National Citrus Scion Breeding Program

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CSIRO Plant Industry

Project Number: CT04007

CT04007

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HAL PROJECT NUMBER *CT04007*

THE NATIONAL CITRUS SCION BREEDING PROGRAM

(July 2004 – June 2007; extended by mutual consent until July 2008[1])

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[1] The project was originally funded until June 2007. However, a delay in starting the project due to contractual issues led to an agreed finish date of July 2008. Subsequent to a successful new project application with an anticipated start date of July 2007, it was agreed to finish project CT04007 in September 2007 so that there was no overlap between the projects. The unfinished work in CT04007 will be continued in the new project. A requirement prior to formal commencement of the new project has been to complete the final report for project CT04007.

HAL PROJECT NUMBER *CT04007*

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Statement of purpose:

The purpose of this document is to report formally the progress made by the research conducted from July 2004 until September 2007 (although data collected during October 2007 have been included) which formed HAL project CT04007, the National Citrus Scion Breeding Program.

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Date of report: December 2007

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1. Media and technical summaries

Media summary

The National Citrus Scion Breeding Program is a long-term program that has been supported by the Australian Citrus Industry since 1991 through a series of discreet one-to-four year projects funded by Horticulture Australia Limited and the research providers CSIRO Plant Industry and DPI&F Queensland. Since 1996 the program has been funded as a fully coordinated project and since 2004 the research has focused in three main areas of activity, namely conventional diploid hybridisation (CSIRO Plant Industry, Merbein), the production of triploid hybrids for seedlessness (DPI&F Queensland, Bundaberg), and mutation breeding (Merbein and Bundaberg).

The development of new scion varieties through breeding, selection and introduction is a high priority for the Australian Citrus Industry. The National Citrus Scion Breeding Program is focused to address industry priorities for new fresh fruit varieties. Major characteristics targeted are seedlessness, easy peel, flavour and size, internal and external quality, and agronomic characteristics such as ease-of-harvest, amongst others. The breeding program aims to produce new varieties adapted to Australia's varied regional conditions and the research has been designed to provide marketing, processing and production advantages to the Australian Citrus Industry.

Key outcomes of the program will be the adoption of innovative new varieties that will address the needs of key industry-identified market windows of opportunity resulting in increased profitability for Australian citrus growers. Key windows of opportunity identified during the program’s development have been for early and late maturing, seedless, sweet, easy-to-peel varieties primarily for export.

Research conducted in project CT04007 is producing results that have application to industry in the form of new varieties, as well as having immediate application to the breeding program itself in the generation of better parent material and genetic information. Two new varieties have been nominated for release from the conventional diploid breeding component of the program while a new triploid, which was developed from research started before HAL funding contributed to the resourcing of the program, has been established in commercial plantings in Queensland. Future R&D will continue to focus on the objectives detailed in the breeding plan and will see more varieties nominated for release to industry.

Technical summary

Technically the National Citrus Scion Breeding Program is divided into three major components. These reflect the breeding approaches being adopted to achieve the goals outlined in the breeding plan and are conventional diploid hybridisation, triploid hybridisation and mutation breeding. The first component is based at CSIRO Plant Industry, Merbein, the second at QDPI&F's Bundaberg Research Station, and the third at both locations.

Conventional diploid hybridisation component

Conventional citrus breeding through hybridisation with diploid parents is a long term proposition requiring a clear and dedicated commitment by both industry and R&D agencies. The strategic hybridisation program has been in progress at Merbein since 1984 and has received support from industry via HAL funding since 1991. During this period the genetic foundations of the program have been built so that crosses are made to accommodate industry requirements for new varieties as documented in the breeding plan.

The hybridisation program is essentially a pipeline approach for the delivery of new varieties. Activities are now at a high level for all phases of the pipeline with the nomination of two varieties for release during 2006. This was a highlight amongst a number of significant achievements during CT04007.

An announcement was made in March 2006 of the pending release of Merbeingold 2336, which yields seedless, juicy fruits that mature early-to-mid-season in the Murray Valley, and Merbeingold 2350, which produces low-seeded to seedless fruits with highly coloured robust yet thin easy-peel rinds that mature mid-season in the Murray Valley. Seediness of fruits of Merbeingold 2350 is dependent on the chances of cross-pollination from surrounding citrus varieties. The decision to release the varieties set in train a release and commercialisation strategy with guidance from the project's reference committee. At the time of finalising this report an agreement on terms has been reached with a commercialiser to manage the release and distribution of the varieties in Australia. The two varieties have been granted provisional Plant Breeders Rights in Australia and Plant Patent applications have been filed in the USA.

A series of new crosses aimed at developing a population of families from which hybrids that produce very early and early-maturing seedless fruits was completed during CT04007. This series addresses one of the major objectives in the breeding plan and has been conducted using parents bred and selected from previous crosses made at Merbein as part of the National Citrus Scion Breeding Program.

The value of developing new parents was demonstrated in pollination experiments conducted with a population of families generated in an earlier project. This population was bred specifically to transmit characteristics that contribute to the seedless phenotype and so far around 50% of the hybrids assessed have been shown to be capable of producing seedless fruits. This is an increase from 30% of the original population from which parents were selected and supported earlier data indicating that parthenocarpic fruit development is under the control of three complementary genes.

The initial or phase 1 evaluation of families of hybrids in the breeding fields has provided new genetic data on seedlessness and other fruit quality characteristics. This phase of evaluation in the pipeline also leads to the identification of a number of new selections for potential entry to phase 2 evaluation where the performance of selections as replicated grafted trees is investigated. Two trees of 39 new selections have been propagated in CT04007 so that budwood will be available from physiologically mature daughter trees when final selection is made for entry into regional test-plots and comparative trials.

Second phase evaluation trials and test-plots with cooperating growers have been established with 7 new selections, six of which are capable of producing seedless fruits even when self-pollinated. The seven selections have produced juicy, sweet (°Brix > 11.0) seedless fruits that mature from March through to September. Selections entered into phase 2 evaluation trials and test-plots during the previous project (CT00012) have performed well with regard to fruit quality, but early yields have been variable. Trees on some properties have produced high yields while on other properties the same selections have had low yields. This may be a result of using buds collected from the original hybrid such that within tree variability for juvenility may have carried over to daughter trees. For this reason, daughter trees are being propagated early as a bud source for selections with potential for entry into phase 2 evaluation.

Four selections currently being investigated in phase 2 evaluation trials have been identified for entry into the mutation breeding program. These selections all have a high capacity for parthenocarpic fruit development but can all self-pollinate. Their yields and fruit quality have been high, but seediness has been a problem. The success achieved from the irradiation program with Kara mandarin, which also has a high capacity for parthenocarpy, indicates that these selections are suitable for a bud irradiation program aimed at developing seedless variants.

Finally, the performance of multiplied trees propagated with buds of hybrid 88-09-28, which produces sweet, easy-to-peel and seedless grapefruit-like fruits, has indicated that this selection should be entered into regional test-plots in the next project. This hybrid is highly parthenocarpic and pollen sterile and its fruits have reasonably high sugar levels (>12° Brix). When its acidity declines in late winter to less than 1.5%, the juice has a refreshing, pleasant flavour and taste tests have indicated a potentially high consumer acceptance over more traditional white grapefruit varieties.

Triploid hybridisation component

Significant achievements have been made at various stages of the triploid breeding component of the project. Most importantly, the first commercial planting of triploid mandarins in Australia occurred in early 2007, utilising a hybrid bred at Bundaberg Research Station. Although this hybrid was produced prior to the commencement of HAL support for the triploid hybridisation research, it provides a clear demonstration of the capacity of the Australian citrus breeding effort to generate superior commercial germplasm, and to see it through to commercial use by growers. Not only is this the first triploid mandarin to be grown commercially in Australia, it is also the most advanced germplasm in the triploid breeding cycle. Activities are now spread across all the different stages of breeding triploids, from controlled crossing and hybrid establishment to the identification of hybrids with superior fruit quality and commercial testing.

During the three years of the project, 10,925 pollinations were performed, 3,787 seeds sown and 30,050 embryos rescued via tissue culture. Six hundred and five fruiting hybrids (from pre-2005 pollinations) were assessed and 16 of these selected for multiple propagation and further assessment. These selections are predominantly aimed at the mandarin segment of the Australian citrus industry, but five hybrids of potential value to the orange and grapefruit/pomelo segments of the industry have also been selected.

Seventy-two families in the triploid program have now produced at least one fruiting hybrid and some patterns in terms of progeny performance are beginning to emerge. Valuable information about the principal faults of culled hybrids has been developed and incorporated into the crossing program to optimise the chances of developing superior commercial citrus varieties. It is clear that the principal characteristics that form the selection criteria in the program are under additive genetic control, supporting the need to develop and utilise phenotypically superior parents as the mainstay of the crossing activity.

Hybrids that fruited during project CT04007 showed a surprisingly large proportion of low-seeded genotypes. Without a flow cytometer, the triploid breeding team has been forced to plant all hybrids without knowledge of what proportion is actually triploid (and therefore likely to be seedless). As these hybrids now commence fruiting it would appear that a large proportion are in fact triploid, based on the frequency of low-seeded hybrids being assessed. Preliminary screening of around 500 hybrids has shown that more than 50% of them have less than three plump seeds per fruit, 36% have no plump seeds, and around 12% are completely seedless. This has important implications for the success of the program because it creates the potential to select hybrids with good fruit quality from a large population of low seeded genotypes.

Triploid breeding is now moving into a potentially productive phase with pollination activities based around progeny-tested parents, material entering Stage Two testing, and thousands of maturing hybrids soon to commence fruiting.

Mutation breeding

At Merbein, the seedless or low-seeded characteristics of two budlines derived from a Kara mandarin bud that was exposed to 60gy gamma irradiation were confirmed and the stability of this trait after one generation of vegetative propagation has been demonstrated. The two seedless (or low-seeded) budlines appeared to have arisen as chimeric mutations which affected both female and male fertility. With interest from domestic and international citrus industries in a seedless Kara mandarin, these two lines have been entered into regional evaluation through the establishment of grower-based test plots. Third generation daughter trees are also being propagated to further establish bud line stability. The results have justified the approach taken and have demonstrated the value of using induced mutagenesis to affect seediness in a highly parthenocarpic variety.

At Bundaberg, potential commercial selections of Fremont and Ellenor have been developed via mutation breeding. These show significantly reduced seed numbers while retaining the capacity for reasonable fruit size. Four low-seeded selections of Fremont have been assessed and average 4-6 seeds per fruit compared with the normal number of around 17 seeds per fruit. One of the Ellenor selections averages around 0.4 seeds per fruit compared with the normal number of 22, and potentially has a mutation for female sterility.

While the intention of the mutation breeding effort was simply to develop genotypes with reduced seed number, there are early indications that we have been successful in producing a mutant with increased disease resistance. A selection of Daisy, which is normally regarded as one of the most susceptible mandarins to Emperor Brown Spot disease, is showing field-resistance to this disease and is being further investigated. If confirmed it will be a world-first – the only known instance of disease resistance development in citrus via mutation breeding.

2. Introduction

With fresh fruit, as for all other horticultural produce, change is ever present and producers and markets can no longer rest assured that traditionally favoured varieties, or indeed existing crops, will continue to command premium prices. It is important that those trading in fresh citrus are continually innovative like others in the horticultural sector. Innovation should be effective at all stages in the market chain from planting materials through to packaging and presentation to the consumer. The use of genetic improvement techniques, whether conventional or bio-technological, offers great opportunity for the generation of novelty. New varieties and types of seedless citrus with novel colour, size, taste, texture and other quality characteristics that address market requirements, or perhaps even alter market perceptions, provide innovation through genetic improvement that will maintain or improve market share and thus command premium prices.

The development of new scion varieties through breeding, selection and introduction is a high priority for the Australian Citrus Industry. Project CT04007 continued the breeding research that forms part of a nationally coordinated citrus improvement program. This national program involves varietal improvement projects covering breeding, evaluation and repository maintenance. The breeding component through the National Citrus Scion Breeding Program is primarily focused to address industry priorities for fruits consumed as fresh products. Major characteristics targeted are seedlessness, easy peel, flavour and size, internal and external quality, and agronomic characteristics such as ease-of-harvest, amongst others. The breeding program aims to produce new scion varieties adapted to Australia's varied regional conditions and the research has been designed to provide marketing, processing and production advantages to the Australian Citrus Industry.

During CT04007 traditional breeding approaches have been pursued in the environments in which the varieties will be grown. Each line of research in CT04007, and indeed in previous projects conducted under the umbrella of the National Citrus Scion Breeding Program, has had specific, short- and long-term goals and thus has been designed to be flexible in response to changing industry and market requirements. Innovation is important for competitiveness in the global market and new varieties need to be developed which grow well in Australia and ship well to provide the industry with an export edge. The research in the project has been tailored for market needs and an important aspect of this research has been focused on producing seedless varieties and breeding lines. The breeding program is designed to generate outstanding new varieties which can be tested in the market place where their novel features can capture consumer interest and thus gain the industry a unique competitive advantage.

By coordinating traditional breeding methods such as hybridisation and mutation breeding, the research team has ensured that the best approach is adopted within the resources available. In this way each targeted aim can be achieved within the overall framework of producing improved, locally adapted citrus scion varieties for the Australian citrus industry.

Project CT04007 has continued the research of CT00012 (2000-04) and CT96014 (1996-2000), each funded as coordinated projects, and before that projects CT111, CT206, CT225, CT315, CT319 & CT522, and so has built on the successes of previous citrus scion breeding projects supported by HAL. As a coordinated breeding program, the components have complimented and not duplicated the research effort and contributed collectively to the overall goal of innovative and improved Australian varieties that address market requirements leading to expanded market opportunities.

The project was originally funded until June 2007. However, a delay in starting the project due to contractual issues led to an agreed finish date of July 2008. With an application to start a new project in July 2007, it was agreed to finish project CT04007 in September 2007 so that there was no overlap between projects. The unfinished work in CT04007 will be continued in the new project. Before formally commencing the new project by agreeing to a new contract, it has been necessary to complete this final report.

This final report outlines progress in the research undertaken in project CT04007 from July 2004 until September 2007. However, while the report was in preparation, data collected during October became available and has been included where appropriate, namely the second phase evaluation trials reported in Chapter 3.

3. Diploid hybridisation

3.1 Introduction

The conventional hybridisation program at CSIRO Plant Industry Merbein is based on crossing diploid parents to yield hybrid progenies, which are evaluated for key characteristics. The data generated are used to identify promising hybrids for:

- entry into second phase replicated evaluation plantings from which new varieties can be identified for release to industry, and
- use as parents in future breeding, thus building on the genetic foundations of the program.

The data are also used to study the inheritance of key traits to develop breeding and selection strategies. As such, the program is dynamic, can be responsive to changing industry priorities, and takes the form of a pipeline approach for the delivery of outputs to achieve the overall industry outcome of successful new scion varieties.

Citrus breeding research at Merbein commenced in the 1960s when CSIRO's citrus germplasm arboretum was established. However, it was not until 1991, when industry supported the research through matching HAL funding, that breeding for new scion varieties received a much higher profile. Before 1991, industry had assisted with in-kind support for testing new selections and with funds from the Citrus Management Company (now Murray Valley Citrus Board) for purchasing isozyme analytical equipment. This equipment was used in HAL project CT111 (1991-92) to identify new seedless Satsuma mandarin hybrids, and in other projects to identify zygotic from nucellar seedlings where female parents have been polyembryonic.

In breeding new Australian varieties, the hybridisation program at Merbein has sought to provide industry with new material for testing and at the same time build on the genetic foundations underpinning the program. In this way, the direction taken by the research can respond to current as well as future industry priorities for new varieties without the need to adopt a hit-or-miss approach in making new crosses.

As with other components of the project, the aims of the diploid hybridisation program are based firmly on the goals documented in the breeding plan with guidance centred on the product specifications detailed therein. The breeding plan was recently updated during August 2007.

This section outlines the progress made in the hybridisation and associated research at Merbein since July 2004. Only summaries of data are reported here for the sake of brevity. Large data sets have been generated and are used for making key decisions in the program. Progress was also documented in 6-monthly milestone reports submitted to HAL during the course of the project and are available for further information.

3.2 Crossing program

A significant outcome from the research conducted over the last decade at Merbein has been to generate new parent material specifically for use in breeding new Australian varieties. This research has recombined and fixed characteristics deemed essential in easier-to-use parents for the development of new varieties to address current, and more importantly, future market requirements.

Historically, both in Australia and overseas, breeding new citrus varieties by hybridisation has involved pair crosses between common knowledge varieties, often repeating the same cross year after year without learning much about the characteristics targeted in the program. In conducting a strategic hybridisation program to develop new parents, the research at Merbein has made a departure from this approach. Resources are used to understand the way that key fruit characteristics are inherited. In this way, breeding and selection strategies are smarter. To achieve this, the evaluation of progenies has to be extensive and detailed. Progenies are screened extensively for the characteristics that contribute to the complex seedless trait. Pollination experiments are conducted to assess the effects of different pollen sources on fruit set and seediness, and observations made on pollen fertility and other flower characteristics. As a result we know more about the genetic control of the characteristics that lead to seedlessness under Australian conditions so we can plan breeding activities with greater certainty of outputs.

Now seedless genes are firmly established in our breeding parents, the hybridisation program has progressed to place greater emphasis on other quality traits such as fruit size, sugar and acid levels, and rind characteristics. These, along with maturity season, are mostly quantitative traits influenced by the actions of many genes. Crosses conducted in CT96014 were aimed at using parents identified in CT319 that would transmit seedless characteristics. The progenies from these crosses, as described later, were planted out during CT00012.

Activities focused on new crosses in CT04007 were divided into two areas. The first concerned completing the establishment in the breeding field of progenies from crosses aimed at late maturing, seedless fruits made in CT00012. The second was to conduct and complete a new program, which was started during 2003-04, aimed at producing families of hybrids that will yield early maturing, seedless fruits.

3.2.1 Crosses from CT00012

Crosses made during CT00012 were aimed at varieties required by industry as identified at a series of grower fora held during 1999, which highlighted the need for improved seedless, late and early maturing varieties. The following describes these.

3.2.1.1 Crosses aimed at late-maturing hybrids

The first hybrids from these crosses, which were outlined in the final report for CT00012, were established in the breeding field at CSIRO during December 2003. The remaining hybrids were planted during May 2005 and this brought the total population size to 1406 hybrids from 65 families. Family size varied from 1-to-303 hybrids. As with previous crosses using CSIRO-bred hybrids, which have been selected because of their potential to transmit seedless traits, seed numbers per cross were low in some instances leading to small families. However, the crossing design employed means that full- and half-sib relationships can be exploited in analysing the data at a future stage of the program.

3.2.1.2 Imperial mandarin x Miho satsuma

Progeny 01-101 (Imperial mandarin x Miho satsuma) was generated in CT00012 and described in the final report for that project. This progeny has been used to partially adopt a recommendation made by Luis Navarro in his review of CT00012. Navarro suggested that hybrids should be grafted *in-situ* to orchard-established citrange rootstocks to provide more uniform planting materials for assessment. He also suggested that by grafting *in-situ*, the hybrids would establish faster and possibly flower sooner. *In-situ* grafting as suggested is a labour consuming exercise and current resources allocated to the research really prevents this approach; own-rooted hybrids require less maintenance in the field both in terms of establishment and not having to be grafted, as well as after-planting care such as dis-budding of rootstocks. Nevertheless, it was decided that this approach should be explored on a limited basis using this Imperial x Miho progeny.

Rather than establishing the rootstocks in the breeding field and grafting the hybrids in-situ, they were budded to Carrizo citrange (CC) and Symons sweet orange (SWO) under glasshouse conditions. Grafted trees and the original hybrid seedling were subsequently maintained under glasshouse conditions until the grafted trees were ready for planting in the field. At planting, each hybrid was established as randomized three-tree plots, ie one tree on own-roots (OR) and two trees grafted to rootstocks under drip irrigation. The trees were planted in autumn 2006 and will be evaluated in future projects. Similar trees of the two parents used to generate this progeny have been included in the planting for comparative purposes.

3.2.2 Crosses aimed at early- and very early- maturing hybrids

The breeding plan highlights the export market window of opportunity that exists for very early maturing fruits. The feasibility for breeding improved very-early maturing hybrids was demonstrated by a hybrid between Clementine and Imperial mandarin, which was first reported in a milestone report for project CT00012 (31/08/02). Hybrid 91-03-04 reaches full maturity in early April at Merbein and, as described later, was fast-tracked into second phase evaluation. As this hybrid was selected from a small family of ten hybrids, and also since other hybrids in the family have been shown to have seedless traits and good fruit quality, a priority was to repeat the cross and also generate other new families with Clementine, Imperial and their hybrids that produce early maturing fruits. A series of crosses were designed with the aim of generating a population of families that will yield early maturing, seedless hybrids and controlled pollinations commenced during 2003. This series of crosses was continued during CT04007 and progress was reported in milestone reports. The following summarises the crosses made and the hybrids that have been established for evaluation in future projects.

Series 1. The aim here was to repeat the Clementine Marisol x Imperial cross that resulted in the selection of 91-03-04. In addition to repeating this cross, other Clementine varieties were included and reciprocal crosses were conducted (Table 3.1).

Table 3.1. Details of Clementine x Imperial crosses and number of hybrids retained per combination.

Female	Male	no. seeds	no. hybrids	% seedling emergence and survival
Clementine – Marisol	Imperial mandarin	85	68	80.00
Clementine – Fina	Imperial mandarin	88	80	90.91
Clementine – De Nules	Imperial mandarin	107	90	84.11
Clementine - Oroval	Imperial mandarin	173	151	87.28
Clementine – Old clone	Imperial mandarin	878	706	80.41
Imperial mandarin	Clementine – Marisol	5	5	100.00
Imperial mandarin	Clementine – Fina	89	64	71.91
Imperial mandarin	Clementine – De Nules	42	24	57.14
Imperial mandarin	Clementine – Oroval	14	1	7.14
Imperial mandarin	Clementine – Old clone	30	20	66.67
Totals		1511	1209	80.01

Series 2. The second series of crosses aimed at early maturing seedless easy-peels involved hybrids bred at Merbein that have been identified as potential parents based on seedless traits, fruit maturity and quality, particularly high juice sugar concentrations. The crossing plan designed is presented in Table 3.2.

Table 3.2 . Summary of crosses made during 2003-06 aimed at generating families that will segregate for early fruit maturity, seedlessness and high fruit quality.

Female	Male	no. seeds	no. hybrids	% emergence
88-02-07	21-03	48	9	18.75
88-02-21	21-03	46	41	89.13
88-02-44	21-03	11	5	45.45
88-04-11	21-03	151	73	48.34
88-05-08	21-03	82	53	64.63
Clementine – Old clone	21-03	1	1	100.00
88-02-07	21-28	36	18	50.00
88-02-21	21-28	50	44	88.00
88-02-44	21-28	32	14	43.75
88-03-08	21-28	19	16	84.21
88-04-11	21-28	301	192	63.79
88-05-08	21-28	179	126	70.39
88-22-41	23-09	1	1	100.00
91-03-01	23-09	3	1	33.33
91-03-04	23-09	3	1	33.33
91-03-07	23-09	7	1	14.29

Table 3.2 contd.

Female	Male	no. seeds	no. hybrids	% emergence
91-03-09	23-09	18	0	0.00
91-03-10	23-09	30	7	23.33
92-01-02	23-09	12	6	50.00
92-01-07	23-09	12	10	83.33
92-01-24	23-09	112	94	83.93
92-01-31	23-09	257	254	98.83
88-22-30	23-26	31	20	64.52
88-22-41	23-26	3	1	33.33
91-03-01	23-26	14	10	71.43
91-03-04	23-26	34	12	35.29
91-03-07	23-26	2	0	0.00
91-03-10	23-26	48	5	10.42
92-01-02	23-26	45	31	68.89
92-01-07	23-26	75	69	92.00
92-01-24	23-26	30	27	90.00
92-01-31	23-26	31	25	80.65
93-05-09	23-26	6	0	0.00
Clementine – De Nules	23-26	134	59	44.03
Clementine – Fina	23-26	87	22	25.29
Clementine – Marisol	23-26	12	10	83.33
Clementine – Old clone	23-26	84	54	64.29
Clementine - Oroval	23-26	82	50	60.98
91-03-01	29-57	1	1	100.00
91-03-04	29-57	20	2	10.00
91-03-07	29-57	17	7	41.18
91-03-09	29-57	6	2	33.33
91-03-10	29-57	70	2	2.86
92-01-02	29-57	53	38	71.70
92-01-07	29-57	42	27	64.29
92-01-24	29-57	104	78	75.00
92-01-31	29-57	76	72	94.74
93-05-09	29-57	9	3	33.33
91-03-01	23-36	3	3	100.00
21-28	Clementine – Old clone	14	5	35.71
21-03	Clementine De Nules	20	18	90.00
92-01-31	Valencia orange	6	5	83.33
Totals		**4090**	**2835**	**69.32**

Coded parents bred at CSIRO Merbein were as follows:

21-03 (Imperial x Ellendale)
21-28 (Imperial x Ellendale)
21-03 (Imperial x Ellendale)
23-09 (Imperial x Ellendale)
23-26 (Imperial x Ellendale)
23-36 (Imperial x Ellendale)
29-57 (Imperial x Ellendale)

88-22-30 (Clementine – Old clone x Valencia orange)
88-22-41 (Clementine – Old clone x Valencia orange)

91-03-01 (Clementine Marisol x Imperial)
91-03-04 (Clementine Marisol x Imperial)
91-03-05 (Clementine Marisol x Imperial)
91-03-07 (Clementine Marisol x Imperial)
91-03-09 (Clementine Marisol x Imperial)
91-03-10 (Clementine Marisol x Imperial)

88-02-07 (Silverhill satsuma x Joppa sweet orange) 88-02-21 (Silverhill satsuma x Joppa sweet orange) 88-02-44 (Silverhill satsuma x Joppa sweet orange) 88-03-08 (Silverhill satsuma x Red Siletta sweet orange) 88-04-11 (Silverhill satsuma x White Siletta sweet orange) 88-05-08 (Silverhill satsuma x Norris Hamlin sweet orange)	92-01-02 (Clementine Fina x Silverhill satsuma) 92-01-07 (Clementine Fina x Silverhill satsuma) 92-01-24 (Clementine Fina x Silverhill satsuma) 92-01-31 (Clementine Fina x Silverhill satsuma) 93-05-09 (Imperial x Clementine Old clone)

3.2.3 A note on seed germination.

Prior to project CT04007, germination rates with Clementine seeds had been poor from both open-pollinated and controlled cross generated seeds. This had been the case for seeds collected from Clementine Oroval x Imperial and Clementine Old clone x Imperial crosses made in 2003. In an effort to maximise germination and seedling recovery rates, a new approach was tried and seeds were germinated in Growool® at a constant 28°C with a 16/8h light/dark regime rather than the normal procedure using a sand bed over potting mix under glasshouse conditions. As the seeds germinated and seedlings emerged (see Figure 3.1), they were transplanted to potting mix in a container, hardened-off and transferred to the glasshouse. This has resulted in much higher germination rates and seedling survival rates, particularly with seeds collected from Clementine mother plants.

Figure 3.1. Emergent seedlings from the crossing program after germination in Growool® under controlled conditions.

3.3 Phase 1 evaluation

Progress in the phase 1 evaluation of new hybrids as they flower was summarised in milestone reports. As the processes involved in conducting this phase of the diploid hybridisation component of the breeding program has been reported extensively in previous final reports and also detailed in the breeding plan, only aspects of phase 1 evaluation will be presented here.

3.3.1 New seedless hybrids

Pollination and pollen exclusion experiments conducted using hybrids as they flower within a population of families generated in 1996-98, have shown that the frequency of hybrids combining traits contributing to the seedless phenotype has increased as the program progresses into second generation crosses using hybrids generated at Merbein as parents.

The population that has been investigated in CT04007 was generated using parthenocarpic selections from families generated from crosses performed between 1984 and 1988 and comprises of 111 full-sib combinations with family size ranging between 1 and 52. There are 27 half-sib families based on the maternal parent and these relationships will be used to analyse the data when collection is complete.

Of the hybrids that have flowered and been tested so far, there is evidence that around 50% are parthenocarpic (185 parthenocarpic to 171 non-parthenocarpic). This is an increase over the population from which the parents used were selected where 144 out of 493 hybrids were parthenocarpic. On an individual full-sib family basis the proportion of parthenocarpic to non-parthenocarpic hybrids varies, although the juvenile period differs between families and for some combinations a majority of hybrids are yet to flower. While it is difficult to speculate too much as a large number of hybrids are yet to flower and be assessed for parthenocarpic ability, within family ratios have indicated that three dominant complementary genes are responsible for segregation of this characteristic, and this supports data from previous crosses.

Of particular interest are two half-sib families involving two Imperial x Hamlin sweet orange hybrids. These monoembryonic hybrids were selected as parents based on a high capability for parthenocarpic fruit development, pollen sterility and a short (4 year) juvenile period. Unlike other half-sib families, almost 50% of the hybrids from the crosses involving these two hybrids have flowered and a high proportion of these are parthenocarpic, segregating 52:15 (hybrid 88-13-11, female parent) and 45:39 (hybrid 88-13-15, female parent) for parthenocarpic to non-parthenocarpic fruit development, respectively.

In addition to the generation of a higher proportion of parthenocarpic hybrids, there has been an increased proportion of new hybrids that yield open-pollinated seedless fruits.

With variability in flowering time both between and within families, screening for seedless traits in this population will continue into the next project. This component of phase 1 evaluation is providing much useful data on the inheritance of seedlessness in citrus and the results so far, which indicate an increase in seedless progeny, support the approach adopted at Merbein in developing improved parents to transmit the characteristics which contribute to the seedless phenotype.

3.3.2 Fruit quality and new selections

Again, milestone reports have outlined the progress made in evaluating fruit quality during phase 1 evaluation. At the time of preparing this final report thirty nine hybrids have been identified with potential to be advanced to phase 2 evaluation test-plots and trials. These 39 hybrids have produced good quality seedless or low-seeded fruits under the conditions of open-pollination in the breeding field. They have been selected from the population of families produced in project CT96014 from 1996 until 1998.

As described later, based on the performance of trees in some of the current plantings in phase 2 evaluation, a decision has been reached to propagate 2 daughter trees of each of these new selections using buds collected from mature fruiting shoots. This adds a refinement to the fast-tracking method employed for entering potential selections into phase 2 trials. While additional phase 1 data are being collected, buds from a greater number of selections showing potential will be used to propagate bud source trees earlier. Then, when a final decision is made with regard to their entry into phase 2 trials, second generation buds will be used to propagate third generation trees with improved precocity for distribution to growers for regional evaluation and for a comparative trial.

One other hybrid was identified with potential during CT04007. This hybrid was identified in a family that was produced during 1988 and had failed to flower and produce fruit when its siblings were being evaluated. Hybrid 88-19-03 (Imperial mandarin x Mediterranean Sweet Orange) produces seedless or low seeded fruits (mean <1) under open-pollination, which mature at the end of May at Merbein. It has high sugar levels and will set fruit parthenocarpically. This selection has been top-worked to eight established orchard trees for further evaluation.

3.4 Phase 2 evaluation

Phase 2 evaluation is conducted with hybrids selected after completing phase 1 evaluation of families within new populations. Hybrids are selected based on their performance as individual trees growing on their own root system under high density (hybrids in phase 1 are grown routinely at a density of 1m intra- and 5m inter-row spacings). As a consequence, selections are mostly made on fruit quality traits before they are entered into phase 2 evaluation trials. Phase 2 trials are conducted with replicated trees of each selection either as nursery-propagated trees or trees produced by top-working to established trees already in an orchard.

In project CT00012, 20 selections from an Imperial mandarin x Ellendale tangor progeny were propagated and entered into phase 2 evaluation. Ten of these selections, considered superior to the other ten, were distributed via a network of growers who were prepared to maintain up to ten trees of each for testing under regional conditions. Before receiving nursery-propagated trees or bud wood for top-working, each grower signed an agreement, which covered a number of issues in relation to testing selections from the breeding program. The main function of this agreement is to emphasise a need to maintain confidentiality as well as prevent further propagation and distribution of the selections at this stage of their development. Most, but not all growers who volunteered to cooperate were happy to sign the agreement. Those reluctant to agree to the conditions were not included in the network.

The other ten hybrids from the Imperial x Ellendale progeny that were deemed to be less promising than those distributed to growers were included in a replicated, comparative trial conducted at one of CSIRO's research farms. This trial included six common knowledge varieties as comparators as well as the twenty selections grafted to three rootstocks, *viz*. Carrizo citrange, Symons sweet orange and Cleopatra mandarin. This trial was established to provided comparative data in the event that one or more selections warranted IP protection, either through PBR or patent, and also provide additional data concerning the performance of the second string selections.

At the start of project CT04007 it was agreed following discussions with the project reference group, that any hybrid considered to have potential after just one season of fruit quality evaluation should be fast-tracked into second phase evaluation at the CSIRO research farm while additional data are collected from the original hybrid. This would allow for a faster appraisal of the selection as a replicated tree once a decision has been made to enter it into regional testing through the grower cooperator network. As a consequence, 17 new hybrid selections were entered into replicated trials at CSIRO in order to generate additional data to those being collected for them as individual trees in phase 1 to identify which would be entered into grower test plots. By the end of CT04007, seven of these hybrids had been identified as worthy of entry into regional test plots and trees were propagated for distribution to cooperating growers willing to sign testing agreements. The trials established on the CSIRO farm will be maintained and the ten not chosen for entry to regional evaluation will also be observed during the next project.

This section of the diploid hybridisation chapter provides further information concerning the second phase evaluation conducted during CT04007.

3.4.1 Regional evaluation of Imperial x Ellendale hybrids

3.4.1.1 Introduction and trial details

When seeking cooperators to assist in the regional evaluation of the ten most promising selections from the Imperial x Ellendale progeny, growers were offered up to ten trees of each selection as nursery-propagated trees or buds to top-work up to ten trees of each selection. Initially growers were sought via the Cittgroup coordinators network, but when this approach failed cooperators were sought by making an announcement at an annual ACG conference and by making contact directly with individuals or local grower groups. This resulted in 8 trial sites being commenced in which between 2 and all ten selections were established. There were three sites in the Riverland of SA, three in the Sunraysia district of NW Victoria/SW NSW, one in the Riverina district of NSW and one in Queensland’s Central Burnett region. Five of these sites accommodated all ten selections while only two selections were grown at each of the other three properties. As a result, one selection was represented at 7 sites, four at 6 sites and the other five at 5 sites. At four of the sites, all ten selections were represented as both nursery-propagated and top-worked trees.

Although trial designs were supplied to grower cooperators when trees or buds were delivered, all cooperators chose to establish the trees according to either anticipated fruit maturity time or their codes supplied. One of the purposes of distributing trees to growers was to have the selections grown as commercial trees. Thus, planting and tree management decisions were left to the growers to make. This approach not only has clear advantages, but also disadvantages, which were manifest in many ways, some examples of which were:

- One grower decided to re-locate the nursery-propagated trees two years after they were established. This resulted in a mix-up when they were re-planted, although project team members were able to identify each selection and adjust the plan. The result was that production on these trees commenced 2 years after other sites.
- On one property, trees were planted in a virgin orchard on mounded rows under fertigation. This led to excellent tree growth and high early yields. Unfortunately,

this grower only selected two selections and they were planted between two heavy pollen producing varieties, which led to quite seedy fruits. Pollen exclusion experiments at this site, however, demonstrated the capacity for both selections to produce seedless fruits.

- Location of trees relative to pollen sources was something that was out of the control of the project team and this had a bearing on seed numbers in the different selections.
- Ownership of two sites changed during the course of the project. In one instance, the new owner did not wish to be part of the network and agreed to destroy the trees as was his prerogative. This clearly set the evaluation of the selections back in the region where this occurred, especially as it was one of the regions where only one site was established, even though all ten selections had been included in the planting. Fortunately, another grower cooperator agreed to join the network and all ten selections were established. However, this set the program back three years in this region with only a few fruits being borne on some of the trees during the final year of CT04007.
- In the second case of ownership change, the new proprietor agreed to maintain the trials, which included all ten selections as both top-worked and nursery-propagated trees, and worthwhile data have been collected. Unfortunately, however, the nursery-propagated trees were neglected from the outset of the trial and the nurse limbs left on the top-worked trees were never removed, meaning that these trees were only half the canopy intended. The grower, however, was able to harvest the other side of the trees for profit. Interestingly, this property has recently changed hands again and the new owner has agreed to maintain the trials and has indicated that he will prune away the half of the tree that grew from the nurse limb.
- Amongst other issues that affected the trials were:
  - Inter-planting amongst established trees that were destined for removal, although this did not occur. As such the young nursery-propagated trees were affected by competition. Fortunately, this occurred at a site where only two selections were planted and these were represented at 6 and 5 other sites, respectively.
  - Failure by the grower to dis-bud top-worked trees on a regular basis without seeking assistance from the project team. As a result these top-worked trees probably failed to demonstrate their full potential.
  - Nursery-propagated trees were planted at the end of rows of large established trees and as such received management more appropriate for large trees and suffered competition effects.
  - As already indicated, some trees received state-of-the art management and thrived while others received lesser management, but nonetheless appropriate attention.
  - Planting density varied between properties and this probably affected tree performance.
  - The effect of reduced water allocations for irrigation in the Murray Valley, mainly in season 2006-07, for some properties.

In spite of the differing levels management the trees received, the data collected have been valuable and assisted in making a number of decisions concerning the fate of the selections included in the trials. One point of concern is the trial in Queensland which had to be replanted and at this stage data have not been collected for the selections there. This will be attended to in the next three years as the trees in the Central Burnett planting bear fruit. Similarly, the other trials will be continued over the next

3-4 years and additional data will be collected, especially where local conditions or the management has resulted in slower tree establishment and growth.

3.4.1.2 Materials and methods

The trees in the regional test plots were either propagated in the nursery at CSIRO and distributed to growers or top-worked to existing trees on grower properties during project CT00012. The details of the trials are presented in Table 3.3.

The period between tree establishment and first fruit harvest varied between sites. Although some trees produced a small number of fruits in 2003, the first complete harvest at some sites occurred during 2004, while for other sites the harvest was delayed until 2005 or 2006.

Table 3.3. Summarised details of regional based trials established on grower properties.

Region	Site	Date established	Tree type & nos.*		Selections	Years harvested
			NPT	TWT		
Sunraysia	1	Oct 2000	5	2	All	2004-07
	2	Oct 2000	5	5	All	2004-07
	3	March 2000	7	-	2127, 2552	2005-07
Riverland	4	Oct 2000	2	1	All	2006-07
	5	Oct 2000	10	-	2127, 2336	2006-07
	6	Oct 2000	10	-	2103, 2105	2004-07
Riverina	7	Nov 2001	2	3	All	2005-07
Central Burnett	8	Oct 2000	10	-	All	never**[1]
	9	Oct 2003	10	-	All	never**[2]

* NPT = nursery-propagated trees; TWT = top-worked trees
** Trees were not harvested either because they were removed[1] or were too young[2]

Fruit harvest

Following tree planting or top-working, the various sites were visited regularly to monitor development or the growers were contacted to check against progress. Table 3.3 gives details when trees were harvested first, which was the year after which the first few fruits were recorded on trees at the respective sites. When trees produced harvestable quantities of fruits, the sites were visited by the project team on a number of occasions to first sample fruits to gauge fruit maturity and then to complete the harvest. Where the trial site was located within the same fruit fly exclusion zone (FFEZ) as Merbein, ie sites 1-6, the harvested fruits were taken to the laboratory for grading, weighing and quality analysis. In 2006, fruits harvested in the Riverland were weighed in the orchard and a sample packed in boxes for cool storage locally to provide fruit for grower displays. Fruits harvested at site 7 in the Riverina, which was outside the Sunraysia/Riverland FFEZ, were weighed and counted at the site to obtain yield and fruit size data. Juice samples were retained for twelve fruits per tree and transported to Merbein for sugar and acid determinations.

Pollination experiments

Insect exclusion

Insect exclusion experiments were superimposed on trees at two sites in 2005 to demonstrate to growers and obtain seedless fruits for display at grower meetings. Two sites were chosen where cross-pollination pressure was high, namely sites 1 and 6 where trees were situated alongside Imperial, Valencia and Mineola (site 1) and Murcott and Mineola (site 6). At site 1, three limbs per tree were bagged on one top-worked and one nursery-propagated tree of selections 2128, 2350 and 2103. At site 6, two limbs were bagged on each of the ten nursery-propagated trees of selection 2103. Both 2103 and 2128 had generated interest from growers at fruit displays, although the seediness of open-pollinated fruits was noted by most as a disadvantage.

Bags were removed from trees after physiological fruit drop in December and limbs were labelled. Fruits were harvested according to maturity tests when all other open-pollinated fruits were harvested. Samples of 6 fruits collected from the bagged limbs were dissected and seeds counted while the remaining fruits were retained for display to growers.

Cross-pollination test with selection 2336

As described later, selection 2336 was nominated for release as Merbeingold 2336. A number of growers have expressed an interest in growing it commercially especially as it produces very little pollen to the point that it can be described to all intents and purposes as being pollen sterile. Controlled crosses with its pollen to other low-seeded parthenocarpic varieties in breeding with Imperial x Ellendale hybrids during project CT614 resulted in very low seed set. Enquiring growers have asked if pollen from 2336 could cross-pollinate Afourer tangor, which is an increasingly popular variety that will produce seedless fruits in the absence of cross-pollination. Thus, in order to be able to answer such enquiries, a number of trees in a commercial Afourer orchard were cross-pollinated with 2336 pollen during spring 2006.

Pollen from 2336 was collected the day before it was required by enclosing its flowers within glassine bags before buds opened. Flowers that opened within the bags were collected the day after and taken to the orchard. Here, Afourer flowers about to open were de-petalled and emasculated before anthers from 2336 were gently brushed against the stigma of naked gynoecia. At de-petalling, any Afourer flowers in which pollen had dehisced within the bud were discarded. Cross-pollinated flowers were labelled with swing tickets and left to develop. Experience has shown that pollinating insects are not attracted to naked gynoecia.

Fruits from cross-pollinated flowers were collected during July 2007 and transported to the laboratory were they were weighed, dissected and seeds counted.

Sprays to manipulate rind quality

Selection 2127 was identified during 2005 as having potential for release as a late maturing seedless variety. Being late maturing (September in Sunraysia and the Riverland), however, deterioration in its rind quality in terms of puffiness was observed to be a potential problem. To evaluate its reaction to rind age delaying sprays, a number of formulations being tried by one of the growers were applied to trees of 2127, as well as 2336 and 2350, during the 2005-06 growing season. The grower has asked that details of the formulations used remain as confidential information.

3.4.1.3 Results and discussion

Voluminous data have been collected from these trials during the course of the project, but it is not an intention to reproduce all this here in this report. A summary only is provided.

Due to the nature of the test plots, some details of which have been given, it has been difficult to compare the results from different sites. Thus, the data have been used for indicative purposes. The fruits harvested from the test plots have also been used in grower and other displays, and for taste tests. Rather than present data for each site individually, for the purposes of this report, and for brevity, the data have been summarised across sites to provide an overview on the performance of the selections.

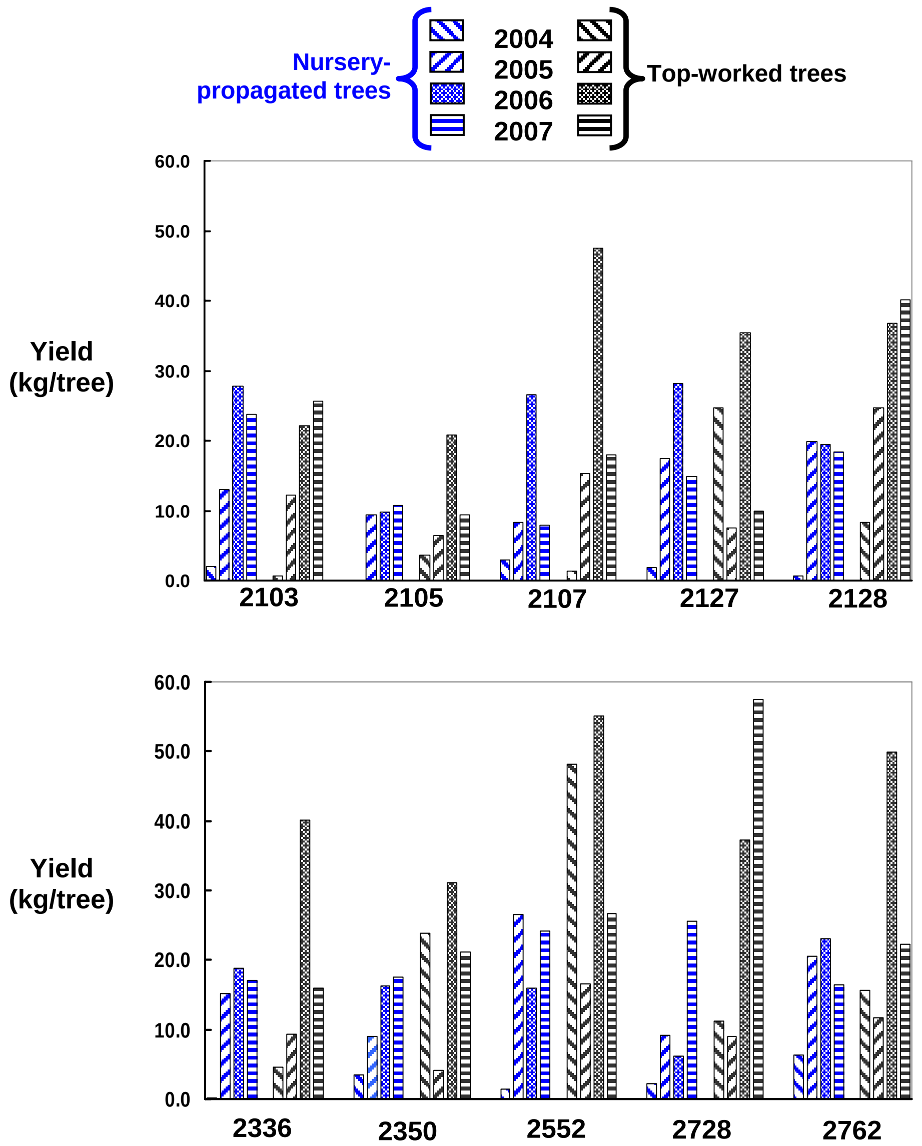
Yield
As might be expected for young trees and equally young budlines, yields increased across sites with time.

Yields were generally higher for top-worked trees (Figure 3.2) although this varied between sites. For example, yields of selection 2350 at Sunraysia site 1 were higher for top-worked trees, reaching 65kg in year 3, than for nursery-propagated trees, while at Sunraysia site 2, yields for nursery-propagated trees reached 30kg in years 3 and 4 compared to the maximum mean of around 10kg for top-worked trees at the same site. It is unclear why this should be the case, although the possibility of viral load in existing trees that were top-worked may have varied between sites and this could account for such differences. Rootstocks varied between sites and complex interactions between different rootstocks and interstocks and combinations of these could also have been important in causing variability between sites, especially between sites within the same region. Similarly, planting densities varied between sites and could also have contributed to variations between tree types within sites and between sites. For example, the nursery propagated trees at sites 1 and 2 were planted at 2.5x2.5m and 5x6m, respectively.

The highest and lowest yielding selections were 2552 and 2105, respectively (Figure 3.2). Yields from all selections increased with time and all, with the exceptions of top-worked trees of 2128 and 2728, were showing some degree of biennial bearing by the fourth year of harvest. No attempt was made to manipulate flower densities or thin crop loads during the course of the project with the trees in the regional test plots.

While it is difficult to account for variations between sites for yields of the selections, yields were particularly high for some seelctions in certain years and the range in yields between and within sites make it hard at this stage to draw any firm conclusions about yield capacities of the selections. For example, top-worked trees of 2336 at sites 1 and 2 had mean yields of 17kg and 9kg, respectively in 2005 followed by 95kg and 46kg in 2006, respectively. Mean yields from nursery propagated trees at the same sites were 6 and 25kg, respectively in 2005 followed by 37kg and 15kg in 2006, respectively. Similar yield fluctuations and differences occurred for all selections at all sites. Additional data from these and other trials over the next 3-4 years will allow a better assessment of yield potentials and biennial bearing patterns for the various selections.

Figure 3.2. Mean yields for harvest seasons 2004-07 for nursery propagated and top-worked trees over all sites used in regional testing of the selections.



It is difficult to draw too many conclusions about yield potentials after just 4 years data. The trees were propagated both in the nursery and by top-working in orchards using the first daughter buds removed from the original seedling trees. As such they were the first daughter trees propagated from the selected hybrids. Seedling trees retain some juvenile characteristics and the position from which buds were taken for propagating trees may have had a bearing on the early yield capacities of the trees in the regional test plots. For example, buds removed from a strong shoot growing from a basal region of a seedling tree may retain and thus display greater juvenile characteristics than one removed from behind a fruit on a mature shoot higher in the tree. The original seedling trees were pruned lightly to stimulate new vigorous growth during the season prior to buds being cut for propagating the trees for second phase evaluation. While trees would have been propagated at random from the buds collected, the physiological age of buds with regard to phase change between juvenile and mature wood may have had an influence on early yield capacity of the trees propagated. This is a consideration now adopted in the program and new selections identified early in phase 1 screening with possible potential for entry into phase 2 at a later stage are being propagated from buds collected from mature fruiting shoots to propagate a bud-source daughter tree. Once this tree flowers, it will be used to provide second generation daughter buds for propagating additional trees if the selection is entered into replicated phase 2 evaluation trials and testing plots.

Fruit quality

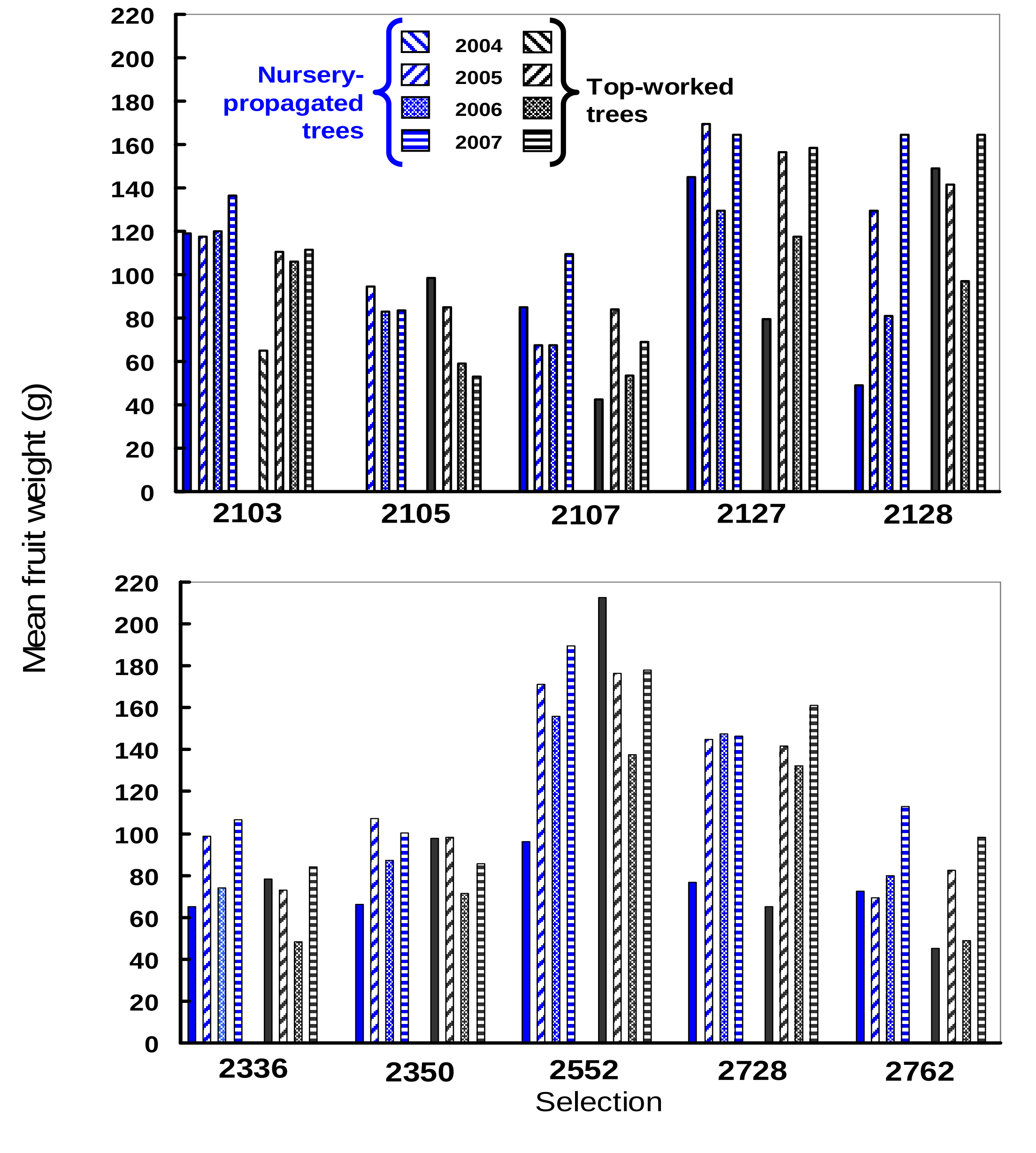
Fruit quality was assessed in many ways by collecting and analysing both objective and subjective data. The type of data was similar to that collected for trees in phase 1 ranging from visual appearance, to weight, rind characteristics, seediness, and juice characteristics. As with yield, a large volume of data has been collected and only a summary will be presented here.

Fruit weight

Mean fruit weight (Figure 3.3) was determined from total harvested yield and fruit numbers. As with yield, mean fruit weights varied between selections, tree type, year and site, although as described already, site-to-site differences are difficult to explain. Selections with the largest fruits were 2127, 2128 and 2552, for which mean fruit weights exceeded 200g. Selections with the lowest mean fruit weights were 2105, 2107, 2336, 2350 and 2762, which were in a similar size range between 60 and 120g depending on site and year. Clearly within selections, fruit weight varied such that selections 2552 and 2128 and 2127 produced individual fruits in the higher range of 300-350g. Such fruits, however, tended to have thick puffy rinds and were atypical of the selection. Similarly, selections with lower mean fruit weights produced a range of sizes with some fruits exceeding 200g, but the larger fruits of these selections tended not to have thick, puffy rinds.

As with yields, mean fruit weights varied with season and while in some instances this appeared to be associated with yields, eg in top-worked trees of 2107, 2127 and 2552 in years 2005-07, it was not a strong association. Indeed for some selections, eg 2103, 2128, 2336 and 2350, fruit weights either increased with time or stabilised to an extent. As with yield, the variation in fruit weights may have been more a function of young tree or bud-line performance and additional data over the next 3-4 years will be important in obtaining a better appreciation of the range of fruit weights for these selections.

Figure 3.3. Mean fruit weight for ten Imperial mandarin x Ellendale tangor selections for harvest seasons 2004-07 from nursery propagated and top-worked trees across all sites.



Visual appearance

Rind colour (Figure 3.4.1) was measured by using visually ranked scores based on a Japanese system (Yamazaki and Suzuki1980), as described in previous final reports for the breeding program, in which the higher the number assigned to a fruit, the more intense the orange/red colour of the rind. Using this system, Imperial mandarin is often assigned a score of 5-6. The selections were all identified originally as having a higher score than Imperial and this was borne out in most seasons for fruits from the regional test plots. Exceptions occurred for a few selections during their first year of harvest and in 2007 when conditions in the Murray Valley resulted in delayed rind maturity and early internal juice maturity. For example, fruits from top-worked trees of 2105, 2336 and 2552 had poorer rind colour in 2007 than for previous seasons.

Figure 3.4. Variation in fruit quality for ten Imperial mandarin x Ellendale tangor selections grown as nursery-propagated and top-worked trees across a range of grower-operated sites. Means are presented for 2004-07 harvests.

Figure 3.4.1 Mean rind colour.

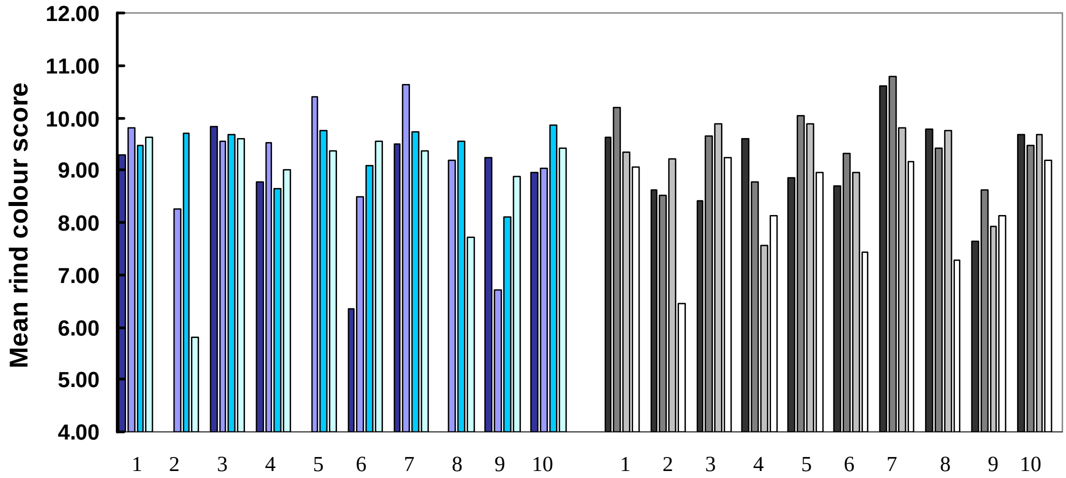


Figure 3.4.2 Mean percentage juice content

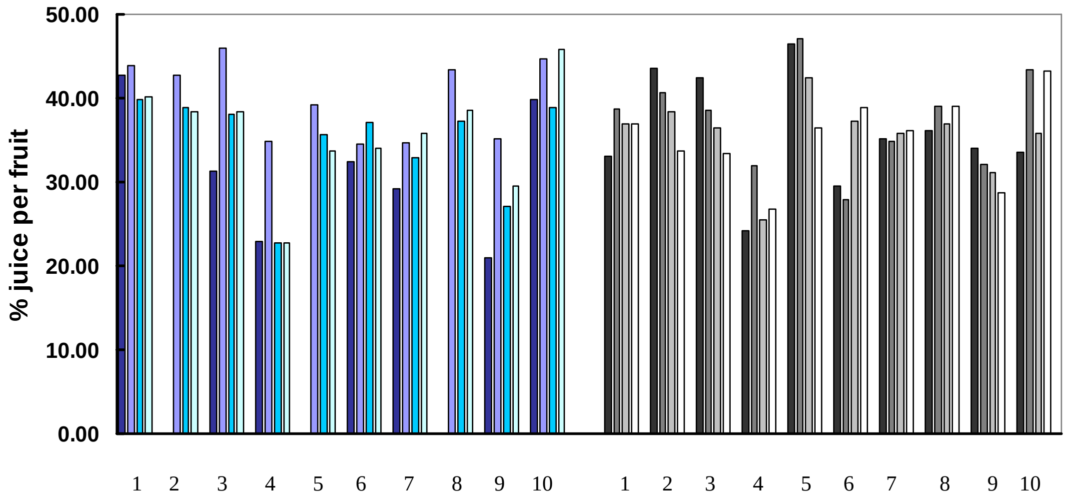


Figure 3.4.3 Mean sugar concentration (° Brix).

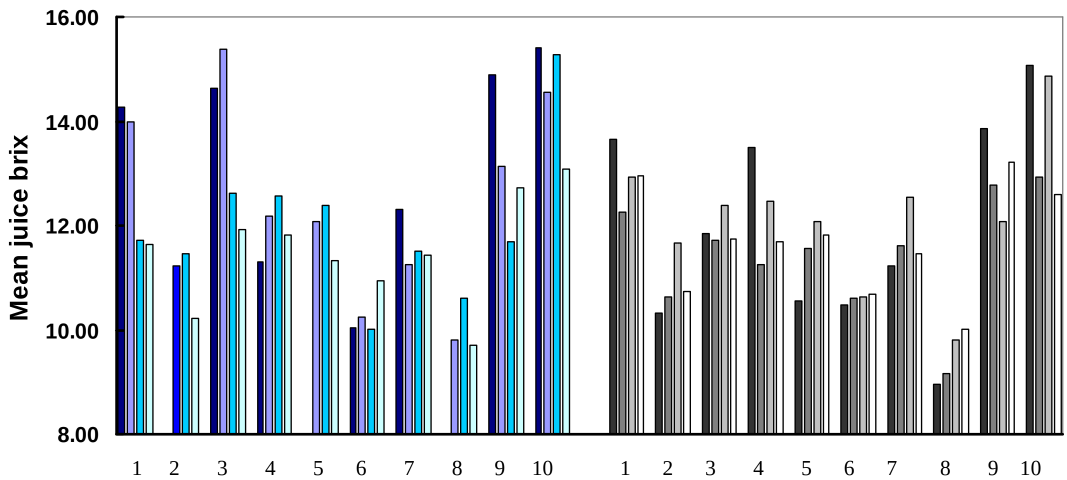


Figure 3.4 contd.

Fig 3.4.4 Mean sugar:acid ratios

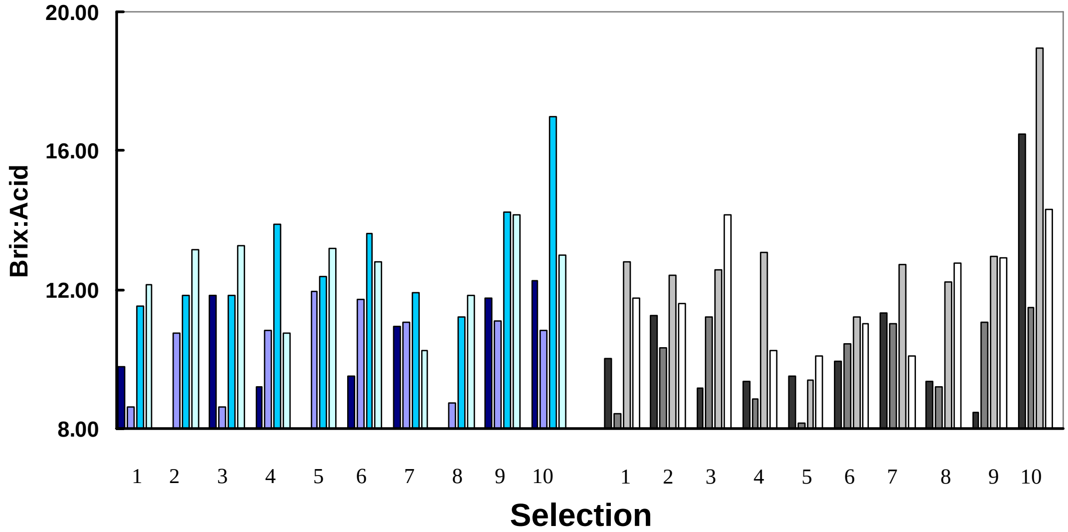
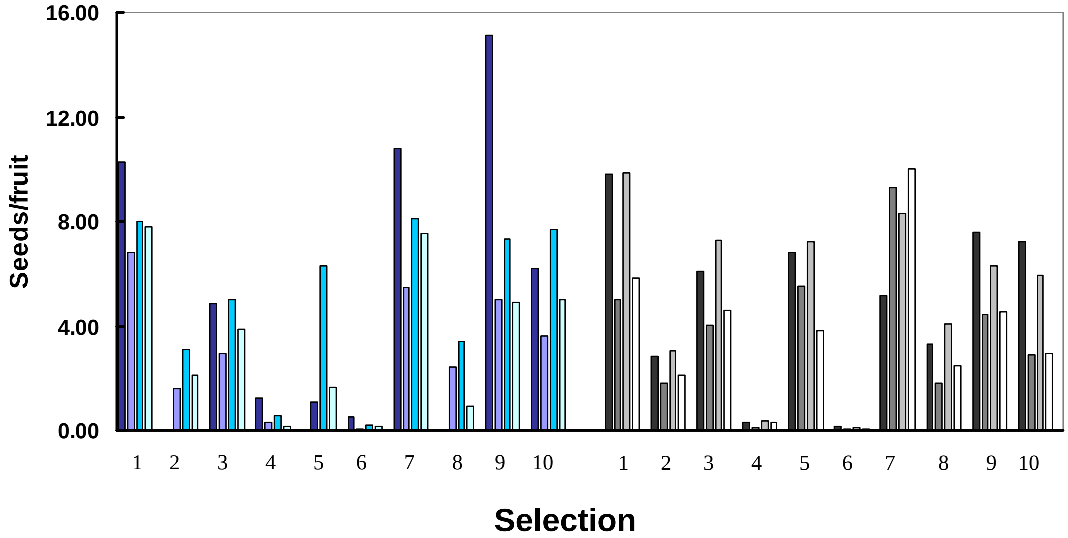


Figure3.4.5 Mean number of seeds per fruit.



Key:

Tree type and year of harvest:

Nursery-propagated trees	**2004**	**2005**	**2006**	**2007**
Top-worked trees	**2004**	**2005**	**2006**	**2007**

Selections:
1, 2103; **2**, 2105; **3**, 2107; **4**, 2127; **5**, 2128;
6, 2336; **7**, 2350; **8**, 2552; **9**, 2728; **10**, 2762

Otherwise, rind colour was generally good and met expectations based on the data that had been collected for the trees in phase 1 evaluation. Fruits of selection 2350 had very good rind colour, a feature noted often by members of the industry when they have been displayed.

Data for other visual characteristics, such as fruit shape and rind texture, are not presented here, but were as expected from the data collected and presented in previous final reports.

Juice characteristics
Mean percentage juice contents (Figure 3.4.2) of fruits were generally as predicted from data collected for the individual hybrids during phase 1 evaluation. For example, selection 2127 had a % juice range of 27-35% during phase 1 evaluation and had the lowest mean % juice in 2004, 06 and 07. Similarly selection 2728 had lower than average juice percentages in phase 1. Conversely, selections 2128 and 2762 had higher than average mean % juice in phase 1 and had similarly high % juice across the trials. With the exception of fruits of selection 2127, juice percentages were generally around or above the recognised minimum level for mandarins, namely 35%, especially as the trees got older.

As with % juice, juice sugar concentrations (Figure 3.4.3) expressed as °Brix from fruits harvested from the regional test plots reflected the data collected for the hybrids in phase 1 evaluation. Thus, mean juice sugar was around 10.5 for selections 2336 and 2552 in phase 1 and were similar in the regional trials although the top-worked trees of 2552 were less than this during the first two years fruits were harvested. Similarly, selection 2762 produced fruits with a mean sugar concentration of around 14.5 °Brix in phase 1 and had high mean juice sugar in the regional plots. Fruits of selection 2762 have been singled out in tastings as being of a particularly pleasant, intensely rich flavour.

With regard to flavour and taste, sugar:acid ratios in extractable juice were all satisfactory and exceeded the recognised minimum of 7.5:1 for mandarins (Figure 3.4.4). Clearly some selections had higher ratios which reflected in favourable comments received at tastings, particularly for selections 2103, 2336, 2350 and 2762.

Seediness
While all selections entered into phase 2 were capable of producing seedless fruits because of their ability for parthenocarpic fruit development, under the conditions of open-pollination they all at some stage contained seeds to varying degrees (Figure 3.4.5). Seedless fruits were harvested from all selections, although both the proportion of seedless fruits and the number of seeds per fruit varied between sites and years.

Fruit seediness appeared to reflect surrounding pollen sources, or lack of pollen at the various sites. Where there were sources of viable pollen, eg site 6, and the trees were established amidst plantings of Murcott tangor and Mineola tangelo, mean seed numbers were higher. For example in 2005, selections 2105, 2107, 2127, 2128, 2336, 2350, 2552 and 2728 at site 2 had mean seed numbers less than 1. At site 1, which is also in the Sunraysia region, for the same year, mean seed numbers for these selections ranged from less than 1 (2336) up to 16 (2128). The trees at site 1 were surrounded by Imperial mandarin, Mineola tangelo and Valencia orange trees, all of which produce viable pollen. The trees at site 2 were located such that they were adjacent to navel orange trees on two sides and native scrub elsewhere. Navel orange flowers are pollen sterile and thus the chance for cross-pollination was limited at site 2 to that occurring between the selections themselves, which presumably occurred due to the presence of a low number of seeds in the fruits of the selections listed.

Selection 2336 stood out from all other selections in that nearly all fruits were seedless. All fruits harvested from nursery-propagated trees in 2006 and from top-worked trees in 2005 and 2007 were seedless. In other years occasional fruits were harvested with seeds, although the mean across all sites was close to zero for this selection. Selection 2127 also had very low mean seed numbers while selections 2103 and 2552 generally had 4 or less seeds per fruits and, as already stated, this varied such that a high proportion of their fruits were seedless at some sites where the chances for cross-pollination were reduced.

Insect exclusion

All fruits that developed on limbs of 2103 (sites 1 and 6), 2128 and 2350 (site 1) that had been bagged prior to flowering were seedless as expected from data produced in earlier projects (Table 3.4; Figure 3.5). While this relatively simple experiment confirmed earlier data with regard to parthenocarpic ability of the selections in question, it provided samples of seedless fruits from these selections for display at industry meetings.

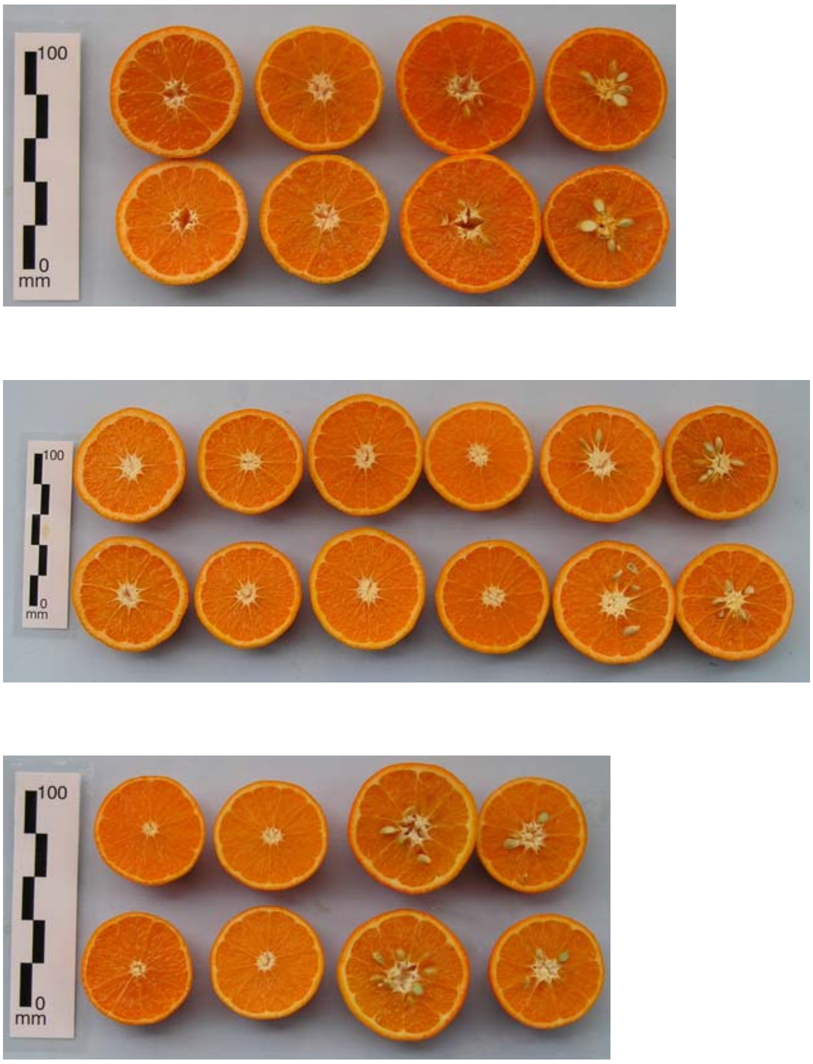
Table 3.4 Mean seed numbers (± sd) in fruits harvested from limbs that had been bagged or left open during flowering. Data are for fruits harvested on July 7, 2006 from trees site 1

Selection	Tree type	Bagged limbs	Open-pollinated
2103	TW	0	11.50 ± 4.50
	NPT	0	17.00 ± 5.29
2128	TW	0	12.33 ± 3.14
	NPT	0	7.25 ± 5.93
2350	TW	0	8.83 ± 2.34
	NPT	0	13.67 ± 3.45

Cross-pollinations between Afourer and Merbeingold 2336

Twenty five terminal flowers on leafy shoots of Afourer were cross-pollinated as described. Nine fruits were harvested on July 19, 2007. Seed numbers within these ranged from 0 to 2 with a mean of 1.33 ± 0.71 (Table 3.5). Fruit weight ranged between 41-to-159g with a mean of 100 ±41. There was no correlation between seed number and fruit weight (r = -0.21).

Figure 3.5. Fruits from bagged (left) and open-pollinated limbs (right) of three selections grown in regional test-plots.



2103 TW

2128 TW

2350 NPT

A visual inspection of freshly opened 2336 flowers generally reveals dull, dry pollen on the anthers (see Figure 3.6). Occasionally, however, a small quantity of bright yellow pollen is present and presumably it is this pollen that resulted in the seeds extracted from the Afourer fruits collected. This indicates that planting 2336 adjacent to Afourer could result in cross-pollination and seeds, although in this investigation a large amount of pollen was deposited on Afourer stigmas and this only resulted in a 2 seeds at most. Under natural pollinating conditions, less seeds would be the most likely scenario as there would be a very low chance of effective cross-pollination occurring between the two varieties.

Table 3.6. Fruit weight and seed numbers in Afourer tangor fruits harvested after their flowers were pollinated with 2336 pollen. Fruits were harvested on July 19, 2007.

Fruit no.	Weight	Seed number
1	131.66	1
2	97.6	2
3	59.67	0
4	155.41	1
5	73.47	2
6	158.65	2
7	104.66	1
8	79.85	2
9	40.87	1
Total	**901.8**	**12**
mean	**100.20**	**1.33**
Sd	**41.49**	**0.71**

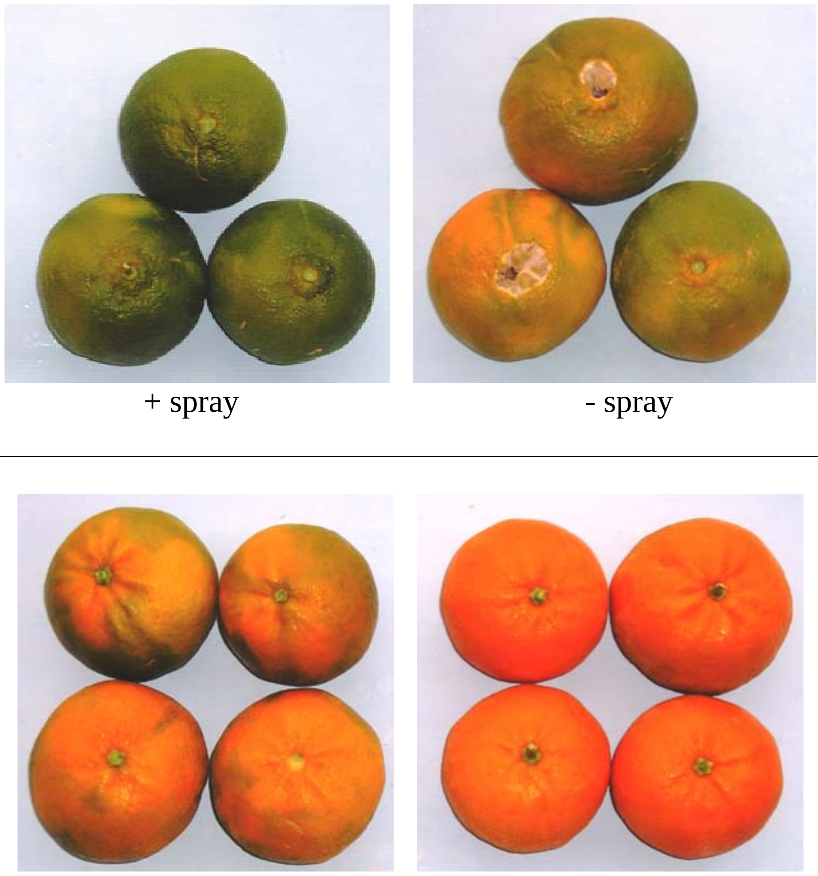
Correlation between fruit weight and seed number; $r=0.21^{ns}$

Figure 3.6. Flower of selection 2336 showing dry, dull pollen on anthers. Occasionally, as can occur with Satsuma mandarin, a small amount of viable, bright yellow pollen is seen.

Sprays to manipulate rind quality

The sprays used for these grower-based trials resulted in delaying rind maturity in the selections used (Figure 3.7) and as a result reduced rind puffiness was evident, especially for selection 2127, which is a late maturing seedless genotype. These tests demonstrated potential to explore this further especially for selection 2127, which may be released at a future date. This selection, as discussed later was nominated for possible release providing control of its rind problems could be addressed. As a result a larger planting of the selection has been established to investigate its agronomy further and also provided larger quantities of fruits for market appraisal.

Figure 3.7. The effects of plant growth regulator sprays on rind colour development of fruits harvested from top-worked trees of selections 2127 and 2336 during June 2006. Sprays caused delays in rind colour development, which in the case of 2127 translated into reduced puffiness when the fruits were harvested during September. The delay in colour development in 2336 was a clear disadvantage as fruits were mature internally at this stage.



2127: + spray; - spray

2336: + spray; - spray

3.4.2 Comparative PBR trial

In addition to establishing regional test plots of selections as part of phase 2 evaluation, a comparative trial that included the selections distributed to growers along with others that were selected for further observation, as well as common knowledge mandarin/tangor varieties for reference purposes, was established at CSIRO. In addition to evaluating the selections, this trial served an additional purpose of providing data to support Plant Breeders Rights (PBR) or Plant Patent applications.

3.4.2.1 Materials and methods

Trial design

Trees of 20 selections and 6 common knowledge varieties (*viz*. Clementine Nules, Imperial mandarin, Ellendale tangor, Sunset mandarin, Encore mandarin and Murcott tangor) were propagated by budding to 3 rootstocks (*viz*. Carrizo citrange, Cleopatra mandarin and Symons sweet orange) during project CT00012 in the nursery at CSIRO.

The trial was planted during spring 2001. The trees were irrigated by overhead sprays and fertilised using a standard citrus N:P:K (12:3:3) formulation. Fertiliser was applied to young trees at 2-monthly intervals and to trees 2 years and older at six-monthly applications at a rate such that they received 800 kg/ha/year. Trace elements, primarily manganese and zinc were applied as foliar sprays as required.

The trial was laid out as two randomized blocks with a three-tree plot for every scion/rootstock combination within each block. Rootstocks were randomized within plots. Trees were maintained vegetatively for the first three years and allowed to retain fruits from season 2004-05 onwards. This allowed for greater uniformity in early tree growth and development. Being from older budlines, some of the common knowledge varieties started to flower in their second year, which was later reflected in the yields they produced relative to the selections.

Trees were harvested according to fruit maturity, which was gauged by sampling fruits and recording juice sugar:acid ratios. Harvest commenced in May with Clementine Nules and some of the hybrid selections and went through until October for Murcott, Encore and hybrid selection 2952. Fruits were harvested by snapping them from the tree to assess the need to clip, graded for size, weighed and counted. A sub-sample of six fruits was taken from the three median grades for each tree and analysed for a range of characteristics as described in previous reports. These included, rind colour, rind texture, fruit size, shape, rind thickness, ease-of-peeling, rind strength in terms of being able to be snapped from the tree, juice content, seed numbers, juice sugar, acid and sugar:acid ratios.

Vegetative descriptive data and observations were also collected for the trees in the trial. The purpose of this was for PBR and Plant Patent applications. These data are not included in this report.

Copper sprays

Mesejo *et al* (2006) reported that weak copper sprays during the bloom period significantly reduced seed numbers in Clemenules mandarin and Afourer tangor by inhibiting pollen tube growth and thus ovule fertilisation. Thus, to investigate if a similar effect could be obtained for the some of the parthenocarpic selections being evaluated, a preliminary experiment was conducted with trees maintained at CSIRO to see if copper sulphate sprays would have a similar effect on seed numbers in selections 2103, 2128, 2350, 2552 and 2952. Trees were sprayed with a solution of copper sulphate (25mg/l) during the 2006 bloom period. Trees were sprayed every second day between September 26 and October 12. Imperial mandarin and Ellendale tangor trees were included as controls. There were three trees of each grafted to one of three rootstocks, namely Carrizo citrange, Symons sweet orange and Cleopatra mandarin. Only one side of each tree, which was selected at random, was sprayed with copper sulphate. Fruits were harvested according to documented and seasonally monitored maturity, returned to the laboratory, weighed, dissected and seed numbers recorded.

3.4.2.2 Results and discussion

As with regional test plots, voluminous data were collected and again only a summary is provided here.

Fruit maturity varied between the varieties and selections and was similar to that predicted from the performance of the selections during phase 1 evaluation.

Yield

There were no significant rootstock or block effects on fruit yield whether it was analysed as cumulative yield over the period 2005-07 (Figure 3.8) or as yield per tree in each year (Figure 3.9). There were significant differences ($P<0.001$) between varieties and selections with the named varieties generally having higher yields than the selections. The highest yielding varieties over the three year period were Nules clementine and Ellendale. The highest yielding selections over the three years were 2103, 2107, 2128, 2309 and 2916. A number of selections failed to produce fruits in 2005. As with the regional-based test plots, this was probably a reflection of the age of the budlines and that the trees were propagated with the first daughter buds cut from the original hybrid. As discussed earlier, steps are now being taken to propagate a daughter tree earlier when a hybrid looks to have some potential in an attempt to avoid issues related to tree precocity. As a consequence, and as discussed in relation to the trees in the regional test-plots, data over the next 3-4 years will be needed to gauge better the yield potential of the selections.

Figure. 3.8 Cumulative yield (kg/tree ± se) for twenty selections and 6 common knowledge varieties for years 2005-07.

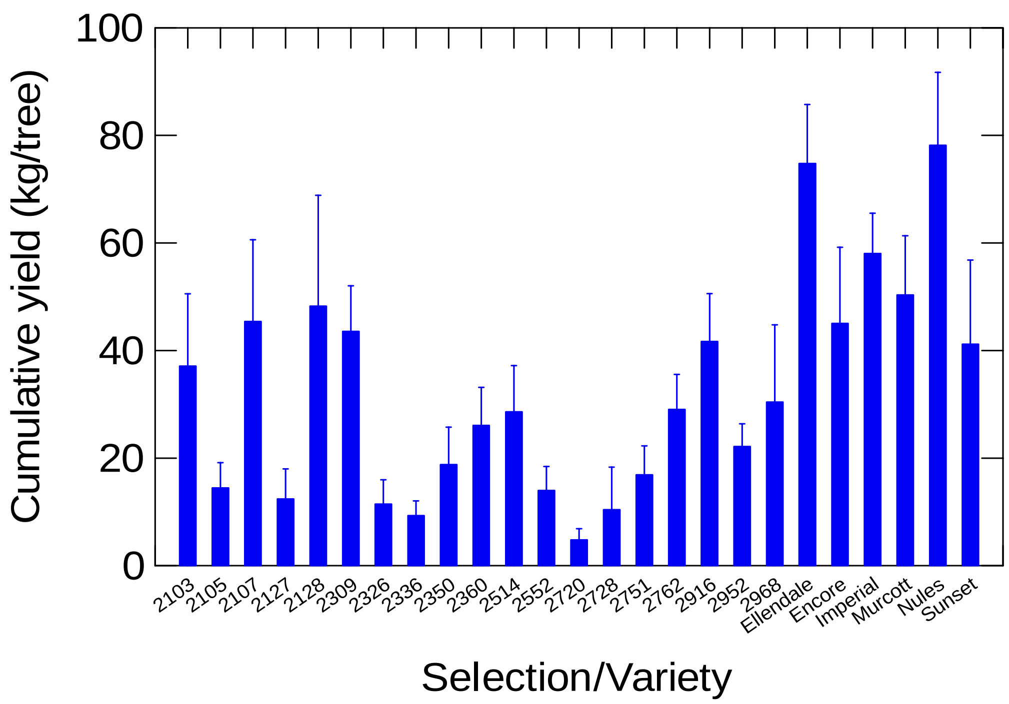
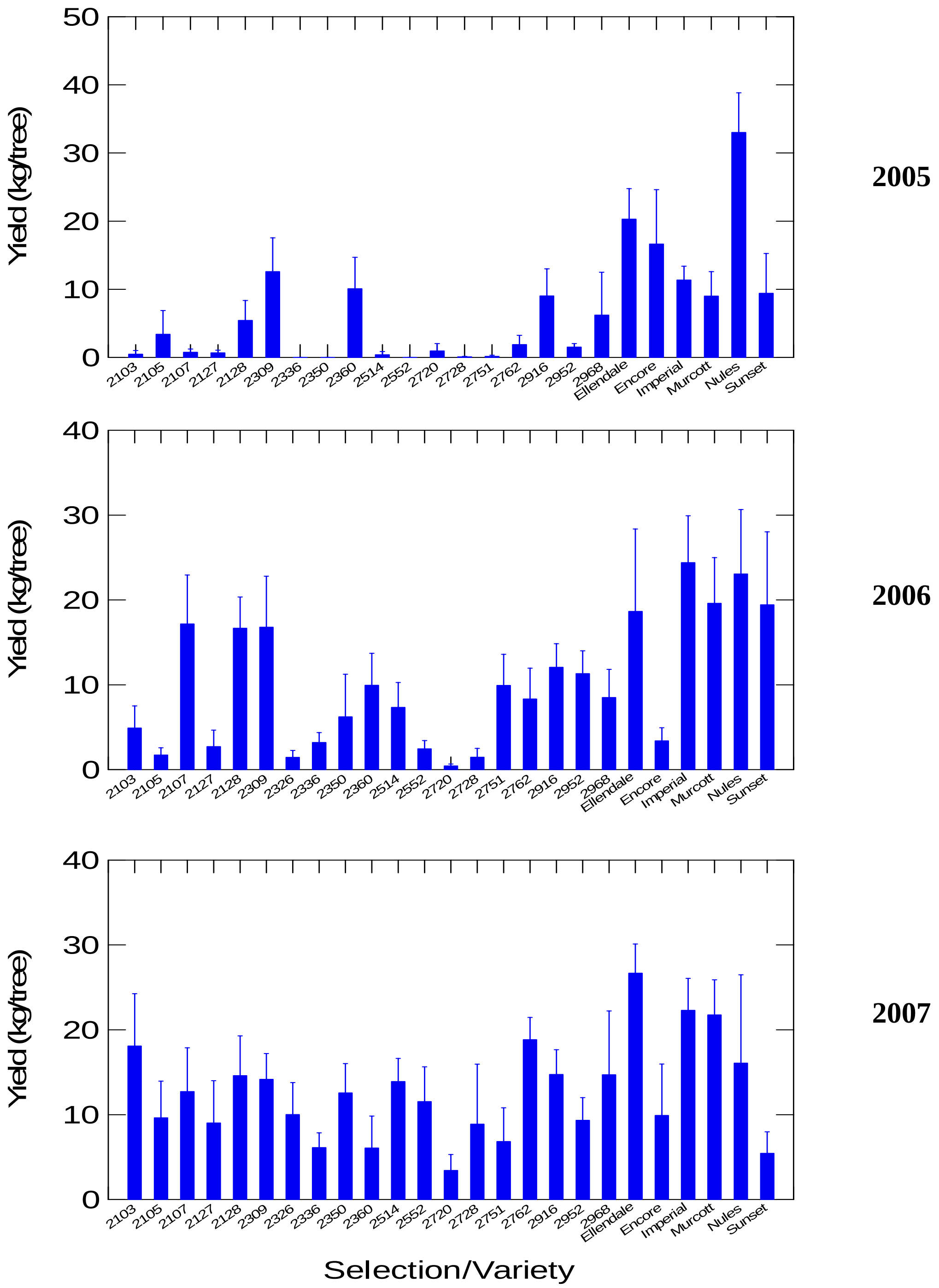


Figure 3.9 Mean yields (n=6) for selections and common knowledge varieties in comparative trial.



Fruit quality

As for the regional test-plots, fruit quality was assessed in many ways and both objective and more subjective data were collected. Again, the range of data was similar to that collected for trees in phase 1 ranging from visual appearance, to weight, rind characteristics, seediness, and juice characteristics. Again, only a summary will be presented here.

Fruit weight

As for yield, there were significant differences (P<0.001) for fruit weight between varieties (Figure 3.10), but no rootstock or block effects in each of the three years that fruits were harvested. Overall mean fruit weight increased with time rising from 100.1 in 2005 to 117.2 in 2007. There was no clear relationship between overall yield and fruit weight. This increase in mean fruit weight was observed in some selections, eg 2103, 2128, 2336, 2350, 2552, but not in others. These increases may again have been a reflection of the age of budlines for some of the selections and the division between vegetative and reproductive growth in some genotypes. As with yield, it will be important to maintain this trial over the next 3-4 years to obtain a clearer indication of fruit weight potential of the selections relative to the varieties included in the trial.

Visual appearance

There were significant differences (P<0.001) for rind colour between varieties within each year as scored using the colour chart referred to earlier (Figure 3.11). There were no significant differences attributable to either rootstocks or blocks. Of the common knowledge varieties included, Imperial mandarin fruits scored lower than the others for rind colour. In 2005 there were two selections that had poorer rind colour than Imperial and in 2006 there were none. In 2007, four selections had rind colour scores less than Imperial and this was most likely a reflection of the warmer than normal autumn experienced in Sunraysia that delayed rind maturity in the district. A number of selections maintained their relatively high scores for rind colour in 2007 (eg 2350, and 2128), although none achieved the score of Sunset mandarin, which is a variety noted of its distinctive bright red/orange rind.

As with fruits harvested from the regional test-plots, other visual characteristics were as expected from the data collected for the original hybrids during phase 1 evaluation.

Juice characteristics

As with rind colour, there were significant differences (P<0.001) between genotypes for percentage extractable juice (Figure 3.12), but no effects due to rootstocks or blocks. Imperial mandarins had the lowest % juice of 6 common knowledge varieties and were the lowest amongst all genotypes in 2005 and 2007. Ellendale and Encore fruits were the juiciest in each year and, along with Sunset, Murcott as well as a number of the selections, exceeded the recognised maturity standard of 35%.

Juice sugar (as °Brix), acid (% citric) and sugar:acid ratios all varied significantly (P<0.001) between varieties (Figures 3.13 and 3.14). Fruits of Murcott consistently had the highest sugar concentrations. A number of selections had juice sugar concentrations exceeding 12 °Brix each year, which has been considered as a minimum in the project breeding plan for addressing the need for sweet fruits in Asian export markets. Amongst these were selections 2103, 2128 and 2762, which have been highlighted from the regional test plots as having high juice sugar concentrations. The lowest sugar concentrations were around 10° Brix (including

Figure 3.10. Mean fruit weights (n=6) for selections and common knowledge varieties in the comparative PBR trial.

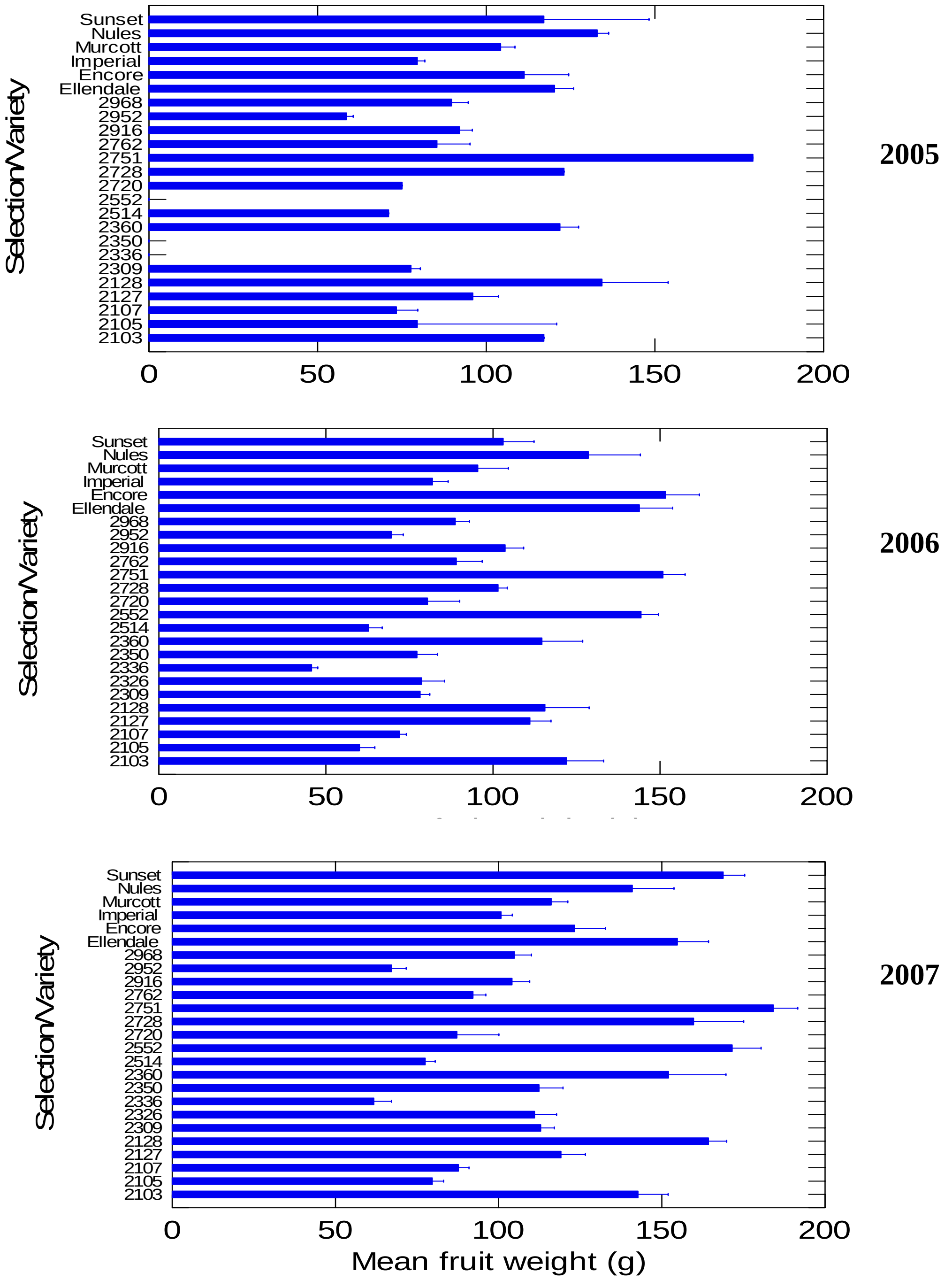


Figure 3.11. Mean rind colour scores (n=6) for selections and common knowledge varieties in the comparative PBR trial.

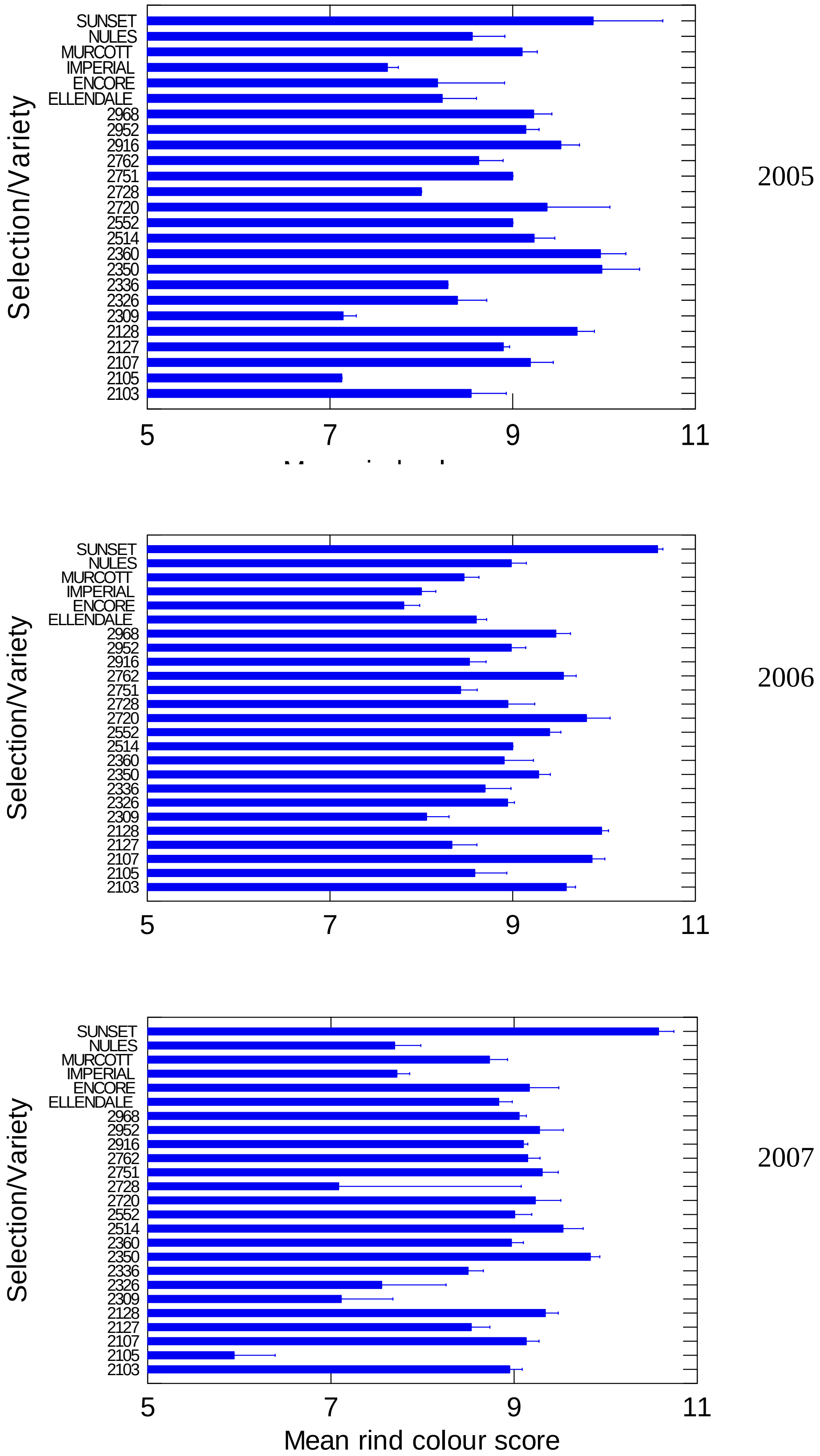


Figure 3.12. Mean percentage juice contents (n=6) for selections and common knowledge varieties in the comparative PBR trial.

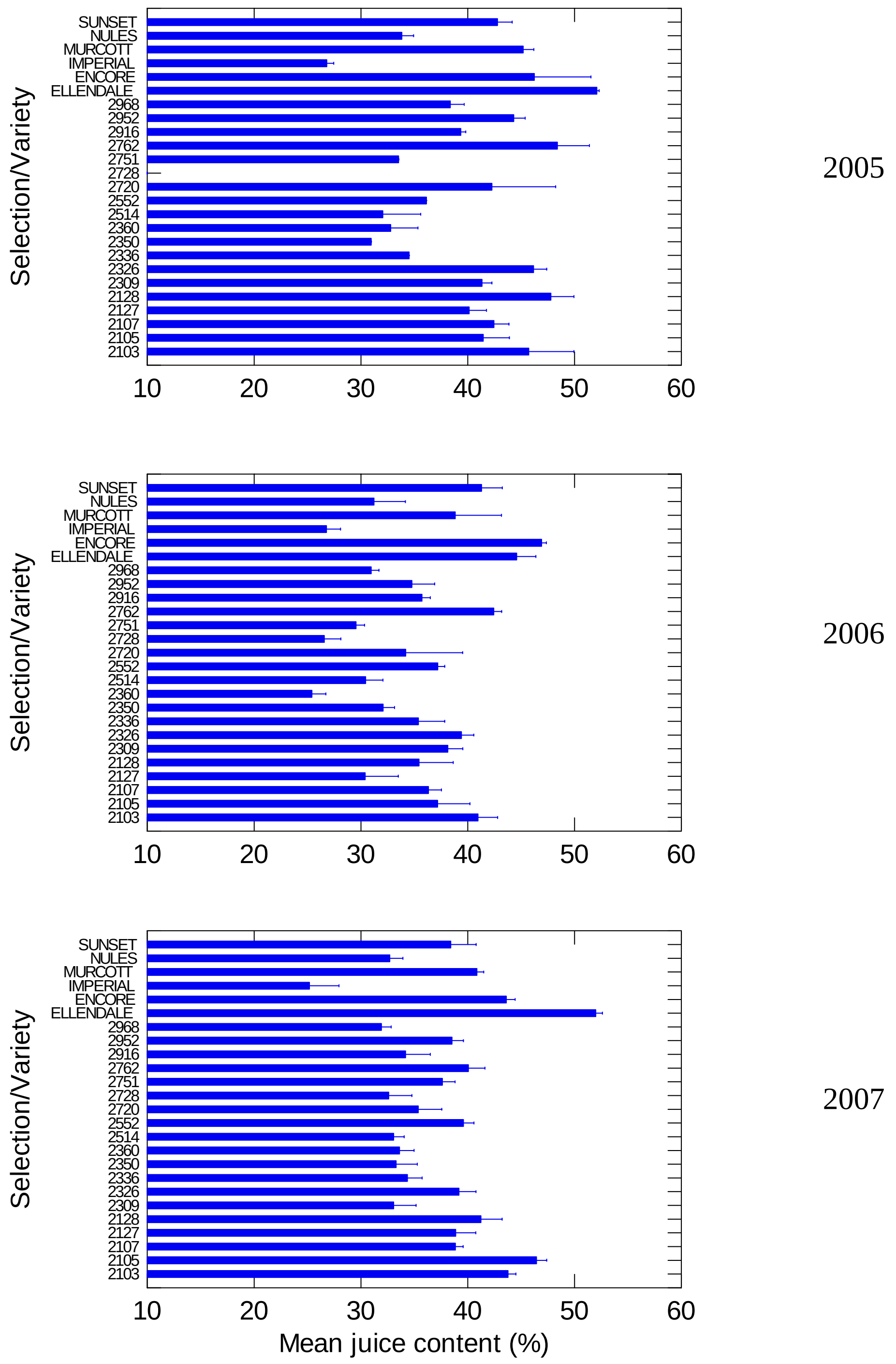


Figure 3.13. Mean (n=6) juice sugar concentrations (as °Brix) for selections and common knowledge varieties in the comparative PBR trial.

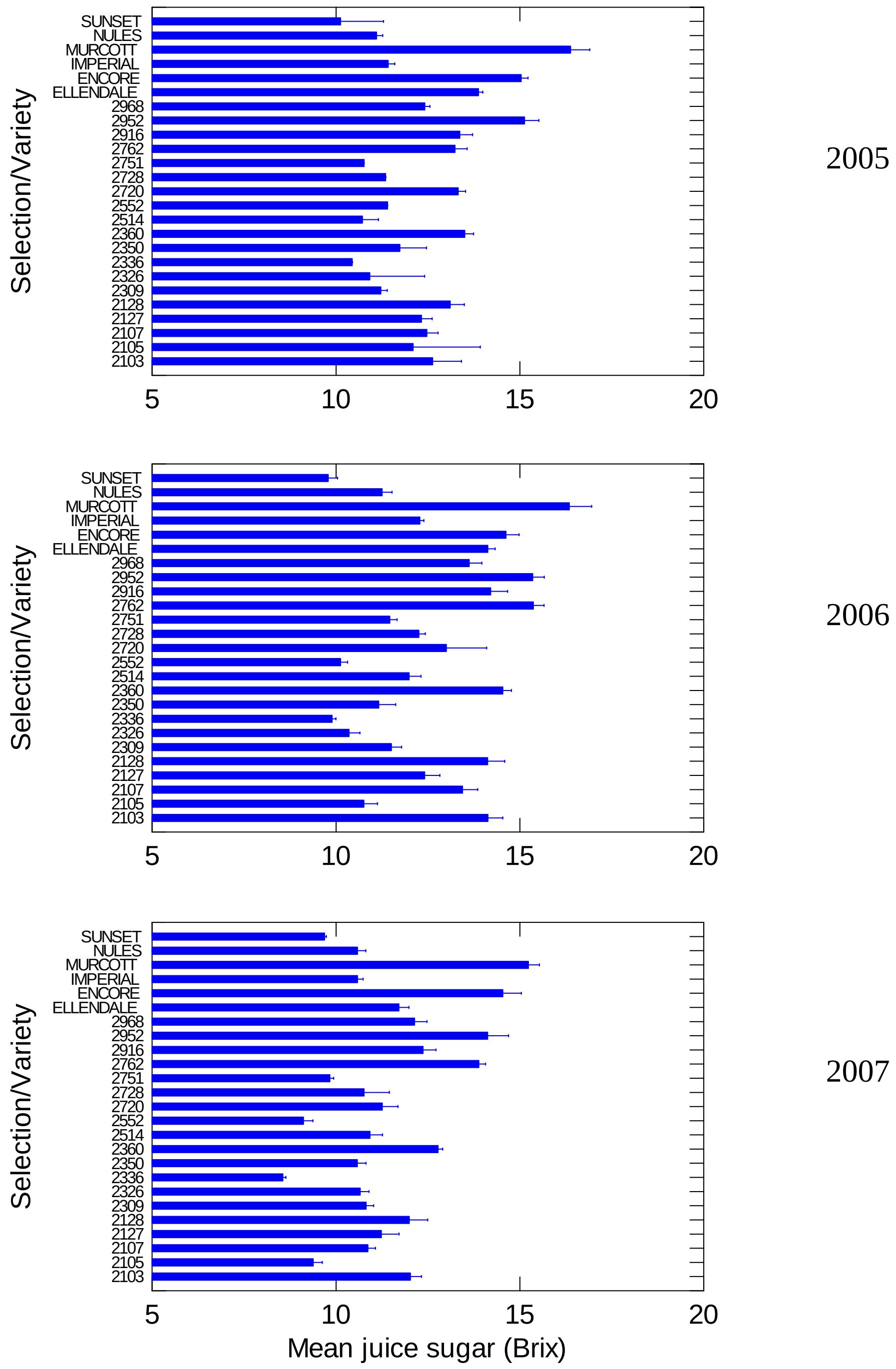


Figure 3.14. Mean (n=6) juice sugar:acid ratios(°Brix:% citric acid) for selections and common knowledge varieties in the comparative PBR trial.

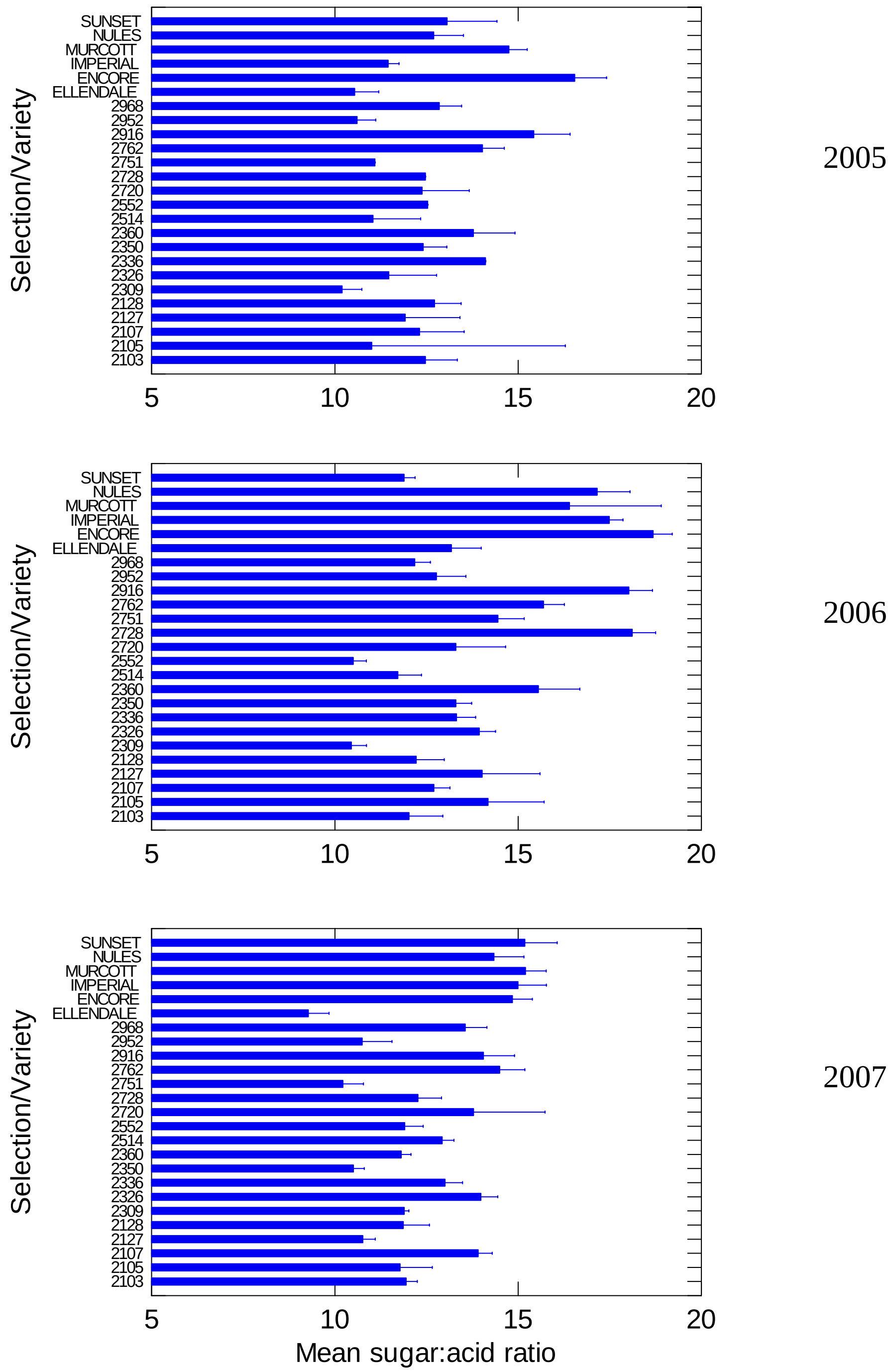
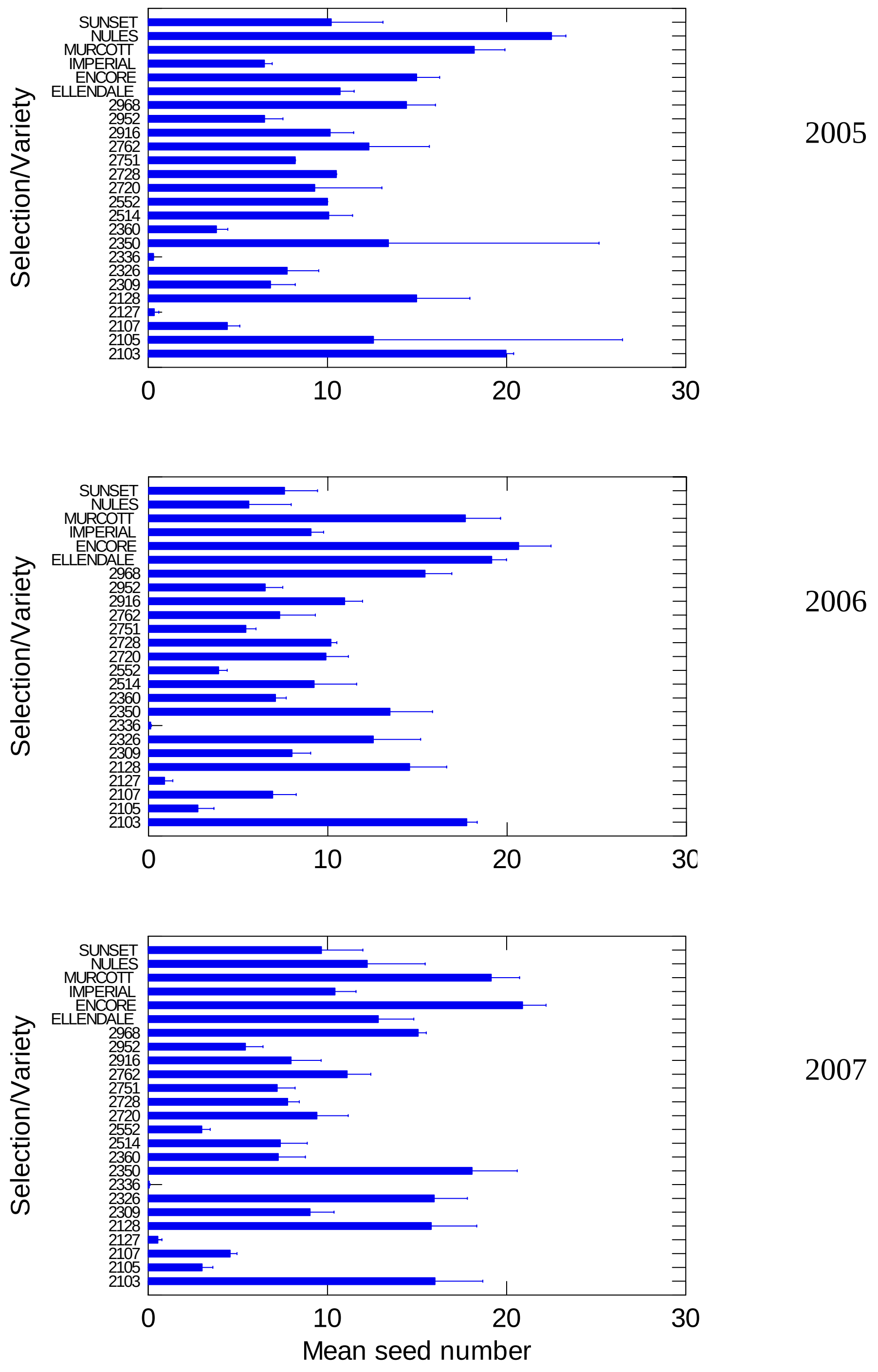


Figure 3.15. Mean seed numbers per fruit (n=6) for selections and common knowledge varieties in the comparative PBR trial.



selections 2336, 2552), but these fruits still developed good flavours due to an appropriate acidity giving a balanced sugar:acid ratio. At maturity all the selections had sugar:acid ratios exceeding the recognised minimum of 7.5:1 for mandarins.

Seediness
There were significant differences (P<0.001) for mean seed numbers between the genotypes in the trial (Figure 3.15) and as with other characteristics there were neither rootstock nor block effects.

The trial is situated within a progeny block at one of CSIRO's research farms. As such, the trees within the trial are surrounded by highly mixed and variable pollen sources. This was reflected in mean seed numbers, which were generally high, especially for some of the selections that had lower seed numbers in regional test plots where they were surrounded by plantings of pollen sterile varieties. Selections 2127 and 2336 stood out in that they had low seed numbers (<1/fruit) and many fruits were seedless. Selection 2552 also had low seed numbers, especially in 2005 and 2006, with a high proportion of fruits seedless. Of the common knowledge varieties, Imperial and Sunset fruits generally had the lowest numbers of seed with means in each year around 8-10. Nules clementine fruits had low seed numbers in 2006, which contrasted with those in 2005 and 2007. Murcott, Encore and to a lesser extent Ellendale had quite high seed numbers per fruit in each year.

Effect of copper sprays on seed numbers

Fruits were harvested from each side of the trees that were sprayed with copper sulphate and kept separate for analysis. Copper sprays had no effect on yields or fruit weights. Similarly, there was no effect on mean seed number, although in 10 scion/rootstock combinations there were less seeds in fruits harvested from the side of the tree that had received copper sprays (Table 3.6). This, however, contrasted with the 8 combinations that had more seeds per fruit.

From the report of Mesejo *et al.* (2006), it appears that copper has an inhibitory effect on the growth of the pollen tube providing the spray is applied before the pollen grain arrives at the stigma. It is possible that timing of sprays were ineffective with regard to pollen that had already been deposited on the stigma and had already germinated. When sprayed on Afourer trees during bloom, copper reduced seed numbers by around 50% although the mean for unsprayed trees was only 2.5 and 4.4 in consecutive years. The tests conducted by Mesejo *et al.* (2006) were also with trees in a commercial orchard where the availability of cross-pollen would have been greatly less than in the trial conducted here. It may be worthwhile to conduct this again in an orchard where there are less sources of mixed pollen. The semi-commercial planting that has been established with selection 2350 (see later) may present an opportunity to do so.

Table 3.6 The effect on mean seed numbers of $CuSO_4$ sprays applied during flowering.

Variety Rootstock	Mean ± SD seed nos.	
	+ copper spray ($25mg\ l^{-1}$ $CuSO_4$)	- copper spray
2103/SWO	15.6 ± 4.9	20.2 ± 5.7
2128/CC	11.5 ± 7.9	13.7 ± 5.5
2128/CLEO	12.6 ± 9.5	13.9 ± 7.4
2128/SWO	8.5 ± 4.6	7.0 ± 5.4
2350/CC	12.5 ± 4.9	11.3 ± 8.6
2350/SWO	14.2 ± 6.4	12.2 ± 5.7
2552/CC	4.8 ± 2.6	3.4 ± 0.5
2552/CLEO	4.1 ± 2.3	5.0 ± 3.2
2552/SWO	3.3 ± 2.3	3.8 ± 2.2
2952/CC	5.6 ± 4.0	7.9 ± 2.6
2952/CLEO	6.3 ± 2.8	5.2 ± 2.4
2952/SWO	7.7 ± 3.6	6.4 ± 2.7
Ellendale/CC	11.6 ± 7.0	14.6 ± 7.4
Ellendale/Cleo	14.2 ± 8.7	17.3 ± 9.0
Ellendale/SWO	13.0 ± 5.5	9.5 ± 4.8
Imperial/Carrizo	7.5 ± 4.2	8.5 ± 4.2
Imperial/Cleopatra	8.7 ± 4.2	8.6 ± 3.1
Imperial/SWO	8.1 ± 4.1	11.0 ± 3.8

3.4.3 Hybrid 88-09-28 – a potential grapefruit substitute

Details concerning hybrid 88-09-28 (Figure 3.16) were reported in previous final reports. During CT00012, ten daughter trees of this hybrid were established at one of CSIRO's farms for further evaluation before deciding if it should be entered into additional phase two trials. Hybrid 88-09-28 produces a grapefruit-type fruit which, because it is strongly parthenocarpic and sterile, are seedless in the absence of cross-pollination. Even under the conditions in which it has been planted at CSIRO, i.e. the breeding field where there are many sources of viable pollen, most of its fruits have been seedless.

As a grapefruit replacement, its fruits are sweet and once the acidity drops at the start of spring, its juice has a good balance with a pleasant flavour. This has been borne out at industry, public and marketer tastings, where response has been favourable. A very positive reaction was received from a fruit marketing company based in Sydney when fruits were supplied for tasting and comment. This same company presented fruits to Coles and Woolworths supermarket buyers who were also impressed with the quality and flavour of the samples they received. The performance of 88-09-28 over the last two seasons indicates that it should be entered into regional evaluation with grower assistance, especially in warmer regions.

Figure 3.16. Grapefruit-like fruits from a new hybrid selection (right) compared with similar of Marsh grapefruit (left). The seedless fruits of this hybrid have juice sugar concentrations of around 12° Brix and once their acidity drops to around 1.5% during September in the Murray Valley, they have a refreshing, pleasant flavour.

3.4.4 Fast-track and new regional trials

In CT00012, seventeen hybrid selections were identified after 1 or 2 seasons' data to have some potential. As a result, these selections were either propagated by budding to seedling rootstocks of Carrizo citrange, Cleopatra mandarin and Symons sweet orange, or top-worked to existing trees. The nursery-propagated trees were planted during spring 2004 and both trials have been maintained during CT04007 while evaluation of the original hybrids continued. These trials commenced flowering during 2006 and analysis of early data from these plus the additional data from the original own-rooted seedling trees has led to seven of the selections being propagated for entry into regional evaluation test-plots with grower cooperators.

For entry into grower-based test-plots, trees of each selection were budded to each of the three rootstocks listed above ready for distribution. New cooperators were sought at a series of Cittgroup meetings held over winter 2006. Letters seeking expressions of interest were distributed to growers who indicated that they may be able to assist with the evaluation, and also to growers who were already part of the network involved with testing the ten Imperial x Ellendale hybrids distributed in CT00012. This resulted in a number of new growers joining the network and after signing the testing agreement, which was reviewed and updated during 2006, nursery propagated trees or budwood of the seven selections have been distributed during spring 2007. Due to the water situation in the Murray Valley, some growers have indicated that they will receive trees in 2008, rather than in 2007.

The characteristics of the selections were detailed in a booklet, which was provided to growers who expressed an interest in cooperating with their further evaluation. These details are summarised in Table 3.17.

Table 3.17. Characteristics of the seven new hybrid selections established under testing agreements with grower cooperators for regional testing.

	88-13-23	**88-14-03**	**88-14-18**	**88-18-02**	**92-01-23**	**91-03-04**	**91-03-09**
							
harvest date	May - June	August/September	August	July – August	Aug – September	March – April	May - June
yield (kg)	26.3 – 97.7	6.5 – 50.4	2.8 – 15.5	7.4 – 15.7	6.9 - 66.8	2.8 – 26.6	2.5 – 16.7
colour	7.6 ± 0.5	9.5 ± 0.7	9.0 ± 1.4	8.5 ± 0.7	10.6 ± 0.7	7.1 ± 2.0	10.0 ± 1.2
easy-peel	Yes	Yes	Yes	Yes	Yes	Yes	Yes
surface texture	Smooth/pebbled	Smooth/pebbled	Smooth/pebbled	Pebbled	Pebbled	Smooth/grainy	Pebbled/grainy
mean fruit diameter (mm)	58.4 ± 4.2	48.7 ± 5.4	65.4 ± 3.8	62.6 ± 4.7	63.9 ± 5.9	44.8 ± 4.4	53.8 ± 9.1
mean fruit weight (g)	89.9 ± 12.9	64.9 ± 18.2	129.7 ± 16.1	102.3 ± 13.7	110.4 ± 25.5	49.1 ± 12.1	70.6 ± 29.7
% juice	31.6	32.3	33.3	26.3	37.5	31	39.1
rind thickness (mm)	3.1 ± 0.6	4.2 ± 1.3	4.7 ± 1.2	5.0 ± 0.6	3.8 ± 0.8	2.5 ± 0.5	3.2 ± 0.4
open-pollinated seed number	1.5 ± 1.3 (range 0 – 5)	5.2 ± 2.6 (range 0 – 13)	8.9 ± 3.7 (range 4 – 23)	8.4 ± 4.6 (range 0 – 18)	7.9 ± 6.3 (range 0 – 20)	4.0 ± 3.9 (range 0 – 16)	2.2 ± 3.1 (range 0 – 13)
juice °brix	11.2 ± 1.0	12.2 ± 1.6	11.5 ± 0.1	12.7 ± 1.0	13.6 ± 1.6	11.5 ± 1.3	12.3 ± 0.6
juice acid (%)	0.7 ± 0.3	1.4 ± 0.2	1.2 ± 0.1	1.1 ± 0.2	1.3 ± 0.1	0.9 ± 0.1	1.0 ± 0.1
brix:acid	15.2	8.9	9.9	11.8	10.2	11.1 – 14.9	12.4
Auto-parthenocarpic	Yes	Yes	No evidence	Yes	Yes	Yes	Yes
self-incompatibility	Yes – self-pollination has given seedless fruits	Yes – self-pollination has given seedless fruits	Yes – self-pollination has given seedless fruits	Yes – self-pollination has given seedless fruits	No	Yes – self-pollination has given seedless fruits	Yes – self-pollination has given seedless fruits

3.4.5 Concluding comments concerning the second phase evaluation trials and test-plots

Several important comments can be made concerning the second phase evaluation trials reported here. First, the establishment of a network of cooperating growers has proved an invaluable asset to the project. Not only does this approach provide valuable in-kind resources to the project, it enables selections to be tested under a range of differing conditions and management styles, which can have advantages and disadvantages as already outlined. From these test-plots maintained by growers, selections have been identified for release as new varieties (Merbeingold 2336 and 2350), while others have shown potential, but require additional work. Thus, selection 2127 needs to be evaluated further using plant growth regulator sprays to slow rind aging and improve its external quality.

Similarly, data from the trials so far, along with comments received from industry when fruits have been displayed, indicate that if the seediness of four selections, namely 2103, 2128, 2552 and 2762, could be reduced or indeed eliminated, then they would warrant nomination for release. These four selections are all strongly parthenocarpic and, as discussed later in the chapter concerning mutation breeding, they are candidates for entry into an irradiation program similar to that conducted at Merbein from which two seedless lines of Kara mandarin have been developed. A new mutation program will commence with these selection during the next project.

Bud-line age and the development of mature as opposed to juvenile buds have also been highlighted in the trials conducted with the selections from the Imperial x Ellendale progeny. As a result, two daughter trees are being propagated as soon as a new hybrid appears to have some potential so that mature grand-daughter buds with a greater propensity for precocity will be used to propagate trees for distribution to grower cooperators.

3.5 Release and conmercialisation activities

3.5.1 Domestic release and commercialisation of Merbeingold varieties

During project CT04007, a nomination was made via the CSBRC to release three varieties from the diploid hybridisation component of the breeding program, This decision was made in late 2005 after two years' data had been collected for the nominated selections from regional evaluation plots in Sunraysia, Riverland and the MIA; after fruits from the selections had been displayed to growers in Sunraysia; after positive feedback had been received from growers at meetings when the fruit had been displayed; and after a meeting was held with fruit marketers from three major citrus packers.

At the meeting with marketers, the representatives of the three companies suggested that while the strategy for commercialisation of the varieties was initiated and executed, a semi-commercial planting of each of the varieties should be established so that when the varieties are finally released, larger volumes of fruit would be available to conduct test market shipments. Accordingly, three top-worked plantings were established for each of the selections during late 2005. As there were additional IP complications with establishing such trials, it was necessary to formulate a new testing agreement that would take into account changing events with selections as they progressed through the commercialisation process.

In setting the commercialisation strategy in motion, the ACG board and the Citrus IAC have been kept fully informed via seminars, informal meetings, visits to Merbein and documentation. The decision made in November 2005 to nominate three selections for commercial release was communicated to the wider industry during the 2006 Annual ACG Conference in Western Australia and via an article in the Australian Citrus News (Sykes, 2006; in which details concerning the varieties and information on the commercialisation strategy were presented). This was followed up with a series of articles in other industry publications and local industry briefings held during winter 2006 as part of a communication plan developed in collaboration with the ACG communications manager, Lee Byrne.

In developing the strategy for release and commercialisation of varieties from the breeding program, a key consideration was the direction received during a meeting involving the Citrus IAC and researchers from organisations involved in breeding, introduction and evaluation at DPI NSW, Dareton (2004), that the industry was neither willing nor in a position to fund large-scale, exhaustive variety evaluation trials of new varieties, be they from the introduction or breeding initiatives they were supporting. The message delivered was that the industry would prefer to have new varieties made available as soon as possible for growers to plant and thus absorb some of the risks associated with the successful adoption of new varieties.

The decision in November 2005 set in motion the strategy that had been developed in conjunction with the CSBRC for the release of varieties from the program. This strategy has been outlined in the breeding plan, communicated to industry during project CT00012 and will not be elaborated on any further here. The following, however, provides a summary of activities conducted since the decision to nominate the three selections for release was made. (The activities undertaken have also been reported in project milestone reports.)

- Establishment of the Citrus Scion Breeding Reference Committee (CSBRC) in March 2000 – the committee has been a key resource in developing the commercialisation strategy as a consultative process.
- Series of industry briefings in the major production regions to outline and receive feedback on the breeding plan and commercialisation strategy (Aug – Oct 2003).
- The Breeding Plan, including the commercialisation strategy, was submitted to IAC (2004).
- Article in Australian Citrus News outlining breeding plan and commercialisation strategy (Feb/March 2004).
- Meeting with marketers from three major citrus packers to view the three selections with potential for release as well as to discuss the release and commercialisation strategy (July 2005).
- Meeting with IAC to provide information concerning progress, outline the commercialisation strategy, and receive feedback (Sept 2005).
- Meeting with IAC/ACG to provide greater detail of commercialisation strategy and receive feedback (November 2005).

- Based on the performance of trees propagated with the selections in grower test-plots in the Riverland, Sunraysia and MIA regions in 2004 and '05, three selections nominated for release (November 2005); contract with Auscitrus for bud multiplication signed – (November 2005).
- Imminent release of Merbeingold 2336 and 2350 announced at the ACG conference (March 2006). A decision was made to hold back on the release of selection 2127 because of rind quality problems.
- Expressions of interest for a commercialiser sought (April 2006).
- Australian Citrus News article published announcing selections for release and outlining the commercialisation strategy (April/May 2006).
- Expressions of interest from commercialisers received. CSBRC sub-committee formed to review expressions of interest and recommend a short list to the CSBRC – June 2006.
- Sub-committee meeting with short-listed commercialisers to progress the selection of successful applicant (26/7/06).
- Selection of the commercialiser continued as an exercise involving the full CSBRC.
- Decision to advise successful applicant made December 22, 2006.
- February 8 2007, selected applicant withdrew.
- Meeting of CSBRC February 14, 2007 agreed to re-open negotiations with second choice applicant. February 17, 2007 face-to-face meeting with second choice applicant. Negotiations to continue as a matter of urgency.
- Negotiations continued with second choice applicant from February 2007. These negotiations progressed to the formulation of a terms sheet, which led to further lengthy negotiations culminating in an acceptance of mutually agreed modified terms in December 2007.
- As of December 12, 2007 contractual arrangements are being finalised by respective legal teams.

During all phases of this process the members of the CSBRC has been kept fully informed of developments and the collective wisdom of this committee has been sought in making decisions. During the negotiations held for most of 2007, the committee has been involved in many telephone conferences as well as in the case of some members, face-to-face meetings. The project team at CSIRO expresses its gratitude to the members of the committee.

3.5.2 International activities

Activities in the area of international commercialisation have been detailed in milestone reports.

With the decision to release the Merbeingold varieties for domestic use, the CSBRC agreed that a strategy should be developed for international release and commercialisation of varieties from the breeding program. During the course of the breeding program, interest in potential varieties from the program has developed from variety managers, nurseries and industries based in other countries. Information concerning the diploid hybridisation component of the breeding program and promising selections has been shared with international visitors to Merbein and during overseas visits by the principal investigator at Merbein. The announcement made concerning the Merbeingold varieties during 2006 stimulated increased interest from other countries.

As a result of this increased interest in the varieties and activities of the breeding program, a sub-committee of CSBRC was formed in July 2006 to progress international commercialisation of varieties. A draft strategy was developed and shared with other members of the committee for review. This strategy remains as a working document and activities will be stepped up in this area during the next project.

One important area for consideration, however, relates to cost resourcing for the release and commercialisation of new varieties. Costs involved in commercialisation activities have been met largely by the project budget, for example health status indexing costs incurred by Auscitrus. However, as the budget became tighter and activities intensified during the course of CT04007, the CSBRC identified the need to explore alternative innovative options to resource these activities. This was seen as being particularly important for the international release and commercialisation strategy. Notwithstanding innovative approaches to secure additional funding, however, it has become clear, as raised by one of the industry representatives on the CSBRC, that in reality the commercialisation process reached a stage whereby costs could not be met by the budget drawn up for CT04007. Solutions to this predicament were adopted as a challenge for the CSBRC with a number of avenues being pursued, especially by the industry members of the committee. Attempts, however, to obtain extra funding from outside of the project by committee members were unsuccessful and remains an exercise to be tackled during the next project.

3.6 Extension and information delivery to industry

Information concerning progress and developments in the diploid hybridisation component of the project has been extended to industry via the media (print and radio) and face-to-face briefings meetings, including Cittgroup meetings. These have been detailed in milestone reports and mentioned in the preceding sections of this chapter.

Information concerning the project has also been extended internationally via visitors at Merbein and also during visits made by Dr Sykes to Spain in 2005 and to the PR of China in 2006 to deliver presentations on the citrus breeding research in Australia.

3.7 References

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4. Triploid hybridisation

4.1 Introduction

Triploid breeding is the principal project activity conducted by DPI&F at Bundaberg Research Station (BRS). Since the late 1990s there has been significant effort in generating large progeny blocks from which high-quality fruit selections can be made. Importantly, the triploid program has now reached the stage where material spans almost the whole breeding cycle, from generating new hybrids through to commercial production of varieties produced at BRS. The following sections examine project activities over the last three years for the various stages of the breeding cycle, including the Crossing program; Assessment of fruiting hybrids; Families that have produced fruit; Stage One selections; Benchmarking selections; and Commercial adoption of new triploid mandarins.

4.2 Crossing Program

4.2.1 Materials and methods

Citrus breeding is a long-term activity. Many years elapse between when the initial pollination is carried out and when the resulting hybrid is selected for commercial production. Therefore it is essential to carefully document breeding activities so that others can accurately review the breeding program and where necessary carry it forward. With a three or five year funding cycle it is not possible to assess a project's merit based on varieties released, but it is possible to estimate merit based on likely future releases from the crossing conducted during the project period. Consequently all pollinations/embryo rescues/field-plantings that occurred in each of the three project years 2004-2006 are detailed in the extensive tables found in the Appendix.

4.2.2 Results and discussion

From Tables 4.1a-c it can be seen that 10,925 pollinations were carried out in the three year period with overall fruit set in each year ranging from 23 to 36%. Arrufatina consistently had high fruit set (a high 64% in 2004) while Daisy was generally one of the poorest (a low of 12% in 2005). Twelve tetraploid pollen parents were used in 2004 with only four used in 2005. The 2006 pollinating season saw the reintroduction of tetraploid oranges to the crossing program – aimed at developing hybrids suitable as orange substitutes. It can also be seen that the tetraploid pomelos were not used in the three years of this project. This is because there are already substantial numbers of hybrids from these parents growing at BRS awaiting first fruiting and assessment.

Large numbers of seeds were sown and embryos rescued in each year of the project. Almost four thousand seeds from 4X x 2X have been directly sown, and an additional 30,050 embryos rescued. Clementine's (including Arrufatina, AustClem, Corsica 1&2 and DeNules) have been important parents in the program and collectively make up a large proportion of the hybrids that have been generated. Efforts have also been made to generate large families with Imperial and Ellendale parentage. For example from 2004 pollinations almost 500 Imperial seeds were direct-sown and more than 1,000 embryos rescued. Thus the program has been able to:

1. utilise parents with high fruit quality
2. include parents with known parthenocarpic ability
3. generate large numbers of hybrids from which to make selections.

From the tables in the Appendix it can be seen that old pollen (more than 12 months in storage) was utilised in 2005 and 2006. This was necessary because flowering times of different varieties often do not overlap and some pollen parents start flowering when the desired seed parent variety has finished flowering. Fresh and stored (12 months) pollen of tetraploid Murcott was used in 2005 and 2006. It can be seen that the percentage fruit set using fresh and stored pollen was very similar. For example in 2005 (Table 4.1b) fruit set on AustClem was 22% with fresh pollen and 15% with stored pollen, and fruit set on Ellendale was 13% with fresh pollen and 22% with stored pollen. Similarly in the 2006 season (Table 4.1c) fruit set on three seed parents (AustClem, Fina, Imperial) for fresh tetraploid Murcott pollen was 26%, 40% and 30% respectively, while for the same seed parents the fruit set with stored pollen was 37%, 43, and 22% respectively. Similar results were obtained with stored tetraploid Excelsior pollen in 2006. Therefore it might reasonably be concluded that pollen can be stored for 12 months and used without any significant reduction in fruit set.

However, the situation in relation to seed production from stored pollen is very different. Fruit resulting from pollinations using stored pollen contain significantly less seed, both in terms of plump seed and flat seeds (used to extract embryos). For example in 2006 (Table 4.1c, 4.2c), 20 pollinations with fresh tetraploid Murcott pollen on Fina resulted in five plump seeds and 118 rescued embryos, but four times as many pollinations with stored Murcott pollen resulted in only four plump seeds and 68 rescued embryos. Consequently the preference in the breeding program is always to use fresh pollen wherever possible.

4.3 Assessment of Fruiting Hybrids

4.3.1 Material and Methods

Hybrids resulting from the triploid breeding work have been field planted on three trellis systems at BRS. Planting on Trellis One started in March 2001 and the latest planting on Trellis Three occurred in May 2005. Hybrids are established as a single row at high density (4m between rows, 0.3-0.5m within row). Detail on the trellis system is contained in the Final Report for CT00012. During the period of this current project some hybrids on both Trellis One and Trellis Two commenced fruiting and were assessed (hybrids on Trellis Three are still too young to fruit). As hybrids flowered and fruited they were identified and assessed. During the harvest season trees were inspected at regular intervals to account for potential variations in fruit maturity times.

When fruiting hybrids were considered ready for harvest, fruit was picked and assessed in the field to determine whether the hybrid should be:
1. culled
2. selected or
3. re-assessed next season.

A system of colour paint spots on the trunk and pin-tags was used to record the assessment decision. For example trees with poor quality fruit were red-spotted on the trunk. Trees that fruited in a particular year but had insufficient fruit to make a select/cull decision were blue-spotted. This made it possible in subsequent seasons to recognise trees that had fruit previously and to know how the hybrid had performed.

Hybrids with outstanding fruit quality were selected at the time of in-field fruit assessment. A physical description was made for each of these selections detailing characteristics of commercial significance as well as factors important to the breeding program. These descriptions cover characteristics such as: age at first fruiting, fruit size, shape, skin texture, ease of peeling, external and internal colour, seediness, Brix, taste, commercial applicability and an ‘overall acceptance’ rating.

4.3.2 Results and Discussion

The above system is proving an efficient method of assessing a large number of hybrids with limited resources. However, while it is consistent with the breeding principle of “spending the most effort with the best genetic material”, it has significant limitations in terms of generating publishable objective information on the breeding program.

Table 4.3a shows the number of hybrids that fruited (and were assessed) for the first time in each project year. There is a general trend of increasing numbers of new hybrids fruiting each season and this trend will continue as the trees mature. Because all trees are retained on the trellis, many hybrids have now been assessed for more than one season. For example in 2007, although only 280 new hybrids were assessed, the total number of hybrids assessed was actually closer to 600 because many of the hybrids that had first fruited in 2005 and 2006 were also fruiting (and therefore re-examined) in 2007.

Table 4.3a: Number of hybrids fruiting for the first time in 2005 to 2007 seasons, for each of two trellises.

	2005	2006	2007	Total No.	% of Trees
Trellis One	56+14	152	128	350	26.6
Trellis Two	48	65	152	265	11.8
Total	118	217	280	615	17.3

To date, some 615 hybrids have been assessed, representing 17.3% of the trees that are present on these two trellises.

Despite the large numbers of hybrids assessed each season, most were totally unsuitable for commercial production (based on fruit quality) and have been marked for culling. The principal reason for culling each of these hybrids has been recorded and is shown in Table 4.3b.

Table 4.3b: The principal reason for culling 493 hybrids from two trellises at BRS from 2005 to 2007.

Principal Fault	Trellis One Culls	Trellis Two Culls	Total No.	% of Trees
Rough skin texture	114	56	170	34.5
Small size	113	32	145	29.4
Poor external colour	19	19	38	7.7
Too acid	13	14	27	5.5
Bad shape	6	19	25	5.1
Soft/puffy	17	7	24	4.9
Seedy	11	12	23	4.7
Odd smell/taste	13	6	19	3.9
Thick rind	8	4	12	2.4
Granulation	6	2	8	1.6
Low Brix	1	1	2	0.4
Total	321	172	493	100

It can be seen that more than half of the hybrids were culled for just two fruit characteristics – skin texture and fruit size. However it must be remembered that this is the ‘principal fault’ and not necessarily the only fault.

Because of the large number of hybrids that have now been assessed in more than one season, and because the principal fault has been recorded for each cull, it has been possible to formulate some understanding of the consistency of characteristics of newly fruiting hybrids from one season to the next. For the most part faults are consistent each season such that a hybrid with say rough skin in 2005 also showed this characteristic as the principal fault in 2006 and/or 2007. Another advantage of recording the principal fault for each cull is that in a small percentage of cases trees that have been marked for culling in one season go on to produce promising looking fruit in subsequent seasons. By having some understanding of the reason for suggesting culling it has been possible to make better judgments of whether hybrids subsequently warrant selection.

Triploid breeding is aimed at generating citrus hybrids that produce seedless fruit. Consequently all fruiting hybrids have, where possible, been assessed for seediness. This involves counting both the number of plump seeds and the number of flat seeds in a fruit. Table 4.3c shows the number of hybrids and their level of seediness. This table is based on the number of plump seeds and not on the number of flat seeds.

It seems clear from Table 4.3c that the triploid breeding approach is proving successful in generating a large proportion of hybrids with little or no seed. This is occurring without any screening for ploidy level prior to planting – all hybrids obtained are field planted because a flow cytometer is not available to measure ploidy. More than half of the hybrids that have been assessed to date have less than three plump seeds and around a third have no plump seeds. Of the 177 hybrids with no plump seeds, 61 were also completely free of flat seeds (12% of the population).

Table 4.3c: Seediness of 494 hybrids grown on two trellises at BRS 2005-2007.

Plump Seeds (per fruit)	Trellis One	Trellis Two	Total No.	% of Trees
0	104	73	177	35.8
1	41	35	76	15.4
2	50	25	75	15.2
3	29	12	41	8.3
4	19	7	26	5.3
5	17	5	22	4.5
6	7	3	10	2.0
7	7	3	10	2.0
8	4	1	5	1.0
9	2	0	2	0.4
10	5	5	10	2.0
11	5	1	6	1.2
12	3	0	3	0.6
13	1	0	1	0.2
>13	23	7	30	6.1
Total	317	177	494	100

While this seed data is a promising result from the breeding program, caution is needed once high quality selections have been made. The seed data above is generally based on just a single piece of fruit in a single season, and to confirm the seediness of a selection would require an assessment of far larger numbers of fruit and to do this in multiple seasons. The intention of the triploid program is to generate hybrids that are productive as well as seedless (or nearly so) under intense pollination pressure. This requires more detailed investigation than the initial screening attempt described above.

4.4 Families that have produced fruit

4.4.1 Materials and methods

By the end of the 2007 season, some 72 different parental combinations (families) had produced at least one fruiting hybrid. As the number of fruiting hybrids in a family increases, it becomes possible to form an opinion of the value of the parents being used.

4.4.2 Results and discussion

Table 4.4 shows some of the families that have to date produced a reasonable number of fruiting hybrids. It also shows the percentage of the family that has fruited (not including Trellis Three), and those combinations that have resulted in Stage One selections (see section 4.5).

Table 4.4: Numbers of fruiting hybrids from different parental combinations. Figures in **bold** are the number of hybrids that have been selected, while figures in *italics* are the percentage of the family that has fruited to date. For Trellis One and Two, BRS.

Female Parent (seed)	Male Parent (pollen)						
	4X Joppa	4X Parra	4X Emperor	4X Murcott	4X PomeloA	4X PomeloB	Total
AustClem	17 *44%*	21 *37%*	11 *21%*	12 **2** *44%*	2 **1** *20%*	2 **1** *20%*	65 **4** *33%*
DeNules	11 *27%*	4 *17%*	83 **2** *31%*	25 **3** *35%*	0 28	0 27	123 **5** *27%*
OtherClems	31 *24%*	7 *7%*	5 *13%*	5 *50%*	0 81	0 30	48 **0** *13%*
Ellendale	14 **1** *44%*	4 *44%*	5 *14%*	5 **1** *18%*	0 3	0 7	28 **2** *24%*
IM111	14 **1** *47%*	1 *20%*	20 *7%*	1 *2%*	3 *5%*	1 *3%*	40 **1** *9%*
Imperial	6 *33%*	2 *13%*	8 *4%*	3 *13%*	6 *20%*	8 *13%*	33 **0** *10%*
Wilking	16 *20%*	11 *17%*	56 *23%*	19 **1** *29%*	3 *4%*	0 63	105 **1** 18%
Total	109 **2** *30%*	50 **0** *19%*	188 **2** *17%*	70 **7** *25%*	14 **1** *5%*	11 **1** *5%*	442 **13** *17%*

From Table 4.4 it can be seen that only 17% of the hybrids on Trellis One and Two have fruited to date and from these 442 fruiting trees, 13 have been selected. There is a strong tendency for hybrids with tetraploid Murcott to be selected (seven selected out of 70 assessed) and the converse is true for tetraploid Joppa where only two selections have been made from 109 hybrids assessed. Similarly with seed parents, AustClem has produced four selections from 65 trees assessed, while only one selection has been made from 105 Wilking hybrids. It is disappointing that no selections have yet been made with Imperial (although only 33 hybrids have been assessed, representing just 10% of the populations on these two trellises). Hybrids with Imperial (assessed to date) have tended to be small and puffy, which is a similar problem to that seen with tetraploid Emperor. The tetraploid pomelos (A and B) have produced hybrids that are slow to fruit with only 5% of trees having fruited to date. Similarly, Imperial has produced hybrids that are a little slower to start fruiting than other seed parents.

4.5 Stage One selections

4.5.1 Materials and methods

A number of fruiting hybrids were recognised during the three years of this project as being of potential commercial merit and have been propagated for more detailed examination. These initial selections are classified as 'Stage One' selections and two trees of each are propagated onto conventional citrus rootstocks and grown under normal orchard conditions. Stage One selections are grown within the high-security compound at BRS to allow detailed fruit quality assessment without the risk of genetic material being stolen.

4.5.2 Results and discussion

Table 4.4 above identifies 13 selections made from different parental combinations during the course of the project. In addition to these 13 selections a further three selections have been made using tetraploid PomeloB as a seed parent (not shown in the table because of space restrictions). These three selections are hybrids with Daisy, Fremont and Murcott. Table 4.5 gives a description of the characteristics of all 16 hybrids, as determined at the time they were first identified as having potential and warranting further assessment. These hybrids have been propagated and will be grown in the high security compound for more detailed assessment. Experience from the conventional breeding program at BRS (non-HAL funded) suggests that another round of culling once these Stage One selections recommence fruiting will be necessary in order to identify genotypes that are consistently outstanding.

These Stage One selections (Table 4.5) have been made for a number of different reasons, consistent with the objectives of the project. Two of the selections (06N001 & 07N003) have strong orange-like characteristics and may prove suitable for the orange industry. Not surprisingly they have a tetraploid orange as one of their parents, and these characteristics are strongly evident in most hybrids. The tetraploid oranges Joppa and Parramatta were used extensively in the pollinating program in the late 1990s but then used infrequently in the last few seasons (instead favouring some of the newly available mandarin-type tetraploids). Now that the merit of these tetraploid orange parents is known, along with the better seed parents to cross them with, they have been reintroduced into the hybridisation program.

Similarly, hybrids that have tetraploid pomelo as a parent have very strong pomelo characteristics. Indeed it is surprising how little the mandarin parent has influenced the overall characteristics of these hybrids. For example 07N006 is a hybrid between the distinctly different parent 4X PomeloB and Fremont, and yet this hybrid displays few characteristics associated with Fremont. This is consistent with previous experience in breeding citrus at Bundaberg where often one parent dominates the characteristics of hybrids, rather than having hybrids with intermediate characteristics.

Most of the 16 selections made in 2006 and 2007 are intended for the mandarin segment of the Australian citrus industry. On occasions, some of these selections have appeared very promising, but the fruit characteristics need to be assessed across a number of seasons. Other problems might also hold-back these selections, despite excellent fruit quality. For example 06N007 produced fruit with excellent appearance and taste and about three seeds during the 2006 season, but seemed to be considerably seedier in 2007. Furthermore this selection is extremely thorny and in its present form would be totally unsuitable for commercial production. Citrus hybrids become less thorny as they mature and pass through additional generations of vegetative propagation – but it remains to be seen how effective this will be on hybrids that start out as thorny as 06N007.

Table 4.5: Parentage and fruit characteristics of 16 hybrids selected in 2006 and 2007 from the triploid breeding program BRS. Characteristics determined at the time of selection. All hybrids grown on their own roots at high density.

Code	Seed Parent	Pollen Parent	Size	Shape	Ext.Colour	Int.Colour	SkinTexture	Brix	Plump seeds	Flat seeds	Priority 1=high	Comments
06N001	IM111	4XJoppa	v.large	round	orange	orange	moderate	10	4	4	2	orange substitute
06N002	AustClem	4XPomB	Small/medium	Imperial	orange	orange/red	moderate	12	12	0	1	poor in 2007
06N003	AustClem	4XMurcott	Small	oblate	bright red	orange	smooth/shiny	13	4	0	2	bit soft?
06N004	Ellendale	4XMurcott	Large	Murcott	orange/red	deep orange	smooth	13	2	0	?	v.thorny
06N005	Wilking	4XMurcott	medium	Murcott	orange/red	orange/red	smooth	13	2	1	2	tastes good
06N006	DeNules	4XMurcott	medium	Murcott	red/orange	deep orange	moderate/smooth	13.5	4	0	1	v.good taste
06N007	DeNules	4XMurcott	medium	flat	red	deep orange	smooth	15	3	1	1	rich taste
06N008	AustClem	4XMurcott	medium	Murcott	deep red	deep orange	moderate/smooth	12.5	3	2	1.5	early colour
07N001	DeNules	4XMurcott	Large	Murcott	deep orange	deep orange	smooth/shiny	11	5	0	?	big crop
07N002	AustClem	4XPomA	not described									pomelo & mandarin
07N003	Ellendale	4XJoppa	Large	round	yellow	yellow/orange	moderate/coarse	11	0	2	2	orange substitute
07N004	DeNules	4XEmperor	medium	flat	orange	orange	moderate/coarse	12	0	1	3	taste okay
07N005	DeNules	4XEmperor	Small	Imperial	yellow/orange	orange	smooth	13.5	2	0	1	late Imperial
07N006	4XPomB	Fremont	Large	round	golden	yellow	moderate/coarse	11.5	5	0	?	tastes good
07N007	4XPomB	Murcott	Large	round	yellow	yellow	smooth	11.5	?	?	?	taste okay
07N008	4XPomB	Daisy	v.large	ovoid	smooth	yellow	yellow	12	4	7	?	good texture flesh

4.6 Benchmarking selections

4.6.1 Materials and methods

Europe is recognised as the main market currently demanding seedless mandarins, and it is the Spanish citrus industry that has been most successful in meeting this demand. Consequently when a group of leading nurserymen and variety managers from Spain visited BRS September 2006 the opportunity was used to benchmark some of the selections from the breeding program and selection criteria, with their detailed understanding of the expectations of the European market. The procedure used was to present the panel of Spanish visitors with a range of fruit from the breeding program and arboretum collection and to then ask them to assess and rate each sample. From this it was possible to determine the relative importance of different characters, and the extent to which certain faults limit the potential of existing selections.

4.6.2 Results and discussion

The visiting nurserymen were enthusiastic participants in the process and provided valuable input to the breeding program. They spent considerable time discussing the various selections and were clear in their view of the relative merits of each sample of fruit. Such enthusiasm and thoroughness is not surprising given the importance of mandarins to their livelihood, their many years of commitment to citriculture and the enormous scale of citrus nursery production that they practise. They recognise the role of new varieties in keeping their industry vibrant and competitive.

Figure 4.6: Spanish citrus nurserymen and variety managers conduct a benchmarking exercise at BRS to align early stage selections from the breeding program with European market requirements, September 2006.

Although it was late in the season and fruit were past their prime, the panel were impressed with selections 06N006 and 06N007. They were only interested in ‘outstanding’ fruit, that is, varieties had to have excellent appearance and taste, as well as being easy to peel and seedless.

4.7 Commercial adoption of new triploid mandarins

4.7.1 Materials and methods

Australia's first commercial planting of a triploid mandarin occurred on a property in Gayndah in early 2007. The variety 'EL3' was bred at BRS in the early-1990s prior to the commencement of HAL support for the Queensland breeding program, and was subject to semi-commercial testing at Gayndah and Mundubbera from 1998 to 2002. As a result, two growers approached QDPI in June 2005 for permission to produce the variety on a commercial scale.

4.7.2 Results and discussion

Growers believe that this triploid hybrid will have significant market appeal in its attractive colour, ease of peeling, sweet taste and seedlessness. However the breeder has some concerns with skin texture and fruit firmness and consequently a limit has been placed on the number of trees that can be grown, until the variety has performed satisfactorily throughout the supply-chain. The variety has been established in commercial plantings of new nursery trees as well as top-working an existing block of Hickson mandarin. It is expected that the first commercial supply of fruit from this new triploid variety will occur in the 2009 season.

Although this variety is not part of the HAL sponsored program, it is the first product from the triploid breeding work at BRS to enter commercial production. It is also the first triploid mandarin to be grown commercially in Australia. As such it demonstrates that Australian citrus breeding is producing varieties that growers recognise as the best available and that will meet changing market requirements.

4.8 Tables referred to in triploid hybridisation chapter

See next page.

Table 4.1a Number of pollinations, and percentage fruit set, for crosses performed in the 2004 season, BRS.

Female	Male Parent (pollen)																													
Parent	4XWilking x Murcott4X 83		4X Fremont		4X Emperor No1		4X Emperor No2		4X Murcott		4XWilking x Murcott4X 95		4XWilking x Murcott4X 96		4X Minneola		4X Burgess		4X Orlando No1		4X Orlando No2		4X Excelsior		4X Dancy		4X Bakers Sweet		Total	
(seed)	No.	%set	No.	%set	No.	%set	No.	%set	No.	%set	No.	%set	No.	%set	No.	%set	No.	%set	No.	%set	No.	%set	No.	%set	No.	%set	No.	%set	No.	%set
Arrufatina	25	76	14	50			30	87	30	67	30	73	30	77	24	83	30	100			40	40	10	50					263	63.91
Aust.Clem	20	45	30	17	35	57			30	87	30	23	30	67	30	20	30	40			25	16							260	37.2
Corsica1	30	10	30	20	30	7			25	12	30	10	30	70	30	60	30	30											235	24.33
Daisy	30	7	27	26			30	47	30	27	30	23	30	33	30	43	30	40			30	0	30	27	5	20	30	0	332	22.54
DeNules			30	3			30	33			30	20	45	64	30	7	30	33			25	0							220	20
Ellendale	14	50	22	41	30	17			30	40	30	30	30	57	30	20	30	47			30	13	20	20			30	7	296	28.5
Encore	20	10	20	30	10	10	20	35	30	13	30	20	30	27	9	11	?	**3**			30	0							199	14.45
Fallglo							19	11	20	0					14	14	30	0											83	5
Fortune	30	17	30	20			30	20	30	13	16	0	10	0	15	13	30	20			30	7							221	11
Hickson	30	7	20	10			40	13	30	0	48	6	50	30	40	0	40	0	35	0			10	10					343	6.909
IM111	30	33	30	23			30	20	30	10	30	13	30	13	30	7	30	3			30	0							270	12.2
Imperial	12	33	23	26			30	57	7	86	30	67	40	63	30	37	30	57	12	67	20	45							234	48.91
Temple	20	0	19	0	20	0	20	0	23	48	20	0	25	20	20	0	20	0	20	0									207	6.182
Wilking	10	10	30	13			27	15	20	35	30	0	30	30	30	17	35	23	?	**9**	?	**2**							212	14
Total	271	24.83	325	21.46	125	18.2	306	30.73	335	33.69	384	21.92	410	42.38	362	23.71	395	28.29	67	19	260	12.3	70	26.75	5	20	60	3.5	3375	22.51

Table 4.1b Number of pollinations, and percentage fruit set, for crosses performed in the 2005 season, BRS.

Female Parent (seed)	Male Parent (pollen)													
	4X Emperor No1		4X Emperor No2		4X Excelsior		4X Fremont		4X Murcott		4X Murcott 04 Pollen		Total	
	No.	%set	No.	%set	No.	%set	No.	%set	No.	%set	No.	%set	No.	%set
Arrufatina					50	50	46	30	171	48			267	45
Aust.Clem					10	10	115	30	130	22	60	15	315	23
Corsica1							45	22			80	26	125	25
Corsica2					20	20							20	20
Daisy					100	5	220	12	100	17			420	12
DeNules							41	15	243	31			284	29
Ellendale					81	9	116	21	85	13	50	22	332	16
Encore	207	10	75	7			38	18	187	15			507	12
Fina							25	36	100	27			125	29
IM111					100	21	110	27					210	24
Imperial					74	49	135	36	300	36			509	38
Marisol									100	41			100	41
Oroval					5	0			50	40			55	36
Total	207	10	75	7	440	23	891	24	1466	30	190	22	3269	28

Table 4.1c Number of pollinations, and percentage fruit set, for crosses performed in the 2006 season, BRS.

Female Parent (seed)	Male Parent (pollen)																			
	4X Bakers Sweet		4X Burgess		4X Emperor 05pollen		4X Excelsior		4X Excelsior 05pollen		4X Fremont		4X Joppa		4X Murcott		4X Murcott 05 Pollen		Total	
	No.	%set	No.	%set	No.	%set	No.	%set	No.	%set	No.	%set	No.	%set	No.	%set	No.	%set	No.	%set
Arrufatina			30	50							130	26			190	45			350	38
Aust.Clem			50	24							100	27			50	26	43	37	243	28
Corsica1			60	35					50	42	100	50					150	72	360	56
Daisy			100	22			70	13			180	20			50	30			400	21
DeNules			60	47			50	42			137	32			120	47			367	41
Ellendale					30	40			110	40	113	23			40	0	130	38	423	31
Encore			50	38			40	30			120	28			125	18			335	26
Fina			40	55							100	83			20	40	80	43	240	61
IM111	50	38	135	36			60	35			170	51	50	44	110	35			575	41
Imperial			100	30			50	24			217	27			218	30	50	22	635	28
Marisol									50	36	10	30					100	43	160	40
Oroval			45	36			40	58			60	28					48	10	193	32
Total	50	38	670	35	30	40	310	32	210	40	1437	35	50	44	923	33	601	44	4281	36

Table 4.2a Number of plump seeds sown and total embryos rescued, for crosses performed in the 2004 season, BRS.

Female	Male Parent (pollen)																													
Parent	4X WilkingxMurcott4X 83		4X Fremont		4X Emperor No1		4X Emperor No2		4X Murcott		4X WilkingxMurcott4X 95		4X WilkingxMurcott4X 96		4X Minneola		4X Burgess		4X Orlando No1		4X Orlando No2		4X Excelsior		4X Dancy		4X Bakers Sweet		Total	
(seed)	Plump	Res	Plump	Res	Plump	Res	Plump	Res	Plump	Res	Plump	Res	Plump	Res	Plump	Res	Plump	Res	Plump	Res	Plump	Res	Plump	Res	Plump	Res	Plump	Res	Plump	Res
Arrufatina		323	1	92			7	439		291	2	308	8	399	11	370	4	430			23	39		78					83	2769
Aust.Clem	1	66		34	14	359			3	43	1	28	11	398	5	25	15	232				8							645	1193
Corsica1	2	39		31		39			2	22		11	11	449	28	341	3	115											46	1047
Daisy	1	38	10	118			20	268	6	139	5	118	5	181	30	229	30	207					16	130	1	21			124	1449
DeNules				3				189			1	83	9	810	1	17	7	230											18	1332
Ellendale		191	5	246	7	126			2	365	6	291	5	522	6	171	10	379			9	23	3	51			4	40	57	2405
Encore		55	1	140		16		181	2	114	6	116		204		17	3	**70**											17	913
Fallglo							4	67							1	57													27	124
Fortune	5	112	2	100			2	184	5	127					1	55	4	152			13	5		25					5	735
Hickson	1	26		28			3	88			2	58	9	279															16	504
IM111	33	221	1	145			16	108	6	48	10	78	10	64	4	24	17	31											147	719
Imperial		50	16	11			21	183	6	77	18	215	33	274	2	34	6	152	6	69	22	13							493	1078
Temple			1						11	245			5	109															16	354
Wilking	47	6	3	73			6	75	11	174			15	185	7	67	35	161	47	**215**	11	**9**							136	965
Total	44		40	1021	21	524	79	1593	54	1645	51	1306	121	3874	96	1407	134	1780	53	284	78	97	19	284	1	21	4	40	1830	15587

Table 4.2b Number of plump seeds sown and total embryos rescued, for crosses performed in the 2005 season, BRS.

Parent	4X Emperor No1		4X Emperor No2		4X Excelsior		4X Fremont		4X Murcott		4X Murcott 04 Pollen		Total	
(seed)	Sown	Rescued	Sown	Rescued	Sown	Rescued	Sown	Rescued	Sown	Rescued	Sown	Rescued	Sown	Rescued
Arrufatina					11	324	31	120	11	997			53	1441
Aust.Clem						6	14	530	18	430	3	13	35	979
Corsica1							2	73			3	16	5	89
Corsica2					2	15							2	15
Daisy					1	25	41	155	21				63	180
DeNules							1	116	31	1116			32	1232
Ellendale					7	112	10	289	1	132	1	12	19	545
Encore	3	0	3	11			114	7	10	16			130	34
Fina							6	102	9	319			15	421
IM111					67	150	72	287					139	437
Imperial					43	142	53	309	130	481			226	932
Marisol									13	227			13	227
Oroval									12	208			12	208
Total	3	0	3	11	131	774	344	1988	256		7	41	744	6740

Table 4.2c Number of plump seeds sown and total embryos rescued, for crosses performed in the 2006 season, BRS.

Female Parent (seed)	Male Parent (pollen)																			
	4X Bakers Sweet		4X Burgess		4X Emperor 05pollen		4X Excelsior		4X Excelsior 05pollen		4X Fremont		4X Joppa		4X Murcott		4X Murcott 05 Pollen		Total	
	Plump	Rescued	Plump	Rescued	Plump	Rescued	Plump	Rescued	Plump	Rescued	Plump	Rescued	Plump	Rescued	Plump	Rescued	Plump	Rescued	Plump	Rescued
Arrufatina			16								3				14	499			33	499
Aust.Clem			10	152							2	308			5	229	1	30	18	719
Corsica1			4	141					0	10	5	416					17	310	26	877
Daisy			54				34				98				16				202	
DeNules			35	248			11	176			5	489			16	712			67	1625
Ellendale					2	40			9	12	5	240					4	194	20	486
Encore			6				5				15	42			7	10			33	52
Fina			26	193							35	1191			5	118	4	68	70	1570
IM111	75		132				99				163	573	72	0	40	257			581	830
Imperial			30	145			7	51			49	365			33	368	0	13	119	942
Marisol									1	19	0	20					2	41	3	80
Oroval			9				12				8	43					12		41	43
Total	75		322	879	2	40	168	227	10	41	388	3687	72	0	136	2193	40	656	1213	7723

5. Mutation breeding

5.1 Introduction

Induced mutation in *Citrus* and in particular irradiation of buds has been shown to be effective in producing low-seeded mutants as well as affecting other fruit quality characteristics of existing varieties. Research conducted during projects CT315, CT319, CT614 and Ct00012 investigated irradiation as a means of altering current commercial varieties with regard to seediness. This research continued as part of project CT04007.

5.2 Irradiation research at CSIRO Merbein

5.2.1 Seedless Kara mandarin lines

Induced mutation has been used successfully to generate new seedless variants of citrus (eg Hearn, 1986). The aim of the research in this component of the project was originally to investigate whether floral phenotype could be altered through induced mutagenesis to capitalise on the parthenocarpic nature of three candidate varieties.

As Ellendale tangor (unpublished data), Imperial (Sykes and Possingham, 1992) and Kara (Sykes *et al.*, 1994) mandarins will set seedless fruits in the absence of pollination, the original aim of the work was to generate and thus investigate if pollen sterile variants of these varieties would produce seedless fruits when grown in isolation from other sources of viable pollen. Seedless variants of lemons and Minneola tangelo were recovered following gamma irradiation of buds (Spiegal-Roy and Vardi, 1989) and in the case of Eureka and Villafranca lemons, the variants were pollen sterile.

In project CT319, buds and small trees were treated either with gamma irradiation or short-wave (254nm) UV light. The final report for CT 319 provides further details. This research continued in CT614 when trees propagated from gamma irradiated buds or UV-treated rooted cuttings were assessed for seedlessness and fruit quality. The main finding regarding this component of the research in CT614 was that two M2 trees derived from buds cut from an M1 tree grown from a Kara bud treated with 60gy gamma irradiation had very low mean seed numbers in their fruits (2.1 ± 1.5 and 1.4 ± 1.5 respectively compared to 18.8 ± 7.4 and 20.1 ± 5.9 for two other M2 trees growing next to them). The research continued in CT00012 focused on these two M2 lines and led to the propagation of a number of M3 daughter trees. Fruit development on these trees while they remained in pots during the last year of CT00012 showed that the daughter trees had retained the ability seen in the M2 trees for seedless fruit production (see final report for CT00012). This result was encouraging, but further data were required from the M3 trees to confirm the stability of these two budlines with regard to seed development. Thus, these M3 trees were established under orchard conditions during CT04007 with the aim of testing their ability for seedless fruit production in the field under strong cross-pollination pressure.

The M3 trees were planted out in the research orchard during October 2004. They were allowed to establish without fruits for two years by removing developing fruits. In spring 2006, the trees were allowed to flower and pollen observations made. Pollen production was very low and was different from normal Kara mandarin. The anthers of the M3 trees were pale yellow and produced little pollen and in this regard they resembled Satsuma mandarin and pollen sterile hybrids that have been bred in the diploid hybridisation program. When flowers were self-pollinated, only seedless fruits resulted.

Fruits from open-pollinated flowers were mostly seedless although occasionally fruits were observed with between 1 and 3 seeds. In this respect the M3 trees have performed in a similar manner to the M2 trees from which they were propagated. These trees will be observed during the next project for yield and fruit quality.

These variants of Kara have stimulated interest both domestically and internationally, with a number of enquiries concerning their availability from other countries. A group of Spanish nurserymen that visited during spring in 2006 were particularly interested in a late seedless Kara mandarin.

As a result of the limited data obtained so far for the M3 trees in the research orchard, the consistent performance of the M2 trees, and the interest in a seedless Kara that has been shown both locally and internationally, a decision was made to enter the two seedless lines into regional evaluation via the grower-testing network established in the diploid hybridisation component of the project.

A new testing agreement was drawn up specifically for these two lines and expressions of interest sought from growers. From this, testing plots, based on top-worked trees, were established during October/November 2007 with cooperating growers in the Sunraysia, Riverland and Riverina regions.

While Kara mandarin is not a major variety, it is grown to a limited extent in the Murray Valley, especially the Riverland of SA. Seediness is one of its limitations and a rough, coarse rind is another. It is a late maturing variety that stores well, suggesting that it could fulfill one of the aims of the breeding plan, namely a late maturing variety for export. A seedless variant of Kara would help towards this goal and a finer rind is one fruit characteristic observed for the budlines developed at Merbein. Data to be obtained from the second phase evaluation of these Kara lines will further test the stability of the budlines, as well as potential yields, fruit maturity and quality, and the stage whereby a decision on nominating one or both of the lines for release should be reached by the end of the next project.

5.2.2 New mutation breeding at CSIRO

As part of the next project in the National Citrus Scion Breeding Program, diploid hybrids selections that have performed well in regional evaluation plots will be entered into an irradiation program. These hybrids, namely selections 2103, 2128, 2552 and 2762, are all capable of parthenocarpic fruit production, but they are also pollen fertile and can self-pollinate. The success that has been achieved with Kara mandarin, which has similar characteristics with regard to pollen and fruit development, indicates that these genotypes are suitable for modification via gamma irradiation. If this proves to be true, then one of the original reasons for conducting the mutation research with Kara, Imperial and Ellendale will have been demonstrated and the use of gamma irradiation to supplement the diploid hybridisation program will become routine.

5.2.5 References

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5.3 Mutation breeding research at DPI&F, Bundaberg

5.3.1 Materials and methods

The project included a small component of mutation breeding in which trees resulting from buds irradiated in a previous project (CT00012) were assessed for low-seeded limbs, and from which buds were taken to produce daughter trees. Pollen and fruit from these daughter trees were also assessed during the three years of the project. No additional irradiation was carried out during the period.

Nine citrus cultivars were identified in CT00012 as good candidates for mutation breeding. It was felt that all of these might be more attractive commercial propositions if they were less seedy. The rationale for choosing each of these nine cultivars is given in the previous Final Report (pg. 57).

Budwood was irradiated in 2000 and the resulting trees field planted in late 2001. Some fruit were produced in 2002, and all fruiting limbs assessed for seed number and fruit size in each season from 2003 to 2006. Selections from these were propagated and the resulting daughter trees field planted in January 2005. These daughter trees were assessed for pollen viability, fruit size, and seed number in 2006 and 2007. In the 2007 season 20 fruit were harvested from each daughter tree and assessed for weight, diameter, rind thickness and plump and flat seeds. Two composite juice samples were taken from 10 fruit and the Brix and acid level determined. Fruit from the progenitor cultivars (normal seedy variety) were included as controls.

Pollen viability of irradiated selections and their progenitor varieties was determined for daughter trees in 2006 and 2007. Pollen viability was assessed using two methods: acetocarmine staining and pollen-tube germination. Ten measurements were made using each method for each daughter tree. The relationship between pollen viability and fruit seediness was examined.

5.3.2 Results and discussion

Table 5.3 shows the results of fruit assessments on daughter trees (and progenitor cultivars) obtained in 2007. It can be seen from these results that a number of low-seeded selections have been developed in the program, though only one selection seems to be completely seedless. In some cases, selections have proven to be just as seedy as the original variety which indicates that the putative mutant limb, from which the daughter trees were propagated, were producing low seeded fruit for reasons other than a mutation. An example of this is the selection ‘R2P9’ of IM111 which is just as seedy as the parent variety.

Four low-seeded selections of Fremont have been developed and all warrant further testing. They have about ¼ of the seed number normally found in Fremont and fruit size has apparently not been affected. This variety has been popular commercially on-and-off over the last 20 years in Australia and is particularly well suited to hot production areas in northern Australia. Fruit size and high seed numbers have been major obstacles preventing the sustained commercial production of this variety. At least one of these low-seeded selections should be virus-indexed and made available to Australian citrus growers as an alternative to the current seedy variety.

Ellenor does not suffer the same fruit size problems of Fremont, but its rough skin and high seed numbers are significant problems that have seen its commercial significance decline. It is regarded by many as one of the finest flavoured of all mandarins. Both low-seeded selections warrant further testing and then entry of the best into the Auscitrus budwood scheme.

Pollen viability has long been recognised as a useful predictor of seediness in irradiation breeding. In our work, the fastest and most efficient method of assessing viability is via acetocarmine staining. Pollen-tube germination takes longer and counting is more difficult. Figure 5.3a shows the relationship between pollen viability (acetocarmine method) and seediness of 29 trees in the irradiation program.

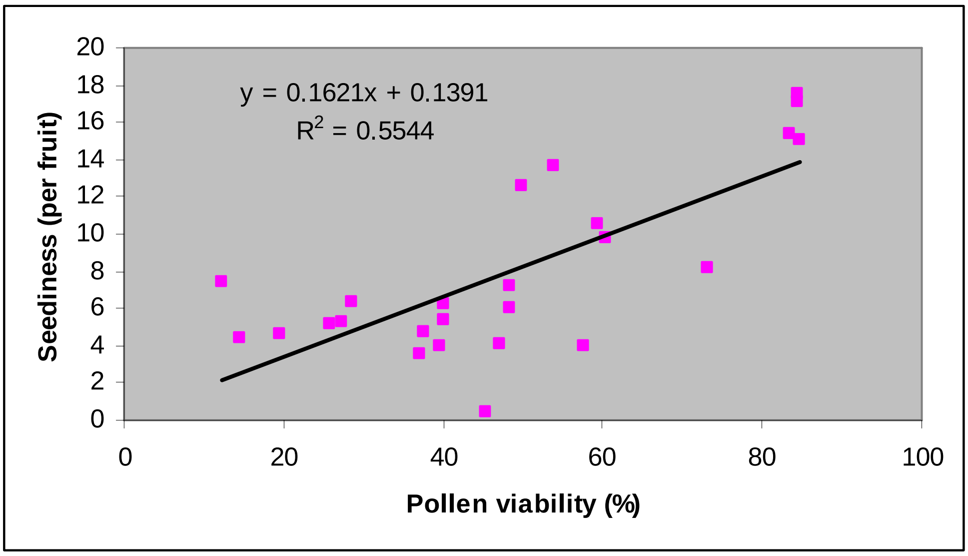

Figure 5.3a: Relationship between seediness and pollen viability for 29 trees of six different mandarin varieties and their mutant derivatives. BRS 2007.
Seed numbers based on 20 fruit per tree and pollen viability on 10 counts of stained/unstained pollen.

Although there is a general trend of increasing pollen viability as the seediness of selections increases, the relationship is not strong enough to be used as a predictor. There are a number of possible explanations for this including the fact that seed number was the result of pollen viability in 2006, and that there was a mixture of six different progenitor varieties used to generate the relationship. However a more likely explanation is that pollen viability tests only assess male fertility whereas seediness is a function of both male and female fertility. Furthermore the impact of reduced male fertility is lessened in our experimental area by the close presence of many alternate pollen sources. With this in mind it is interesting to note one tree that has pollen viability of around 45% but produces no seed. This is an Ellenor selection (see Table 5.3 above) and these results suggest that it may have a mutation for female sterility that could be used in the conventional breeding program. This is being further investigated.

Potentially, one of the most interesting outcomes of the irradiation research at BRS is a Daisy mutant that is apparently resistant to Emperor Brown Spot (EBS) diseases. Figure 5.3b shows the leaf of the apparently resistant mutant alongside a leaf of normal Daisy showing typical EBS symptoms.

Table 5.3: Seed numbers and fruit quality parameters of daughter trees from low-seeded selections of six varieties, compared with the original progenitor variety (control). All fruit assessed 23rd July 2007, BRS. Values are the mean of 20 fruit. Note: control trees were not in the same planting or of the same age, but selected from nearby orchards.

	Tree	Plump seeds	StdDv	Flat seeds	Fruit Wt.	Rind	Brix	Acid	Brix:Acid
Afourer									
R3P59	1	8	2	1	133	4	11.9	0.70	17
Control	1	15	6	2	317	6	10.7	0.53	20
Daisy									
R3P30	1	4		2	117	4	12.2	0.69	18
R3P33	1	4	2	2	168	5	12.0	0.86	14
R6P40	1	11	3	2	140	4	12.5	0.87	14
	2	13	4	1	151	4	11.5	0.84	14
Control	1	18	3	6	258	6	11.1	0.51	22
	2	15	5	8	183	5	13.0	0.67	20
Ellenor									
R3P24	1	0	1	3	121	4	12.0	1.02	12
R6P4	1	4	4	6	148	5	12.0	0.89	13
	2	7	4	7	142	5	12.7	1.10	12
Control	1	22	9	7	162	4	13.4	0.96	14
Fremont									
R2P31a	1	4	2	1	101	4	14.6	1.07	14
R2P31b	1	4	2	1	98	4	15	0.83	18
	2	5	2	1	89	4	15.4	1.04	15
R2P32	1	5	2	1	95	4	15.6	0.97	16
	2	6	2	1	69	4	16.3	1.21	13
R5P1	1	6	2	1	94	5	14.3	0.93	15
	2	5	2	1	97	4	15.5	0.98	16
control	1	17	4	2	87	4	12.6	0.54	23
IM111									
R2P18	1	5	2	2	149	4	9.5	0.85	11
	2	7	2	1	175	5	9.6	0.93	10
R2P9	1	10	5	5	217	5	9.1	0.98	9
	2	14	4	3	231	5	10.0	0.79	13
control	1	15	5	7	185	5	9.7	0.80	12
IrM1									
R6P68	1	6	2	0	148	3	13.0	1.24	11
	2	5	1	1	156	3	12.1	1.13	11
control	1	5	2	1	137	2	15.4	1.14	14
	2	7	2	1	164	3	14.4	1.10	13

Figure 5.3b: Apparent field-resistant mutant of Daisy mandarin (LHS) alongside a leaf from normal Daisy showing typical EBS symptoms, September 2007, BRS.

The two daughter trees of this selection have consistently shown freedom from symptoms in the field while all surrounding trees are infected. The disease status of this selection is currently being further tested using a detached leaf assay. There is no other known case in citrus where disease resistance has been induced via mutation breeding, so care is needed to ensure that the observed field resistance is not simply a disease escape. Daisy is grown commercially and has potential as an export variety. In Queensland it is highly susceptible to EBS, and a disease resistant selection would be of significant economic/environmental value to the citrus industry.

6. Project-wide activities

6.1 The citrus scion breeding reference committee

An initiative put in place towards the end of project CT96014 was the establishment of the Citrus Scion Breeding Reference Committee (CSBRC) consisting of industry, CSIRO, QDPI&F and HAL representatives. The overall aim of this committee has been to act as an industry/agency steering group operating in a consultative manner to ensure that the breeding program remains focused on short- and long-term strategic industry priorities. The committee assists in setting targets for the breeding program so outcomes are defined clearly and understood by all parties. The committee has assisted in communicating activities, research outputs and industry outcomes to the wider citrus industry. During project CT04007, the CSBRC has played an invaluable role in assisting with the selection of a commercialiser to manage the release of two new varieties from CSIRO.

The CSBRC held regular meetings via telephone conference during project CT04007. Formal minutes of twelve full meetings of the CSBRC were recorded and circulated during the course of CT04007. In addition, a number of shorter meetings were held involving those members of the committee who were available at the time to discuss and make decisions concerning the commercialisation of the two Merbeingold varieties nominated for release. Records were maintained from these shorter meetings, but were not recorded as formal minutes for circulation to the committee. During periods of intense activity concerning events relating to the commercialisation of these varieties, the committee was meeting at fortnightly intervals.

6.2 Project teleconferences

With the reduction in size of the breeding program following the decision by the IAC not to continue the funding support for the gene technology component that was undertaken up until the end of project CT00012, project teleconferences have been held on a less formal basis. With just two principal investigators involved in the project, discussions concerning the project have been conducted by telephone on a regular needs basis without formal agenda or minutes.

6.3 The breeding plan

Project CT04007 has been conducted with reference to the breeding plan, which was first drafted in project CT96014. The plan is reviewed regularly and updated accordingly. The most recent revision in August 2007 was made in response to a request from HAL as part of the approval process for the new project. The breeding plan is maintained as a confidential working document available for consultation by the ACG Executive, the IAC, the CSBRC, HAL, CSIRO, QDPI&F and the project team.

In addition to preparing an update of the breeding plan in August 2007, the breeding team also completed a "**Best Practice Breeding Program Review and Assessment**" with help from Bill Blowes of BeeBill Enterprises P/L, Tumut, NSW during June 2007. Also as part of meeting HAL requirements for the approval of the new project, the team with assistance from Andrew Collins of HAL completed a Benefit-to-Cost analysis of the breeding program.

6.4 Extension activities

During the course of the project many opportunities have been accepted to extend information concerning the progress being made and the delivery of specific results to the industry. These have been reported in the preceding chapters and have included presentations at Cittgroup meetings, specific briefings hosted by CSIRO and QDPI&F to highlight various aspects of the project, displays at field days (which have also included tastings of fruits in season), presentation of fruit samples to citrus fruit marketers for feedback, presentations to industry meetings other than Cittgroup functions (eg at ACG annual conference), and via the media including radio and print (publications in industry journals and newsletters are listed below).

Activities associated with the breeding program have also been extended to visitors at Merbein and Bundaberg over the course of the project and the principal investigators have also presented seminars and conference talks both within their organisations and to outside institutions. Dr Sykes was invited to present details of the research at Merbein in Spain (February 2005) and the Peoples Republic of China (December 2006) during the course of the project.

6.5 Publications

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In addition, applications have been prepared for Plant Breeders Rights (PBR) for Merbeingold 2336 and 2350, a CSIRO fact sheet was prepared concerning these two varieties and a US Plant Patent application was prepared and field in 2006. The application for PBR will be published in the PBR office's Plant Varieties Journal.

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