Final Report

National Citrus Scion Breeding Program

CSIRO Plant Industry

Project Number: CT07000

CT07000

This project has been funded by Horticulture Innovation Australia Limited using the citrus industry levy and funds from the Australian Government.

ISBN 0 7341 3650 1

Published and distributed by:
Horticulture Innovation Australia Limited
Level 8, 1 Chifley Square
Sydney NSW 2000
Tel: (02) 8295 2300
Fax: (02) 8295 2399

HAL PROJECT NUMBER *CT07000*
(May 18, 2012)

THE NATIONAL CITRUS SCION BREEDING PROGRAM

STEPHEN SYKES[a] AND MALCOLM SMITH[b]

[a] CSIRO Plant Industry

[b] Agri-Science Queensland - a service of the Department of Employment, Economic Development and Innovation

HAL PROJECT NUMBER *CT07000*

Project leaders:

STEPHEN SYKES

(CSIRO Plant Industry, PMB, Merbein, Vic 3505)[1]

MALCOLM SMITH

(Agri-Science Queensland DEEDI, Bundaberg Research Station, MS108, Ashfield Road, Bundaberg, Qld 4670)

Statement of purpose:

The purpose of this document is to report formally the progress made by the research conducted from September 2007 until December 2011, which formed HAL project CT07000, the National Citrus Scion Breeding Program.

Acknowledgments:

- Project CT07000 has been funded by HAL using the citrus industry levy and matched funds from the Federal Government.
- Project CT07000 was also supported by funds from CSIRO and Agri-Science Queensland DEEDI.
- Project CT07000 was supported in-kind by cooperating citrus growers who hosted trial plantings.

Date of report: May 18, 2012

[1] Address at which the research was conducted. Address at time of report preparation: Honorary Post-retirement Research Fellow, CSIRO Plant Industry, Irymple, PO Box 905, Mildura, Victoria 3502.

CONTENTS

1. 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 projects funded by Horticulture Australia Limited and the research providers CSIRO Plant Industry and Agri-Science Queensland DEEDI. 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 (Agri-Science Queensland DEEDI, 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 CT07000 has produced 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 (Merbeingold 2336 and Merbeingold 2350) have been released 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. Many other promising selections, combining seedlessness or low seed numbers, attractive internal and external appearance and high eating quality, have been identified and daughter trees are being evaluated in a range of locations. These selections have been derived through diploid, triploid and mutation breeding initiatives. Of particular note are three selections from the mutation breeding program (two low-seeded Kara mandarins from Merbein and a low-seeded Fremont from Bundaberg) that are in the process of being entered into large scale semi-commercial trials with industry cooperators.

With the withdrawal by CSIRO from citrus research, including breeding, the future of the program will need to be reviewed by industry. Regardless of this, the program is in a healthy position and it is anticipated that further R&D will see more varieties nominated for release to industry due to improved germplasm that will underpin an Australian citrus breeding program into the future and so address the goals documented in the breeding plan.

2. Technical Summary

The breeding approaches adopted to achieve the goals outlined in the breeding plan are conventional diploid hybridisation, triploid hybridisation and mutation breeding.

Conventional diploid hybridisation and mutation breeding at CSIRO

The research conducted at CSIRO during CT07000 covered all facets of the pipeline approach to citrus breeding.

Progenies from crosses conducted aimed at the selection and development of new early, seedless mandarins were established in the breeding orchard. At the same time crosses aimed at reducing seedling juvenility, which is one of the major impediments to rapid genetic improvement via conventional cross-breeding were conducted resulting in the selection of new hybrids that flowered within two years of seeds being sown. This crossing program, aimed at reducing juvenility, is now assessing 2nd and 3rd generation crosses involving short juvenile period germplasm originally sourced from China and Vietnam during an ACIAR-funded project conducted in the 1990s.

Screening for autonomic parthenocarpic fruit set, which is a key trait contributing to the seedless phenotype further reinforced the hypothesis developed at Merbein that this characteristic is under the control of three dominant complementary genes. Further evidence was obtained from crosses made in the mid-1990s specifically to investigate this characteristic. Evidence also came from a family derived from a cross between Imperial mandarin X Miho wase satsuma. Previous research led to a prediction that this family would segregate 3:1 in favour of parthenocarpy and the data obtained did indeed support this. From the early work at Merbein where progenies segregated 1:3 at best for autonomic parthenocarpy, parents are now available that should produce progenies with all hybrids being parthenocarpic.

This Imperial x Miho family was also used to demonstrate that there is little value in propagating all breeding progenies to a standard rootstock to shorten seedling juvenility. There were no significant effects on flowering time due to rootstock over own-rooted hybrids.

New selections were made and daughter trees propagated either for further evaluation or for use as new parents after 1st phase evaluation of two progeny groups. Twenty four low-seeded, potentially seedless hybrids were selected from a population of families generated in the 1990s. A further 14 selections were established in the field from part of a progeny bred for new late maturing seedless mandarins.

A number of promising selections made in previous projects were evaluated as multiplied daughter trees at CSIRO. A number of selections from these proved to be promising, while others can now be eliminated from the program. Amongst the promising types was a very early mandarin hybrid that matures its fruit at the end of March-to-early April. Fruit size is an issue with this selection, however, although preliminary work has demonstrated that it is amenable to judicious thinning to improve size.

Following the announcement made in March 2006 of the pending release of Merbeingold 2336 (which yields seedless, juicy fruits that mature early-to-mid-season) and Merbeingold 2350 (which produces low-seeded to seedless fruits with highly coloured, robust yet thin easy-peel rinds), a commercialiser was licenced to develop the varieties in Australia. This resulted in the first commercial trees being planted in spring 2011. Semi-commercial trials of the varieties were monitored and harvested throughout CT0700 leading to further data to optimise harvest times for the two varieties. Consumer evaluation of the two varieties was also undertaken by the commercialiser.

Four selections that were investigated in phase 2 evaluation trials during CT04007 were entered into the mutation breeding program. These selections all have a high capacity for parthenocarpic fruit development, but can all self-pollinate. Buds of each selection were irradiated and by the end of CT07000 first and second generation daughter trees had been propagated and established in the breeding orchard for evaluation. It is hoped that new pollen sterile or anther dysfunctional variants will be identified from these trees that will allow further testing of the selections as seedless genotypes.

Finally, the performance of two variants of Kara mandarin developed in the mutation program at Merbein warranted the recommendation from the Citrus Scion Breeding Reference Committee that they should now be entered into large-scale semi-commercial trials. Bud lines from the two variants have been shown to be stable with dysfunctional anthers persisting at least through to 4th generation daughter trees. The lines have also come through rigorous trials where they were embedded in different orchards where cross-pollination pressures have varied. The maximum number of seeds found in any fruit was 4 with the vast majority of fruit of the two lines being seedless, even where the trees were growing within Valencia orchards. The two lines also vary in maturity time with fruit of one being harvested 4 weeks after the other. Their lateness should help provide an alternative late-to-very late maturing seedless mandarin.

Triploid hybridisation component at Agri-Science Queensland DEEDI

Triploid breeding activities have seen major shifts in the parentage used to generate progeny blocks. This has been driven by:

1. results from existing progeny blocks as they start to produce fruiting hybrids, and
2. the development of better diploid and tetraploid germplasm.

The long generation time of citrus means that many years elapse and much effort is expended before the merit of parental combinations can be determined. To manage this problem we have used fruit quality data - from the first hybrids to fruit within progeny blocks - to suspend poor performing parents from use in future crossing programs. This strategy has proven successful in quickly transitioning the breeding population toward better parental combinations. As an example, tetraploid Emperor was used extensively as a pollen parent each flowering season from 1998, and in 2007 there were almost 200 hybrids fruiting for the first time. Concerns over excessively rough skin texture resulted in the immediate suspension of tetraploid Emperor pollinations in 2007, a decision vindicated in 2011 when more than 1,100 field-grown trees were culled. Conversely, tetraploid Murcott represented only 12% of pollinations in 1998, but in recent years it has been as high as 70%, recognising the fact that it is already a parent in 42% of existing selections even though it was less than 7% of hybrids planted prior to 2009. The availability of new tetraploids like Fremont, and the tandem breeding of high fruit quality diploids has greatly aided the transition to better parental combinations[2]. Indeed, in the 2011 season, 93% of seed parents were diploid hybrids bred at BRS, where as four years earlier there had been none. Future work will continue to focus on breeding better quality diploid and tetraploid parents, with an additional focus on disease resistance.

Strong seasonal variations in fertility and fruit set have frustrated the breeding team, with the last three seasons being some of the worst since 1998. Consistent annual production of certain parental combinations has enabled the comparison of up to 14 consecutive years' data on fruit set. As an example, Ellendale x 4XMurcott crosses during this period have averaged 14% fruit set but ranged from 0 to 40%. There is a tendency for all crosses to set well in certain seasons (e.g. 2004), but more often the seasonal affect is complicated by variation in the fertility of both

[2] NB A separate diploid hybridisation program is conducted at BRS with local Queensland industry funding support. This program is different to that conducted at CSIRO Merbein, which is reported here as a major component of the National Citrus Scion Breeding Program.

female and male parents. More research is needed on seasonal/climatic effects on pollen fertility as a step toward improving fruit set and hybrid recovery in triploid breeding.

The issue of thorniness of triploids has been examined and found to be strongly cultivar dependent. Within the population of 30 hybrids selected so far there is no detectable link to parentage, with hybrids from the same family producing widely different levels of thorniness. This wide segregation for the thorniness trait demonstrates the capacity to select against this trait from within any parental combination of interest.

Many progeny blocks from the best parental combinations are now reaching maturity and it is anticipated that the number of annual selections will increase dramatically in the next few years. Daughter trees of existing selections are also now fruiting, creating the opportunity for the triploid breeding to showcase new hybrids to growers, associated industry, and for testing with consumer groups.

3. 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 has been a continuing high priority for the Australian Citrus Industry. Project CT07000 continued 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 CT07000 traditional breeding approaches have continued to be pursued in the environments in which the varieties will be grown. Each line of research in CT07000, 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 CT07000 has continued the research of CT04007 (2004-07), 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.

CT07000 was funded for a different period than previous projects in the National Citrus Scion Breeding Program. In formulating the new project in 2006, the breeding team argued that as long-term research, a three year cycle is short and the team focuses much time on initiating, formulating and closing a project when a longer project time frame would allow greater efficiency and use of resources in actually conducting research. This is especially so if the breeding program is viewed as a long term commitment by industry. The team argued that a five-year project time frame was more suited to a breeding program, especially if reviews are conducted towards the end of or soon after each project. The team argued also that as a winter harvested product, a breeding project funded on the basis of the Australian financial year of July-through-June was less than appropriate and that a better cycle for funding a breeding project would be on a calendar year basis, i.e. from January–through-December. Thus, Project CT07000 commenced on July 1 2007 and was destined for completion on December 31 2011 to set up for potential future funding cycles to be for five years based on a calendar year cycle.

Having reached agreement on the new format for funding projects within the National Citrus Scion Breeding Program, CSIRO, however, as a consequence of budget cuts announced by the Australian Federal Government in 2008, announced that it would be closing the Merbein Research Station and that the organisation would be withdrawing from all citrus research from June 30, 2009. Although this decision to withdraw from all citrus research (and, thus, to terminate CSIRO's involvement in citrus breeding) was modified later such that the work in CT07000 could be completed, this report is the final one in a series dating back to CT111 that documents the research conducted at CSIRO Merbein.

This final report outlines progress in the research undertaken by CSIRO and Agri-Science Queensland DEEDI in project CT07000 from July 2007 until December 2011. While the report goes in depth where appropriate, other areas may be treated with what may seem to be a degree of brevity. In such instances, the reader is referred to the milestone reports that were submitted to HAL during the course of the project or should contact either of the authors for additional information.

4. Diploid hybridisation

4.1 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. This would also include new candidates for entry into mutation breeding to alter one or more specific characteristics.

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 isoenzyme 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 updated during August 2007.

This section outlines the progress made in the hybridisation and associated research at Merbein during CT07000. 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.

4.2 Crossing program

4.2.1 Crosses from CT04007

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 (see final report for CT04007 for details). The breeding plan highlights the export market window of opportunity that exists for very early maturing fruits. At the start of CT07000, the aim was to establish the progenies from these crosses in the breeding field at CSIRO's experimental farm in NW Victoria once they had grown to a suitable stage of development under glass- and shadehouse conditions. The drought that gripped much of the country over the past decade-or-so, however, and which led to severe restrictions on irrigation allocations, meant that these progenies were held in pots in a shadehouse for much of CT07000. It was deemed that their survival was better safeguarded in a situation where they could be hand watered than in the breeding orchard where they could suffer severe water stress.

The progenies from the crosses conducted in CT04007 aimed at early-maturing, seedless varieties were finally planted at CSIRO's experimental farm in NW Victoria during 2010. Although CSIRO will cease its involvement in citrus breeding research at the end of CT07000 in December 2011, it has agreed that these hybrid families will be maintained at CSIRO's experimental farm in NW Victoria so as to allow alternative arrangements for their eventual evaluation.

4.2.2 Crosses aimed at reducing seedling juvenility

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. One such departure has been to investigate, through controlled crosses, the possibilities for reducing seedling juvenility and thus improve citrus breeding efficiency.

This component of the breeding research at CSIRO started as a result of germplasm that was introduced to Australia in a project funded by ACIAR in the 1990s. Within the different accessions that were introduced in this associated research, two genotypes were identified as having very short seedling juvenility with their nucellar seedlings often flowering within a year of germination. One of these introductions was a lemon-type called Con lemon. Exploratory crosses with Con lemon using Clementine mandarin as the female parent resulted in two hybrid seedlings that flowered within 2 years of germination. As a result, more crosses were conducted as a CSIRO appropriation project with other monoembryonic seed parents and from these, a range of hybrids that flowered within 3 years of seed germination were retained for further breeding.

During CT07000, further crosses were conducted using the original Con lemon, hybrids that CSIRO had generated using Con lemon as a male parent and other parents developed in the diploid hybridisation program of the National Scion Breeding Program to introgress reduced seedling juvenility into the breeding population.

4.2.2.1 Materials and methods

Controlled crosses were made during springs 2007-to-2009 inclusive (Table 4.1). Unfortunately, a severe heat wave during early November in 2009 caused all developing fruitlets on female parents to drop prematurely and no seeds were harvested from crosses conducted in 2009.

Table 4.1. Controlled crosses using either Con lemon or hybrids bred with Con lemon aimed at introgressing reduced seedling juvenility into the scion breeding population at CSIRO Merbein.

Year of cross	**Female[a]**	**Male[a]**	**Location of female parent**	**No seedlings** (& selections made)
2007	Ellendale	00-200-01	Orchard	-
	Imperial	00-200-01	Glasshouse	11
	Pummelo CS28	00-200-01	Orchard	-
	Clementine – Fina	Con lemon	Orchard	9
	Clementine – Marisol	Con lemon	Orchard	5
	Clementine – Nules	Con lemon	Orchard	44
	Clementine – old	Con lemon	Glasshouse & orchard	14 (1)
	Clementine – Oroval	Con lemon	Orchard	-
	Ellendale	Con lemon	Orchard	-
	Imperial	Con lemon	Glasshouse & orchard	80 (2)
	Pummelo CS28	Con lemon	Orchard	9
	00-200-01	Imperial	Shadehouse	-
	88-08-46	00-200-01	Orchard	-
	88-08-46	Con lemon	Orchard	-
	88-02-21	00-200-01	Orchard	-
	88-02-21	Con lemon	Orchard	-
	88-02-07	00-200-01	Orchard	-
	88-02-07	Con lemon	Orchard	-
	2127	00-200-01	Orchard	-
	2127	Con lemon	Orchard	-
	2350	00-200-01	Orchard	-
	2350	Con lemon	Orchard	-
	2552	00-200-01	Orchard	-
	2552	Con lemon	Orchard	-
	2762	00-200-01	Orchard	-
	2762	Con lemon	Orchard	-
2008	00-200-01	03-106-27	Shadehouse	-
	2103	00-200-01	Glasshouse	8
	2103	03-106-27	Shadehouse	-
	2128	00-200-01	Glasshouse	-
	2350	00-200-01	Glasshouse	-
	Clementine 813	03-106-27	Glasshouse	28
	Fina	03-106-27	Orchard	-
	Fina	03-106-53	Orchard	3
	Fina	03-106-66	Orchard	1
	Imperial	00-200-01	Glasshouse	-
	Imperial	03-106-27	Glasshouse	1
	Imperial	03-106-27	Orchard	1
	Imperial	03-106-53	Glasshouse	8
	Imperial	03-106-53	Orchard	2
	Marisol	03-106-27	Orchard	-
	Marisol	03-106-53	Orchard	1
	Marisol	03-106-66	Orchard	-
	Nules	03-106-27	Orchard	15

Nules	03-106-53	Orchard	23
Nules	03-106-66	Orchard	34 (1[b])
Oroval	03-106-27	Orchard	1
Oroval	03-106-53	Orchard	5
Oroval	03-106-66	Orchard	-

[a] Parents that were not named varieties were as follows:

2103, 2127, 2128, 2350, 2552 and 2762 are Imperial x Ellendale (strongly parthenocarpic)
00-200-01 is Clementine 813 x Con lemon
03-106-27, 03-106-53, 03-106-66 are Pummelo CS28 x Con lemon
88-02-07 and 88-02-21 are Silverhill Satsuma x Joppa sweet orange
88-08-46 is Silverhill Satsuma x Pummelo CS28

[b] One seedling flowered 5 months after germination, but failed to survive.

Fruit from the controlled pollinations were harvested when colour break occurred and the seeds were extracted, washed, surface dried and stored in polyethylene bags at 4°C until required. When all seeds had been collected in each year, the outer seed coats were removed and they were sown in soaked Growool® held in large plastic containers and placed in a growth room for germination (see final report for CT04007).

Emergent seedlings were transferred to a standard potting mix, hardened off in the growth room and transferred to a glasshouse. After 3 months in the glasshouse, seedlings were placed in a shade house under ambient conditions and observed for flower development.

Any seedlings that flowered within 3 years of germination were retained and ultimately established in the orchard at CSIRO's experimental farm in NW Victoria. All other seedlings, i.e. those that failed to flower within 3 years of germination were destroyed.

4.2.2.2 Results and discussion

Seed germination was generally poor (data not presented) even though seeds were sown under conditions that have maximized citrus seed emergence for other seed batches. This has been a feature of seeds of Con lemon and its progeny when generated from controlled crosses. Though the data cannot lend much more support than that afforded by previous work, it appears that Con lemon carries lethal or sub-lethal genes that affect seed, or more likely embryo germination.

Coupled with this poor germination, once the radical emerges from a seed, the developing seedling often ceases to grow further or grows very slowly and eventually dies (Figure 4.1). Again this has been seen with other crosses involving Con lemon and hybrid progeny bred with it at CSIRO.

Figure 4.1. Germination typical of seeds from a controlled cross-pollination involving Con lemon or one of its hybrid seedlings produced at CSIRO Merbein. Embryo germination, emergence and subsequent seedling growth varies as depicted in the image. Only the 5 strong seedlings at the bottom of the image survived.

The seven crosses made in 2007 that resulted in 172 viable seedlings (Table 4.1) were produced from 1472 seeds; and the 14 crosses made in 2008 that yielded 128 good seedlings (Table 4.1) came from 1364 monoembryonic seeds.

Nevertheless, a proportion of seedlings from both years was strong and these grew as expected. Numbers in parentheses in Table 4.1 after the number of seedlings that developed from any one cross indicates the number of seedlings that flowered within 2 years of germinations. Three hybrids with reduced juvenility have been established in the orchard at CSIRO's experimental farm in NW Victoria and thus, added to the collection of short juvenile period hybrids generated at Merbein. This collection of hybrids will be maintained pending decisions in relation to the future of breeding material beyond December 2011 and in particular the intellectual property associated with this component of the project.

4.3 Phase 1 evaluation

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

4.3.1 Screening for parthenocarpic ability

Pollination and pollen exclusion experiments conducted using hybrids as they flowered continued during CT07000 with the aim of finalizing data sets for hybrids generated in previous projects while also generating new data for more recent progenies as they commenced fruit production. With regard to the latter, a key progeny was an Imperial mandarin x Miho wase satsuma family generated in 2001, and is dealt with in detail separately in the next section.

The main thrust of pollination experiments in CT07000 was with a population of families generated in 1996-98. This population was generated using parthenocarpic hybrids selected from families of crosses performed between 1984 and 1988 and comprised of 111 full-sib combinations with family size ranging between 1 and 69.

Screening for parthenocarpic ability commenced with this population in CT04007, during which it was shown that the frequency of hybrids combining traits contributing to the seedless phenotype had increased as the program progressed into second generation crosses using hybrids generated at Merbein as parents. This trend continued in CT07000, although as has been noted many times both in our program and in others around the world, the juvenility characteristic of citrus seedlings has prevented the total completion of data sets, as in each family there still remains individuals that did not flower, even 14 years after germination. Interestingly, all but 5 of 111 hybrids in the Imperial x Miho family referred to above have failed to flower and produce fruit. Previous work with Satsuma mandarin parents have shown that its progenies generally have a shorter mean juvenile period than other varieties that have been used as parents.

Progress updates with this population of families were detailed in project milestone reports and so only a final summary is presented here.

Of the hybrids that have flowered and been tested so far, there is evidence that 336 are parthenocarpic and 449 are 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 varied, although as stated above, 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 segregation ratios (e.g. Table 4.2) have indicated that three dominant complementary genes are responsible for the expression of this characteristic and, thus, support data from previous crosses.

Of particular interest were 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.

Table 4.2. Segregation ratios for parthenocarpy in selected families demonstrating that a model based on three dominant complementary genes can account for differences between different parents. From these ratios it has been possible to assign genotypes for the different parents used in the program and be able to predict the outcome of future crosses in terms of the ability for progeny to yield seedless fruit.

Cross	Observed P:NP*	Fit	χ^2
Clementine x 2539	9:12 (*37*)	1:1	0.42 ns
		7:9	0.007 ns
		3:5	0.24 ns
		27:37	**0.003 ns**
Fina x 2539	10:16 (*35*)	1:1	1.38 ns
		1:2	0.30 ns
		7:9	0.30 ns
		3:5	0.01 ns
		27:37	**0.15 ns**
88-13-15 x 2920	8:9 (*10*)	**1:1**	**0.06 ns**
		27:37	0.16 ns
88-13-15 x 2939	24:10 (*0*)	3:1	0.35 ns
88-13-15 x 2332	13:17 (*6*)	1:1	0.54 ns
		7:9	**0.009 ns**
		27:37	0.012 ns
88-13-15 x 2511	12:6 (*2*)	3:1	0.67 ns
		2:1	**0.00 ns**
88-13-15 x 2552	2:7 (*5*)	**1:3**	**0.04 ns**
88-13-15 x 2916	16:26 (*15*)	1:3	3.84 p=0.05
		1:1	2.38 ns
		1:2	0.43 ns
		7:9	0.55 ns
		27:37	**0.29 ns**
2539 x 2332	3:14 (*6*)	1:3	0.49 ns
		1:7	**0.44 ns**
2539 x 2552	13:17 (*13*)	1:1	0.52 ns
		7:9	**0.009 ns**
		27:37	0.015 ns
2539 x 2939	16:23 (*29*)	1:1	1.26 ns
		7:9	0.13 ns
		27:37	**0.02 ns**
2939 x 2916	2:24 (*15*)	1:7	0.55 ns
		1:15	**0.086 ns**
2939 x Pummelo CS1	2:12 (*15*)	1:3	0.85 ns
		1:7	**0.05 ns**
		1:15	1.57 ns

* Number of hybrids that failed to flower in parentheses.

With variability in flowering time both between and within families, screening for seedless traits in this population may continue into the future depending on whether another project is funded and the extent to which resources available allow for detailed and time consuming pollination experiments. Regardless of whether or not this component of the research is funded into the future, however, this component of phase 1 evaluation has provided 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.

4.3.2 Progeny 01-101- (Imperial mandarin x Miho satsuma)

Progeny 01-101 (Imperial mandarin x Miho satsuma) was generated in project CT00012. This progeny has served many purposes in addition to screening and selection for potential new varieties, especially for the production of early, maturing seedless, easy-peel fruit with potential as Imperial substitutes. Both mandarin types yield early maturing parthenocarpic fruit and earlier work had shown that pollen sterility may be transmitted to their sexual progeny. This had indicated that a cross between Imperial mandarin and a Satsuma would yield valuable data to help verify some earlier observations concerning the inheritance of parthenocarpic fruit development. Previous data from other crosses suggested that the progeny from these two parthenocarpic parents would segregate 3:1 for autonomic parthenocarpy based on a three complementary dominant gene hypothesis.

The main issue with using these parents centered on Satsuma, which is pollen sterile, highly polyembryonic, yielding mostly nucellar seedlings, and characterised by frequent embryo abortion, a key trait leading to its seedless phenotype. This makes satsumas difficult to use as a parent; low seed numbers and polyembryony make it a poor female parent, while dysfunctional pollen generally precludes its use as a male. This latter characteristic, however, proved less of a barrier when in winter 2001 viable pollen was discovered on a Miho wase tree growing alongside a number of other varieties of Satsuma in the glasshouse at CSIRO Merbein. Thus, this cross was made in 2001 by serendipitously forcing early flowering in the Miho wase satsuma under glasshouse conditions causing the production of viable pollen, which was stored for a short term at 4°C until it was used to cross-pollinate Imperial mandarin flowers in the arboretum.

In addition to screening the hybrids within this progeny for key production and quality traits, they have 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. As fairly unique and interesting progeny, it was advantageous to multiply the individual hybrids early to ensure their survival and also provide replicate plants for experimental purposes, thus they were used to test Navarro’s recommendation under the conditions at CSIRO's experimental farm in NW Victoria.

4.3.2.1 Materials and Methods

Trees
Rather than establishing rootstocks in the breeding field and grafting the hybrids in-situ as suggested by Luis Navarro, they were budded to nucellar seedlings of Carrizo citrange (CC) and Symons sweet orange (SWO) under the same glasshouse conditions that the hybrids seedlings had been raised. To ensure tree age was as close as practicably possible, the rootstocks seeds were sown at the same time as the seeds from the Imperial mandarin parent. Grafted trees and the original hybrid seedlings were subsequently maintained under glasshouse conditions until the grafted trees were planted in the orchard during autumn 2006. Trees were heavily mulched with composted wood chips and irrigated via drippers. The planting design was such that each hybrid was established as randomized three-tree plots, i.e. one tree on own-roots (OR) and two trees grafted to the rootstocks (CC or SWO). Trees of the two parents grafted to CC and SWO were included in the planting for comparative purposes.

Flowering and fruiting
Trees were observed throughout the year for the development of flowers and subsequently fruit. Flowering time was recorded as years from seed germination.

Seedless traits
When flowering commenced, standard treatments (*viz*. limb bagging with insect proof mesh bags, emasculation without pollination, emasculation with controlled self-pollination, and open-pollination) were applied to assess for parthenocarpic ability. These treatments were applied for at least two seasons where possible within the constraints caused by hybrid juvenility and the timeframe of the project. The treatments were also applied to as many trees in each three-tree replicate plots for each hybrid.

Fruit yield and quality
Routine fruit sampling, harvesting and quality assessments were conducted in every season that the hybrids flowered and produced fruit. These measurements were recorded for every fruiting tree in each three-tree plot.

4.3.2.2 Results and discussion

Flowering and fruiting
As has been the case with other satsuma progenies generated at Merbein, where mean time to first flowering has been less than that for many other parent combinations, the first hybrids in this progeny flowered in 2008 and by spring 2010 only 5 of the hybrids had not flowered. Of those that flowered, all but 5 produced sufficient fruit for analysis.

The effect of rootstock vs. own roots on first flowering varied between hybrids and there was no clear advantage for either rootstock or own roots, although overall, more hybrids had flowered by spring 2010 on their own roots (80.1%) than on either rootstock (73% and 59.8% for SWO and CC, respectively).

Thus, at least for this Imperial x Miho family, there was no benefit achieved by grafting the hybrids to either of the rootstocks to promote earlier flowering. Indeed in terms of human resources available for the program, the use of own-rooted hybrids would be more efficient since time taken in dis-budding and removing suckers is eliminated. Similarly time expended in producing a grafted tree is avoided. There are occasions where grafting a new hybrid to a rootstock may be advantageous, e.g. in a replant situation where disease may be a factor in seedling survival, but in terms of using resources more efficiently, own-roots for initial or first phase evaluation of breeding progenies is probably more advantageous.

Seedless traits
The pollination experiments were designed to provide evidence for autonomic parthenocarpic fruit production. From this, 60 hybrids were shown to be parthenocarpic without the need for any external stimulation. There was no clear evidence for autonomic parthenocarpic fruit development for 46 hybrids, although there was evidence from self-pollination that 27 of these were capable of stimulative parthenocarpic fruit production. There was no evidence for any form of parthenocarpic fruit production for 19 hybrids. Under previous circumstances, these experiments would be conducted over several years so that each hybrid would be tested at least twice. However, with the end of the project and CSIRO's withdrawal from citrus research from December 2011 onwards, this is unlikely to occur. It may be justified, however, to assume that autonomic parthenocarpy would be demonstrated for some of the hybrids for which evidence was not obtained this year. Clearly the hybrids for which stimulative parthenocarpy was demonstrated have the capacity for seedless fruit and it may well be that some if not all of these are autonomic parthenocarps. With this assumption, segregation for parthenocarpic fruit based on just one year's data agreed with the 3:1 predicted ratio ($\chi^2 = 2.82$, p = 0.10-0.05).

Seventy seven and 21 hybrids produced seedless or only seeded fruit, respectively, under open-pollination. Of the 77 that yielded seedless fruit, 69 also produced seeded fruit under the conditions of open-pollination. Four hybrids yielded only seedless fruit regardless of whether or not pollination occurred.

Fruit yield and quality
While fruit developed on some trees in 2009 and 2010, only visual assessments were made for most of these in these years because priorities were directed to other progenies. Full tree harvests were conducted in 2011 and yields recorded. Fruit were sampled and fruit quality traits recorded.

Where fruit appeared promising in 2009 and 2010, samples were collected and fruit quality was assessed. From the data collected for 9 hybrids, 6 of which yielded seedless fruit under open-pollination, one (01-101-30; see figure 4.2) appeared promising and 2 trees were propagated to be established in the orchard for further evaluation. Fruit from hybrid 01-101-30 were large and seedless with better internal colour than either parent. These fruit were harvested during early May with juice sugar and citric acid concentrations of 9.9 – 11.5 °Brix and 1.0 – 1.2%, respectively.

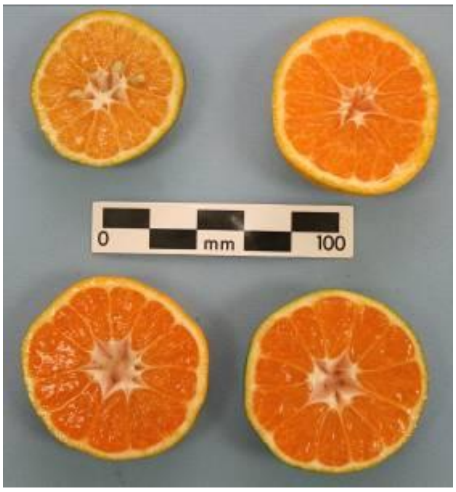


Figure 4.2. Seedless fruit harvested on May 1 2009 from an Imperial mandarin x Miho wase satsuma hybrid that shows promise as an early variety. The image shows fruit of the parents (Imperial top left, Miho wase top right) and of the hybrid (bottom left and right) that were collected from orchard-grown trees under conditions of high cross-pollination pressure.

Fruit yield and quality were measured for as many trees as possible during the 2011 harvest season. There were highly significant differences (P<0.001) between hybrids within the family for all yield parameters and quality characteristics measured. The only significant rootstock effect was for seed number, which is difficult to explain unless rootstocks affected flowering time and thus cross-pollination by different pollen sources. This, however, would be difficult to establish, especially as the data were for just one season and could potentially change in other years.

Table 4.3 presents progeny mean data for yield and fruit quality compared to the data collected for six trees of each parent grafted to sweet orange rootstock, which were planted alongside the Imperial x Miho progeny.

Table 4.3. Mean progeny values for a range of key traits compared to data for trees of the parents from a cross between Imperial mandarin x Miho wase satsuma for fruit harvested during 2011.

Trait	Progeny	Imperial mandarin	Miho wase
Yield (kg)	8.99 ± 9.7	25.03 ± 6.02	22.18 ± 12.40
Mean fruit weight (g)	150.2 ± 55.3	85.3 ± 8.3	140.4 ± 27.9
Rind colour	8.6 ± 0.5	7.8 ± 0.2	6.4 ± 2.1
Fruit diameter	74.8 ± 11.1	61.4 ± 2.6	70.2 ± 5.5
% Juice	26.9 ± 8.7	24.6 ± 2.1	41.5 ± 3.1
Rind thickness	6.1 ± 1.5	3.7 ± 0.2	3.8 ± 0.6
OP Seed number	3.2 ± 3.2	5.6 ± 27	0.0 ± 0.0
Juice Brix	10.2 ± 1.2	11.8 ± 08	9.2 ± 1.4
Juice % citrate	0.8 ± 0.3	0.7 ± 0.1	0.7 ± 0.1
Brix : Acid	14.9 ± 5.6	17.7 ± 2.4	13.8 ± 2.6

For each of the traits listed in Table 4.2, the variation between hybrids was large. Thus, the ranges in yield, fruit weight, percentage juice, open-pollinated seed number and juice sugar were 0.1-52.7kg, 40-351g, 5.8–46.8%, 0–15.5, and 7.5–15.3 ºBrix, respectively. These ranges indicated that potentially useful selections could be made from this progeny for both entry into second phase evaluation and also for parents. While it will be necessary for further data to be obtained before such selections can be considered, potential selections have been highlighted in Table 4.4 to indicate the possible value of this progeny to the breeding effort.

Table 4.4. Fruit characteristics for nine Imperial mandarin x Miho wase satsuma hybrids that with further data may have potential for entry into phase 2 evaluation trials. Data are means ± s.d. for three trees grown either on own roots or grafted to Carrizo citrange and Symons sweet orange. For most trees, 2011 was the first year they carried fruit. OP = open-pollinated.

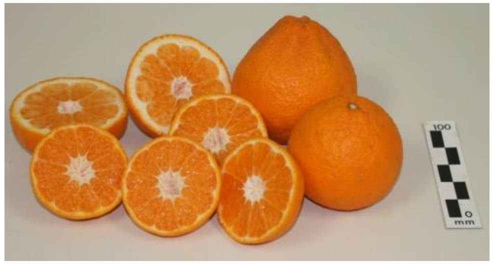
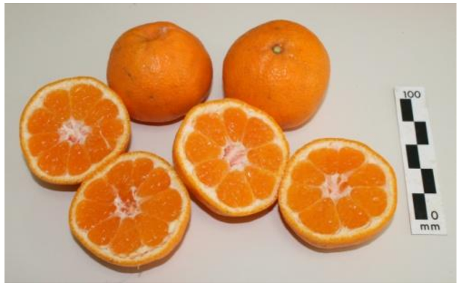
Trait	**Hybrid 01-101-14**	**Hybrid 01-101-16**	**Hybrid 01-101-17**
Image			
Yield (kg)	3.3 ± 2.6	6.8 ± 3.0	5.1 ± 3.7
Fruit weight (g)	169.7 ± 12.4	250.4 ± 26.0	167.2 ± 0.1
Rind colour[a]	8.8 ± 0.2	8.5 ± 0.5	8.3 ± 0.1
% Juice	37.3 ± 3.1	28.6 ± 3.8	40.1 ± 2.1
Rind thickness	3.9 ± 0.6	9.0 ± 0.5	4.0 ± 0.7
OP Seed number	7.7 ± 2.5	0.0 ± 0.0	1.5 ± 0.5
Juice Brix	10.8 ± 0.7	9.2 ± 0.2	11.0 ± 0.3
Brix : Acid	9.6 ± 0.1	15.8 ± 1.6	14.7 ± 2.9

Table 4.4, contd.			
Trait	**Hybrid 01-101-21**	**Hybrid 01-101-31**	**Hybrid 01-101-39**
Image			
Yield (kg)	14.2 ± 19.0	9.7 ± 8.9	22.2 ± 1.7
Fruit weight (g)	152.4 ± 10.0	88.9 ± 11.6	114.5 ± 62.7
Rind colour	8.6 ± 0.1	8.4 ± 0.2	8.8 ± 0.1
% Juice	18.6 ± 4.3	33.6 ± 3.3	40.5 ± 1.2
Rind thickness	6.5 ± 1.2	4.7 ± 0.5	4.1 ± 0.6
OP Seed number	0.0 ± 0.0	0.9 ± 0.2	2.4 ± 0.8
Juice Brix	10.8 ± 0.1	10.1 ± 0.5	10.5 ± 1.1
Brix : Acid	12.7 ± 3.9	7.6 ± 0.4	11.9 ± 1.0

Table 4.4, contd.			
Trait	**Hybrid 01-101-45**	**Hybrid 01-101-54**	**Hybrid 01-101-101**
Image			
Yield (kg)	22.2 ± 1.8	27.6 ± 4.7	5.8 ± 1.1
Fruit weight (g)	114.5 ± 36.4	169.4 ± 1.9	180.8 ± 16.8
Rind colour	9.0 ± 0.0	8.3 ± 0.3	8.8 ± 0.2
% Juice	24.5 ± 4.3	41.2 ± 0.3	38.8 ± 3.5
Rind thickness	5.8 ± 1.0	6.7 ± 0.1	6.6 ± 0.4
OP Seed number	1.2 ± 1.4	0.4 ± 0.6	1.3 ± 0.8
Juice Brix	12.5 ± 0.5	10.4 ± 0.1	10.0 ± 0.6
Brix : Acid	19.2 ± 2.26	9.4 ± 0.6	10.7 ± 2.6

[a] Rind colour was scored using a visual rind colour ranking based on a Japanese system (Yamazaki and Suzuki, 1980). The higher the number assigned to a fruit, the more intense the orange/red colour of the rind. Imperial mandarin would generally score a mean of 7.

4.3.3 New selections

Evaluation of hybrids growing on their own roots continued in CT07000. While the data collected provides information that can be used to investigate the inheritance of key traits, the main purpose of this work is to identify promising new recombinants for entry into second phase evaluation where selections are tested as grafted trees.

Previously, much effort has been devoted to harvesting complete trees in all families to obtain a complete as possible data set. Thus, hybrids have been harvested and analysed even though a quick subjective assessment of their fruit quality would eliminate them from being selected for the next phase of evaluation. This approach allows for the collection of complete data sets for each family. In CT07000, such complete and extensive analysis of each hybrid did not occur. There were two main reasons for this. The first was that with increasing numbers of trees being evaluated in phase 2 trials, insufficient time was available to harvest all hybrids in phase 1. Second, with the announcement by CSIRO in May 2008 that the Merbein laboratory was to be closed and that all CSIRO citrus research would stop, initially by June 30, 2009, but which was subsequently revised to December 31, 2011, there was a priority need to assess the hybrid families that were planted at Merbein so that preliminary selections could be made, propagated and retained. This was necessary as the sale of the site was to be made with all research plantings removed.

Again for brevity, details of the selections made are presented here only and other details concerning the different families are omitted. Selections were made from two main progeny groups being progeny groups 10 and 11, which have been described in the final report of previous HAL-funded projects.

Progeny group 10

As already described, progeny group 10 is a population of families from crosses conducted in CT96014 aimed at generating hybrids that segregate for seedless characteristics and provide further data to investigate the inheritance of these traits. This progeny group is largely from second generation crosses using parents bred and selected in the program.

Evaluation of this population continued in CT07000 as hybrids flowered and produced fruit. The hybrids were assessed for the characteristics that contribute to the seedless phenotype and fruit quality.

After season 2010, 39 hybrids were identified as possible selections for entry into phase 2 evaluation. Accordingly, these were propagated by grafting to Carrizo citrange rootstocks. Following the completion of data collection for these hybrids during 2011, this number was reduced to 24 hybrids and summary data for these are presented Table 4.5 with fruit and flower images presented in Figure 4.3.

From pollination experiments, all but three of the 24 hybrids selected are capable of yielding seedless fruit in the absence of cross-pollination, even though under breeding field conditions only a proportion have produced open-pollinated seedless fruit due to the high cross pollination pressure. At this stage, two trees of each of the 24 hybrids selected have been established in the orchard at CSIRO's experimental farm in NW Victoria for further investigation and potential use as parents in future breeding.

Table 4.5. Summarised fruit quality data collected in 2011 for 24 selected hybrids identified from Progeny Group 10 either for entry into further evaluation trials or for use as breeding parents. Data are mean ± standard deviation.

Selection code	Parents	Rind colour	Fruit diameter	Fruit weight	Open-pollinated seed number	Juice sugar (°Brix)	Evidence for parthenocarpy.
97-12-25	2939 x 2916	11.0 ± 0.0	38.5 ± 6.8	33.1 ± 14.6	0.3 ± 0.5	12.2 ± 1.4	Yes
97-22-26	88-13-15 x 2332	9.8 ± 0.4	68.0 ± 3.2	141.0 ± 14.4	9.2 ± 3.2	13.5 ± 0.4	Yes
98-12-01	Imperial x 2762	11.67 ± 0.5	58.0 ± 3.4	72.0 ± 10.7	5.7 ± 2.1	12.0 ± 0.7	?
98-10-11	Imperial x 2935	9.7 ± 0.5	58.2 ± 6.7	92.0 ± 24.4	13.7 ± 7.3	11.5 ± 0.6	?
98-07-10	Imperial x 2511	7.8 ± 0.4	57.5 ± 8.1	86.6 ± 24.7	9.3 ± 3.4	13.6 ± 1.2	Yes
96-02-02	Oroval x 2539	8.5 ± 0.6	73.0 ± 10.4	165.3 ± 63.8	3.5 ± 5.8	11.8 ± 1.3	Yes
96-06-16	Fina x 2539	7.0 ± 0.0	46.8 ± 2.9	35.0 ± 4.4	0.8 ± 1.3	11.5 ± 1.5	Yes
96-06-31	Fina x 2539	10.2 ± 0.4	51.3 ± 5.3	67.3 ± 14.9	7.0 ± 5.4	10.8 ± 0.6	Yes
96-06-34	Fina x 2539	10.0 ± 0.0	58.5 ± 4.4	67.0 ± 8.5	9.2 ± 6.7	13.6 ± 1.2	Yes
96-06-39	Fina x 2539	8.0 ± 0.0	58.2 ± 6.3	68.8 ± 15.5	9.6 ± 4.0	10.8 ± 1.2	Yes
96-20-01	Marisol x 2342	9.0 ± 0.0	66.3 ± 6.8	105.8 ± 29.4	2.2 ± 3.5	11.7 ± 1.4	Yes
96-34-04	Imperial x 2535	9.5 ± 0.7	53.5 ± 3.5	71.3 ± 11.6	10.0 ± 2.8	11.1 ± 0.7	Yes
96-15-01	? X ?	10.8 ± 0.8	66.3 ± 8.6	119.8 ± 30.6	8.5 ± 7.3	11.5 ± 0.5	Yes
96-16-09	Clementine x 2539	11.0 ± 0.0	66.3 ± 9.2	116.7 ± 35.4	7.0 ± 4.4	12.6 ± 1.0	Yes
96-16-35	Clementine x 2539	11.2 ± 0.4	52.5 ± 4.0	79.4 ± 18.1	5.8 ± 3.8	11.9 ± 0.9	Yes
96-42-01	2939 x 2336	11.5 ± 0.6	51.0 ± 5.0	70.2 ± 15.5	5.0 ± 7.0	12.4 ± 0.8	Yes
96-01-10	88-13-15 x 2332	7.8 ± 0.4	56.0 ± 6.8	67.1 ± 19.9	5.2 ± 3.3	11.6 ± 1.1	?
96-10-02	88-13-15 x 2939	8.5 ± 0.6	57.7 ± 6.0	86.1 ± 19.2	0.0 ± 0.0	12.5 ± 0.4	Yes
98-08-01	2539 x 2939	11.0 ± 1.3	66.0 ± 5.6	122.8 ± 21.9	0.5 ± 0.6	12.5 ± 1.1	Yes
96-11-07	88-13-15 x 2916	8.0 ± 0.0	63.0 ± 2.8	110.5 ± 10.8	0.3 ± 0.5	10.5 ± 1.4	Yes
96-108-01	88-02-21 x 2539	10.8 ± 1.0	75.5 ± 8.1	216.2 ± 59.9	15.0 ± 7.9	11.2 ± 0.4	Yes
96-14-08	2539 x 2552	10.7 ± 0.5	69.8 ± 4.9	118.9 ± 14.7	3.5 ± 3.6	13.6 ± 1.1	Yes
97-22-20	88-13-15 x 2332	9.0 ± 0.0	67.5 ± 5.7	150.5 ± 29.7	5.2 ± 4.4	11.0 ± 1.0	Yes
96-11-35	88-13-15 x 2916	9.0 ± 1.0	53.0 ± 4.6	76.1 ± 14.3	10.0 ± 8.2	11.7 ± 1.2	Yes

Figure 4.3. Images of fruit and flowers of 24 new selections from crosses made during 1996-98. While the original hybrids are still being maintained at CSIRO's experimental farm in NW Victoria, two daughter trees of each have been established grafted to Carrizo citrange rootstock for further evaluation

97-12-25 (2939 x 2916)

97-22-26 (88-13-15 x 2332)

96-10-02 (88-13-15 x 2939)


96-108-01 (88-02-21 x 2539)

96-11-07 (88-13-15 x 2916)

96-11-35 (88-13-15 x 2916)

Figure 4.3. contd.

96-14-08 (2539 x 2552)

97-08-01 (2539 x 2939)

97-22-20 (88-13-15 x 2332)

96-01-10 (88-13-15 x 2332)

96-02-02 (Oroval x 2539)

96-06-16 (Fina x 2539)

Figure 4.3. contd.

96-06-31 (Fina x 2539)

96-06-34 (Fina x 2539)

96-06-39 (Fina x 2539)

96-15-01 (? X ?)

96-16-09 (Clementine x 2539)

96-16-35 (Clementine x 2539)

Figure 4.3. contd.

96-20-01 (Marisol x 2342)

96-34-04 (Imperial x 2535)

96-42-01 (2939 x 2336)

98-07-10 (Imperial x 2511)

98-10-11 (Imperial x 2935)

98-12-01 (Imperial x 2762)

Progeny group 11

Progeny group 11 comprises families from crosses made during the period 1999-2002 aimed at generating new dual purpose (fresh fruit and juicing types) seedless sweet orange varieties and late maturing mandarins. The crosses were designed and made using hybrids selected from the program that possess seedless genes and produce large-fruited, monoembryonic tangors. These crosses were made in response to individual industry requests, the outcome of a series of grower fora held in 1999 and to address a key priority identified in the breeding plan, namely late maturing, easy-to-peel, seedless fruit.

The families from these crosses were planted at CSIRO, Merbein and at CSIRO's experimental farm in NW Victoria. As stated earlier, with the closure of the Merbein laboratory by December 31, 2011, there was a priority need to assess the hybrid families that were planted at Merbein so that preliminary selections could be made, propagated and retained. Thus, the progenies at Merbein were treated with priority and as individual hybrids flowered and fruited they were assessed for their potential for entry into second phase trials. Initially, this was conducted in a fairly subjective way after which those trees that had been identified as producing good quality fruit were harvested and their fruit analysed in the normal manner.

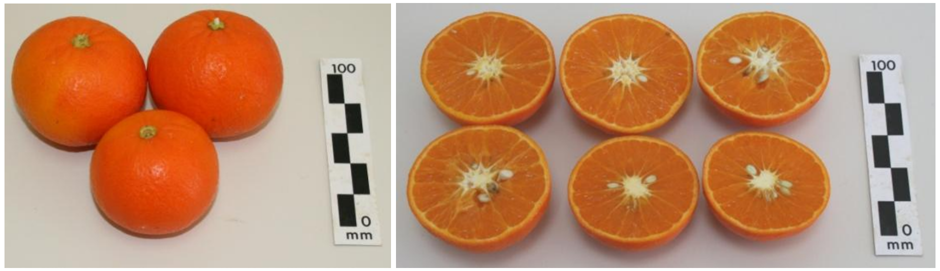
By the end of the 2011 harvest season, 19 hybrids had been identified from the part of the population planted at Merbein that displayed some potential for selection later for entry into phase 2 trials (Table 4.6). Fourteen of these hybrids (Figure 4.4) had been propagated successfully in previous seasons and were thus planted at CSIRO's experimental farm in NW Victoria for future observation. Five of the hybrids had proved difficult to propagate and at the time of preparing this report they remain under shadehouse conditions for further growth prior to also being established in the orchard.

Table 4.6. Characteristics of preliminary selections made from a population bred to select and develop new dual purpose (fresh fruit and juicing types) seedless sweet orange varieties and late maturing mandarins. These hybrids were established at CSIRO's Merbein farm and they were assigned priority for evaluation before the research station was closed in November 2011.

Code	Cross	COLOR	DIAM	WT	%juice	SEEDS	BRIX	Parthenocarpic
The following selections have been established in the breeding field for further evaluation:								
01-16-35	88-02-18 x 2916	9.9 ± 0.3	55.7 ± 10.3	69.0 ± 33.8	25.9	3. 7 ± 5.4	11.1 ± 1.2	Yes
01-36-02	88-02-21 x 2360	7.8 ± 0.4	62.2 ± 6.9	112.9 ± 28.1	41.4	12.8 ± 4.6	13.0 ± 0.5	?
01-37-13	88-02-21 x Encore	11.1 ± 0.3	62.7 ± 5.0	106.9 ± 21.9	45.4	4.2 ± 2.2	14.9 ± 1.6	Yes
01-37-24	88-02-21 x Encore	11.0 ± 0.0	58.7 ± 1.9	95.5 ± 9.7	47.1	1.5 ± 1.1	14.5 ± 2.1	Yes
01-38-32	88-02-21 x 2952	8.8 ± 1.2	64.8 ± 8.3	126.0 ± 43.3	51.8	7.8 ± 5.2	13.4 ± 1.6	?
01-39-04	88-02-21 x Fortune	10.8 ± 0.7	64.9 ± 2.5	130.9 ± 13.0	46.2	5.2 ± 4.5	13.6 ± 0.6	Yes
01-39-09	88-02-21 x Fortune	10.0 ± 0.5	51.9 ± 2.5	66.6 ± 10.6	39.9	4.0 ± 4.5	14.7 ± 0.8	Yes
01-39-15	88-02-21 x Fortune	10.4 ± 0.5	56.4 ± 4.5	88.8 ± 18.9	46.2	3.0 ± 3.3	15.3 ± 1.0	Yes
01-39-16	88-02-21 x Fortune	9.2 ± 0.5	45.8 ± 5.1	53.4 ± 16.9	39.6	2.8 ± 2.5	16.4 ± 1.1	Yes
01-39-23	88-02-21 x Fortune	10.0 ± 0.0	60.0 ± 1.3	95.3 ± 6.9	50.8	3.3 ± 4.1	15.3 ± 0.9	Yes
01-39-44	88-02-21 x Fortune	8.7 ± 0.5	55.3 ± 3.4	81.6 ± 13.1	56.4	0.3 ± 0.8	14.1 ± 0.8	Yes
01-39-51	88-02-21 x Fortune	8.7 ± 0.5	60.7 ± 3.4	121.0 ± 22.3	47.1	11.7 ± 8.1	13.5 ± 0.9	?
01-40-02	88-02-21 x 2751	9.8 ± 0.4	67.5 ± 5.4	140.9 ± 31.0	46.2	11.3 ± 3.7	12.0 ± 0.3	?
01-40-05	88-02-21 x 2751	9.0 ± 0.3	76.1 ± 5.3	183.0 ± 34.5	55.8	20.0 ± 6.7	12.6 ± 1.0	Yes
The following trees proved difficult to propagate and are still maintained in pots:								
01-16-40	88-02-18 x 2916	7.7 ± 0.5	53.8 ± 8.3	82.9 ± 31.3	33.8	13.9 ± 8.6	12.6 ± 1.2	Yes
01-17-07	88-02-18 x Fortune	10.2 ± 0.4	67.2 ± 4.8	142.6 ± 35.5	48.7	12.4 ± 8.2	11.6 ± 1.2	Yes
01-17-09	88-02-18 x Fortune	9.9 ± 0.8	62.1 ± 5.0	106.6 ± 23.1	45.0	5.8 ± 5.5	12.1 ± 0.8	Yes
01-39-34	88-02-21 x Fortune	9.9 ± 0.3	69.6 ± 4.3	140.3 ± 23.2	51.1	16.1 ± 4.5	11.7 ± 1.0	yes
01-17-04	88-02-18 x Fortune	10.2 ± 0.4	64.8 ± 6.9	146.4 ± 46.6	46.3	7.3 ± 6.4	12.0 ± 1.1	?

Figure 4.3. Images of fruit from preliminary selections made from a population bred to select and develop new dual purpose (fresh fruit and juicing types) seedless sweet orange varieties and late maturing mandarins. These hybrids were established at CSIRO's Merbein farm and they were assigned priority for evaluation before the research station was closed in November 2011. Daughter trees of these selections have been established at CSIRO's experimental farm in NW Victoria for further evaluation before final selections can be made.

01-37-24 (88-02-21 x Encore)

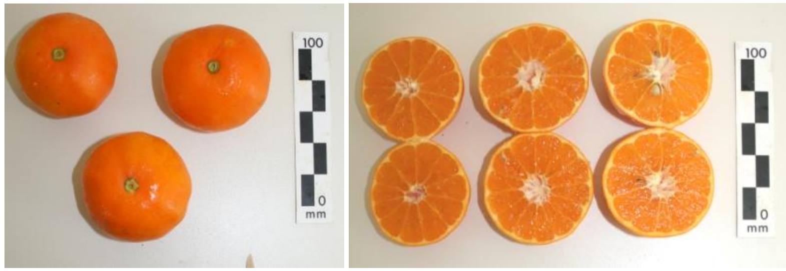


01-39-04 (88-02-21 x Fortune)

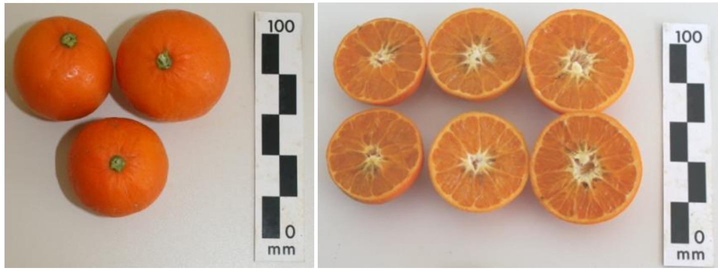
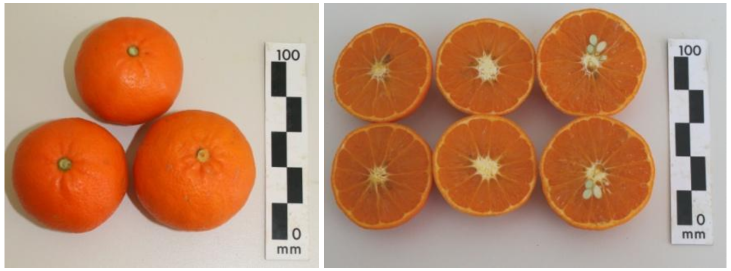
01-40-05 (88-02-21 x 2751)

01-16-35 (88-02-18 x 2916

01-39-51 (88-02-21 x Fortune)



01-39-44 (88-02-21 x Fortune)

 01-39-23 (88-02-21 x Fortune)	 01-39-16 (88-02-21 x Fortune)
 01-39-15 (88-02-21 x Fortune)	 01-39-09 (88-02-21 x Fortune)
 01-38-32 (88-02-21 x 2952)	Images unavailable for: 01-40-02 (88-02-21 x 2751) 01-36-02 (88-02-21 x 2360) 01-37-13 (88-02-21 x Encore)

4.4 Phase 2 evaluation

4.4.1 Introduction

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 under high density on their own root system. 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. Trials with these selections have been conducted on CSIRO land and also as regional based test plots with a network of cooperating citrus growers.

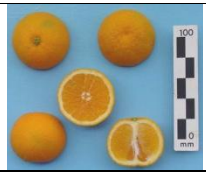
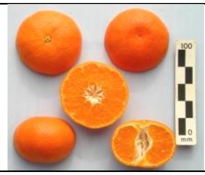
Before receiving nursery-propagated trees or bud wood for top-working, each grower signed an agreement, which covered a number of issues in relation to testing selections from the breeding program. The main function of this agreement is to emphasise a need to maintain confidentiality as well as prevent further propagation and distribution of the selections at this stage of their development.

In CT04007, it was agreed following discussions with the project reference group, that any hybrid considered to have potential after just one or two seasons 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 being worthy of entry into regional test plots and trees were propagated for distribution to cooperating growers willing to sign testing agreements. The characteristics of the selections (Table 4.7) were detailed in a booklet, which was provided to growers who expressed an interest in cooperating with their further evaluation.

This section of the diploid hybridisation chapter reports data collected from the trials at CSIRO with all the hybrid selections and those from five grower cooperator trials in the Murray and Murrumbidgee valleys. Data has yet to be collected from trials in Queensland as notification of tree growth and progress from the growers concerned did not warrant visits to these sites. Only nursery propagated trees were sent to Queensland and their dispatch was delayed at the request of the growers.

Table 4.7. Characteristics of the seven new hybrid selections established under testing agreements with grower cooperators for regional testing. The data presented were obtained from the original hybrid seedling trees growing on their own roots over at least three seasons.

	Selection A	**Selection G**	**Selection H**	**Selection I**	**Selection B**	**Selection C**	**Selection J**
							
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

4.4.2 Evaluation of 17 advanced selections from CT04007

4.4.2.1 Trials at CSIRO's experimental farm in NW Victoria

4.4.2.1.1 Trial background details

In CT04007, seventeen hybrid selections were identified from the breeding fields as own-rooted seedlings to have potential for future release as new varieties. 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 at CSIRO's experimental farm in NW Victoria. The nursery-propagated trees were planted during spring 2004 and both trials were maintained during CT04007 while evaluation of the original hybrids continued. These trials commenced flowering during 2006 and analysis of early data plus the additional data from the original own-rooted seedling trees led to seven of the selections being propagated for entry into regional evaluation test-plots with grower cooperators. Evaluation of the seventeen selections continued in CT07000 and the data are reported here.

4.4.2.1.2 Materials and methods

Nursery propagated trees - each of 16 selections was grafted to one of three rootstocks in the nursery and planted as two randomised blocks with each selection randomised as a three tree plot in each block. Each plot had a tree grafted to each of the three rootstocks. A seventeenth selection, 92-01-31, was not propagated in the nursery, but was only included in the top-worked component of this trial.

Top-worked trees - each selection was top-worked by budding into a new shoot of approximate pencil-thickness, which was generated by pruning the trees and allowing a new shoot to grow. The trees used for top-working were from a previous planting of Kara mandarin on Symons sweet orange rootstock. As mentioned in the previous paragraph, selection 92-01-31 was included in the top-worked component because fruit from the original tree varied in rind characteristics according to whether or not they contained seeds. Seedless fruit had delayed rind maturity based on colour development and their rinds were smoother. If this characteristic could be shown to be consistent for multiplied trees, it would be a useful marker to identify seedless fruits in the orchard. The purpose of including this selection was more for breeding purposes than for variety development at this stage.

Trees were harvested according to the results of fruit analysis of samples. These samples were from the previous season, which gave an indication of maturity time for the selections, and also from the current season, which were collected during the lead up period before the anticipated time for optimum maturity. Thus, harvest time was fine-tuned each season. At harvest, each tree was picked separately and fruit numbers and weight recorded. Fruit were snapped rather than clipped, which assessed their ease of harvest without rind damage or plugging. Mean fruit weight was calculated from tree yield and fruit number. At least one sub-sample of six fruit were retained from each tree at harvest and used to assess fruit quality, which was measured as percentage extractable juice, juice sugar, acid and sugar:acid ratio, seed number, rind thickness and coarseness, fruit shape, external and internal colour, and peeling ability.

As the trial was affected by limited water for irrigation during seasons 2007 and ‘08, only data collected for seasons 2009-2011 are presented after being analysed according to the experimental design.

4.4.2.1.3 Results and discussion

4.4.2.1.3.1 Nursery-propagated trees

Yield varied significantly (P<0.001) between selections and across years. As such mean yields per selection are presented for each year (Fig. 4.4a). Neither rootstock nor block had any significant effect on tree yields from 2009-2011. There was some evidence for year-to-year fluctuations in yield for some selections, e.g. 91-02-23, Selection I and 87-03-05, while others increased yield progressively over the 3 years, e.g. 87-03-26, Selection C and 88-22-55. Some selections failed to yield much in any year and should probably be eliminated from further evaluation, e.g. Selection H and 87-03-14. The highest yielding selection was Selection B in 2011, which was also promising for other characteristics recorded, although its yields in 2009 and 2010 were poor. Selection 87-03-26 was the highest yielding over the three years with Selection B second. Selections 87-03-26, B, G, I, 88-18-18, C, A and 87-03-05 all exceeded 20kg fruit per tree per year over the three seasons.

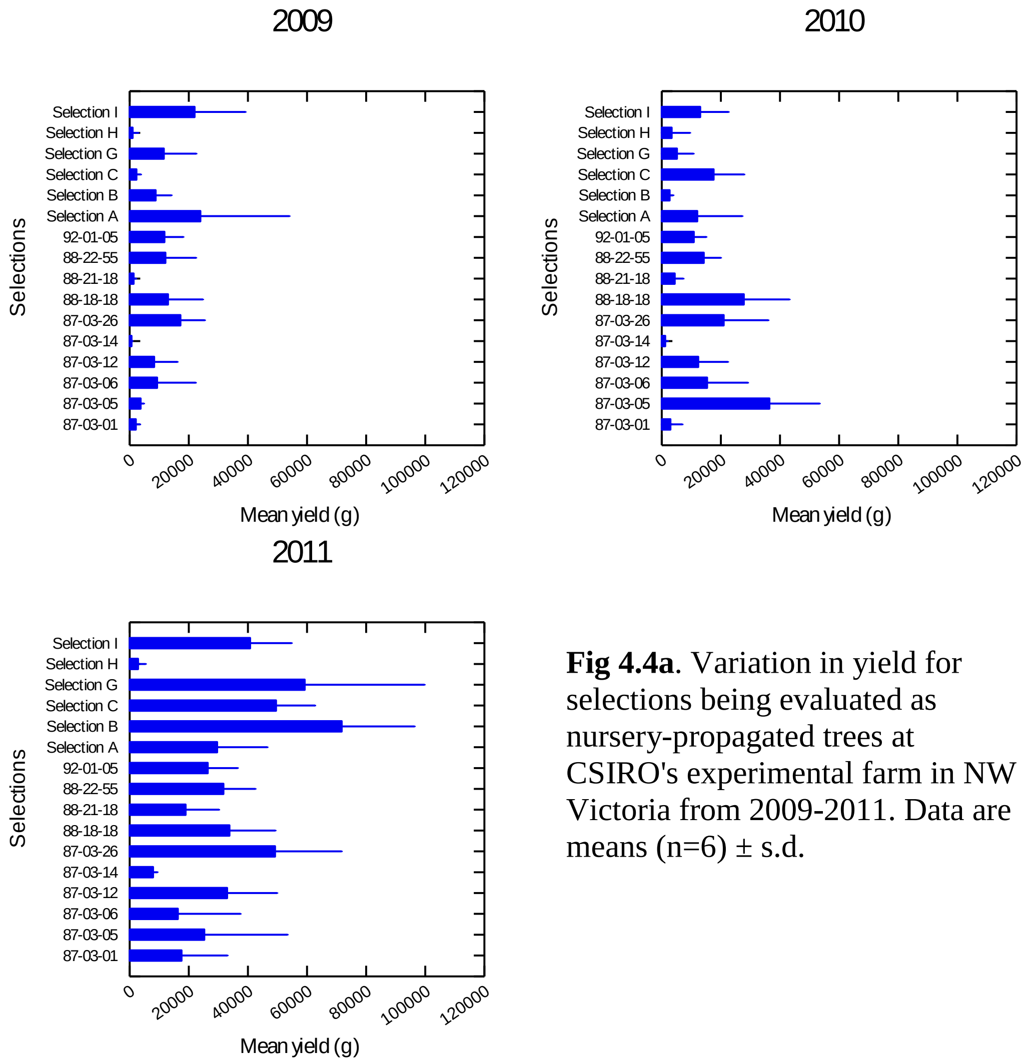
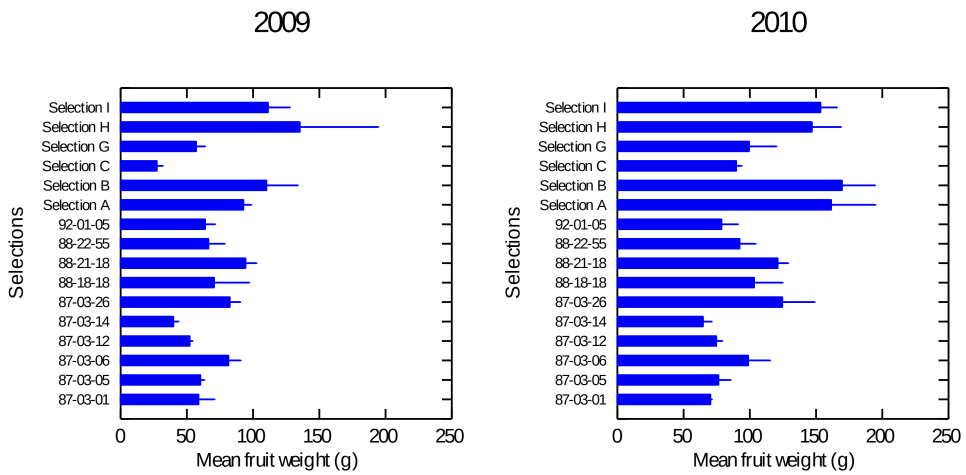


Fig 4.4a. Variation in yield for selections being evaluated as nursery-propagated trees at CSIRO's experimental farm in NW Victoria from 2009-2011. Data are means (n=6) ± s.d.

Mean fruit weight, as determined from the total crop harvested, also varied significantly (P<0.001) between selections and across years and similarly means are presented for each year (Fig. 4.4b). Fruit weight was generally greater for the selections in 2010 than in 2009 and 2011. Yields were higher in 2011, which may have affected fruit weight. Mean fruit weight ranged from around 50g for some selections up to greater than 150g for some selections in some years. Selection H produced the largest fruit, but also the smallest yields, which were probably connected. Neither rootstock nor block had any significant effect on fruit weight within selections in any year. The largest mean fruit across seasons were produced by selections H, I, B, A and 88-21-18, which all exceeded 100g per fruit per year.

There were significant (P<0.001) year-to-year variations in all fruit quality characteristics recorded and, thus, data are presented for each year. There were significant differences between selections for all fruit quality characteristics recorded between 2009 and 2011 and these are described in brief below. Rootstocks and blocks had minimal effect on fruit quality. There were significant (P<0.05) rootstock effects for rind thickness, juice sugar and juice acid in 2009, for percentage juice in 2010 and for seeds in 2011. Collectively, across all years, rootstocks had no significant effects on any fruit quality parameter recorded.



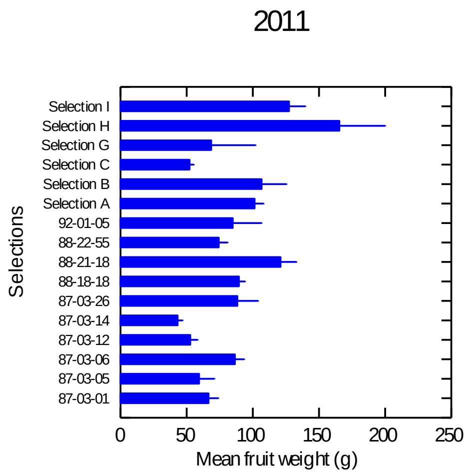


Fig 4.4b. Variation in mean fruit weight (g) for selections being evaluated as nursery propagated trees at CSIRO's experimental farm in NW Victoria from 2009-2011. Data are means (n=6) ± s.d.

Open-pollinated seed numbers varied between selections and years ranging from less than one for selection A in 2009 to in excess of 20 for 87-03-05 in 2010 (Figure 4.4c). All selections had been chosen based on their ability for parthenocarpic fruit development, but clearly in a situation where many pollen sources exist and cross-pollination was not prevented, seediness was greater than expected for some selections. Selections A, 87-03-01, G, 87-03-12, B, H and C were the most consistent with regard to low open-pollinated seed numbers. There were significant block effects for seed numbers in 2010 and 2011, which may indicate pollen flow availability due to adjacent and various pollen sources, but it was beyond the scope of normal sampling of harvested fruit to determine if this was indeed a factor.

Percentage extractable juice varied significantly (P<0.001) between years and selections (Figure 4.4d). Most selections produced fruit with more than 30% by weight juice extracted by a single half-fruit rotating reamer. Selection 88-21-18 consistently produced the juiciest fruit while selection 88-03-14 produced fairly dry fruit in 2010 and 2011. Fruit harvested in 2011 were generally less juicy than fruit from the previous two years.

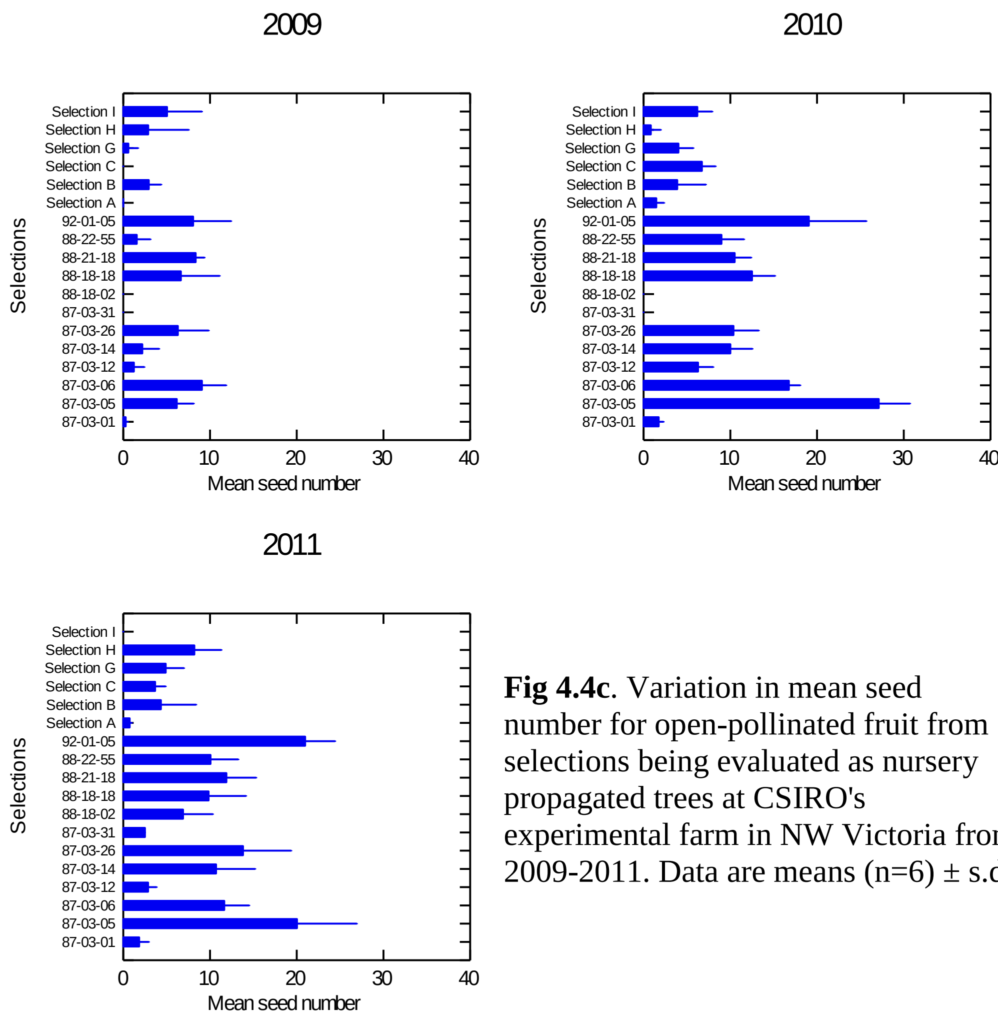


Fig 4.4c. Variation in mean seed number for open-pollinated fruit from selections being evaluated as nursery propagated trees at CSIRO's experimental farm in NW Victoria from 2009-2011. Data are means (n=6) ± s.d.

Juice sugar concentrations (Figure 4.4e) varied significantly between selections (P<0.001) and years (P<0.05), but were unaffected by rootstock or position (block). Selection 87-03-05 had the lowest juice sugar concentrations while 88-22-55, 88-21-

18, 87-03-01, B, 87-03-06 and 92-01-05 were consistent with juice sugar exceeding 12° Brix each season.

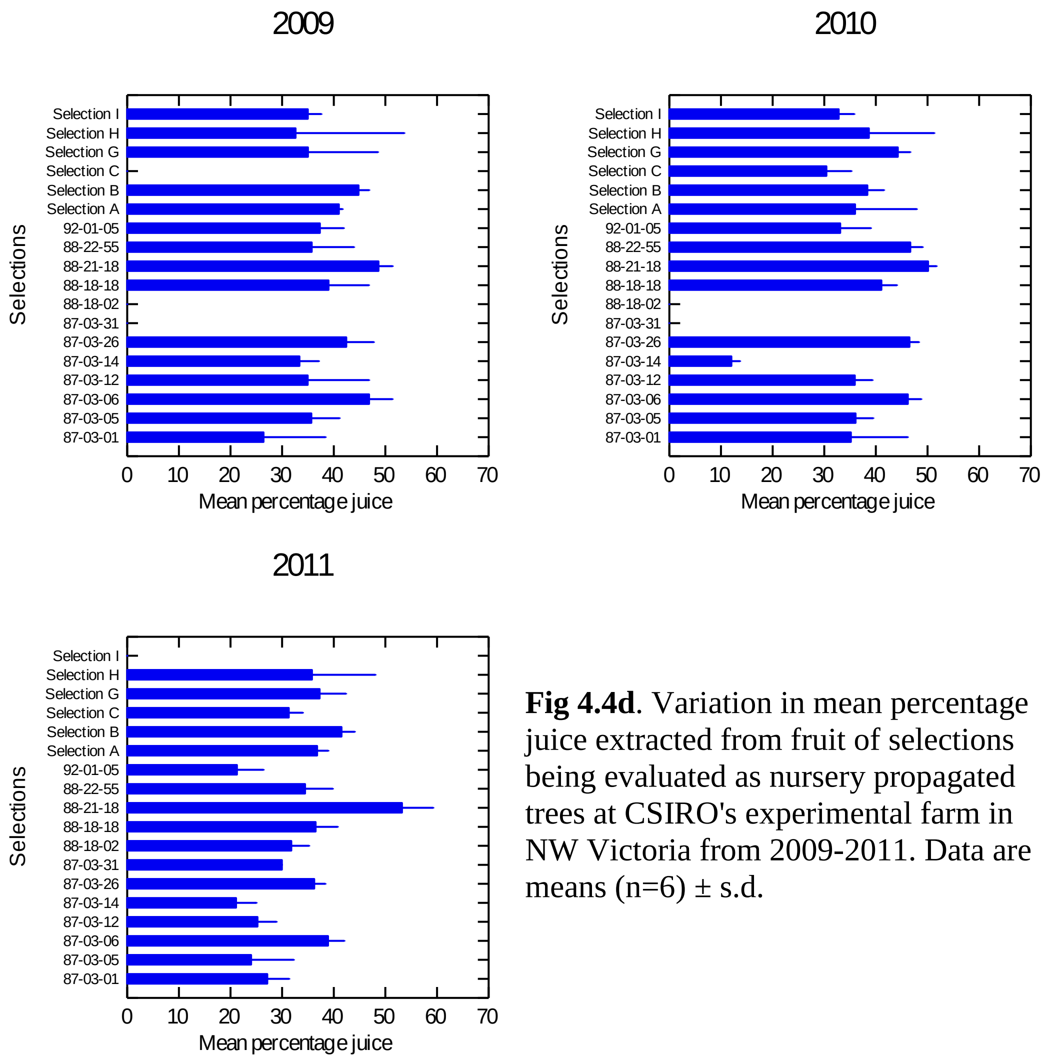
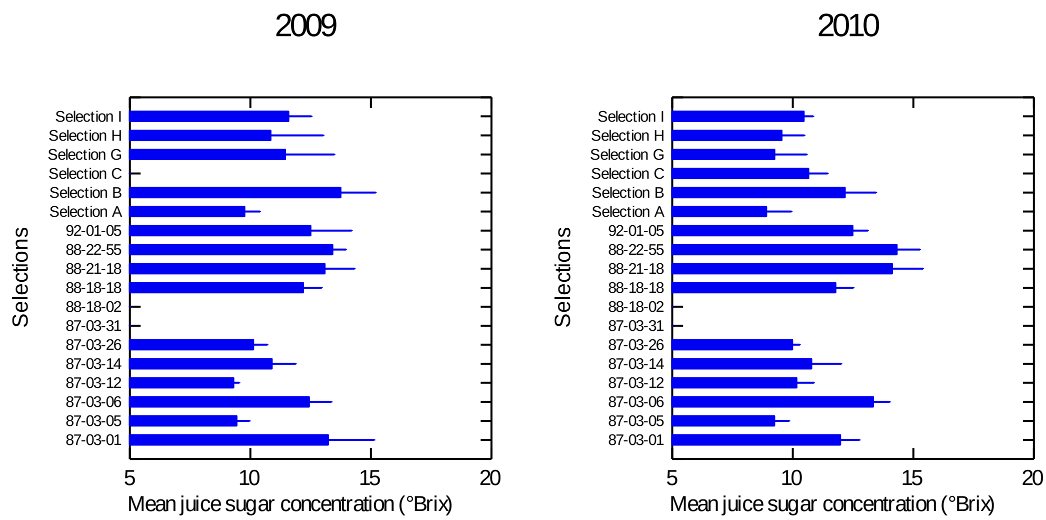


Fig 4.4d. Variation in mean percentage juice extracted from fruit of selections being evaluated as nursery propagated trees at CSIRO's experimental farm in NW Victoria from 2009-2011. Data are means (n=6) ± s.d.

By ranking the mean values for the data collected without any weighting, the best performing selection over the three years was B. Selection H came second based on fruit quality, yet its poor yield over the three years would question a need to pursue this hybrid. It may, however, be worthwhile persisting with as a parent for its quality attributes. Based on the collective data, some selections would not warrant much further evaluation, but when their season of maturity is considered, then persistence with them is warranted. An example here is selection C, which matures its fruit at the end of March-to-early April in the Murray Valley. Though selection C produces small fruit, it had thin, robust rinds and produced sweet fruit with low seed numbers. With the earliest maturing fruit, selection C needs further evaluation especially as with judicious thinning, which was conducted at one of the grower properties (see later), fruit size was increased significantly without impacting on yield.

From the trial with nursery-propagated trees, further evaluation is warranted for B, C, A, G and I.



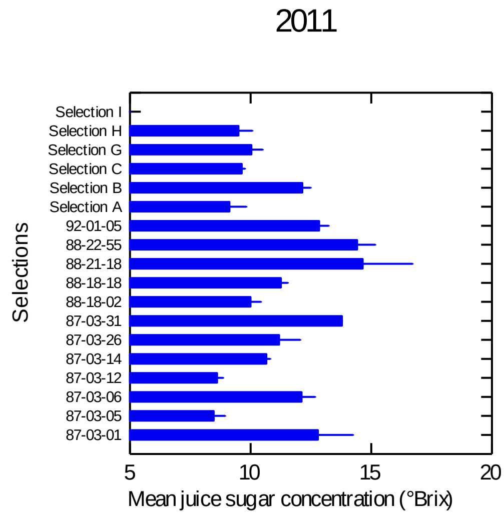


Fig 4.4e. Variation in mean sugar concentration of juice (ºBrix) extracted from fruit of selections being evaluated as nursery propagated trees at CSIRO's experimental farm in NW Victoria from 2009-2011. Data are means (n=6) ± s.d.

4.4.2.1.3.2 Top-worked trees

As for the nursery-propagated trees, yield varied significantly (P<0.001) between selections and across years. As such, mean yields per selection are presented for each year (Figure 4.5a). Again, as for the nursery-propagated trees, there were no significant effects of blocks on tree yields from 2009-2011. There were year-to-year fluctuations in yield for some selections, e.g. 91-01-05, C, 87-03-06, 87-03-12, 91-02-23, 92-01-31, 88-18-18, 88-21-18, 88-22-55 and 87-03-05, while others increased yield progressively over the 3 years, e.g. 87-03-26, 87-03-01, G, I, H and A. In these respects there were some differences between top-worked and nursery-propagated trees for some selections. Some selections failed to yield much in any year and should probably be eliminated from further evaluation, e.g. 87-03-01, H and 87-03-14. Yields for top-worked trees were generally lower than for nursery-propagated trees over the three seasons, with only three selections 88-22-55, 88-18-18 and 88-03-26 exceeding 20kg fruit per tree per year over the three seasons.

Mean fruit weight, as determined from the total crop harvested, also varied significantly (P<0.001) between selections and across years, and similarly means are presented for each year (Figure 4.5b). Fruit weight was generally greater for the selections in 2010 than in 2009 and 2011 and this was to a degree associated with crop load, although mean fruit weight was greater in 2011 compared to 2009, which was not associated with crop load. This may have been more a factor of tree age and thus canopy volume. The location of the trees in the trial (block) had no effect on fruit weight within selections in any year. The largest mean fruit sizes (across seasons) were produced by selections H, I, B and 88-21-18, which all exceeded 100g per fruit per year.

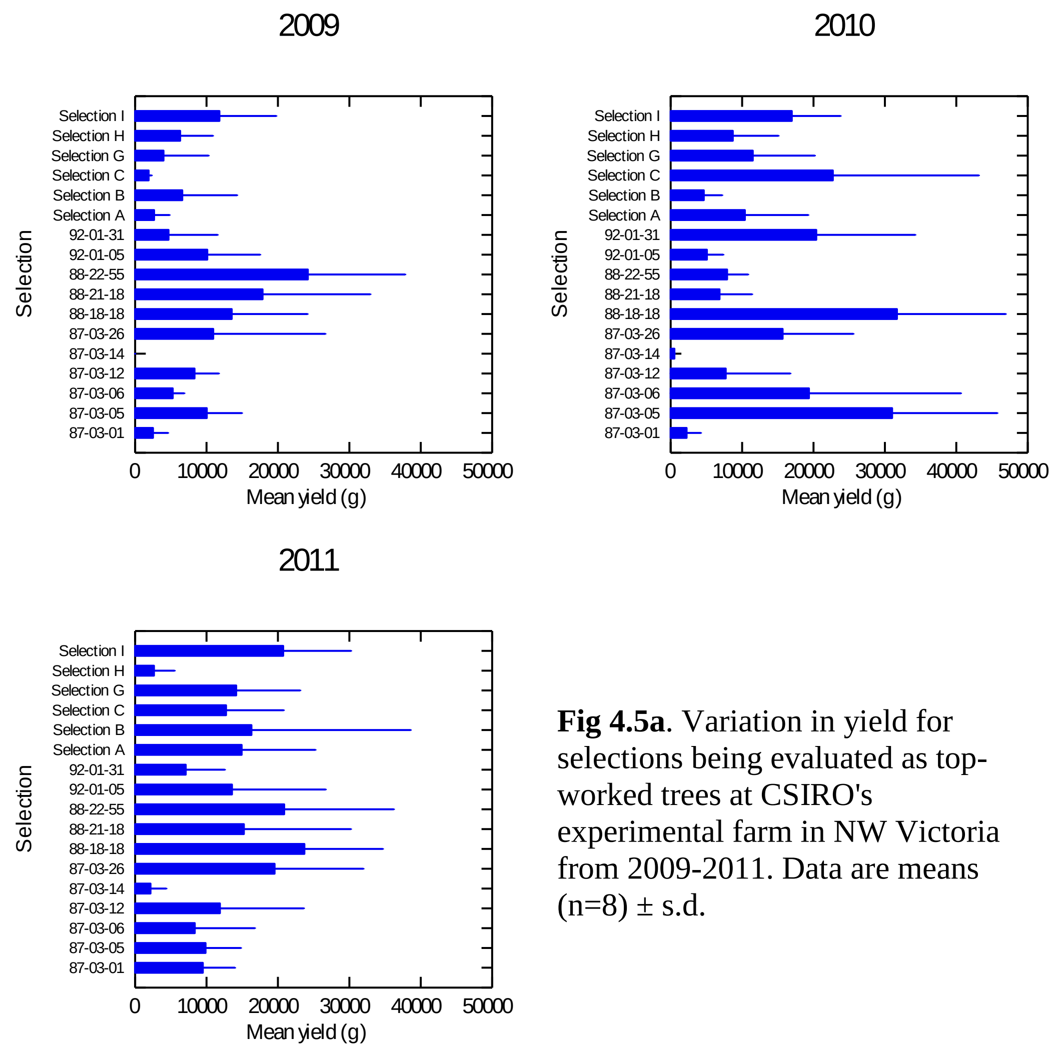
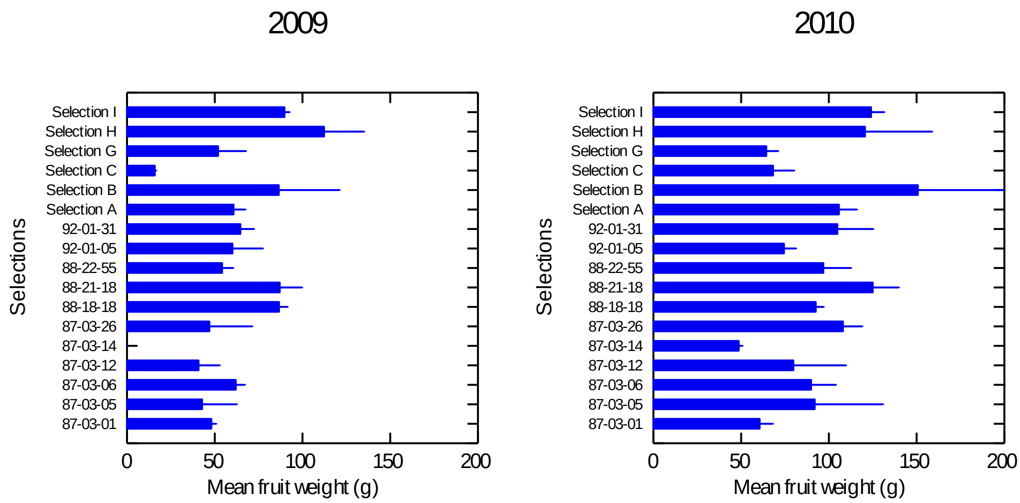


Fig 4.5a. Variation in yield for selections being evaluated as top-worked trees at CSIRO's experimental farm in NW Victoria from 2009-2011. Data are means (n=8) ± s.d.

As with the nursery-propagated trees, there were significant (P<0.001) year-to-year variations in all fruit quality characteristics recorded and, thus, data are presented for each year. There were significant differences between selections for all fruit quality characteristics recorded between 2009 and 2011 and these will be described in brief below. Location (blocks) had minimal effect on fruit quality in most years, but there were significant (P<0.05) block effects for sugar:acid ratios in 2009, for fruit diameter, fruit weight and percentage juice in 2010 and for seeds (P<0.01) in 2011.



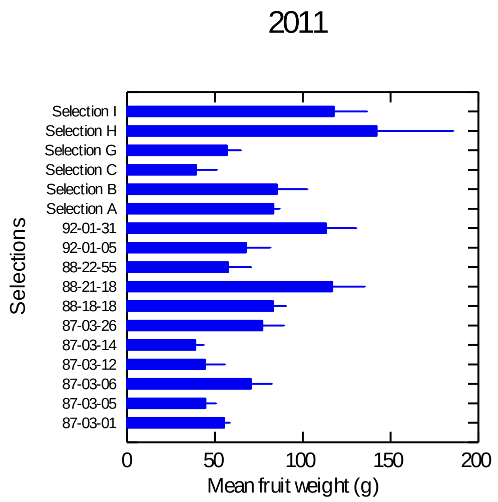
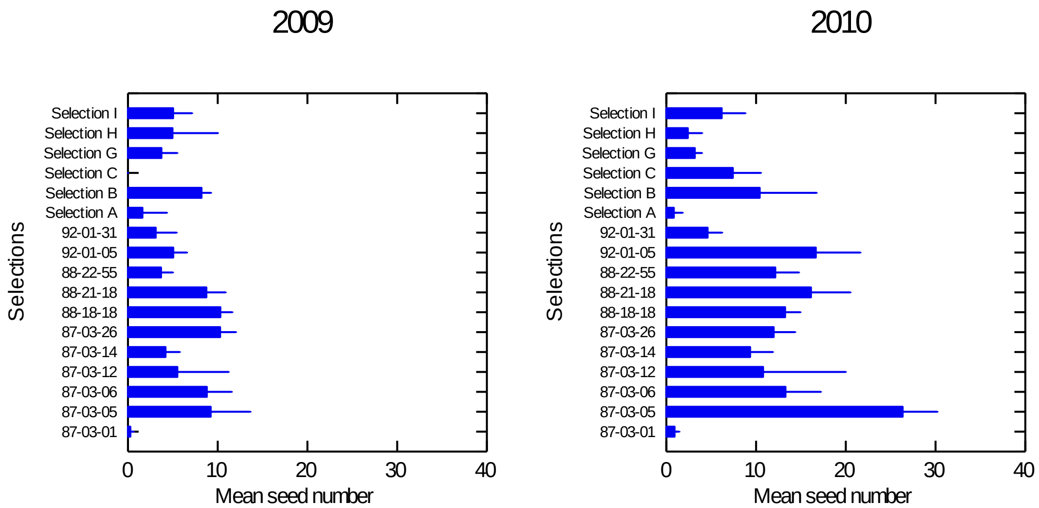


Fig 4.5b. Variation in mean fruit weight for selections being evaluated as top-worked trees at CSIRO's experimental farm in NW Victoria from 2009-2011. Data are means (n=8) ± s.d.

Open-pollinated seed numbers varied between selections and years ranging from less than one for selections 87-03-01 and A in 2009 to in excess of 26 for 87-03-05 in 2010 (Figure 4.5c). All selections had been chosen based on their ability for parthenocarpic fruit development, but clearly in a situation where many pollen sources exist and cross-pollination was not prevented, seediness was greater than expected for some selections. Selections A, 87-03-01, G, 87-03-12, B, H, C and 92-01-31 were the most consistent with regard to low open-pollinated seed numbers. There were significant block effects for seed numbers in 2011, which again may have indicated changes in pollen flow between years due to adjacent and various pollen sources, but it was beyond the scope of normal sampling of harvested fruit to determine if this was indeed a factor.

Percentage extractable juice varied significantly ($P<0.001$) between years and selections (Figure 4.5d). Most selections produced fruit with more than 30% by weight juice extracted by a single half-fruit rotating reamer. Selections 87-03-26 and 88-21-18 consistently produced the juiciest fruit while selection 92-01-31 produced fruit with the lowest percentage of extractable juice. As with the nursery-propagated trees, fruit harvested in 2011 were generally less juicy than fruit from the previous two years.



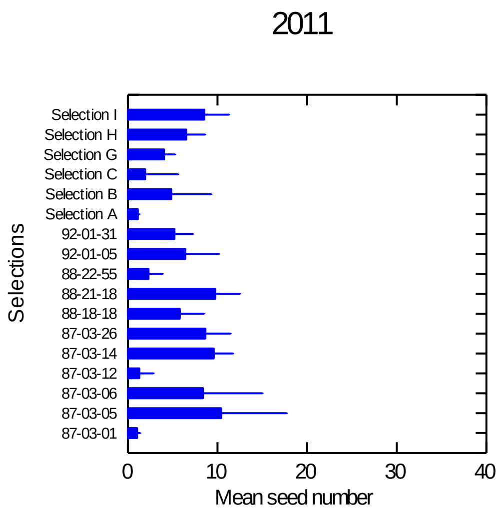


Fig 4.5c. Variation in mean seed number for selections being evaluated as top-worked trees at CSIRO's experimental farm in NW Victoria from 2009-2011. Data are means (n=8) ± s.d.

Juice sugar concentrations (Figure 4.5e) varied significantly between selections (P<0.001) and years (P<0.05), but were unaffected by position (block). As with the nursery-propagated trees, selection 87-03-05 had the lowest juice sugar concentrations while 88-22-55, 88-21-18, 87-03-01, B, 87-03-06, 92-01-05, 92-01-31 and 88-18-18 were consistent between seasons with juice sugar in each year exceeding 12° Brix.

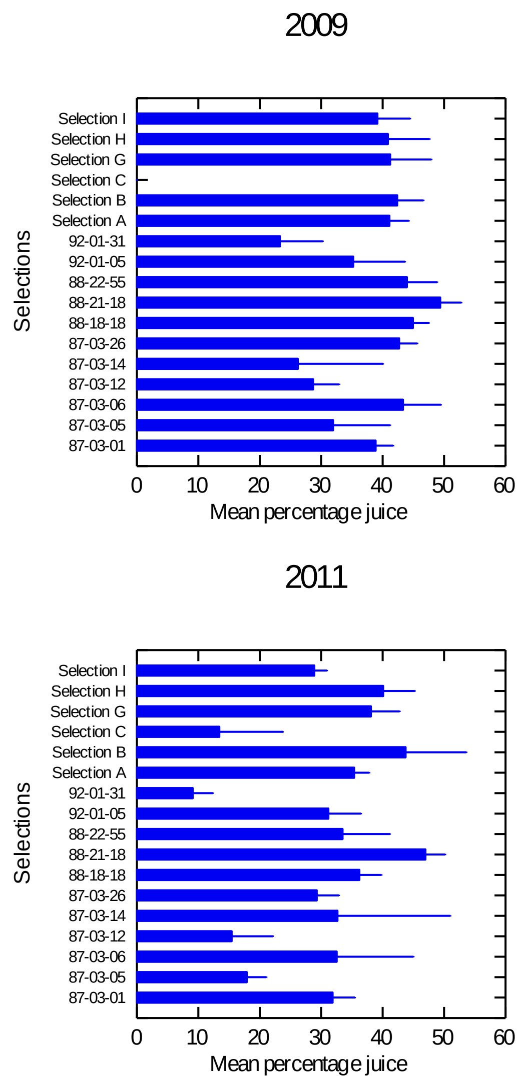
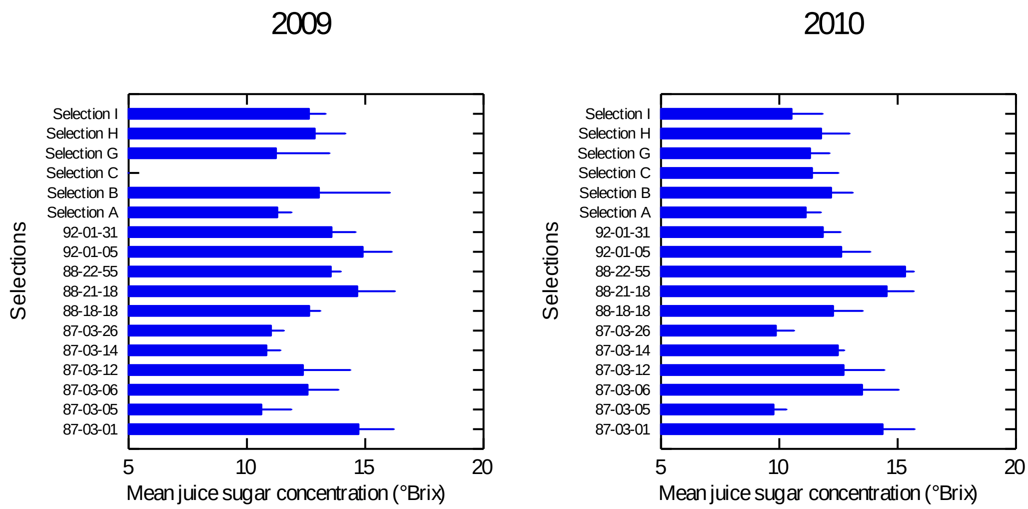


Fig 4.5d. Variation in mean percentage juice extracted from fruit of selections being evaluated as top-worked trees at CSIRO's experimental farm in NW Victoria from 2009-2011. Data are means (n=8) ± s.d.

By averaging and ranking means across all quality data, 88-21-18 was the best performing selection. However, when performance was assessed just on fruit size, % juice, seediness and juice sugar concentration, selection H was ranked first ahead of 22-21-18, A, 88-18-18, 92-01-31, I, 87-03-26 and B.

Again, based on its very early ripening, Selection C, with its acceptable juice sugar concentration, low seediness (it is parthenocarpic and pollen sterile and, thus, seedless when isolated from viable pollen), deserves continued evaluation for the reasons already discussed. The small fruit size of Selection C is a disadvantage, but further developmental work on controlling crop load to improve size would be warranted on the basis that it outperforms all other available germplasm on earliness. Selection C is very strongly parthenocarpic and sets a high proportion of it flowers. The use of winter GA application to promote vegetative over reproductive growth may be beneficial, especially as it would not be carrying fruit when spraying would be optimal.

Interestingly and as already described, during the latter stages of project CT04007, 17 selections were identified warranting further evaluation and by the start of CT07000, 6 of these were selected for entry into regional grower-based trials. This approach was pursued due to a perceived need to speed up the entry of the most promising material into industry-based testing. From the results for fruit quality presented here for the second phase replicated trial with these 17 selections based at CSIRO's experimental farm in NW Victoria, the inclusion of selections H, I and 91-01-23 were justified. The early maturing and seedless characteristics of selections C and A also justified their inclusion in grower trials. The low yield of selection H, however, suggests that its inclusion in the trials on the basis of the performance of the original own-rooted hybrid was not justified. Conversely, the performance of some of the other selections within this group of seventeen, suggests that selections 88-21-18, 87-03-26 and 88-18-18 should have been included in grower-based trials. Fortunately these selections have been maintained in the CSIRO-based trial and could be available for further evaluation in grower-based trials.



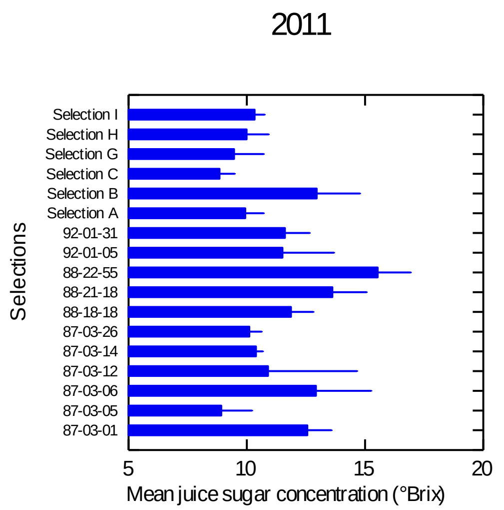


Fig 4.5e. Variation in mean sugar concentration of juice (°Brix) extracted from fruit of selections being evaluated as top-worked trees at CSIRO's experimental farm in NW Victoria from 2009-2011. Data are means (n=8) ± s.d.

4.4.2.2 Trials with grower cooperators

For entry into grower-based test-plots, trees of seven selections (Table 4.7) were budded to each of the three rootstocks used in the nursery-propagated trees in the trial at CSIRO's experimental farm in NW Victoria for distribution to grower cooperators, who 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 some new growers joining the network and after signing the non-propagation and non-distribution testing agreement. Nursery propagated trees or bud wood of the seven selections were distributed during spring 2007. Due to the water allocation restrictions for irrigation in the Murray Valley, some growers delayed tree receivals until 2008.

4.4.2.2.1 Trial details

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 in the numerical order of their codes. 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 discussed in the final report for CT04007. Of particular note in assessing tree performance, the location of trees relative to pollen sources was something that was out of the control of the project team and this would clearly have a bearing on seed numbers in the different selections.

4.4.2.2.2 Materials and methods

Six growers were enlisted to assist with the second phase evaluation of the 7 selections offered and trees or bud wood were distributed (Table 4.8).

Table 4.8. Location and the dates that trees or bud wood were distributed to grower cooperators for second phase trials involving seven selections made during CT04007.

Site	Location	Year trees/buds dispatched[a]
1	Lower Darling Valley Irrigation region, NSW	November 2007
2	Lower Darling Valley Irrigation region, NSW	November 2007
3	Mid-Murray Irrigation region, Vic.	November 2007
4	Murrumbidgee Irrigation Area, NSW	November 2007
5	Central Burnett Irrigation region, Queensland	March 2008
6	Emerald citrus growing region, Queensland	May 2009

[a] Though dispatched on these dates, some growers decided to hold trees in their own nursery area until more favourable planting conditions occurred.

The grower at site 1 also received buds to top work trees of each selection in addition to planting nursery-propagated trees. All growers, except the grower at site 4, received all seven selections offered. The grower in the MIA only received trees of selections A, H and J. This latter selection (J) was offered in the seven selections even though it had not been included in the trials at CSIRO. The performance of the original hybrid during the period between establishing the trials at CSIRO and distributing selections to growers suggested that it should be entered into second phase evaluation.

Trees were inspected at least once a year in the southern regions and from these it was agreed to allow the trees to grow vegetatively until spring 2010 when any fruit set following flowering were allowed to remain for harvesting in 2011. This delay in allowing fruit to be retained by the trees was in part due to the effects of reduced irrigation allocations during the severe drought experienced up until 2010. Water restrictions in the Murray-Darling basin meant that trees did not perhaps develop to their full potential in their first two years after planting. The team relied on advice from the Queensland growers when trees carried sufficient fruit to warrant a visit to harvest them. As at 2011, no advice had been received and it is anticipated that these tress will carry fruit in 2012.

Thus, the first harvest of these seven selections from grower properties occurred in 2011 and consequently only one year's data are presented here. Depending on the future of the research, ideally fruit should be harvested from these trees for at least a further 3 years to evaluate their performance.

At maturity, trees were harvested individually and fruit weight and number recorded. Fruit quality data were collected from samples of 6 fruit from the mid-size range for each tree and the data averaged across all trees at each site. As fruit fly restrictions prevented the return of whole fruit from the MIA site to the laboratory at Merbein, juice samples were collected in the orchard and returned to the laboratory for analysis.

4.4.2.2.3 Results and discussion

Due to the nature of the grower-based test plots, it is difficult to compare the results from different sites. Thus, the data have been presented as means for each selection at each site.

Not all trees at all sites produced fruit in 2011 (Table 4.9). This was perhaps a function of being very young bud lines in which some buds collected to propagate daughter trees may have been more juvenile than others. This would result in varying degrees of daughter tree precocity giving variable yields in the first years of production within a batch of trees. Fruit were harvested from all nursery propagated trees of selection A at all sites. Interestingly, the 2 top worked trees of selection A at site 1 failed to produce fruit, which was contrary to expectations. All nursery propagated and top worked trees of selection C produced fruit, although this selection was not established at the MIA site. The only selection that failed to produce fruit at any site was selection B at site 3. Otherwise, fruit were harvested from at least one tree for each selection at all sites at which they were established.

The most productive selections were A, which was quite consistent across sites, C and J. Selection H was the least productive, which was also the case for the trees in the two trials at CSIRO's experimental farm in NW Victoria.

While the yields that the trees produced would have affected fruit size, especially for H, which, as in the CSIRO-based trials, had low yields, the ranges in fruit size between the selections in the grower-based trials was similar to those seen for the trees at CSIRO. Selection H produced the largest mean fruit weight, although as stated it was the least productive and if data from subsequent years do not show an improvement, it is probably not worthwhile persisting with this hybrid. Fruit weight for selections A, G, I and B were in an acceptable range and these hybrids were ranked in a similar order as for the CSIRO-based trials.

Table 4.9. Yield and fruit weight for seven selections at 4 grower-cooperator sites in the irrigated regions. Data are presented as means ± sd.

Selection	Site[a]	Harvest date	Tree type	Rootstock[b]	No. trees yielding fruit	Yield (kg)	Mean fruit wt. (g)
A	1	25/05/2011	NP	CC	5/5	12.6 ± 6.6	115.8 ± 3.9
	2	25/05/2011	NP	CC	10/10	4.2 ± 4.0	107.7 ± 14.1
	3	13/04/2011	NP	CC	5/5	14.2 ± 9.8	87.5 ± 3.6
	4	06/07/2011	NP	Cleo	5/5	5.4 ± 4.6	118.8 ± 10.1
	4	06/07/2011	NP	CC	5/5	9.5 ± 4.7	121.6 ± 13.6
G	1	13/09/2011	NP	CC	5/5	2.6 ± 3.3	105.8 ± 11.5
	2	13/09/2011	NP	CC	6/10	2.2 ± 4.5	102.7 ± 24.6
	3	14/09/2011	NP	CC	5/5	2.2 ± 1.7	79.6 ± 21.6
H	1	2/08/2011	TW	Val/CC	1/2	4.0	284.3
	2	2/08/2011	NP	CC	1/10	0.2	235.0
	3	4/08/2011	NP	CC	4/5	0.3 ± 0.3	149.7 ± 23.3
	4	06/07/2011	NP	Cleo	1/5	0.1	149.7
	4	06/07/2011	NP	CC	1/5	0.5	151.2 ± 5.6
I	1	6/07/2011	NP	CC	4/5	1.4 ± 0.7	163.0 ± 25.2
	2	6/07/2011	NP	CC	5/10	4.1 ± 3.9	121.5 ± 60.5
	3	7/07/2011	NP	CC	5/5	1.7 ± 1.9	123.8 ± 8.3
C	1	12/04/2011	NP	CC	5/5	13.8 ± 3.1	61.6 ± 8.1
	1	12/04/2011	TW	Val/CC	2/2	24.0 ± 33.0	72.0 ± 18.3
	2	12/04/2011	NP	CC	10/10	4.5 ± 2.6	67.4 ± 8.9
	3	13/04/2011	NP	CC	5/5	8.4 ± 5.4	58.3 ± 2.5
J	1	14/06/2011	NP	CC	4/5	5.7 ± 3.3	103.7 ± 13.4
	1	14/06/2011	TW	Val/CC	2/2	16.4 ± 22.9	51.6 ± 47.2
	2	15/06/2011	NP	CC	8/10	5.8 ± 3.5	103.3 ± 14.0
	3	16/06/2011	NP	CC	5/5	8.8 ± 5.2	95.9 ± 13.0
	4	06/07/2011	NP	Cleo	9/10	3.4 ± 3.0	106.7 ± 9.6
B	1	19/08/2011	NP	CC	5/5	5.8 ± 8.2	172.1 ± 15.9
	2	19/08/2011	NP	CC	9/10	1.9 ± 2.0	188.7 ± 13.3

[a] Sites:

1 – Lower Darling Irrigation area site 1
2 – Lower Darling Irrigation area site 2
3 – Mid-Murray Irrigation area site
4 – MIA site

[b] Rootstock

CC = Carrizo citrange; Cleo = Cleopatra mandarin; Val/CC = Valencia interstock on Carrizo citrange rootstock.

Selection C again produced the smallest fruit, but again its earliness coupled with other quality traits indicate that it needs to be persisted with and efforts made to improve its fruit size. One of the nursery-propagated trees at site 1 was thinned in mid-January 2011 such that approximately 60% of the fruit was removed. Though the yield of this tree was not different relative to the other 4 trees, the mean weight of the fruit harvested was somewhat larger (Table 4.9). While this was a simple and non-replicated test, it suggested that further work would be warranted with this selection to improve fruit size as discussed earlier.

Selection J, which was not included in the CSIRO-based trials, produced the second smallest fruit with a range between sites of 52-107g, although the smallest fruit were produced on the two top-worked trees at site 1. Mean fruit weights for nursery-propagated trees at all sites were acceptable, however. One of the top-worked trees of this selection at site 1 produced only 11 small fruit with a mean weight of 18g compared to the other top-worked tree that had a yield of 32.5kg with a mean fruit weight of 85g. It is possible that there was late flowering for the first top-worked tree or there was an incompatibility with the Valencia interstock due to some reason. This is something that will need to be followed up in subsequent years.

Table 4.9. Fruit number, yield and mean fruit weight for individual nursery-propagated trees of selection C harvested from site 1 on April 12, 2011. Trees were grafted to Carrizo citrange rootstock.

Tree	Fruit No.	Yield (g)	Mean Fruit Weight (g)
1	237	13350	56.33
2	310	16440	53.03
3	182	13490	74.12
4	146	9030	61.85
5	269	16850	62.64

Approximately 60% of the fruit on tree 3 were removed during mid-January 2011.

Rind colour was poor for the two early selections, C and A (Table 4.10), which may have been a result of the unusual, almost tropical weather experienced at the sites in the latter part of summer into autumn in 2011. Rind colour was a problem for other early-season fruit at these sites in 2011. Rind colour for the other, later maturing selections was as expected being better than that expected for Imperial mandarin, which has been used as a minimum benchmark in the selection process. As expected, selection B had good deep coloured rinds. As with other quality characteristics, rind colour will need to be monitored in coming seasons, especially with more normal climatic conditions.

Fruit size traits for the samples were as expected from the mean fruit weights determined from the crop harvested (Table 4.10). Rind thickness varied between selections as expected with fruit of selection C having the thinnest rinds and those of selection H the thickest, reflecting the difference in fruit size (Table 4.10).

Even though there were opportunities for cross-pollination at all sites, the low mean seed numbers of selections A, C and J were encouraging (Table 4.11). Seedless fruit were harvested from all these selections at every site, although the mean seed number for C at site 3 was higher than for the other sites. Fruit of selection B had higher mean seed numbers that anticipated, especially when compared to the other selections at the sites it produced fruit. Nonetheless, seedless fruit were harvested from the trees of selection B at these sites indicating its potential to produce seedless fruit.

Percentage extractable juice was for the most part acceptable although selections A, H and I had quite low % juice at site 2 (Table 4.11). Again, this trait needs to be monitored further over coming seasons, especially if site differences continue to be a feature of the data.

Juice sugar concentrations were lower for all selections than anticipated from other data (Table 4.11). This may have been a reflection of the unusual wet and humid climatic conditions experienced in the summer/autumn of 2010-2011, or a factor associated with the first harvest from young trees. Again, further monitoring over coming seasons will be important here. Juice acidity was acceptable and resulted in good sugar:acid ratios.

Table 4.10. Rind and size characteristics for fruit harvested in 2011 from trees of 7 selections grown at different grower sites in the Murray, Darling and Murrumbidgee irrigation areas. Data are means ± s.d..

Selection	Site[a]	Harvest date	Tree type[b]	Stock[c]	Rind colour[d]	Fruit diameter (mm)	Fruit weight (g)	rind thickness (mm)
A	1	25/05/2011	NP	CC	6.4 ± 1.7	67 ± 5	130 ± 24	3.4 ± 0.6
	2	25/05/2011	NP	CC	5.2 ± 2.2	67 ± 7	128 ± 34	3.3 ± 0.8
	3	13/04/2011	NP	CC	6.1 ± 0.7	62 ± 3	103 ± 13	2.9 ± 0.3
G	1	13/09/2011	NP	CC	9.8 ± 0.4	61 ± 4	108 ± 19	5.5 ± 0.7
	2	13/09/2011	NP	CC	9.3± 0.6	62 ± 7	111 ± 30	6.2 ± 1.1
	3	14/09/2011	NP	CC	9.1 ± 1.5	56 ± 4	84 ± 20	5.3 ± 1.0
H	1	2/08/2011	TW	Val/CC	11.5 ± 0.5	90 ± 3	289 ± 16	6.2 ± 1.5
	2	2/08/2011	NP	CC	11.0	85	233	8.0
	3	4/08/2011	NP	CC	10.0 ± 0.0	71 ± 4	149 ± 23	6.8 ± 0.4
I	1	6/07/2011	NP	CC	8.9 ± 0.6	78 ± 7	165 ± 30	8.3 ± 1.4
	2	6/07/2011	NP	CC	9.4 ± 0.6	78 ± 11	174 ± 49	8.4 ± 1.5
	3	7/07/2011	NP	CC	9.4 ± 0.3	69 ± 4	128 ± 13	7.3 ± 0.6
C	1	12/04/2011	NP	CC	6.8 ± 2.2	52 ± 3	72 ± 12	2.5 ± 0.6
	1	12/04/2011	TW	Val/CC	6.8 ± 2.5	53 ± 2	75 ± 7	2.6 ± 0.5
	2	12/04/2011	NP	CC	4.7 ± 2.1	52 ± 4	72 ± 16	2.1 ± 0.7
	3	13/04/2011	NP	CC	6.0 ± 1.0	53 ± 2	72 ± 8	2.0 ± 0.2
J	1	14/06/2011	NP	CC	10.6 ± 0.5	63 ± 6	104 ± 25	3.8 ± 1.0
	1	14/06/2011	TW	Val/CC	10.0 ± 0.7	62 ± 6	111 ± 19	3.0 ± 0.6
	2	15/06/2011	NP	CC	10.4 ± 0.5	63 ± 8	111 ± 34	3.3 ± 1.0
	3	16/06/2011	NP	CC	10.7 ± 0.3	63 ± 3	101 ± 10	3.6 ± 0.4
B	1	19/08/2011	NP	CC	11.1 ± 0.6	77 ± 6	174 ± 27	4.6 ± 0.8
	2	19/08/2011	NP	CC	11.4 ± 0.5	81 ± 7	197 ± 38	5.6 ± 0.9

[a] Sites were as follows

1 – Lower Darling Irrigation area site 1
2 – Lower Darling Irrigation area site 2
3 – Mid-Murray Irrigation area site
4 – MIA site

[b] Tree type: N P = nursery-propagated; TW = top-worked

[c] Stock = rootstock: CC - Carrizo citrange; Val/CC - Valencia orange interstock on Carrizo citrange rootstock.

[d] Rind colour was scored using a visual based scale where the higher the number the redder the rind. Imperial mandarin as reference scores 7 on such a scale.

Table 4.11. Seediness and juice characteristics for fruit harvested in 2011 from trees of 7 selections grown at different grower sites in the Murray, Darling and Murrumbidgee irrigation areas. Data are means ± s.d..

Selection	Site	Harvest date	Tree type	R/S	Seeds per fruit	%juice	Juice sugar (ºBrix)	% citrate	Brix:acid ratio
A	1	25/05/2011	NP	CC	2.1 ± 1.7	32 ± 2	9.5 ± 0.7	0.9 ± 0.1	10.8 ± 1.2
	2	25/05/2011	NP	CC	1.3 ± 1.7	18 ± 5	8.2 ± 0.5	0.8 ± 0.1	10.1 ± 1.0
	3	13/04/2011	NP	CC	2.1 ± 0.3	31 ± 4	8.5 ± 0.5	0.8 ± 0.1	11.2 ± 1.3
	4	6/07/2011	NP	Cleo	0.7 ± 0.8		9.4 ± 0.4	0.7 ± 0.1	12.8 ± 0.7
	4	6/07/2011	NP	CC	0.4 ± 0.6		9.6 ± 0.3	0.7 ± 0.1	13.6 ± 1.0
G	1	13/09/2011	NP	CC	9.6 ± 3.1	46 ± 1	9.2 ± 0.9	1.3 ± 0.2	7.5 ± 1.2
	2	13/09/2011	NP	CC	7.2 ± 5.1	39 ± 4	8.0 ± 1.0	1.1 ± 0.2	7.2 ± 1.3
	3	14/09/2011	NP	CC	8.8 ± 1.4	42 ± 11	8.0 ± 2.0	1.0 ± 0.3	8.2 ± 1.1
H	1	2/08/2011	TW	Val/CC	12.3 ± 2.9	42 ± 0.0	10.7 ± 1.0	1.3 ± 0.1	8.0 ± 0.0
	2	2/08/2011	NP	CC	0.0	28	8.5	1.0	8.5
	3	4/08/2011	NP	CC	9.4 ± 6.0	27 ± 5	8.8 ± 0.9	0.9 ± 0.1	9.9 ± 1.6
	4	6/07/2011	NP	Cleo	3.0 ± 1.0		9.0 ± 0.1	1.6 ± 0.1	5.5 ± 0.0
	4	6/07/2011	NP	CC	0.0 ± 0.0		8.4 ± 0.1		
I	1	6/07/2011	NP	CC	11.7 ± 6.5	28 ± 3	9.4 ± 0.5	1.1 ± 0.2	9.2 ± 1.6
	2	6/07/2011	NP	CC	5.6 ± 5.9	25 ± 4	9.1 ± 0.6	0.9 ± 0.1	10.7 ± 1.7
	3	7/07/2011	NP	CC	11.1 ± 3.7	30 ± 1	8.7 ± 0.4	0.8 ± 0.1	11.3 ± 2.0
C	1	12/04/2011	NP	CC	2.8 ± 2.1	29 ± 6	9.2 ± 0.6	0.7 ± 0.1	13.9 ± 1.1
	1	12/04/2011	TW	Val/CC	0.8 ± 1.1	29 ± 8	9.4 ± 0.8	0.7 ± 0.1	14.5 ± 0.6
	2	12/04/2011	NP	CC	2.1 ± 3.0	34 ± 5	8.6 ± 0.6	0.7 ± 0.0	12.2 ± 1.0
	3	13/04/2011	NP	CC	6.9 ± 3.8	34 ± 2	8.5 ± 0.2	0.7 ± 0.0	12.8 ± 0.7
J	1	14/06/2011	NP	CC	2.1 ± 2.7	29 ± 3	10.1 ± 0.6	1.0 ± 0.1	10.0 ± 1.0
	1	14/06/2011	TW	Val/CC	4.3 ± 2.9	41 ± 1	9.7 ± 1.2	1.0 ± 0.1	9.9 ± 1.3
	2	15/06/2011	NP	CC	0.4 ± 1.1	31 ± 4	9.6 ± 0.7	1.0 ± 0.1	10.1 ± 0.7
	3	16/06/2011	NP	CC	2.2 ± 0.7	34 ± 2	10.3 ± 0.6	1.0 ± 0.1	10.8 ± 0.9
	4	6/07/2011	NP	Cleo	0.1 ± 0.6		10.3 ± 0.7	1.0 ± 0.2	10.6 ± 1.9
B	1	19/08/2011	NP	CC	7.1 ± 6.6	40 ± 4	10.9 ± 1.7	1.0 ± 0.2	11.0 ± 2.6
	2	19/08/2011	NP	CC	8.2 ± 8.8	32 ± 4	10.7 ± 1.2	0.9 ± 0.2	12.0 ± 2.8

Sites, tree types and stocks were as described in the footnote for Table 4.10.

Due to fruit fly issues preventing fruit from being taken to the laboratory at Merbein, % juice was not determined for fruit harvested at site 4 in the Murrumbidgee Irrigation Area.

4.4.3 Hybrid selection F – a potential grapefruit substitute

Information concerning the selection of hybrid selection F was reported in previous final reports. During CT00012, ten daughter trees of this selection were established at one of CSIRO's farms for further evaluation before deciding if it should be entered into additional phase two trials. Hybrid selection F produces 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, although it has been observed that often the very large fruits have more seeds than the smaller fruits.

As the pressure of completing other tasks accelerated during CT07000 due to the announcement made by CSIRO in 2008 that it was closing Merbein and withdrawing from citrus research, little further practical work was conducted with the small trial of selection F at CSIRO's experimental farm in NW Victoria other than to use it in order to provide fruit samples to collaborating parties for testing and evaluation. Fruit were, however, harvested in 2009 to quantify any association between seed numbers and fruit size in an open-pollination situation. Fruit from the ten trees at CSIRO's experimental farm in NW Victoria were harvested on October 28, 2009 and graded on fruit diameter to provide 3 distinct sizes. These fruit were then weighed, dissected and their seed counted.

Table 4.12. The relationship between fruit size and seed numbers for hybrid selection F when grown under conditions favourable to a high cross-pollination pressure with viable pollen. Fruit were harvested on October 28, 2009. Data are means ± s.d.

Size	Weight (g)	Diameter (mm)	seed nos. per fruit
Large	820 ± 157	126 ± 7	22 ± 21
Medium	346 ± 22	92 ± 5	1 ± 2
Small	135 ± 7	65 ± 1	2 ± 4

Large fruit generally had more seeds than smaller fruit, but because even very large fruit were often seedless, this difference was not significant (Table 4.12). Under the conditions for strong cross-pollination pressure at CSIRO's experimental farm in NW Victoria due to the proximity of breeding orchards in which hybrid families are segregating for pollen sterility, there were seeds present in some fruit in all size grades. Smaller seeded fruit, however, had generally fewer seeds than seeded large fruit. As smaller fruit are likely to be more acceptable to the consumer and also likely to be more consistently seedless, this is a case where effort may need to be expended in promoting smaller rather than larger fruit development. This suggests that crop regulation will be important if this hybrid is released commercially and research will be needed to investigate how optimum fruit size can be achieved.

As a grapefruit replacement, fruit of selection F 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. This feedback in addition to the orchard performance of selection F indicated that it should be entered into regional testing with the cooperating grower network. Accordingly a booklet, including a fact sheet (Figure 4.6) was produced and circulated to growers seeking expressions of interest in evaluating the hybrid.

Growers were offered 5 nursery-propagated trees grafted to Carrizo citrange and following signing of non-propagation and non-distribution testing agreements, trees were distributed to growers in Victoria, NSW and Queensland during 2010 for the first two states and in 2011 for the latter. Apart from one site in the MIA, where additional trees were supplied in 2011 after an accident with a herbicide, these trees have established well and their first fruit should be ready for harvesting from 2013.

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

Several comments can be made concerning the second phase evaluation trials reported here. First, the establishment of a network of cooperating growers, which was started in earlier projects, has continued to provide 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 Merbeingold 2350), while others have shown potential, but require additional work. Thus, the seven selections provided to growers during CT07000, which have only been harvested once in the regional sites, will require further monitoring in the immediate future.

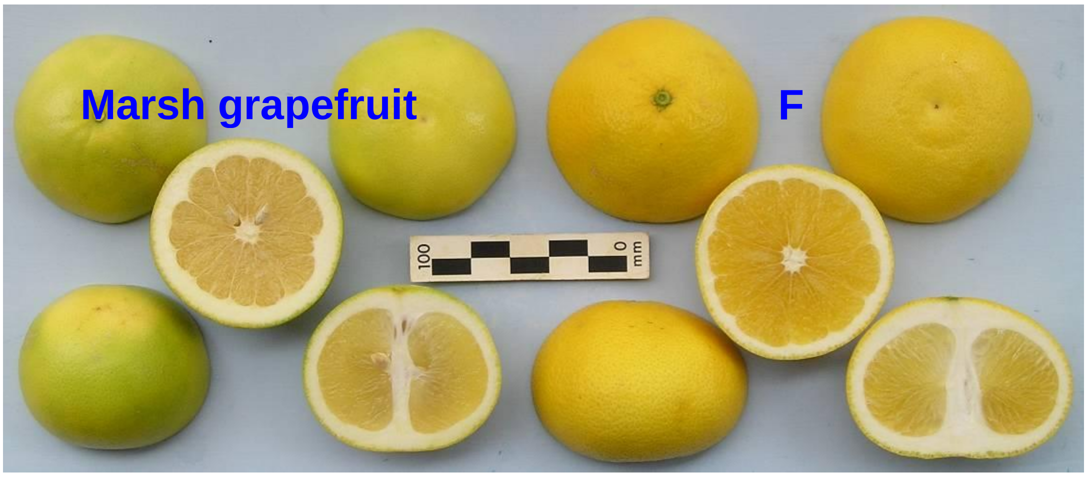
Further data from these trials coupled with the information from those conducted at CSIRO's experimental farm in NW Victoria may also indicate if any should be entered into an irradiation program as has been done with four selections identified previously and which are described in a later chapter.

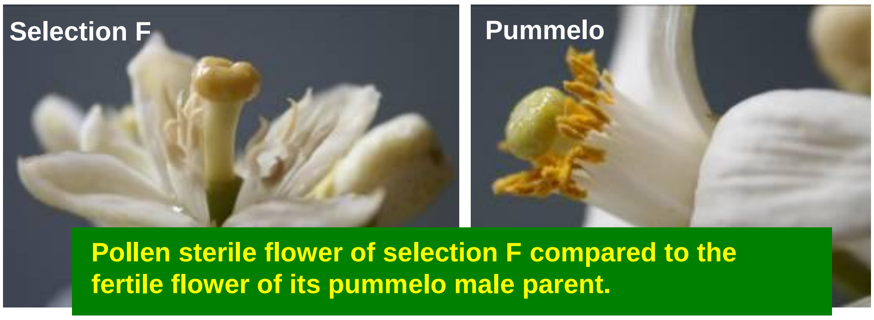
Bud-line age and the development of mature as opposed to juvenile buds were highlighted in a previous final report, and as a result, daughter trees were propagated to yield buds for propagating trees to be entered into grower trials. The aim being that grand-daughter buds would have a greater propensity for precocity and that the trees distributed would come into productive bearing faster. The first yields of selections A, C and J (Table 4.9) suggest that this has been moderately successful, although there were no controls propagated from first generation daughter buds for comparison. The yields of selection H88-13, however, suggest that there was no benefit for this hybrid. As is often the case in breeding highly heterozygous woody perennial fruit trees, the variability extends to many traits when recombinants are produced.

The National Citrus Scion Breeding Program - CSIRO Plant Industry, Merbein component

Notice of new selection available for regional testing by grower cooperators

Selection F (Silverhill satsuma x Pummelo CS28)





HARVEST DATE	September – December (Murray Valley)
YIELD (kg)	Up to 85kg for 4 year-old daughter tree
COLOUR	Yellow
SHAPE	Oblate
BUTTON REMOVAL	No, can be snapped from tree
EASY-PEEL	Yes, for a grapefruit
SURFACE TEXTURE	Smooth/pebbled
MEAN FRUIT DIAMETER (mm)	76 – 140
MEAN FRUIT WEIGHT (g)	360 – 700
% EXTRACTABLE JUICE	35 – 40
RIND THICKNESS (mm)	6 – 16
OPEN-POLLINATED SEED NUMBER	0 – 50 (depending on cross-pollination)
SEED NUMBER IN ABSENCE OF POLLINATION	0
JUICE °BRIX	8.4 – 16.0
JUICE ACID (%)	1.9 – 2.5
BRIX:ACID	5 – 8
PARTHENOCARPIC	Yes, strongly
POLLEN STERILITY	Yes

Figure 4.6. Fact sheet providing the characteristics of selection F

4.5 Release and commercialisation activities

4.5.1 Semi-commercial top-worked trial of Merbeingold varieties.

During project CT04007, a nomination was made via the Citrus Scion Breeding Reference Committee (CSBRC) to release three varieties from the diploid hybridisation component of the breeding program (see final report for CT04007). Two of these selections were ultimately released as Merbeingold 2336 and Merbeingold 2350. In reaching this decision to release, a meeting was held with fruit marketers from three major citrus packers who 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 early 2006 after a new testing agreement was signed in late 2005 with the grower who volunteered to establish the plantings. As there were additional IP complications with establishing such trials, it was necessary to formulate a modified testing agreement that would take into account changing events with selections as they progressed through the commercialisation process.

Buds of the three selections were supplied to the grower hosting the trials in January 2006, which turned out to be less than optimal for tree propagation due to climatic conditions that prevailed just after top-working was completed.

One selection, 2127, a sibling of the two Merbeingold varieties, was a late maturing, seedless variety. Problems associated with rind maturity and puffiness saw this selection subsequently dropped from the program.

Selection 2336, which became Merbeingold 2336, was top-worked to some Satsuma trees on citrange rootstock. These trees were located on a sand hill surrounded by varieties that were pollen fertile and thus capable of cross-pollinating the 2336 trees. This was acceptable as extensive pollination tests had established that 2336 will be seedless even when cross-pollinated. Unfortunately, a number of the top-works failed and as a result only 146 of the 252 trees propagated survived to produce fruit.

Selection 2350, which became Merbeingold 2350, was top-worked to some Fallglo mandarin trees on citrange rootstock. These trees were located on flat ground in a different part the farm characterized by a heavier sandy-loam soil and were surrounded by navel orange trees, which would not be a source of pollen to cross-pollinate the 2350 flowers. The nearest pollen source for the 2350 trees were some Imperial mandarin trees 150m away, along with a small collection of different citrus types, also 150m away planted around the grower's house. As for the 2336 trees, a number of top-works failed and only 138 of the 222 trees propagated have produced fruit.

These two top-worked trial plantings have proved extremely valuable during the early years of commercialisation of the two Merbeingold varieties. Data have been collected every year to monitor fruit maturity to advise when harvest should occur and to track changes with season. When trees were harvested, total yields were recorded. At harvest, randomly selected trees were harvested separately so that a full tree analysis could occur. These complete tree harvests provided data concerning yield components and fruit quality. As a result, voluminous data have been collected for these trials. For the sake of brevity, however, only selected, summarised data are provided here.

Fruit maturity
Fruit maturity in the two trials was monitored using semi-randomly selected trees each year within different areas of each trial. Thus, for the Merbeingold 2336 trial, which was located on a sand hill, trees were selected at random in two zones near the base and near the top of the of the hill. The number of trees selected ranged from 2 through 4 to 6 in seasons 2009, 2010 and 2011, respectively.

Fruit were collected at regular intervals from early-to-mid May from both within and on the outside of these trees at all points of the compass at a height of approximately 1.25m. Fruit from each tree were returned to the laboratory where total sugar (°Brix) and % citrate were determined for extracted juice. These data along with the sugar:acid ratio were used to monitor fruit maturity and so decide on a harvest date for each variety.

The data for fruit maturity are presented in Figures 4.7a-c with the various measurements plotted against days from January 1 in each year. Juice sugar for Merbeingold 2336 varied between seasons with the concentrations in 2011 being the lowest. Season 2011 was unusual in the amount of rainfall that occurred in January, February and March and the lower-than average mean temperatures coupled with higher humidities. This more tropical weather experienced in 2011 may have affected juice composition, especially sugar. Overall the sugar of 2336 juice was more-or-less constant during each season, although it did vary quite widely between samples in 2010. Juice sugar increased with time in Merbeingold 2350 fruit, although there was a drop off in 2010. As such, there was a difference in sugar accumulation between the two varieties, although as indicated, they were located in different soil types and under different topographical conditions.

The situation with regard to juice acidity was equally different between the two varieties with acidity of 2336 dropping quite markedly over time until harvest. In fact, juice acidity being lower in 2011, dropped away quite quickly and was perhaps lower than desirable by the time the trees were harvested on June 14. Though not reported here, this resulted in a less sprightly flavour, which was commented upon in taste tests conducted by the commercialiser. Again, this may have been a factor of the tropical, more humid conditions in 2011 and emphasised the importance of harvesting fruit when the sugar:acid ratio is at its optimum.

The acidity of 2350 juice behaved in a different manner to that of 2336 and while it did drop away during the course of the season, its reduction was far less dramatic allowing for greater latitude in harvest date. Coupled with a higher sugar, juice of 2350 has a higher acidity, but still yields a well balanced juice in terms of the sugar:acid ratio. In seasons 2010 and 2011, the acidity of 2350 juice appeared to stabilise around 1.0%, which meant that the fruit had a pleasant flavour from quite early, although the eating quality improved and was at best in mid-to-late July. This does mean that 2350 has greater latitude with regard to harvest date and to an extent the fruit can be stored on tree better than its sibling variety, Merbeingold 2336.

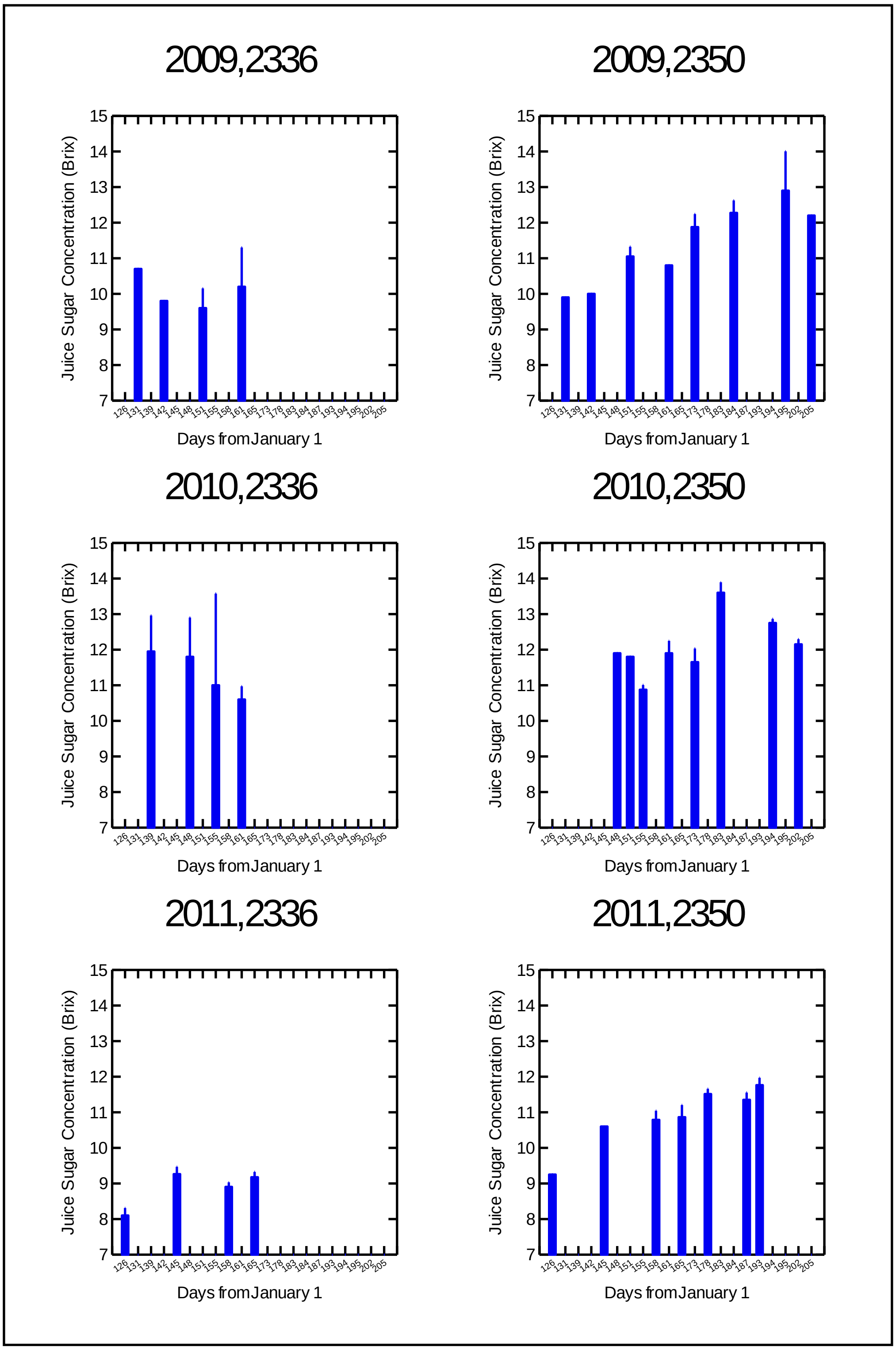


Figure 4.7a. Variation with time from May until harvest for juice sugar concentrations of Merbeingold 2336 (2336) and Merbeingold 2350 (2350) varieties as top-worked trees in a large semi-commercial scale trial. Data are means ± s.d and are for seasons 2009-2011 inclusive.

At harvest, juice sugar:acid ratios exceeded 10 for both varieties. The higher juice sugar concentration for 2350 fruit, however, resulted in a richer overall flavour, which can be used as another characteristic to determine the optimum time for harvesting its fruit.

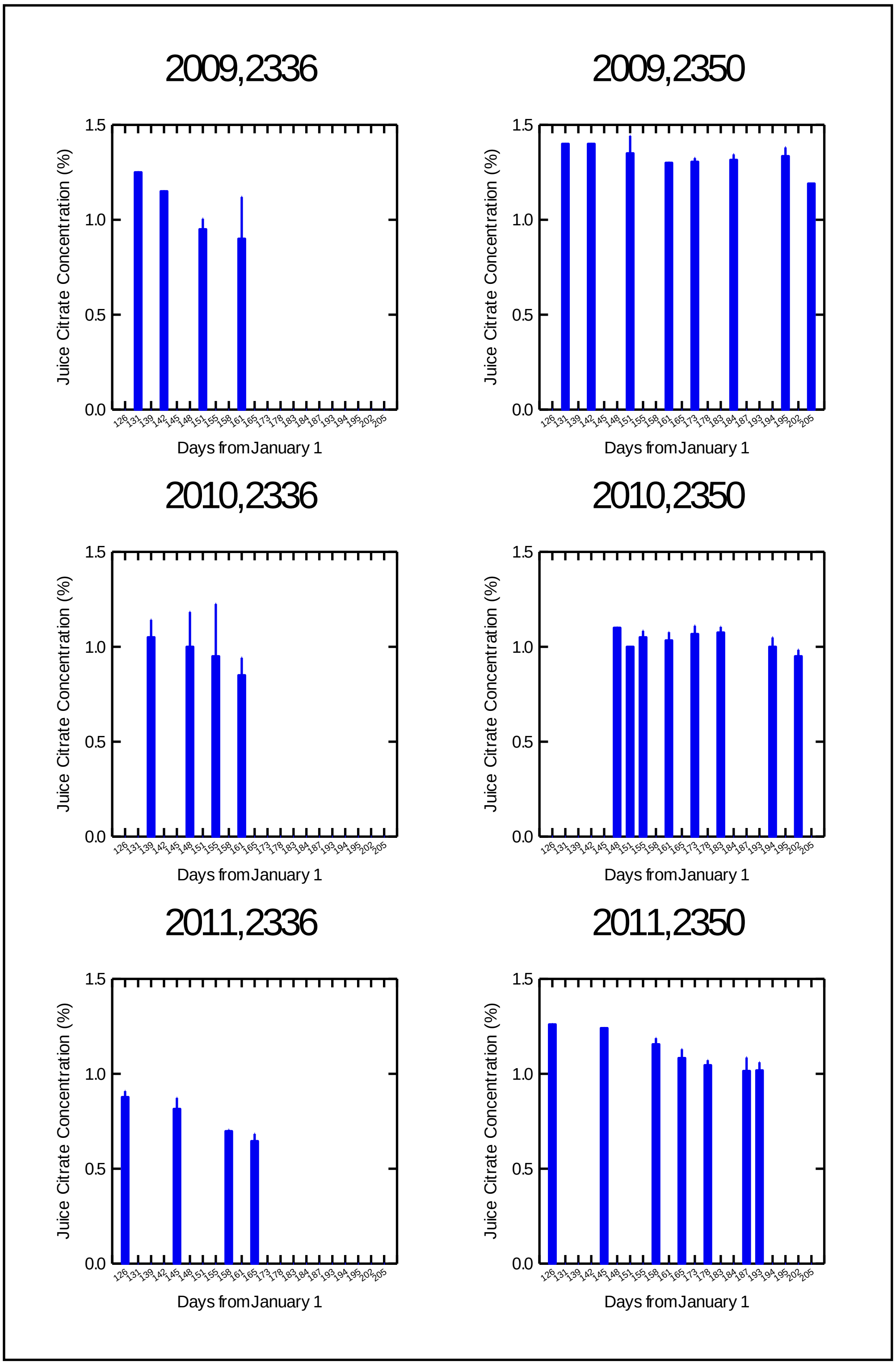


Figure 4.7b. Variation with time from May until harvest for juice citrate concentrations of Merbeingold 2336 (2336) and Merbeingold 2350 (2350) varieties as top-worked trees in a large semi-commercial scale trial. Data are means ± s.d and are for seasons 2009-2011 inclusive.

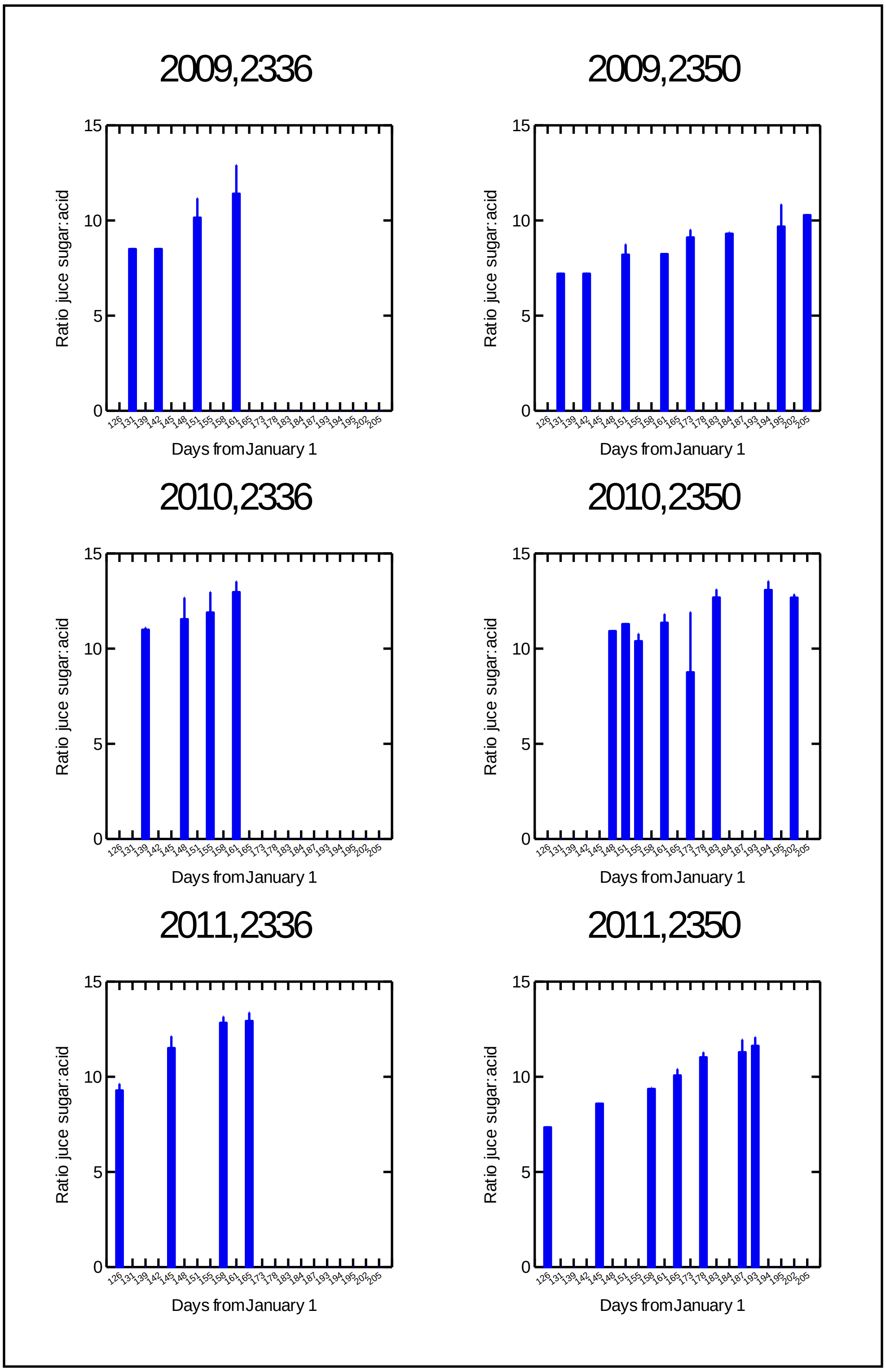


Figure 4.7c. Variation with time from May until harvest for juice sugar:acid ratios of Merbeingold 2336 (2336) and Merbeingold 2350 (2350) varieties as top-worked trees in a large semi-commercial scale trial. Data are means ± s.d and are for seasons 2009-2011 inclusive.

Yield

Trees in these trials were first harvested in 2008 (Table 4.13) after their second spring. Fruit was harvested from 57 and 26 trees of 2336 and 2350, respectively, and were used for industry displays and tastings. At this stage, the commercialiser had not been appointed and consequently this first-harvested fruit was not used for marketing purposes. Fruit were harvested in subsequent years and sent to a packing house for grading, packing and shipping to a distribution centre for use by the commercialiser for marketing purposes. All trees of both selections yielded fruit from 2009 onwards, which increased progressively for 2350. The yield of the 2336 decreased markedly in 2010. This was due to an unseasonal heat wave experienced during November 2009 when a significant fruit drop occurred, not only with the 2336 trees in the trial, but also for many other plantings in the Darling and Murray irrigation areas. At harvest, fruit were mainly picked from the NE sector of the trees and only 0.75T of fruit was sent for packing compared to 2T and 7T in 2009 and 2011, respectively. Being located on a sand hill, the 2336 trees were probably more prone to the hot, dry winds that accompanied the November 2009 heat wave. The trees of Merbeingold 2350 did not suffer in the same way being located on a flat section of the farm in a heavier soil type. Interestingly, two ten-year-old top-worked trees of selection 2336 located under similar conditions near to the 2350 trial, produced 83 and 104 kg of fruit, respectively, in 2010 with mean fruit weights of 143g and 153g.

Table 4.13. Yield per tree for top-worked trees of Merbeingold 2336 and Merbeingold 2350 from 2008 onwards. The amount of fruit sent to for commercial packing in each year is also given.

Selection[a]	Year	Mean yield per tree (kg.)	Fruit sent for packing (T)
2336 (146 trees)	2008 (57 trees) [a]	4.0	-
	2009	13.7	1.90
	2010	8.7	0.75
	2011	47.3	6.75
2350 (138 trees)	2008 (26 trees) [a]	4.2	-
	2009	24.8	3.38
	2010	33.6	4.25
	2011	75.4	10.12

[a] The number of top-worked trees for each selection is presented in parentheses. Note in 2008, the first year that trees carried fruit, the number of trees from which fruit were harvested was less than the number top-worked successfully and is presented in parentheses in the year column.

Apart from the effect of the heat wave experienced in November 2009 on the 2010 crop of Merbeingold 2336, the yield of these trees has been satisfactory and from recent inspections of the trees in January 2012, higher yields are anticipated for the 2012 season.

Fruit Quality

A random sample of fruit was taken for each variety from within the bins into which fruit were placed by the picking team, and which were destined for the packing shed in 2009, 2010 and 2011. A random sample was retained from the fruit harvested in 2008. These samples were taken to the laboratory where routine fruit quality analyses were conducted (Table 4.13).

Overall, the data for fruit quality across seasons, with annual variations, were as expected from other smaller plantings reported in previous reports. Rind colour for Merbeingold 2350 was more intense than for 2336, which is less red. Fruit weight for 2336 was larger than 2350, except for the fruit analysed in 2010, which may have been a sampling error or a factor of the November heat wave experienced in 2009. Nonetheless, fruit size was appropriate for seedless or low seeded dessert fruit. The rind thickness for 2336 was as expected greater than for 2350, which though thinner is generally more robust. Juice percentages were above 35% and were greater for 2350, again as expected.

The vast majority of Merbeingold 2336 fruit were seedless, even though they were situated adjacent to a viable pollen source that could have cross-pollinated its flowers. Only seedless fruit were harvested in 2009 and 2010. Some seeds were detected in fruit in seasons 2009 and 2011, but these were rare and never exceeded 2 per fruit. There were seeded Merbeingold 2350 fruit, although the majority was seedless. Occasional fruit had up to 12 seeds per fruit, but these were rare and were no doubt due to a cross-pollination event resulting from the pollen viable trees growing within the vicinity of the trial. Seed numbers were very low for 2350 in 2009 and indeed 82% of the sample analysed were seedless in this season, thus, meeting the requirements for premium grade seedless fruit acceptable in the European market.

As a result of the seeds found in the 2350 fruit, further samples were collected and dissected to obtain a better profile of seediness in 2009 and 2010. In 2009, 300 fruit were collected at random from the harvested fruit, weighed individually before seeds were extracted and counted. In 2010, 50 fruit were collected, weighed and dissected from 6 randomly selected trees from across the trial. Any association between fruit weight and seediness was explored through standard correlation and regression analyses.

The large sample sizes led to significant correlations between fruit weight and seed numbers for all 7 samples of fruit analysed (Table 4.15) suggesting a positive association between these two characteristics for Merbeingold 2350. However, the range in r^2 indicated that at best only 30% of the variation in fruit weight could be associated with the variation in seed number, and for one sample this was only 14%. As there were large and small seedless fruit in all the samples analysed, there were clearly other more important factors that determine fruit weight.

Juice quality in terms of sugar, acid and their ratio was largely as expected from the data collected to monitor fruit maturity. From the data and feedback from tastings in 2009 and 2010, when Merbeingold 2336 fruit were better accepted (see comments in Table 4.16), an optimum juice acid level should be around 0.8-0.9 % with a juice sugar greater than 10 ° Brix when fruit are harvested. Merbeingold 2350 was perhaps less demanding with regards timing of harvest as its juice quality was less prone to deteriorate quickly.

Table 4.14. Fruit quality of Merbeingold 2336 and Merbeingold 2350 determined from a random sample of fruit taken from a fruit bin at harvest of top-worked trees in the semi-commercial trial. Data are means ± standard deviations for sample sizes that varied according to year.

Variety	Harvest date	Rind colour	Fruit weight	Fruit diameter	Rind thickness	% juice	Mean seed no.	Juice quality		
								° Brix	% citrate	Sugar:acid
Merbeingold 2336	June 6, 2008	5.8 ± 1.9	135.4 ± 6.7	67.8 ± 2.2	4.2 ± 0.4	37.1	0.3 ± 0.5	10.0 ± 0.4	0.6 ± 0.1	16.1
	June 10, 2009		120.9 ± 24.8			33.3 ± 3.5	0.0 ± 0.0	10.2 ± 0.7	0.9 ± 0.1	11.4 ± 0.9
	June 10, 2010		146.3 ± 20.0			37.6 ± 4.5	0.0 ± 0.0	10.6 ± 1.3	0.8 ± 0.1	13.0 ± 0.4
	June 14, 2011	8.8 ± 1.0	104.0 ± 25.9	61.2 ± 6.2	5.9 ± 1.2	37.1 ± 0.7	0.1 ± 0.3	9.2 ± 0.6	0.6 ± 0.1	14.4 ± 1.7
Merbeingold 2350	June 12, 2008	9.8 ± 0.8	108.8 ± 5.5	64.8 ± 2.3	3.3 ± 0.5	34.8	3.8 ± 1.9	13.0 ± 1.4	1.2	10.9
	July 29, 2009	11.1 ± 0.5	87.5 ± 32.0	56.8 ± 8.9	2.3 ± 0.9	42.7 ± 4.7	0.3 ± 1.1	12.0 ± 0.8	1.2 ± 0.1	10.4 ± 0.5
	July 21, 2010	11.2 ± 0.6	158.4 ± 29.2			42.7 ± 2.7	2.5 ± 3.5	12.1 ± 0.8	1.0 ± 0.1	12.7 ± 0.3
	July 22, 2011	10.8 ± 0.4	101.5 ± 25.3	61.7 ± 6.0	3.3 ± 0.9	43.8 ± 3.4	3.2 ± 0.9	11.7 ± 0.8	0.9 ± 0.1	13.3 ± 1.3

Missing values are where these data were not recorded.

Table 4.15. Weights and seed numbers for random samples of Merbeingold 2350 fruit collected during the 2009 and 2010 harvests of the semi-commercial trial. Data are means ± standard deviations.

Year	Tree	Mean fruit weight (g)	Mean seed no.	n	r^2	Sig.
2009	Combined sample	120.9 ± 28.1	1.9 ± 3.2	300	0.24	***
2010	1	122.6 ± 26.6	2.3 ± 3.4	50	0.25	***
	2	102.8 ± 19.1	1.6 ± 3.0	50	0.18	**
	3	108.2 ± 20.3	1.0 ± 2.7	50	0.16	**
	4	145.3 ± 26.0	3.6 ± 4.2	50	0.30	***
	5	121.9 ± 28.8	1.5 ± 2.7	50	0.25	***
	6	124.2 ± 26.7	1.5 ± 2.5	50	0.14	**

r^2 is the degree of association between fruit weight and seed number being the square of the correlation coefficient (r)
Sig. is the significance of the estimate of r for each sample or tree (** $P<0.01$; *** $P<0.001$)

Table 4.16. Selection of comments sent from CSIRO scientific, administrative and executive staffs in Canberra who were offered fruit of Merbeingold 2336 in 2010.
Really delicious fruit from Merbein - I will definitely buy that when it becomes available Very good – it had everything I look for in a mandarin – full of flavour, nice acidity edge, colour and skin quality (appearance) excellent, no seeds is great, – plus it was free, what more could we want! Only problem is I need to wait a couple of years for the next ones. Should be a winner. *Thanks so much for sending the mandarins, they are the most delicious mandarins I have ever tasted. They are like bursts of sunshine in your mouth, which is so refreshing on the palate.* *It must be so exciting to be able to taste the end result of all your hard work and have it turn out so well.* *Once again, they are absolutely gorgeous!* *Good luck for the future.* The mandarins are great, fantastic taste and easy to deal with. Sure they will be a hit. cheers also to confirm I tried one on my 10 year old son, who loved them and thought they were a ray of sunshine and very sweet and easy to deal with. No annoying pips. Cheers Thanks for the taste of the new crop. Excellent. you've done a great job. Hopefully you will be rewarded with a great success for this variety. I have just had an opportunity to taste my mandarins. They're great. Thanks and well done to everyone. I know that this has been the result of many years of effort and it must be very satisfying to see the fruit coming to final stages of commercialisation I'd like to add my thanks and positive feedback here as well – they mandarins have been shared around the office and the feedback has been terrific! **Just tasted your new release and thought it was terrific. Well done and I hope it is successful in the marketplace.** It was very juicy and sweet! Nice it is seedless. Found it softer (mushier) than the usual mandarins I normally have. I took home 2 of your new mandarins so the family could try them. I thought you might enjoy the feedback: • my husband said: "I've eaten a lot of mandarins but that is by far the nicest I have ever tasted" • my 17 year old daughter took hers to school and shared it with friends. They all raved about it (and it is pretty hard to impress 17 year-olds). However one did say: "Can you ask the researcher dude to do something about the white stringy bits". I guess the work never ends.... For my part, I thought they were absolutely beautiful and I'm looking forward to being able to buy them at my local fruit market

4.5.2 Merbeingold 2350 vs. Afourer

As commercial production of Afourer mandarin in the Murray-Darling Basin irrigation regions increased during the course of CT07000, and with more fruit available of Merbeingold 2350 for tasting by industry and consumer groups, the similarity in their fruit appearance was noted (Figure 4.8). Comments were received on a number of occasions concerning this, often with an opinion on the relative taste and flavour attributes of fruit from the two varieties. Most comments with regard to flavour and ease of eating favoured Merbeingold 2350 over Afourer fruit.

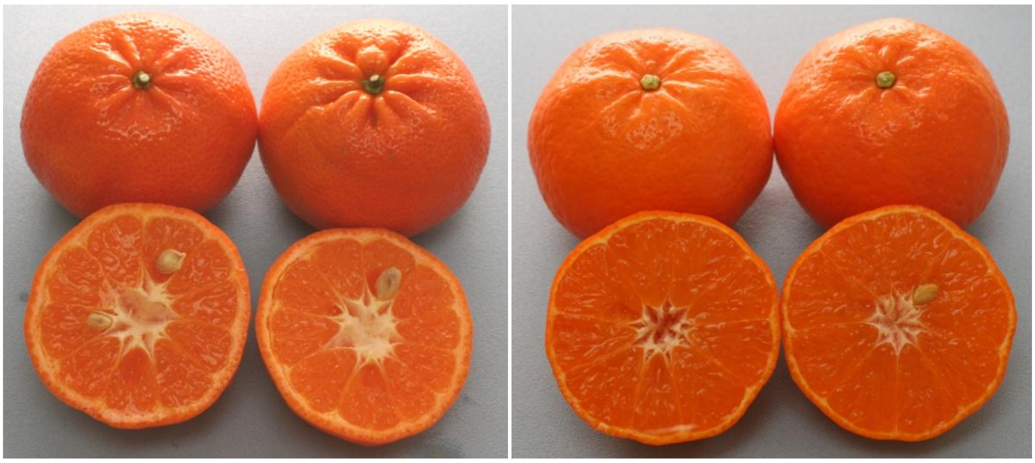


Fig. 4.8. A comparison of Afourer (left) and Merbeingold 2350 (right) fruit.

Mindful of comments about the similarities between the two varieties, and since the grower who hosted the Merbeingold semi-commercial trails also grows Afourer in a situation such that it is remote from pollen sources, fruit from the two varieties were compared in 2010 and 2011.

Fruit were sampled at random from those harvested from the two varieties in both years. In 2010, these samples were size graded into four classes and fruit quality analysed in the laboratory. In 2011, similar samples were taken at harvest and a sample analysed from each before the fruit was divided up into sample bags containing two fruit of each distinguished by an arbitrary label such as M for Afourer and N for Merbeingold 2350. The fruits in the bags were of median size for the sample and were comparable in this respect. The bags were then distributed amongst a wide range of people including citrus growers, other citrus industry representatives, staff at CSIRO and other contacts in the wider community to gauge consumer opinion of the two varieties. Two hundred sample bags were distributed and the tasters, who in some instances included friends or family groups and not just the individuals to whom the fruit were given, were asked to assess overall flavour, texture and ease of eating of the fruit and to nominate which of the two varieties they preferred based on these parameters. Tasters were asked to email or phone-in their preferences.

The data collected in 2010 demonstrated some clear similarities between fruit of the two varieties (Table 4.17). Afourer generally produced heavier fruit, probably a reflection of its higher juice content. Rind colour was similar as was juice sugar concentration. The acidities of the smaller Afourer fruit were generally higher, indicating that they should perhaps have been harvested a week-or-so later. The 2350 fruit were more uniform with regard to juice acidity, which also gave a more consistent sugar:acid ratio between the 4 sizes of fruit.

Mean segment number was a distinguishing difference between fruit of the two varieties. Though not presented, the carpellary membranes of Merbeingold 2350 were also softer than those of the Afourer fruit. Mean seed number of the largest Merbeingold 2350 fruit were higher than expected for the sample analysed and was greater than that for the Afourer fruit. There was little difference in mean seed number between the two varieties in the other three grade sizes. While the Afourer trees on the property were grown in an area surrounded by pollen sterile navel orange trees, the Merbeingold 2350 trees, as noted earlier, were located closer to viable pollen sources, which were approximately 150m away. This suggests that seedless fruit development for Merbeingold 2350 may be less affected by the potential for cross-pollination than for Afourer mandarin.

The fruit that were harvested for taste testing in 2011 had similar fruit quality characteristics as the fruit analysed in 2010 (Table 4.18). Although there was a gap of 6 days between harvesting the 2350 and Afourer fruit, the juice acidity of the Afourer fruit was greater giving a sugar:acid ratio of around 10 for the Afourer fruit compared to 13 for the 2350 fruit. This may have been a factor in the responses received from tasters.

The result of the taste test, in which tasters were asked to nominate the fruit they preferred, was that 66% preferred Merbeingold 2350 and 34% preferred Afourer. While comments were not sought, some respondents volunteered subjective observations. From these it was clear that some people thought that the Afourer fruits were more acidic, but that this was a positive for them. For others, the acidity was a negative. Those that commented on the mouth feel were all positive about the Merbeingold 2350 fruit with their softer membranes observing that they were easier to chew. Comments were also received stating that the Merbeingold 2350 fruit were less fibrous with larger, plumper juice sacs.

While these results supported comments made at earlier tastings of Merbeingold 2350, the difference in juice acidity probably affected the outcome. Nevertheless, there were no adverse comments received about the Merbeingold 2350 fruit.

Table 4.17. Comparison of fruit quality between Merbeingold 2350 and Afourer fruit collected on July 21, 2010 from the site where the semi-commercial top-worked trials of the Merbeingold varieties were conducted (July 21, 2010). Fruit were collected from 4 trees, bulked together and size graded.

Variety	Sample[a]	Mean rind colour[b]	Mean fruit weight (g)	% juice[c]	Mean number of segments	Mean seed no.	Juice quality: °BRIX	% Citric acid	Brix:acid ratio
2350	A	11.2	187.6	39.0	9.3	6.5	12.2	0.9	13.0
Afourer	A	10.8	206.1	49.4	10.2	0.7	12.4	0.9	13.9
2350	B	11.0	170.7	42.3	9.3	1.5	11.8	0.9	12.6
Afourer	B	10.8	173.8	49.1	9.7	0.5	12.0	1.1	11.2
2350	C	11.5	155.6	44.8	8.8	1.5	12.3	1.0	12.8
Afourer	C	11.0	151.9	50.7	10.3	0.3	12.1	1.2	10.5
2350	D	11.0	119.7	44.7	8.5	0.3	12.3	1.0	12.3
Afourer	D	11.0	121.4	52.1	10.2	0.3	12.8	1.2	10.9

[a] Four samples of 6 fruits, separated on size, were analysed.
[b] Rind colour was scored using a visual rind colour ranking based on a Japanese system (Yamazaki and Suzuki, 1980). The higher the number assigned to a fruit, the more intense the orange/red colour of the rind. Imperial mandarin would generally score a mean of 7.
[c] % juice was calculated from the weight of juice extracted using a domestic reamer without squeezing the pulp.

Table 4.18. Characteristics of the fruit collected from Afourer and Merbeingold 2350 trees used in a taste test during 2011. Two similar sized fruit of each variety were distributed to 200 recipients for their (and sometimes their friend's and family's) opinion on flavour, texture and ease of eating. Data are means (n=20) ± standard deviation.

Variety	Harvest Date	Weight	% juice	Seed No.	Brix	Acid	B:A
Afourer	July 28	116 ± 32	48.4 ± 2.5	2.5 ± 2.8	12.2 ± 0.9	1.2 ± 0.2	9.8 ± 1.3
Merbeingold 2350	July 22	102 ± 25	43.8 ± 3.4	3.2 ± 0.9	11.7 ± 0.8	0.9 ± 0.1	13.3 ± 1.3

4.5.3 Release and commercialisation of Merbeingold varieties

4.5.3.1 Domestic

The final report for CT04007 outlined the process employed for the selection of a commercialiser to manage the release and development of the two varieties in Australia. To re-cap briefly, an announcement was made in March 2006 of the pending release of Merbeingold 2336 and Merbeingold 2350. The decision to release the varieties set in train a release and commercialisation strategy with guidance from the project's reference committee, the Citrus Scion Breeding Reference Committee (CSBRC). At the time of finalising the report for CT04007, an agreement on terms had been reached with a commercialiser to manage the varieties in Australia, by which time the two varieties had been granted provisional Plant Breeders Rights in Australia and Plant Patent applications had been filed in the USA.

By May 2008, the commercialisation agreement had been prepared ready for signing between CSIRO and the chosen commercialiser. As this was being processed, however, following the release of the Australian Federal Budget in early May, CSIRO announced the closure of the Merbein Research Station and a decision to withdraw from citrus research. Initially, the withdrawal from citrus research was to be by June 30, 2009. This decision caused the chosen commercialiser to withdraw its interest in managing the release and marketing of the two Merbeingold varieties.

Negotiations, however, continued with interested parties and at the 2009 Citrus Australia Limited national conference it was announced that Perfection Fresh Australia (PFA) was the commercialiser of the two Merbeingold varieties.

Subsequent to this announcement, PFA have been driving the release of the varieties with reporting obligations to CSIRO. CSIRO has reported activities to the CSBRC on a regular basis and feedback, where warranted within the terms of the agreement, was acted upon. CSIRO receives annual reports from PFA and activities are measured against key performance criteria in annual meetings between these two entities.

During the first two years of the commercialisation of the varieties, CSIRO has provided technical support to PFA, as per the examples of data presented earlier, and has provided bud wood to AusCitrus, which is the propagator of buds for distribution to nurseries contracted by PFA to produce trees for growers. The first nursery-propagated trees for growers were available in spring 2011 (Figure 4.9).

Fig. 4.9. A new commercial planting of Merbeingold 2336. The trees were planted in November 2011 and these images were captured 3 months later.

4.5.3.2 International

At August 6, 2010, CSBRC meeting, committee members were asked to comment on a draft Expression of Interest document that was prepared by CSIRO for distribution worldwide to seek parties interested in commercialising the varieties in other territories. This document also included other advanced selections, which may be released as new varieties in the future. The draft document was discussed and suggestions from the committee recorded and acted upon accordingly. Following this, and after due clearance of the modified draft by HAL and CAL, the document was distributed. This resulted in 9 applications and a selection panel comprising representatives of CAL, HAL and CSIRO met during November 2010 to identify potential international commercialisers. Acting on a short list agreed to by the selection panel, Lionel Henderson (Director - Business Development, CSIRO Plant Industry) visited parties identified as suitable for the licences during December 2010 and January 2011 to acquire additional information based on questions that arose during the selection discussions. Following this, the selection panel re-convened and agreed on the successful applicants and the regions in which they would be allowed to operate. Once the decisions were endorsed by CAL and HAL, the successful and unsuccessful applicants were duly notified. The CSBRC was briefed on this at its next meeting.

CSIRO then proceeded to develop terms sheets with the successful applicants, who also visited CSIRO during the harvest period to view the varieties and other selections that were made available through the EOI. From successful negotiations of terms, the commercialisation, materials transfer and testing agreements have been or are in the process of being signed at the time of writing this report. Import permits have been received from some countries where the germplasm is to be grown and procurement of phytosanitary certificates is progressing so that bud wood can be exported.

Further developments will occur after the end of CT0700 and CSIRO has indicated that it will continue to commercialise this material and report progress at regular intervals to CAL and HAL.

4.6 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 and meetings, including Cittgroup meetings. These have been detailed in milestone reports and mentioned in the preceding sections of this chapter.

4.7 References

Yamazaki, T. and Suzuki, K. (1980) Color charts: Useful guide to evaluate the fruit maturation. 1. Colorimetric specifications if color charts for Japanese pear, apple, peach, grape, kaki and citrus fruits. *Bull. Fruit Tree Res. Stn. A.*, **7**, 19-44.

5. Triploid hybridisation

5.1 Introduction

Triploid breeding aims to generate new citrus varieties that are seedless on account of infertility resulting from uneven chromosome numbers. It is the same technique successfully used by cucurbit breeders to produce seedless watermelons, and there are many similarities that warrant examination at this early point as background information for the use of the technique in citrus. Seedless watermelons have almost completely replaced seeded varieties in the commercial marketplace over the last two decades. The increasing popularity of seedless watermelons has been driven by improvements in flesh colour and eating quality, not just the absence of seeds. Fruit are not strictly seedless, often having small soft seed 'traces' or the occasional fully-formed seed. Many of the earlier varieties were of poor eating quality and appearance and this hindered the commercial adoption of seedless watermelon. Production issues have also been an obstacle as many earlier varieties had poor seed viability, low yields and required significant inputs such as pollinator varieties and bees to achieve profitable yields.

Extensive experience has been developed at Bundaberg Research Station (BRS) in the application of triploid breeding to citrus improvement, particularly in combining seedlessness with the other characteristics essential for commercial success. This includes attention to the four obstacles discussed above in relation to seedless watermelon breeding, as well as the additional obstacle of thorniness.

This chapter describes breeding activity in the triploid component of the national project in CT07000 and focuses on significant obstacles and achievements that have occurred during the course of the project.

5.2 Crossing program

A diverse range of crosses were conducted during the four flowering seasons covered by this project. Data from the 2007 season have also been included because project end dates prevented its full description in the past report. Details of pollinations, parents, fruit set, and embryos rescued in each season are detailed in Tables 5.1a-i, which are presented in the Appendix to this chapter.

A total of 16,807 pollinations were performed during this period representing a major logistical effort to perform the required crosses during the short flowering seasons.

5.3 Parentage

5.3.1 Existing parents

Tables 5.2a-b indicate which parents (pollen and seed parents) were employed in each of the five seasons.

Table 5.2a: Tetraploid parents (pollen) used in crosses from 2007 to 2011.

	2007	2008	2009	2010	2011
*4X?Afourer	✓				
4XBakersSweet		✓	✓	✓	
*4X?Ellenor	✓			✓	
4XFremont	✓	✓	✓	✓	✓
4XJoppa	✓	✓	✓	✓	✓
4XMurcott	✓	✓	✓	✓	✓
4XParra				✓	✓
4XPomA			✓		
*4X?07N002		✓			
*4X?642W/S		✓			
*4X?05C023		✓			

* The ? indicates an original suspicion concerning the ploidy of these genotypes, which was later found to be diploid not tetraploid.

Table 5.2b: Diploid parents (seed) used in crosses from 2007 to 2011.

	2007	2008	2009	2010	2011
Arrufatina	✓	✓	✓	✓	
AustClem	✓	✓			
Corsica 1	✓	✓		✓	
DeNules	✓	✓	✓	✓	
Ellendale	✓	✓	✓	✓	✓
Fina	✓				
IM111	✓	✓	✓	✓	
Imperial	✓	✓			
Marisol	✓				
Oroval	✓	✓			
01C011		✓	✓	✓	✓
01C049				✓	
02C059		✓	✓	✓	✓
02C061		✓	✓	✓	
02C065			✓	✓	✓
02C100			✓		
02C110				✓	
02C114					✓
02C122				✓	✓
03C024					✓
03C048					✓
03C066		✓			
05C001					✓
05C016					✓
05C020			✓		✓
05C028					✓
07C007					✓
11C031					✓
11Q023					✓

5.3.2 Identifying better parents and excluding the poor ones

A significant body of knowledge now exists at BRS concerning the breeding merit of different parents (and parent combinations). Application of this knowledge has been central to the efficient generation of large populations likely to yield new commercial hybrids. The biological reality of citrus breeding dictates that much effort, over many years, ultimately leads to the conclusion that many parents should never have been used! By establishing the relative merit of parents and quickly applying this to each season's crossing program we can avoid wasting increasingly scarce breeding resources. There are three parents that warrant mention as examples of varieties that have previously been used in the program but then dropped once their progeny started fruiting.

This issue is best illustrated by 4XEmperor, which was enthusiastically included in the crossing program each year from 1998 to 2007. It was a variety with strong mandarin characteristics, well adapted to warm production areas, and had been used (in its diploid form) to breed the high quality variety Fremont. Many hybrids with 4XEmperor were produced and field-planted at BRS commencing in March 2001. Almost 200 of these hybrids were fruiting by 2007. Concerns over excessively rough skin on these hybrids caused us to quickly drop 4XEmperor from the crossing program from 2007 onwards. However, by this time a large number of hybrids were still making their way through the nursery phase and in young plantings. These were retained to enable the observation of larger numbers of fruiting hybrids (to confirm that the decision to drop 4XEmperor was not premature). By 2011 we had field planted 1,114 hybrids with 4XEmperor parentage and assessed fruit from 272 of these. Only five selections had been made and even these were considered of marginal quality (primary fault being skin texture). Consequently, nearly all remaining progeny with 4XEmperor parentage were selectively bulldozed in 2011 (Figure 5.1). This created more room for adjacent rows of hybrids of better parentage.

The two tetraploid pomelos (4XPomA and 4XPomB) used in the program at BRS have also proven disappointing, though again it is not until much effort had been expended and many hybrids fruited and assessed that this conclusion could be reached. Initially they held great promise for the program, not least of all because they were monoembryonic and so could be used as seed parents. Resulting hybrids were seedless but all lacked sufficiently high Brix levels to be of commercial merit. The pomelo parent exerted a very strong influence on all characteristics of their progeny so-much-so that crosses with mandarins bore practically no resemblance to mandarins. This inability to share traits with the other parent, and their intrinsically low Brix meant that progeny were unsuitable as anything other than potential new pomelo types. Even so, they were really were unsuitable because their fruit didn't taste sweet enough, which is one of the primary requirements for pomelo varieties. Thus, they were dropped from the crossing program from 2004 onwards (with limited use in 2009). From a practical view point, these pomelo hybrids also created field management problems because of their extremely high vigour and the crowding they created in progeny blocks. The vast majority of these hybrids (1,250 trees) were removed in late 2011. There are five selections resulting from these pomelo families and they will continue to be assessed as daughter trees to see if Brix levels improve.

Aside from these two poorly performing tetraploid parents, the disappointing performance of Wilking as a diploid monoembryonic seed parent is also worth mentioning. It was used in the crossing program from 1998 to 2004 and demonstrated very high levels of fruit set and successful embryo recovery. The first of these hybrids fruited in 2005, but we immediately made the decision to suspend its use in the crossing program. By 2007, over 100 hybrids had fruited but only one was considered worthy of selection. The remainder were coarse-skinned and had poor external and internal colour. Only five selections with Wilking parentage have been made, and in 2011 all remaining hybrids (of which 854 had been generated by the program) were culled.

Figure 5.1a-b: Complete removal of certain families from the program was carried out in 2011 in three progeny blocks at BRS. While many of these hybrids had not yet fruited and been assessed, there was sufficient information to show that these parental combinations were unlikely to produce commercially successful varieties. Culling of these families creates more room for adjacent families and reduces ongoing maintenance and assessment costs.

Conversely, the program has identified some parents that are outstanding in terms of their ability to transmit commercially desirable traits to their offspring. One of the best examples is 4XMurcott, which is present in 15 of the selections made to date (42% of all selections). It has been used in the crossing program every season since 1998 and increasingly so, as its value as a parent became apparent (as progeny started to fruit ~2005). For example in 1998 it represented only 12% of all pollinations performed but in the last four seasons has made up from 55 to 70% of annual pollinations. There are now 1,083 hybrids with 4XMurcott parentage field-planted at BRS, with 65% of these planted since 2009. This means that most of them have not yet fruited and been assessed. 4XMurcott was a relatively insignificant parent in the early progeny blocks from the program (7% of hybrids planted prior to 2009) and yet it is already a parent in 42% of existing selections. This suggests that a large number of high quality selections are likely to appear in the next few years.

5.3.3 Using new tetraploid parents (pollen parents)

Inclusion of new and better parents has always been a key objective of the triploid program. Most important among these has been 4XFremont which has been included to the maximum extent that pollen availability will allow. Pollen source trees are now maintained in the field as well as in large pots in the nursery. It has been used each pollinating season from 2007 with 1,959 pollinations incorporating 11 different seed parents. Its varietal limitations in terms of fruit size and peelability have been recognised in developing the crossing program and consequently it is only used in crosses with seed parents likely to address (and not aggravate) such limitations. It has, for example, been crossed with Ellendale each season (except 2009) with the expectation of boosting fruit size and ease of peeling.

As a result, there are now 703 hybrids growing in the field at BRS that have 4XFremont parentage, 96% of which were planted since 2008. Importantly, nine of the hybrids that were planted in 2009 have already flowered and some fruit will be available for assessment in 2012.

5.3.4 Developing new tetraploid parents

Because the tetraploid parent generally contributes two-thirds of the genetic make-up of new triploid hybrids, it is important to be continually developing better tetraploid parents. This need has long been recognised in the program at BRS and is the reason new parents like 4XFremont have been developed and incorporated (see discussion above). The main technique used in breeding new tetraploid parents relies on the spontaneous occurrence of autotetraploid seedlings from polyembryonic varieties. Seed from polyembryonic varieties are bulk-sown and the resulting plants visually screened for the occurrence of morphological distinct individuals (thicker, broader darker-green leaves, and short stubby roots). Such seedlings are then checked using flow cytometry to confirm whether or not they are tetraploid. Plants are then grown through to maturity so that pollen can be collected from them and used in triploid breeding.

Past work at BRS has relied on existing commercial varieties like Murcott, Emperor, Fremont and various sweet oranges to generate new tetraploid parents. However, during the course of this current project the technique has been extended so that BRS-bred polyembryonic varieties have now been used. This has been made possible by the DEEDI and local Queensland citrus industry funded diploid breeding project that runs in tandem to the triploid work. Many of the parents used in the diploid project are polyembryonic which results in a significant number of the resulting hybrids also being polyembryonic. Hence any newly selected hybrids that are polyembryonic can then be used themselves to develop new tetraploid parents for triploid breeding. Most of these hybrids have outstanding fruit quality.

Seed from the five best polyembryonic selections (00C018, 02C048, 02C062, 02C063, 02C104) were bulk-sown in August 2008 and the seedlings allowed to grow for 11 months. The resulting plants were then bare-rooted and individually examined for morphological traits associated with autotetraploids (mainly thicker leathery leaves, more rounded leaves, and stubby roots). Identified seedlings were then potted-up and allowed to grow for a further month. Fresh leaf material was then macerated in extraction buffer, stained with propidium iodide, filtered and freighted overnight to the Queensland Brain Institute (Brisbane). Collaborating scientists then analysed these samples to establish ploidy using techniques jointly developed in the previous project.

Thirty five seedlings were selected from the five seed parents. One hundred leaf samples were processed at BRS and analysed at QBI on seven occasions between 18th August and 14th December 2009. Two new tetraploids were confirmed for the selections 00C018 and 02C063, based on the flow cytometry outputs presented in Figure 5.2a-b.

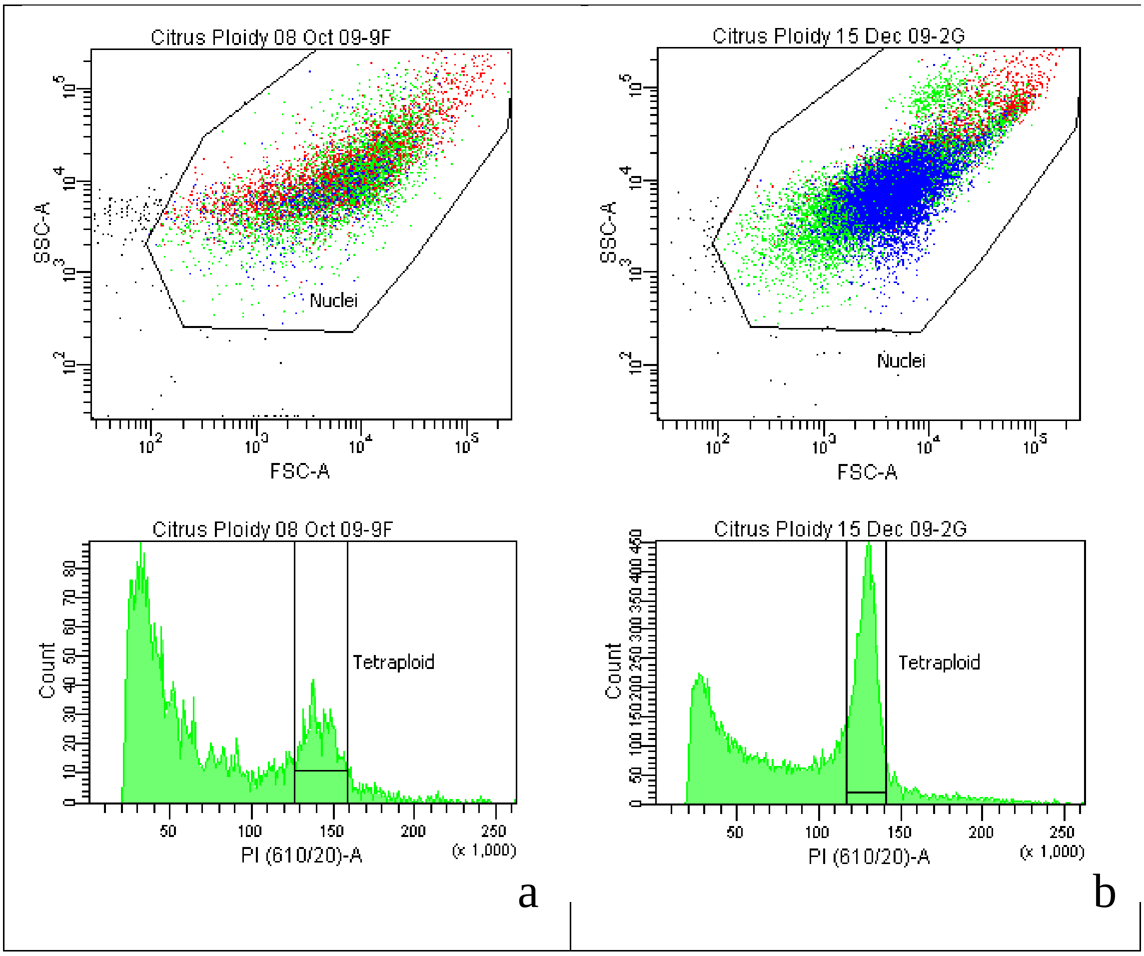


Figure 5.2a-b: Flow cytometry outputs for two new autotetraploids, 09Q062(c) and 11Q007(d), developed by the project. Progenitor varieties were high fruit quality polyembryonic selections from the diploid breeding program at BRS. Samples were processed at BRS and analysed by the Queensland Brain Institute, Brisbane, Oct/Dec 2009.

The original seedlings of these newly-confirmed tetraploids were maintained in the nursery until April 2010 at which time sufficient bud wood was available to top-work trees in the high security compound. Additional nursery trees were also propagated and later transplanted into 25L pots. Figure 4.3a-b shows the results some 18 months later for one of these new tetraploids. It is hoped that some flowers may be produced in August 2012, and if so the pollen will be immediately used in the triploid crossing program.

The exercise of identifying new autotetraploids from the BRS-bred polyembryonic hybrids proved more challenging than previously experienced with conventional commercial varieties. The frequency of morphologically distinct seedlings, and the extent to which their morphology differed from diploid seedlings, was far less than expected. While there is no obvious genetic explanation for this, in practical terms it necessitates the screening of larger numbers of seedlings than previously used. There are still many high quality diploid selections at BRS which could prove very useful to the triploid breeding program if they were available at tetraploids. Consequently, in August 2011 large numbers of seed from the diploid selections 00C029, 02C109 and

03C046 were sown in readiness to repeat the process described above. This is now an annual process with potential new progenitor selections ear-marked during each fruiting season. High quality polyembryonic hybrids from the diploid breeding project will continue to be used to develop better parents for triploid breeding.

Figure 5.3a-b: Newly developed autotetraploid 09Q062, 18 months after identification and multiplication via top-working (e) and large potted trees (f), BRS.

An unexpected outcome of using BRS-bred polyembryonic varieties was the occurrence, in high numbers, of triploid progeny amongst the seedlings sampled for ploidy confirmation. These were seedlings that we visually identified as distinct amongst their nucellar sibling, but they turned out to be triploid rather than tetraploid. It is normal to expect a percentage of seedlings from polyembryonic varieties to be zygotic, but the expectation is that these would be diploid rather than triploid. Repeated testing of these nine seedlings via flow cytometry continued to indicate their triploid status. While these triploid plants are not the desired autotetraploids, they have been kept and field-planted; as triploids derived from at least one parent of high fruit quality, they have the potential to be commercially desirable in their own right. Code numbers for these plants are 09N006 to 09N014 and they were field planted in November 2010.

5.3.5 Breeding better diploid parents (seed parents)

While the triploid program has long been hindered by the availability of high quality tetraploid parents, and the extended time period required to develop them, the importance of finding better diploids to use as seed parents has not been overlooked. Many diploids cannot easily be used as seed parents because of polyembryony, thus restricting the range of genetic material available to the breeder. However, the great benefit of running the triploid program at BRS is that a DEEDI and local Queensland citrus industry funded diploid breeding program has been running in tandem. Aside from generating useful commercial hybrids, this diploid breeding program has also proven a highly valuable source of seed parents for triploid breeding. During the course of this project, 19 new high quality seed parents were incorporated (see Table 5.1b), representing significantly improved parental material in terms of characteristics like skin texture, colour, Brix and fruit size. These better quality diploid parents will be used increasingly in future seasons.

5.4 Fruit set

We have observed wide variation in the level of fruit set following controlled crosses. The causes of this are unknown but it creates considerable difficulties in the embryo rescue phase because fruit numbers can vary widely between seasons. For some key parental combinations we have made the same crosses for many years and so have access to a data set illustrating variation in fruit set from season to season. The best data set is for the cross Ellendale x 4XMurcott, which has been made in every season since 1998. A minimum of 25 pollinations were made in each season, with at least 100 pollinations in seven of the 14 seasons. The variation in fruit set is illustrated in Figure 5.4.

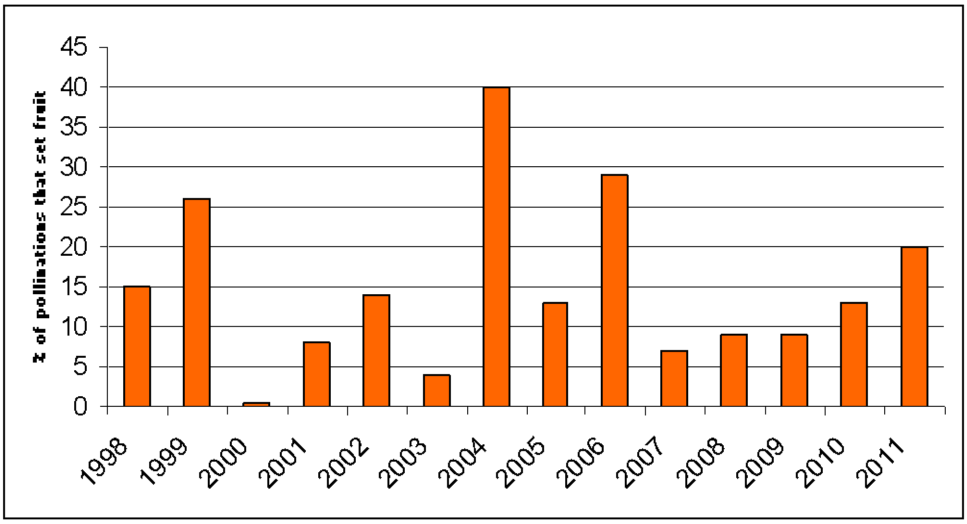


Figure 5.4: Seasonal variation in fruit set for the cross Ellendale x 4XMurcott, for crosses performed in 1998 through to 2011.

Fruit set averaged 14% over this period but ranged from 0 to 40%. The practical implications of this variability to the breeding program are very significant because it is impossible to predict how many pollinations are needed in order to generate the required number of fruit. For example, in 2004 it took only 30 pollinations to produce 12 fruit, whereas in 2008, increasing the number of pollinations by almost 100 fold (290 pollinations) resulted in barely more than twice as many fruit (26 fruit). There is no apparent seasonal trend or alternating trend in this data.

Another important parental combination, involving the same tetraploid parent, is DeNules x 4XMurcott, and it has been made every season since 1998 excepting 2004. Fruit set has ranged from 0 to 47% but averaged 28% (more than twice that of Ellendale x 4XMurcott). More importantly, fruit set rates have been less variable between seasons with nine of the eleven seasons giving at least 25% set. Another

Clementine that has regularly been crossed with tetraploid Murcott is Arrufatina, with pollinations from 2001 to 2010, excluding 2002. Fruit set averaged an impressive 41% and ranged from 15 to 67%. Figure 5.5 shows the seasonal variation in fruit set of Ellendale, DeNules and Arrufatina when crossed with 4XMurcott.

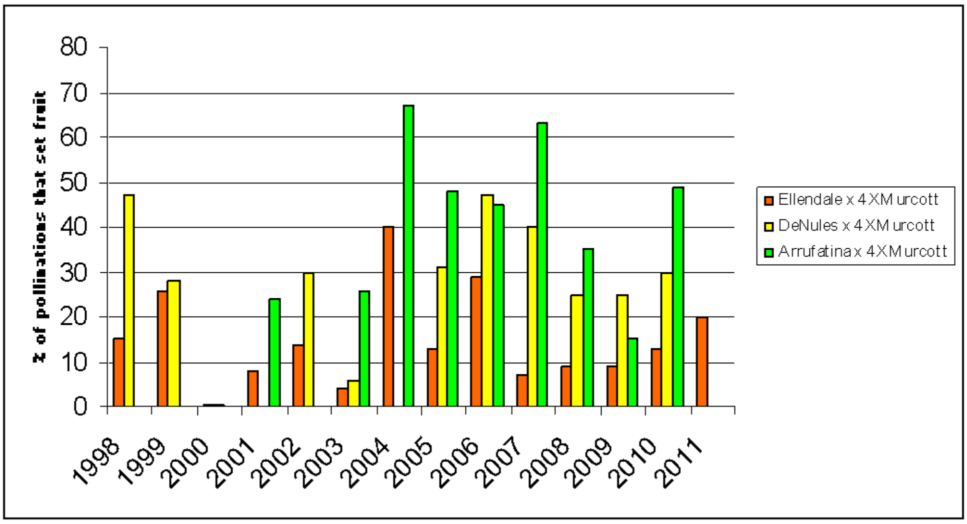


Figure 5.5: Comparison of seasonal fruit set for three crosses made regularly during the period 1998 to 2011.

There is some seasonal consistence, with 2004 being a particularly good year and 2003 being one of the poorest. However, seasons do not totally account for fruit set variation because these three seed parents show different trends.

Ellendale has also been crossed with 4XFremont for the last nine seasons (excluding 2009) and set has ranged from 3 to 41% (average 21%). As with the three families described above, the 2004 season was the best while 2003 was one of the worst. The relationship between set on Ellendale using 4XMurcott and 4XFremont pollen is shown in Figure 5.6.

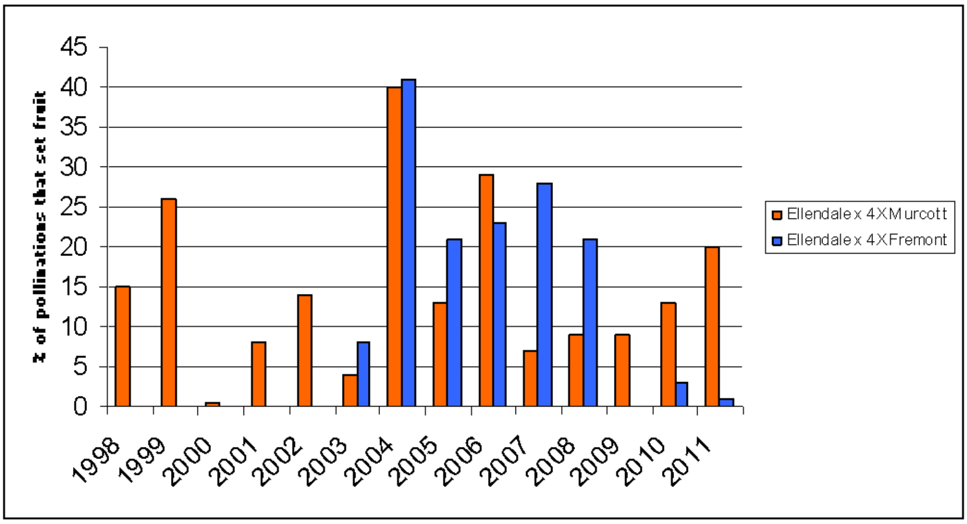


Figure 5.6. Seasonal variation in fruit set on Ellendale when pollinated with two different tetraploid pollen sources between 1998 and 2011.

Seasonal variations are more consistent with the two pollen parents than with different seed parents (Figure 5.6) suggesting that it is the seed parent which is driving seasonal variation rather than the pollen. Even though the pollen parent has a major impact on fruit set, its effect is not under such strong seasonal effects.

We have no explanation for why fruit set varies widely between seasons despite its important practical significance to the breeding program. To understand why 2004 was such a good season, and be able to replicate this every year would be a major step forward. This is particularly so given that three of the four seasons covered by this current project were characterised by low set (with the notable exception of Arrufatina x 4XMurcott). Seasonal variation in male and female fertility has been studied in the process of breeding bananas via ploidy manipulation. Ortiz *et al*. (1998) found strong seasonal effects in some varieties but not others and was able to relate this to climatic conditions. While fruit set is not a limitation in banana breeding it is possible that the variations we are seeing in citrus relates to this same phenomena of fertility variation caused by climatic conditions in different seasons.

Fortunately, the above analysis of long-term data suggests that recent low set rates are not a consistent trend and we should expect 'normal' rates to return as the breeding program proceeds. Gibberellic acid and synthetic auxins were also sprayed on pollinated trees during the 2011 flowering season to promote ovule retention. Further research aimed at improving fruit set after hand-pollination is warranted and will be incorporated into future project work as resources permit.

5.5 Embryo rescue and plantlet recovery

The process of triploid breeding results in poorly developed embryos which must be recovered via tissue culture in order to obtain hybrid plants. The efficiency of the embryo rescue process and the successful transition of plantlets to the nursery and field is one of the biggest challenges in breeding triploid citrus. It continued to cause much frustration during the course of this project. However a number of obstacles were identified and overcome and new practical techniques developed. These advancements will continue to increase the efficiency with which triploid hybrids can be established in the field.

5.5.1 Embryo recovery

Modifications were made to the embryo recovery protocol during the course of this project. This was in part due to a transition to more 'difficult' parents that produced fewer or weaker embryos as well as the need to generate higher hybrid numbers for field planting. The use of rockwool has proven highly effective in transitioning plantlets from tissue culture media. Its use stemmed from discussions in 2007 with Dr Mike Smith, an experienced practitioner in the tissue culture of woody perennial species. He considered the fundamental problem in tissue culturing these species to be the development of 'proper' roots. Although plantlets on agar grow roots, these roots differ morphologically from normal citrus roots. Regardless of how long plantlets remain on agar they seldom develop strong root systems. Consequently, it was decided to try transferring the germinated embryos onto different support media.

We evaluated a range of support media including rockwool, peat blocks and potting mix, the first of these proving the most effective. It enables plantlets to develop 'normal' roots rather than the stubby roots that occur in agar. As a result, embryos now spend mostly less than two months under tissue culture conditions (germination media in Petri dishes) before they are placed on rockwool blocks. Curiously, the other component of this project at Merbein had also commenced using rockwool (Sykes and Smith 2007), but as a way of boosting seed germination percentages rather than addressing a root development problem. It is an interesting example of simple and old technology overcoming a current obstacle in the breeding work.

5.5.2 Plantlet desiccation

A significant number of plantlets were lost in 2011 during the transition from the growth room to the protected nursery. Most of these losses were the result of desiccation from hydrophobic potting mix. This problem was caused by incorrectly composted pine bark (see Smith *et al.* 2008) and greater care is now taken during this transition to the nursery.

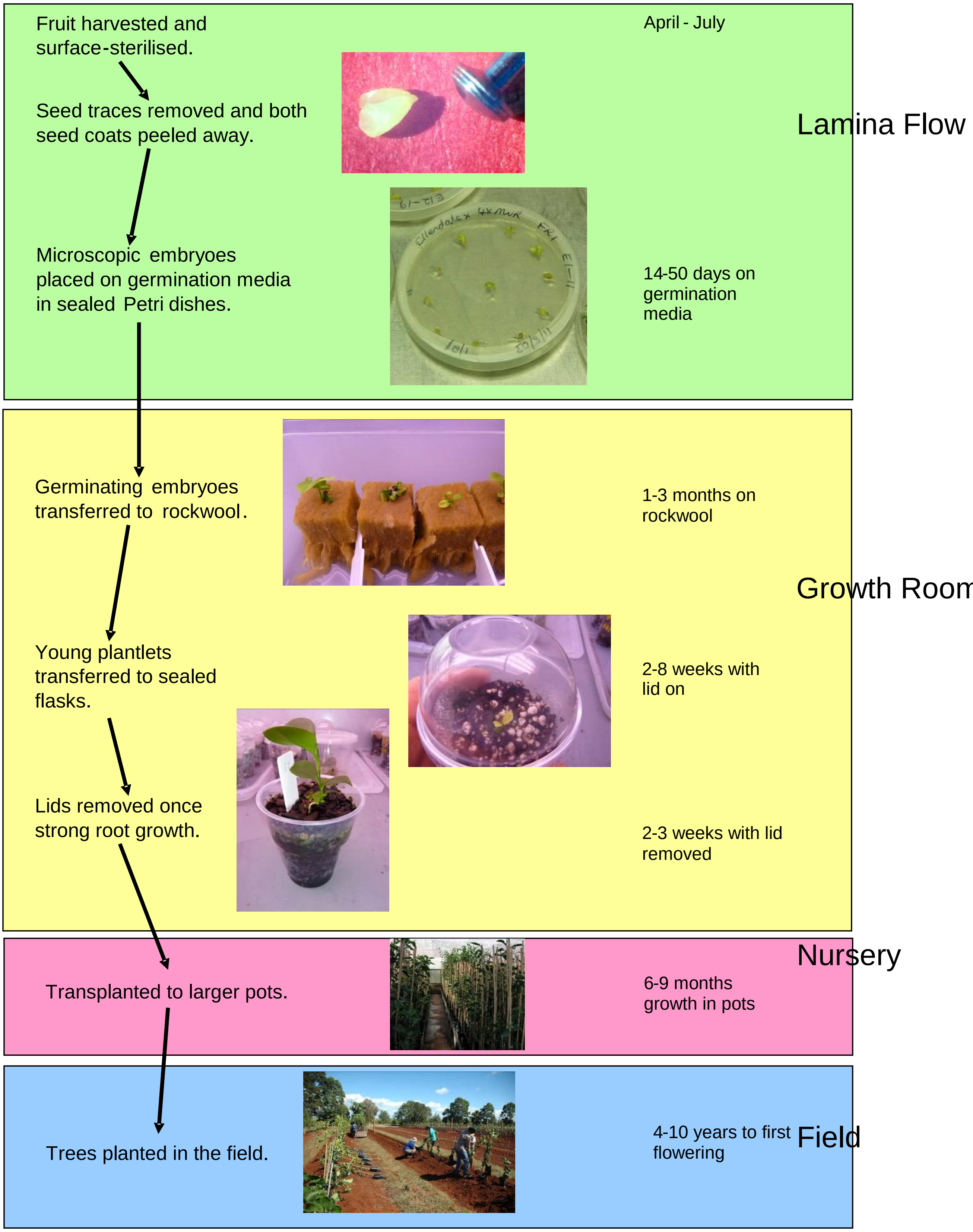
A new process was recently instigated to address this problem based on horticultural principles surrounding the production of cuttings. It is well recognised that cuttings can produce shoot growth under high humidity but unless this is also accompanied by root development the shoot growth will die when transferred to lower humidity. Similarly, plantlets derived from tissue culture will die under low humidity if they do not have a strong root system. Consequently, plantlets are now transferred from rockwool to potting-mix within *sealed* containers that generate high humidity. Lids on these containers are only removed once strong root growth can be observed through the clear plastic. Losses have been greatly reduced.

5.5.3 Improved triploid recovery protocol

The procedural changes discussed above have now become standard practice during the annual cycle of breeding in the triploid program. While the current protocol is a relatively simple process in itself, it is the result of many years of trial-and-error and much frustration for the technicians and breeder involved. So many techniques have been used unsuccessfully, despite their apparent effectiveness in other breeding programs and endorsement by visiting scientists and experts. This has included use of various agars, including floating 'boats' on liquid media, numerous plant growth regulator additions and modifications, multiple sizes and types of culture tubes, multiple procedures for hardening-up plantlets both in the laboratory and nursery, and various timeframes for transition through the different stages of the process.

Simplicity has prevailed, and the procedure is now based around fundamental horticultural principles of stimulating germination, encouraging the production of normal roots, keeping plantlets actively growing, and managing transpiration until a proper root system develops. Figure 5.7 illustrates this protocol.

Figure 5.7. Improved Triploid Recovery Protocol, BRS.



5.5.4 Fungal gnat

Large numbers of young hybrid plantlets became sick and many died during 2009 as a result of problems initially thought to be caused by *Phytophthora* spp. (Figure 5.8)

Figure 5.8. Damage to the root of a citrus seedling originally thought to be caused by Phytophthora but subsequently shown to be caused by the feeding of fungal gnats (*Bradysia* spp.).

More detailed examination revealed that a small insect (*Bradysia* spp.) was living in the potting mix and feeding off the roots. Small flying adults could sometimes be seen running on the surface of the potting mix or flying away when disturbed. These fungal gnats were particularly difficult to control, and many conventional soil-applied insecticides (e.g. chlorpyrifos) proved ineffective. Control was finally achieved with Mesurol® (a.i. methiocarb) at 1.2g/L applied as a drench to the potting mix. However, this chemical is highly toxic and its continued use is not consistent with OH&S requirements for nursery use at BRS. An effective, but safer, alternative is still being sought, while in the meantime careful monitoring is employed to detect the insect before it causes plant losses.

5.5.5 Use of Rhizotonic®

A problem with citrus triploid breeding, that is common to many tissue culture protocols for woody perennials, is the challenge of keeping plants actively growing. It is not uncommon to have plantlets that ‘sit’ at a growth stage for 12 months or more, neither growing or dying. Renewing the growth media often fails to do anything, and adding nutrients can cause toxicity. We have trialled many new ideas and procedures to speed-up plantlet growth and establishment, but one of the few that has proven effective is the use of Rhizotonic® a marine algae based liquid product. At 4mL/L (adjusted to a pH of 5.8) it is used to initially drench the rockwool blocks and then misted onto the plantlets at approximately two week intervals. It has improved survival rates through reduced transplant shock and stimulates growth. It also results in less algal growth on the rockwool blocks compared with other nutrient solutions.

5.6 Field plantings

Major field plantings of new hybrids have occurred during the current project. The first of these occurred in February 2009 and included hybrids generated in the 2005 to

2007 pollinating seasons. Included were many families that had never been planted before because of the recent inclusion of new parents. Just as importantly from a resource allocation point-of-view is the fact that these new plantings also exclude certain parents that the project had discovered as poor-performing (particularly the tetraploid pomelos and Emperor, and diploid Wilking). Some of the parents appearing for the first time in field plantings include 4XExcelsior as well as greatly expanded numbers of hybrids with 4XBurgess and 4XFremont. Figure 5.9a-b shows part of the field plantings made and maintained during the course of this current project.

Figure 5.9a-b. Hybrids from the triploid breeding program established during the course of the current project, a) at time of planting, Feb 2009, b) 2¾ years later, Dec 2011, BRS Some of these plants flowered for the first time in 2011 and a few will carry fruit in the 2012 season (three years after planting). The matrix below show those families from the 2009 planting that produced some flowering hybrids in the 2011 season.

Field plantings during the course of this current project also included, for the first time, hybrids generated from BRS-bred diploid parents (see discussion above). These were planted in May and November 2010 and in October 2011. The matrix below (Tables 5.3 a and b) shows which parental combinations are now field-established.

Table 5.3a: Parental combinations (families) that have produced some flowering hybrids after 2¾ years in the field (planted Feb. 2009, BRS).

Female \ Male	Burgess	Emperor	Excelsior	Fremont	Minneola	Murcott	Orlando	Wilk/Mur83	Wilk/Mur95	Wilk/Mur96
Arrufatina	✓		✓		✓	✓	✓	✓	✓	✓
AustClem				✓		✓				
Corsica1					✓					✓
Daisy	✓	✓		✓						
DeNules	✓					✓				✓
Ellendale				✓	✓					✓
Fina	✓			✓						
Fortune						✓	✓	✓		
Hickson										✓
Imperial							✓		✓	✓
IM111	✓		✓	✓						
Temple										✓
Wilking						✓				

Table 5.3b: Parental combinations (families) now field established at BRS that have parents which are selections from the BRS diploid breeding program.

Female Parent	Male Parent					
	4XB'sweet	4XFremont	4XJoppa	4XMurcott	4xPomA	4X?07N002[a]
01C011	✓	✓	✓	✓		
02C055	✓					
02C059	✓	✓		✓		
02C061	✓	✓	✓			✓
02C065	✓	✓	✓	✓	✓	
02C100			✓	✓		
03C066				✓		
05C020				✓		

[a] ? indicates putative tetraploid parent.

5.7 Hybrid selection and Stage 2 testing

5.7.1 Selections made during the project period

Twenty new hybrids were selected during this project from 11 different parental combinations. Details of these selections are given in Table 5.4. Descriptions are those made at the time of selection from within the progeny blocks. It can be seen that seed numbers were low, often zero with the exception of 09N005, which was selected as a potential late juicing orange. The number and quality of selections made in the last year of this project were below expectations. Many new families are now approaching an age where hybrids are old enough to start fruiting and it was expected that we would have seen some of these in 2011. However, the record-breaking summer rainfall dramatically reduced fruit quality and hampered management inputs. Despite these constraints, five selections were still made and it is anticipated that many more will be identified in the next few seasons.

5.7.2 Thorniness of triploid selections

As mentioned in the introduction, the potential for thorniness to obstruct commercial uptake of new triploids from the program has long been recognised. While this problem is not restricted to triploids, it is certainly the case that triploids are more likely to be excessively thorny than diploids generated from the same parentage. All young citrus hybrids are thorny but the problem can be particularly prevalent in triploids. Thorniness decreases as clones mature and there are various ways to hasten this process (see (Cameron and Frost 1968) p326-330). Extent of thorns is not a primary selection criteria at BRS but hybrids with excessive thorns are noted and carefully monitored.

To examine this issue of thorniness in more detail we inspected daughter trees of 30 selections and rated each tree on a scale from 0 (no thorns) to 5 (extremely thorny). The rating was done by two experienced field technicians working without knowledge of selection codes or parentage. Two separate trees of each selection were rated. These daughter trees were of different ages and had been growing in the field from between four months and five years. Results were subject to statistical analysis to determine whether:

a. some selections were more thorny than others
b. young trees were more thorny than older trees
c. parentage had any impact on extent of thorns.

Table 5.5 shows results for all triploids growing as daughter trees in the field at BRS.

Table 5.4: Parentage and fruit characteristics of 20 hybrids selected between 2007 and 2011 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	Rind Colour	Flesh Colour	Rind texture	°Brix	Plump seeds	Flat seeds	Priority	comments
08N001	AustClem	4XMurcott	medium	Murcott	orange/red	orange/red	smooth	14	3	0	2	Tastes good
08N002	DeNules	4XMurcott	medium	Murcott	orange/red	orange/red	smooth	14	0	0	3	Good, bit acid
08N003	4XPomB	Daisy	small	round	Yellow	yellow	moderate/smooth	11	0-10	0	2	Not acid, fine flesh
08N004	Ellendale	4XMurcott	large	Murcott	orange/red	orange/red	moderate/smooth	13	3.5	0	3	Taste okay
09N001	AustClem	4XMurcott	medium	flat	Red	red	rough	11	3	0	3	Little rag & albedo
09N002	DeNules	4XEmperor	small	flat	Red	red	smooth	11.5	0	0	4	Might travel well
09N003	AustClem	4XMurcott	medium	Murcott	Red	red	moderate/smooth	12	3	0	3	Nice taste
09N004	Wilking	4XEmperor	small	round	Red	red	moderate/smooth	12	3	0	4	Best of family
09N005	Wilking	4XParra	medium	round	Orange	orange	moderate/coarse	12	15	0	4	Late orange type
10N001	DeNules	4XEmperor	medium	round	Orange	yellow/orange	smooth/pebbly	12	0	0	3	Taste okay
10N002	4XPomB	Murcott	medium	round	lemon yellow	yellow/green	smooth	10	7	3	3	Thin rind
10N003	4XPomB	Murcott	small	round	Yellow	yellow	smooth		5	0	2	Grapefruit
10N004	Ellendale	4XMurcott	large	Murcott	Orange	orange	smooth	11	5	0	3	Low thorns
10N005	Ellendale	4XMurcott	large	Murcott	Orange	orange	smooth	11	5	0	3	Nice taste
10N006	Wilking	4XParra	medium	round	Orange	orange	moderate/smooth	11.5	5	0	3	Thorny
11N001	Wilking	4XJoppa	medium	round	Orange	orange	moderate	12.5	0	1	3	Orange substitute
11N002	AustClem	4XJoppa	med/small	round	deep orange	deep orange	moderate/smooth	10.5	2	7	3	Taste ordinary
11N003	Ellendale	4XMurcott	large	Murcott	Red	red	smooth	11	3	0	3	Thin skin, firm
11N004	AustClem	WilkMur96	medium	Murcott	Red	orange	moderate/smooth	11.5	0	0	2	No albedo
11N005	DeNules	WilkMur96	medium	flat	Red	orange	moderate/smooth	12	1	1	3	Thin skin, low rag

Table 5.5: Average thorniness, tree age and parentage of 30 triploid hybrid selections, grown as daughter trees, BRS.

Triploid Hybrid Code	Thorniness (0=none, 5=extremely)	Field age of daughter trees	Parentage	
			Female	Male
06N008	0.5	4	AustClem	4XMurcott
07N005	1	2.8	DeNules	4XEmperor
06N003	1.5	4	AustClem	4XMurcott
09N003	1.5	2	AustClem	4XMurcott
10N001	1.5	1.5	DeNules	4XEmperor
10N003	2	1.5	4XPomB	Murcott
07N003	2	2.8	Ellendale	4XJoppa
08N004	2	2	Ellendale	4XMurcott
09N002	2.5	2	DeNules	4XEmperor
10N004	2.5	1.5	Ellendale	4XMurcott
10N005	3	1.5	Ellendale	4XMurcott
06N005	3	4	Wilking	4XMurcott
07N006	3.5	2.8	4XPomB	Fremont
08N001	3.5	2	AustClem	4XMurcott
07N004	3.5	2.8	DeNules	4XEmperor
06N004	3.5	4	Ellendale	4XMurcott
09N005	3.5	2	Wilking	4XParra
07N008	4	2.8	4XPomB	Daisy
08N003	4	2	4XPomB	Daisy
10N002	4	1.5	4XPomB	Murcott
07N002	4	2.8	AustClem	4XPomA
06N007	4	4	DeNules	4XMurcott
08N002	4	2	DeNules	4XMurcott
07N007	4.5	2.8	4XPomB	Murcott
06N006	4.5	4	DeNules	4XMurcott
06N001	4.5	4	IM111	4XJoppa
07N001	5	2.8	DeNules	4XMurcott
09N004	5	2	Wilking	4XEmperor
10N006	5	1.5	Wilking	4XParra
09N001	5	2	AustClem	4XMurcott
Significance	<0.001			
LSD	1.44			

Statistical analysis showed that the effect of selection was highly significant, meaning that the extent of thorns was strongly related to the variety. Tree age was not important in determining thorniness with average thorniness of selections in each of the four age categories from youngest to oldest being 3.0, 3.4, 3.4 and 3.1. However, it should be noted that even the oldest daughter trees were just four years in the ground, so thorniness at this point in time represents the extreme of what might be expected in older orchards. The analysis also showed that thorniness was not dependant on parentage. This is self-evident from the table above where it can be seen that parentage is spread throughout the range of thorniness. The best example of this is AustClem x 4XMurcott (highlighted in yellow) which has produced selections ranging from very low thorns (0.5) through to extremely thorny (5) selections. Figure 5.10a-d below shows two selections from this family that vary widely in the extent of thorns.

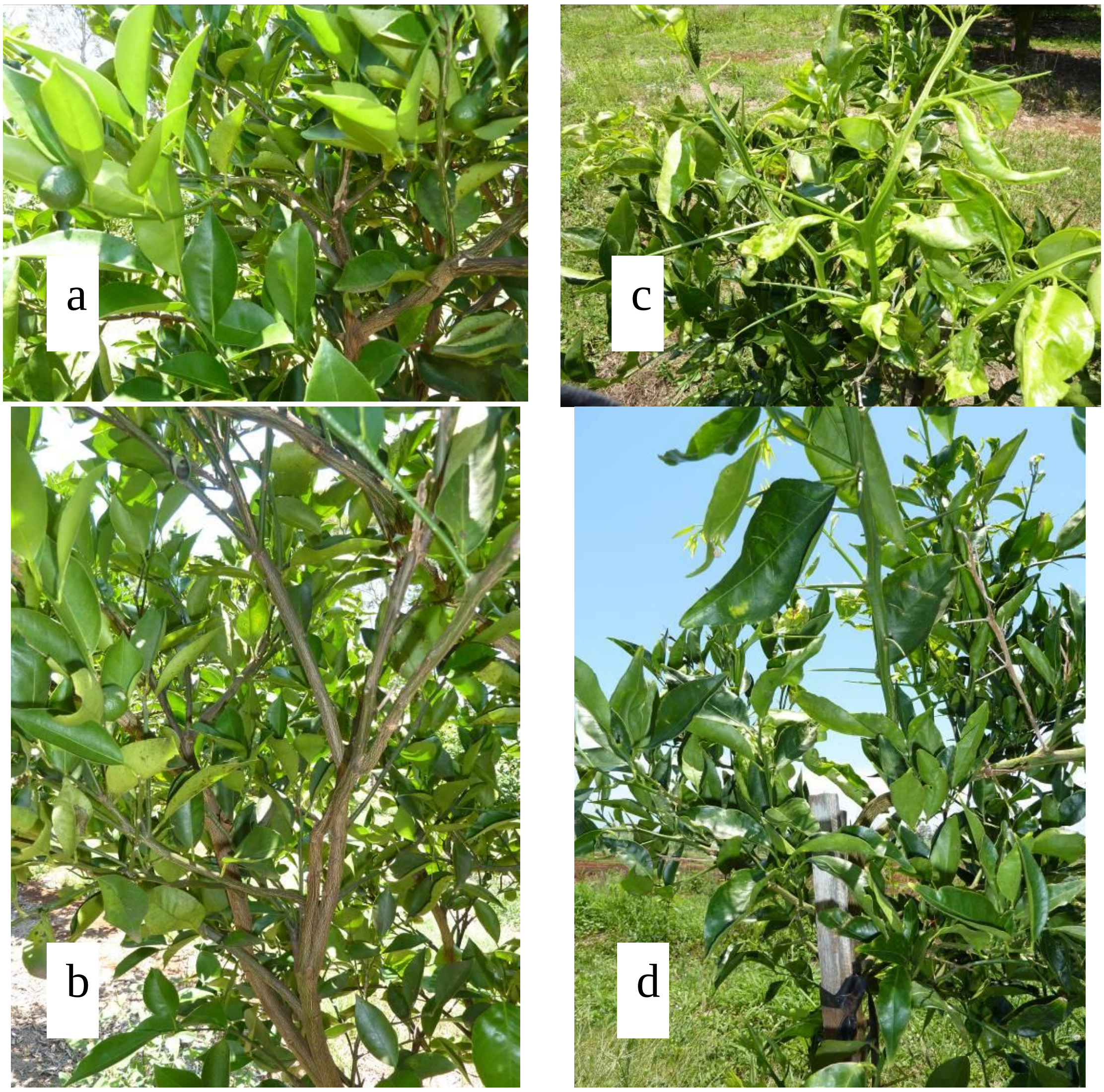


Figure 5.10a-d: Branches of 06N008 (a, b) and 09N001 (c, d) demonstrating the wide segregation for thorniness within the same family (AustClem x 4XMurcott).

These results have important practical implications for the breeding program. They demonstrate that the thorn ‘problem’ with triploids can easily be tackled by selecting against the trait. Doing so does not exclude using particular families or parents because there is wide segregation for the trait within families. The results also show that thorniness is a strong varietal characteristic because it was possible to categorise varieties using just two young replicated trees.

Such results confirm our view that the thorniness issue in triploid breeding can be managed through selection, and that it will not present an obstacle to commercial adoption. This view is supported by the semi-commercial planting of triploid mandarin ‘H3’ which occurred in Gayndah in 2007; growers considered it to be no different to other varieties in terms of thorniness.

5.7.3 Managing fruit production from daughter trees

Daughter trees fruited for the first time during the course of this current project. Some of the selections made in 2006 carried a good crop on their daughter trees in the 2010 season. One of the more promising was 06N005 which is shown below (Figure 5.11)

Figure 5.11. First crop of fruit on a daughter tree of the selection 06N005, July 2010, BRS.

Based on these promising results from 2010, it was anticipated that 2011 would be even better.   However the severe wet weather of the 2010-11 summer caused havoc and planned activities, including fruit displays for growers and industry leaders, had to be postponed to 2012.

Daughter trees of all selections from this project were assessed in December 2011 to determine which have flowered and set fruit, and are likely to produce mature fruit in the 2012 season. Table 5.6 shows the number of hybrids that were selected in each season since 2006, and how many of these are currently carrying fruit.

Table 5.6. Fruiting of daughter trees for selections made in each year of the project, Dec 2011, BRS.		
Year of Selection	No. Hybrids Selected	Daughter Trees Fruiting
2006	8	8
2007	8	8
2008	4	4
2009	5	4
2010	6	0

5.7.4 Multiplication of 06N006 and semi-commercial evaluations

Everyone involved in the project recognises the importance of making new material available commercially as soon as possible. For this reason, multiple trees of 06N006 have been propagated and will be ready for field planting in 2012 (Figure 5.12). While there is still only limited information available on the performance of this variety, the original parent tree has consistently produced good yields of high quality fruit every season since it first fruited in 2006. It also has low seed numbers, but daughter trees have been thorny even though the parent tree is not particularly so. Additional information from fruiting daughter trees will be available in April 2012 and the decision can then be made whether or not to proceed immediately with field-planting these nursery trees on a commercial property.

Figure 5.12. Nursery trees of 06N006 in the nursery at BRS, Dec 2011, ready for field planting in a semi-commercial evaluation dependent on performance of fruiting daughter trees in 2012.

5.8 References

Cameron JW, Frost HB (1968) Genetics, Breeding and Nucellar Embryony. In 'The Citrus Industry Vol 2: Anatomy, Physiology, Genetics, and Reproduction'. (Eds W Reuther, LD Batchelor, HJ Webber) pp. 325-370. (University of California: Riverside).

Ortiz R, Ulburghs F, Okoro JU (1998) Seasonal variation of apparent male fertility and 2 n pollen production in plantain and banana. *HortScience* 33, 146-148.

Smith MW, Gultzow DL, Newman TK, Miles AK (2008) Better growing media in a citrus research nursery. In '8th International Congress of Citrus Nurserymen'. Chongqing, China. (Ed. C Zhou). (Poster No. 26 (C5)).

Sykes S, Smith MW (2007) The National Citrus Scion Breeding Program July 2004 - June 2007. CSIRO and QDPI&F, HAL Project Final Report No. CT04007.

5.9 Appendix for Chapter 4: Tables of pollinations, fruit set, plump seeds sown and embryos rescued for 2007 to 2011

Table 5.1a: Number of pollinations, and percentage fruit set, for crosses performed in the 2007 season at Bundaberg Research Stn. (BRS).

Female Parent (seed)	Male Parent (pollen)															
	4X? Afourer		4X? Ellenor		4X Fremont		4XJoppa 06pollen		4X Joppa		4XMurcott 06pollen		4X Murcott		Total	
	No.	%set	No.	%set	No.	%set	No.	%set	No.	%set	No.	%set	No.	%set	No.	%set
Arrufatina			120	0									216	62	216	63
Aust.Clem	70	0	96	0							341	12			341	12
Corsica 1											270	33			270	33
DeNules	85	0	150	0							230	7	75	40	305	21
Ellendale	30	0	70	0	322	28					274	7			596	18
Fina			60	0							90	21			90	21
IM111			65	0	150	20	100	5	180	16			30	17	460	16
Imperial	26	0	329	0									9	56	9	56
Marisol											355	2			355	2
Oroval			100	0									230	4	230	4
Total	211	0	990	0	472	25	100	5	180	16	1560	10	560	33	2872	19

Table 5.1b: Number of pollinations, and percentage fruit set, for crosses performed in the 2008 season at BRS.

Female Parent (seed)	Male Parent (pollen)																			
	4X Bakers Sweet		4X Fremont 07 pollen		4X Fremont		4X Joppa		4X Murcott 07 pollen		4X Murcott		4X? 07N002		4X? 642W/S		4X? 05C023		Total	
	No.	%set	No.	%set	No.	%set	No.	%set	No.	%set	No.	%set	No.	%set	No.	%set	No.	%set	No.	%set
Arrufatina											100	35	46	20			40	8	186	25
Aust.Clem									320	4	36	8							356	5
Corsica 1									322	1									322	1
DeNules									270	10	40	25							310	12
Ellendale					110	21			290	9			30	13					430	12
Imperial									50	16	320	28							370	26
IM111	170	5					250	13											420	10
Oroval									250	3									250	3
01C011			20	5	50	0					45	0							115	1
02C059					75	0					100	7							175	4
02C061	35	0			78	5	40	3			50	6	30	3	2	0			235	4
03C066											50	6							50	6
Total	205	4	20	5	313	9	290	11	1502	6	741	20	106	13	2	0	40	8	3219	10

Table 5.1c: Number of pollinations, and percentage fruit set, for crosses performed in the 2009 season at BRS.

Female Parent (seed)	Male Parent (pollen)											
	4X Bakers Sweet		4X Fremont		4X Joppa		4X Murcott		4X PomA		Total	
	No.	%set	No.	%set	No.	%set	No.	%set	No.	%set	No.	%set
Arrufatina							300	15			300	15
DeNules							400	25			400	25
Ellendale							550	9			550	9
IM111					21	0					21	0
01C011	40	23	48	0	40	20	200	9	40	0	368	9
02C059	100	1	35	3	50	2	140	5	80	0	405	2
02C061	105	2	115	1	140	0	225	1	100	0	685	1
02C065	70	23	55	4	90	16	155	8	100	2	470	10
02C100	70	0	80	1	90	1	170	2	90	0	500	1
05C020							275	14	150	0	425	9
Total	385	7	333	2	431	6	2415	11	560	0	4124	8

Table 5.1d: Number of pollinations, and percentage fruit set, for crosses performed in the 2010 season at BRS.

Female Parent (seed)	Male Parent (pollen)															
	4X Bakers Sweet		4X? Ellenor		4X Fremont		4X Joppa		4X Murcott 09 pollen		4X Murcott		4X Parra		Total	
	No.	%set	No.	%set	No.	%set	No.	%set	No.	%set	No.	%set	No.	%set	No.	%set
Arrufatina											360	49			360	49
Corsica 1									165	1	50	52			215	14
DeNules											320	30			320	30
Ellendale			58	10	100	3			120	13	380	13			658	11
IM111	154	5			60	2	100	2					182	2	496	3
01C011	30	0			19	0	50	6			50	0	50	8	199	4
01C049					42	0					274	10			316	9
02C059	40	0			40	0	40	0			95	0	70	0	285	0
02C061	50	0			60	0	50	0			60	0	40	0	260	0
02C065					20	0	20	0			40	10	30	13	110	7
02C110	30	0					40	0			55	0	40	0	165	0
02C122			4	0	47	4					55	11			106	8
Total	304	2	62	10	388	2	300	2	285	6	1739	22	412	3	3490	13

Table 5.1e: Number of pollinations for crosses performed in the 2011 season at BRS. Percentage fruit set will be determined in May 2012.

Female Parent (seed)	Male Parent (pollen)									
	4X Fremont		4X Joppa		4X Murcott		4X Parra		Total	
	No.	%set	No.	%set	No.	%set	No.	%set	No.	%set
Arrufatina										
DeNules										
Ellendale	140				454				594	
IM111										
01C011	50				155				205	
01C049										
02C059					340				340	
02C061										
02C065	45				130				175	
02C110										
02C122	35				197				232	
02C114	53				125				178	
03C024	50				97		20		167	
03C048			50				60		110	
05C001			520				21		541	
05C016	30								30	
05C020	20				95				115	
05C028	30								30	
07C007			28		95				123	
11C031			140				102		242	
11Q023					20				20	
Total	453		738		1708		203		3102	

Table 5.1f: Number of plump seeds sown and total embryos rescued, for crosses performed in the 2007 season at BRS.

Female Parent (seed)	Male Parent (pollen)											
	4X Fremont		4X Joppa 06pollen		4X Joppa		4X Murcott 06pollen		4X Murcott		Total	
	Plump	Rescued	Plump	Rescued	Plump	Rescued	Plump	Rescued	Plump	Rescued	Plump	Rescued
Arrufatina									26	1741	26	1741
Aust.Clem							2	107			2	107
Corsica 1							2	109			2	109
DeNules							5	52	2	433	7	485
Ellendale	24	1722					0	71			24	1746
Fina							1	12			1	12
IM111	51	395	5	44	53	289			10	56	119	784
Imperial							1	12	7	16	8	28
Marisol							0	0			0	0
Oroval									1	81	0	81
Total	75	2117	5	44	53	289	11	363	46	2327	189	5093

Table 5.1g: Number of plump seeds sown and total embryos rescued, for crosses performed in the 2008 season at BRS.

Female Parent (seed)	Male Parent (pollen)																			
	4X Bakers Sweet		4X Fremont 07 pollen		4X Fremont		4X Joppa		4X Murcott 07 pollen		4X Murcott		4X? 07N002		4X? 642W/S		4X? 05C023		Total	
	Plump	Rescued	Plump	Rescued	Plump	Rescued	Plump	Rescued	Plump	Rescued	Plump	Rescued	Plump	Rescued	Plump	Rescued	Plump	Rescued	Plump	Rescued
Arrufatina											1	296	17	10			27	0	43	306
Aust.Clem									2	33	0	27							5	60
Corsica 1									1	3									1	3
DeNules									10	31	3	150							38	181
Ellendale					10	384			0	27			5	13					15	424
Imperial									1	4	51	384							52	388
IM111	29	59					118	214											147	273
Oroval									3	3									3	3
01C011			1	0	0	0					0	0							1	0
02C059					0	0					1	16							1	15
02C061	0	0			5	30	1	14			2	13	3	3	0	0			11	60
03C066											18	39							18	39
Total	29	59	1	0	15	414	119	228	17	101	76	925	25	26	0	0	27	0	335	1752

Table 5.1h: Number of plump seeds sown and total embryos rescued, for crosses performed in the 2009 season at BRS.

Female Parent (seed)	Male Parent (pollen)											
	4X Bakers Sweet		4X Fremont		4X Joppa		4X Murcott		4X PomA		Total	
	Plump	Rescued	Plump	Rescued	Plump	Rescued	Plump	Rescued	Plump	Rescued	Plump	Rescued
Arrufatina							2	139			2	139
DeNules							22	1382			21	1382
Ellendale							6	914			6	914
IM111					0	0					0	0
01C011	28	67	0	0	18	48	9	96	0	0	46	211
02C059	1	11	0	2	1	4	0	44	0	0	2	61
02C061	2	15	2	4	0	0	0	20	0	0	4	39
02C065	31	95	1	4	21	74	10	11	2	2	40	186
02C100	0	0	0	0	1	10	3	25	0	0	4	35
05C020							28	170	0	0	28	170
Total	62	188	3	10	41	136	80	2801	2	2	153	3137

Table 5.1i: Number of plump seeds sown and total embryos rescued, for crosses performed in the 2010 season at BRS.

Female Parent (seed)	Male Parent (pollen)															
	4X Bakers Sweet		4X? Ellenor		4X Fremont		4X Joppa		4X Murcott 09 pollen		4X Murcott		4X Parra		Total	
	Plump	Rescued	Plump	Rescued	Plump	Rescued	Plump	Rescued	Plump	Rescued	Plump	Rescued	Plump	Rescued	Plump	Rescued
Arrufatina											13	1697			13	1697
Corsica 1									5	0	1	104			6	104
DeNules											15	1532			15	1532
Ellendale			all plump not 4X		1	25			2	15	9	698			12	738
IM111	23	37			1	0	0	2					11	24	35	63
01C011	0	0			0	0	3	16			0	0	2	17	5	33
01C049					0	0					3	170			3	170
02C059	0	0			0	0	0	0			0	0	0	0	0	0
02C061	0	0			0	0	0	0			0	0	0	0	0	0
02C065					0	0	0	0			1	19	4	24	5	43
02C110	0	0					0	0			0	0	0	0	0	0
02C122					4	18					3	25			7	43
Total	23	37			6	43	3	18	7	15	45	4245	17	65	101	4423

6. Mutation breeding

6.1 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, CT00012 and CT04007 investigated irradiation as a means of altering current commercial varieties with regard to seediness. This research continued as part of project CT07000 and included new highly parthenocarpic selections that had been shown from previous research to yield high quality fruit.

6.2 Irradiation research at CSIRO Merbein

6.2.1 New mutation breeding at CSIRO

As suggested in the final report for CT04007, diploid hybrid selections that had performed well in regional evaluation plots were entered into a new irradiation program in CT07000. These hybrids, namely selections 2103, 2128, 2552 and 2762 (all Imperial mandarin x Ellendale tangor, see previous final report for the National Citrus Scion Breeding Program for details), are all capable of parthenocarpic fruit production, but they are also pollen fertile and can self-pollinate. The success that has been reported previously and again here in the next section with Kara mandarin, which has similar characteristics with regard to pollen and fruit development, suggested that these genotypes may be modified via gamma irradiation to produce new seedless variants. It was argued in the final report for CT04007 that if this could be demonstrated, then one of the original reasons for commencing the mutation research with Kara, Imperial and Ellendale back in CT319 will have been demonstrated and the use of gamma irradiation to supplement the diploid hybridisation program would become routine.

6.2.1.1 Materials and methods

The methods used in this component of the project followed the protocol that had been used previously with buds of Ellendale tangor, Imperial and Kara mandarins. That is, Symons sweet orange rootstocks were propagated under glasshouse conditions by sowing seeds in sand beds and raising the resultant seedlings until they were ready to receive irradiated buds via T-cuts in the bark to generate M1 trees. M1 trees that grew and survived were maintained in the glasshouse until they had grown sufficiently to cut buds from them in order to propagate M2 generation trees, which would then be established under field conditions and observed for anther and pollen development as well as the incidence of seedless fruit. In fact, due to the severe drought experienced during the project in the Murray Valley and the resultant restrictions in water availability for irrigation, all trees were maintained in large pots under glass- and shadehouse conditions until spring 2011, when they were finally established at CSIRO's experimental farm in NW Victoria.

Development and growth of the rootstock seedlings propagated were such that they were ready for budding in autumn 2008. Buds of the candidate selections were sent to the Australian Nuclear Science and Technology Organisation (ANSTO) in mid-February 2008 for irradiation at 3 gamma dosages (Table 6.1). These dosage levels were chosen as the seedless Kara lines arose from a bud that had received 6ogy of gamma irradiation while higher rates had killed mandarin buds.

Table 6.1. Gamma dosage used to irradiate buds and number of trees propagated[a] for four highly parthenocarpic hybrid selections.			
Genotype	Gamma dosage		
	40gy	50gy	60gy
2103	40 trees	40 trees	40 trees
2128	40 trees	40 trees	40 trees
2552	40 trees	40 trees	40 trees
2762	40 trees	40 trees	40 trees

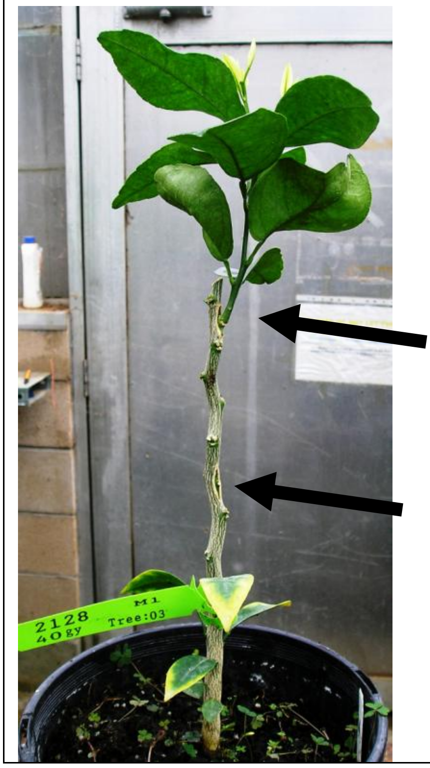
[a] two buds were T-budded to each rootstock.

Forty M1 trees were propagated for each genotype/dosage during the period February 27 to March 7, 2008. Bud survival/mortality varied between the four selections (Table 6.2) and growth rates of those buds that survived was variable. Some irradiated buds remained dormant while others developed into small shoots (Figure 6.1).

Table 6.2. Survival of gamma irradiated buds, expressed as a percentage of all buds per genotype per dosage, at 1, 14, 30 and 100 days after being T-budded into rootstocks under glasshouse conditions.

	Gamma Dosage	Days from T-budding			
Genotype	(gy)	1	14	30	100
2103	0	100	100	100	100
2103	40	100	5	5	5
2103	50	100	3.75	3.75	3.75
2103	60	100	7.5	6.25	6.25
2128	0	100	100	100	100
2128	40	100	38.75	36.25	35
2128	50	100	23.75	22.5	22.5
2128	60	100	0	0	0
2552	0	100	100	100	100
2552	40	100	35	30	30
2552	50	100	8.75	6.25	6.25
2552	60	100	1.25	1.25	1.25
2762	0	100	100	100	100
2762	40	100	1.25	1.25	1.25
2762	50	100	6.25	3.75	3.75
2762	60	100	1.25	1.25	1.25

Following budding, the growth of the M1 trees was unexpectedly slow and this prevented any M2 trees from being propagated until spring 2008 when surviving M1 trees had grown sufficiently well enough and had hardened off such that buds could be removed to propagate the M2 trees. There were no surviving M1 trees propagated from buds that had been irradiated at the 60gy dosage. While in previous work, ten buds were removed from nodes 3-through-12 on the shoot that developed and used to propagate the M2 trees, growth of the trees in this instance was so variable that as many buds as possible were taken from node 3 and above and budded into the rootstocks. Buds were removed from thirteen M1 trees to propagate the M2 trees (Table 6.3).



Upper bud developed

Lower bud dormant

Figure 6.1. A small M1 tree propagated by T-budding a Symons sweet orange rootstock with two gamma irradiated buds (40gy) of selection 2128. The image was captured 130 days after budding and while both buds have survived, only the upper bud has grown.

Table 6.3. Surviving M1 trees and number of M2 trees propagated from these. The number of trees surviving in December 2011 is presented.

Original hybrid	dose	M1 tree no.	M2 tree code	no. M2 trees propagated	M2 trees at Dec 2011
2103	40gy	12 ✓	A	15	2
2128	40gy	3✓	B	23	11
2128	40gy	10✓	C	15	7
2128	40gy	13✓	D	8	-
2128	40gy	16✓	E	16	8
2128	40gy	19✓	F	16	10
2128	40gy	20✓	G	15	-
2128	40gy	35✓	H	11	2
2128	50gy	11✓	J	4	-
2128	50gy	12✓	K	14	3
2128	50gy	17✓	L	12	-
2552	40gy	29✓	M	12	1
2552	40gy	30✓	N	15	-

Following propagation, M2 bud survival, growth and tree development was variable ranging from 0-to-68.7%. The pattern of bud mortality was similarly variable; for example, in the cases of 2128G and 2552N, the buds and accompanying wood/bark simply died; in other cases, e.g. 2128L, the bud itself died back, but the surrounding wood and bark remained viable (Figure 6.2).

Figure 6.2. Unusual bud mortality in M2 trees propagated from M1 shoots that developed from irradiated buds of hybrid selection 2128. The two images left and centre show dieback of the bud, leaf scar area and a thorn while the remaining tissue from the M1 tree remains green. The image on the right hand side shows a shoot developing from a similar bud taken from the same M1 shoot.

Following budding, the M2 trees were maintained under glasshouse conditions at a temperature range of 20-35°C and fertilised regularly to promote vigorous growth in order to speed up their evaluation, especially as the restrictions on planting due to the effects of the drought on irrigation water availability had prevented their establishment in the orchard. As a result, the trees remained vegetative and flowering failed to occur in 2009.

To continue to maintain their growth before being able to plant them in the orchard, the trees were re-potted into 33 L containers in late spring 2009 and re-located to ambient conditions in a shade house where they were maintained for the last two years of the project. However, they failed to flower again in 2010 and only 2 trees each produced one flowering shoot in spring 2011, which was insufficient to assess any changes in floral characteristics. In addition, these few flowers also failed to set any fruit.

These trees have now been established in the breeding orchard at CSIRO's experimental farm in NW Victoria and should commence flowering in spring 2012 onwards when their capacity for producing seedless fruit under the conditions of open-pollination can be assessed. Thus, data have not been collected as planned and to date no M3 daughter trees have been propagated.

As with other aspects of the CSIRO component of the National Citrus Scion Breeding Program, however, the continuation of this research is uncertain after closure of Merbein and CSIRO's exit from citrus research after December 2011. Nevertheless, the candidate selections that were irradiated are all highly parthenocarpic and it can still be anticipated that pollen sterile, and possibly ovule/embryo aborting variants of them will be obtained from this or a further irradiation program in a similar manner to that achieved with the two seedless Kara lines.

6.2.2 Seedless Kara mandarin lines

6.2.2.1 Trials with grower cooperators

6.2.2.1.1 Background to the trials

Induced mutation has been used successfully to generate new seedless variants of citrus (e.g. 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 bud lines 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 performed in a similar manner to the M2 trees from which they were propagated.

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 data obtained 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.

Expressions of interest were sought from growers to evaluate these two lines under a confidential, non-propagation and non-distribution agreement. 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 bud lines developed at Merbein. The aim of the work conducted during CT07000 was to collect data from second phase evaluation test plots with grower cooperators to test the stability of the bud lines, as well as potential yields, fruit maturity and quality. It was hoped that by the end of the project a decision would be made in conjunction with the CSBRC either on further evaluation via large-scale semi-commercial trials or the release of one or both of the lines.

6.2.2.1.2 Materials and Methods

Grower cooperators were sought either amongst the network that had been established in earlier projects or by approaching new participants at grower information meetings where the characteristics of the two lines were described. In selecting growers, a key consideration was to establish trees within orchards that would provide different levels of cross-pollination pressure. Earlier experiments at Merbein with container grown plants had shown that even when cross-pollinated under controlled conditions with Valencia orange pollen, fruit of these lines were either seedless or contained less than 4 seeds. It was important that their ability to yield seedless or low-seeded fruit be tested under conditions favouring natural cross-pollination.

Initially seven growers were enlisted to evaluate these lines. In order to speed the process, these growers were asked to top-work existing trees within established orchards so that the effects of cross-pollination could be investigated within the timeframe of the project.

Six growers were able to top-work trees and accordingly bud wood was supplied after they had signed confidential testing agreements. The sites offered by these growers were in the Murray, Darling and Murrumbidgee irrigation regions. All 6 growers successfully top-worked the trees, but growth was very poor on one sandy site at which the trees were exposed to prevailing winds and these trees have subsequently been eliminated for the investigation. The grower who maintained these trees decided to remove the orchard around the trees soon after top-working and, although the Kara lines were left in place, they struggled to become established trees, especially with irrigation allocation restrictions that were enforced at the time. Top-working by another grower was successful and vegetative growth exceeded expectations. These trees, however failed to produce fruit during the course of the project. From flowering observations in spring 2011, these trees will yield fruit during the 2012 harvest season, however. This left trees at four sites in the southern irrigation production regions.

Two growers in Queensland also offered to establish trees of the Kara lines, but were unable to top-work any existing trees. In these cases, nursery-propagated trees were supplied. One grower was able to plant trees in 2008 and the other in 2010, but so far there has been insufficient fruit produced by these trees to warrant harvesting and thus data are not presented.

Thus, for the purpose of this report, data are presented for fruit harvested from 4 sites. The first harvest was in 2010, 2 years after the trees were top-worked. Harvests were conducted at the time when the two lines were anticipated to be at optimum maturity, although some sites were sampled ahead of schedule to ascertain that fruit were developing as anticipated – these sampling data are not presented here. From observations on trees at CSIRO's experimental farm in NW Victoria, where the original source trees and daughter trees were established, fruit maturity for Kara Selection E was around early October and for Kara Selection D around early November. In the case of the site in the Murrumbidgee Irrigation Area (MIA), trees of both lines were harvested during mid-October in both years.

Fruit were harvested following the normal procedure of snapping rather than clipping the pedicel. Fruit were weighed and counted for every tree before being taken to the laboratory for analysis. The standard analysis was conducted for a minimum sample of six fruits per tree harvested.

As with most grower test plots, tree positioning in the field was determined by each grower according to that which suited their operations. Thus, the data are presented as means ± standard deviations.

6.2.2.1.3 Results and Discussion

Yields and fruit weight

Fruit yields 2 and 3 years after top-working were encouraging (Table 6.4). Mean fruit weights in 2010 were high, but reduced in 2011 as yield increased. In considering fruit size (weight), it needs to be pointed out that these trees were established within existing orchards under an irrigation/fertilizer regime developed for the citrus type that surrounded them. The aims of the growers would be to manage the surrounding trees for optimum fruit size needed for the market purposes of the respective crops. These management strategies would not necessarily be appropriate for a newly top-worked tree.

Table 6.4. Yields and mean fruit weight for two lines of Kara mandarin established as top-worked trees at four locations. Trees were harvested in 2010 and 2011, 2 and 3 years after top-working[a], respectively.

Site[b]	Name/Sel.	Harvest date	yield (kg)	Mean fruit wt. (g)
1	D	8/11/2010	18.5 ± 7.7	252.1 ± 33.0
		8/11/2011	60-70 (est)[c]	-
	E	5/10/2010	23.0 ± 9.9	234.7 ± 13.0
		7/10/2011	69.8 ± 21.5	156.07 ± 3.87
2[d]	D	9/11/2010	10.4 ± 8.8	249.3 ± 23.5
		4/11/2011	24.1 ± 8.9	173.5 ± 11.0
	E	6/10/2010	8.9 ± 3.7	188.7 ± 17.1
		14/10/2011	17.5 ± 11.7	147.5 ± 8.2
3	D	5/11/2010	12.5 ± 11.5	258.9 ± 21.6
		4/11/2011	50.0 ± 10.1	192.7 ± 7.6
	E	7/10/2010	13.1 ± 9.3	210.1 ± 10.2
		12/10/2011	59.1 ± 11.3	153.0 ± 14.0
4	D	19/10/2010	10.0 ± 9.2	162.2 ± 22.1
		18\10\2011	60.6 ± 13.8	189.0 ± 7.0
	E	19/10/2010	15.1 ± 7.6	156.4 ± 30.2
		18\10\2011	41.9 ± 25.2	140.0 ± 7.2

[a] Rootstock/interstocks were as follows:

Site 1 – Valencia orange/citrange
Site 2 – Valencia orange/citrange
Site 3 – Navel orange/citrange
Site 4 – Valencia orange/trifoliata

[b] Sites were:

1. Trees alongside an Afourer orchard in the Sunraysia Murray Irrigation Area (12 trees of each)
2. Trees within a Valencia orchard in the Lower Darling Irrigation Area (10 trees of each)
3. Trees within a Navel orchard in the Mid-Murray Irrigation Area (5 trees of each)
4. Trees within a Valencia orchard in the Murrumbidgee Irrigation Area (5 trees of each)

[c] Estimated yield in September 2011; the trial was stripped of fruit by a water-borne avian pest before harvest.

[d] A nurse limb of Valencia orange remained in place since these trees were top-worked and occupied approximately 2/3 of the canopy when they were harvested. Thus, yields are for only approximately one third of normal canopy size for 2 and 3-year-old top-worked trees.

Fruit quality
Fruit quality at all sites was generally acceptable (Table 6.5). As mentioned above, fruit size was perhaps larger than the market would wish, but, as discussed, the trees were not managed for mandarin production, especially for young trees. Similarly rinds were relatively thick, although in proportion with fruit diameter. There was some evidence of albedo breakdown, especially for the later maturing line. Again, however, the trees had no specialist management treatments and neither GA nor other rind improving sprays were applied at any stage of the trials. It would be valuable to investigate the effects of rind-improvement sprays in the next stage of evaluation of these lines.

Rind colour was good exceeding the score of 7 that would normally be allocated to Imperial mandarin using the scale employed at Merbein. Similarly, percentage juice levels were excellent and exceeded the industry standard for mandarins.

Juice sugar and acid levels varied between sites with º Brix ranging from around 10 at some sites to a high of 14 for Selection D at the MIA site in 2011. The higher sugar coupled with higher percentage acidity at the MIA site was probably a function of rootstock (see footnote in Table 6.4) and also because this region is recognized as being a later maturing area for citrus in general. The trees in the MIA site were top-worked to Valencia orange interstocks with *Poncirus trifoliata* rootstocks. The trees at the other sites were all on citrange rootstocks with either Valencia or Navel orange interstocks. The *Poncirus* rootstock used for the MIA trees probably also contributed to the smaller size of the fruit harvested there. The sugar:acid ratios for the fruit harvested in both years were acceptable and contributed to refreshing flavour that was appreciated by both growers and consumers in informal tastings that were conducted with the fruit of both lines.

Perhaps the most noteworthy characteristic measured in these trials was seediness of fruit harvested. Mean seed numbers were generally less than one, even where trees were established within Valencia orange orchards. In this regard, one of the main aims of the trials was achieved and the data supported earlier controlled cross-pollination work. The results for seed numbers were significant and warrant further details as follows.

6.2.2.2 Seediness of Kara lines Selection D and Selection E

Of particular interest were the data concerning fruit seediness in relation to surrounding polleniser varieties. The main aim of the bud irradiation program was to induce pollen sterility in parthenocarpic mandarin varieties. Trees propagated from these Kara lines develop flowers with dysfunctional pollen and from controlled cross-pollination studies appear to have ovules that for the most part abort. Even under controlled cross-pollination using viable pollen, seed numbers are low and very often zero. With the possibility of cross-pollination resulting in some seed development, the trials were established by top-working to trees within established orchards to ensure varying cross-pollination pressures. Across the various sites, the trees were surrounded by Afourer mandarin trees (site 1 - pollen fertile), by navel orange trees (site 3 - pollen sterile) and Valencia orange trees (sites 2 and 4 - pollen fertile).

Table 6.5. Quality of fruit harvested from top-worked trees of two lines of Kara mandarin at four sites during 2010 and 2011, 2 and 3 years after top-working, respectively. Data are means ± standard deviations for 6-fruit samples for all trees at each site (n = 30-to-60 depending on site)..

Site [a]	Name /Selection	Harvest date	Rind colour	Fruit diameter (mm)	Fruit weight (g)	%juice	rind thickness (mm)	Seed number	Juice:		
									º Brix	% Citrate	Sugar:acid
1	*E*	*5/10/2010*	*8.9 ± 0.4*	*87.6 ± 7.4*	*230 ± 42*	*36.4 ± 3.7*	*6.5 ± 1.8*	*0.7 ± 0.9*	*11.0 ± 1.3*	*1.1 ± 0.1*	*10.0 ± 0.9*
	D	8/11/2010	8.8 ± 0.5	90.2 ± 6.0	251 ± 5	45.5 ± 3.0	6.3 ± 0.9	1.2 ± 1.3	11.2 ± 1.2	1.2 ± 0.2	9.2 ± 1.3
2	*E*	*6/10/2010*	*8.8 ± 0.4*	*75.5 ± 8.0*	*181 ± 45*	*44.3 ± 4.8*	*4.2 ± 1.1*	*0.6 ± 0.7*	*10.3 ± 1.6*	*0.9 ± 0.1*	*11.2 ± 1.1*
	D	9/11/2010	8.7 ± 0.5	88.3 ± 7.4	253 ± 45	47.7 ± 2.8	5.1 ± 1.0	0.8 ± 0.8	10.1 ± 1.9	0.9 ± 0.2	11.8 ± 1.3
3	*E*	*7/10/2010*	*8.8 ± 0.4*	*86.3 ± 6.1*	*222 ± 4*	*40.8 ± 2.9*	*6.2 ± 1.5*	*0.3 ± 0.6*	*10.9 ± 1.1*	*1.0 ± 0.0*	*11.0 ± 1.0*
	D	5/11/2010	8.3 ± 0.5	91.1 ± 7.5	254 ± 43	47.0 ± 3.1	6.5 ± 1.4	0.0	10.6 ± 0.9	1.1 ± 0.1	9.6 ± 0.8
4	*E*	*19/10/2010*	*9.0* ± 0.0	*77.3 ± 8.6*	*156 ± 30*	*45.5 ± 3.3*	*5.7 ± 1.0*	*0.1 ± 0.3*	*13.2 ± 0.5*	*1.6 ± 0.1*	*8.5 ± 0.6*
	D	19/10/2010	9.0 ± 0.0	78.8 ± 3.8	162 ± 22	45.3 ± 3.4	5.9 ± 0.8	0.0	14.0 ± 0.3	1.9 ± 0.1	7.3 ± 0.1
1	*E*	7/10/2011	9.8 ± 0.2	76.6 ± 3.4	174 ± 15	42.5 ± 2.0	5.4 ± 0.5	0.2 ± 0.3	10.2 ± 0.7	0.9 ± 0.1	10.8 ± 0.9
	D[b]										
2	*E*	14/10/2011	8.4 ± 0.3	76.3 ± 1.6	175 ± 11	45.5 ± 2.1	4.5 ± 0.3	0.1 ± 0.2	9.7 ± 0.4	0.8 ± 0.1	12.5 ± 1.6
	D	4/11/2011	8.6 ± 0.3	82.0 ± 2.9	200 ± 14	50.5 ± 2.2	4.8 ± 0.4	0.3 ± 0.3	9.8 ± 0.7	0.9 ± 0.1	11.2 ± 1.2
3	*E*	12/10/2011	8.7 ± 0.1	72.9 ± 2.2	156 ± 16	42.5 ± 1.5	5.1 ± 0.3	0.0	10.6 ± 0.8	0.8 ± 0.1	12.7 ± 2.1
	D		8.5 ± 0.3	81.6 ± 1.4	195 ± 11	47.8 ± 1.9	5.7 ± 0.3	0.0 ± 0.1	10.7 ± 0.5	1.2 ± 0.1	9.3 ± 0.6
4 [C]	*E*	19/10/2011			140 ± 7			0.1 ± 0.4	12.2 ± 1.3	1.2 ± 0.1	10.0 ± 1.1
	D	19/10/2011			189 ± 7			0.1 ± 0.4	12.2 ± 0.4	1.4 ± 0.1	8.9 ± 0.5

[a] Sites were:

1. Trees alongside an Afourer orchard in the Sunraysia Murray Irrigation Area
2. Trees within a Valencia orchard in the Lower Darling Irrigation Area
3. Trees within a Navel orchard in the Mid-Murray Irrigation Area
4. Trees within a Valencia orchard in the Murrumbidgee Irrigation Area

[b] the trial was stripped of fruit by a water-borne avian pest before harvest.

[C] Due to quarantine restrictions caused by Queensland Fruit Fly, fruit could not be returned to the laboratory for full analyses and only field weights were collected along with juice samples for lab analysis.

Seediness data were collected for fruit from trees harvested during 2010 and 2011 by dissecting a sample of fruit from all the trees harvested (Figure 6.3). The number of fruit sampled across sites varied from 331-to-12 (Table 6.6). Data concerning seediness obtained for the original M2 trees located at CSIRO's experimental farm in NW Victoria, where trees were surrounded by many sources of pollen from progenies in the breeding program, are also presented in Table 6.6.

Figure 6.3. Fruit harvested from Kara line Selection D cut in the orchard at site 4 (MIA) to assess seediness.

At all sites, and over two seasons, the maximum number of seeds in any one fruit of either line was 4 (c.f. 20+ for normal Kara mandarin) and the mean number of seeds per fruit was less than 1. In many markets, these Kara mandarin fruit would qualify as seedless, although because there is always a chance of a fruit containing up to 4 seeds, a more appropriate marketing descriptor would be to label them as low-seeded.

At site 1, 50% of fruit harvested were seedless in 2010, while at sites 2, 3 and 4 this was around 50%, 90% and 90%, respectively, for line Selection D, and 60%, 85% and 98%, respectively, for line Selection E (Table 6.6). In 2011, these were higher for both lines at sites 1, 2 and 3, but, similar for Selection D and lower for Selection E at site 4. Seasonal variations in conditions at flowering influencing bee behavior may have accounted for these seasonal differences, but this would require further investigation.

The variation in seed number between sites was anticipated for sites 1, 2 and 3, but not for site 4 where a greater proportion of the fruit were seedless even though the trees were surrounded by Valencia orange. It is unclear why this may have occurred, but clearly demonstrated the potential for these two lines to yield seedless fruit even where the potential for cross-pollination is high.

Table 6.6 Seediness of two Kara mandarin selections at 5 sites offering different cross-pollination pressures

Kara Selection	Site[a]	Year	No. fruit	% seedless	Mean seed no.	Range
D	**1**	**2010**	**331**	**49.5**	**0.75 ± 0.88**	**0 - 4**
		2011	**12[b]**	**83.3**	**0.25 ± 0.62**	**0 – 2**
	2	2010	289	51.9	0.69 ± 0.86	0 - 4
		2011	120	85.8	0.20 ± 0.59	0 – 4
	3	**2010**	**115**	**89.6**	**0.13 ± 0.41**	**0 - 2**
		2011	**200**	**99.0**	**0.01 ± 0.10**	**0 - 1**
	4	2010	72	91.7	0.08 ± 0.28	0 - 1
		2011	150	90.0	0.12 ± 0.38	0 - 2
	CSIRO	**2010**	**79**	**65.8**	**0.52 ± 0.85**	**0 - 4**
		2011	**200**	**88.0**	**0.17 ± 0.50**	**0 - 3**
E	1	2010	246	48.4	0.74 ± 0.86	0 - 4
		2011	215	84.7	0.20 ± 0.53	0 – 3
	2	**2010**	**179**	**63.1**	**0.53 ± 0.79**	**0 - 3**
		2011	**230**	**91.3**	**0.10 ± 0.36**	**0 – 2**
	3	2010	252	85.7	0.18 ± 0.48	0 - 3
		2011	220	98.6	0.02 ± 0.16	0 – 2
	4	**2010**	**71**	**98.6**	**0.03 ± 0.24**	**0 - 2**
		2011	**150**	**90.7**	**0.11 ± 0.38**	**0 – 2**
	CSIRO	2010	27	44.4	0.74 ± 0.81	0 - 3
		2011	181	93.9	0.07 ± 0.27	0 – 2

[a] Sites were:

1. Trees alongside an Afourer orchard in the Sunraysia Murray Irrigation Area (12 trees of each)
2. Trees within a Valencia orchard in the Lower Darling Irrigation Area (10 trees of each)
3. Trees within a Navel orchard in the Mid-Murray Irrigation Area (5 trees of each)
4. Trees within a Valencia orchard in the Murrumbidgee Irrigation Area (5 trees of each)

CSIRO – surrounded by many pollen sources

[b] Data from a sample of 12 fruit collected 7 ahead of anticipated harvest date. Fruit were stripped from trees by water fowl before harvest.

The data for site 3, where the trees were surrounded by pollen sterile navel oranges were interesting in that it was anticipated that nearly 100% of fruit would have been seedless. However, in 2010 around 10-15% was seeded. While it was assumed when the trees were top-worked that they were far removed from any viable pollen source, a walk after harvest in 2010 revealed that the grower had also top-worked some trees approximately 50m away with a new mandarin variety that produces viable pollen. These trees were probably the reason for greater than anticipated seed numbers in fruit harvested in 2010.

The seediness of the fruit harvested from the original M2 trees of the two lines were as expected, especially as they are surrounded by a very mixed population of genotypes producing viable pollen.

6.2.2.3 Line stability

A potential issue with any variety developed as a variant of another via mutation breeding is that a reversion can occur in daughter, grand-daughter or subsequent vegetative propagated generations of trees. This has been noted recently with seedless Daisy mandarin from California (Roose *et al.*, 2010) and most likely occurs due to the selected lines being a chimera with the chance of reversion being a characteristic of such bud lines.

The Kara lines described here may equally revert to the original type and produce seeded fruits rather than the seedless or low-seeded types harvested in the trials that have been conducted so far. The manner in which the two lines were selected originally from M2 trees may mean that this is less likely to occur, however, than if they had been selected as M1 trees and buds collected from these to propagate subsequent generations. Kara Selection D and Kara Selection E have defined bud line pedigree. Nevertheless, should one or both become new varieties, the stability of daughter trees must be a consideration in to the future.

So far, from the trees propagated for regional and PBR DUS trials, there has been no evidence for any reversion to a seeded type, however.

While for brevity further data are not presented here, seedless fruits have been harvested from the M2 trees and also from M3 trees generated from them indicating that these lines are stable. This has been seen with trees at CSIRO and in the regional grower-based trials and has been reported in the project milestone reports.

Evidence for line stability has come from a small trial that was established at CSIRO farm in NW Victoria involving M3 generation trees and a PBR DUS trial that was maintained in large pots under shadehouse conditions at Merbein.

The vast majority of fruit harvested from the trial at CSIRO's experimental farm in NW Victoria in the last 3 seasons (2009-to-2011) were seedless with 4 being the most seeds in any fruit. The site of this trial is such that cross pollination with viable pollen is extremely likely and there is a high cross-pollination pressure. Nevertheless, and in comparison to the control unselected Kara trees, from which only seeded fruit (many having 30+ seeds) were harvested, the results obtained indicated that line stability with regard to the production of seedless fruit has been maintained in the daughter trees.

The floral characteristics of the seedless Kara lines have been assessed using the trees propagated for the PBR DUS trial This trial also includes M4 generation trees. Here, as expected, the Kara control and M1 trees produced viable pollen and the M2 trees dysfunctional anthers, which failed to dehisce viable pollen (Fig. 6.4), as had been the original observation when the two lines were first selected. The M3 and M4 trees also produced flowers with dysfunctional anthers (Fig. 6.4), which like those of the M2 trees failed to produce viable pollen. This further demonstrated the stability of the selected seedless lines of Kara generated from the original irradiated buds.

Fig 6.4. Normal viable pollen produced from the anthers of flowers from the control Kara and the M1 trees compared to dysfunctional anthers from flowers of the M2, M3 and M4 generation daughter trees which do not release pollen. As a consequence the M2, M3 and M4 trees are unable to self pollinate and when planted as a solid block, they yield seedless fruit.

6.2.2.4 Further development of Kara lines Selection D and Selection E

Late-to very late maturing seedless mandarins are being sought by citrus breeding programs around the world. As such, these seedless Kara lines are promising and they have generated significant interest amongst citrus variety commercialisers internationally, some of whom are in the process of being licensed to evaluate them further in production regions in other parts of the world.

Within Australia, fruit from the Kara lines have been displayed on a number of occasions and have generated interest, which other than when grower cooperators were recruited for the evaluation trials described here, has been handled by informing interested parties that the selections are still under evaluation and may be released at a later date.

Fruit were again displayed to interested growers during October 2011 in the Murray and Murrumbidgee production regions. Particular interest was shown in the two lines at the AGM of Riverina Citrus Growers at which the principal investigator delivered a presentation describing the history of the two lines and data from the grower trials. A number of growers registered an interest in the varieties as they could see an opportunity for late seedless mandarins for the region, which is traditionally a late maturity area. Included in these growers was the host of the trial in the MIA who specifically asked if he could propagate more trees to be able to produce fruit for commercial purposes.

This interest shown by producers was discussed at a meeting of the CSBRC during November 2011 at which data from the trials were tabled for consideration. It was agreed that these two low-seeded lines of Kara mandarin should now be entered into large-scale semi-commercial top-worked trials on cooperator grower properties. This would provide further fruit for market evaluation before a decision to release the lines could be reached.

Initially, expressions of interest in establishing 0.5-1.0 ha top-worked trails should be sought from the growers who have participated in the evaluation of the lines described here. Depending on their response, other cooperators should also be sought. These trials, as for previous semi-commercial trials with advanced selections, will be conducted under confidentiality arrangements with growers who sign a non-propagation, non-distribution agreement (NPNDA).

In anticipation that Plant Breeders Rights may be sought for these two lines of Kara mandarin, a distinctness, uniformity and stability (DUS) trial (as reported earlier) has been propagated and data collected. This trial was maintained as container-grown trees under shadehouse conditions, but with the closure of CSIRO Merbein, this trial has now been established under drip irrigation at CSIRO's experimental farm in NW Victoria.

6.2.3 References

Hearn, C.J. 1986, Development of seedless grapefruit cultivars through budwood irradiation. J. Amer. Soc. Hort. Sci. **111**, 304-6

Roose M.L., Williams, T.E. and Federici, C.T. 2010, Production of seedy fruit by ‘DaisySL’ mandarin. See: http://plantbiology.ucr.edu/faculty/DaisySL-seedy%20fruit%20032310.pdf

Spiegal-Roy P. and Vardi, A (1989) Induced mutation in citrus. Proc. 6th Int. Congress of SABRAO, Tsukuba, Japan, 773-5

Sykes, S., Koltunow, A. and Lee, S. (1994) The genetic improvement of mandarins in Australia. Aust. Citrus News (July), 8-12.

Sykes, S.R. & Possingham, J.V. (1992). The effect of excluding insect pollinators on seediness of Imperial mandarin fruits. Aust. J. Exptl. Agric. **32**, 409-11.

6.3 Mutation breeding research at DEEDI, Bundaberg

Mutation breeding represents a small component of national breeding project activities at Bundaberg, though it has been employed extensively in the past to generate the now commercial varieties IrM1 and IrM2. It is also currently being used in the DEEDI and local Queensland citrus industry funded diploid breeding under CT09023 to reduce seed numbers in selected hybrids. The mutation breeding work within CT07000 stems from the irradiation of budwood of nine cultivars which occurred in 2000, from which resulting nursery trees were field-planted in late 2001. Limbs of trees propagated from irradiated buds of all nine cultivars were identified from seed counts conducted in 2003 to 2006. Budwood was taken from the best of these to generate daughter trees, which were field-planted in 2005 and 2007 and assessed for seed number, fruit size, Brix, acid, and pollen viability until 2010.

As a result we have identified two selections of Fremont with greatly reduced seed numbers and that have retained the normal fruit size of seedy Fremont. The reduction in seed number following irradiation is often accompanied by reduced fruit size. This was a critical issue for Fremont because it is a variety that is already considered of marginal size for commercial markets. We obtained some low-seeded selections with less seeds, but the reduction in fruit size made them commercially unacceptable. Figure 6.5 shows seed numbers for R2P31a and R2P31b in each of the four seasons 2007 to 2010, compared with normal Fremont.

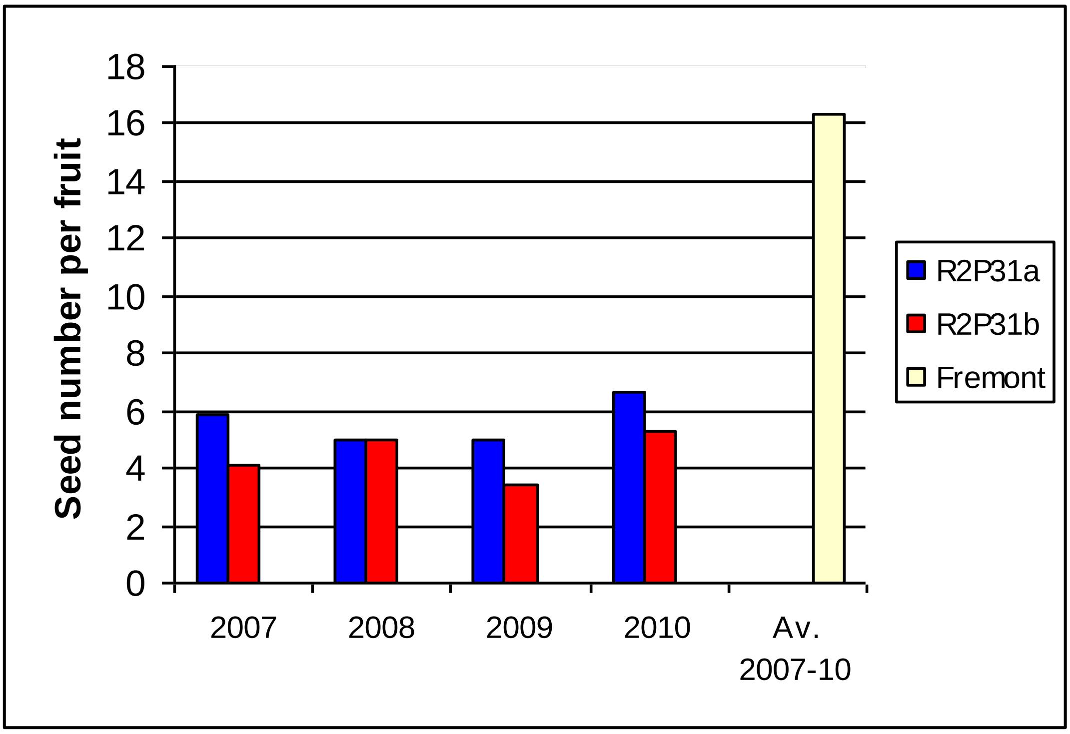


Figure 6.5: Average seed number per fruit of two low-seeded selections of Fremont compared with normal Fremont over four seasons. From daughter trees grown in a mixed pollen environment, BRS.

It can be seen that these two selections have less than one-third of the seed number normally found in Fremont, even when grown in an orchard with high pollination pressure. It has been demonstrated that normal Fremont can be totally seedless in a commercial orchard when grown in isolation (Hoult *et al.* 2008), so it is reasonable to expect that our new low-seeded selections will have even lower seed numbers than in Figure 6.3 when grown in solid blocks under commercial conditions.

Grand-daughter trees of both selections are currently being used to generate meristems for shoot-tip-grafting. Once CTV-free accessions have been produced, they will be made available to AusCitrus for re-testing and entry into their budwood scheme. While Fremont is still grown commercially, and is important on some export markets, it is not considered of sufficient merit for the expensive process of PBR protection and commercialisation. Instead it is proposed to make R2P31a and R2P31b freely available to Australian growers who are considering planting Fremont so they can obtain the additional advantage of less seeds.

Reference

Hoult MD, Moore C, Connelly M, Hearnden M, Smith MW (2008) Early performance of Fremont mandarin on several rootstocks in a hot, monsoonal environment. In 'Proceedings of the International Society of Citriculture 11th International Citrus Congress'. Wuhan, China. (Ed. X Deng). 216.

7. Technology Transfer

7.1 The citrus scion breeding reference committee
7.2 The breeding plan
7.3 Extension activities

7.1 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 played an important role in technology transfer by assisting in communicating activities, research outputs and industry outcomes to the wider citrus industry. During project CT07000, 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 and in evaluating expressions of interest from off-shore entities for the evaluation and commercialisation of these varieties and other advanced selections.

The CSBRC held regular meetings via telephone conference during project CT07000. Formal minutes of the meetings of the CSBRC were recorded and circulated during the course of the project.

7.2 The breeding plan

As has been the case in other recent projects, CT07000 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 this 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** a**nd 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.

7.3 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 project milestone reports and also in the preceding chapters where appropriate. Activities 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, 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.

A dot point summary follows:

*Joint meeting of AusCitrus and Queensland Citrus Improvement Committees, February 2008.

* Annual Mildura Field Days, May 2008, 2009, 2010 and 2011.

* Annual Riverland Field Days, September 2008, 2009, 2010 and 2011.

* Article, HAL Citrus Annual Industry Report, 2008.

> Sykes S and Smith M (2008) New project started to breed new Australian fruits with consumer appeal. HAL Citrus Annual Industry Report, 2008.

* QDPI&F Citrus R&D Consultants Forum, Mundubbera, Qld, September 2008.

* CITTGROUP visit to CSIRO Merbein, October 2008.

* 11th International Society of Citriculture Congress, Wuhan, China

> Sykes S and Smith MW (2010) Australia's National Citrus Scion Breeding Program. In: Proceedings 11th International Citrus Congress, Wuhan, China, Vol. 1, 220-221. China Agricultural Press, China.

> Smith MW, Gultzow DL, Newman TK, Chapman JC, Hofman HJ and Johnson TV (2010) Breeding for climate change in sub-tropical Australia. In: Proceedings 11th International Citrus Congress, Wuhan, China Vol. 1, Paper 130. China Agricultural Press.

* Peer reviewed publications

> Sykes SR (2008) The effect on citrus fruit of excluding pollinating insects at flowering and implications for breeding new seedless cultivars. Journal of Horticultural Science and Biotechnology 82, 713-718.

> Sykes SR (2008) Segregation in an 'Imperial' mandarin x 'Ellendale' tangor family for characteristics that contribute to the seedless phenotype. Journal of Horticultural Science and Biotechnology 83, 719-724.

* QDPI&F Industry Extension Meetings, February and March 2009.

> Smith MW (2009) Rootstocks and New Scions for North Queensland Citrus Growers. In: Presentation to North Queensland CITTGROUP meeting, 19 February 2009. Ed. S. Powell.

Smith MW (2009) Developments in Local Breeding Programs. In: Presentation at the Citrus Research and Development Seminar. Greenhaven Orchard, 5 March 2009. Eds. H Hofman, A. Miles, S Powell.

* Article on ‘Pollination and Seediness in Citrus Easy-peels’. In: Australian Citrus News, 2009.

* Citrus Australia Conference, Presentation on Commercialisation Strategy, November 2009.

* Citrus Australia Conference, Field Walk and Breeding and Evaluation Update, November 2009.

* Public tastings of the Merbeingold varieties, Mildura, July and August 2010.

* Article titled ‘Breeding Program Moves Forward’ in HAL Annual Report to the Citrus Industry, 2010.

* Imperials/Easy Peel Mandarin Fora, in various citrus producing regions, 2010.

* National Citrus Pathology Workshop, Genetic Solutions to Commercial Citrus Disease Problems, 2010.

* Citrus Australia Ltd., Grower Pre-Season Quality Workshops, 2011.

* Citrus Australia Ltd., Varieties Committee, Breeding Program Update and Commercialisation Strategy, Melbourne, March 2011.

* Article, HAL Citrus Annual Industry Report, 2011.

8. Recommendations

8.1 Scientific

- Continue evaluation of promising selections, combining seedlessness or low seed numbers, attractive internal and external appearance and high eating quality
- Enter three selections from the mutation breeding program (two low-seeded Kara mandarins from Merbein and a low-seeded Fremont from Bundaberg) into large scale semi-commercial trials with industry co-operators
- Utilise parents, identified from knowledge that autonomic parthenocarpic fruit set is under the control of three dominant complementary genes, to produce progenies consisting of all parthenocarpic hybrids,
- Utilise short juvenility hybrids identifided from 2nd and 3rd generation crosses involving short juvenile period germplasm to further shorten the breeding cycle.

- Continue to focus on breeding better quality diploid and tetraploid parents, with an additional focus on disease resistance.
- Undertake more research on seasonal/climatic effects on pollen fertility as a step toward improving fruit set and hybrid recovery in triploid breeding.

8.2 Industry

- Provide technical information on the production and fruit quality characteristics of Merbeingold 2336 and Merbeingold 2350, as presented in this report, to the Australian national commercialiser of the varieties to assist communication of performance data to potentially interested growers and to retailers.
- Showcase new hybrids from the triploid program to growers, associated industry, and for testing with consumer groups
- Seek industry support to continue the triploid breeding program through Agri-Science Queensland.
- Seek industry support to identify ways to see through to completion the evaluation of promising hybrid progeny from the southern diploid and mutation breeding program.

9. Acknowledgements

The following are duly acknowledged for valuable and significant inputs made to project CT07000:

Toni Newman, Debra Gultzow, Jamie Fischer and Rachael Langenbaker have provided excellent technical skills at Bundaberg Research Station.

Shona Duffield, Suze Cox, Phil Cupper and Margaret Symens have provided excellent technical skills at CSIRO Plant Industry, Merbein and also liaised with cooperating growers in the maintenance and harvest of regional test-plots in the Murray Valley.

The farm team at CSIRO, Merbein, led by Mr Grant McCarthy and more recently by Mr. Arryn Clarke, have skilfully maintained CSIRO's citrus plantings.

The members of the Citrus Scion Breeding Reference Committee, especially the industry representatives, have willingly devoted much valuable time and effort in attending meetings. Their contribution to the project is highly valued and their respective inputs have been highly respected and very much appreciated by the project team and other committee members from HAL, QDPI&F and CSIRO.

Citrus growers who have cooperated in the regional evaluation of hybrid selections from CSIRO. As signatories to testing agreements, these growers have to remain anonymous, but are duly acknowledged here for the valuable in-kind contribution they have made to the project.

Dr Rob Walker and Virginia Barber provided input to completing the final stages of this report.