

Horticulture Innovation Australia

Final Report

Low-chill Stonefruit Cultivar Breeding and Evaluation

Dr Bruce Topp
The Department of Agriculture and Fisheries (DAF)

Project Number: SF07003

SF07003

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PURPOSE OF THE REPORT:

The purpose of this report is to describe the breeding and associated research, development and extension that occurred during the SF07003 Horticulture Australia Limited project that was conducted from 2007-2014.

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MEDIA SUMMARY

Peaches and nectarines grown in subtropical Australia are called low-chill stonefruit. They require only a small number of cold nights each winter to break dormancy which allows them to thrive in the subtropics. Low-chill stonefruit are mostly grown in northern NSW and southern QLD. They produce peaches and nectarines that are fully ripe in September and October, well before there are any other Australian stonefruit available for consumers. The low-chill sector of the Australian stonefruit industry produces peaches, nectarines and plums with an annual wholesale value of \$35M.

Low-chill stonefruit quality is of high importance to the wider national industry as it sets the standard for the start of the domestic season. Consumers purchasing high quality low-chill peaches in October and November will continue purchasing high-chill peaches during summer. Conversely, consumers will not purchase high-chill fruit if they have bad experiences with low-chill varieties. In the future, low-chill cultivars may have direct benefit in temperate regions of Australia that are affected by global warming.

The low-chill peach breeding program is located at Nambour in Queensland and was initiated in 2001 in response to industry requests for new peach and nectarine cultivars adapted to subtropical Australia. The program is developing a range of high quality peach and nectarine cultivars that will grow well in subtropical Australian conditions.

From 2007-2013, over 10,000 new peach and nectarine trees were field planted at Nambour and evaluated for fruit and tree traits. Fruit characteristics being selected are large size, high flavour, firm flesh and early ripening. Tree characteristics being selected are low chilling requirement, moderate crop load and compact tree habit. We selected 64 elite trees and these have been sent for grower testing.

Growers play a major role in the breeding program through their involvement in a two-stage grower testing scheme. The first stage is a small-scale test in which a limited number of grower co-operators receive and evaluate a large number of selections. Results from this testing are used to define the very best selections. The second stage is a large-scale test in which the elite selections are made available to any grower in Australia who agrees to participate in the scheme. A total of 64 selections were sent for testing in the small-scale scheme during this project. A total of 16 elite selections and 21,796 trees have been released to industry for the large-scale testing from 2007 to 2013. Three selections from this breeding program are currently in a Plant Breeders Rights trial.

Industry and researchers have been informed of the results from this project through 16 industry meetings and field days, 5 local, national and international conferences and the publication of 26 articles in grower magazines and scientific journals. The most significant extension results have occurred from the close liaison between the project team and voluntary grower organisation Low Chill Australia Inc.

The current breeding project has developed a strong base of new peach and nectarine varieties for use by the low-chill stonefruit industry. This material may assume greater national significance if winter temperatures in southern states increase. A new 5-year breeding project has been submitted to HAL in which low-chill varieties will be further developed and more superior cultivars released to industry.

TECHNICAL SUMMARY

The low-chill peach (*Prunus persica* (L.) Batsch) breeding program at Maroochy Research Facility (MRF), Nambour, Queensland Australia was initiated in 2001 in response to industry requests for new peach and nectarine cultivars adapted to subtropical Australia. The program aims to develop a range of high quality peach and nectarine cultivars with both melting and non-melting flesh, with chilling requirements of 100 to 400 chill units (CU) but an emphasis on 150 CU or less.

The program's breeding strategies have included collection and evaluation of germplasm from Australia, Europe, America and Asia, hybridisation with the industry standard 'Tropic Beauty' peach, inter-crossing of subtropical and temperate parents and incorporation of non-melting flesh.

Hybridisation of selected parents resulted in production of 18,843 seed of which 23% were produced using embryo culture. Our embryo culture method is a two stage process involving an ovule pre-culture growth medium with high sucrose followed by stratification in a germination medium. We refined these methods by use of a plant tissue culture biocide which significantly reduced contamination in the ovule growth phase and resulted in a 63% increase in plant height 4 weeks after de-flasking compared to controls.

From 2007-2013, 10,912 progeny seedlings were field planted at MRF and each population was evaluated for two years commencing the year after planting. Fruit characteristics being selected are large size, high flavour, firm flesh and early ripening. Tree characteristics being selected are low chilling requirement, moderate crop load and compact tree habit. Of the 10,912 progeny planted, 10,286 were old enough to produce fruit and we selected 122 elite genotypes which represents a 1.2% selection rate. A total of 821 fruit and tree evaluations were conducted at MRF consisting of 389 peach, 368 nectarine, 51 plum and 13 other *Prunus* which included apricot, cherry, almond, nume and interspecific combinations.

Three elite selections, 'Q17-20' peach, 'Q32-59' nectarine and 'Q53-4' peach, were propagated for Plant Breeders Rights trial. The PBR trial was planted at MRF in 2010 and will be completed in 2014. It is a randomised complete block design with 6 replicates of the candidate cultivars and the comparators 'UFSun' peach, 'SunWright' nectarine and 'Rayon' peach. Part 1 PBR has been granted for these three selections.

Expressions of interest to commercialise seven low-chill novel fruit selections were publicly called for in 2010 and a commercial partner was selected by a panel of representatives from HAL, LCA and DAFF in 2011. The novel selections were two flat (or peento) peaches, one flat nectarine, two nectarines lacking in red skin pigmentation and two peaches lacking red skin pigmentation. It is considered that subject to more extensive commercial-scale testing these selections will meet niche, Australian marketing requirements.

A significant achievement of this project is the development of a two-stage grower testing scheme. It combines the attributes of industry involvement, rapid wide-spread testing, stratified evaluation and low-project cost. Its use is being considered by other horticultural breeding projects. The first stage is a small-scale test in which a limited number of grower co-operators receive and evaluate a large number of selections. Results from this stratum of testing are used to define a subset of elite selections. The second stage is a large-scale test in which the elite selections are made available to any grower in Australia who agrees to participate in the scheme. A total of 64 selections were sent for testing in the small-scale scheme over 6 years. A total of 16 elite selections and 21,796 trees have been released to industry for the large-scale testing from 2007 to 2013. The three selections currently in PBR trial were the first candidates in the large-scale scheme.

Extension of the results from this project have included members of the project team speaking about the breeding project at 16 industry meetings and field days, 5 local, national and international conferences and the publication of 26 articles in grower magazines and scientific journals. The most significant extension results have occurred from the close liaison between the project team and Low Chill Australia Inc.

The current breeding project has developed a useful base of germplasm for the low-chill stonefruit industry. This material may assume greater national significance if winter temperatures in southern, temperate states increase. A new 5-year breeding project has been submitted to HAL in which further development of the low-chill germplasm base will be explored and more superior cultivars released to industry.

1.0 INTRODUCTION

Low-chill stonefruit accounts for 15% of Australian stonefruit production and has a value of \$35M. The main low-chill production regions are in northern New South Wales and southern Queensland where less than 300 chill units (CU) are accumulated each winter. Low-chill stonefruit varieties are also grown in medium chill regions (receiving 300 to 500 CU each winter) in inland Queensland and NSW and colder regions near Swan Hill, Riverland, Sydney and Perth where spring frosts do not limit production. These low-chill cultivars produce the first stonefruit ripening in Australia from September to November. Fruit ripening in this time period receive high prices but are 46% more expensive to produce than fruit from traditional temperate growing regions (Slack and Ullio, 1999).

Fruit quality in subtropical peaches affects profitability of the wider national stone fruit industry as it sets the standard and subsequent demand for the stonefruit season. Consumers purchasing high quality peaches, nectarines and plums in October and November will continue purchasing during summer. Conversely, consumers may not purchase temperate peaches if they have bad experiences with subtropical cultivars. To consumers they are all just stone fruit regardless of their production in low-chill or high-chill regions.

Growers need new peach, nectarine and plum varieties that are subtropically adapted and reduce costs of production. Conducting the breeding program in Australia allows selection under local conditions for the characteristics required by the Australian industry. The industry specifies the characteristics that most need improvement. With breeding conducted in Australia, new selections are tested at industry test sites without the delay.

The current subtropical peach breeding program is located at Maroochy Research Facility (MRF), Nambour, Queensland Australia, latitude 26.64 S, longitude 152.94 E, elevation 53m (Topp et al., 2012). It was initiated in response to grower requests for new low-chill cultivars with fruit of high eating quality and trees adapted to local growing environments. A scoping study conducted in 2001-2002 provided an overview of the Australian market, the major segments within the market, the potential for new segments and a strategy for developing new cultivars (Topp et al., 2002). The recommended strategy involved breeding new cultivars under local conditions and allowing the industry to have control in setting the breeding objectives and with wide access to the new cultivars.

Germplasm enhancement is a critical pre-breeding strategy and involves the introgression of foreign germplasm into existing breeding populations in order to introduce specific genes and to broaden the genetic base (Topp and Sherman, 2000). Part of our prior research has involved evaluation of foreign germplasm and identification of lines that may be of use as parents in the current cycles of breeding (Russell and Topp, 2002).

Parental germplasm has been obtained from Australia, Brazil, Mexico, California, Georgia, Florida and China. The majority of seedlings have been produced by intercrossing low-chill genotypes predominately from the University of Florida but also from Brazil and Mexico (Topp et al., 2008). 'Tropic Beauty', the most planted low-chill peach in Australia, was used extensively as a parent in the early years of our breeding. It has provided high quality melting flesh types with a balanced flavour, but earlier ripening cultivars are required by industry. A small number of hybrids between subtropical and temperate genotypes have been produced and grown out in medium- and high-chill regions. The seedlings generally require 400 to 600 CU, but a few lower chill genotypes have been produced. The seedlings invariably have many blind nodes (Boonprakob and Byrne, 2003) and produce elongated and pointy fruit (Topp and

Sherman, 1989) when grown in the subtropics. Backcrossing to subtropical parents is required to lower the chilling requirement and improve fruit shape and cropping.

Domestically, it is important that quality fruit are produced by the low-chill sector of the industry in order to start consumers enjoying their spring purchases of peaches. There is also the prospect of expanding Australia's export to SE Asia from September to December as there is a marked reduction in peach volume in Asia at this time (Nissen et al., 2000; Wei, 2001).

The specific objectives for the SF07003 project that were listed in the project application were:

Release to the Australian industry, new peach and nectarine selections with high fruit quality and adaptation to Australia's subtropics. Specifically, we will release 5 new selections for large scale testing from 2007-2012 with a total of over 10,000 trees to be propagated by 2012.

Create a series of low-chill, non-melting, yellow and white flesh, peaches and nectarines that have improved eating quality compared to standard cultivars. By 2012 we will have propagated a total of 12 selections comprising 3 superior genotypes for each series (i.e. 3 non-melting white flesh peaches; 3 non-melting yellow flesh peaches; 3 non-melting white flesh nectarines; and 3 non-melting yellow flesh nectarines).

Build a diverse germplasm base that allows rapid response to changes in breeding direction due to changes in market requirements or new opportunity development.

2.0 POLLINATION AND HYBRID SEED PRODUCTION

2.1 Methods

Hybrid seed were produced using controlled hand pollinations of selected parents (Scorza and Sherman, 1996) in June, July and August each year. Seed with fruit development periods (FDPs) over 80 days were stratified at 4⁰C in moist perlite until radicles had elongated over 1 cm. Germinated seed were planted in seedling trays and grown in shadehouses until field planting in autumn.

Embryos were cultured when the FDP of the seed parent was 80 days or less (Hamill et al, 2005). The protocol for embryo culture is included as Appendix 12.1. The efficiency of embryo culture was improved by experiments we conducted during this project. These experiments are described in Chapter 3.

2.2 Results and discussion

A total of 18,843 seed were produced from the pollinations in 2007 to 2013 which resulted in 8,262 seedling trees field planted at MRF at Nambour (Table 1). Seedling establishment ranged from a high of 63% in 2012 to a low of 30% in 2011. The low establishment generally corresponded to years which featured many seed parents with short fruit development periods (FDPs).

Approximately 23% of the seed were stratified using embryo culture. This allowed us to select for short FDP in both the pollen and also the seed parent and thus improve the rate of genetic gain for early ripening.

Table 2.1. Numbers of seed produced by regular stratification and embryo culture and the number of seedlings established in field trials for each year of the project.

Year of pollination	Seed number stratified in perlite ^z	Seed number embryo cultured	Total seed number	Field planted seedlings ^y	Total establishment ^x
2007	4,547	500	5,047	1,881	37%
2008	4,381	0	4,381	2,162	49%
2009	2,857	1,660	4,517	2,658	59%
2010	0	0	0	0	0%
2011	1,621	1,482	3,103	935	30%
2012	1,000	0	1,000	626	63%
2013	102	693	795	Not yet planted	
	14,508	4,335	18,843	8,262	46%

^z The 2012 seed number is an estimate.

^y Field planting occurred in autumn in the year after pollination.

^x Establishment percentage is the ratio of seedlings field planted to the total seed number and does not include the 2013 seed which are currently in the glasshouse.

Parents are selected on the basis of high levels of complementarity and commonality. Complementarity, such that high levels of each trait are present in at least one parent, is required to ensure acceptable levels of this trait in the population. Parents must complement each other in the sense that low levels of a trait in one parent must be balanced by high levels of that trait in the other parent. It is highly unlikely that acceptable levels of a quantitative trait will be obtained in elite selections if not found in one or both parents. Commonality, such that both parents have acceptable levels of as many traits as possible, is needed to increase the probability of the desired alleles with the extreme case being fixation in the new population. The theory for the commonality requirement is that, where both parents have the required level of an attribute, the necessary alleles have a high probability of being common to both parents and may not segregate in the breeding population. The frequency of individuals with all the required alleles is increased as fewer desirable alleles segregate (P. Brennan, pers. comm.).

The project was expected to produce at least 5,000 progeny seedlings. We have produced three times this amount due to improved efficiencies in our hybridisation and seedling production methodologies and the cooperation we have received from visiting students and scientists who have contributed to the program at no cost during their visits.

3.0 EMBRYO CULTURE

Embryo culture is used in our subtropical peach breeding program for germination of seed from parents with short fruit development periods (Hamill et al. 2005, Topp et al. 2008). These cultivars are commercially desirable because of high prices for early season fruit and reduced time of fruit on the tree. Embryo culture has been extensively used to rescue embryos from early maturing cultivars where the flesh matures before the seed and conventional germination methods provide low recoveries (Ramming, 1983; Ramming 1990). Embryo culture is required to improve germination and ultimately increase population sizes for evaluation in the field.

Ramming (1985) and Chaparro and Sherman (1994) described the use of a two stage embryo culture technique involving an ovule pre-culture medium with 6% sucrose followed by stratification in germination medium that increased the germination of early-ripening *Prunus* genotypes. However using a similar two stage embryo culture process with Knops media (Chaparro and Sherman, 1994) we have found significant losses due to contamination. In this method where the first stage embryos were incubated in the high sucrose media at 20°C high levels of contamination, mainly due to bacteria, caused losses of about 25% (data not presented).

While the two stage process improved embryo development, contamination must be reduced before the method can be used reliably. A method to reduce embryo contamination under conditions amenable to bacterial growth is needed. The objective of this experiment was to develop a method to reduce contamination in a two stage embryo culture technique by using the plant tissue culture biocide Plant Preservation Media, PPM (PhytoTechnology Laboratories, Inc.). The results from this study were presented at the Australasian Plant Breeding Conference in 2009.

3.1 Methods

Fruit were collected from an elite nectarine genotype grown under subtropical conditions with a fruit development period (FDP) of 85-90 days. The fruit were surface sterilised in 70% ethanol for 2 min, followed by 5 min in 2% Sodium Hypochlorite and Tween 20 solution with a final rinse in 70% ethanol to remove excess Sodium Hypochlorite. Damaged fruit were discarded to reduce potential contamination. The embryos were aseptically extracted in a laminar flow cabinet by cutting through stem-end of the fruit into the stony endocarp, cracking the endocarp open and gently removing the seed with sterile forceps. Seed cutters and forceps were resterilised between each seed extraction, with cutters dipped in 70% ethanol and forceps dry heat sterilised. Previous experiments performed showed that leaving the testa intact around the embryo resulted in better growth of the embryo in culture with a higher proportion of germination and survival (unpublished data). Therefore, the testa was left intact for this experiment.

Four treatments were investigated, all based on modified Knops medium two stage tissue culture protocol involving a pre-culture stage on Knops ovule growth media (KOGM) with 60g/L sucrose (Chaparro and Sherman, 1994) and stratification stage on Knops germination medium (KGM) with 30g/L sucrose (Chaparro and Sherman, 1994). All media were solidified with 12 g/L agar, and the pH adjusted to 6.0 prior to autoclaving at 121°C for 20 minutes. 100 ml of media was dispensed into 500 ml tissue culture tubs. The first (pre-culture) stage involved culturing embryos onto KOGM followed by incubation at 20°C for four weeks in darkness. Embryos were then aseptically transferred onto KGM before stratification at 5°C in darkness until they showed signs of germination (approx. 12weeks). To assist in acclimatisation of

embryos, the incubation temperature was gradually increased over 5 days to 20°C in darkness when germination was observed in the majority of treatments.

The four treatments were: 1. Knops two stage culture media only. (Control) 2. Knops two stage culture media - with each embryo dipped into a 2ml/L PPM solution before culturing onto ovule and germination growth media. (KPD) 3. Knops two stage media – with addition of 1ml/L PPM to both ovule and Germination media. (KPM) and 4. Knops two stage media – with each embryo dipped both into a 2ml/L PPM solution before being cultured onto ovule and germination media supplemented with 1ml/L PPM (KPDM).

Each treatment consisted of five tubs each containing 10 embryos. The embryos were placed into the media radicle end down. Tubs were placed in randomised design on a single shelf within a refrigerated incubator in five rows.

Following incubation, tubs were visually inspected for contamination, with a yes/no rating given for each embryo, with an overall percentage of contamination generated for analysis. Embryos were deflasked and embryo, radicle and shoot length recorded. Percentage of embryo area with discoloration was also recorded. Embryos were planted into seeding trays containing a perlite based medium and placed into a temperature controlled glasshouse at approx. 26°C. Only viable embryos were planted out. After 4 weeks, the height of each seedling was measured and overall survival recorded.

Residual maximum likelihood was used to compare measurements of embryo, radicle and shoot lengths and plant height. Binary data of germination and contamination percentages were analysed by generalised linear models, using binomial distribution and logit link. Data were analysed using the statistical package Genstat 11.1.

3.2 Results

The embryos dipped into PPM had a similar high level of contamination as the control, whereas media amended with PPM had no contamination. (Table 1). 76% and 78% of embryos in control and KPD, respectively, had visible contamination surrounding the embryo while no contamination was observed surrounding embryos in KPM and KPDM.

To investigate the potential phytotoxicity of PPM on early embryo development the percentage of embryos with radicle elongation at time of deflasking, and the total germination were recorded (measured by percentage of overall survival after 4 weeks). KPM and KPDM had significantly higher earlier embryo radicle development (94%) compared to control and KPD (60% and 45% respectively) (Table 1). All treatments using PPM produced significantly better overall survival than the control (Table 1). It is interesting to see that even the KPD treatment with high contamination at deflasking had significantly higher survival than the control and produced similar total germination to the treatments that had no contamination. (Table 1.).

Early embryo development was assessed by measuring embryo, radicle and shoot lengths and was used to measure early germination and any phytotoxic effects from PPM. There was no significant difference in embryo length between treatments for surviving embryos (Figure 1). The radicle lengths were significantly shorter in the KPD treatment (where PPM was applied as an embryo dip only) compared with PPM amended media. Shoot lengths were significantly longer in the PPM amended media (KPM, KPDM) compared to control or PPM embryo dip (Figure 1.). The effect of the improved radicle and shoot

development from treatments was still evident at 4 weeks of growth where KPM produced significantly taller plants (Figure 2).

3.3 Discussion

Reports on the use of two stage embryo culture have described better germination from early maturing fruit but have not reported on losses from bacterial contamination (Ramming, 1985 and Chaparro and Sherman, 1994). Contamination is a significant constraint as it readily occurs due to the high sucrose concentration and incubation temperature required for the two stage process despite the use of aseptic techniques (unpublished data).

This report describes how PPM amended media can eliminate bacteria and improve embryo germination and growth without phytotoxicity. However the PPM as a pre-treatment dip did not prevent contamination. The addition of PPM as a direct dip may delay radicle elongation which was significantly lower in this treatment compared with addition to media alone (Table 1). Shoot growth was also slightly less where PPM was used both in media and directly (Figure 1). This may be due to PPM being evenly dispersed in the media and active during incubation. In contrast, dipping results in higher concentration in direct contact with the embryo. This may have some negative effect on subsequent embryo development and will have a less residual effect on bacteria that may come from within the embryo.

Embryos in treatments with PPM incorporated into the media (KPM and KPD) had a higher percentage of early embryo development and longer shoots than the control and KPD. This was probably due to the PPM inhibiting the growth of bacteria rather than PPM itself promoting radicle elongation and shoot growth. This is supported by the fact that although dipping only the embryos (KPD) did not prevent contamination, it significantly increased survival compared with the control. This may be due to suppression rather than elimination of bacteria. These results also suggest that direct dipping with PPM does not eliminate bacteria and may reduce growth. Media amended with PPM eliminated bacteria and was not phytotoxic.

The most important part of any embryo rescue protocol is maximising the number of plants reaching the field. In this chapter we have described an effective method using PPM incorporated into the media that eliminated contamination and provided high germination rates. Therefore, PPM amended media should be used in future embryo culture protocols in low-chill breeding programs.

3.4 Summary

Two stage embryo culture is used to increase germination of embryos from varieties of stonefruit with short fruit development periods. A downside to this process is contamination of embryos, mainly from bacteria, since they are cultured at temperatures amenable to bacterial growth at high concentrations of sucrose. Contaminated embryos have reduced germination and survival which lowers the number of seedlings available for use in conventional breeding programs. This study investigates the use of the plant tissue culture biocide Plant Preservation Media, PPM (PhytoTechnology Laboratories, Inc.) for control of embryo culture contamination. There were four treatments: Knops two stage culture media; Knops two stage culture media - with each embryo dipped into a 2ml/L PPM solution before culturing; Knops two stage media – with addition of 1ml/L PPM to both ovule and Germination media; Knops two stage media – with each embryo dipped into a 2ml/L PPM solution before culture onto media supplemented with 1ml/L PPM. Embryos were evaluated for contamination and embryo growth as well as phytotoxic effects from PPM for a low-chill nectarine cultivar. Significantly lower levels of contamination were achieved by treatments that

incorporated PPM into the media. From this study we have identified that PPM amended media can eliminate contamination from a two stage embryo culture technique which increases germination and survival of a low-chill nectarine genotype without any phytotoxicity.

Table 3.1. Mean¹ percentage of embryos with visible contamination and germination for Control, KPD, KPM and KPDM treatments²

Treatments	% of contaminated embryos	% of embryo radicle elongation	% of total survival
1.Control	76 a	60 b	72 b
2.KPD	78 a	45 b	98 a
3.KPM	0 b	94 a	100 a
4.KPDM	0 b	94 a	98 a

¹Means within a variable not followed by a common letter are significantly different (P<0.05)

²Treatments

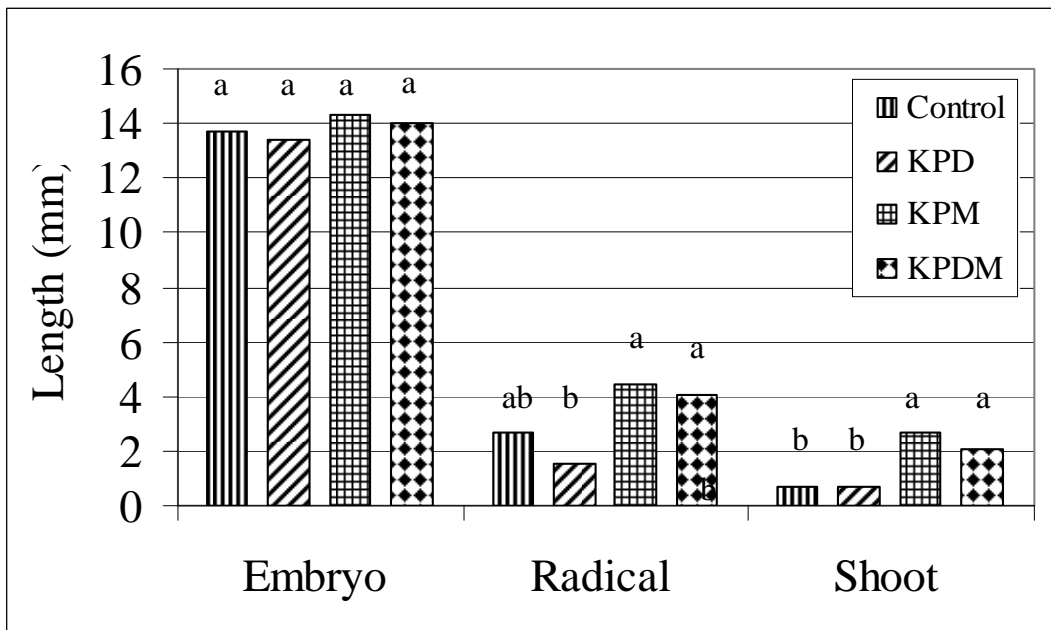
Control = Knops two stage culture media only.

KPD = Knops two stage culture media - with each embryo dipped into a 2ml/L PPM solution before culturing onto ovule and germination growth media.

KPM = Knops two stage media – with addition of 1ml/L PPM to both ovule and Germination media.

KPDM = Knops two stage media – with each embryo dipped into a 2ml/L PPM solution before being cultured onto ovule and germination media supplemented with 1ml/L PPM.

Figure 3.1. Mean¹ early embryo development for Control, KPD, KPM and KPDM treatments²



¹Means within a variable not followed by a common letter are significantly different ($P < 0.05$)

²Treatments

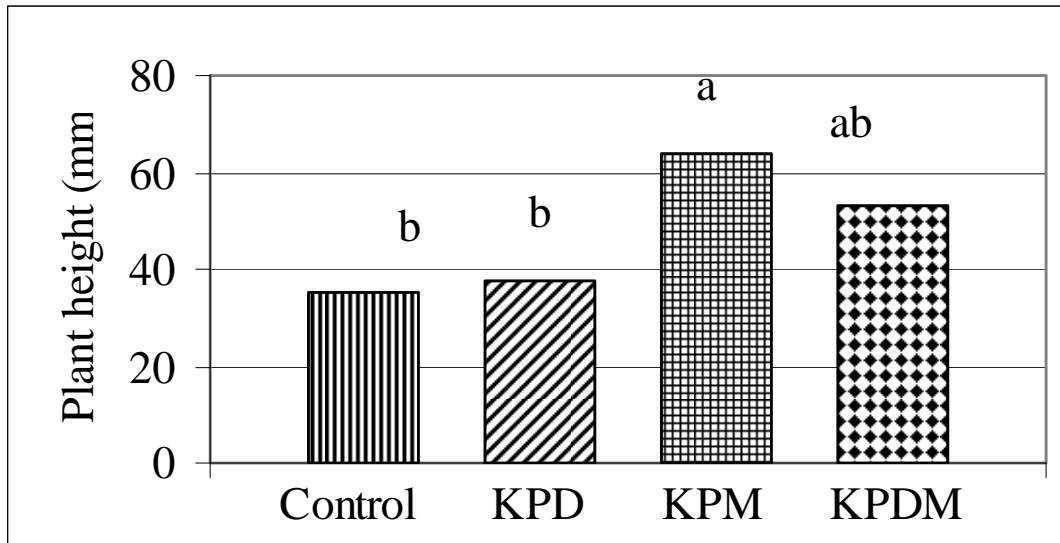
Control = Knops two stage culture media only.

KPD = Knops two stage culture media - with each embryo dipped into a 2ml/L PPM solution before culturing onto ovule and germination growth media.

KPM = Knops two stage media – with addition of 1ml/L PPM to both ovule and Germination media.

KPDM = Knops two stage media – with each embryo dipped into a 2ml/L PPM solution before being cultured onto ovule and germination media supplemented with 1ml/L PPM.

Figure 3.2. Mean¹ plant heights 4 weeks after deflasking for Control, KPD, KPM and KPDM treatments²



¹Means within a variable not followed by a common letter are significantly different ($P < 0.05$)

²Treatments

Control = Knops two stage culture media only.

KPD = Knops two stage culture media - with each embryo dipped into a 2ml/L PPM solution before culturing onto ovule and germination growth media.

KPM = Knops two stage media – with addition of 1ml/L PPM to both ovule and Germination media.

KPDM = Knops two stage media – with each embryo dipped into a 2ml/L PPM solution before being cultured onto ovule and germination media supplemented with 1ml/L PPM.

4.0 REDUCING JUVENILITY WITH PACLOBUTRAZOL

Long juvenile periods are a major impediment to cultivar improvement in fruit tree crops. They slow the annual rate of response to selection by increasing the length of the selection cycle and increase breeding cost due to longer times for trees in the field (Hansche, 1983).

Peaches and nectarines often have juvenile periods of at least 3 years in temperate regions (Hansche, 1986) but this can be reduced through breeding and by orchard management that promotes rapid growth of young seedling trees (Sherman and Lyrene, 1983).

Trees in the juvenile phase cannot be induced to flower until they exceed a threshold number of nodes (Zimmerman, 1972). There is a phase change between juvenile and adult stages during which external stimuli may promote the change from juvenile to adult growth phase.

Snowball *et al.* (1994) grew 'West Indian' lime and 'Meiwa' kumquat seedlings under rapid growth conditions for 5-8 months to reach 100 nodes and then treated them with paclobutrazol. Juvenility was reduced from 14 months in the untreated controls to 11 months for the paclobutrazol treatments and flowering intensity was increased.

On grafted peach cultivars, paclobutrazol has been reported to reduce tree vigour and increase fruit numbers and fruit size (Erez, 1984, George and Nissen, 1987, Martin *et al.*, 1987). The current study was conducted to determine the effect on juvenility of paclobutrazol application to non-bearing peach seedling trees. The field experiments were conducted during SF02015, the analysis was conducted during SF07003 and the results were presented at the Australasian Plant Breeding conference in 2009.

4.1 Methods

Experiment 1 – Comparison of foliar spray and trunk paint application of paclobutrazol

Seedling trees were grown in a greenhouse and then planted in high density progeny rows when 11 months old from seed. Trees were planted in mounded, double-row beds with 30cm separating trees within each row, 30 cm between rows and 3 m between beds. Trees were irrigated using T-tape and managed to promote rapid growth (Sherman *et al.*, 1973).

Paclobutrazol (Austar®, Westsilver Pty Ltd, Australia) was applied to the seedling trees as a foliar spray or a trunk in autumn when the trees were 18 months old. The foliar spray concentration was 500 mg active ingredient per plant and was applied in 250 ml of water and 0.05% wetting agent per plant. The trunk paint was applied using 500 mg active ingredient mixed with undiluted Nu-Film 17® (Miller Chemical and Fertilizer Corporation, USA) per plant and painted onto a 50 cm length of the trunk from 10 to 60 cm above ground level. Nu-Film 17® is a highly viscous sticker-extender used as a surfactant (Fitzell and Baker, 1979). Control plants were neither sprayed nor painted.

Treatments were applied systematically to a total of 36 trees consisting of 4 families, 9 trees per family and 3 trees per family-treatment plot. Within each family group the first 3 trees were sprayed, the next 3 painted and the final 3 were not treated.

Total numbers of flowers and fruit for each tree were recorded during the fruiting season immediately after paclobutrazol application (about 6 months after application). Shoot length was recorded on 4 randomly selected shoots during the fruiting season.

Treatment means and standard errors were calculated using family as a blocking factor.

Experiment 2 – Comparison of trunk painting concentrations of paclobutrazol

Seedling trees were planted and managed as described in experiment 1. Four treatments were systematically applied to 2 families in November when trees were 13 months old. A total of 84 trees were treated consisting of 10 single tree replicates of each treatment for the N5-55 family and 11 replicates for N6-25 family. The trees were planted in a long row with separation between the 2 families. Treatments were applied in the same order to each group of 4 trees.

A mixture of paclobutrazol and undiluted Nu-Film 17® was painted on to the trunk of seedling trees for a length of 10 cm, 20 cm or 40 cm commencing at 10 cm above ground level. The control treatment consisted of painting undiluted Nu-Film 17® onto 20 cm of trunk.

In February 2004, the number of flower buds per node and nodes with flower buds were recorded on 40 cm sections of two randomly selected branches per tree. Tree trunk circumference was measured in 12 months after treatment application.

The families were analysed separately. Flower buds per node, % nodes with flower buds and % change in butt circumference were each modelled by initially fitting a single spline (Payne *et al.*, 2008) of order 3 to the data, then adding the treatment effect, followed by the interaction between position and treatment and finally separate splines of order 3 for each treatment. Significance of terms was tested at $P=0.05$ and terms dropped until only significant terms remained in the model.

4.2 Results

Experiment 1

Paclobutrazol application by spray and paint reduced shoot extension growth by 37% and 53% respectively (Table 1). Spray and paint application of paclobutrazol resulted in two and four fold increases in flower numbers and three and tenfold increases in fruit numbers compared to the control. The control treatment resulted in 21 flowers per tree and 2.5 fruits per tree. The paclobutrazol paint treatment produced the highest numbers of flowers and fruits per tree, 80 and 26 respectively (Table 1). The paclobutrazol spray treatment produced results that were intermediate between the control and the paint treatment.

For the paint treatment all 12 trees produced fruit whereas for the spray treatment 8 of 12 trees produced fruit (67%) and for the control 5 of 12 trees produced fruit (42%).

Variability was high with all coefficients of variation over 65% (Table 1). High variability was expected because each seedling is genetically distinct. This genetic variability within each treatment will increase the within treatment error components of variance.

Experiment 2

The results for the two families were analysed separately because they were planted in two separate sections and thus effects of family and position were confounded. The N5-55 family had lower numbers of flowers per node and % of nodes with flowers than the N6-25 family, 0.26 versus 0.61 and 14.7 versus 34.2 respectively.

For % change in butt circumference for both families, treatment was the only significant term in the model. For family N5-55, trunk painting with paclobutrazol significantly reduced trunk butt circumference in all treatments. For family N6-25, trunk butt circumference was significantly reduced by the 20 and 40 cm trunk paint applications but there was no significant difference between the control and the 10 cm trunk paint treatment (Table 2).

For family N5-55, for both average number of flowers per node and % nodes with flower buds, the model was reduced to a single spline and so no significant difference among treatments. However there was a trend present, with the average number of flower buds per node being 0.16 for control trees compared with 0.29 for the remaining treatments and the % nodes with flower buds averaging 9.9 % for control trees compared with 16.4% for the remaining treatments.

For family N6-25, there were significantly more flower buds per node and % of nodes with flower buds for the 20 and 40 cm paclobutrazol treatments than for the 10 cm treatment or control (Figures 1 and 2).

4.3 Discussion

In the first experiment it was demonstrated that spray and trunk paint application of paclobutrazol were effective in increasing the number of flowers and fruit per tree and in increasing the percentage of trees that produce fruit in their first year of bearing. Trunk painting of paclobutrazol was more effective than spray application in increasing flower and fruit numbers and % fruit set.

Treatments that increase the number of fruits on each seedling tree in the first year of bearing will improve breeding efficiency. A sample of 5 to 10 fruits are harvested for evaluation of fruit traits in our peach seedling populations. Hansche (1986) recommended a sample of 5 fruit per seedling for desired level of precision in estimating fruit quality traits in his breeding plots. In this regard, the spray and paint application of paclobutrazol which resulted in mean fruit numbers of 8 and 26 are useful in allowing adequate fruit sample sizes compared to the control of 2.5 fruit per tree.

Trees with small numbers of fruits are evaluated by breeders but the results can be biased. In particular, trees with very few fruit per tree provide upwardly biased estimates of fruit size. Fruit thinning prior to stone hardening is used to even out crop loads but bias will still occur for trees with very few fruit. Paclobutrazol treatments should reduce the magnitude of this problem by reducing the number of trees with small fruit numbers.

Paclobutrazol treatments in experiment 1 increased the number of seedlings that fruited in the first year the population commenced bearing. Increasing the percentage of seedling trees that produce fruit in a given time will increase the annual rate of genetic gain because response to selection per year is inversely related to the length of the selection cycle (Hansche, 1983). In some tree crops, many years of evaluation of the fruit is required before selection of elite parents for the next generation. For example, macadamia seedlings are evaluated for 5 years of fruiting in order to accurately predict yield. In these instances greater gain in annual response to selection may be obtained by altering the selection methods rather than increasing the % of seedlings that fruit in the first cropping year.

Soil drenching is the recommended method of applying paclobutrazol to commercial peach orchards (George *et al.*, 1993). This method was not trialled in the current study because the progeny blocks in our breeding program are constantly rotated and reused. Paclobutrazol persists in soil and may reduce growth during the first growing season of replants thus reducing precocity.

The second experiment showed that not all families of peach seedlings will respond to paclobutrazol in the same manner. The N5-55 family did not show a significant floral response to paclobutrazol application, although the trend to increased flower bud number was consistent with the N6-25 family. It is difficult to make conclusions regarding family effects as family differences were confounded with row position.

Paclobutrazol alters tree growth and fruit quality traits that may limit its use in breeding programs. Trees treated with paclobutrazol are less vigorous and develop a weeping growth habit compared to non-treated trees. Some breeding programs have tree architecture as a major goal and use of paclobutrazol in the breeding plots may prevent selection of the desired genotypes.

Fruit size is increased and maturity is enhanced with paclobutrazol application (Allan *et al.*, 1993). It would therefore be difficult to compare results from treated and non-treated blocks or years or with standard cultivars grown without paclobutrazol treatment. The shorter fruit development period reported in paclobutrazol treated fruits may also reduce embryo dry mass (Allan *et al.*, 1993) and thus reduce germination rates of hybrid seed in breeding plots.

Managing peach seedlings to obtain high levels of precocity involves growing the plants vigorously to attain the critical height for fruiting in the shortest possible time (Sherman and Lyrene, 1983). This is done by optimising pest and disease management, water and fertiliser application and pruning to a single central leader with constant removal of suckers (Scorza and Sherman, 1996, Topp *et al.*, 2008). Paclobutrazol treatment to reduce juvenility should be used in conjunction with these techniques not as an alternative.

The results from this study suggest that it is possible to use paclobutrazol painting in peach seedling progeny to increase the number of fruits per tree and the number of trees bearing fruit. An increase in both or either of these traits will increase breeding efficiency. It will not be suited to breeding programs where tree growth habit is a primary selection characteristic because of the strong influence of paclobutrazol in reducing tree vigour and altering tree growth habit. It is likely to be of most benefit where rapid turnover of generations is required accompanied by selection for simply inherited traits or markers that will be uninfluenced by the paclobutrazol.

4.4 Summary

A limitation to genetic improvement in peach is the length of the juvenile period. Reduction in the length of the juvenile period of individual seedling trees or an increase in the percentage of seedling trees that flower in a given time will increase the annual rate of genetic gain in a peach breeding program.

The effect of paclobutrazol on peach seedling juvenility was investigated in a subtropical peach breeding program at Maroochy Research Facility at Nambour, Queensland. Paclobutrazol was applied to one year old seedling trees in late summer by either foliar spraying or painting the trunk and compared with untreated trees. Flower and fruit numbers and shoot growth were recorded the following growing season.

Paclobutrazol application increased the number of flowers and fruits produced on the seedling trees with bark painting being more effective than foliar spraying.

Table 4.1. Means (\pm SEM) of number of flowers and fruits per tree, % fruit set and shoot length measured 6 months after application of paclobutrazol (PBZ) spray and paint applied to 18 month old peach seedlings.

Treatment	Number of flowers per tree	Number of fruits per tree	Fruit set (%)	Mean shoot length (mm)
Control	21 (\pm 6)	2.5 (\pm 0.8)	8 (\pm 2)	30 (\pm 2.2)
PBZ spray	38 (\pm 15)	8 (\pm 3.5)	29 (\pm 8)	19 (\pm 1.3)
PBZ paint	80 (\pm 22)	26 (\pm 7)	53 (\pm 7)	14 (\pm 1.3)

Table 4.2. Percentage increase in trunk circumference 4 months after application with paclobutrazol (PBZ) trunk paint treatments.

Treatment	% change in trunk circumference	
	Family N5-55	Family N6-25
Control	25 a	27 a
PBZ 10	18 b	23 ab
PBZ 20	13 b	17 bc
PBZ 40	13 b	13 c
SEM	2.4	2.0

Means not followed by a common letter are significantly different at P=0.05.

Figure 4.1. Flower buds per node for N6-25 family modelled by 4 parallel splines. Treatments are control, and paint application of paclobutrazol (PBZ) on 10 cm (PBZ10), 20 cm (PBZ20) and 40 cm (PBZ40) of trunk.

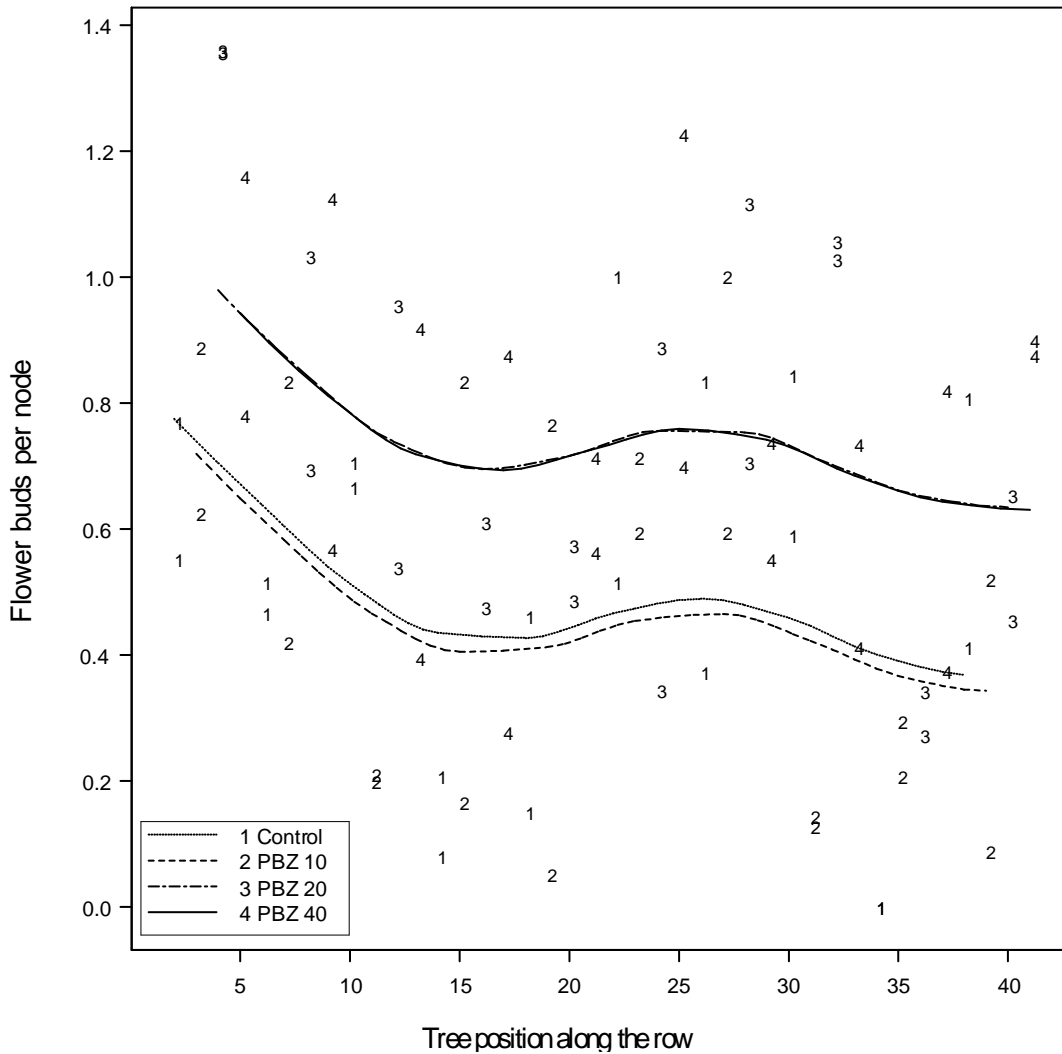
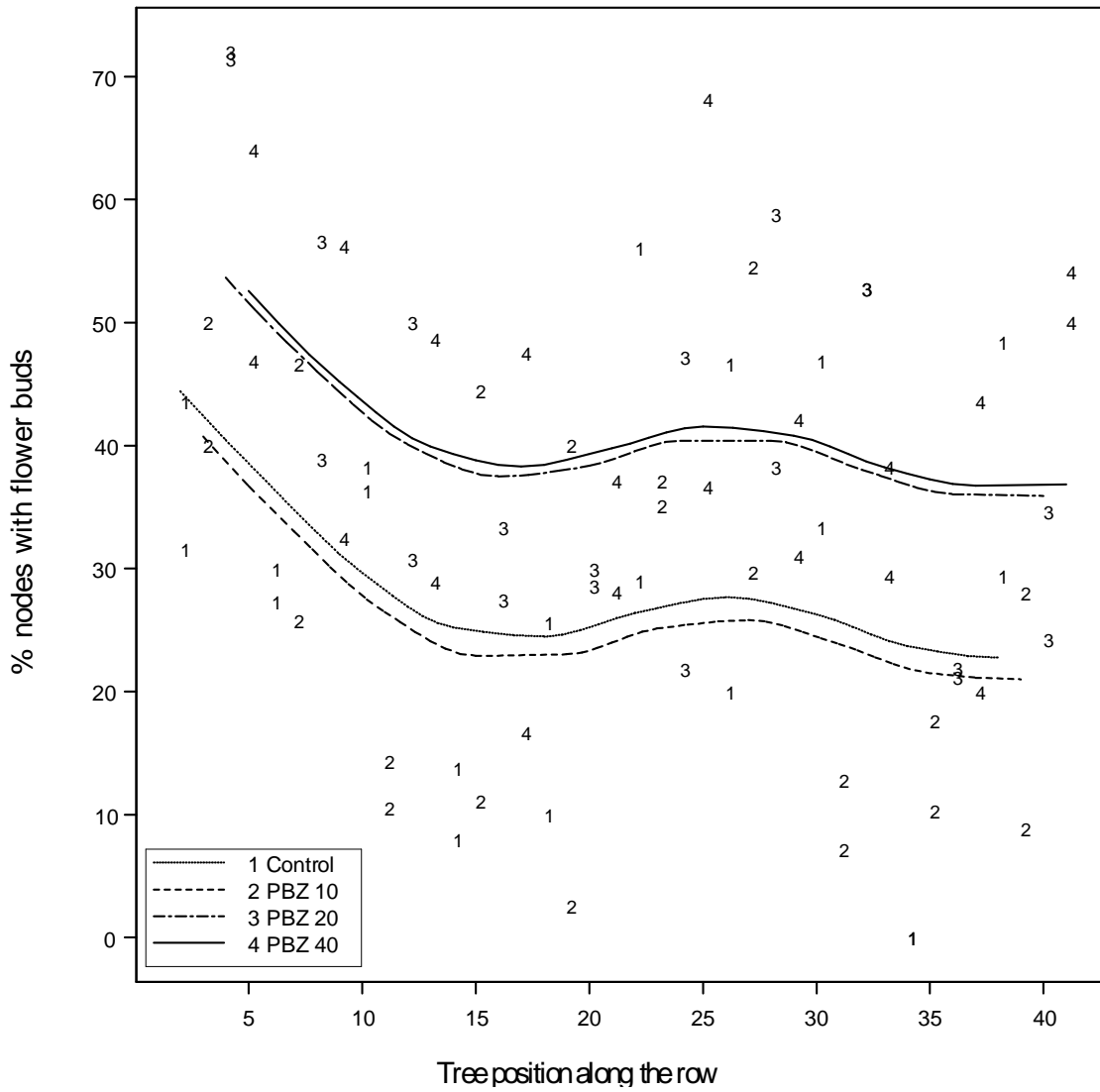


Figure 4.2. Percentage of nodes with flower buds for N6-25 family modelled by 4 parallel splines. Treatments are control, and paint application of paclobutrazol (PBZ) on 10 cm (PBZ10), 20 cm (PBZ20) and 40 cm (PBZ40) of trunk.



5.0 EVALUATIONS AT MAROOCHY RESEARCH FACILITY (MRF)

There are three sets of breeding material that we evaluate at MRF. The first is the populations of hybrid seedling progeny. Each seedling is a unique genotype and only one tree of each genotype is planted and evaluated. The second set of material is the elite genotypes that are selected from the seedling population and then propagated by budding. Commercial cultivars are also propagated as comparators. The third set of material is the Plant Breeders Rights (PBR) trial in which data is collected on candidate cultivars that are about to be released to industry. Results for the evaluation of all three sets of material at MRF are presented in the following sections.

5.1 Evaluation of seedling progeny populations

Seedlings were planted in early autumn, about five months after fruit harvest, in mounded double-row beds at 22,000 trees per ha. Trickle irrigation was applied under plastic mulch. Trees were trained to a central leader and enclosed in netting to prevent damage by birds and bats. The trees were grown using standard commercial pest, disease and fertiliser management (Vock and Campbell, 1998).

Tree characteristics evaluated were harvest date of first eating ripe fruit, fruit development period (FDP) in days from 50% full bloom to ripe fruit and crop load rated on a 1 (light) to 9 (heavy) scale. Chilling requirement was estimated as chill units (Table 5.1) by comparison to flowering time of known standard peach and nectarine cultivars ‘Okinawa’ (150 CU), ‘Sunred’ (250 CU), ‘Flordagold’ (350 CU) and ‘Sunlite’ (450 CU) (Sherman and Lyrene, 1998).

Table 5.1. Full bloom dates from 2008-2013 at Nambour, Queensland for standard peach and nectarine cultivars with their estimated chill units.

	Standard cultivar			
	Okinawa	Sunred	Flordagold	Sunlite
Estimated Chill Units	150	250	350	450
YEAR				
2008	07-Jul-08	21-Jul-08	18-Aug-08	08-Sep-08
2009	10-Jul-09	23-Jul-09	14-Aug-09	9-Sep-09
2010	12-Jul-10	26-Jul-10	17-Aug-10	7-Sep-10
2011	4-Jul-11	25-Jul-11	15-Aug-11	29-Aug-11
2012	10-Jul-12	30-Jul-12	14-Aug-12	31-Aug-12
2013	01-Jul-13	21-Jul-13	19-Aug-13	07-Sep-13

The date of full bloom occurred as early as 1st July in 2013 for ‘Okinawa’ and as late as 9th September in 2009 for ‘Sunlite’. This 2 month difference in bloom time is a source of variation in ripening time in the seedling populations.

Fruit were harvested when eating ripe and rated on a 1 (least desirable) to 9 (most desirable) scale for attractiveness, amount of red blush, flavour, firmness, juiciness, amount of fuzz and stone freedom.

Soluble solids were measured with a hand held refractometer. Fruit weight data was the mean of 4 to 10 fruit per tree.

A total of 122 new genotypes were selected from the seedling populations from 2007 to 2013 (Table 5.2). The percentage selected ranged from 0.2% to 2.5%. The seedling population planted in 2007 contained a particularly successful cross that involved the use of one of our very high flavour, melting flesh, medium size fruit selections crossed with a very large, non-melting flesh selection. This combination produced a range of high quality, very attractive nectarine selections segregating for both melting and non-melting flesh.

Table 5.2. Number of genotypes selected from each of the original seedling progeny populations

Year planted	Number of seedlings	Year seedlings were evaluated	Number of genotypes selected	% of seedlings selected
2007	2,650	2008	67	2.5
2008	1,881	2009	11	0.6
2009	2,162	2010	5	0.2
2010	2,658	2011	31	1.2
2011	0	2012	0	0.0
2012	935	2013	8	0.9
2013	626	2014	NYF ^z	NYF
Total	10,912		122	1.2

^z NYF not yet fruiting

5.2 Evaluation of budded elite selections

Each elite selection from the seedling progeny population was propagated onto virus tested ‘Okinawa’ rootstock in November immediately after fruit evaluation. Two trees of each new selection were planted at MRF and these produced fruit one year after planting. Selections that performed well in this test were distributed for grower evaluation. Grower testing occurs concurrently with testing at MRF and occurs in two separate schemes, a small-scale test of one tree per selection and a large-scale test of up to 2,000 trees per selection. These testing schemes are described in chapter 6.

Table 5.3. Number of evaluations completed from 2007-2013 on cultivars and budded elite selections of peach, nectarine, plum and other Prunus crops (apricot, cherry, mume and hybrids).

Year	Crop type				
	Peach	Nectarine	Plum	Other	
2007	67	59	9	1	136
2008	74	67	21	2	164
2009	87	91	10	6	194
2010	81	69	5	2	157
2011	29	53		1	83

2012	35	22	5	1	63
2013	16	7	1	0	24
	389	368	51	13	821

A total of 821 fruit samples from the budded elite selections and cultivars were evaluated at MRF during the project (Table 5.3). Peach and nectarine were the most studied crops and were roughly equal in the number of samples evaluated. This is in keeping with the project objective to focus 85% or greater of the breeding efforts on standard peach and nectarine. Our breeding work on other crops includes developing subtropical adapted cherry, apricot and plum and using interspecific combinations to obtain novel fruits.

5.3 Evaluation of Plant Breeders Rights trial

The PBR trial was planted at MRF in winter 2010 on mounded rows, with trickle irrigation at a spacing of 4m x 2m. Trees were propagated by budding on 'Okinawa' rootstock. The experimental design was a randomised complete block with 6 replicates. The candidate selections were 'Q17-20' peach, 'Q32-59' nectarine and 'Q53-4' peach and their corresponding comparators were 'UFSun' and 'UFBeauty' peaches, 'SunWright' nectarine, and 'Rayon' peach. Data was collected as per the International Union for the Protection of New Varieties of Plants, UPOV guidelines (UPOV, 2010) in 2011, 2012 and 2013. Characters used in selection were fruit size, flavour, shape, colour, firmness, flesh texture, yield, tree habit, flowering time, ripening time and adaptation to subtropical growing conditions.

A description of the three candidate cultivars is provided in Appendix 12.2. The trial results will be compiled and submitted for final PBR in June 2014. The following is a description of the origin and testing of the selections.

Q17-20 peach

'Q17-20' resulted from open pollinated seed of 'UFSun' that were collected from Gainesville, Florida, USA in 1998. These seed were stratified and germinated in AQIS post-entry quarantine facilities at Eagle Farm in Brisbane, Queensland. The resulting seedlings were field planted at Maroochy Research Facility, Nambour. 'Q17-20' was selected from this population of seedlings in 2000. Fruit and tree characteristics on the original seedling tree were observed from 2000-2002. Two trees of 'Q17-20' were propagated by budding onto peach rootstock and planted at Maroochy Research Facility in 2002. A further seven trees of 'Q17-20' were distributed to test sites in NSW and QLD. Evaluation of fruit and tree characteristics of these propagated trees were collected from 2003-2007 and resulted in the decision to apply for PBR. 'Q17-20' is higher chill than 'UFSun' (200CU vs. 100CU) and has more red skin blush (80% vs. 40%). 'Q17-20' differs from 'UFBeauty' in that it has reinform not globose leaf glands.

Q32-59 nectarine

'Q32-59' resulted from open pollinated seed of the pollen-sterile nectarine selection 'Fla82-25n' that were collected from Gatton, Queensland in 1999. These seed were stratified, germinated and grown in a glasshouse over winter 2000 at Applethorpe Research Station, Stanthorpe, Queensland. The resulting seedlings were field planted at Maroochy Research Facility, Nambour in October 2000. 'Q32-59' was selected from this population of seedlings in 2002. Fruit and tree characteristics on the original seedling tree were observed from 2002-2004. Two trees of 'Q32-59' were propagated by budding onto peach rootstock and planted at Maroochy Research Facility in 2003. Twelve trees of 'Q32-59' were planted at Maroochy Research Facility in 2005. A further five trees of 'Q32-59' were distributed to test sites in NSW and QLD in 2006. Fruit and tree characteristics of the propagated trees were collected from 2003-2007. It is considered a replacement for 'SunWright' and produces larger fruit.

Q53-4 peach

'Q53-4' peach resulted from a controlled pollination of 'Rayon' and 'UFGold' conducted in winter 2001 resulted in hybrid seed which were stratified, germinated and grown in a screenhouse during summer 2001-2002. The resulting seedlings were field planted in September 2002 at Maroochy Research Facility, Nambour. 'Q53-4' was selected from this population of seedlings in 2003. Fruit and tree characteristics on the original seedling tree were observed from 2003-2005. Twenty trees of 'Q53-4' were propagated by budding onto peach rootstock and planted at Maroochy Research Facility in 2004. A further 10 trees of 'Q53-4' were distributed to test sites in NSW and QLD. Fruit and tree characteristics of the propagated trees were collected from 2005-2008. Compared to the seed parent 'Rayon', it has a cling rather than a free stone and compared to the pollen parent 'UFGold' it has melting rather than non-melting flesh.

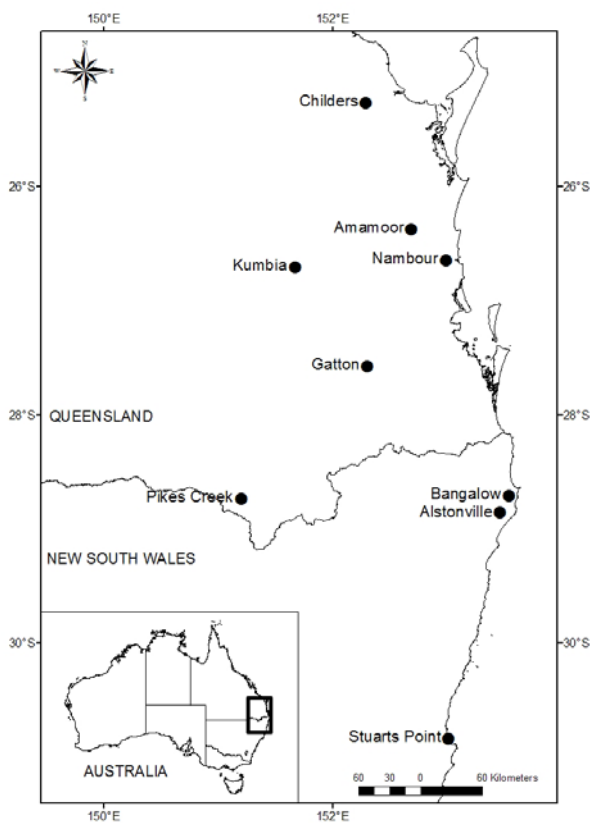
6.0 EVALUATIONS AT GROWER ORCHARDS

6.1 Small-scale testing

Small-scale tests occurred at eight commercial orchard sites in Queensland and New South Wales (Figure 6.1). The five coastal sites are all less than 50 m above sea level and have mean June temperatures ranging from 13.5⁰C at Stuarts Point to 16.5⁰C at Childers. The three inland sites are elevated and have mean June temperatures as low as 11.9⁰C at Pikes Creek. It is estimated that the warmest site, Childers, receives about 150 CU and the coldest site, Pikes Creek, about 650 CU.

The growers evaluating the new material are generally members of the breeding project steering committee and provide their time voluntarily. Growers are trained in varietal evaluation to ensure uniform data collection and ratings. Grower cooperation for this testing is important in allowing wide spread testing at low project cost over a short time. One tree of each selection is provided to the growers along with a tree of 'Tropic Beauty' peach which is used as the standard comparator. Performance of the new selections is evaluated by the growers who meet annually with the breeding team to discuss which selections should progress to large-scale evaluation.

Figure 6.1. Location of the eight grower small-scale evaluation sites relative to the primary breeding site at Maroochy Research Facility, Nambour.



A total of 64 selections have been distributed for small-scale grower testing during six years (Table 6.1) at an average of 10-11 new selections per year. The selections consisted of 34 nectarines and 30 peaches. The nectarines were evenly divided amongst yellow and white flesh types whereas there were slightly more yellow (n=18) than white (n=12) flesh peaches. Approximately 56% of the selections had non-melting flesh texture. The market requirement for flesh colour and crop type (peach or nectarine) varies with ripening time. Currently there is a demand for yellow flesh peaches in the early season prior to ‘Tropic Beauty’ season. Supermarkets require a selection of both yellow and white flesh, peaches and nectarines during stonefruit season. Large plantings of ‘Tropic Beauty’ in the past decade have skewed production towards yellow flesh peach in the main low-chill production time slot of October. More recent plantings of ‘Polar Lite’ are likely to result in a peak of white flesh nectarine production in October.

Table 6.1. Classification of the small-scale test nectarine and peach selections by flesh colour and flesh texture.

	Colour	Texture		
		Melting	Non-melting	
NECTARINE	White	6	11	17
	Yellow	4	13	17
PEACH	White	9	3	12
	Yellow	9	9	18
TOTAL		28	36	64

The outputs listed in the SF07003 project proposal stipulated that we create a series of low-chill, non-melting, white and yellow flesh, peach and nectarine selections with at least 3 selections in each category. These selections have been created in each of these categories for non-melting flesh and in addition also for melting flesh (Table 6.1). Reid et al. (2006) found there was no significant difference for consumer ratings of flavour, juiciness and purchase intent for melting versus non-melting nectarines. It is therefore important to provide growers with a range of selection options that encompass both flesh texture types.

6.2 Large-scale testing

The large scale testing scheme was established to test elite selections from the low-chill breeding project. The scheme requires participating growers to test between 100 and 2000 trees of each selection, provide evaluation data and pay a testing fee per tree, which partly funds the breeding project. Elite selections are available to all Australian growers and require a materials transfer (non-propagation) agreement to be signed. Tropic Beauty peach (industry standard) is used as a comparator when evaluating elite selections. Large-scale field testing of elite selections generates evaluation data on which to base a general commercial release decision.

From 2007 to 2013, 16 elite selections have been released for large-scale testing and 21,796 buds have been propagated for evaluation at 11 sites in New South Wales, Queensland and Western Australia (Table 6.2).

Table 6.2. The total number of trees and location of elite selections from 2006-2013.

Location	State	Number of trees
Alstonville	NSW	780
Bangalow	NSW	2,330
Childers	QLD	6,000
Dandaragen	WA	4,000
Gatton	QLD	3,950
Hogarths Range	NSW	110
Karagullen	WA	1,500
Knockrow	NSW	1,030
Kumbia	QLD	700
Malanda	QLD	96
Nambour	QLD	1,300
Total		21,796

The 16 elite selections consist of 9 peach and 7 nectarine genotypes with both yellow (n=7) and white (n=9) flesh types represented (Table 6.2). Nearly 70% of our selections have a chilling requirement less than or equal to 150 CU and have non-melting flesh texture. The high proportion of these characteristics is a reflection of the objectives of the project. Low chilling requirement is necessary to allow dormancy to be completed in the subtropics where winters are warm. The non-melting flesh trait increases productivity by reducing wastage from soft fruit. It provides the advantage for consumers that the fruit can be left on the tree until physiologically ripe, with full development of sugars and flavour, before harvest. Traditional low-chill melting flesh cultivars could not be harvested at full ripe stage because the flesh was too soft for transport to market.

The most recent releases include three white flesh nectarines with the prefix Q223 (Table 6.3). These produce well sized fruit with excellent flavour and attractive skin colour. This population consisted of both melting and non-melting flesh selections and it is of interest to note that the three selected by growers for large-scale test were all non-melting.

Table 6.3. Elite selections released to industry for large-scale testing 2007-2013

Selection	Year	Crop^z	Flesh colour^y	Flesh texture^x	Flesh acidity^w	Chill (CU)^v	Future	Comments
Q53-4	2006/07	P	Y	M	M	150	PBR	large fruit; late ripening
Q17-20	2007/08	P	Y	NM	M	200	PBR	early to mid-season
Q32-59	2007/08	N	Y	M	M	150	PBR	SunWright replacement
Q115-67	2008/09	P	W	NM	L	250	still testing	good quality but ripens late; export potential
Q70-53	2008/09	N	Y	NM	M	125	discard	excellent flavour and appearance but too small
Q219-20	2009/10	P	W	NM	M	150	still testing	looks promising at Gatton test site
Q77-46	2009/10	N	Y	NM	M	250	still testing	bright red skin; very attractive
Q219-25	2009/10	N	W	NM	M	150	discard	light cropper
Q566-15	2009/10	N	Y	NM	L	350	still testing	excellent flavour; test in medium chill regions
Q52-18	2010/11	P	W	M	H	150	still testing	large fruit; balanced flavour with acid
Q68-64	2010/11	P	Y	M	M	125	still testing	crops well in very low-chill regions
Q113-21	2011/12	P	Y	M	L	150	still testing	growers like appearance and flavour
Q58-70	2011/12	N	Y	NM	M	150	still testing	early ripening
Q223-45	2012/13	N	W	NM	M	150	still testing	high quality; mid-season
Q223-20	2012/13	N	W	NM	M	250	still testing	high quality; mid-season
Q223-31	2012/13	N	W	NM	M	150	still testing	high quality; mid-late season

^z N, nectarine; P, peach.

^y W, white; Y, yellow.

^x M, melting; N, non-melting.

^w L, low; M, moderate; H, high acidity.

^v Chilling requirement measured in chill units (CU).

Feedback from grower testers on the performance of selections in the large-scale testing scheme.

Q17-20 Peach (released 2007/08)

This peach is the most widely tested variety under the large scale testing scheme. Over 4 tonnes of saleable fruit was produced in Childers in the 2009 season. Ripening time ranged from mid to late October. 17 tonne of Q17-20 was harvested in Childers for the 2010 season resulting in 11.7 tonne of saleable fruit. Fruit was harvested between 12th October and 10th November (Figure 6.2). Tray sizes ranged from 28 to 45 counts however the majority of fruit was packed as 10kg bulk boxes (Figure 6.3).

Figure 6.2. Harvest dates and kilograms harvested of Q17-20 in Childers 2010

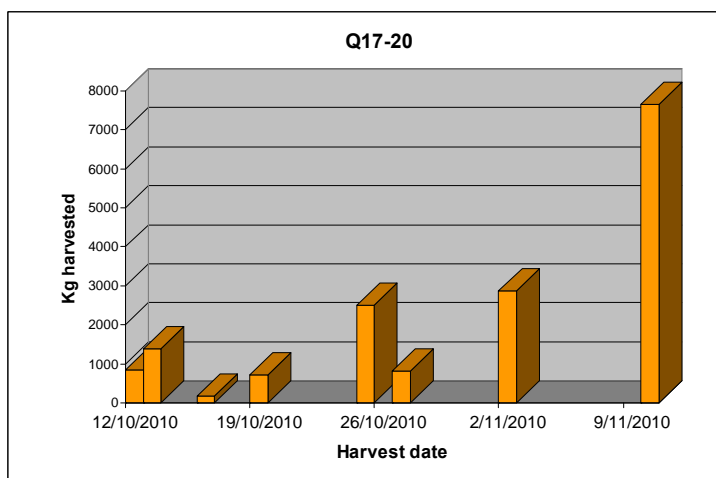
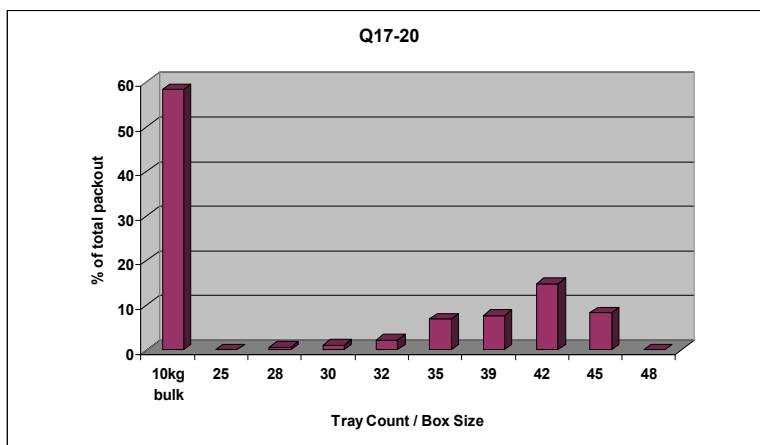


Figure 6.3. Distribution of tray/box size for 11.7t of Q17-20 grown in Childers 2010



Q52-18 Peach (released 2010/11)

This peach performed very well in Nambour but was late ripening due to cool, wet weather. Good size fruit and easy to harvest despite the rain. Well received at market and withstood the weather better than expected. Good taste and brix.

Q53-4 Peach (released 2006/07)

14 tonnes of this selection was produced in Childers in the 2009 season. Ripening time ranged from the end of October to mid-November. Production in Childers increased to 34 tonne of fruit resulting in 24 tonne saleable fruit. Fruit was harvested between 15th and 26th November (Figure 6.4) with tray sizes ranging from 25–45 counts (Figure 6.5). 47% of fruit was sold in 10kg bulk boxes.

Figure 6.4. Harvest dates and kilograms harvested of Q53-4 in Childers 2010

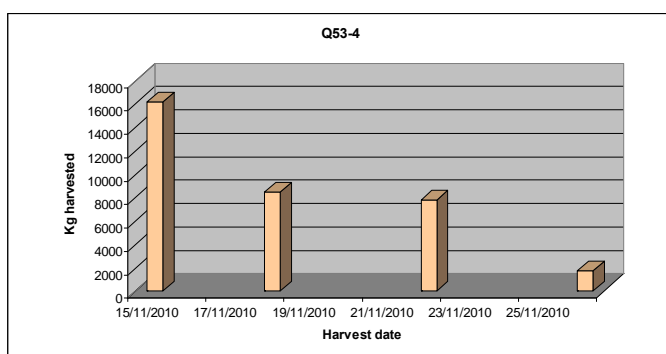
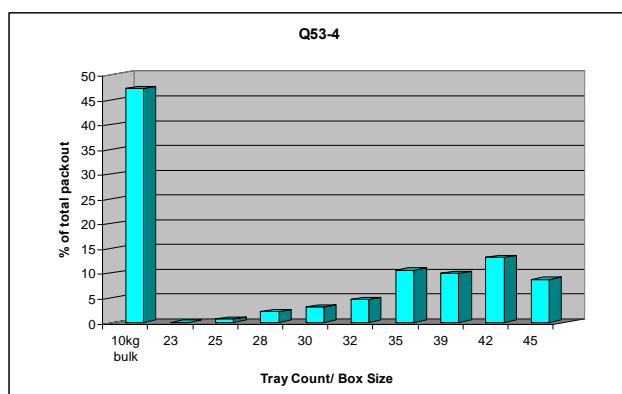


Figure 6.5. Distribution of tray/box size for 24t of Q53-4 grown in Childers 2010



Q68-64 Peach (released 2010/11)

This selection is currently tested in Malanda in North Queensland. Grower has described Q68-64 as very low chill with early/even flowering followed by good leaf bud development even in a failed winter like 2010. Fruit is well shaped with good flavour/sweetness. Some negative comments were fruit slightly on the small side and very dark in colour and looked sun burnt even though they weren't.

Q115-67 Peach (released 2008/09)

Q115-67 has been tested in Kumbia and Karragullen. Kumbia produced 4 pellets of this selection in the 2010 season. Very positive feedback was received from market agent and more of this fruit was requested. Grower found Q115-67 to be a little late for his liking but attributed this to leaving the leaves on too long. Grower indicated that care will need to be taken to not pick too early because it develops a full red skin colour before mature.

Q219-20 Peach (released 2009/10)

219-20 has been planted in Gatton in 2010. This selection has the desirable fruit traits of early ripening, attractiveness, good flavour, firm flesh and even shape. Grower indicated the crop in 2013 showed good potential.

Q32-59 Nectarine (released 2007/08)

This variety is being tested in Childers, Bangalow and Dandaragan.

1.5 tonnes of saleable fruit was produced in Childers in the 2009 season with an average brix level ranging from 11 to 13% and average size of 73 to 75 mm. Ripening time ranged from mid to late October. 23 tonne of Q32-59 was harvested in Childers between 22nd October and 20th November 2010 (Figure 6.6). 13 tonne of saleable fruit was produced from the 23 tonnes harvested with tray sizes ranging from 28-50 counts (Figure 6.7). Only 21% of Q32-59 was sold as 10kg bulk boxes.

Figure 6.6. Harvest dates and kilograms harvested of Q32-59 in Childers 2010

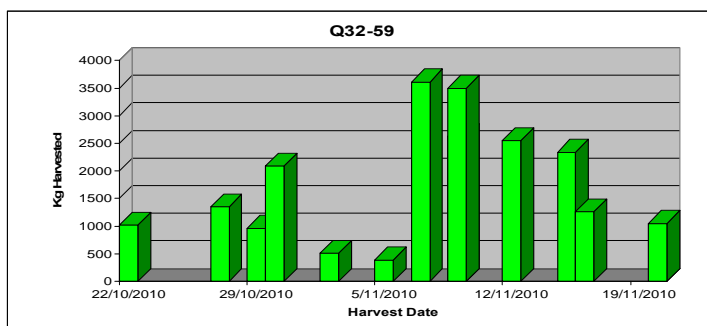
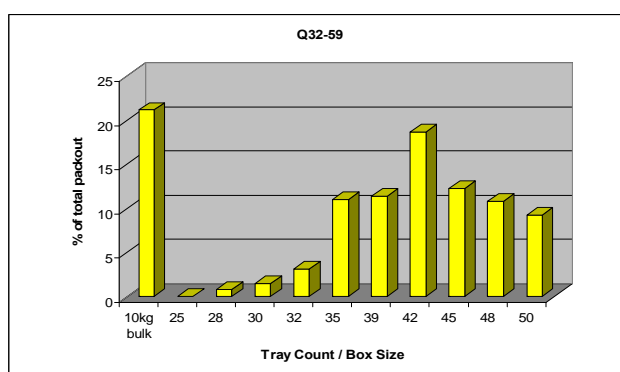


Figure 6.7. Distribution of tray/box size for 24t of Q32-59 grown in Childers 2010



Q70-53 Nectarine (released 2008/09)

This variety is being tested in Gatton and Karragullen. Trees were planted at Gatton in July 2010. Very early ripening, small fruit, bright red skin. Initial results indicate the fruit is too small for standard commercial use. The highly attractive appearance and good eating quality warrant testing of this selection as a novel fruit.

Q77-46 Nectarine (released 2009/10)

Q77-46 is under test in Nambour and Karragullen. Trees were also planted in Bangalow but were removed before any fruit data was available. Test trees at Nambour had a very small crop despite good size trees (not a very good set) which was attributed to wet weather at flowering time. Grower has concerns about the size of the fruit. Taste was OK.

Q219-25 Nectarine (released 2009/10)

Q219-25 is being tested in Nambour, Bangalow, Alstonville and Karragullen. Growers describe it as being a late but a good quality nectarine. Further reports have indicated that it produces light crops and so it has been discarded.

GB566-15 Nectarine (released 2009/10)

GB566-15 is currently being tested in Kumbia. Grower reported fruit as being the best tasting nectarine for the season. More data on this selection will be available this year. This is a higher chill selection that requires 350 CU and should be tested in southern locations.

Summary

The large scale testing scheme has provided vital information on elite selections from various growing regions. Evaluations of plantings greater than 100 trees allows decisions to be made on a selection's potential as measured under commercial management conditions. Data on Q17-20, Q32-59 and Q53-4 from large-scale grower tests has led to the future commercialisation of these selections. The close involvement of growers with this testing scheme provides a high level of interaction between the breeders, the growers and marketers and allows for practical outcomes in the selection of new varieties.

7.0 NOVEL FRUITS

The breeding project was directed by industry to concentrate 85% of its effort on development of new tropical peach and nectarine cultivars to improve on the current standards in terms of productivity and fruit quality. The remaining 15% of effort is directed at breeding other types of tropically adapted stonefruit such as apricots, plums, cherries, hybrids and alternative fruit types. Alternative fruit types have included peaches and nectarines with flat shapes (*peentos*) and with no anthocyanin pigmentation.

New cultivars from the former category are defined as regular peach and nectarine cultivars and will be commercialised using a non-exclusive, once-off tree royalty scheme. New cultivars from the latter category are the novel fruit types and in this chapter their characteristics and commercialisation are described.

7.1 Description of novel fruits

Seven peach and nectarine selections were determined by the steering committee as being novel fruits (Table 7.1). Selections are considered novel because of their unusual fruit characteristics or tree growth habit. These seven novel selections were peaches and nectarines that had flat rather than round shape, commonly called *peento*, saucer or doughnut peaches/nectarines, and other selections had a regular round shape but lacked red pigmentation in the skin. It was considered that subject to more extensive commercial-scale testing, these novel fruits are likely to meet niche, Australian marketing requirements.

The new selections are described in detail in Appendix 12.3. In summary they comprise of:

- one yellow-fleshed peach and one white-fleshed peach that lack red skin pigmentation, each with trees having approximately 150 chill units
- one *peento* (flat) nectarine with yellow flesh, the tree having approximately 150 chill units
- two *peento* (flat) peaches, one with white flesh, one with yellow flesh and both with trees having approximately 250 chill units
- two yellow-fleshed nectarines lacking red skin pigmentation.

All varieties are early maturing, have fruit total soluble sugars (TSS) in the range of 12 to 14% and have been shown to have a pleasant, well-balanced flavour in limited-scale assessment conducted at Nambour.

These selections will be protected using Plant Breeders Rights (PBR). Prior to application for PBR new material is protected by material transfer agreements (MTA) that restrict distribution and use of the germplasm.

7.2 Commercialisation of novel fruits

The principal aim of the commercialisation was to maximise the potential of the new selections in the Australian stonefruit market and to achieve a profitable return to the commercialisation partner. A full copy of the Expression of Interest (EOI) document with details of the selection criteria, terms and

conditions of the contract and the requirements involved in granting an exclusive licence are included in Appendix 12.4

DAFF advertised for expressions of interest to commercialise the 7 selections in November 2010. The EOI responses were examined by a panel of stakeholders and a report on the EOI process was prepared and recommendation on the commercial partner made in February 2011. The outcome was that Blackboy Ridge Pty Ltd (BBR) was recommended as the commercial partner as it satisfied or exceeded all the evaluation criteria.

Table 7.1. Description of seven novel fruit selections compared with the industry standard peach 'Tropic Beauty'.

Selection number	Crop type	Fruit shape	Fruit size (g)	Harvest cw Tropic Beauty (+/- days)	Chill (chill units)	Skin Colour	Flesh colour	Flesh type	Sweetness TSS (%)	Stone Freedom
Q74-69	Nectarine	Oblong/Round	55-65	-21	150	Yellow with traces of red	Yellow	Non-melting	14	Cling
Q10-58	Nectarine	Ovate/Round	65-80	+14	350	All yellow	All yellow	Non-melting	12	Cling
Q58-67	Peach	Ovate/Round	80-100	-21	150	All cream	White	Non-melting	12	Cling
Q74-30	Peach	Ovate/Round	75-85	+4	150	All yellow	All yellow	Non-melting	13	Cling
Q73-50	Peach	Flat	45-55	+4	250	Light Red	White	Non-melting	12	Cling
Q73-58	Peach	Flat	45-65	+1	250	Medium Red	All yellow	Non-melting	13	Cling
Q108-65	Nectarine	Flat	60-70	+1	150	Bright Red	All yellow	Non-melting	13	Cling
Tropic Beauty (standard)	Peach	Round	75-100	0	150	Medium Red	Yellow	Melting	11	Semi-cling

8.0 TECHNOLOGY TRANSFER

Meetings and Field Days

A summary of the breeding project was presented at the LCA annual general meeting in Bangalow in December 2007.

The industry steering committee met on 6th March 2008 at Nambour to discuss and review the project progress.

A stakeholder meeting was held in October 2008 to draft the commercialisation plan.

A summary of the breeding project was presented to the LCA annual general meeting in Bangalow in December 2008.

An industry steering meeting was held in January 2009 at which the commercialisation plan was ratified.

Two project steering meetings in March 2009 and May 2009 reporting on project progress and planning.

A report on the breeding project was presented to the Low Chill Australia annual general meeting at Bangalow in December 2009.

The industry steering committee for the project met in February 2010 to review the project and discuss the performance of new selections.

A report on the breeding project and the new peach selections Q52-18 and Q68-64 was presented by Bruce Topp at the Low Chill Australia AGM at Bangalow in December 2010.

The industry steering committee for the project met in February 2011 to review the project. The major topic of discussion was the review of the large scale testing scheme.

A report on the breeding project was presented by Bruce Topp at the Low Chill Australia AGM at Bangalow in December 2011.

The industry steering committee for the project met at Wollangbar, NSW in February 2012 to review the project. A summary of the peach and nectarine selections distributed in 2011-12 through the large-scale testing scheme was presented to the group.

A talk on the breeding project was presented at the Low Chill Australia AGM at Bangalow in December 2012.

A meeting of Low Chill Australia and the breeding project steering committee was held at Maroochy Research Station on 15 February 2013.

A handout describing four of our new peach and nectarine selections was prepared for use by a local nurseryman in providing information to growers on the selection's characteristics October 2013.

An update on the breeding program was provided to growers at the Annual General Meeting of Chill Australia on 10 December 2014.

Conference presentations

Topp, B. L., G. W. Bignell, D. M. Russell, and P. Wilk. 2009. Breeding low-chill peaches in subtropical Queensland. Paper presented at the 7th International Society of Horticultural Science Peach Congress, Lleida Spain, June 2009.

A talk was presented at the National Low Chill Stonefruit Conference in Ballina in April 2010. The invited presentation was by Topp B, Bignell G, Russell D, Wilk P., titled “Breeding new varieties of low chill stone fruit for the Australian market”.

Invited speaker at the HortLink Agronomy Forum in May 2013 on topic of “Low Chill Stonefruit breeding program”.

“Use of the Mexican Peach ‘Nieve’ for Breeding New Cultivars in Australia” paper by B. Topp, D. Krolow, E. Correa, D. Russell, J. Neal and S. Perez at the 8th International Peach Symposium, June 2013, Matera, Italy.

“Testing Low-chill Peach Selections in Subtropical and Temperate Queensland” poster presented at 8th International Peach Symposium, June 2013, Matera, Italy by D. Russell, G. Bignell, J. Neal and B. Topp.

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9.0 RECOMMENDATIONS –SCIENTIFIC & INDUSTRY

1. It is recommended that breeding from 2014 to 2019 should focus on shorter harvest periods and targeted early-ripening periods.

A major shift has occurred in cultivar requirements of the Australian low-chill industry due to two factors:

- Use of fenthion for fruit fly control has been drastically changed. Growers can no longer apply cover sprays of fenthion and then harvest the fruit 3 days later. Standard cultivars such as ‘Tropic Beauty’ peach require harvesting of ripe fruit every 3-4 days for a period of 6-8 weeks. This equates to 10 to 18 harvests from each tree. With the altered fruit fly control measures it will be very difficult to prevent fly damage over such a long harvest period. Continual insecticide application during long harvest periods will no longer be possible. New cultivars are required that can be picked in a short time period with only a few harvests per cultivar rather than the current 10-18 harvests.
- Traditionally, the earliest temperate zone peach production started in late November. With new, early ripening cultivars the temperate regions are producing fruit in mid and early November. Imported Californian peach and nectarine fruits are now being sold in Australia in winter and through to September. Thus the low-chill peach industry is being squeezed at both ends of its time-slot. Our new cultivars need to focus on production in the October time-slot.

It is recommended that future breeding be used to assist in addressing these two issues by having short fruit development periods and therefore early ripening; and non-melting and stony-hard flesh to reduce the number of harvests and wastage. This research will result in reduced costs of production through reduced harvest costs. It will enable the industry to better adapt to the new insecticide regime that has occurred with the loss/reduction of fenthion use.

2. It is recommended that the small-scale testing be modified to improve the quality of data and the distribution of this data to industry.

The small-scale testing scheme has the advantages of rapid, wide-spread testing of many new selections at low-project cost. It could be improved in several ways and it is recommended that methods to improve efficiency of the testing scheme be evaluated with industry and introduced into the scheme.

Possible methods by which the scheme may be improved are to:

Introduce replication at each test site (currently only one tree of each selection is tested)

Review data collection procedures to improve harmonisation of results.

Publish results of the small-scale tests more widely.

3. It is recommended that the testing of our selections be expanded to include cooler, temperate regions of Australia.

A small but important percentage of our new selections are medium-chill as opposed to low-chill. These medium-chill selections are not adapted to the subtropics and have potential to perform well in traditional temperate fruit growing regions. It is recommended that testing in cooler regions of Australia occur. Prime locations for this test would be locations such as Renmark in the Riverland of South Australia that have interest in early ripening cultivars.

Our low-chill selections should also be tested in the cooler regions. It is possible to grow low-chill peach cultivars in temperate regions in micro-climates where the probability of spring frost damage is low. Southern growers are seeking earlier and earlier ripening cultivars and low-chill is an important method of obtaining this goal.

5. Breeding should be expanded to include the production of new rootstocks.

A major obstacle in low-chill peach production is the extreme vigour of the low-chill trees. During one growing season a single shoot can grow 3m. The high vigour is detrimental to profitability because of the additional pruning costs and the potential reduction of crop yield at the expense of vegetative growth (low harvest index). Currently, there are no low-chill rootstocks that provide dwarfing. Paclobutrazol is used commercially in Australia to control vigour but its use is prohibited in some countries and is not suitable for use on all soil types within Australia.

It is recommended that germplasm screening be initiated in order to produce rootstocks that are adapted to low-chill environments and reduce tree vigour.

6. DNA technologies should be investigated for ways in which their inclusion may increase breeding efficiency particularly with reference to the narrowing of the genetic stocks that occurs can occur in breeding.

Western peach breeding populations suffer from a narrow genetic base (Scorza et al., 1985; Byrne, 2003) due to a relatively small number of founding parents that were imported from China in the initial breeding and a lack of infusion of new germplasm in subsequent generations. Feral peach seedling populations exist in Australia's subtropical regions. There are anecdotal suggestions that some of these populations are derived from seed originally imported by Chinese immigrants who came to Australia during the Gold Rushes of the late 1800s.

It is recommended that a study is conducted of these feral peach populations using DNA markers to compare them with legacy and commercial subtropical peach genotypes from the current breeding programs. Results from this experiment will provide evidence of a potentially novel source of peach germplasm for use in future breeding. It will also highlight segments of the peach genome that have been altered during breeding and thus identify target regions for future breeding.

The benefits of this research go beyond the specific outcomes of the experiment. Benefits also will arise from the process of incorporation of the molecular technologies into the breeding project.

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12.0 APPENDICES

12.1 Protocol for Prunus embryo rescue

Sharon Hamill 2014

IN CLEAN AREA OUTSIDE LABORATORY

Use green hard fruit NOT ripe soft fruit

It is VERY important in order to reduce contamination that ALL soft or damaged fruit are removed from fruit that will have embryos rescued.

Isolate green sound fruit and place into mesh bag with written label. (Plastic pot tag written with pencil). Do not use pen or this will dissolve. Write information in laboratory book.

Sterilise whole fruit in 70% ethanol for 2 minutes

Followed by 2% Sodium hypochlorite with Tween surfactant for 5 minutes

Finally rinse off Sodium hypochlorite with 70% ethanol.

Drain

INSIDE LABORATORY

Apply sterile technique working in laminar flow cabinet

Extraction of embryos from fruit is performed in a cabinet separate to where embryos are placed into media.

Stonefruit seed (stone or endocarp) cutting secateurs are used.

Use two pairs of secateurs per person and store secateurs in beakers of 80% ethanol and place in solution between each seed extraction.

Using special secateurs crack open the stone (the endocarp) to remove embryo. Do this by cutting down the middle of the fruit (suture line) down the groove and twist as cutting.

Leave testa on seed and place into labelled sterile petri dish in preparation for embryo's being placed into media.

Placing embryos into media done in a separate cabinet

Excised embryos are placed into ovule /embryo development media with 1.5 ml/L Plant Preservation Media (PPM) in deep petri dishes (5 per plate). PPM is a biocide and is an essential step to prevent and reduce bacterial contamination which will otherwise occur on the sugar rich media. 1.5ml /Litre is effective but this can be increased to 2ml/L if needed.

Plates are cultured at 20-22 degrees C in the dark for a minimum of 4 weeks for ovule /embryo development. At end of this cycle embryos are observed and when seen to have grown and plumped are then placed into germination media. If embryos/ovules have not plumped up then they will need to stay on the media for longer. Observation is important at this step.

Using sterile technique transfer embryos into germination media with 1.5ml/L PPM and stratify in dark at 5 to 6 degrees C. If possible set 6 degree day temp and 4 degree night temp. Remove when most of the embryos in the plate are JUST starting to germinate. It is important that the embryos do not start to grow and produce true leaves as this needs to happen in the glasshouse environment of lower humidity and higher temperature with sunlight. Acclimatise in a glasshouse under double shade and in a plastic tent under high humidity so plants germinate and produce leaves and roots in the glasshouse environment.

Prepare Ovule Development and Germination media

Stock solutions

Solution A: KNO_3 – 237.95 g/L (Potassium nitrate)

Solution B: NH_4NO_3 – 20.625 g/L (Ammonium nitrate)

Solution C: Na_2EDTA – 0.930 g/L (Ethylenediaminetetraacetic acid -disodium salt)
 $\text{FeSO}_4 \cdot 7\text{H}_2\text{O}$ – 0.70 g/L (Ferrous sulphate heptahydrate)

Solution D: $\text{CaCl}_2 \cdot 2\text{H}_2\text{O}$ – 89.80 g/L (Calcium chloride dihydrate)
 $\text{Ca}(\text{NO}_3)_2 \cdot 4\text{H}_2\text{O}$ – 28.00 g/L (Calcium nitrate)

Solution E: KH_2PO_4 – 34.00 g/L (Potassium dihydrogen orthophosphate)

Solution F: H_3BO_3 – 1.240 g/L (Boric acid)
 Na_2MoO_4 – 0.05 g/L (Sodium molybdate)
 $\text{CoCl}_2 \cdot 4\text{H}_2\text{O}$ – 0.005 g/L (Cobaltous chloride)
 KI – 0.166 g/L (Potassium iodine)

Solution G: $\text{MnSO}_4 \cdot 4\text{H}_2\text{O}$ – 4.460 g/L (Manganese sulphate monohydrate)
 $\text{MgSO}_4 \cdot 7\text{H}_2\text{O}$ – 74.00 g/L (Magnesium sulphate)
 $\text{CuSO}_4 \cdot 5\text{H}_2\text{O}$ – 0.005 g/L (Copper sulphate)
 $\text{ZnSO}_4 \cdot 7\text{H}_2\text{O}$ – 1.720 g/L (Zinc sulphate)

Murashige & Skoog Vitamin Solution

Use 10ml stock per litre

Nicotinic acid 0.5 g/L
Thiamine HCl 0.1 g/L
Pyridoxine HCl 0.5 g/L
Glycine 2 g/L
Myo-inositol 100 g/L

STONE FRUIT OVULE/EMBRYO DEVELOPMENT MEDIA

	1 L	2L
Vitamin Solution	1 ml	2ml
Stock solutions A, B and C (each)	20ml	40ml
Stock solutions D, E, F, and G (each)	5 ml	10ml
Sucrose	60g	120g
PPM	1.5 - ml	3ml
Phytigel	2.5g	

STONE FRUIT OVULE/ EMBRYO GERMINATION MEDIA

	1 L	2L
Vitamin Solution	1 ml	2ml
Stock solutions A	0.8 ml	1.6ml
Stock solution C	40ml	80ml
Stock solutions D, E, F, and G (each)	5 ml	10ml
Sucrose	30g	60g
PPM	1.5 - 2.0ml	3ml
Phytigel	2.5g	

Add stock solutions to cylinder; make up required volume with distilled water.


Add to beaker, add sucrose and PPM + heat (if needed). Adjust to pH 6.2


Measure 2.5g phytigel per 1L bottle, add solution and autoclave.


Check pH close to 5.5 - 5.7 after autoclaving.

Cool to 55oC and pour into deep petrie dishes (~20plates/L)

12.2 Descriptions of selections in Plant Breeders Rights trial

Q17-20 peach		
FRUIT	PARAMETER	COMMENT
	Ripening Time	Ripens early, about 90 days after bloom, and overlapping with the harvest of Tropic Beauty peach. Harvested in early to mid-October at Nambour.
	Size	Small to medium size, averaging from 70-120g per fruit.
	Shape	Ovate at Nambour but round at Stanthorpe. Has been noted to produce suture bulges in warm years
	Skin colour	Red blush covers about 70% of the skin surface. Yellow background colour.
	Fuzz	Moderate amount of skin pubescence.
	Flesh	Yellow, non-melting flesh that has a standard sugar-acid balance with a mild peach flavour.
	Stone freedom	Clingstone
TREE		
	Vigour	Vigorous growth, intermediate growth habit between upright and spreading.
	Yield	Moderate to heavy crops, will require thinning to obtain adequate fruit size.
	Chilling	Bloom period overlaps and is slightly later than Tropic Beauty so is rated at 150-200 chill units.
SUMMARY 		<p>This is a low-chill, non-melting, yellow flesh peach that may have some commercial potential due to its early ripening period and firm, non-melting flesh.</p> <p>The above information is from limited testing of 1 to 2 trees at one location (Nambour) over 4 years. Further testing to verify these observations and to provide information on post-orchard characteristics will occur in a large-scale testing scheme starting in 2006. Planting these selections during the large-scale testing entails commercial risk.</p>

Q32-59 nectarine	PARAMETER	COMMENT
FRUIT		
	Ripening Time	Ripens early, overlapping with SunWright nectarine. Harvested in early to mid-October at Nambour, about 90-100 days after bloom.
	Size	Medium to large size for season; averages from 80 to 120g per fruit.
	Shape	Ovate to oblong shape with no tip and a slight suture bulge.
	Skin colour	Red blush covering approx 70% of the skin surface.
	Flesh	Yellow, melting flesh that has a sugar-acid balance and pleasant flavour.
	Stone freedom	Clingstone
TREE		
	Vigour	Vigorous growth; with a semi-upright growth habit.
	Yield	Sets moderate to heavy crops that will require thinning.
	Flowering period and Chilling	Bloom period overlaps with Tropic Beauty peach and is rated at approx 150 chill units. Blooms in early to mid July at Nambour.
SUMMARY		<p>This is a low-chill nectarine that produces yellow, melting flesh fruit. It may have some commercial potential due to its early ripening period and low chilling requirement.</p> <p>The above information is from limited testing at one location (Nambour) over 4 years. Further testing to verify these observations and to provide information on post-orchard characteristics will occur in the large-scale testing scheme. Planting these selections during the large-scale testing entails commercial risk.</p>
		

Q53-4 peach		
FRUIT		
	Ripening Time	Ripens mid to late season, about 110-120 days after bloom. Harvested in early to mid-November at Nambour.
	Size	Medium to large size, averages from 100 to 200g per fruit.
	Shape	Ovate with a slight tip at Nambour.
	Skin colour	Red blush covers about 60% of the skin surface. Yellow background colour.
	Fuzz	Light to moderate amount of skin pubescence.
	Flesh	Yellow, melting flesh that has a standard sugar-acid balance with a pleasant peach flavour.
	Stone freedom	Semi-clingstone
TREE		
	Vigour	Vigorous growth, intermediate growth habit between upright and spreading.
	Yield	Light to moderate crops, will probably not require much thinning to obtain adequate fruit size.
	Chilling	Bloom period overlaps with Tropic Beauty so is rated at approx 150 chill units.
SUMMARY		
	<p>This is a low-chill, melting, yellow flesh peach that may have some commercial potential in the subtropical peach fruit season due to its medium to large fruit size and later ripening period.</p> <p>The above information is from limited testing of 1 to 12 trees at one location (Nambour) over 3 years. Further testing to verify these observations and to provide information on post-orchard characteristics will occur in a large-scale testing scheme starting in 2006. Planting these selections during the large-scale testing entails commercial risk.</p>	

12.3 Novel fruit descriptions

Important note: These characteristics are from limited testing at one location (Nambour) over two to five years depending on the variety. Further testing to verify these observations and to provide information on post-orchard characteristics will occur in larger-scale testing. Planting new selections in larger-scale testing may involve commercial risk.

Q74-69 nectarine

FRUIT

Ripening time

Ripens early, around 21 days earlier than Tropic Beauty. Harvested in late September to early October at Nambour, about 80-90 days after bloom.

Size: Medium size for season; averages from 55 to 65g per fruit.

Shape: Oblong to round shape with no tip.

Skin colour: Yellow skin with a slight orange to red blush covering approx 10% of surface.

Flesh: Yellow, non-melting flesh that has balanced sugar and acid, pleasant flavour and TSS averaging 14%.

Stone freedom: Clingstone.

TREE

Vigour: Vigorous growth; with a semi-upright growth habit.

Yield: Sets moderate to heavy crops that will require thinning.

Flowering period and chilling: Bloom period overlaps with and is slightly earlier than Tropic Beauty peach. Rated at approx.150 chill units. Blooms in early to mid-July at Nambour.

SUMMARY

This is a low-chill nectarine with yellow skin and yellow, non-melting flesh. It is potentially useful commercially because of its attractive yellow skin, early ripening period and low chilling requirement.

This information is from limited testing at one location (Nambour) over four years.



Q10-58 nectarine

FRUIT

Ripening time: Ripens around 14 days later than Tropic Beauty. Harvested in late October to early November at Nambour, about 80-85 days after bloom.

Size: Medium size for season; averages from 65 to 80g per fruit.

Shape: Ovate to round.

Skin colour: Yellow skin with a slight orange to red blush covering approx 10% of surface.

Flesh: Yellow, non-melting flesh that has balanced sugar and acid, pleasant flavour and TSS averaging 12%.

Stone freedom: Clingstone.

TREE

Vigour: Vigorous growth; with a semi-upright growth habit.

Yield: Sets moderate to heavy crops that will require thinning.

Flowering period and chilling: Bloom period is 33 days after Tropic Beauty peach and is rated at approx. 350 chill units. Blooms early to mid-August at Nambour.

SUMMARY

This is a low-chill nectarine with fruit with yellow skin and yellow, non-melting flesh fruit. It is potentially useful commercially because of its all attractive yellow skin, early ripening period and low chilling requirement.

This information is from limited testing at one location (Nambour) over 5 years.



Q58-67 peach

FRUIT

Ripening time: Ripens around 20 days earlier than Tropic Beauty. Harvested in late September to early October at Nambour, about 75 days after bloom.

Size: Medium size for season; averages from 80 to 100g per fruit.

Shape: Ovate to round.

Skin colour: Pale yellow skin usually with no red blush.

Flesh: White, non-melting flesh that has a balanced sugar-acid ratio and pleasant flavour and TSS averaging 12%.

Stone freedom: Clingstone

TREE

Vigour: Vigorous growth; with a semi-upright growth habit.

Yield: Sets light to moderate crops.

Flowering period and Chilling: Bloom period is similar to Tropic Beauty peach and is rated at approx. 150 chill units. Blooms in early to mid-July at Nambour.

SUMMARY

Q58-67 is a low-chill, pale yellow skinned peach that produces white, non-melting flesh fruit. It is potentially useful because of its attractive skin colour and early ripening, and the tree has low chilling requirement.

The above information is from limited testing at one location (Nambour) over 5 years.



Q74-30 peach

FRUIT

Ripening time: Ripens around 4 days later than Tropic Beauty, in mid to late October at Nambour, about 90-100 days after bloom.

Size: Medium size for season; averages from 75 to 85g per fruit.

Shape: Ovate to round shape.

Skin colour: Yellow skin; no red blush observed.

Flesh: Yellow, non-melting flesh that has balanced sugar and acid, pleasant flavour and TSS averaging 13%.

Stone freedom: Clingstone

TREE

Vigour

Vigorous growth; with a semi-upright growth habit.

Yield

Sets moderate crops that will require thinning.

Flowering period and chilling

Bloom period overlaps with Tropic Beauty peach and is rated at approx. 150 chill units. Blooms in early to mid-July at Nambour.

SUMMARY

This is a low-chill peach that produces all-yellow fruit with yellow, non-melting flesh. It is potentially useful commercially because of its attractive yellow skin, early ripening period and low chilling requirement.

This information is from limited testing at one location (Nambour) over four years.



Q73-50 peento (flat), white-fleshed peach

FRUIT

Ripening time: Ripens early, around 4 days later than Tropic Beauty and 10 days earlier than UFO. Harvested in mid to late October at Nambour, about 80-90 days after bloom.

Size: Small to medium size for season; averages from 45 to 55g per fruit.

Shape: Flat.

Skin colour: Light red

Flesh: White, non-melting flesh that has balanced sugar and acid, pleasant flavour and TSS averaging 12%.

Stone freedom: Clingstone

TREE

Vigour: Vigorous growth; with a semi-upright growth habit.

Yield: Sets light to moderate crops.

Flowering period and chilling: Bloom period is 18 days later than Tropic Beauty peach and is rated at approx. 250 chill units. Blooms in mid to late July at Nambour.

SUMMARY

This is a low-chill, flat peach with white, non-melting flesh. It is potentially useful commercially because of its flat shape, white flesh, early ripening period and low chilling requirement.

This information is from limited testing at one location (Nambour) over four years.



Q73-58 peento (flat), yellow-fleshed peach

FRUIT

Ripening time: Ripens around 1 day later than Tropic Beauty and 13 days earlier than UFO. Harvested in mid to late October at Nambour, about 90-100 days after bloom.

Size: Medium size for season; averages from 45 to 65g per fruit.

Shape: Flat.

Skin colour: Medium red.

Flesh: Yellow, non-melting flesh that balanced sugar and acid, pleasant flavour and TSS averaging 13%.

Stone freedom: Clingstone



TREE

Vigour: Vigorous growth; with a semi-upright growth habit.

Yield: Sets moderate crops that will require thinning.

Flowering period and chilling: Bloom period is 18 days later than Tropic Beauty peach and is rated at approx. 250 chill units. Blooms in mid to late July at Nambour.

SUMMARY

This is a low-chill, flat peach with yellow, non-melting flesh. It is potentially useful commercially because of its all early ripening period and low chilling requirement.

This information is from limited testing at one location (Nambour) over five years.

Q108-65 flat nectarine

FRUIT

Ripening time: Ripens early, around 1 day later than Tropic Beauty and 13 days earlier than UFO. Harvested in mid to late October at Nambour, about 90-100 days after bloom.

Size: Medium size for season; averages from 60 to 70g per fruit.

Shape: Flat.

Skin colour: Bright red.

Flesh: Yellow, non-melting flesh that has balanced sugar and acid, pleasant flavour and TSS averaging 13%.

Stone freedom: Clingstone

TREE

Vigour: Vigorous growth; with a semi-upright growth habit.

Yield: Sets light to moderate crops.

Flowering period and chilling: Bloom period overlaps with Tropic Beauty peach and is rated at approx 150 chill units. Blooms in early to mid-July at Nambour.

SUMMARY

This is a low-chill, flat nectarine with yellow, non-melting flesh. It is potentially useful commercially because of its early ripening period and low chilling requirement.

This information is from limited testing at one location (Nambour) over two years.



12.4 Expression of Interest document used for commercialisation of novel fruits



**AGRI-SCIENCE QUEENSLAND,
DEPARTMENT OF EMPLOYMENT, ECONOMIC DEVELOPMENT AND
INNOVATION**

**EXPRESSIONS OF INTEREST
DEEDIO0055**

Applications are to be received by: 3 December 2010, 4pm AEST.

Late or incomplete applications will not be considered.

All applicants are also required to consider and sign the Disclosure Statement at Attachment A, which covers confidentiality and conflict of interest matters.

Seven novel, low-chill peach and nectarine varieties for commercialisation

Agri-Science Queensland, a service of the Department of Employment, Economic Development and Innovation (DEEDI), is inviting expressions of interest to commercialise the following seven (7) novel varieties of low-chill, early-ripening peaches and nectarines which are important outcomes of its breeding program.

All varieties are novel because of their fruit quality, appearance or shape, blossoming and fruit harvest times and low chilling requirements. Subject to more extensive commercial-scale testing, these are likely to meet niche, Australian marketing requirements.

The new varieties are:

- one yellow-fleshed peach and one yellow-fleshed nectarine, each with trees having approximately 150 chill units;
- a *flat* nectarine with yellow flesh, the tree having approximately 150 chill units;
- two *peento* (flat) peaches, one with white flesh, one with yellow flesh and both with trees having approximately 250 chill units;
- a yellow-fleshed nectarine, the tree having approximately 350 chill units.

All varieties are early maturing, have fruit total soluble sugars (TSS) in the range of 12 to 14% and have been shown to have a pleasant, well-balanced flavour in limited-scale assessment conducted at Nambour, south-east Queensland.

The new varieties are all briefly described in the **attached factsheet** which includes a comparative chart of fruit and tree characteristics.

Background

The department's low-chill stone-fruit breeding program, based at Nambour, is achieving its goal of generating high-quality fresh-market varieties that have novel fruit characteristics and mature early in order to capture that high-priced fruit trade.

Novel fruit types are important because their distinguishing attributes would be sought-after by consumers in comparison with standard, early season varieties. The seven varieties offered for commercialisation have distinctive combinations of fruit colours, shape and size coupled with the trees' low chilling requirements.

The breeding project's industry-based, steering committee has assessed the seven varieties as worthy of wide-scale testing in low-chill production areas with a view to effective commercialisation of one or more of them by the commercialisation partner.

Intellectual property

The intellectual property rights in the seven varieties vest in The State of Queensland (acting through the Department of Employment, Economic Development and Innovation) and Horticulture Australia Limited.

Scope of expression of interest

Expressions of interest are invited from organisations interested in obtaining an exclusive licence to:

- manage evaluation trials of the varieties of sufficient size in Australia for adequate time and locations in order to document commercial potential including market acceptance; and
- exercise the first right of refusal to undertake planned commercialisation of selected varieties in Australia for fresh market production including legal protection of the intellectual property, annual reporting and royalty payments.

The principal objectives are to:

- maximise the varieties' potential in Australian stone-fruit markets and production regions; and
- achieve profitable financial returns to the commercialisation partner.

Terms and conditions will be recorded in an executed licence agreement.

Requirements

The exclusive licence agreement granted to the successful licensee would involve:

- clonally propagating the seven varieties onto disease-free, preferably virus-tested rootstocks;
- undertaking appropriate commercial evaluation trials of the varieties including market acceptance;
- registering selected varieties with IP Australia and maintaining registration on behalf of DEEDI;
- undertaking planned commercialisation of selected varieties including:
 - a successful marketing plan to advertise, promote and sell varieties,
 - propagating or sub-licensing the propagation of disease-free trees in sufficient quantity for planned commercialisation,
 - entering into sub-licensing agreements through the production supply chain to securely manage the intellectual property in the varieties,
 - reporting progress and sales volumes to DEEDI for compliance and royalty purposes;
- a royalty on production which will be paid on the basis of:

- a single, once-only payment per tree propagated or annual tree-rental fee for trees used in the supply chain,
- a royalty as a percentage of income from wholesale sales of fruit, or
- a combination of the above.

Criteria

The following criteria will be used in the selection of the successful licensee. It is important that interested entities address and clearly document how they meet these criteria in sufficient detail and under separate headings:

1. Demonstrated experience in the production of a range of disease-free, clonal stone-fruit varieties in sufficient quantity for licensed commercial evaluation and commercial development.
2. Understanding and demonstrated experience in sub-licensing agreements to facilitate evaluation of the commercial potential of the plant intellectual property.
3. Understanding and demonstrated experience in thorough evaluation and documentation of commercial potential of selected stone-fruit varieties through the supply chain.
4. Understanding and demonstrated experience in the effective management and commercialisation of protected plant intellectual property in Australian markets in regard to:
 - 4.1. legally protecting selected varieties under the *Plant Breeder's Rights Act 1994*;
 - 4.2. sub-licensing agreements with plant propagators and/or growers to grow selected varieties in sufficient numbers to promote and meet market expectations;
 - 4.3. marketing plans to advertise, promote and sell selected, licensed varieties;
 - 4.4. an outline of indicative performance targets that will be binding and enforceable irrespective of actual performance, including:
 - a firm indication of the minimum number of plants to be propagated and distributed under licence for initial, commercial evaluation in-orchard and with sufficient volume of fruit in major consumer markets;
 - anticipated minimum number of plants to be propagated for use in the supply chain following the selection and legal protection of selected varieties; and
 - a firm indication of the minimum per tree royalty, fruit production royalty, or combination of the two forms of royalty to be paid to DEEDI.
5. Description of the applicant's business organisation and practices, including timely and appropriate record keeping and reporting practices, and experience in efficient collection and payment of royalties.

Terms and conditions of contract

The following is a summarised description of the key terms and conditions that will be included in the licence agreement.

- **Intellectual Property:** Ownership of the intellectual property in the relevant varieties will remain with DEEDI and equity partners.
- **Extent and term of licence granted:** The licence will be for Australia and a term to be stipulated at the time of agreement.

- **Royalty payments:** DEEDI envisages a royalty payment based on trees propagated, fruit production or an appropriate combination of the two forms of royalty. A per-tree royalty would be paid either as a once-only fee per tree or annually as part of a tree rental scheme. A fruit production royalty would be represented by a percentage of wholesale prices received.
- **Reporting requirements:** The licensee will be expected to report to DEEDI on progress against milestones and performance targets. DEEDI reserves the right to audit the licensee's records.
- **Performance criteria:** The licensee will be expected to meet the stipulated performance terms, including the milestones for commercialisation of the varieties as stated in the licensing agreement. DEEDI will reserve the right to recover royalties based on the stipulated performance measures and, if appropriate, terminate the licence agreement upon default of agreed performance or other contractual obligations.
- **Confidentiality:** It will be expected that DEEDI, its equity partners and the licensee will keep all information owned by each party confidential.
- **Jurisdiction:** The licence agreement will be governed and interpreted in accordance with the laws of Queensland, Australia.

Process following submission

The Tender Evaluation Committee will evaluate applications against the responses provided under the criteria. Short-listed applicants may be asked to provide a presentation to the Tender Evaluation Committee before a final decision is made.

Contact: **Consultant**
Department of Employment, Economic Development and Innovation
Ph 07 3225 1673
Email: procurement@deedi.qld.gov.au

Address & Manner of Response: Applications must be submitted as one (1) hardcopy with the Front Cover form (provided at Attachment B); and one (1) electronic copy (pdf format).

Late and/or incomplete applications will not be considered.

The written application is to be submitted to:
The Queensland Government Tender Box
GPO Box 2482
Brisbane QLD 4001

The electronic copy is to be emailed to:
procurement@deedi.qld.gov.au

Disclosure Statement

Acknowledgement of Obligation

EXPRESSION OF INTEREST DEEDIO0055: Seven novel, low-chill peach and nectarine varieties for commercialisation

By this declaration dated the [insert DD/Month/YYYY]

<i>Name</i>	<i>Position</i>
-------------	-----------------

acknowledge and agree to the following:

1. CONFIDENTIALITY OBLIGATIONS

- 1.1 In the course of submitting a response to the **Department of Employment, Economic Development and Innovation** ('the Department'), relating to this Expression of Interest (EOI) process; I may be exposed to information which is confidential.
- 1.2 Improper use or disclosure of that information could jeopardise or invalidate the EOI process and may severely damage the Department's ability to perform its governmental/statutory functions.
- 1.3 I agree to treat all information provided to me in the course of submitting an EOI response; described in Clause 1.1 herein as confidential information, which must not be divulged to any person without the prior written consent of the Department.
Confidential information includes information of a sensitive, personal, commercial or political nature made available to you that could cause harm to individuals or the State if disclosed other than in accordance with its intended purpose to target audience.

2. CONFLICT OF INTEREST

- 2.1 I warrant that before signing this declaration, I have disclosed on this document all the past, current and anticipated interests which may conflict with my impartial involvement in this EOI process.
- 2.2 I agree that during the course of this EOI process, I will not engage in any activity or obtain any interest likely to conflict with my impartiality in respect of this project. In the event that a real or apparent conflict of interest arises, I shall immediately disclose it to the Department.

Declaration of Conflict of Interest

I declare that the following are all the past, current and anticipated interests which may give rise to a real or apparent conflict with my impartial involvement in the EOI process.

I note that conflicts of interest may arise under the following situations:

- an event or situation and the context in which it occurs;

- the nature of my work;
- any personal or private interests that may directly or indirectly influence and/or benefit me or others;
- my relationships with, or the names of other parties;
- a conflict of interest may relate to both pecuniary and non-pecuniary interests.

The reason/s why I consider the situation may be a conflict of interest, or be perceived by others as a conflict of interest is below (if none, write NONE).

Signature: _____

Dated:

Witness Name: _____

Witness

Signature: _____

Dated:

Attachment B

Front Cover for Submissions

This section is to be completed and attached to the front of all submitted applications.

EOI Name: DEEDIO0055 Seven novel, low-chill peach and nectarine varieties for commercialisation		Closing Date: 3 December 2010, 4 pm AEST
Name of PERSON / BUSINESS / CORPORATION:		
Address:		
	Post Code:	
Australian Company Number (ACN):		
Australian Business Number (ABN):		
Business Number (BN):		
Are you registered for GST?	YES <input type="checkbox"/> / NO <input type="checkbox"/>	
State of Australia/Overseas where Business Name or Corporation is Registered:		
Name of Holding Company or Corporate Group:		
Enquiries To:		
E-Mail Address:		
Telephone No.:		
Mobile No.:		
Fax No.:		
Signature:	Date:	