocks. This would then provide some early indications of rootstocks that may be the best choice for future commercial plantings, as well as alert us to any incompatibility problems as early as possible. In total, some 23 different rootstock cultivars have been incorporated into the six semi-commercial plantings established during this project. In the 2014 season it was decided to examine rootstock effects on scion performance at field site B, where trees of five different Advanced Selections had been planted on multiple trees of 6 different rootstocks. We chose to sample three of these scion cultivars (00C018, 01C011 and 02C063) and 5 of the rootstocks (Benton, Troyer, Fraser, Swingle and US812). Fruit were sampled from a total of 68 trees with the tree numbers for each combination as shown in Table 4.1 below. Trees had been field planted for five years at the time of sampling.

Table 4.1: Numbers of trees of each scion/rootstock combination from which fruit samples and crop load information were collected.

	00C018	**01C011**	**02C063**
Benton	7	5	9
Fraser	4	4	1
Swingle	2	5	3
Troyer	6	7	7
US812	4	1	3
Total	22	23	23

Because these three scion cultivars vary in their maturity time, samples were collected on three different dates, corresponding with their optimum maturity time. Fruit of 01C011 were collected on the 4th June 2014, fruit from 00C018 on the 27th June 2014 and finally fruit of 02C063 were collected on the 6th August 2014. Fifteen fruit were collected from each datum tree, selected at shoulder height from the eastern side of the canopy. These were transported back to BRS where they were chlorine dipped (quarantine protocol), then weighed and processed. Data on average fruit weight, percentage juice, acidity, Brix, Brix:acid, and BrimA were generated for all samples. For fruit of 00C018 we also rated skin texture of each sample (using a 6 point scale where 0=completely smooth and 5=extremely rough) and counted seed numbers from 5 fruit. For fruit of 02C063 we assessed skin texture, as above and estimated external colour on a 6 point scale (where 0=pale and 5=bright red). Crop load of trees in the field had been rated on the 22nd May 2013 (using a 6-point scale where 0=no fruit and 5=overcropping), and was again rated on this same scale in the 2014 season (4th June 2014) before fruit samples were collected. All trees were also give a rating for tree health on the 7th August 2014 using a scale from 0=dead to 7=extremely green. A coding system was implemented prior to all sampling, processing and rating to ensure assessors had no knowledge of the particular rootstock they were examining.

Data was analysed using Residual maximum likelihood (REML) in GenStat by Dr Joanne DeFaveri, QDAF Mareeba. Table 4.2 provides a summary of P values for the 12 traits measured.

Table 4.2: Significance values (P) for 12 traits measured in an experiment with 3 scion cultivars on 5 rootstocks. Values are presented for the Rootstock, Scion, and Rootstock:Scion interaction, with significant values (P<0.05) presented in bold.

Trait	**Rootstock**	**Scion**	**R:S**
Average fruit weight	0.510	0.132	**0.005**
Juice percentage	0.363	<0.001	<0.001
Juice acidity	0.302	<0.001	0.115
Juice Brix	**0.002**	<0.001	0.790
Brix:acid ratio	0.230	<0.001	0.188
BrimA	**0.016**	<0.001	0.262
Skin texture[1]	0.099	0.234	0.834
External fruit colour[2]	0.874		
Seeds per fruit[3]	0.483		
Crop load in 2013	0.639	<0.001	0.071
Crop load in 2014	0.231	0.637	0.793
Tree health in 2014	**<0.001**	0.150	**0.021**

1. Measures only on 00C018 and 02C063, 2. Measured only on 00C018, 3. Measured only on 02C063.

Rootstock effects were detected for juice Brix, BrimA and tree health, with juice content approaching significance (0.070). Not surprisingly, most traits were significantly different between scions demonstrating that it is scion genetics that have the strongest influence on these characteristics. One possible exception to this is tree health, where significant rootstock effects were detected, but not scion effects. This is consistent with good horticultural thinking, in which rootstock genetics is primarily targeted at disease tolerance and improving scion health. It also supports the strategy of the BRS breeding effort in which both scion and rootstock genetics are being improved but via a focus on different traits for each. In the case of rootstock breeding the BRS effort (CT 13004) has a major focus on disease resistance.

Because this experiment was designed to examine rootstock effects, only these will be examined in detail. Rootstock effects on juice Brix are illustrated in Figure 4.1.

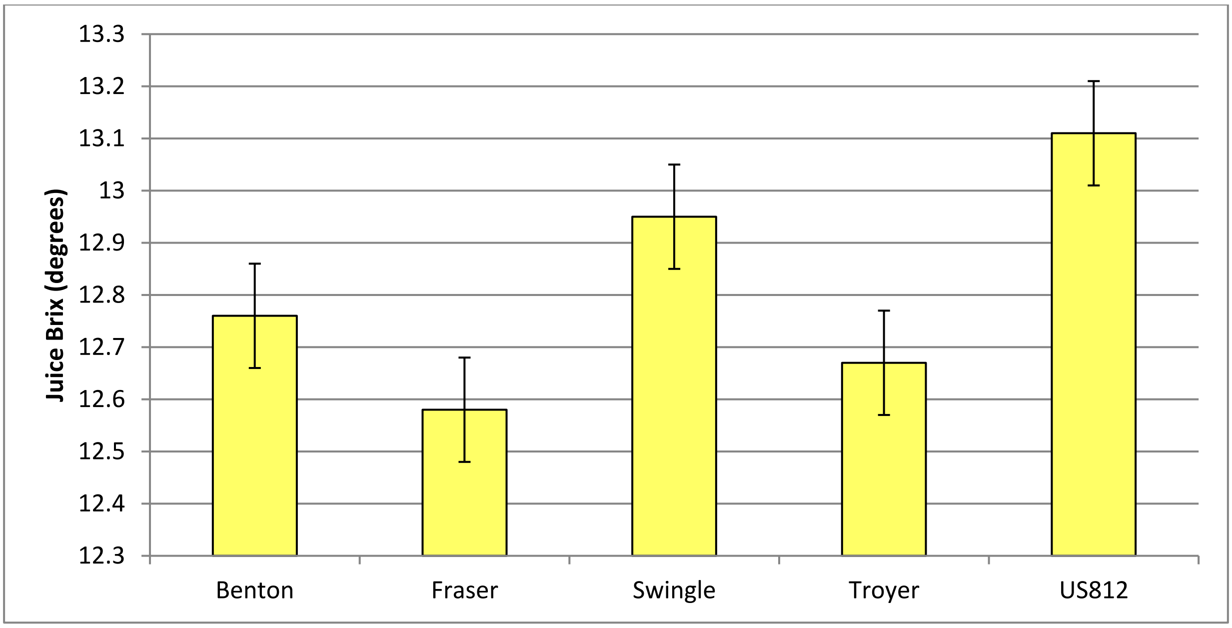

Figure 4.1: Rootstock effect on juice Brix levels, averaged across three scion cultivars. Error bars represent the average standard error.

These results suggest that rootstocks like US812 may help to increase the Brix content of fruit, while stocks like Fraser and perhaps Troyer do not compare favourably. While these differences are relatively small (particularly when compared to Brix differences induced by different scions) they are still of economic value provided the rootstocks are equivalent in all other traits. Figure 4.2 shows the average rootstock effect on the BrimA value of the juice.

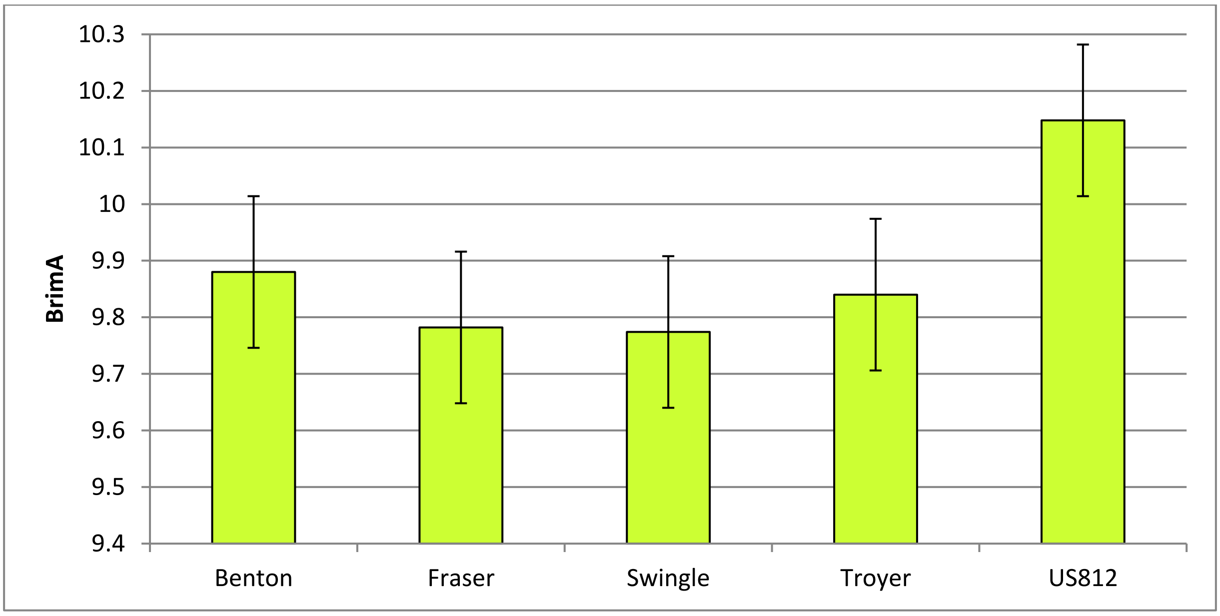

Figure 4.2: Rootstock effect on juice BrimA value, averaged across three scion cultivars. Error bars represent the average standard error.

BrimA, and the associated values for the Californian Citrus Standard and Australian Citrus Standard (BrimA x 16.5) is used as a means of predicting consumer satisfaction with taste and has been shown to be a better predictor than the conventional Brix:acid ratio. With this in mind, it is interesting that US812 appears to hasten the maturity time compared with the other rootstocks. Conversely, Swingle is often considered to be a rootstock that induces later fruit maturity but this has not been confirmed in our current study.

There was a highly significant rootstock effect on tree health (<0.001) which is illustrated in Figure 4.3.

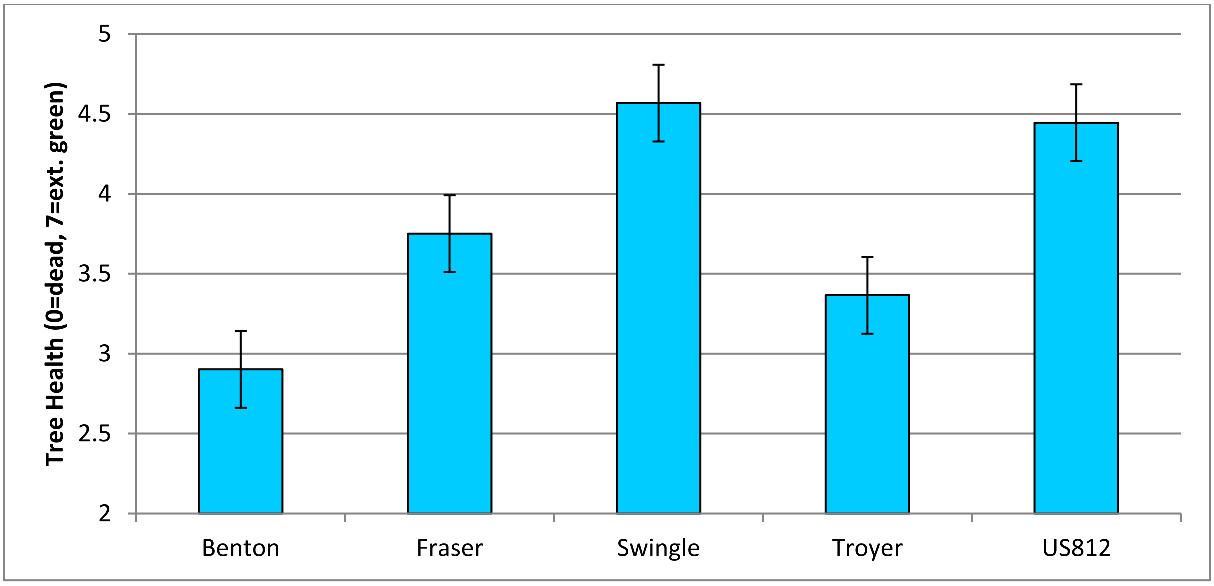

Figure 4.3: Rootstock effect on Tree health, averaged across three scion cultivars. Error bars represent the average standard error.

These results suggest that Swingle and US812 produced a healthier looking tree canopy than stocks like Benton and Troyer. The reason(s) for this need closer examination since they could be related to mild disease impacts or simply to greater nutrient depletion caused by heavier cropping in past seasons. There was also a significant interaction with scion (0.021) and on closer inspection of the data this took the form of 00C018 tree health being largely uninfluenced by rootstock whereas the tree health of 02C063 was affected to a very large extent. In particular, trees of 02C063 on Swingle rootstock were far healthier than trees of this same scion grown on Benton

Overall, this examination of 68 trees at five years of age shows that any of these five rootstocks would be suitable for these three Advanced Selections. This is not surprising given that all 5 rootstocks are of some commercial importance internationally. The promising results from US812 (all be it with limited replication of this treatment) are encouraging enough to prompt a more detailed and broad scale assessment of this rootstock. Although all five rootstocks performed adequately, there are still important aspects such as budunion incompatibility that need to be assessed when the trees are somewhat older. Already we are seeing the common benching problem on Swingle and unless future results reveal some particular advantage, then it would be wise to avoid this stock for the time being. Observations of the other 18 rootstocks contained in the semi-commercial plantings will continue, and the above detailed assessment of fruit quality effects may be repeated if these observations suggest it is warranted.

Chapter 5: Postharvest performance of Advanced Selections

Concerns have been expressed for many years that our breeding strategy of selecting for high Brix, and consequently high eating quality, was likely to lead to fruit with a short post-harvest life prone to development of off-flavours during their passage through the supply chain. There was little to allay these concerns as some literature supported the belief that high Brix:acid ratio fruit were more prone to off-flavour development (e.g. Arpaia and Obenland, 2011). Further amplifying these concerns was some preliminary post-harvest studies conducted by the breeding team at BRS in 2009, in which hybrids from the program were put through a supply chain simulation based on the Chinese market and then subject to taste panel assessments. This work showed that all fruit had developed significant levels of off-flavours. In 2012 an opportunity arose to study this problem more carefully via collaboration in a scoping conducted by Helen Hofman (Hofman et al., 2013). We were able to included Advanced Hybrids from the breeding program in this postharvest research project, "CT12000 Internal quality of exported mandarins: scoping study".

Large quantities of 01C011 and 00C018 were harvested from the Mundubbera semi-commercial trial site (Site B, Table 3.1) on the 6th July 2012 and transported to Nambour were they were washed and sanitized. Fruit were individually wiped with a microfiber cloth after a three minute soak in a mild chlorine solution (Turco-san at 200ppm, 2g/10L). This removed dirt and mould. Following this, the fruit were dipped in sanitizer (sodium orthophenol phenate) for 3 minutes followed by an 80 second dip in a fungicide mix of Zancotine (1.3mL/L water) and Magnate (0.68g/L water). Fruit was then air-dried prior to the application of various coating treatments. An identical process was followed about three months later when fruit of 02C063 was harvested from the same trial site on the 30th August 2012.

The fruit of the three Advanced Selections were subject to different coating/bagging treatments and various steps in a supply chain simulation [based on a study tour of the Chinese market, (Smith and Campbell, 2008)], and their performance compared with Murcott, Hickson and IrM1 (a low-seeded Murcott). Fruit of 00C018 and 01C011 received five different waxing treatments consisting of a non-waxed control, two shellac-based coating and two carnauba-based coatings all applied at the rate of 1l/tonne of fruit. The two shellac-based coatings were Decco Citrus LustrR 402A, and Castle 873 while the carnauba-based coatings were Decco Carnauba Premium and Colin Campbell Chemicals Natural Shine 960. Fruit of 02C063 received these same five coating treatments plus an additional four treatments used a lower rate of 700 mL/tonne of each of the four coatings. All coatings were applied by individually pipetting the required quantity of coating to each fruit and then manually rubbing over the surface of the fruit using latex gloves. Coated fruit were air dried, with the assistance of an electric fan, prior to being allocated to their different storage treatments.

Figure 5.1: Fruit of 00C018 and 01C011 being treated with various coatings ('waxes') prior to storage under five different temperature regimes, designed to simulate various stages of the mandarin supply chain from Australia to China.

Six different storage temperature regimes that might apply in Asian markets were examined, ranging from ideal conditions to prevent off-flavour development (Treatment 1) through to severe temperature conditions unfavourable for fruit storage (Treatment 6):

Treatment 1: 30 days at 1.5°C

Treatment 2: 30 days at 1.5°C, then 11 days at 5°C.

Treatment 3: 30 days at 1.5°C, then 2 days at 25°C

Treatment 4: 30 days at 1.5°C, then 2 days at 25°C, then 9 days at 5°C

Treatment 5: 30 days at 1.5°C, then 2 days at 25°C, then 2 days at 5°C, then 3 days at 25°C

Treatment 6: 30 days at 1.5°C, then 2 days at 25°C, then 2 days at 5°C, then 7 days at 25°C

Fruit from all three Advanced Selections were subject to Treatments 3, 4, 5, and 6. Fruit of 01C011 and 00C018 were also subject to Treatment 1, whereas fruit of 02C063 was instead subject to Treatment 2. This complex mixture of treatments was designed to test how fruit receiving different coatings reacted to different conditions at various points in the supply chain. The performance of fruit was assessed by measurement of ethanol content, and ratings of off-flavour based on smell and taste. Ethanol was measured using a two-step enzyme based testing kit from Megazyme International and a spectrophotometer. Twenty fruit from each treatment were randomly divided into four replicates of five fruit, and 5mL of juice collected and combined from each of the five fruit making up the replicate. Samples were frozen immediately after collection and thawed just prior to ethanol determination. Individual fruit were rated for off-flavour smell and taste (by two assessors) during the process of collecting samples for subjective and quantitative ethanol determination. Each fruit was rated according to the scale:

0 = no detectable problems

0.5 = taint or sour

1 = noticeable off-flavour

2 = strong off-flavour

3 = severe off-flavour.

In addition, juice samples were subject to two taste panels of 58 and 70 untrained participants, primarily aimed at relating measure ethanol levels to consumer perceptions of off-flavour. Fruit respiration rates following different coating and holding conditions were measured via CO_2 concentration changes in sealed containers holding individual fruit. Fruit weight loss was assessed by changes in weight. TSS and acid were determined by titration with standardised sodium hydroxide and a digital refractometer respectively.

The different coating treatments caused effects on the three Advanced Selection that were identical to the effects on the three control cultivars (Murcott, Hickson, IrM1). Shellac-based coatings caused a large increase in ethanol levels and off-flavour development that was further amplified as the temperature simulations became more severe. The carnauba-based coatings caused a significant increase in ethanol levels compared with un-coated fruit but the effect was small and more severe temperature simulations did not amplify the effect. Figure 5.2 shows the typical response to coating treatments, in this example for 02C063.

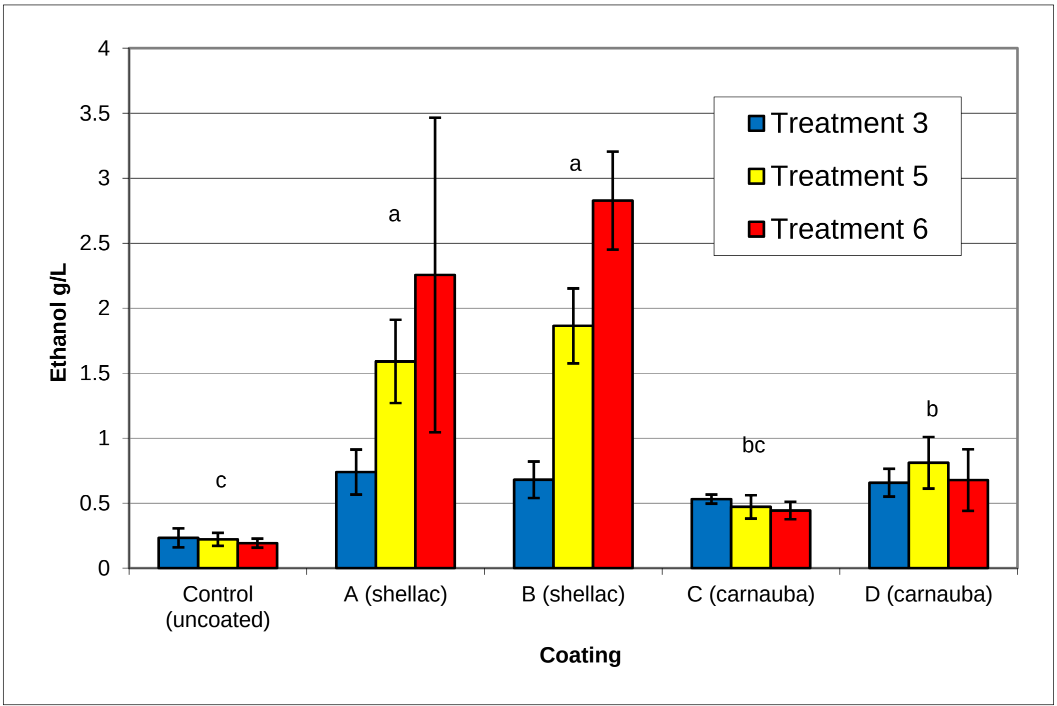

Figure 5.2: Ethanol levels of 02C063 fruit subject to five different fruit coatings (@700mL/tonne) and held under three different temperature regimes.

Figure 5.3 illustrates the effect of a wider range of storage temperature regimes on 02C063 fruit treated with the five different fruit coatings (@1L/tonne). Here it can again be seen that shellac-based coatings dramatically increase fruit ethanol content particularly under more extreme temperature simulations.

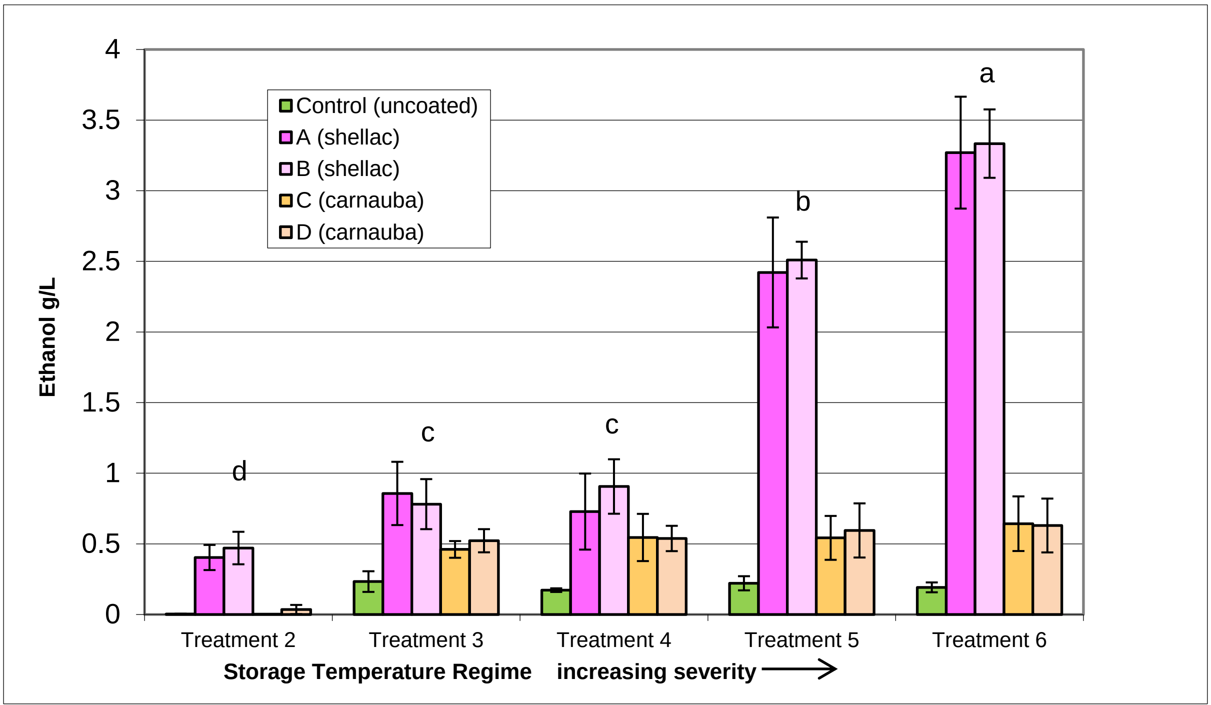

Figure 5.3: Effect of increasingly severe storage temperature regimes on ethanol build-up in 02C063 fruit treated with five different coatings.

Taste panel data, generated during this study, suggested that Australian consumers could detect ethanol once it reached about 1.5-2g/L, entirely consistent with the work of Hagenmaier 2002 who suggested 1.5g/L as the detectable level. Based on this figure, we can see that fruit of 02C063 only reach unacceptably high levels if it was coated with a shellac-based wax AND was subject to 'non-refrigerated' storage. Given that citrus exports are always likely to be exposed to high temperatures during some part of the supply chain, it is essential that shellac-based coatings are not used on mandarins. Even within the domestic market, mandarins are purchased in part for their ornamental appeal and stored at elevated room temperatures prior to consumption. Hence the common phrase 'fruit-bowl-taste' accurately captures this relationship between ornamental value and off-flavour development; a relationship that is exacerbated by using shellac-based coatings. Hofman's study caused a major re-think of fruit waxing within the Australian citrus industry, and illustrated how easily many of the current problems could be solved.

While the type of coating was identified as the main reason for off-flavour development, the study also considered whether different cultivars reacted differently to the various coating and temperature treatments. Figure 5.4 shows the mean ethanol content of six cultivars held under three different temperature regimes when treated with an ethanol-inducing coating (shellac-based at 1L/tonne).

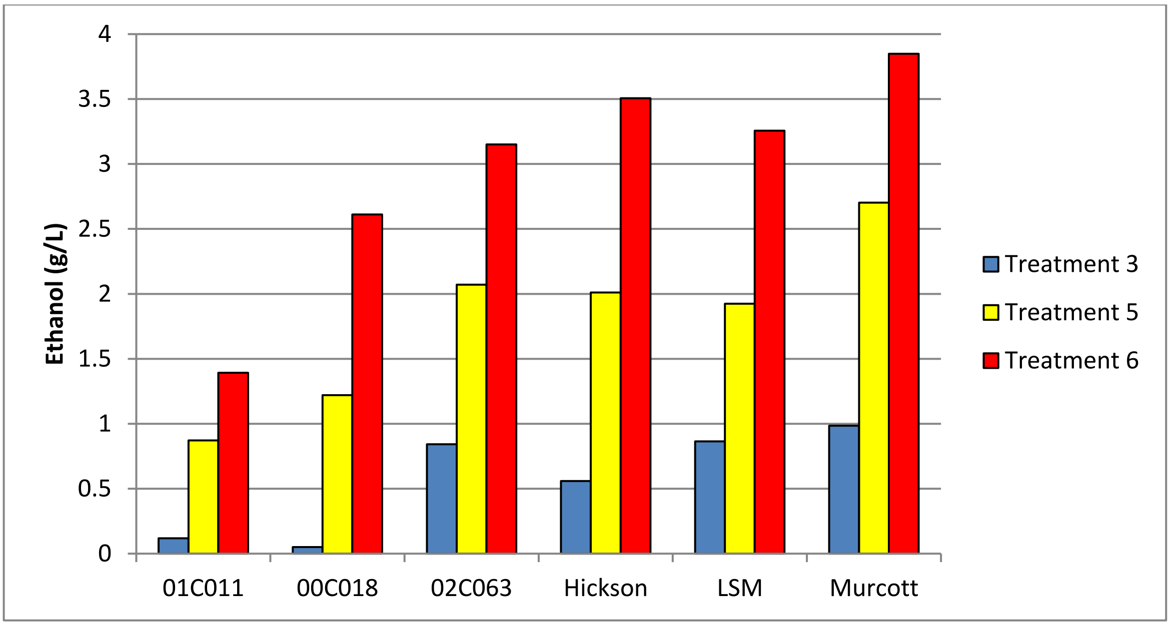

Figure 5.4: Effect of increasingly severe storage temperature regimes on the build-up of ethanol in fruit of 6 different cultivars treated with a shellac-based coating. (Treatment 3= 30d @ 1.5°C + 2d @ 25°C; Treatment 5=30d @ 1.5°C + 2d @ 25°C + 2d @ 5°C + 3d @ 25°C; Treatment 6=30d @ 1.5°C + 2d @ 25°C + 2d @ 5°C + 7d @ 25°C).

It is clear from Figure 5.4, that all cultivars respond in a similar way to increasing storage temperatures and that the three Advanced Selections are no more prone to ethanol accumulation (at any of the three temperature regimes) than the three control cultivars. This is confirmed in Figure 5.5 where fruit have been left un-coated (note the change in scale of the vertical axis).

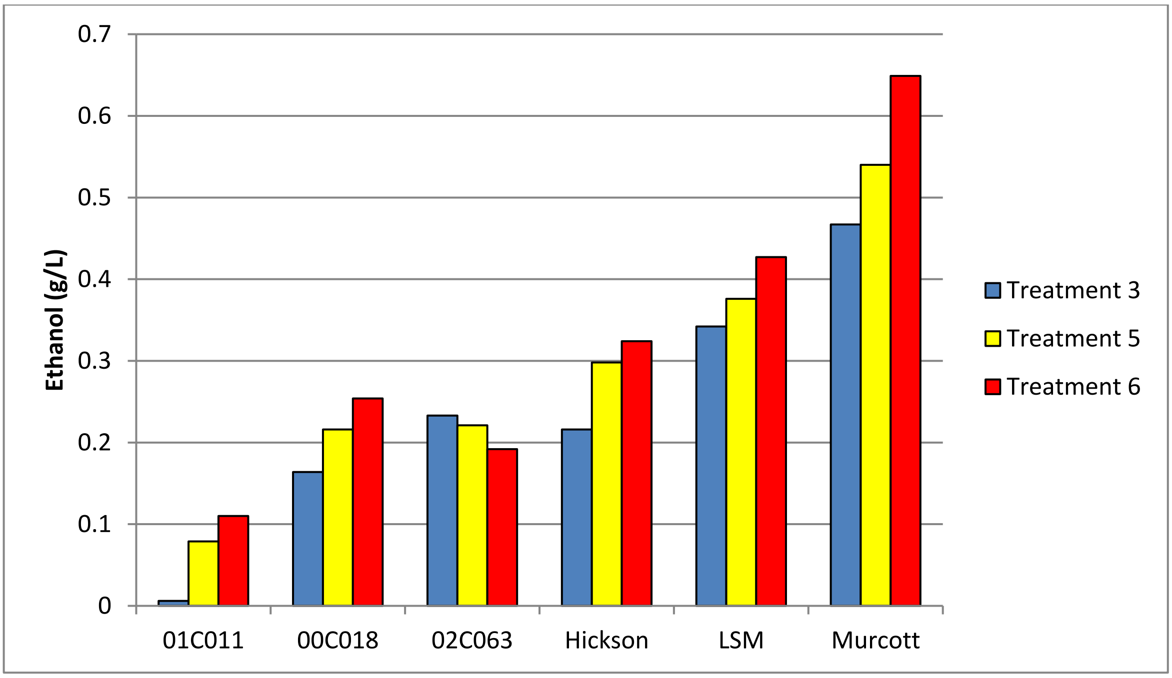

Figure 5.5: Effect of increasingly severe storage temperature regimes on the build-up of ethanol in fruit of 6 different cultivars left un-coated. (Treatment 3= 30d @ 1.5°C + 2d @ 25°C; Treatment 5=30d @ 1.5°C + 2d @ 25°C + 2d @ 5°C + 3d @ 25°C; Treatment 6=30d @ 1.5°C + 2d @ 25°C + 2d @ 5°C + 7d @ 25°C).

While it is clear that the three Advanced Selections are no more prone to ethanol (and off-flavour) build-up than the three currently exported commercial cultivars, it is also obvious from Figures 5.4 and 5.5 that the six cultivars do differ in the amount of ethanol they produce during storage. If we can understand what causes these differences then it may help to ensure any future releases from the breeding program are similarly free of off-flavour development problems. To help address this question the key characteristics of the six cultivars were measured before and during the study. The correlations between these characteristics and subsequent ethanol accumulation (under various coating and temperature treatments) are shown in Table 5.1.

Table 5.1: Correlation coefficients of fruit characteristics with ethanol production under different fruit coatings and storage temperature regimes, for six mandarin cultivars (n=6).

	Brix (°)	Acid (%)	Brix: acid	ACS[2]	Fruit Wt	Ethanol at harvest	Respiration
Brix	1.000						
Acid	0.549	1.000					
Brix:acid	-0.294	-0.938	1.000				
ACS	0.737	-0.160	0.411	1.000			
Fruit Wt	-0.781	-0.147	-0.135	-0.803	1.000		
Ethanol at harvest	-0.137	0.255	-0.387	-0.367	-0.093	1.000	
Respiration	0.701	0.565	-0.407	0.371	-0.363	-0.356	1.000
Ethanol in Uncoated	0.398	0.860	-0.851	-0.225	0.202	-0.139	0.559
Ethanol in Shellac coated	0.222	0.634	-0.715	-0.250	0.390	-0.251	0.637
Ethanol in Carnauba coated	0.313	0.877	-0.947	-0.340	0.253	0.121	0.496
Ethanol at Mild temp.	0.201	0.693	-0.735	-0.322	0.342	-0.242	0.701
Ethanol at Moderate temp.	0.372	0.754	-0.774	-0.169	0.254	-0.252	0.679
Ethanol at Severe temp.	0.244	0.732	-0.842	-0.303	0.373	-0.008	0.455
Mean ethanol	0.280	0.750	-0.816	-0.276	0.338	-0.151	0.610

2 Australian Citrus Standard = (Brix – (%Acid x 4)) x 16.5

It can be seen that ethanol content of fruit is most strongly associated with the Brix:acid ratio and acid contents of the fruit. Thus cultivars with a LOW Brix:acid ratio tended to produce more ethanol during storage, while the same was true of cultivars that were high in acid. On both accounts, this runs counter to the proposition that high Brix fruit are likely to develop more off-flavours. Instead it would seem that cultivars with high acid may be more prone to ethanol build-up (and off-flavour development). The fruit respiration rate is positively correlated with Brix and to some extent with ethanol accumulation, but it does not follow that Brix is then closely correlated with ethanol build-up. It is noteworthy that while Brix:acid ratio is closely correlated with ethanol content, the same is not true for the Australian Citrus Standard (ACS). The ACS has been adopted as a more accurate way of predicting consumer taste satisfaction compared with the previously used Brix:acid ratio (Hancock, 2013), and yet it is the older methodology that relates more closely to off-flavour development. Our negative correlation between Brix:acid ratio and ethanol accumulation also runs contrary to some authors who believe that high Brix:acid ratios favour off-flavour development (Hagenmaier, 2002, Hagenmaier and Shaw, 2002, Arpaia and Obenland, 2011). Fruit ethanol content at harvest has little or no relationship with ethanol build-up during the supply chain simulations, and would therefore be a poor way of predicting off-flavour problems in new mandarin genotypes.

Although of some interest in guiding future breeding and selection work, the above correlation matrix needs to be viewed with caution because it is based on correlations between just six cultivars. It would be useful to repeat the exercise with a far wider range of cultivars to better identify the fruit characteristics most closely linked to off-flavour development during storage. Given the similar correlation coefficients between different coatings and different storage temperature regimes, such an examination of a broader range of mandarin genotypes could be conducted with just one coating and under just one storage temperature regime. In the meantime, it is reasonable to assume that cultivars with high acidity and/or low Brix:acid ratios are likely to be more prone to off-flavour development. A breeding program that selects for high Brix (as has been the case at BRS) is thus likely to reduce the problems of off-flavour, rather than to aggravate the problem as has been suggested in the past. Genetic contributions to off-flavour development have been poorly studied and may yet show that factors such as peel permeability are more important than Brix and acid values. In the absence of such studies it should suffice to simply perform regular export simulations, sampling and tasting of samples of 20 or more fruit of each new genotype prior to commercialisation and well ahead of actual exports (as per recommendations in Hofman et al., 2013).

Taste panel assessments conducted during this study provide the most extensive data yet collected on the acceptability of new hybrids compared with existing commercial varieities. The hybrid 02C063 was compared with Hickson, LSM and Murcott using juice samples extracted from various waxing treatments and storage regimes. Fifty three respondents provided ratings for a total of 385 juice samples. Although the main purpose for this was to determine the acceptability of juices with various ethanol contents, the data also provides an indication of the acceptability of juice from 02C063 (Figure 5.6).

The results in Figure 5.6 suggest that 02C063 is at least as acceptable as the other three cultivars. Importantly, this high level of acceptability did not decline as the storage temperatures became more unsuitable. Standard error bars fell below the ‘neither like nor dislike’ (rating “4”) for just one treatment, and this was only slightly below “4”. By contrast all LSM means and standard deviations were below “4”, while three of the Hickson treatments had standard errors below “4”. The data tend to suggest that cultivars like Hickson (and perhaps LSM) become increasingly unacceptable as storage temperatures become less favourable, whereas cultivars like 02C063 and Murcott retain their acceptability even under more extreme temperature treatments. Clarification of this important aspect of genotype postharvest differences would require a much larger study. None-the-less, the results suggest that hybrids from the breeding program may be quite robust and could be tested under even more severe supply-chain simulations.

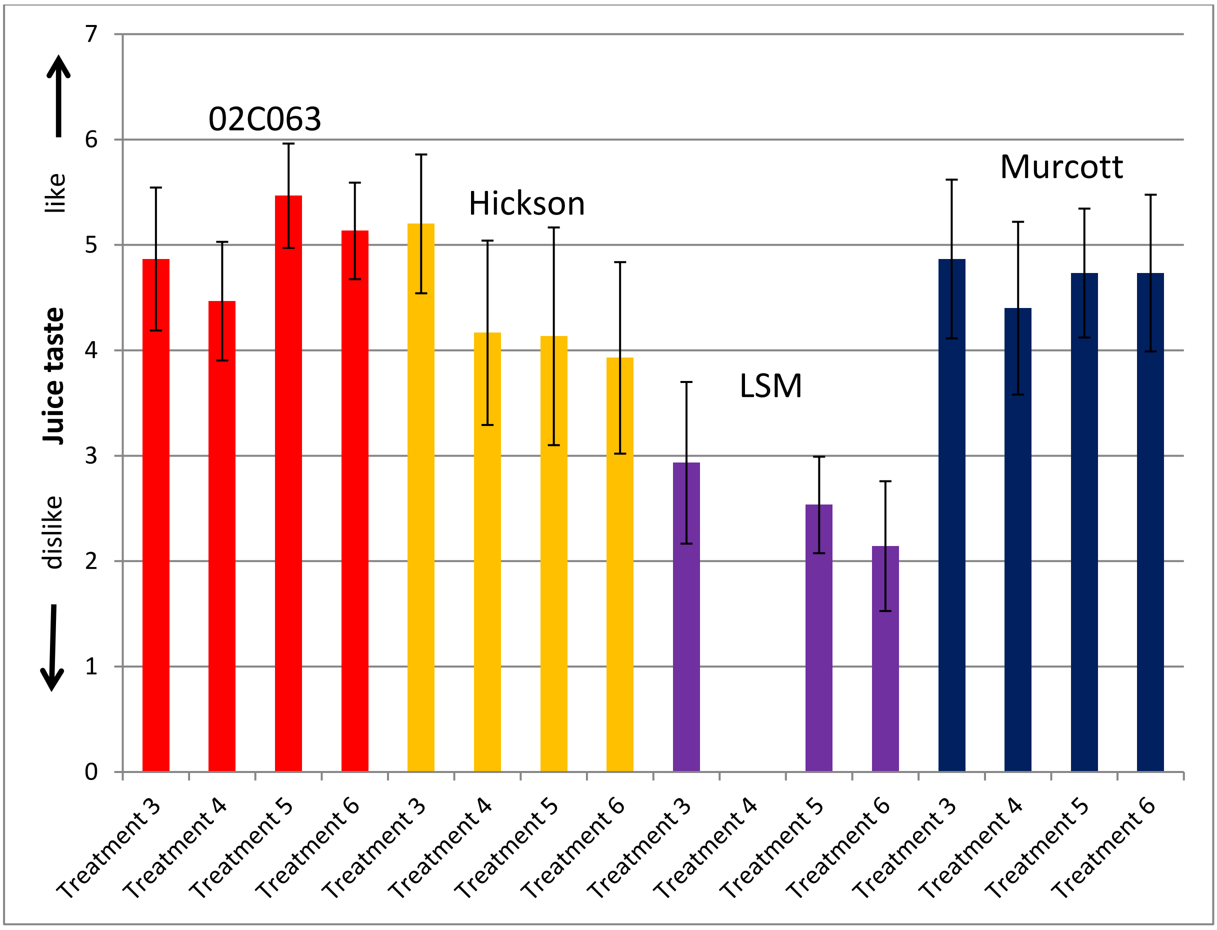

Figure 5.6: Taste acceptability of juice from four different cultivars held under four different storage temperature regimes. Storage conditions become increasingly unfavourable from Treatment 3 through to Treatment 6. The Juice Taste was on a hedonic scale centred at 4='neither like nor dislike'. Each column is the mean of 15 ratings, with error bars representing the standard error.

The opportunity to participate in this post-harvest study and to include three Advanced Selections in the testing protocol provided a significant boost to the breeding program by demonstrating that:

1. The main cause of off-flavours is the use of shellac-based coatings, with greatly amplified effects at higher storage temperatures
2. Selecting for high Brix is unlikely to create off-flavour storage problems, and may even help to reduce them.

Chapter 6: Development, selection and stability testing of low-seeded mutations

The Mandarin Hybridisation Project developed and tested large populations of diploid hybrids with the objective of finding just a few with outstanding fruit quality and productivity. The issue of seeds was not a consideration during the breeding process and almost all Original Selections that resulted were highly seedy (20-30 seeds per fruit). A primary objective of this current project was to breed lower-seeded versions of some of the better quality Original Selections from the Mandarin Hybridisation Project. Thus, it was recognised that the development of new commercially acceptable mandarins would require a 2-stage breeding approach in which high quality segregants were identified from within large segregating populations, followed by treatment and re-selection of this material to identify low-seeded variants. Citrus breeding is like no other tree crop, in that it requires: very large segregating populations in order to find hybrids with all the desired characteristics AND; the disruption of fertility (the very essence of breeding) so that fruit contain few if any seeds.

This project devoted considerable resources and effort toward producing, selecting and testing the stability of low-seeded variants of diploid mandarin hybrids. Unless low-seeded variants could be produced then there was no commercial future for this germplasm, regardless of how outstanding it may have been for all other traits. The previous QDAF success in breeding the low-seeded Murcotts, IrM1 and IrM2, enabled the breeding team to take a pragmatic approach to this task, and with the assistance of commercial growers were able to establish sufficiently large populations from which to select low-seeded variants with greatly improved commercial potential.

The semi-commercial testing sites (see Chapter 3) were used as the breeding populations from which to select low-seeded variants. Although these sites were originally intended just to test the performance of Advanced Selections under commercial conditions, they also created the ideal opportunity to perform mutation breeding because of the large numbers of trees of each Advanced Selection that were to be planted. Consequently, all budwood of Advanced Selections was subject to irradiation (at the Univeristy of Queensland gamma cell) prior to being budded in the nursery at BRS. While this greatly increased the task of producing trees for the semi-commercial sites (because of high bud mortality following irradiation) it enable the establishment of large populations of trees derived from irradiated buds.

Figure 6.1: Part of the thousands of nursery trees propagated from irradiation-treated buds. Those that survived were planted at field sites for eventual selection of low-seeded variants. Where buds died, the rootstock trees were re-propagated a few months later using the same process.

The first of these populations was planted at Gayndah on the 26th March 2009 and shortly afterwards at Mundubbera on the 23rd April 2009. This consisted of 543 trees of five different Advanced Selections, all of which were derived from irradiated buds. Budwood irradiation and subsequent budding of these trees had taken place in January 2008, and for the buds that failed (irradiation effect) the process was repeated in April 2008. Trees at these field sites flowered for the first time in August 2010 (17 months after planting) and when the resulting fruit had achieved sufficient size in January 2011 they were checked for the presence of low-seeded variants. This process was conducted in the field by sampling fruit from every branch of every tree, cutting the fruit equatorially, and visually estimating whether the seed number was less than expected. If so, then more fruit were cut to confirm the consistent production of low-seeded fruit from that particular branch. The low-seeded branch was then traced back to the main trunk to determine which other branches (if any) on the same tree were also low-seeded. A total of 13 putative low-seeded variants were identified in this first seed number assessment, and budwood of all of these was collected and propagated onto conventional rootstocks at BRS in the following days. Being such young trees and often just single branches, it was sometimes the case that few fruit were available to check seed number, and so many selections were made and propagated in the knowledge that they may later prove to be excessively seedy. However, this risk was outweighed by the need to get low-seed variatants available for commercial production as soon as possible.

A second round of seed checking occurred at the same two semi-commercial sites (Sites A and B) in May of the same year and five more putative variants were identified and propagated. One of these would later go on to become a prime candidate for commercialisation. This task of cutting fruit from every limb of every tree occurred each year of the project and a large number of putative low-seeded variants soon began to accumulate. For example, by October 2015 a total of 16 different low-seeded variants of 01C011 had been discovered and propagated.

The process of finding limbs with low-seeded fruit was time consuming. For example, the 2015 Assessment of trees as Site C took a team of six people (the breeding team plus three school-based trainees) 12 hours to check. By the end of the project period a total of 64 low-seeded variants of eight different Advanced Selections (00C018, 00C029, 01C011, 02C059, 02C061, 02C062, 02C063, and 08C004) had been identified and propagated. Table 6.1 shows the number of low-seeded selections made from each of the semi-commercial trial sites during the project period.

Table 6.1: Numbers of putative low-seeded selections made at each of the 6 semi-commercial trial sites established during the project period.

Site	No. of trees	No. of variants selected
A	272	8
B	271	31
C	255	3
D	126	Daughter trees
E	65	Top-worked trees
F	396	22
Total	1,385	64

Having identified putative low-seeded mutations of the Advanced Selections, two important tasks were then to confirm low seed numbers after propagation of daughter trees, and deciding which of the multiple variants of each Advanced Selection was the best. Because mutation breeding relies on a 'random' single-cell event, and seed reduction can result from alterations to any number of the complex pathways involved in fertility, then wide variation occurs in the extent to which seed numbers are reduced AND in non-target changes such as effects on fruit size and productivity. Hence in mandarin mutation breeding it is important to select a large number of low-seeded variants from which the best genotype can be selected after careful comparison. In the rush to progress material toward commercialisation we decided to propagate multiple trees of all low-seeded selections as soon as they were discovered and to then plant multiple trees at different field sites. In doing so we were able to breed low-seeded selections and identify the best variants for commercial production (three generations of trees) all within the time period of this project. The down-side of this approach is that resources were spent propagating and testing low-seeded variants of Advanced Selections that would later be dumped from the program (because of issues with low productivity and disease sensitivity discovered only after the semi-commercial sites were assessed). None-the-less, having performance data from multiple daughter trees grown at different field sites has greatly aided the decisions of which selections to use for the final stage of commercial testing (field planted in early 2016, see Chapter 8).

The Advanced Selection 01C011 is a good candidate to illustrate the issues discussed in the above paragraph. The first low-seeded selections of it were identified and propagated in January 2011 and by the end of the project a total of 16 variants of this original diploid hybrid had been identified. Assessment of daughter trees has been critical in making an accurate decision on the merit of these different low-seeded variants. Data from the original discovery is often based on just a single limb, so it is essential to produce new trees from this chosen limb and confirm that these new trees have the desired phenotype. The project established daughter trees at three different and diverse orchards so that anomalies caused by management or climate were minimised. Figure 6.2 presents data for just three of the low-seeded variants of 01C011 grown at three different locations.

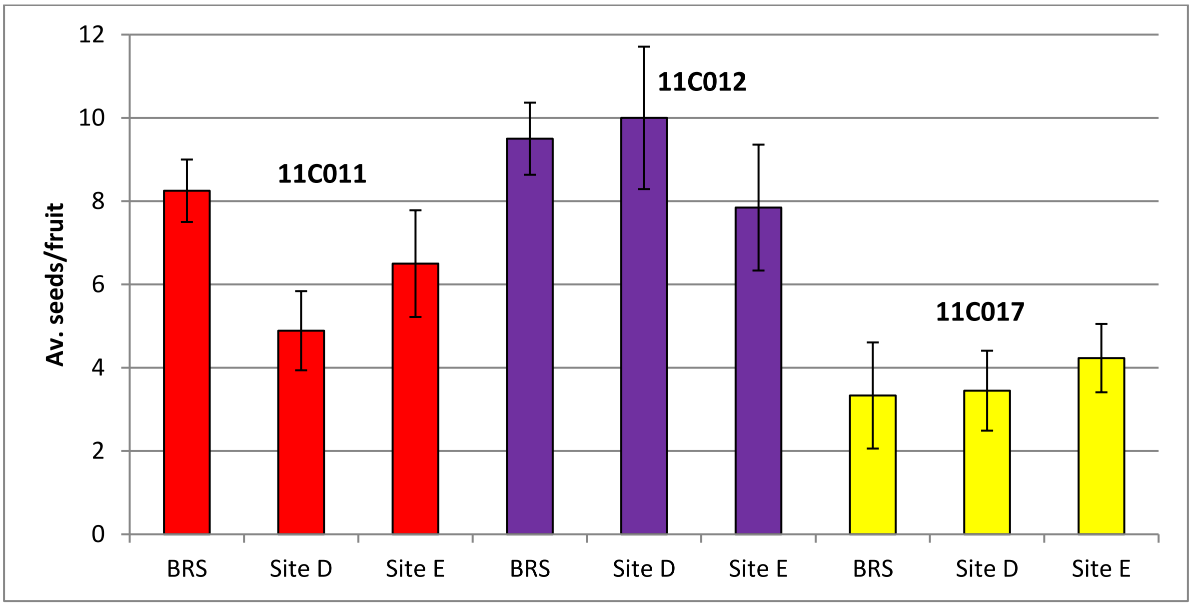

Figure 6.2: Variation in average seed number per fruit of three different low-seeded variants of 01C011 growing at three different locations in the 2015 season. Error bars represent standard deviations.

It can be seen that there are distinct differences between these three selections in terms of their seediness, with 11C017 clearly being better than the other two. Importantly, the low seed number of 11C017 is maintained at all three sites. It should also be noted that Site E is a top-worked site where as the other two are fruit from nursery daughter trees. Thus stability and consistency of seed number have been maintained with the propagation of multiple trees, and using different methods of tree production.

Mutation breeding can cause deleterious effects, and a selection strategy based solely on reduced seed number runs a significant chance of inadvertently selecting these undesirable variants. Because of the association of fertility with seed number and with productivity, there is a chance that low-seeded variants may also have reduced productivity. Such a link has been clearly demonstrated by this project and is illustrated in Figures 6.3 and 6.4 below.

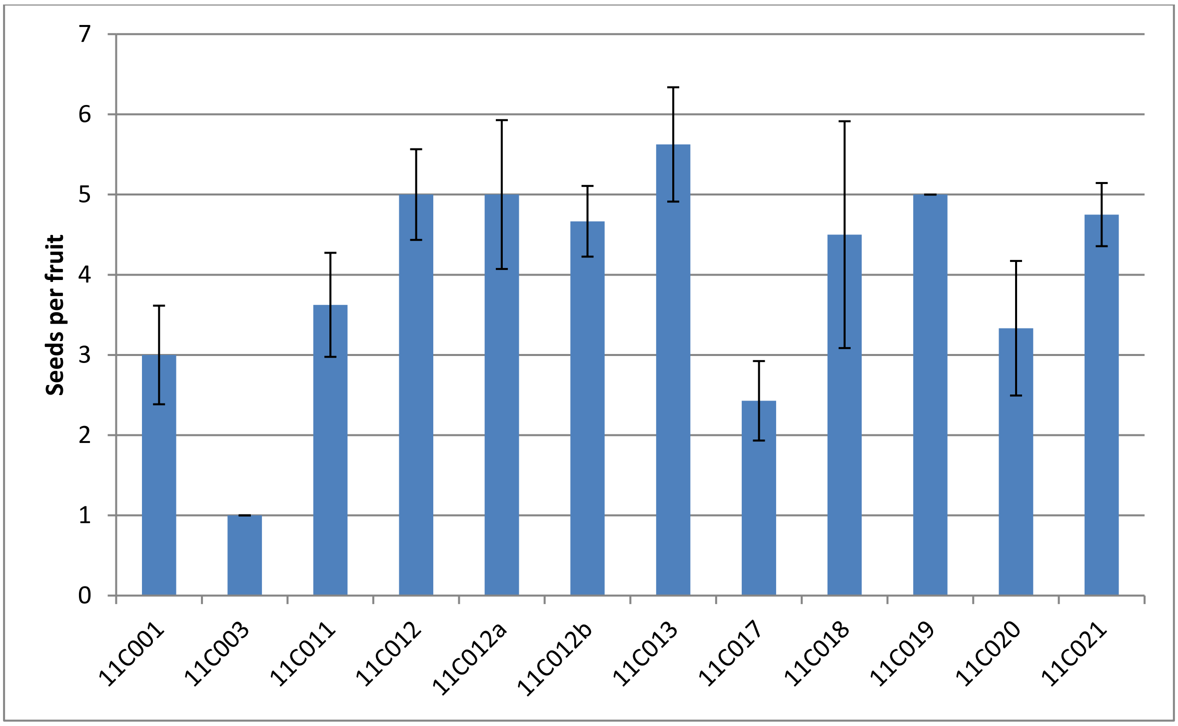

Figure 6.3: Average seed number per fruit of daughter trees of 12 different mutations of 01C011. Data collected from eight daughter trees of each mutation in July 2015 at Site D.

Based solely on seed number per fruit it is clear from Figure 6.3 that the selection 11C003 has very low seed numbers and would be the obvious choice for commercial production. However, when this seed data is combined with productivity data in Figure 6.4, a very different picture emerges.

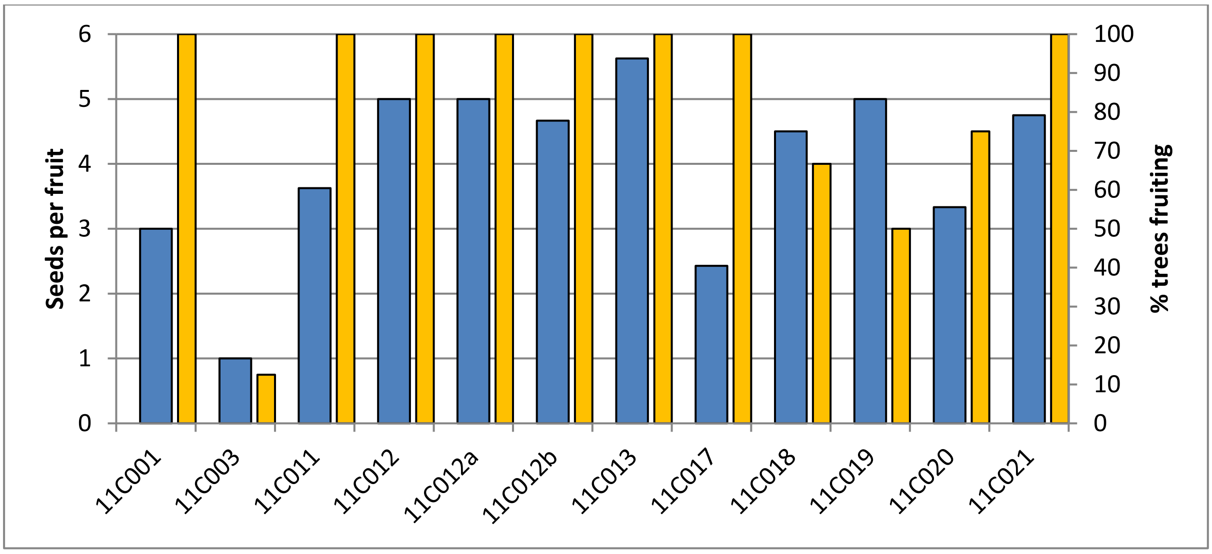

Figure 6.4: Average seed number per fruit and % of trees fruiting, for daughter trees of 12 different mutations of 01C011. Data collected from eight daughter trees of each mutation in July 2015 at Site D.

Here it can be seen that while 11C003 has low seed numbers it also has very few daughter trees actually fruiting. Such a low productivity selection is unlikely to be commercially viable. Instead there are selections like 11C017 which have slightly more seeds per fruit and are very productive. A selection like 11C017 would be far more commercial desirable and viable than 11C003, and this fact only emerges because daughter trees have been properly tested for traits other than just seed number.

The availability of multiple daughter trees has created the opportunity to examine other fruit quality traits that may differ between variants of the same Advanced Selection. Figure 6.5 shows the mean acidity of 12 different selections of 01C011 in the 2015 season.

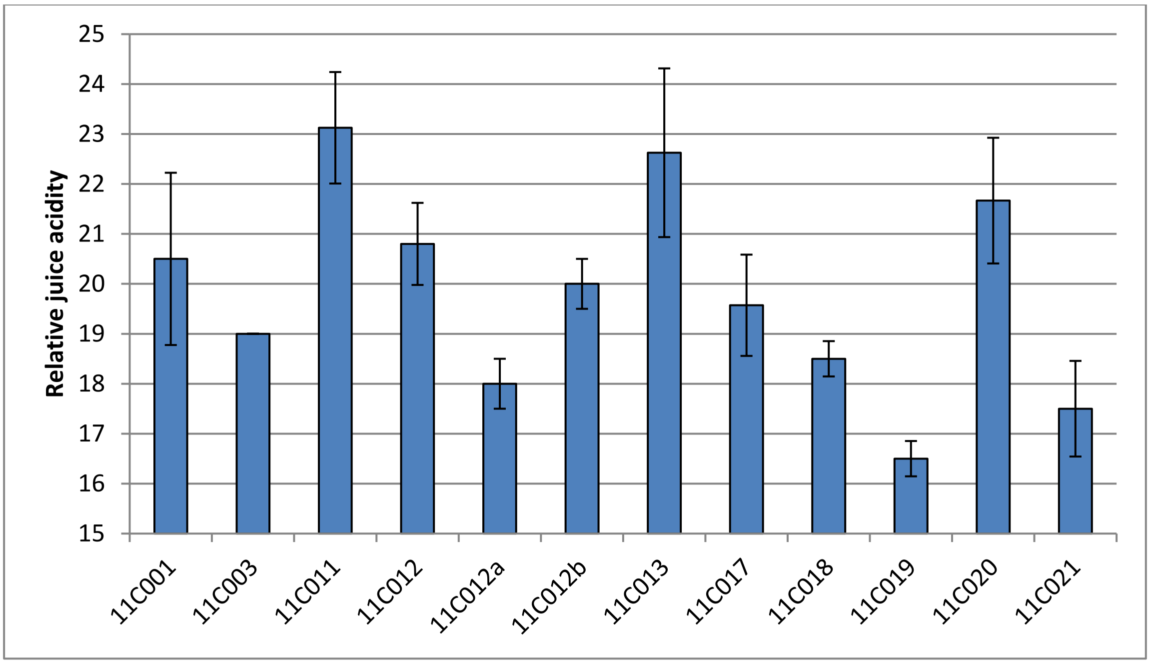

Figure 6.5: Average acidity of 12 different low-seeded variants of 01C011, measured from multiple daughter trees in July 2015 at Site D.

It would seem that selections like 11C019 and 11C021 have lower juice acidity than 11C011 and 11C013. This could have important implications for meeting market maturity standards, harvest times and postharvest storage behaviour, and warrants confirmation in future seasons. We have already removed some low-seeded variants from the program after discovering they had become extremely acid, acquired undesirably tastes, or granulated severely. An example of this is 11C002 (a low-seeded variant of 01C011) which was very low seeded (<3 seeds/fruit) but produced trees with severe leaf deformity from which the fruit were small and granulated. Similarly, 11C003 produced fruit with very low seed numbers but it grew extremely poorly at the top-worked site (Site E) and at the fruit display held on the 5th June 2013 was described as: “bland, bitter and acid, the worst of all the six 01C011 selections on display”. There is now ample evidence that the mutation component of mandarin breeding cannot just focus on finding lower seed numbers.

As the number of available low-seeded and productive variants increase, then it becomes possible to select for other traits that may have been altered during the mutation event. Again, this emphasises the importance of selecting and evaluating multiple variants of the same Advanced Selection.

Chapter 7: Project governance, industry engagement and progress toward commercialisation

This project represents one component of the process of commercialising new mandarin germplasm which commenced in the 1990s. With such long lead times, multiple contributing organisations, and the changes that invariably occur within these organisations, it becomes critical to have stable project governance in order to ensure successful commercial outcomes. Such governance has come from the Management Committee who met regularly throughout the project period to review results, develop strategies, and provide a clear pathway forwards. This has been no easy task, with the Management Committee having to contend with economic problems within the citrus industry, a series of natural disasters, alternative strategies for finding better cultivars, and complex business/political issues such as commercialisation. Despite these complications, the Management Committee have consistently provided clear guidance to the breeding team and dealt with issues before they became problems. They were for example, able to ensure that voluntary contributions continued to be contributed by the two grower organisation despite some angst within these organisations that funds were being contributed without anything being produced. It is a challenging task to sell the benefits of such long-term research, but members of the Management Committee have been able to do it, and thus keep the project on track.

A participatory approach has always been taken in this breeding work, ensuring that future beneficiaries not only contribute to the cost of the work but are also involved in key decisions about the directions it takes. Part of this participatory approach has been to develop modules that document how upcoming issues will be addressed. These modules are developed by the Management Committee and guide the breeding team. Seven such modules had been developed prior to the start of this current project, and the Management Committee were now tasked with the job of developing a Commercialisation Module. As with all other modules, this was developed well ahead of it being needed so that issues could be considered before they hindered progress. The Management Committee met on the 14th December 2011 to develop the Commercialisation Module using techniques such as pin-boarding and scenario planning. The resulting document has been reviewed at all subsequent meetings and been endorsed by all parties to the project. It clearly captures the main issue of concern from the different parties involved in the project, and has often been supplied to individual growers concerned about how any new cultivars will be commercialised from the project. The document is contained in Appendix One.

Another complex issue, which the Management Committee had to contend with in the later stages of this project, was the final stage commercial testing of selections prior to full commercial release. Throughout the project they examined fruit, data and grower feedback to decide whether any material was of sufficient merit to enter this final stage of testing. Annual fruit displays had revealed that growers were ready to start planting some of the material in commercial quantities. Guided by the Commercialisation Module, an open tender process commenced on the 5th June 2014 and a supporting document (see Appendix Two) was supplied to all members of the Gayndah and Mundubbera associations (VC contributors to the project). This was emailed to members on two separate occasions and the open tender process was also advertised at the Citrus Australia pre-season meeting in Gaydah on the 24th February 2015 and the Citrus Technical Field Day held in Mildura on the 17th March 2015. After wide publicity and being held open for 16 months the tender process was closed on the 7th October 2015 and the Management Committee examined tenders. A total of four businesses had submitted expressions of interest, and it was decided that the ~2,000 trees should be allocated evenly between these parties in consultation between the parties and the breeding team. The Management Committee had already decided which of the low-seeded variants showed the most merit (meeting of the 7th October 2015) and it was left to the breeding team to consult with each successful tender as to exactly which selections they wanted to include, what rootstocks they preferred, and their preferred planting dates. The first commercial plantings occurred in February 2016 and it is expected that all remaining plantings will be completed by the end of 2017 (see Chapter 8 for details).

An important component of the participatory approach and industry engagement of this breeding program has been regular fruit displays for project contributors. These have been held every year at multiple locations, and often at multiple occasions within a season coinciding with when selections are at their optimum maturity. They have proven beneficial not only for outsiders to see the type of fruit that is being produced, but also for the breeding team to gain a wider range of feedback of commercial relevance. Initially these fruit displays were intended only for growers of the Gayndah and Mundubbera associations who were making voluntary contributions to the project, but the Management Committee decided in 2012 that future displays would be open to all growers and their marketing associates. This occurred for the first time at the fruit displays held on the 5th June 2013. Another important change to fruit displays and grower engagement was to include fruit of imported cultivars, thanks mostly to the efforts of Graeme Sanderson from NSWDPI in Dareton. Attendees were then able to compare the quality of fruit from their own breeding program with what was being produced in other parts of the world.

Industry engagement has also taken other forms during the progress of this project. This includes regular interactions and discussions with the collaborators hosting semi-commercial plantings as well as other field trials from other citrus projects. This has helped keep the breeding team up-to-date with changes in the industry and markets and provided direct feedback on the merits of particular selections. The breeding team has also presented project updates at local and national industry meetings annually throughout the project life.

This industry engagement culminated in a major citrus varieties field day held at BRS in June 2016, and jointly organised by the Qld Regional Advisory Committee of Citrus Australia Ltd. This event was attended by senior representatives of all contributing organisations and a wide range of growers all of whom had to travel some distance to attend and some even traveling from as far afield as Renmark, Mareeba and Emerald (Figure 7.2).

Figure 7.1: Fruit displays have been held at multiple locations in every year of the project, providing an opportunity for growers and marketers to see new selections ahead of their potential release/availability. Important feedback has also been gained by the breeding team.

Attendees were given the opportunity to taste selections from the breeding program as well as sample fruit from variety commercialisers who also displayed their currently available cultivars. The breeding team also presented a seminar concerning releases from the program, consumer trends driving breeding objectives, implications of climate change, and mutations breeding. They also participated in panel discussions of future variety needs for Australia. Very positive feedback was received concerning both the field-day and the cultivars on display, including comments such as:

"the best field day I have attended for a long time",
"the breeding program is heading in exactly the right direction",
"one of the best research stations I have visited, and I have been to many around the world",
"when can we get access to these varieties", and
"exciting".

Figure 7.2: Participants at the Citrus Variety Field-day held at Bundaberg Research Station on 16th June 2016. (photos curtesy of Nathan Hancock)

In the days following the field day, three companies approached the breeding team wanting to participate in the final stage of cultivar testing. They were disappointed that this opportunity had already passed, but informed that the expressions of interest (see above) had been open for more than 12 months and widely promoted. The need for new and better mandarin cultivars is as great as it has ever been and it seems that material emerging from this breeding project goes some way toward providing the sort of material growers/markets/consumers are looking for.

PBR processes are in place to protect new cultivars generated by this project. There has been a concerted effort to delay the lodging of PBR applications for as long as possible in order to maximise the period of intellectual property protection. The final stage of cultivar testing can take some years, and uptake of new material can be slow at first, so it is important that PBR protection has not expired by the time any new cultivars achieve commercial popularity. A total of six PBR applications have been lodged, the best low-seed variants and their original diploid hybrid progenitors. This should ensure security of the material as emerging issues with mutation breeding and essential derived material are resolved in various law courts around the world.

Chapter 8: Final stage commercial testing

The project in its original form had been pressured into including test marketing of Advanced Selections as one of the tasks. However, it very quickly became apparent that even with large amounts of fruit available from the semi-commercial testing sites (see Table 3.1) such test marketing as envisaged by the then Hort Innovation portfolio manager would not work. The fruit were simply far too seedy. Consideration was given to having trained taste panels instructed to ignore the presence of large numbers of seeds, but this would not capture the full spectrum of marketing issues associated with new cultivars. Annual fruit displays regularly received feedback that "the fruit were too seedy", and there was no point in repeating this exercise on an industrial scale. Instead it was realised that the newly selected low-seeded variants needed to be produced in commercial quantities, so that consumers could test the final product and not be distracted by high seed numbers (20-30 per fruit). With the approval of all parties, funds intended for the test marketing were directed to the production of commercial quantities of trees of the best known low-seeded variants. Following an open tender process (see Chapter 7) the breeding team negotiated with the three successful bidders to progagate the selections they considered would best suit their businesses.

All propagation occurred at BRS which, because of the number of trees and range of material involved, stretched nursery facilities to their limits. The first round of propagation of trees for these commercial sites occurred on the 4th December 2014 and have continued subsequently as budwood and rootstock material permits.

The first consignments of trees were dispatched from BRS for two orchards near Mundubbera on the 11th February 2016. They were planted immediately on arrival by the collaborating growers with the intention of having the trees well established prior to any winter frosts (Figures 8.1 and 8.2).

With such large numbers of trees of the same low-seeded variants being planted, the breeding team took the opportunity to incorporate some mutation breeding experiments within these plantings. These experiments examine the potential of budwood pre-treatments to increase bud survival after irradiation, and to further study whether any increased survival at higher dose rates alters the probability of finding useful mutants. As remarkable as it may seem, we have been unable to find any literature proving whether higher dose rates increase the frequency of useful mutants. Early work from Israel suggests that dose rate is not important, but an examination of some of the more useful variants arising from mutation breeding program around the world tends to suggest that these were exposed to higher doses than is normally used. Conventional diploid crossing with resultant wide segregation, followed by mutation breeding to remove seeds, is still one of the best pathways to better citrus cultivars, and so our new experiments may help to improve the efficiency of the mutation stage of this process.

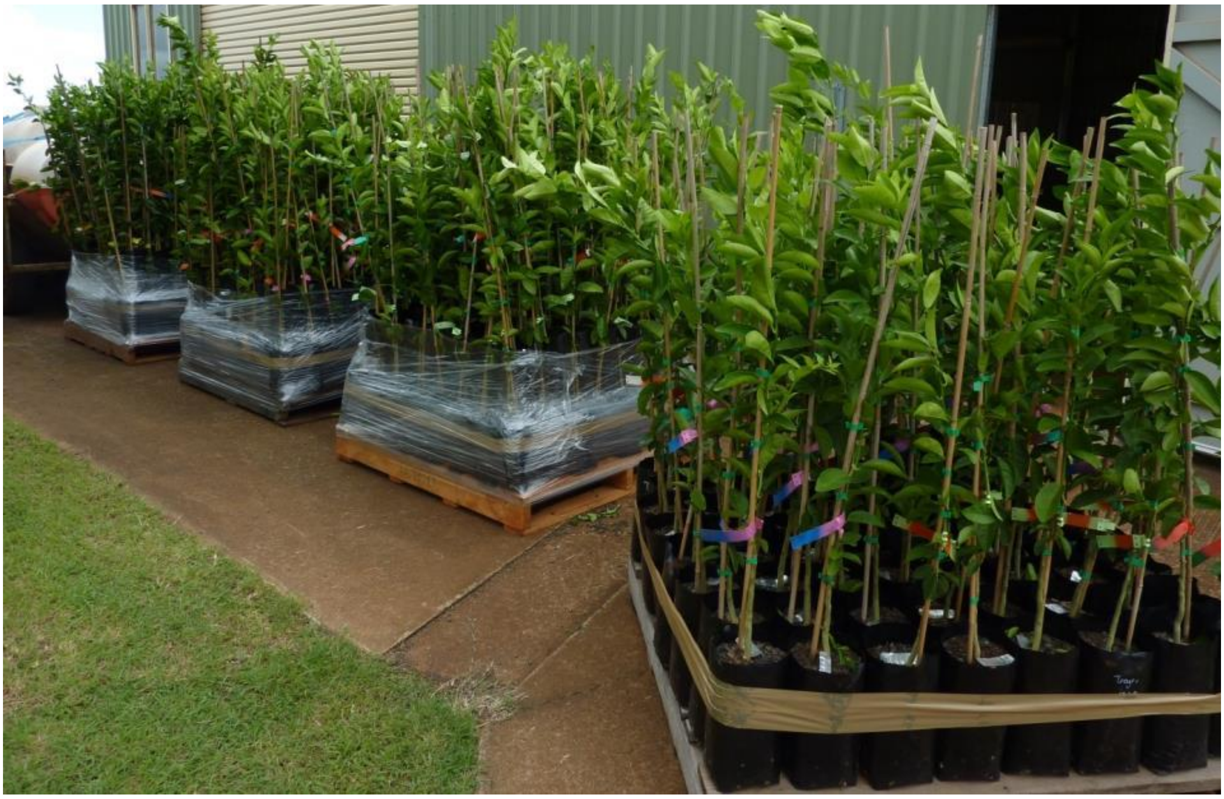

Figure 8.1: Nursery trees propagated at BRS ready for dispatch to two commercial test sites near Mundubbera. This consignment contained the best 14 low-seeded variants of 00C018, 01C011 and 02C063, together with a trial designed to improve efficiency in mutation breeding.

The budding of large numbers of trees for the production of commercial quantities of fruit placed the breeding team under significant pressure to “best-guess” which of the low-seeded variants was likely to perform well. This decision was often based on extremely limited information since even the oldest low-seeded variants had only been discovered just a few years earlier (2011). What appeared to be better selections were sometimes made in subsequent years but performance information on these was even more limited. The rush to find low-seeded variants in this project was also done in the knowledge that “…the first and second crops of only a few fruit may not give reliable estimates of seediness of subsequent crops.” (Hearn, 1986).

Perhaps the best example of finding promising variants toward the end of the project is 15C001. This is a low-seeded variant of 00C018 which was first discovered in 2015, and sparked particular interest because it appeared to be extremely low seeded. Prior to its discovery we had only limited success in finding 00C018 selections with reduced seed numbers. Finding a very low-seeded variant of one of our better hybrids, when there were few other good options, forced us to immediately include this selection in the trees intended for the commercial blocks. It remains to be confirmed whether this selection consistently produces low-seeded fruit, whether it has adequate fruit size, and whether the trees are productive! Such is the pressure to “get material out there”.

Figure 8.2: First commercial-scale field planting of low-seeded variants from the Mandarin Hybridisation Project. Planted near Mundubbera on the 12th February 2016. Consisting of 522 trees mostly of two low-seeded variants of 01C011.

Because the PBR process commenced at the same time as the planting of these commercial blocks, it will now be possible to allow other growers to see orchards of these new cultivars. To date this has not been possible and all previous trial sites have been kept strictly confidential, so that budwood is not stolen. Thus these new commercial blocks will not only produce significant quantities of fruit for market testing, but they will also serve as demonstration sites for other growers contemplating planting these new cultivars.

Recommendations

It is recommended that the genetic material developed and established in test plots during this project be progressed toward commercialisation as a matter of urgency. Existing commercial varieties have major limitations in their climatic adaptation and suitability for a changing consumer market. Introduction of new varieties directly from overseas has not been the easy solution that was hoped. As Australian growers face increasing competition in export markets it is critical that they have access to better germplasm that will give them an edge over their rivals. Germplasm developed by the Mandarin Hybridisation Project and tested in this project has been specifically selected to meet the changing demands of both export and domestic markets.

The rush to get material into commercial production has greatly increased the workload for the breeding program, and this needs to be kept in check. Putative low-seeded variants are being entered into commercial test sites within 12 months of their original selection, without any knowledge of their stability or productivity. The general relationship between reduced seed number and lower productivity requires careful consideration by the breeding team in order to ensure growers get the best balance between seed number and production. It is recommended that alternative techniques for breeding seedless citrus continue to be investigated because of the length of time required to complete the two-stage system of diploid crossing followed by mutation breeding. This need is further amplified by an increasing consumer demand for truly seedless germplasm rather than the low-seeded selections which have been acceptable up until now (and have been relatively easy to develop via mutation treatments).

The large and complex array of low-seeded selections needs to be simplified as soon as possible. This process has already commenced and should be accelerated as the three commercial-scale field sites commence fruiting. It is clear from the results generated in this project that mutation treatments affect more than just seed numbers and the breeding team needs to ensure other traits have not been negatively affected. Selections must be eliminated when there are concerns about productivity and/or fruit quality. Low seed number is not sufficient evidence to ensure commercial suitability.

Industry must continue to be involved in the breeding work. Traits that are critically important to commercial production and consumer acceptability can easily be overlooked by a breeding team working in isolation. The process of breeding citrus is slow and with few successes and a collaborative effort is required to ensure the best chance of success.

Appendix One: Commercialisation module of the MHP breeding plan

Mandarin Hybridisation Project (currently CT09023)
Management Committee Meeting, 14th December 2011

Draft Commercialisation Plan

Background
The Management Committee for the Mandarin Hybridisation Project (MHC) met on the 14th December 2011 to discuss issues associated with commercialisation and develop a draft plan from which to move forward. The meeting was held at the Gayndah Courthouse from 1.00 to 3.30pm and included the nominated members: Mark Trott, Troy Emmerton, Ian Shepherd, Tim Ulcoq and Malcolm Smith.

The deliberations were structured around the areas of: issues, scenarios, timeframes and strategies. Pin-boarding was use to ensure everyone participated and received equal hearing.

Issues
There have been 5 main investors in this project and they have different perspectives on commercialisation. There needs to be a shared appreciation of these different perspectives BEFORE a commercialisation strategy is developed. Consequently, participants were asked to list their key issues/concerns. These issues/concerns were grouped for *grower investors* (Gayndah and Mundubbera associations, and QFVG whose interest in the project was passed to the two associations) and *non-grower investors* (DAFFQ, HAL).

Grower investors

1. Grower control. Growers do not want to grow varieties over which they have no control. They want control of growing, planting and marketing and do not want to see this control being handed to a different entity. This is the whole reason why the two associations have been patiently investing in this research project for almost 20 years. This control is to extend all the way along the chain.
2. Confidence in the variety and incentive to plant. Deciding to plant a new variety represents a significant business risk, as most new varieties fail. Don’t be greedy up-front. Charging royalties before varieties are accepted as commercially viable adds to this risk, and has become a significant disincentive to planting new varieties.
3. Return on investment. Growers have been supporting this research project through voluntary contributions since 1995. As such, it is possibly the longest running voluntary contributions breeding project in Australian horticulture. Growers expect to see some reward for their investment, commitment, and willingness to support such long-term high-risk research.
4. Market relevance and competition. The commercialisation strategy needs to consider what market(s) the variety(s) is directed at. Emerging issues such as disease resistance and seediness need to be considered. The strategy must recognise that there are hundreds of new citrus varieties available from other sources. This includes new varieties from overseas breeding programs, other Australian breeding projects, local selections made by private growers, private commercialisation managers, and old public domain selections that now meet

market requirements. Varieties from this project must out-perform this competition, have high market acceptability, and not be restricted in their adoption by economically unsound commercialisation expectations.

*Non-grower investors (DAFFQ, HAL)**(note:heads of these organisations were not present at the meeting, and points below are based on an assumed understanding of organisations positions).*

1. Royalty stream. Continued investment in breeding is dependent on royalty income. Royalties currently fund the major portion of citrus breeding activities in Australia. Organisations like DAFFQ and HAL invest in long-term research like citrus breeding based on the understanding that part of the cost of the research will be recovered through royalties if the breeding is successful.
2. Industry/economic benefit. The continued international competitiveness of the Queensland citrus industry is dependent on innovation. New varieties that improve competitiveness need to be available and utilised. The commercialisation strategy needs to facilitate both availability and utilisation. Unless Queensland is producing the best varieties available, then export markets will be lost, and domestic markets will be filled by better fruit from overseas. Governments need to see an economic return and improved industry viability if they are to continue investing in horticultural research.
3. Benefit to contributors. For growers to continue to invest voluntarily in R&D they need to gain some advantage over parties looking for "a free ride". It is only fair that the people who have made the commitment, and made the project happen, should benefit from their efforts. Compulsory national levies are far from adequate to fund the level of innovation needed to keep Australian citrus growers internationally competitive. Greater innovation can only come through groups like the Gayndah and Mundubbera associations 'self investing' in research, and this will only occur when their members obtain a benefit from the funds they invest.

Scenarios

The meeting next discussed possible scenarios for commercialisation. The documents "CDI Pinnacle Management (2007) *Commercialisation Casebook*. HAL, AH05007" and "ACIPA (2008) *Plant breeders rights: A guide for horticultural industries*. HAL, HG04020, HG07051" were available at the meeting and provide examples of commercialisation efforts. Past experiences within the citrus industry were discussed including the exorbitant "entrance fee" approaches for totally unproven varieties (i.e. never been grown anywhere in Australia, and scant information available from overseas). These experiences have causes significant financial hardship as businesses payed up-front fees because they would not be able to gain access to the varieties at a later date. Waiting for the variety to fruit meant missing out on being able to ever grow it.
The meeting recognised that there is no ideal template for commercialisation, but incorporating the needs/issues of the different parties involved (see above) is fundamental.

Timeframes and Strategy

The areas of 'timeframe' and 'strategy' were developed together by the meeting.

Partial commercialisation: The MHC will nominate a variety from the research project that they believed has commercial merit. Between 1,000 and 2,000 trees of this variety will be propagated for commercial testing. The minimum number of trees at a commercial orchard will be 500, and preferable occurring in two different zones, but not exceeding a total of 2,000 trees. At each site, these trees will be managed as the orchard owner sees fit (tree spacing, pruning etc). Top-working will be allowed but is probably unlikely to occur because of insufficient budwood availability. The locations of these trial plantings will be decided through merit assessment by the MHC, of written expressions of interest submitted by Association members. Members with a history of hosting previous trials would be given preference. After 3 years growth and preliminary market testing, field days will be held ahead of commercialisation in year 4. The MHC may opt to initiate commercialisation ahead of this timeframe, or postpone it depending on variety performance and industry needs.

Full commercialisation: Having decided that releasing a particular variety is likely to benefit the industry, the MHC will set an initial production-area-target (or tree number) based on the performance and likely market(s) of the variety and call for expressions of interest. If interest exceeds the production-area-target then proportional allocation will occur. In no case will tree numbers less than 100 be allowed. The production-area-target will be reviewed every 2 years by the MHC. Association members will have exclusive access to varieties for the first 5 years. The MHC are prepared to consider all access requests beyond this, on a case-by-case basis including international distribution.

Prior to fruit production, a marketing partner(s) will be appointed by the MHC via written expressions of interest. They will be appointed for an approximately 5 year period, with performance reviewed every 2-3 years.

Royalties will be collected based on market return and orchard production. This will help to ensure an equitable royalty stream while also linking financial outlay to market activity. The formula for calculating royalties will incorporate average return per box, tonnage per hectare (based on tree age), and a nominated royalty fraction . This formula will calculate the required royalty per hectare. For example, if the average return per box is $20, the yield for trees of a particular age is 40t/ha, and the royalty fraction is set at 1%, then the calculation becomes:

$$20_{(\$/\text{box})} \times 67_{(15\text{kg boxes/t})} \times 40_{(\text{t/ha})} \times 0.01_{(\%\ \text{royalty})} = \$536/\text{ha}.$$

Figures will be set for the whole industry, not individual farms, so that growers who achieve higher returns per box and/or better yields are not penalised with higher royalties.

Conclusion

This document is intended as a starting point and will be modified as more input becomes available.

Draft document compiled by Malcolm Smith, waiting endorsement by the MHC as a true and accurate record.

Appendix One: Commercialisation Module as originally developed and endorsed by all parties at subsequent meetings of the Mandarin Hybridisation Committee.

Appendix Two: Expression of Interest document

Invitation to submit Expression of Interest

The **Mandarin Hybridisation Committee** invites expressions of interest from Members of the Gayndah and District Fruit Growers Association and the Mundubbera Fruit Growers Association for the partial commercialisation of varieties from the Mandarin Hybridisation Project (currently CT09023).

Three varieties from this breeding program show promise and there is now a need to test them on a larger scale to determine market acceptability.

Only 2,000 trees are to be established in this first round of partial commercialisation, and the required minimum block size is around 500 trees.

Participating members will pay commercial rates for the nursery trees (~$15) but will be exempt from royalties on these trees for the first 10 years after planting.

The Mandarin Hybridisation Committee are primarily interested in further development of selections derived from "01C011", but selections of "00C018" and "02C063" are also available for partial commercialisation.

Members have had the opportunity, each year, to view these new mandarins at the fruit displays held at Mundubbera and Gayndah. They will be displayed again this season.

"01C011"
A large Murcott-shaped fruit that matures May-June. Smooth skin with no neck, easy peeling with little rag and albedo. Consistently good eating with Brix 10 to 16. Trees commence bearing at a young age and crop heavily. Susceptible to Alternaria, and possibly albedo breakdown in some seasons.

"00C018"
A moderate to small Murcott-shaped fruit that matures June-August. Smooth skin with no neck, moderate to peel with little rag and albedo. Good internal and external colour and appearance. Excellent eating quality with Brix 14.4 to 16. Susceptible to Alternaria.

"02C063"
A large Murcott-shaped fruit that matures July-September. Smooth skin with no neck, easy to peel with little rag and albedo. Good eating quality with Brix 10 to 13.5. A very attractive late season fruit. Susceptible to Alternaria.

These new varieties are unproven and come with significant risk of failure. Members wanting only "proven commercial varieties" should wait until more information becomes available.

Expressions of interest should be submitted through your Association Secretary.

Queensland Government

Mundubbera Fruit Growers Association

Gayndah and District Fruit Growers Association

HAL Horticulture Australia

Appendix Two: The Expression of Interest document sent out to all Association members and widely publicised during the open and transparent tender process conducted by the Mandarin Hybridisation Committee.

Acknowledgments

It is thanks to the efforts of many individuals that this project ran smoothly and achieved its objectives. Deb Gultzow and Toni Newman provided careful and committed technical support throughout the life of the project, as well as Carola Parfitt during her contract period.

Roger Broadley (formerly Director of Subtropical Fruit & Genetic Improvement, now retired) lobbied for support within QDAF and worked hard to ensure the completion of milestones, as did Rod Edmond with contracts and finance, and Jodie Campbell with intellectual property and commercialisation complexities.

Alok Kumar took over the Hort Innovation portfolio shortly after project commencement and provided a welcome pragmatism to the complex business of creating new cultivars in challenging crops like citrus. His constructive dialogue with QDAF management, in particular Roger, Rod and Jodie, allowed the breeding team to spend the bulk of their efforts “in the field” getting on with front-line tasks.

Grower representatives on the project Management Committee of Tim Ulcoq, Mark Trott, Ian Shepherd, and Troy Emmerton have kept the project on track. They have championed the project within industry, sometimes against hostile fellow growers impatient to see results, and taken on the difficult task of “selling” the benefits of long-term research, in an age where everything is supposed to happen instantly. Similarly, Ben Harzer and Judy Shepherd, as Secretaries of the two Associations have kept their members informed of fruit displays and project activities, and ensured the timely payment of project Voluntary Contributions.

Success of the project was entirely dependent on growers making available parts of their orchards for the planting of semi-commercial blocks of Advanced Selections. Without the generosity of the orchard owners Mark, Peter and Sue Trott; Murray, Averial, Matthew and Rachael Benham; Cris Bryant; Troy and Ainsley Emmerton, this breeding project would not have happened. They not only made their orchards and management inputs freely available, and allowed access to the breeding team at all times, but also often helped with tree planting and cultivar assessments.

Research colleagues, in particular Helen Hofman, Andrew Miles and Joanne DeFaveri have provided expert support and advice to the breeding team. The BRS Facility Manager Bruce Boucher has worked hard to help us maintain field plantings with minimal inputs dictated by extremely tight operations budgets.

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