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Final Report

Macadamia Breeding and Conservation

Dr Bruce Topp
The University of Queensland

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Level 8, 1 Chifley Square
Sydney NSW 2000
Tel: (02) 8295 2300
Fax: (02) 8295 2399

PROJECT LEADER:

Dr BL Topp, Principal Plant Breeder
Queensland Alliance for Agriculture and Food Innovation, The University of Queensland
Maroochy Research Facility, PO Box 5083 SCMC, Nambour, QLD 4560 Australia
Phone: 07 54535973 Fax 07 54535901
Email: b.topp@uq.edu.au

PROJECT TEAM:

Dr Jodi Neal, Department of Agriculture and Fisheries, Maroochy Research Facility, Nambour, QLD

Dr Craig Hardner, Queensland Alliance for Agriculture and Food Innovation, The University of Queensland, St Lucia, QLD

Dr Alison Kelly, Department of Agriculture and Fisheries, Maroochy Research Facility, Toowoomba, QLD

Mr Paul O'Hare, Department of Agriculture and Fisheries, Maroochy Research Facility, Nambour, QLD

Dr Olufemi Akinsanmi, Queensland Alliance for Agriculture and Food Innovation, The University of Queensland, St Lucia, QLD

Dr Andre Drenth, Queensland Alliance for Agriculture and Food Innovation, The University of Queensland, St Lucia, QLD

Dr Ruth Huwer, NSW Department of Primary Industries, Wollongbar Primary Industries Institute, Wollongbar, NSW

Mr Craig Maddox, NSW Department of Primary Industries, Wollongbar Primary Industries Institute, Wollongbar, NSW

Dr Andrzej Kilian, Diversity Arrays Technology, University of Canberra, Bruce, ACT

Mr Dougal Russell, Principal Experimentalist, Department of Agriculture and Fisheries, Nambour, QLD

Mr Rod Daley, Technical Officer, Department of Agriculture and Fisheries, Bundaberg Research Facility, Bundaberg, QLD

Mr Edward Howell, Technical Officer, Queensland Alliance for Agriculture and Food Innovation, The University of Queensland, Nambour, QLD

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Summary

Macadamia (*Macadamia integrifolia* Maiden & Betche and *M. tetraphylla* L.A.S. Johnson) is an Australian native, evergreen nut tree adapted to the subtropics. Australia is a major world producer with 17,000 hectares of orchards producing over 40,000 tonnes of nut-in-shell (NIS) in 2015, with a market value of AUD$200 million. Australia is also the home of the macadamia with wild macadamias growing in northern NSW and southern Queensland. Our breeding project is using this valuable native resource to develop new cultivars for a more profitable Australian industry.

Key industry stakeholders were consulted to help guide the objectives of the breeding program. Stakeholders included marketers, growers, agribusiness representatives and pest consultants. Stakeholders nominated and prioritised characteristics required in future macadamia cultivars. The characteristics that received the highest priority from the stakeholders included increased yield, resistance to husk spot, desirable consumer characteristics, resistance to fruit spotting bugs and small tree size. These traits are being evaluated in all the elite selections developed from the breeding program.

Previous breeding by CSIRO had created the B1.2 population which was planted in 14 trials at 9 locations in Queensland and NSW in 2001-2003. We evaluated these 1,961 seedlings for NIS yield, kernel recovery, tree height and tree canopy width from 2009 to 2013. A selection index was used to combine genetic and phenotypic values of yield and tree growth traits to select the top 207 candidates. Detailed agronomic and field observations on nut quality, disease and insect susceptibility, nut drop pattern and other key traits were then used, with industry input, to reduce the elite population to 23 selections. These selections will be evaluated in regional field trials to provide a second release of new cultivars to industry. The 20 elite seedling selections with highest selection index values had cumulative NIS yield 39% greater than five clonally propagated varieties. This is based on single tree data and will require confirmation with replicated testing.

We created a second generation of seedling populations using controlled hand pollinations to combine the elite first generation selections. This recurrent selection strategy builds on the progress obtained in previous breeding to create new cultivars which are another step higher in profitability. A total of 3,555 seedlings using 79 parents were created in this project and have been planted in randomised trials at Nambour and Bundaberg. These trials will be evaluated for eight years and the resulting elite selections will be clonally propagated for regional testing prior to industry release.

Industry identified husk spot and fruit spotting bug as the major disease and pest concerns at the start of this project. The elite selections that are due for release in 2017 were screened for husk spot severity and four selections with moderate to high husk spot were identified. A relationship between husk spot level and sticktights (dried husk that stays in the tree) was found and is now used as a screening tool in our progeny trials.

Fruit spotting bug incidence was examined on wild and cultivated genotypes at *ex-situ* germplasm trials at Alstonville and Tiaro. No resistant material was identified but the wild species *M. ternifolia* was found to be highly susceptible and shows promise for use in hedges as a trap and fruit spotting bug monitoring tool.

We evaluated four rootstock trials at Baffle Creek, Bundaberg, Newrybar and Wollongbar in which 12 cultivars were propagated as seedling or cutting rootstocks and grafted to combinations of the same 12 cultivars as scions. Non-grafted cuttings on their own roots were also included. Scion was more important than rootstock in determining NIS yield and tree size. Rootstock had virtually no influence on kernel recovery. After 12 years the cumulative NIS yield for 'Beaumont' cuttings was 13% higher than the industry standard 'H2' seedling rootstock. We suggest further trial of 'Beaumont' rather than full scale adoption, due to limitations in the current trial design and lack of testing in a wide range of soils and environments. The RVT3 project has both 'H2' seedling and 'Beaumont' cutting used on 30 scion varieties at nine locations and will provide a more robust comparison of the two rootstocks. Full recommendations will be possible at the conclusion of RVT3 in 2017.

Many other activities were completed during this project including:

- Establishment of two breeding arboretums,
- Genetic fingerprinting of 20 elite selections prior to 2017 release,

- Study of oil development in kernel of wild germplasm,
- Identification of incorrectly labelled trial trees through the use of DNA technology,
- Study of the genetic diversity of our elite selections,
- Evaluation of commercial cultivars at remote sites,
- Comparison of four breeding strategies and subsequent establishment of a two-stage breeding trial that will reduce breeding time and increase efficiency,
- Experiments to compare methods of visual estimation of yield which will reduce the evaluation costs of future trials,
- Industry consultation to develop improved methods for future regional testing of elite selections, and
- Numerous industry consultation meetings, presentations, field days and articles in industry media.

The project has successfully re-invigorated the seedling production phase of breeding after a 10 year hiatus in which no new seedling populations were produced. We have used the elite selections developed in previous projects as parents to produce the second generation which will provide further improvement for industry. Industry will benefit from 2017 onwards from the first release of new cultivars from the breeding projects. Future breeding is essential to provide the Australian industry with continued international competitive advantage.

Keywords

Macadamia, breeding, strategy, evaluation, seedling, progeny, nut, quality, germplasm, cultivar, oil, rootstock, yield, kernel recovery, molecular markers, extension, efficiency, husk spot, *Pseudocercospora macadamiae*, fruit spotting bug, *Amblypelta nitida*, *M. integrifolia*, *M. tetraphylla*, *M. ternifolia*, species.

1.0 Introduction

Macadamia is an Australian native, evergreen nut tree adapted to the subtropics. It is the basis of an international industry producing premium kernels for snacks, confectionary, baking, ice-cream and oil. The Australian macadamia industry produces about 40,000 tonnes of nut-in-shell annually with a market value of over AUD$200M. Australia is the second largest macadamia producer in the world, just behind South Africa, with six million trees planted on 17,000 ha.

Compared to most horticultural crops, macadamia is recently domesticated with current cultivars only two to four generations from the wild. Although macadamia is an Australian native it is the breeding and cultivation in Hawaii in the early-mid 1900s that has most impacted on its international commercialisation. Hawaiian cultivars account for over 80% of trees planted in Australia (Jones and Mayer, 2009). The three most commonly planted cultivars are all from Hawaii. These are 'HAES344', 'HAES741' and 'HAES246', which were released in 1971, 1977 and 1948, respectively. The last cultivar released from the Hawaiian breeding program was 'HAES790' in 1990 (Hardner et al., 2009). Breeding and selection of new cultivars under Australian conditions, using our native resource has the scope to increase industry profitability.

An industry supported breeding project, led by CSIRO, commenced in 1996 aimed at producing cultivars for the Australian industry (McConchie et al., 1999). It used a quantitative genetic approach which combined experimental design and pedigree relationships to increase accuracy of genetic value predictions (Hardner et al., 2009). The traits of interest were formally defined and selection of superior genotypes was accomplished by using an economically weighted selection index. A first round of crossing resulted in 1,611 seedlings, designated the B1.1 population, which were planted at Alstonville and Bundaberg in 1997-1998. A second round of breeding resulted in 1,961 seedlings, designated the B1.2 population, which were planted from 2001-2003 in 14 trials at nine locations in NSW and Queensland.

The HIA project MC02054 "Macadamia Improvement and Conservation" (MC02054) finished in 2008. It resulted in the selection of 20 candidate genotypes, selected from the B1.1 population, that are predicted to provide a 30% increase in profitability compared to industry standards (McConchie et al., 2008). These 20 candidates were clonally propagated by grafting onto 'H2' seedling and 'Beaumont' cutting rootstocks and planted in randomised, replicated regional variety trials (RVTs). They are currently being evaluated in the HIA project MC11001 "Regional Variety Trials – Series 3" (MC11001). The trial has only two years to run and the first cultivars are due for release in 2017. Included in the RVT3 trial are five selections from the Australian private breeding company Hidden Valley Plantations and five industry standard cultivars.

In addition to creating seedling progeny trials, CSIRO also planted four rootstock trials at Bundaberg, Baffle Creek, Newrybar and Wollongbar, two *ex-situ* germplasm trials at Tiaro and Alstonville and two non-RVT cultivar trials at Emerald and Pretty Gully (McConchie et al., 2008).

The main objectives of the current breeding project MC09021 were to create new seedling populations and to evaluate and analyse the existing trials.

2.0 Industry Consultation to Determine Key Cultivar Traits

A major industry consultation activity of this project was to determine what characteristics were of most importance in new cultivars. This chapter describes the process of industry consultation used to answer this question and the results from the consultation.

2.1 Methods

Key stakeholders were identified for consultation on important future macadamia cultivar characteristics. The stakeholders included:

- Marketers
- Growers
- Agribusiness representatives (including processors)
- Pest consultants.

A priority setting exercise was conducted with each of these stakeholder groups at industry meetings, including:

- AMS marketing subcommittee meeting
- Seven regional MacGroup meetings (for both growers and agribusiness representatives, Figure 2.1)
- Annual macadamia pest consultants meeting.

Stakeholders were asked to consider the following question at each of the meetings: "What are the most important characteristics (from your perspective) that will be required in new macadamia cultivars in 10 to 20 years?" The 10 to 20 year time frame was important due to the long term nature of macadamia breeding.

Each group of stakeholders was made aware that the project team was consulting with other industry stakeholders and all the results were to be summarised and analysed in consultation with MIVIC before being used to guide the breeding objectives.

Figure 2.1 Alstonville grower meeting for evaluation of cultivar characteristics.

Each stakeholder at the meeting was asked to nominate important future cultivar characteristics from their perspective. A list of nominated potential characteristics was developed. Discussion was limited to clarification of the characteristics rather than debating relative merits. Potential characteristics were consolidated if the meeting agreed they were identical.

Each stakeholder was then given 3 votes to select the characteristics that were most important to them. The number of votes for each characteristic was then tallied at each meeting.

2.2 Results

The detailed results from each meeting are attached in Appendix 1. These results list all the nominated characteristics and the number of votes from each group of stakeholders.

The votes from all the meetings were summarised and closely related specific characteristics were grouped within a broad topic to enable comparison and analysis. For example, the broad topic of yield includes high yields, consistent yields, yield precocity and increased kernel yield through increased kernel recoveries.

There were a total of 503 votes. The nominated desired characteristics that consistently voted the most highly were:

- Yield (148 votes)
- Resistance to husk spot resistance (59 votes)
- Desirable consumer characteristics (52 votes)
- Resistance to fruit spotting bugs (42 votes)
- Smaller trees (37 votes)

Other characteristics that received greater than 10 votes included:

- Tree structure e.g. open canopies
- Absence of sticktight nuts
- Resistance to other biotic factors e.g. trunk canker, nutborer, lace bugs, rats
- Post-harvest quality e.g. long shelf life
- Resistance to other abiotic factors e.g. tolerance of climatic conditions.

2.3 Discussion

2.3.1 Yield

High yield was consistently voted as the highest priority or one of the highest priorities at each of the MacGroup meetings by the growers and the agribusiness representatives. It was also one of the highest priorities amongst the pest consultants.

High yield included both high yield of kernel per hectare and per cubic metre of canopy volume. These two characteristics were combined at some MacGroups and considered separately at others.

Consistent yield from year to year was also considered an important characteristic at several of the MacGroup meetings. Biennial bearing with high yields followed alternately by poor yields was not considered desirable.

Increasing kernel yield through increasing kernel recovery and yield precocity also both received votes at the MacGroup meetings.

2.3.2 Resistance to husk spot

Tolerance or resistance to husk spot was voted one of the top 3 priorities at each of the MacGroup meetings apart from Bundaberg (only two votes out of 64 in Bundaberg). It was also voted the highest priority amongst the pest consultants. This high ranking in many of the groups reflects the perception by the industry of the importance of losses due to husk spot in macadamia orchards, particularly in New South Wales and south-east Queensland.

The high number of votes for no sticktight nuts at MacGroup meetings and amongst the pest consultants is also related to the importance of sticktight nuts as a source of infection for macadamia husk spot.

2.3.3 Desirable consumer characteristics

Desirable consumer characteristics were voted the top priority amongst the marketers. They also received the most votes at the Bundaberg MacGroup meeting and featured highly at several other MacGroup meetings.

The marketers identified taste, shelf life, colour and texture as important aspects of desirable consumer characteristics:

- Taste must appeal to consumers, and must be maintained after the kernel has been stored.
- The kernel quality must be maintained over the required storage period (e.g. 6 or 12 or 24 months).
- Consistent colour is required within a variety, there must be no difference in colour between the top and bottom of the kernel and pale creamy white is preferred as the kernel colour.
- The preferred texture is a soft crunch.
- Consistency is required for taste, colour and texture

The high number of votes for post-harvest quality is also closely related to desirable consumer characteristics. It is important to note that how the kernel is handled both on-farm and through the supply chain can have a major influence on kernel post-harvest quality (e.g. shelf-life).

2.3.4 Tree size

Small, dwarf and/or compact trees received votes at all the MacGroup meetings (particularly in Alstonville, Dunoon and Bangalow) and amongst the pest consultants. This reflects the importance with which canopy management and orchard crowding are regarded amongst Australian macadamia farmers and resulting effects on orchard practices and sustainability.

2.3.5 Fruit spotting bugs

Tolerance or resistance to fruit spotting bugs was voted as one of the top 5 priority characteristics at most of the MacGroup meetings and among the pest consultants. It was consistently voted the most important of the insect pests to target in the breeding of new macadamia cultivars.

Nut borers, lace bugs and rats also received priority votes at MacGroup (particularly in Alstonville and Bangalow) and pest consultant meetings but much less than for fruit spotting bugs.

3.0 Progeny Seedling Production

The first generation of seedlings of the HAL funded Macadamia Breeding Program (B1) were produced by CSIRO between 1997 and 2003. No further seedlings were produced by CSIRO. A primary focus of the current project was to re-invigorate the production of seedling progeny. Under project MC09021, a second-generation seedling population was created using elite selections and high breeding value parents from generations B1.1 (parents crossed 1994/1995, trials planted 1997/1998, selections made 2007) and B1.2 (parents crossed 1997/1999, trials planted 2001-2003, selections made 2014). This approach is expected to result in further improvements for the key selection traits of yield, kernel recovery, kernel quality, small tree size, husk spot resistance and fruit spotting bug resistance. The following chapter describes the seedling production, planting and parents.

3.1 Methods

3.1.1 Parental selection

The genotypes selected as parents for the cross-pollinations varied between trials and years. The precocity trial (BNAMB11) was designed to test the high-density, two-stage (tandem) breeding strategy outlined by Topp et. al, (2012). Maternal genotypes for this trial were selected to include highly precocious genotypes, as well as a number of the B1.1 generation elite selections. All offspring were open-pollinated, collected in 2009.

All other seedling trials were medium density, with the same design as the B1.1 and B1.2 generations. The majority of parents used for the BQBRS12 and BQBRS13 trials were elite selections from the B1.1 progeny population. The remaining trials predominantly included parents selected from the B1.2 progeny population. Specifically, these were trees identified as having high breeding values from the quantitative genetic analysis conducted on this population. Putative dwarf trees and other trees of interest were also included as parents. Crosses of families that performed well in the B1.2 generation but contained few seedlings were also repeated.

3.1.2 Controlled cross-pollinations

Controlled cross-pollinations were conducted annually between 2010 and 2014, in late August and early September. Racemes on both maternal and paternal trees were enclosed in paper bags prior to opening to prevent pollen contamination. Pollen was collected from the paternal trees once the racemes had opened fully. Racemes were inserted into clear plastic tubes and rubbed until a sufficient quantity of pollen coated the inside of the tube. The recipient racemes on the maternal trees were first rubbed in the same manner to remove excess self pollen, then rubbed with tubes containing the donor pollen. Crossed racemes were immediately placed back inside paper bags. After 1-3 months the paper bags were replaced with onion bags, so that the mature nuts would not be lost once they fell from the trees. The nuts were collected once mature, between April and May the year after crossing.

In 2013, cincturing of branches where crosses were present was tested to determine if this increased nut set on the crossed racemes. Cincturing was only undertaken on branches with a large number of leaves (Trueman and Turnbull 1994). Results (not presented) indicated a substantial increase in nut set, and so all crossed trees were cinctured in 2014.

3.1.3 Seed germination

The mature nuts were collected in March-April each year and dehusked within 24 hours of collection. They were soaked in water for 24 hours prior to shallow planting into 60 cell Bowman trays (4.5 cm x 11 cm) containing Searle's Premium Potting Mix. Nuts were planted with the micropile lying horizontal to ensure straight growth of shoots and roots. Trays were placed in a glasshouse at 25-35˚C on benches covered with plastic to maintain high humidity, and watered daily (Figure 3.1).

Figure 3.1. Plastic tunnel used to maintain high humidity around germinating seed.

Once the seedlings grew to the capacity of the planting cells they were potted into 90 mm bottomless square pots in 12-pot-racks and transferred to a shadehouse with 30% shade. Seedlings were fertilised with Osmocote Plus Native eight to nine month slow release fertilizer and for the first few weeks watered with Searl's Flourish Native Plants Soluble Plant Food at lower than the recommended rate (two teaspoons instead of three per nine litres)

3.1.4 Propagation of standards

Cultivar and parental standards were clonally propagated each year as cuttings for inclusion in the progeny trials. The method was adapted from Bell (1996, and 1998), Badgery-Parker (1997) and Oppenheimer and Reuveni (1961). Cuttings 3-5 mm in diameter and 15 to 20 cm long were taken between October and January each year. Stems were cut longer than required in the field and immediately placed in an insulated bag with freezer bricks. Cuttings were sprayed periodically with water to maintain high humidity in the bag.

Cuttings were planted the same day as collection to prevent dehydration. Shoots were trimmed to the required length, and all but the top three whorls of leaves removed together with any lateral shoots. Shoots were cut just below a bud to force roots, scored (2-3 cm) on either side of the base revealing fresh green cambium tissue, then dipped in Rooting Hormone Gel, CLONEX®, "Purple" IBA 3g/l, (O'Conner WA 6163, Australia) before planting.

The cutting media mix used was 50% Chillagoe Perlite (Coarse), 50% Coir Fibre Peat, (GalukuTM Root Zone Media, Sydney 2000, Australia). Square native propagation tubes (50 mm x 125 mm deep) were filled with cutting medium and left in the mist-house under misting jets to allow the mix to become saturated with water. Stems were pushed into the mix and gently firmed around to increase soil contact. Pots were spaced out to allow room for leaves and to contain disease outbreaks.

Cuttings were kept constantly wet in a mist-house (Figure 3.2.) using Toro Waterbirds® PC misting Jets (Toro Australia Pty/Ltd, Banyo QLD, Australia) PC (36-151l/h) with Toro non-drip valves to protect plants from dripping sprinkler heads. Mist timing was controlled by a Sterling 12 timer (Superior Controls Co., Inc., Torrance CA. 90501, USA), set to mist every four minutes for 20 seconds on summer days, and every six minutes for 20 seconds on winter days. At night, the misting was once an hour from 7pm to 5am in summer and once an hour from 5pm to 7am in winter. Lengthy misting ensured the leaves and potting mix stayed wet throughout. Bell (1996) removes cuttings when root tips appear at the base of the pots from six weeks after potting, however very few of our cuttings developed long root systems in this time frame. In this experiment all cuttings were removed from the mist-house after 3-5 months in summer and 5-6 months in winter.


Figure 3.2. Cuttings in the mist-house.

All cuttings were transferred to a shade house with 30% shade and 4 x 15 minutes watering per day. A few grains of Osmocote® Plus TE, 8-9 month Native (Scotts , Bella Vista, NSW 2153, Australia) were spread on top of the pots. After two weeks the cuttings were potted up into 90 mm "bottomless" square pots with Searles Premium Potting Mix (Searles, Kilcoy, QLD 4515, Australia).

3.1.5 Planting

Seedlings and standards were typically planted into the field between April and May the following year, approximately 20 months after crossing and 12 months after nut collection. The BQBRS12 trial (Table 3.1) was an exception to this and was planted an additional 12 months after crossing. CSIRO observed that leaving plants in the nursery for an extra year in this manner resulted in higher survival rates in the field (Craig Hardner pers. comm.), however this was not considered necessary here given that all second-generation trial locations were irrigated. Prior to planting soil tests and improvement were conducted, rows were mounded, irrigation was installed, and inter-row cover crops sown. Trial design incorporated replicated cuttings of commercial cultivars and individual seedlings in incomplete block designs with single tree plots. Trees were pruned, fertilised, irrigated and managed for insect and pest control as per commercial practices (O'Hare et al., 2004).

The precocity trial (BNAMB11) was planted at the Maroochy Research Facility (MRF) in November 2011 at tree spacings of 1 × 4 m. The remaining three trials (BQBRS12, BQBRS13 and BQBRS14) were planted at the Bundaberg Research Facility (BRF) in 2013 and 2014 with spacings of 4 × 6 m. Two additional trials are planned for 2015 and 2016 for crosses performed in 2013 and 2014, and will be planted at BRF and MRF respectively.

3.2 Results

A total of 3,555 seedlings and 405 standards (control trees) have been produced to date under the current project (Table 3.1). This number does not currently include standards for the 2014 crosses. Nuts from the 2014 crosses were collected in April 2015 and will be germinated for planting in 2016.

Table 3.1. Counts of seedling progeny, families, parents, standards and buffer trees produced in MC09021. Seedlings produced from the 2014 crosses are due for planting in 2016, and so final numbers are not yet available for this trial.

Trial code	Location	Year planted	Seedlings	Families	Parents	Seedlings/ family mean (range)	Standards	Standard genotypes	Buffer trees	Total planted
BNAMB11	MRF	2011	720	32	32	23 (6-26)	139	29	127	986
BQBRS12	BRF	2013	432	32	22	14 (1-54)	47	18	52	531
BQBRS13	BRF	2013	769	36	23	21 (1-89)	69	18	101	939
BQBRS14	BRF	2014	477	33	21	14 (1-89)	80	23	107	664
BQBRS15	BRF	2015	556	27	24	19 (1-93)	70	29	28	654
Total planted			2954	155	79	19 (1-103)	405	55	415	3774
2014 crosses *	N/A	2016	601	15	18	28 (4-166)	Unknown	23	Unknown	N/A
**Grand total ** **			**3555**	**155**	**79**	**22 (1-166)**	**405**	**55**	**415**	**4375**

MRF =* Ungerminated nut count, as final seedling count not yet available. ** Excluding unknown values.

A total of 2,954 seedlings, 405 standards and 415 buffer trees were planted into five trials between 2011 and 2015. Tree survival rates for all trials have been very high at 97%. Standards in these trials were selected to include seedling parents, a selection of industry cultivars, and other trees required to ensure adequate overlap of standards between trials (Table 3.2, Appendix 2).

Table 3.2 Counts of standards in common between trials/cross years.

	BNAMB11	BQBRS12	BQBRS13	BQBRS14	BQBRS15	2014 crosses
BNAMB11	29	13	11	6	3	7
BQBRS12		18	11	8	5	8
BQBRS13			18	10	7	9
BQBRS14				23	11	11
BQBRS15					29	18
2014 crosses						23

155 families of seedlings have been produced (Appendix 3), where a family is defined as progeny from a unique combination of female and male parents. The average family size was 22 seedlings, and ranged from 1 to 166 seedlings per family (Table 3.1). The families produced in each cross year typically included little overlap with other years (Table 3.3).

Table 3.3. Counts of seedling families (above centre line) and parents (below) in common between trials/cross years.

	BNAMB11	BQBRS12	BQBRS13	BQBRS14	BQBRS15	2014 crosses
BNAMB11	32/32	1	0	0	0	0
BQBRS12	13	32/22	7	0	0	0
BQBRS13	14	14	36/23	1	1	1
BQBRS14	3	4	5	33/21	4	2
BQBRS15	2	3	3	7	24/27	4
2014 crosses	0	0	1	4	11	18/15

As quantitative genetic approaches were used to select parents with a high breeding value and thus are expected to produce progeny with a higher average performance than the first generation progeny, further improvements in performance for the key selection traits are expected, beyond those observed in the first generation. The precocity trial established in this project will also allow assessment of the tandem selection strategy aimed to reduce breeding time and increase gain per unit cost.

3.3 Conclusions

A total of 3,774 trees have been planted to date, in the precocity trial at Maroochy Research Facility and three trials at the Bundaberg Research Facility (Figure 3.3). There were 986 trees in the precocity trial (created from open-pollinated hybrid seed collected in 2009, 531 trees in the BQBRS12 trial (created from 2010 controlled cross-pollinations), 939 trees in the BQBRS13 trial (created from 2011 crosses), and 664 trees in the BQBRS14 trial (created from 2012 crosses) and 654 trees in the BQBRS15 trial (created from 2013 crosses). An additional 601 nuts will be germinated from 2014 crosses and planted in 2016. The final total number of trees is estimated to be approximately 4,375. A more accurate number will be available in April 2016 following germination of nuts from the 2014 crosses and design of the 2016 trial. The large population and the use of parents with high breeding value will facilitate identification of selections with improved performance over those produce by the first breeding generation.

(A)

(B)

Figure 3.3. Progeny trial at Bundaberg Research Station (A) at planting in April 2013 and (B) after two years in April 2015.

4.0 Arboretum Establishment

Two breeding arboretums were established at Bundaberg Research Facility (BRF) and Maroochy Research Facility (MRF). This is the first time that arboretums have been established under the Macadamia Breeding Program. The arboretums will be a valuable resource for the breeding program, allowing easy access to elite parents and improving efficiency of crossing activities. Trees planted include B1.1 and B1.2 selections, cultivars not currently easily accessible, and putative dwarfing genotypes. An additional high-density arboretum was established at MRF as a repository for plants that were only required for several years. Trees in this block include elite B1.2 selections grown for propagation material, hybrids between various *Macadamia* species to test for desirable kernel traits, and offspring from a self-compatibility experiment for further observation.

4.1 Methods

The trees in the arboretums include seedling trees and trees clonally propagated from cuttings, following the protocols in Chapter 3. Prior to planting, soil tests and improvement were conducted, rows were mounded, irrigation was installed, and inter-row cover crops sown. Planting at BRF was undertaken between 2013 and 2014 as plant material became available. The MRF arboretums were planted in March 2014 and incorporated incomplete block designs with single tree plots. Trees were pruned, fertilised, irrigated and managed for insect and pest control as per commercial practices (O'Hare et al., 2004).

4.2 Results and discussion

126 permanent and 234 temporary trees have been planted across the three arboretums (Figure 4.1, Table 4.1, Appendix 4). The arboretums will continue to be expanded as cuttings from additional elite parents from the B1.2 population become available for planting.

Figure 4.1. Aboretum planting at Bundaberg Research Facility planted in 2010 and photographed in 2015.

Table 4.1. Planting densities and tree numbers for the arboretums.

Arboretum	Spacing (m)	Trees
BRF low density	4 x 6	82
MRF low density	4 x 8	44
MRF high density	1 x 4	234
Total		360

The annual rate of genetic gain from the breeding program is directly related to the generation length. The generation length is defined as the time from seed to seed from one generation to the next and as such depends on how quickly the new seedling selections can be identified and used in the next round of crossing. Rapid propagation of the new seedling selections into the arboretums will be a part of the strategy to increase genetic gain by reducing the generation length.

5.0 B1.2 Progeny Evaluations and Selection of Elite Candidates

The B1.2 progeny trials were planted by CSIRO from 2000 to 2003. The code, B1.2 signifies "B" for breeding trial and "1.2" because it was the second planting of the first progeny generation. The B1.1 progeny trials (first planting of first generation progeny) were planted by CSIRO in 1997 and 1998 and the elite B1.1 trees were planted in the current regional variety trial, RVT3.

This chapter describes the harvest, evaluation and analysis of the B1.2 progeny trials and the outputs, which are the elite selections now ready for industry regional variety trialling. 2018 progeny from 142 families were assessed for four traits (cumulative total nut in shell mass to age eight (CTNM), cumulative total kernel mass to age eight (CTKM), total kernel recovery (TKR), and kernel yield efficiency (YE)) to create an initial shortlist of 207 genotypes of interest. The best phenotypically performing genotypes for each trait showed improvement of between 29% and 43% compared with the top performing cultivar. Shortlisted selections were relatively evenly distributed between trial locations. The family Beaumont x NG18 performed very well in terms of number of progeny selected for CTNM, CTKM and YE. Families NG8 x A199 and NG8 x 705 also performed well for YE.

5.1 Initial selection of the top 207

Macadamia trees naturally drop nuts between March and October and an individual tree can drop nuts from six weeks to five months depending on the variety. Harvesting of the B1.2 progeny blocks began in early March in the Bundaberg region and April in northern New South Wales. Trials were harvested up to three times throughout the season at six to eight week intervals. For the last harvest each tree was stripped of all remaining nuts.

Determining which nut comes from which tree depends on canopy overlap and ground topography. Steep slopes as at East Gympie and Dunoon trial sites can see nuts rolling into neighbouring trees; however in these cases phenotypic traits such as size, shape and maturity assisted in determining tree of origin.

Trees were harvested using a finger-wheel harvester or by hand into a bucket (Figure 5.1). Nuts were collected into 800 mm x 400 mm drawstring onion bags or 50 cm polynet bags depending on the tree crop load. Each bag was labelled and taken back to the shed and dehusked within 48 hours. Bags were dehusked and wet nut in shell weight (WNIS) recorded. Rat, husk spot and Green Vegetable bug damage were sorted out and discarded (Figure 5.2). A polynet bag sample of approximately 100 nuts was labelled, weighed and recorded.

Each bagged sample was dried over six days for 48 hours at 35 ^{o}C, 48 hours at 45 ^{o}C and 48 hours at 55 ^{o}C until 1.5% moisture content was reached (dry nut in shell, NIS). Weights were recorded, the difference between NIS and WNIS calculated and applied to the total for that harvest. Samples were stored in 30 L air-tight drums in ambient air temperature until evaluation of nut and kernel characteristics (Figure 5.3).

Figure 5.1. Nut harvesting by hand and using finger-wheel harvesters.

Figure 5.2. Sorting damaged nuts after dehusking.

Figure 5.3. Drum storage for kernel assessment.

The B1.2 generation of breeding trials included for assessment 2,018 progeny from 142 families and 44 parents. These were planted in 14 trials at nine different locations from Baffle Creek in the North to Newrybar in the South (Figure 5.4), with 60 to 354 progeny per trial. Trees were assessed for yield, height and canopy width from four to eight years of age, and total kernel recovery (TKR) in 2010 (seven to nine years of age depending on trial).

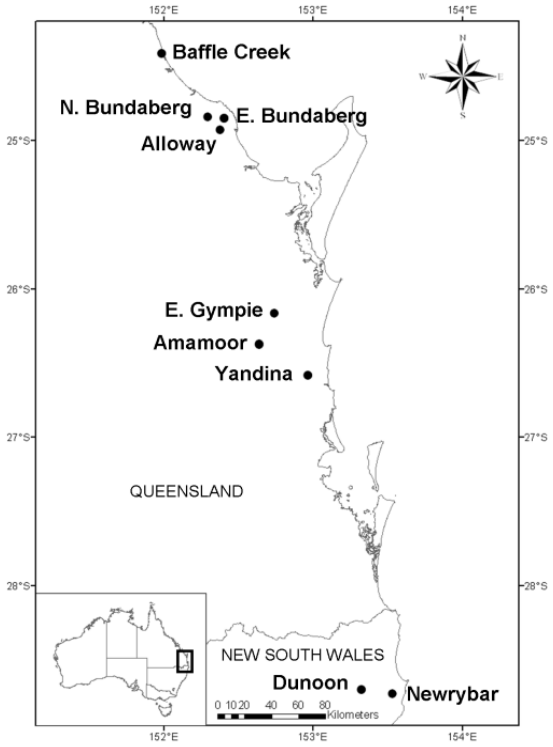

Figure 5.4. Map of trial locations.

Each trial site was managed by the owner as per standard commercial operations which slightly varied from farm to farm. Trees were pruned, fertilised, irrigated and managed for insect and pest control as per commercial practices

(O'Hare et al., 2004). Pre-harvest clean-ups were carried out on most blocks before the season while mowing was usually done by the grower prior to project harvesting.

Tree height and width measurements were taken each year at the end of the harvest season, usually in October/November (Figure 5.5).

Figure 5.5. Height measurements at Dunoon, 2009 using a six metre measuring pole.

Quantitative genetic analyses were used to estimate clonal and breeding values for all trees for four traits. These traits were: cumulative total nut in shell mass to age eight (CTNM), cumulative total kernel mass to age eight (CTKM), TKR, and kernel yield efficiency (YE, kilograms of kernel per cubic metre of canopy). Clonal values provide the best estimate of genotype performance, removing trial and smaller-scale spatial and environmental effects, and incorporating pedigree as a form of replication. These values, as well as phenotypic and standardised phenotypic values of the four traits were used to create an initial shortlist of trees for selection. 207 trees were included in the initial shortlist. Individuals with high breeding values for each trait were also included in the initial shortlist, for the production of the next generation of progeny rather than being considered for placement in RVTs.

5.1.1 Top ranking genotypes

The best performing genotypes phenotypically for each of the traits assessed are summarised in Table 5.1. Across traits, the improvement compared to the best ranking cultivar ranged from 29-43%. This is a large improvement of individual traits. It should be noted, however, that no one individual has been selected that combines superior levels for all traits.

Table 5.1. Phenotypic values of the best performing progeny and cultivars for each of the four traits assessed.

	CTNM (kg/tree)		CTKM (kg/tree)		TKR (%)		YE (kg/m^3)	
	Value	Family/CV	Value	Family/CV	Value	Family/CV	Value	Family/CV
Best progeny (phenotypic)	56.8	NG8 x Yonik	24.5	781 x A4	62	344 x 804	0.87	A16 x 705
Best cultivar (phenotypic)	38.7	A199	16.0	Yonik	44	4/7	0.50	Beaumont
% improvement	31.9		34.6		29.0		42.6	

CTNM = cumulative total nut in shell mass to age eight; CTKM = cumulative total kernel mass to age eight; TKR = total kernel recovery; YE = kernel yield efficiency.

5.1.2 Locations

Productivity varied across the different sites with mean site cumulative NIS yields to age eight ranging from 1.2 kg/tree at Yandina to 19.8 kg/tree at Hinkler (Figure 5.6)

Selections for each trait were relatively evenly distributed between locations, with the possible exception of YE (Table 5.2). Bundaberg consistently produced the most selections across all four traits, and while this location also possessed the largest number of total progeny, it also produced the highest percentage of selections for CTKM and YE. Hinkler Park produced the highest percentage of CTNM selections, and Newrybar produced the highest percentage of TKR selections.

Table 5.2. Total number of progeny assessed, and count of selections and percentage of total progeny for each trait at each of the trial locations.

Location	Total assessed	CTNM		CTKM		TKR		YE	
		Count	%	Count	%	Count	%	Count	%
Alloway	148	1	0.7	3	2.0	1	0.7	9	6.1
Amamoor	262	6	2.3	20	7.6	3	1.1	30	11.5
Baffle Creek	258	4	1.6	9	3.5	2	0.8	21	8.1
Bundaberg	454	15	3.3	39	8.6	8	1.8	55	12.1
Dunoon	230	5	2.2	6	2.6	2	0.9	12	5.2
E. Gympie	141	4	2.8	6	4.3	2	1.4	4	2.8
Hinkler Park	159	6	3.8	6	3.8	7	4.4	8	5.0
Newrybar	88	1	1.1	6	6.8	3	3.4	4	4.5
Yandina	278	8	2.9	11	4.0	2	0.7	10	3.6

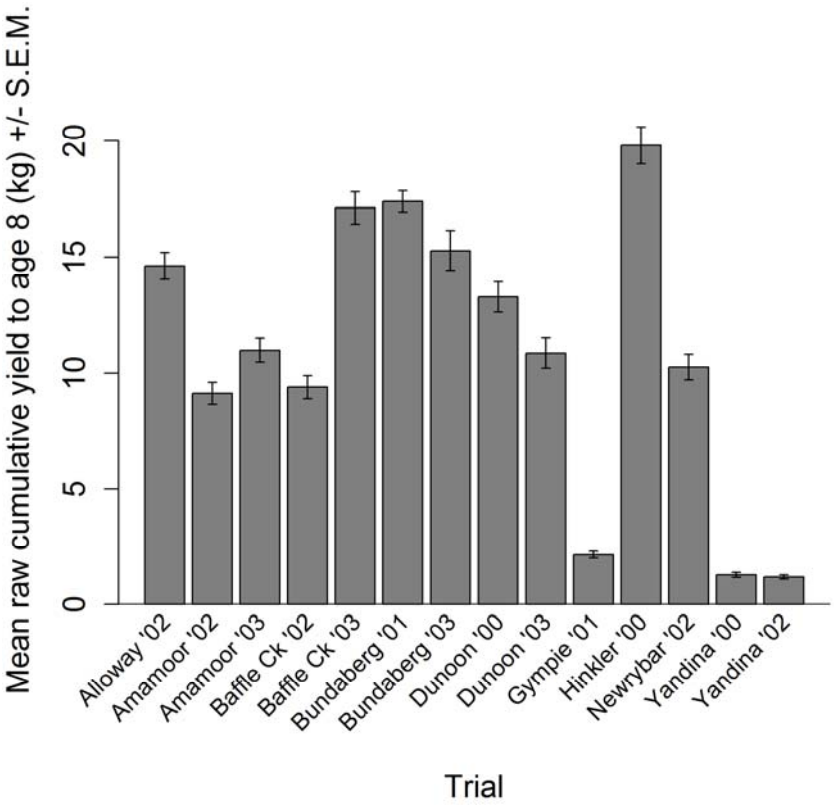

Figure 5.6. Mean cumulative yield to age eight (kg/tree) for the 14 B1.2 progeny trials.

5.1.3 Family representation

Some families were observed to have higher representation in the selection list than others (Table 5.3). Of the 30 total progeny in the family 'Beaumont' x 'NG18' there were ten individuals selected for CTNM, nine for CTKM, and seven for YE. 'HAES 660' x '1/40' performed well for TKR, with five of a total of 20 individuals included in the shortlist. 'NG8' x 'A199' and 'NG8' x '705' are also notable for having over 60% of their progeny in the shortlist for YE (12 out of a total of 18, and six of a total of ten, respectively).

Table 5.3. Number of genotypes selected from each family for the traits cumulative total nut in shell mass to age eight (CTNM), cumulative total kernel mass to age eight (CTKM), total kernel recovery (TKR), and kernel yield efficiency (YE). Total number of progeny per family is also included.

Family	Total assessed	CTNM	CTKM	TKR	YE
Beaumont x NG18	30	10	9		7
NG18 x HAES 660	38	4	6		3
781 x A4	19	3	3		
762 x A199	28	2	3		6
816 x Renown	15	2	2		2
Renown x Beaumont	37	2			3
1/40 x 762	9	1	1		
705 x NG18	18	1	1		
816 x NG7	8	1	3	1	3
A199 x 814	15	1	6	1	3
HAES 246 x A38	38	1	2		1
HAES 333 x A16	31	1	1		
HAES 660 x 1/40	20	1	1	5	1

Family	Total assessed	CTNM	CTKM	TKR	YE
HAES 741 x Renown	18	1	2		
NG18 x 797	23	1	1		
NG4 x Own Venture	31	1	2		2
NG7 x Own Venture	24	1	3	3	6
NG8 x Yonik	19	1	2		4
Own Venture x Renown	24	1			1
Renown x HAES 660	20	1			1
814 x A199	11		4		2
HAES 660 x 762	26		2		2
HAES 772 x Own Venture	21		2		3
NG29 x A199	16		2	1	
NG35 x A199	9		2		1
Own Venture x NG7	10		2	3	2
1/40 x 849	20		1	1	2
797 x A16	33		1		2
842 x 2/12	5		1		
A16 x 705	30		1		1
A199 x NG43	16		1		3
A38 x L64	11		1		
A4 x HAES 791	18		1	1	4
A9/9 x Yonik	14		1		
NG29 x NG43	24		1		
Yonik x 814	17		1		1
L64 x 849	9			2	
NG4 x A4	13			2	1
4/7 x HAES 344	26			1	1
804 x NG18	17			1	
828 x L46	19			1	
A16 x NG4	12			1	
HAES 344 x 804	30			1	
NG8 x A199	18				12
NG8 x 705	10				6
705 x NG4	6				4
L64 x HAES 344	35				3
762 x NG8	33				2
A16 x A38	20				2
NG8 x 797	34				2
NG8 x HAES 344	5				2
Yonik x NG8	9				2
1/40 x Beaumont	5				1
2/12 x 781	31				1
2/48 x HAES 791	11				1
794 x L46	13				1
804 x HAES 772	7				1

Family	Total assessed	CTNM	CTKM	TKR	YE
849 x L64	6				1
A199 x NG35	10				1
A9/9 x 705	27				1
HAES 333 x NG8	30				1
HAES 660 x NG43	16				1
HAES 772 x 849	33				1
HAES 791 x 814	20				1
L46 x 794	5				1
NG35 x HAES 791	26				1
NG7 x 705	3				1

These top 207 selections were additionally evaluated for nut drop pattern, tree shape, foliage density, stick-tight severity, husk spot, susceptibility to canker, wind damage and kernel quality.

5.2 Final selection of the top 23 for industry trial

The list of 207 superior selections that were selected as described in section 5.1 of this report was further reduced to 43 selections and then the 43 selections were evaluated by an industry advisory group to reduce the final list to the top 23.

5.2.1 Methods

On 10 September 2014, invited representatives from growers, nurseries, processors and the AMS met at Nambour to reduce the list of 43 selections to 23. Each participant was provided with three page data sheets for each of the 43 selections and for industry standard cultivars. A sample of the data sheets for one selection is provided in Appendix 5. A summary data sheet was also provided along with a description of the characteristics and both are attached in Appendix 6.

5.2.2 Results

The final 23 selections include representatives from eight of the nine trial locations (Figure 5.7) with East Gympie the only site not represented.

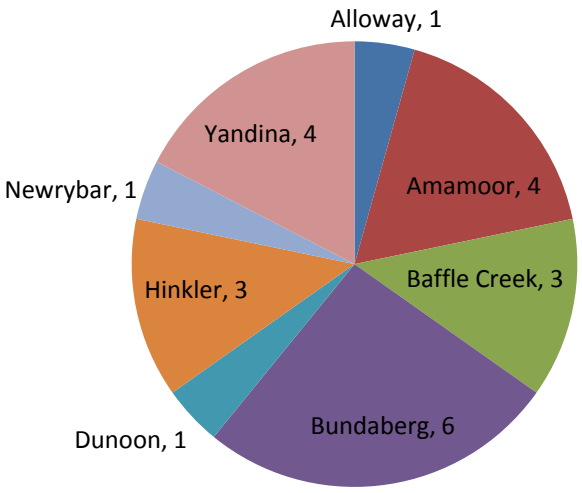

Figure 5.7. Number of selections from each trial location.

The 20 elite B1.2 selections with highest selection index values were compared to the five standard cultivars with highest selection index values. The standard cultivars were 'Yonik', 'M2/12', 'M4/7', 'NG18' and 'A199'. TKR and canopy volume were similar for both groups but NIS yield was 39% greater for the elite selections (Figure 5.8). This is a substantial genetic gain but it will need to be confirmed with replicated testing of the clonally propagated elites.

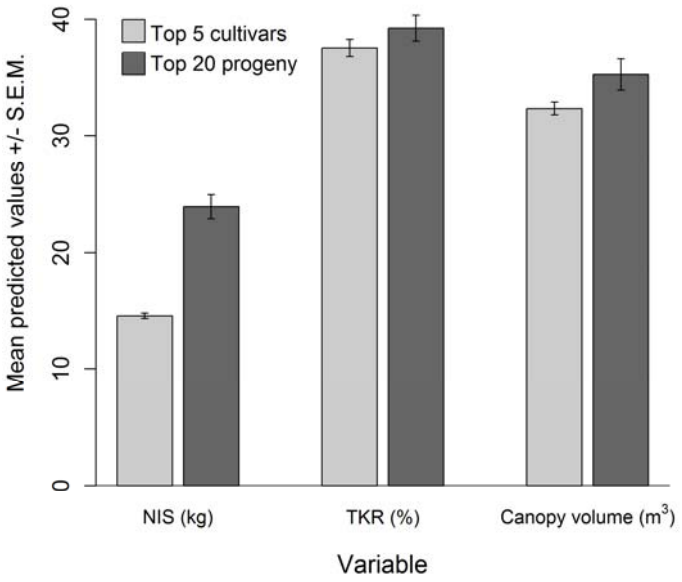

Figure 5.8. Mean predicted values for cumulative nut-in-shell yield to age eight (kg/tree), total kernel recovery (%) and canopy volume (m^3) of the top five cultivars and top 20 progeny from the B1.2 breeding population.

6.0 Rootstock Trials

6.1 Abstract

Twelve cultivars were propagated as open-pollinated seedling and cutting (clonal) rootstocks, and own-rooted cuttings. The same cultivars were also used as scions, grafted to a subset of rootstocks, and planted at four trial locations.

Scion cultivar accounted for more of the variation in all the measured traits (yield, total kernel recovery, tree height, tree canopy width, yield efficiency, leaning and Phytophthora susceptibility) than did rootstock cultivar. Rootstock did contribute significantly to yield, tree height and tree canopy width. There was little evidence for any interaction between rootstock and propagation method (seedling, cuttings, own roots), with the exception of yield and canopy width, where own roots was significantly lower that cuttings or seedlings.

The existing Australian industry standard rootstock, 'H2' seedling, performed well across all traits. 'HAES 695' (also known as 'Beaumont') cutting rootstock produced the highest yields, with 13% higher yield than 'H2' seedling. 'Beaumont' cuttings produced similar sized trees as 'H2' seedling. Twelve percent of 'Beaumont' cuttings at the Newrybar trial were recorded as leaning at some stage during the life of the trial. This was not significantly different to H2 seedling, at 0%.

We suggest further trial of 'Beaumont' rather than full scale adoption, because of limitations in the current trial design and lack of testing in a wide range of soils and environments. The RVT3 project has both 'H2' seedling and 'Beaumont' cutting used on 30 scion varieties at nine locations and will provide a more robust comparison of the two rootstocks. Full recommendations will be possible at the conclusion of RVT3 in 2017.

6.2 Introduction

Clonally propagated plants are typically produced by grafting cuttings from known cultivars (scions) onto either rooted cuttings or germinated, open-pollinated nuts (rootstocks). In macadamias (*Macadamia integrifolia* and *M. tetraphylla*), rootstocks are used to enable selected scions to be vegetatively propagated through grafting or budding, shorten time in the nursery and to reduce the variation that occurs between seedlings (Hardner, 2004).

Open pollinated seedlings of the cultivar 'H2' have been preferentially used as rootstocks in Australia since the early 1990s. It has been reported that this cultivar is favoured because it possesses broad stems and uniform seedlings (O'Hare, Stephenson, et al., 2004, Stephenson, 1990). In South Africa, 'Beaumont' ('HAES 695') cuttings are preferred (Bell, 1996).

In apple, rootstock genotype has been observed to have profound effects on many scion characteristics (Ferree and Carlson, 1987). Different clonal rootstock genotypes are used to alleviate unfavourable soil and climatic conditions, increase resistance to root and scion disease, increase precocity, and reduce tree size (Westwood, 1993).

Little information is available to support rootstock choice in macadamia despite such potential benefits. Newett (1987) investigated the effect on yield of cultivar H2 and *M. tetraphylla* seedling rootstocks for four scion cultivars ('HAES 344', 'HAES 660', 'HAES 741' and 'HAES 800') in a trial west of Rockhampton, Queensland, and found a 24% higher yield for 'H2'. Trochoulias (1992) assessed *M. integrifolia* ('HAES 246') and *M. tetraphylla* ('Gower') seedling and clonal rootstocks for a variety of scion cultivars in a trial near Wollongbar, NSW. Strong winds in 1987 caused significantly more damage to clonal rootstocks than seedling rootstocks. 'Gower' seedling rootstock produced higher yield per unit canopy area than other rootstocks. Rootstock did not affect kernel recovery or grade-one kernel.

The current study was initiated with the Australian macadamia industry to:
- identify elite rootstocks for the industry
- quantify the importance of rootstock effects for production
- quantify the importance of rootstock-scion interactions
- quantify the differences between own rooted, grafted seedling or clonal seedling rootstock

• develop early screening methods for elite rootstock.

Yield, total kernel recovery, tree height and canopy width, yield efficiency, percentage of trees that leaned, and susceptibility to Phytophthora were assessed for a range of rootstock-scion combinations and propagation methods.

6.3 Methods

6.3.1 Experimental design

Twelve cultivars (Table 6.1) were selected to represent a range of genetic material based on DNA profiles (Peace, 2003). These were propagated as clonal and seedling rootstocks, and as own-rooted cuttings (i.e. cuttings growing with their own roots rather than being budded or grafted onto a rootstock), as described by Hardner and McConchie (2006). Seedling rootstocks of three additional cultivars ('A38', 'H2', and 'D4') were also obtained. 'A16' did not propagate well as clonal rootstocks, and was included as seedling rootstocks and own rooted cuttings only. The 12 cultivars were budded onto the different rootstock types (including themselves) in an unbalanced, circular design (Table 6.2).

A tree audit by CSIRO in 2008 identified some trees that did not match their morphological varietal description, suggesting that their cultivar assignment was possibly incorrect. The widespread nature of this error indicates there may have been incorrect identification of cultivars at the outsourced propagation stage. In 2013 we collected leaf material from a sample of these trees for DNA analysis. This analysis confirmed that a number of 'A268' scions had been incorrectly identified, and some '842' scions were actually 'H2' progeny. In light of these issues, a conservative approach was taken with the data, and all trees recorded as having '842' or 'A268' clonal or seedling rootstocks were removed from the analyses. The only exception to this was own-rooted cuttings, where identification could be confirmed by visual examination of tree morphology.

The experimental design (Table 6.2) limited the ability to consider specific rootstock by scion interaction effects. In addition, variability in propagation success and removal of trees from analysis due to identification issues rendered further imbalance to the rootstock by scion structure. The ability to model rootstock by scion interactions was consequently limited.

Table 6.1. Cultivars used as scions and rootstocks.

Code	Cultivar name	Year released	Source	Use in trial	Rootstock type
A16	A16	1988	Hidden Valley Plantation	S, R	Seedling, OR
A38	A38	1994	Hidden Valley Plantation	R	Seedling
A268	A268		Hidden Valley Plantation	S, R	OR
D4	D4; Renown		Norm Greber	R	Seedling
H2	H2; Hinde		Early Australian selection	S, R	Seedling
NG8	NG8; X8		Norm Greber	S, R	Cutting,seedling, OR
246	Keauhou; HAES 246	1948	HAES	S, R	Cutting,seedling, OR
344	Kau; HAES 344	1971	HAES	S, R	Cutting,seedling, OR
695	Beaumont; HAES 695		HAES	S, R	Cutting,seedling, OR
741	Mauka; HAES 741	1977	HAES	S, R	Cutting,seedling, OR
781	HAES 781		HAES	S, R	Cutting,seedling, OR
814	HAES 814		HAES	S, R	Cutting,seedling, OR
816	HAES 816		HAES	S, R	Cutting,seedling, OR
842	HAES 842		HAES	S, R	OR
849	HAES 849		HAES	S, R	Cutting,seedling, OR

HAES = Hawaii Agricultural Experiment Station, S = scion, R = rootstock, OR = own rooted cutting.

Table 6.2. Number of trees for each treatment combination of scions and rootstocks propagated as cuttings, seedlings and own roots.

	Scion													
Rootstock	**A16**	**NG8**	**246**	**816**	**842**	**849**	**781**	**H2**	**741**	**A268**	**695**	**814**	**344**	**Total**
							Own roots							
A16	14													14
NG8		17												17
246			10											10
816				5										5
842					6									6
849						8								8
781							5							5
741									15					15
A268										10				10
695											13			13
814												16		16
344													14	14
Own roots Total	14	17	10	5	6	8	5	0	15	10	13	16	14	133
							Cutting							
A16		1	1											2
NG8		1	1	1		1								4
246			10	4	2	4	1							21
816				4	2	2	2	1	5					16
849						2			4	1	3			10
781							2		5	5		7		19
741									1			1	2	4
695	6	13									13	13	13	58
814		4	4									6	2	16
344	2	1	1	4									1	9
Cutting Total	8	20	17	13	4	9	5	1	15	6	16	27	18	159
							Seedling							
A16	3	4	7	2	1			1						18
NG8		10	12	7		8								37
246			4	1	1	3	6							15
816				3	1	6	5	1	4					20
849						4	3		4	9	1			21
781							5	1	7	4	2	8		27
741									5	7	4	11	4	31
695	5	12									12	11	6	46
814		2	2									2	5	11
344	2	1	2	3									3	11
A38	2	8	4	6										20
D4						2	5		4					11
H2										8	6	3		17
Seedling Total	12	37	31	22	3	23	24	3	24	28	25	35	18	285
Total	**34**	**74**	**58**	**40**	**13**	**40**	**34**	**4**	**54**	**44**	**54**	**78**	**50**	**577**

6.3.2 Trial design

Field trials were established at five locations (Baffle Creek and Bundaberg in Queensland, and Newrybar, Wollongbar, and Maclean in New South Wales, Figure 6.1) in late 2002 and early 2003. There were variable numbers of plants for the different rootstock by scion combinations due to variable strike rate, sowing success, budding success, and tree identification issues.

The trials at Baffle Creek, Bundaberg and Newrybar were planted as two replicate incomplete block designs, with a block size of 4 trees, for the different rootstock by scion combinations. At Wollongbar, a single replicate of 128 plants was established, but sufficient duplicate trees (19) were substituted which allow for estimation of between plot variance. The Maclean trial was similarly planted as a single replicate incomplete block design. It had to be removed in 2005 at three years of age due to a change in property ownership. This trial has subsequently been omitted from analysis and further inclusion in this report. The Newrybar trial was removed in 2012 due to highway construction, therefore age 11 data is missing for this trial. The loss of these trials, in addition to the loss of individual assessment trees due to death and identification issues, resulted in a very large decrease in the number of available data trees by the end of the study (Table 6.3).

The trials were planted with spacings of 8m between the rows and 4m between trees along the row. Trial dimensions for all four sites are shown in Table 6.4. Irrigation was applied at the Baffle Creek and Bundaberg trials, while Newrybar and Wollongbar were rain fed only. Chemical use at the Wollongbar trial was limited to promote inter-row sward as a repository for beneficial insects. The remaining three trials were managed according to standard industry practise.

Table 6.3. Attrition of assessment trees in the trials over time due to trial removal, tree identification issues, and tree sickness and death.

	Original design	**After tree ID check**							
Trial	**Age 1**	**Age 1**	**Age 2**	**Age 3**	**Age 5**	**Age 7**	**Age 8**	**Age 10**	**Age 11**
Baffle Creek	256	177	165	154	150	146	144	141	138
Newrybar	256	188	182	180	176	132	133	133	0**
Bundaberg	256	124*	162	160	160	157	154	152	147
Wollongbar	128	88	88	88	88	80	80	79	77
Maclean	128	NA	NA	NA	0**	0	0	0	0
Total	1024	577	597	582	574	515	511	505	362

* 83 trees were not planted until eight months after initial trial establishment. ** Trial removed.

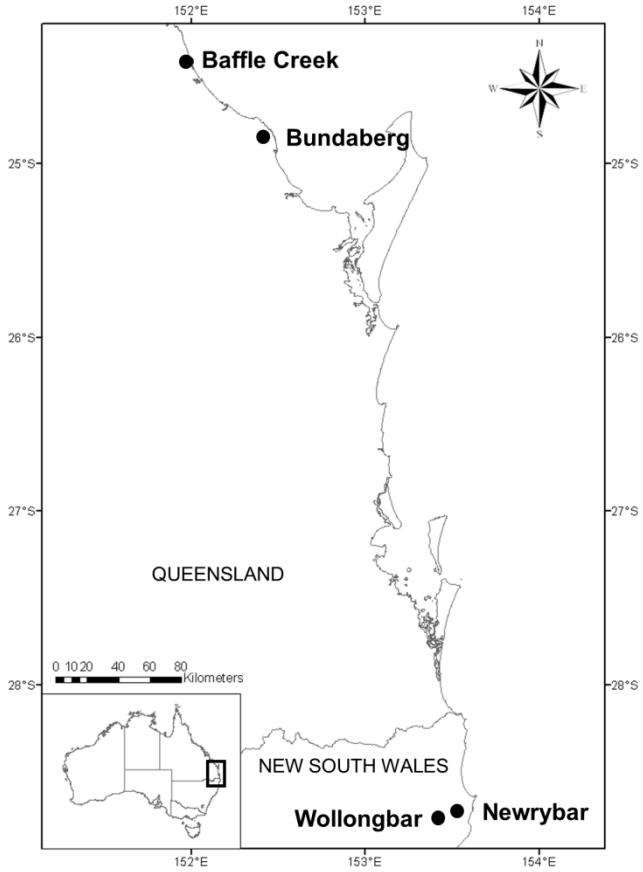

Figure 6.1. Map of analysed trial locations.

Table 6.4. Structure and dimensions of the analysed trials.

Trial	Replicates	Rootstock x scion combinations	Rows	Tree spacings
Baffle Creek	2	55	8	39
Bundaberg	2	52	10	28
Newrybar	2	57	6	44
Wollongbar	1	47	6	24

6.3.3 Assessment methods

A total of 577 trees were assessed. Yield of nut in shell (NIS) was assessed at ages 5, 7, 8, 10, and 11 at all trials. While some trees may have produced prior to age 4, this amount was considered inconsequential for assessment. Nuts were harvested up to three times in a season, and were de-husked within 48 hours of harvest and dried to approximately 1.5 % kernel moisture content using standard commercial drying methods (see Chapter 5.0 for detailed protocol). The dried nuts were weighed, and total NIS yield for each harvest season calculated.

Total kernel recovery (TKR) was assessed for all trees in 2010 (age 8). A sample of 50 nuts per tree was dried to approximately 1.5% kernel moisture content and cracked. Shell and kernels were weighed, and percentage kernel calculated.

Tree height and canopy width across the row were measured most years from 2003 (age 1 from planting) to 2013 using measuring poles and tape measures. Canopy volume was calculated as the volume of a spheroid using height and canopy width measures.

Yield efficiency was derived in two ways:

(1) cumulative NIS yield 2009-2013 / 2009 canopy volume; and
(2) cumulative NIS yield to 2009-2013 / 2013 canopy volume.

The first method allowed the analysis to incorporate all four trials, as Newrybar tree size data was not available after 2009. The second method only comprises the remaining three trials but has the advantage of including the latest canopy volume data.

The propensity for trees to lean or fall over was assessed annually over the lifetimes of the trials. Trees that had ever been recorded as leaning and trees that never leaned were binomially categorised for analysis.

The trees were evaluated in 2012 using a 0 (none) to 4 (severe) scale for both trunk canker and foliage symptoms associated with Phytophthora (*Phytophthora cinnamomi*). The trunk canker severity scale was:
0, no symptoms;
1, very mild; 1 canker, < 10cm long
2, mild, 2-3 cankers, 1-10 cm long;
3, severe, 2-3 cankers, > 10cm long
4, very severe, >3 cankers, >10 cm long

The foliage symptom severity scale was:
0, no symptoms;
1, very mild; mild yellowing, no defoliation or dieback
2, mild, moderate yellowing, < 10% defoliation
3, severe, 10-50% defoliation, mild dieback
4, very severe, > 50% defoliation, severe dieback

6.3.4 Statistical methods

The aim of the study was to compare the ranking among rootstocks across four locations, while allowing for the incomplete allocation of scions to rootstocks. In addition, the relative performance among scions was of interest, and also the effect of the type of rootstock as a cutting, seedling or own root tree.

Yield data across trials and years was analysed in a linear mixed model framework with terms for the first pass of a simple variance component analysis given in structural form as

*Rootstock*Scion**__*Type*Trial*Age*__ *+ Replicate*

where the main effects and interactions for __*Type, Trial, Age*__ were fitted as fixed terms and *Rootstock, Scion* were fitted as random terms, together with their interactions with the fixed terms. *Replicate* was fitted as a random term. Yield data was transformed using a square-root transformation to meet the assumption of homogeneity of variance at each age of recording. All other traits were analysed in standard measured units.

The simple variance component model was extended to include more complex variance models for treatment terms involving rootstock and scion. The terms chosen for variance modelling were determined by the magnitude of the variance parameters in the variance component model, and will be reported separately for each trait. For yield, a factor analytic model (Smith 2001) was included for the scion by age interaction. For the growth measurements of height and canopy width, the response over time was fitted as a random coefficient regression. The factor for age was replaced by a linear term for tree age as a fixed effect and a random term for the deviation from this regression.

A random regression for scion by tree age was also included in the random model terms, following the method of Verbyla (1999). In addition, a spline term for age, both overall and specifically for each scion, was included to allow for a response which was not linear (Verbyla, 1999). Yield efficiency data was analysed using the same terms in the linear mixed model as for yield.

At the residual level, a separable autoregressive process was considered across both dimensions of row and tree spacing in the two-dimensional spatial layout of trees in each trial. Furthermore, the repeated measurements made

over years were modelled using a fully unstructured matrix allowing for heterogeneous residual variances and covariances between years.

A logistic regression was fitted to the binomial leaning data at the Newrybar trial, comparing the main effects of Type, Rootstock and Scion, and the interaction of Type and Rootstock. No other trial produced a large enough number of leaning trees to allow analysis. The Newrybar data was very sparse and so the results of the analysis should be treated with caution.

A logistic regression was fitted to Phytophthora incidence data from both the canopy and the trunk symptoms. The regression was fitted at each site and included terms for Type, Rootstock and Scion, as well as the interaction of Type × Rootstock and Type × Scion. Higher order interaction terms were not considered due to the low levels of disease incidence at most sites, and the low number of trees recorded for any rootstock by scion combination. Significance levels were determined from a chi-square test in the overall analysis of deviance. Comparisons between specific levels of each factor were based on repeated pairwise combinations of levels using the chi-square probability from analysis of deviance.

Phytophthora severity data for both the trunk and canopy symptoms were analysed using a linear mixed model with fixed terms for only the main effects of Type, Rootstock and Scion. Again, higher order interaction terms were not considered due to few datum trees which displayed disease symptoms. Pairwise testing for mean severity rating for each level of main effect factor was conducted using a protected Least significant difference (LSD) test. A lower bound of zero was used for the predicted means.

The linear mixed model for yield, height canopy width, yield efficiency and Phytophthora severity was fitted in ASReml-R (Butler *et al.* 2009). Variance parameters were estimated using Residual maximum likelihood (REML) (Patterson and Thompson, 1971). Best linear unbiased estimates (BLUEs) of the fixed effects of Trial, Type, Age (and their interactions) were given. Best linear unbiased predictions (BLUPs) of the random effects of rootstock and scion are presented, together with their interactions with other fixed and random effects. BLUPs provide the best estimate for varietal selection, and are most relevant for varietal comparison in this study with highly unbalanced rootstock by scion combinations (Robinson, 1991). Individual rootstocks were compared against the H2 standard using a two-tailed test based on the standard normal (z) distribution. The logistic regression analyses were conducted on binomial data using the generalized linear model procedures in Genstat 16.0 (VSN International, 2011).

6.4 Results

6.4.1 Relative importance of rootstock versus scion

How important is rootstock compared to scion in determining yield and tree growth? We can answer this question by comparing the amount of variation that both rootstock and scion explain in the statistical model used in our analysis. The answer depends on the particular trait we are examining. For kernel recovery, the scion is hugely important and rootstock has virtually no effect, whereas for other traits both rootstock and scion have an influence. This section of the chapter explains the influence of rootstock and scion cultivars on the measured traits. The partitioning of variance between the model components was calculated for all traits with the exception of leaning and Phytophthora scores, which were fitted as fixed effects. The results are described below.

6.4.1.1 Yield

Rootstock cultivar and scion cultivar accounted for 19% and 76% of observed variation in yield respectively, averaged across all trials and ages. Only 5% of the variation could be attributed to the interactions between rootstock and scion cultivars. Of the variation due to rootstock, rootstock by type interactions (cutting, seedling, own roots) accounted for 6% of the yield variation. The interactions of scion by age and scion by trial by age were large, accounting for 31% and 28% of the variation.

It was observed that the majority of the age by scion effect could be attributed to 2007 only, when trees were only five years of age. At this age, yield of the scion cultivars 'A268' and '695' were substantially higher than the other cultivars. Given that yield at this age is generally low, treatment variances were also estimated for yield data excluding age 5 (Table 6.5).

When age 5 data is excluded, the percentage variation accounted for by rootstock cultivar increased substantially to 41%, and scion cultivar decreased to 55%. The rootstock by scion interaction remained low at 4%.

Table 6.5. Percentage of the total variation attributed to each treatment for the various measured traits.

Treatment	Yield	Total kernel recovery	Height	Canopy width	Yield efficiency 2009	Yield efficiency 2013
Rootstock	34	0	9	19	0	8
Rootstock x Trial	0	0	4	7	0	0
Rootstock x Age	0		1	0		
Rootstock x Age x Trial	0		0	0		
Rootstock x Type	6	0	0	9	0	0
Rootstock x Age x Type	0		0	1		
Rootstock x Trial x Type	0	1	0	0	0	0
Rootstock x Age x Trial x Type	0		0	0		
Scion	0	67	41	17	79	92
Scion x Trial	12	17	0	0	21	0
Scion x Age	14		16	20		
Scion x Age x Trial	25		2	10		
Scion x Type	0	0	8	0	0	0
Scion x Age x Type	4		2	1		
Scion x Trial x Type	0	0	2	0	0	0
Scion x Age x Trial x Type	0		0	0		
Rootstock x Scion	0	15	4	6	0	0
Rootstock x Scion x Trial	0	0	9	0	0	0
Rootstock x Scion x Age	3		1	8		
Rootstock x Scion x Age x Trial	1		0	1		
Rootstock total	41	1	14	36	0	8
Scion total	55	84	71	48	100	92
Rootstock x Scion total	4	15	14	16	0	0

Yield variance components are for the analysis excluding age 5. Canopy width components are for the analysis excluding ages one to three. Higher order interactions with rootstock by scion were not significant and have been omitted from the table. Variance components are not available for leaning or Phytophthora scores as these were fitted as fixed effects.

6.4.1.2 Total kernel recovery

Rootstock cultivar accounted for only 1% of the variation in total kernel recovery, whereas the effect of scion cultivar was large, accounting for 84% overall (Table 6.5). The rootstock by scion interaction accounted for 15% of the variation. Of the scion variance, scion by trial accounted for 17%.

6.4.1.3 Height

Overall, rootstock cultivar accounted for 14% of the observed variance in tree height, and scion for 71% (Table 6.5). The interaction between rootstock and scion accounted for 14% of the variance and scion by age for 16%.

6.4.1.4 Canopy Width

With all tree ages included, rootstock accounted for only 1% of the variance in canopy width, with scion accounting for 96%. Scion by itself and the interaction between scion and tree age together accounted for the majority of variance in canopy width (36% and 57% respectively). The large variance component observed for scion by age was largely due to the rapid early growth in canopy width of two scion cultivars, '695' and 'A268' (Figure 6.2).

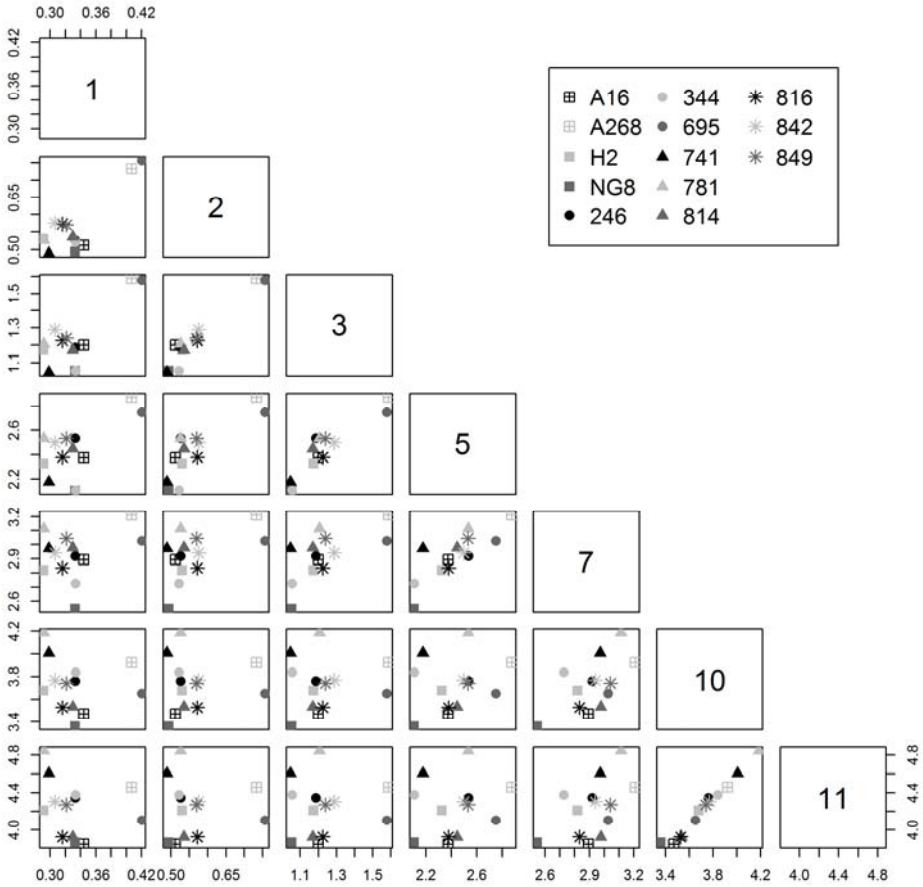

Figure 6.2. Canopy width values (m) for scion by tree age. The numbers in the diagonal boxes show the age of the trees. The boxes on the off-diagonal show the pairwise plots of tree canopy width between each age of measurement. Specifically, the graph shows that 'A268' and '695' have greatest canopy width for young trees at ages 1 and 2, and these scions dominate canopy width for young trees. This changes over time to show that '781' and '741' have the greatest canopy width at ages 10 and 11, and rootstocks 'NG8', 'A16', '816' and '814' have the least canopy width at ages 10 and 11.

Removing ages one to three from the analysis revealed a significantly higher rootstock contribution to canopy width at 36%, with 48% of variance due to scion (Table 6.5). Rootstock by type accounted for 9% of the variation, and rootstock by trial 7%. Six percent of the variation could be attributed to the interaction between rootstock and scion.

6.4.1.5 Yield Efficiency

For yield efficiency calculated using 2009 canopy volumes, scion accounted for 79% of the variation, and the interaction between scion and trial for 21% of the yield efficiency variation (Table 6.5). Rootstock did not contribute, accounting for 0% of the variation. With the 2013 canopy volumes, scion accounted for 92% of the variation and rootstock 8%.

6.4.2 Rootstock effects

6.4.2.1 Yield

BLUP values of the rootstock cultivar effect on yield across all sites and ages are shown in Figure 6.3. Cultivar '695' cutting ranked the highest for cumulative yield, producing 57.3 kg/tree to age 11. This was 13.4% higher than the Australian industry standard, 'H2' seedling, which produced 49.6 kg/tree (Figure 6.3). This was the only cultivar and type combination to yield significantly higher than 'H2' seedling. On an annual yield basis (as opposed to cumulative), '695' cutting yielded an average of 12.8% higher across ages 7 to 11 compared with 'H2' seedling.

Own roots consistently produced lower yields than cutting or seedling rootstocks (Figure 6.3). Rootstock-scion combinations with the same genotype for both were genetically identical to the own rooted plant of that genotype. For example '695' scion budded onto '695' cutting rootstock were genetically identical to the '695' own root treatment. This provided a unique point of comparison; the own roots treatment yielded 23.3% less than cuttings.

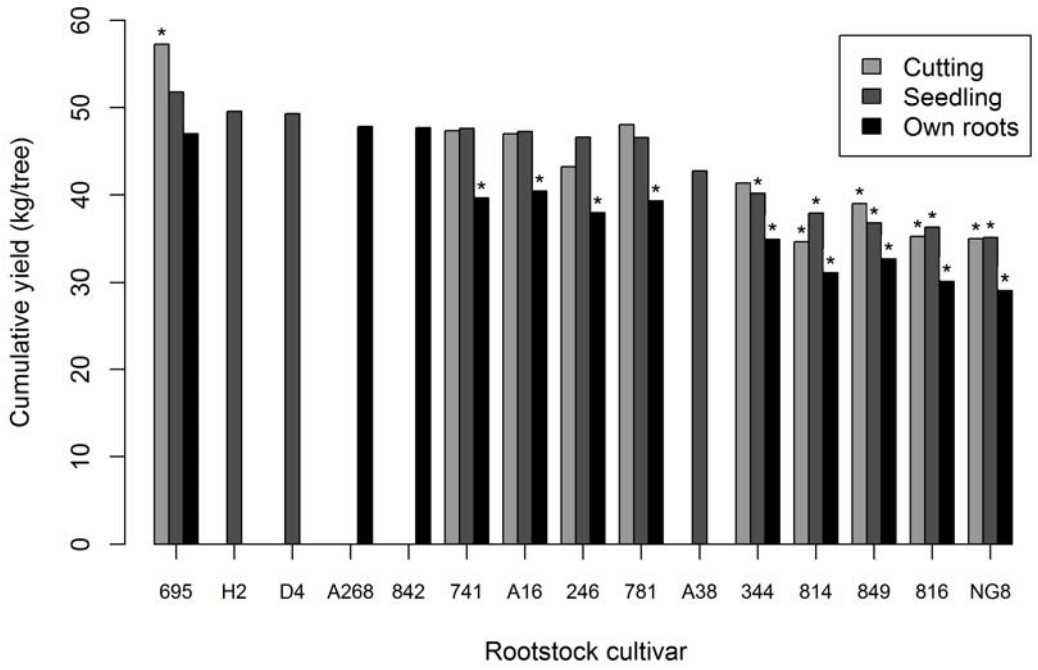

Figure 6.3. Cumulative nut in shell yield (kg/tree) for rootstock cultivars and rootstock type. * = significantly different to 'H2' seedling at $P \leq 0.05$.

6.4.2.2 Total kernel recovery

The majority of scions performed similarly for kernel recovery across all rootstocks. Scion cultivars 'NG8' and '849' varied the most across rootstock cultivars, with a range of 37 to 41% TKR for 'NG8', and 33 to 39% for '849'.

6.4.2.3 Height

Across all trials, '849' rootstock consistently produced the shortest trees (Figure 6.4), with mean heights at age 11 of 5.9m, 3.5m and 5.3m at the Baffle Creek, Bundaberg and Wollongbar trials respectively. 'H2' had mean heights at age 11 of 6.2m, 3.6m and 5.5m at the three trials. Four rootstock cultivar by type combinations were also identified as significantly shorter than 'H2' seedling at age 11 while not significantly different in yield, but the height difference was a maximum of only 0.4m (Table 6.6). Cultivar '695' rootstock produced the tallest trees, with average heights at age 11 of 6.1m, 3.8m and 5.4m at the Baffle Creek, Bundaberg and Wollongbar trials respectively. This was not significantly taller than 'H2' seedling rootstock trees.

Table 6.6. Predicted values and probability of significant differences (2-tailed test) from the industry rootstock standard 'H2' seedling, for cumulative yield, height at age 11 and canopy width at age 11.

Rootstock cultivar [a]	Type	Cumulative yield (kg/tree)	Height at age 11 (m)			Canopy width at age 11 (m)		
			Baff	Bund	Woll	Baff	Bund	Woll
H2	Seedling	49.6	6.2	3.6	5.5	5.0	4.6	5.1
695	Cutting	57.3 *	6.1	3.8	5.4	4.9	4.8	4.8
268	OR	47.8	5.9	3.6 *	5.5	4.6	4.6	4.9
842	OR	47.7	5.8 *	3.5 *	5.4	4.6	4.6	4.8
695	OR	46.9	5.9	3.6 *	5.5	4.7	4.6	4.9
344	Cutting	41.4	6	3.7	5.2 *	4.7	4.7	4.5 *

[a] Only significantly smaller rootstocks that also have significantly higher or not significantly different yield to 'H2' seedling are shown. * Differences from 'H2' seedling at P≤0.05. OR = own roots. Total kernel recovery is not included as there was no rootstock effect for this trait.

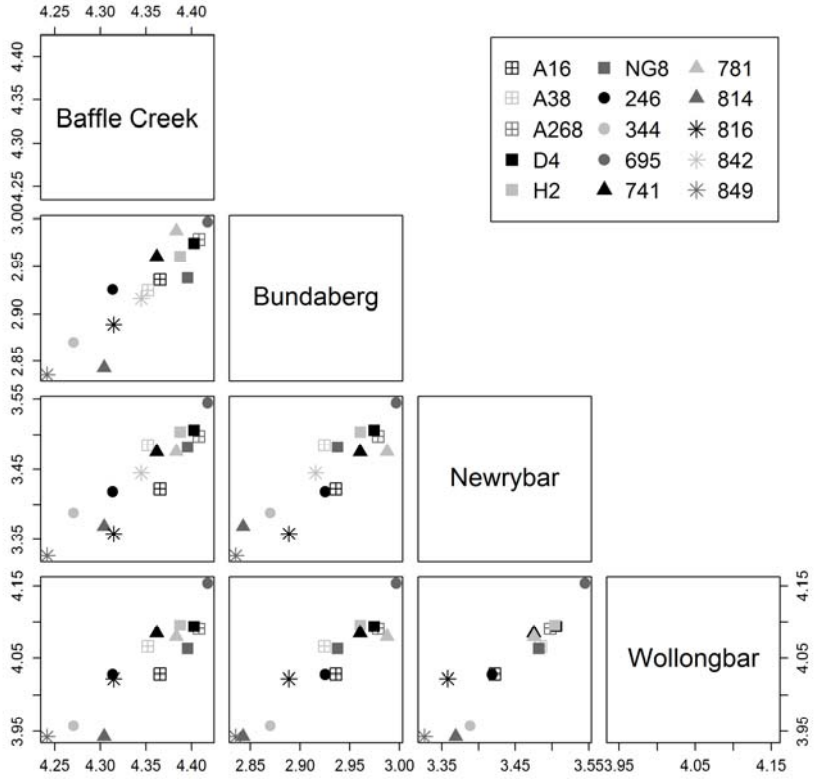

Figure 6.4. Tree height values (m) at age 7 for rootstock by trial combinations. The diagonal boxes show the trial under study and the boxes containing plots show the pairwise graphs for comparing height of trees between each pair of trial locations. The closeness of points to the diagonal line from bottom left to top right in each box shows the consistency of tree height for each rootstock in each pair of trials. Rootstock cultivar '849' consistently produces the shortest trees in all trials, and '814' and '344' are next shortest in most trials.

6.4.2.4 Canopy Width

Rootstock cultivar '814' consistently produced the smallest canopy widths across all rootstock types (Figure 6.5). Cultivar '344' own roots was also identified as significantly smaller in canopy width (Wollongbar trial only) than 'H2' seedling while not being significantly different for yield (Table 6.6). The own roots treatment consistently produced smaller widths than budded trees where the scion and rootstock (cutting) cultivars were identical (7.3% smaller on average, Figure 6.6).

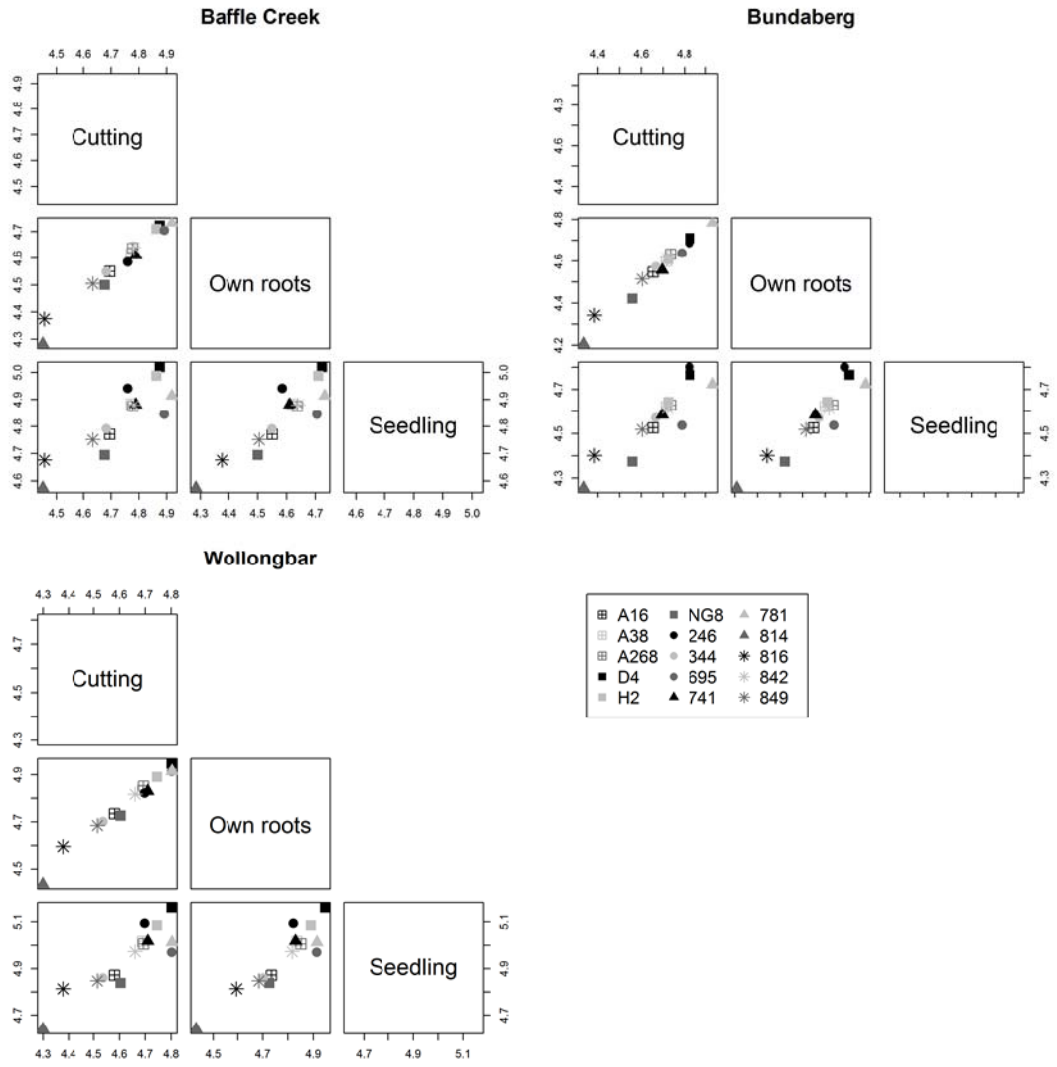

Figure 6.5. Canopy width values (m) for rootstock by trial by type at age 11.The diagonal boxes list the three types of propagation as cutting, own roots and seedling. The boxes on the off-diagonal show the pairwise plots of tree canopy width between each propagation type. Specifically, the graphs show that rootstock '814' has consistently smallest canopy type for each propagation method, and this occurs at all three trial locations.

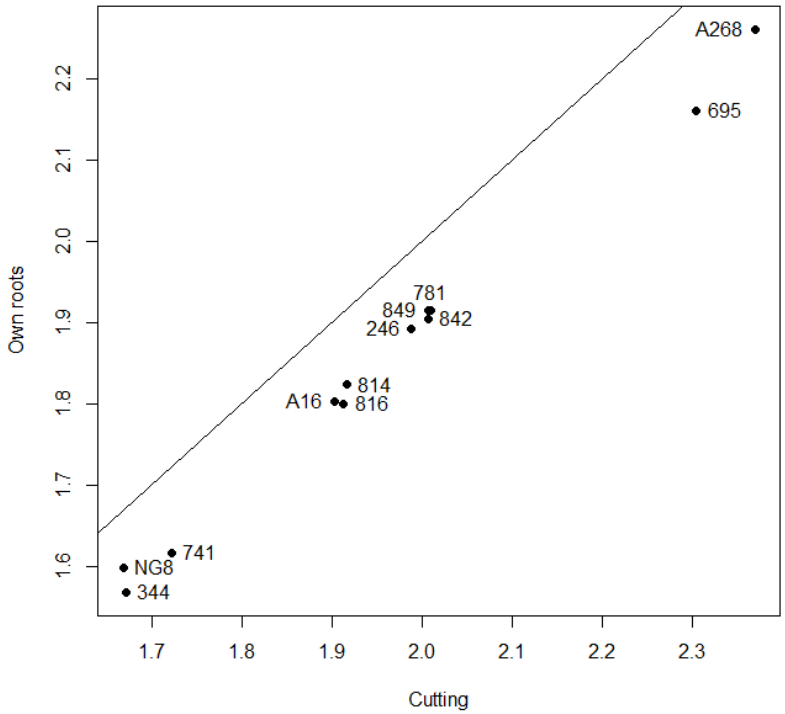

Figure 6.6. Predicted canopy width values (m) for the cutting treatment where scion cultivar is the same as rootstock cultivar, plotted against own roots treatment. Line shows 1:1 position.

6.4.2.5 Yield Efficiency

Minimal variation was observed between rootstock cultivars, with '695' possessing marginally higher yield efficiency than some of the other cultivars at 0.34 kg NIS per cubic metre of tree canopy (Figure 6.7).

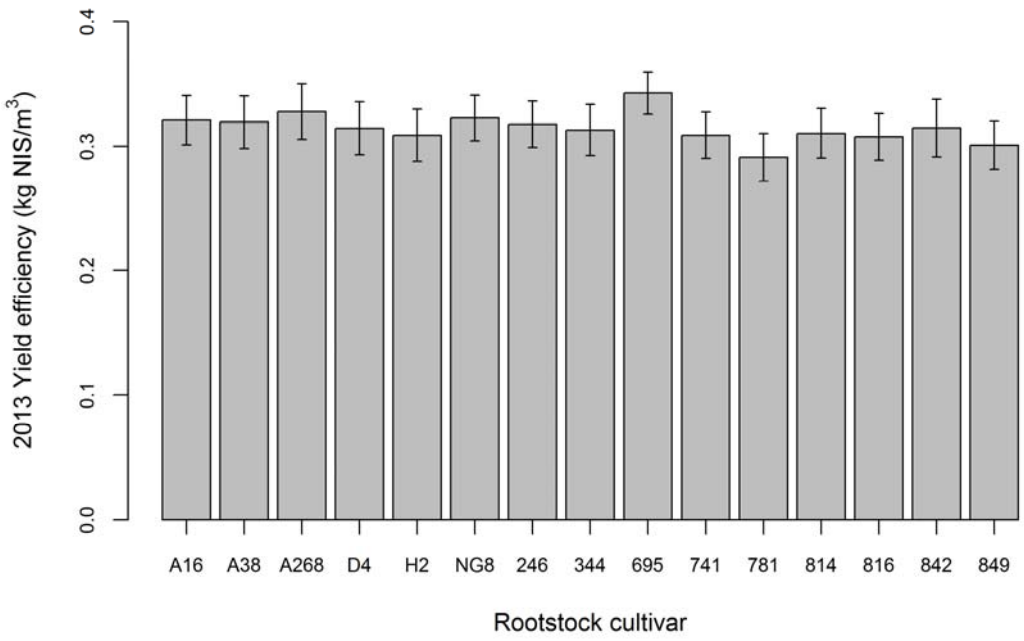

Figure 6.7. Rootstock yield efficiencies for 2013 canopy volumes ± standard error of the mean.

6.4.2.6 Tree Leaning

There was only very limited data for the analysis of leaning among rootstock cultivars and the results should be treated with a high degree of caution. A number of rootstock cultivars ranked highly for percentage leaning, with no consistent trend for rootstock type (Figure 6.8). Cultivar '695' rootstock was observed to have a low percentage of leaning trees across all three rootstock types.

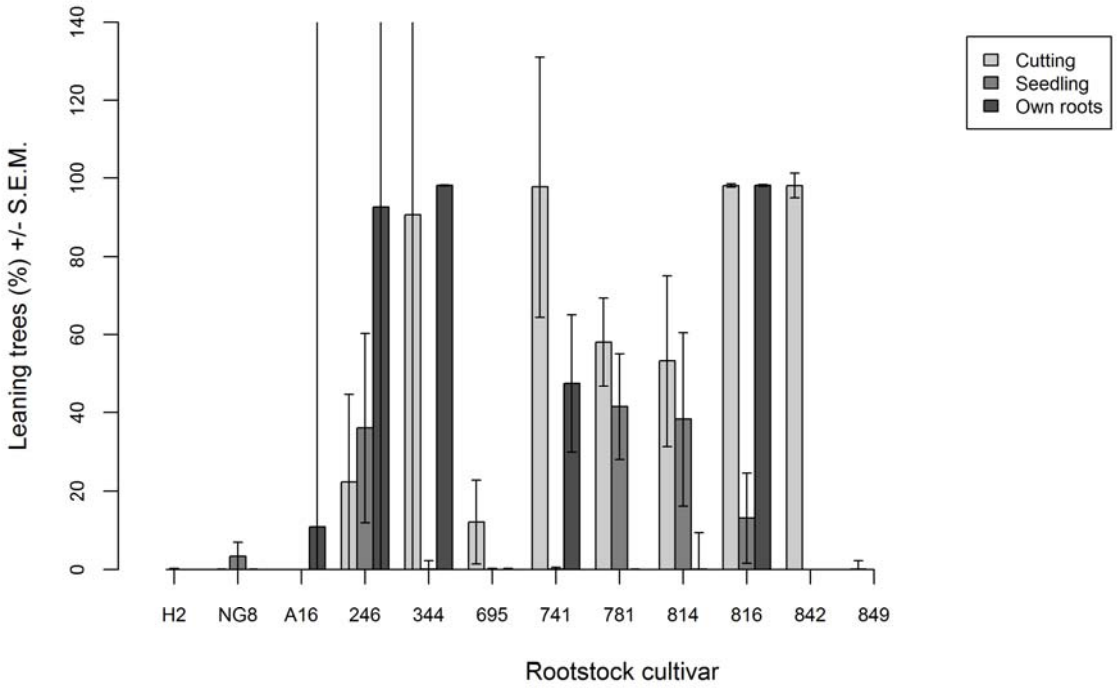

Figure 6.8. Percentage of trees leaning for rootstock cultivars by type at the Newrybar trial.

6.4.2.7 Phytophthora Canopy Score

For the analysis of symptoms of Phytophthora in the tree canopies there were only sufficient trees without the zero rating (i.e. no symptoms) at the Bundaberg trial and this result was non-significant for all effects. Including all zero values, there was a significant type effect at Newrybar, where cuttings showed significantly less canopy symptoms of Phytophthora than seedlings (Figure 6.9). There were no significant rootstock cultivar effects.

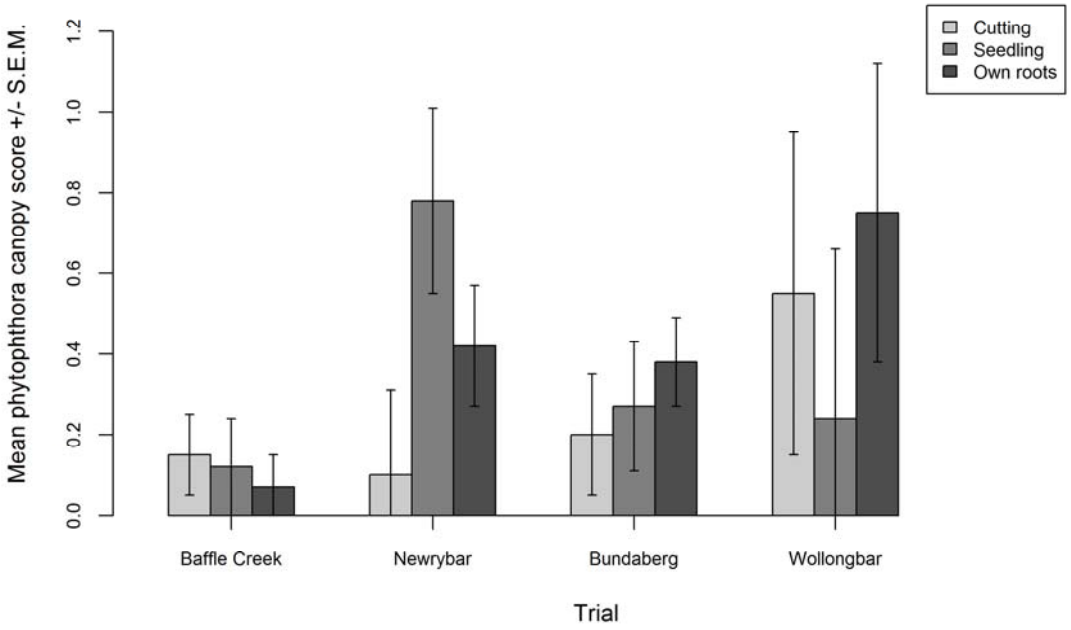

Figure 6.9. Phytophthora canopy scores for rootstock type.

6.4.2.8 Phytophthora Trunk Canker Score

Analysis of trunk canker score was undertaken with and without zero ratings (i.e. no symptoms of trunk canker) included, except for Wollongbar where there were insufficient trees with non-zero ratings for analysis. There were significant rootstock effects at Baffle Creek, Newrybar and Wollongbar. There were also type effects at Newrybar when the data with zero values was excluded.

Rootstock cultivars ranked differently at the different trials for trunk canker score (Figure 6.10). Cultivars 'A38' and '695' possessed consistently low scores across all trials. Cutting rootstocks at Newrybar were observed to possess significantly lower trunk scores than seedlings or own roots when the zero values were excluded (Figure 6.11).

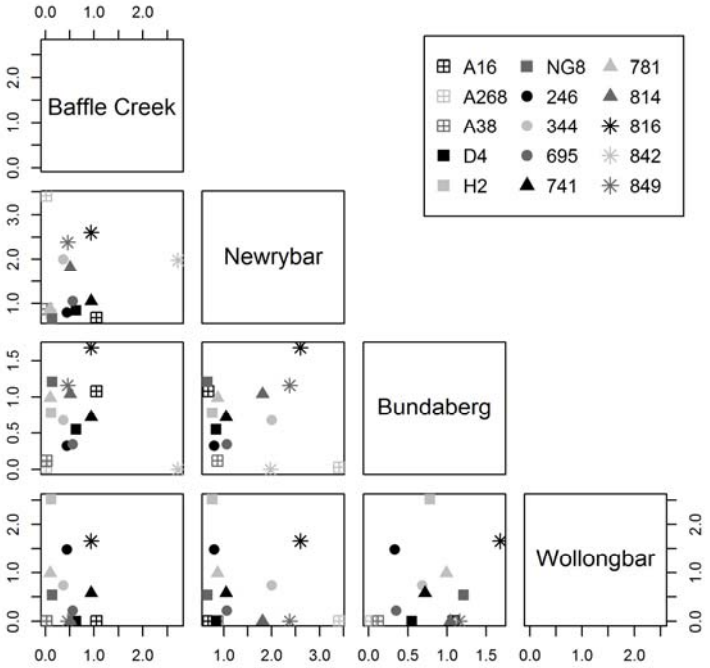

Figure 6.10. Phytophthora trunk canker scores for rootstock cultivars by trial, where zero values have been included in the analysis. The diagonal boxes list the four trial locations, and the boxes on the off-diagonal show the pairwise plots of canker scores for rootstocks between each pair of trial locations. Specifically, the graph highlights the differing canker levels in trees from each rootstock at each trial location. For example, rootstock '842' has highest canker scores at Baffle Creek, moderate scores at Newrybar, low scores at Bundaberg, and was not recorded at Wollongbar.

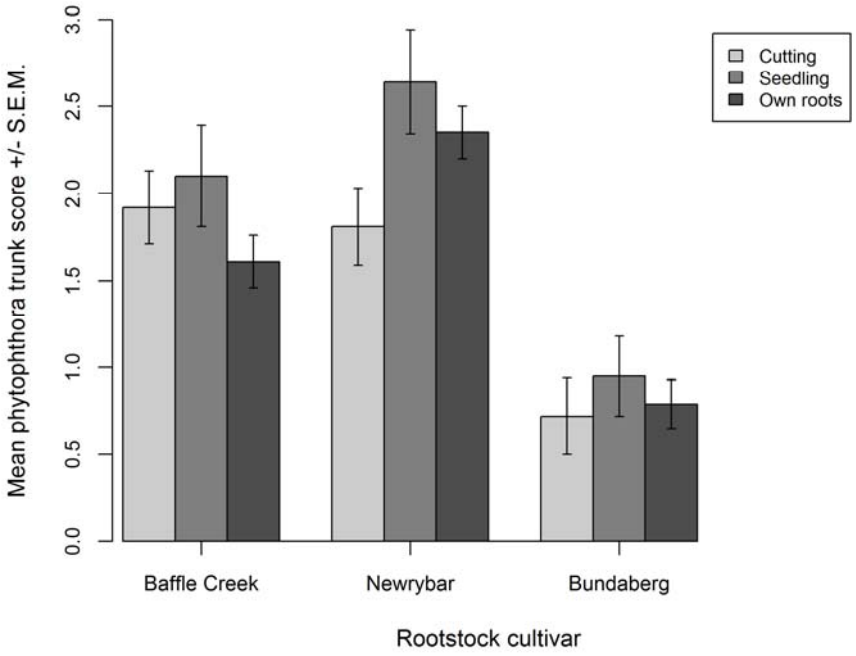

Figure 6.11. Phytophthora trunk canker scores for rootstock type by trial. Zero values were not included in analysis. Wollongbar is not included as insufficient non-zero data was available for analysis.

6.4.3 Scion effects

6.4.3.1 Yield

Scion cultivar performance for yield varied substantially between locations and ages. Cumulative yield at each trial is presented in Figure 6.12. Cultivar 'A268' produced the highest cumulative yields at all trials except Newrybar, where '695' was the top performer.

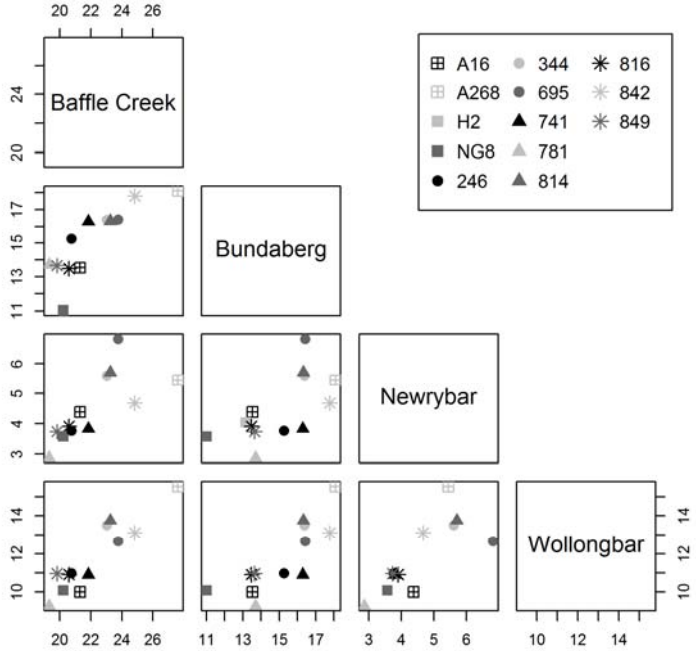

Figure 6.12. Cumulative nut in shell yield (kg/tree) for scion by trial. Each box shows a pairwise plot of scion performance between each pair of trial locations. Consistency of scion rankings can be observed between sites where the points lie close to the diagonal line from bottom left to top right in each plot (e.g. Baffle Creek and Wollongbar). Differential scion performance is most evident at Newrybar with changes in the top four ranked scions.

6.4.3.2 Total kernel recovery

Scion cultivars '816' and 'A16' consistently produced the highest kernel recoveries across all trials (Figure 6.13), with average values of 43% and 42% total kernel recovery respectively. Cultivar '344' possessed the lowest kernel recovery, at 34% averaged across trials.

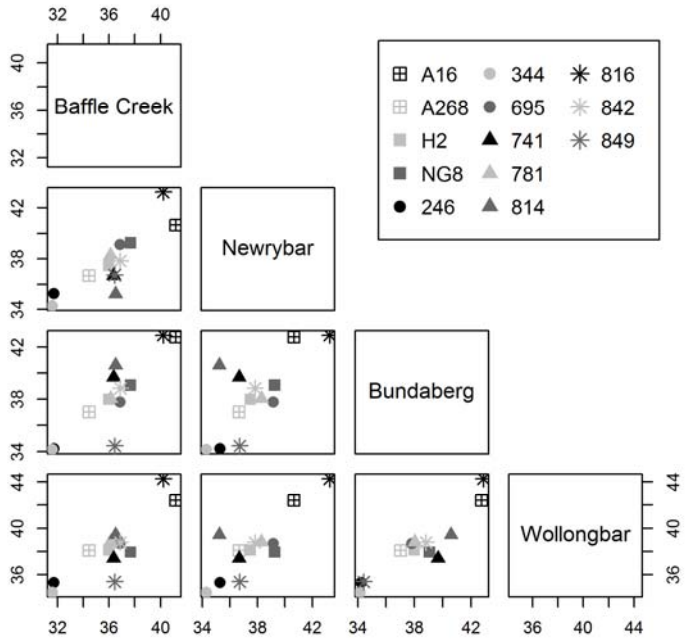

Figure 6.13. Total kernel recovery values (%) for scion by trial combinations. Each box shows a pairwise plot of scion performance between each pair of trial locations. The two top scions for kernel recovery '816' and 'A16' are consistent across all trial locations. Other scions, for example '741' and '814', have higher recovery at Bundaberg, but lower recovery at Newrybar.

6.4.3.3 Height

Rankings of scion height changed substantially with tree age (Figure 6.14). At age 11, scion cultivar 'NG8' produced the shortest tree, with a predicted value of 4.3m. Cultivar '344' produced the tallest trees at age 11, at 5.1m in height. 'Beaumont' ('695') was the tallest scion up to age five at all trials but of only mid-range height from ages seven to 11. In contrast, '344' grew slowly in its early years but was the tallest scion at age 11.

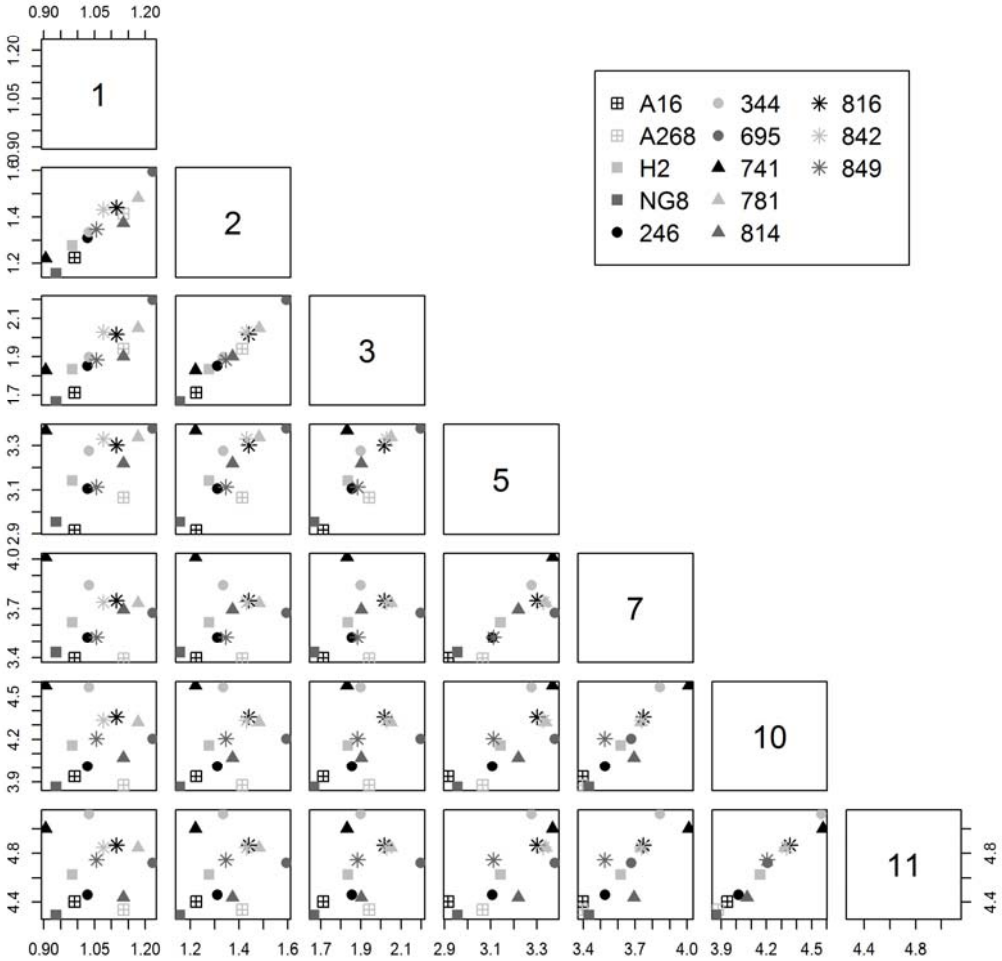

Figure 6.14. Tree height values (m) for scion by tree age. The graph shows the close agreement in scion rankings for tree height in trees at ages 1, 2 and 3 years. It also shows the closeness in scion rankings for tree height in older trees at ages 10 and 11. However, the box on the bottom left shows the lack of agreement in tree height rankings between trees at age 1 and age 11, showing it is difficult to predict mature tree height for scions from early growth patterns.

6.4.3.4 Canopy Width

At age 11 scion cultivars 'NG8' and 'A16' produced the smallest canopy widths across all type treatments, while '781' and '741' were the largest (Figure 6.2).

6.4.3.5 Yield Efficiency

In the 2009 analysis, cultivar '344' scions consistently possessed the highest yield efficiencies (i.e. the highest yield of NIS per cubic metre of canopy) and '781' the lowest (Figure 6.15). For the 2013 analysis a number of scion cultivars possessed high yield efficiencies, the highest being '814' with a value of 0.40 (Figure 6.16). Cultivar '781' scion yield efficiency remained low at 0.22.

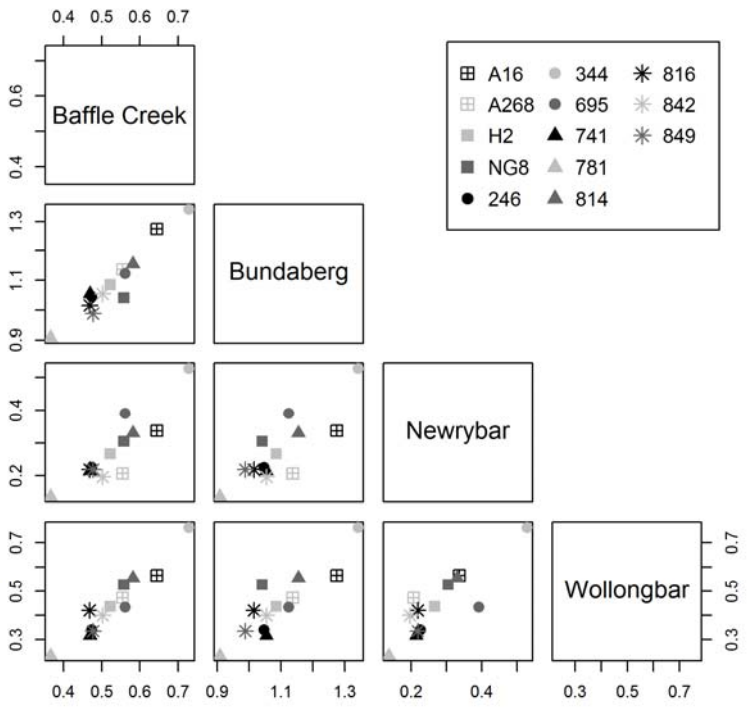

Figure 6.15. Scion yield efficiencies for 2009 (age 7) canopy volumes. The graph shows that scion '344' has the highest yield efficiency consistently across all trial locations. While there is generally close agreement in scion performance between trials, there is some change in rankings between specific pairs of trials. For example Scion '695' has the second highest yield efficiency at Newrybar, is in the top 6 scions at Baffle Creek, Bundaberg and Wollongbar.

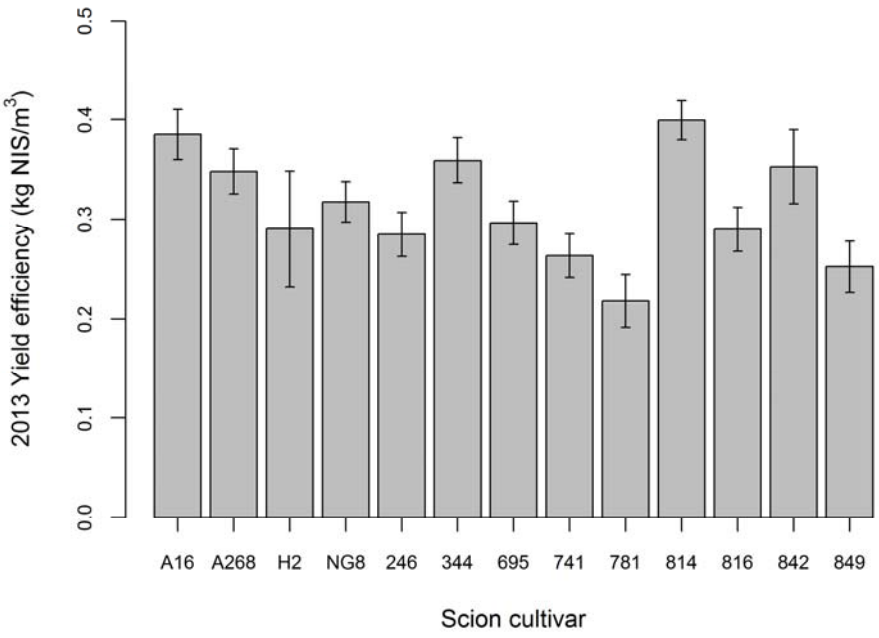

Figure 6.16. Scion yield efficiencies for 2013 (age 11) canopy volumes ± S.E.M.

6.4.3.6 Tree Leaning

Scion cultivars demonstrated a large amount of variation in leaning. The highest percentages of trees leaning were observed for cultivars '781' and '849', with 70% and 55% respectively (Figure 6.17). 'A16' and '842' performed the best for leaning, each with no trees recorded as having leaned during the life of the trials.

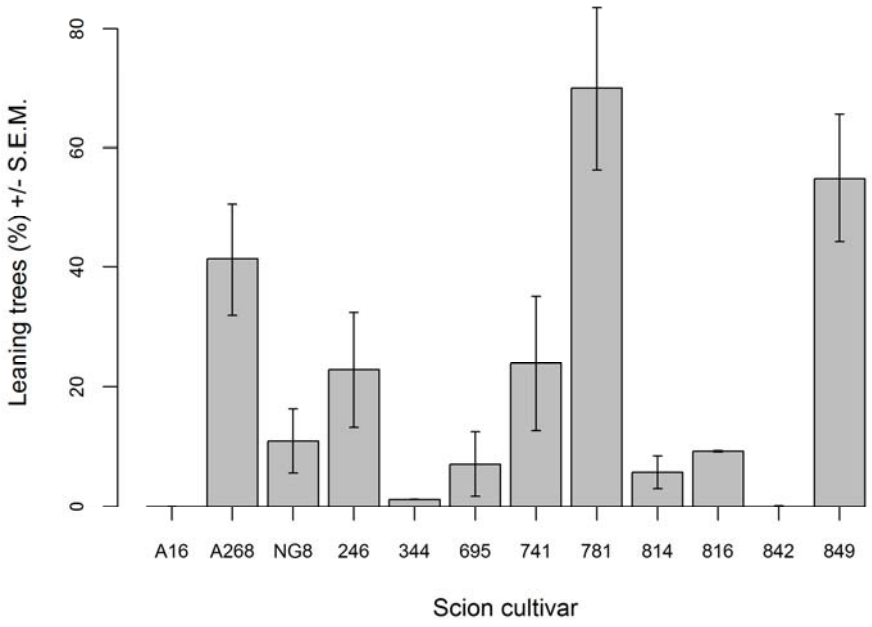

Figure 6.17. Percentage of trees leaning for scion cultivars at the Newrybar trial.

6.4.3.7 Phytophthora Canopy Score

For the analysis of symptoms associated with Phytophthora in the tree canopies there were only sufficient trees without the zero rating (i.e. no symptoms) at the Bundaberg trial and this result was non-significant for all effects. There was a significant scion effect at Bundaberg when all zero values were included. Cultivars 'NG8' and 'H2' possessed the highest canopy severity scores for scion at 1.8 and 1.5 (Figure 6.18). With the exception of 'A16', '814' and '816', the remaining scion cultivars had very low canopy Phytophthora scores.

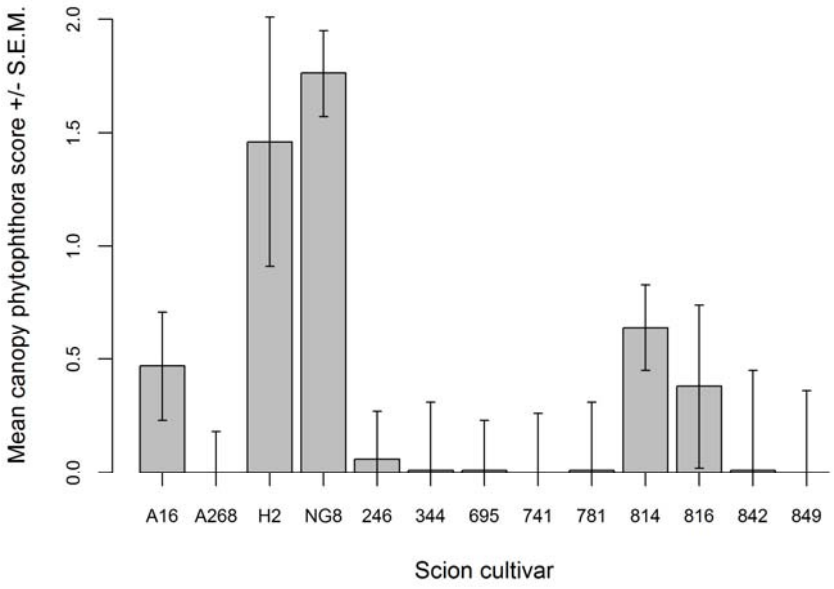

Figure 6.18. Phytophthora canopy scores for scion cultivars at the Bundaberg trial.

6.4.3.8 Phytophthora Trunk Canker Score

There were significant scion effects on Phytophthora trunk canker score at the Wollongbar trial only. At this trial, '781' had the highest trunk canker score of the scion cultivars at 2.3, and 'NG8', '814' and '741' had the lowest score (Figure 6.19).

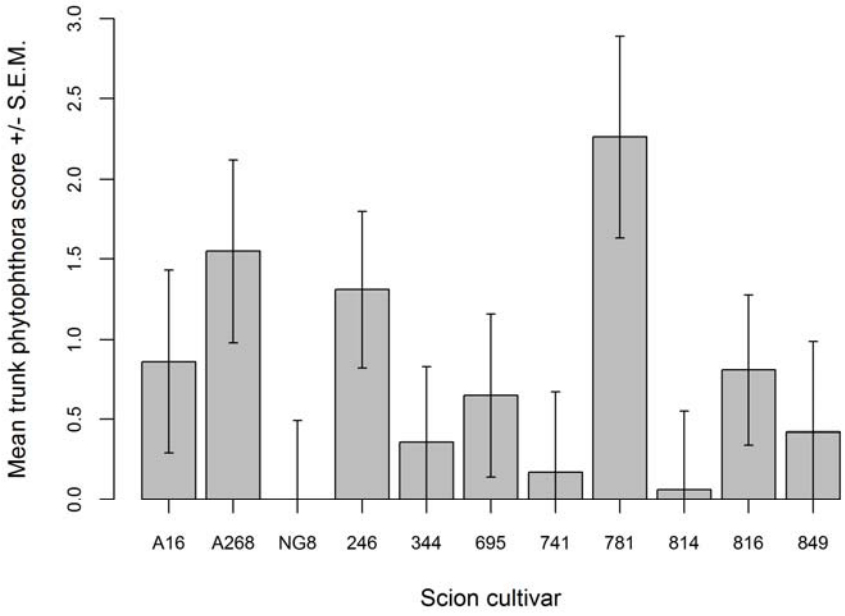

Figure 6.19. Phytophthora trunk canker scores for scion cultivars at the Wollongbar.

6.5 Discussion

6.5.1 The importance of rootstock effects for production, and differences between own rooted, grafted seedling or clonal seedling rootstock

Scion cultivar was the major contributor to observed variances in all traits measured in this study. Rootstock cultivar did affect yield, height, and canopy width but not total kernel recovery or yield efficiency. Rootstock performance for all traits was relatively stable across trials, scions, and tree ages.

Rootstock type did not affect most of the traits measured, and accounted for only a small amount of the variation in yield and canopy width. The major component of this variation was the lower performance of own-rooted cuttings for yield and canopy width compared to grafted cuttings and seedlings. Several processes could be responsible for this, including a difference in the ontogenetic age of material used in the rootstock types, with own roots potentially being less mature, or grafting itself conferring a positive effect on these traits.

Trochoulias (1992) observed that cuttings developed poorer root systems and suffered more from strong winds than seedling rootstocks in a study investigating differences between seedling and cutting rootstocks. This finding was not observed in this study, with no significant difference between the different rootstock types for tree leaning.

Cuttings were observed to have fewer Phytophthora symptoms than seedlings at the Newrybar trial for both canopy and trunk scores, and fewer than own roots for trunk score. No differences between rootstock types were observed at the other trials suggesting that the observed differences at Newrybar may be site-specific.

6.5.2 The importance of rootstock-scion interactions

Interactions between rootstock and scion cultivars were limited for yield and yield efficiency. This suggests that rootstock cultivars can be used on any of the tested scion cultivars with approximately equivalent results for these traits. There was a slightly larger interaction between rootstock and scion for total kernel recovery, height and canopy width. It should be noted that the grafting design employed in this study, along with the loss of trees from identification issues and trial loss, limit the ability to discern rootstock by scion effects with confidence, so these findings should be treated with caution.

6.5.3 Identifying elite rootstocks for industry

It was observed that the current industry rootstock standard, 'H2' seedling, performed well for many of the measured traits, in particular yield and leaning, compared to the other rootstock cultivars and types tested. There were a number of rootstocks that produced shorter trees than 'H2' seedling but most also delivered significantly lower yields. No rootstock was observed to have similar yields to 'H2' seedling while having a substantially smaller canopy. 'H2' seedling rootstock scored poorly for Phytophthora trunk canker scores at the Wollongbar trial, but had only low or average levels of trunk canker at the other two trials.

'Beaumont' ('695') cutting was the best performer overall of all the rootstocks tested. This rootstock produced significantly higher yields than all other rootstocks, and did not differ significantly in height or canopy width from 'H2' seedling. It ranked highest for yield efficiency, and performed well for leaning. It ranked low to average across all trials for the amount of Phytophthora trunk canker. These results suggest that '695' cutting may be a desirable rootstock for Australian conditions.

Cutting (clonal) rootstocks of 'Gower' and '246' were observed to be significantly more susceptible to uprooting by strong wind than their open-pollinated seedling counterparts in a previous study (Trochoulias 1992). This suggested that the root systems of cuttings may not be as well developed as seedlings. This trend was not observed to be the case in this study. Cutting rootstocks had a significantly higher percentage of trees leaning than seedling rootstocks for only two cultivars, '741' and '816'. There were no significant differences between cutting and seedling rootstocks for the remaining cultivars. Caution is recommended in interpreting these results, however, given the sparsity of the leaning data. The low incidence of leaning for '695' cuttings and own roots may be due to the excellent and rapid root development observed in cuttings of this cultivar (Bell, 1996). Cultivar '695' also possessed the highest strike rate of all cuttings propagated in this experiment, at 80% (Hardner and McConchie, 2006). Clonal '695' rootstock is already the industry standard in South Africa (Bell, 1996). Adoption in Australia may provide a small productivity

increase and greater uniformity in performance than the genetically diverse open-pollinated 'H2' seedlings currently in use.

Hardner (2004) suggested that early cutting vigour may have a greater impact on scion performance than other rootstock effects. This trend is supported very generally in this study, with high nursery vigour (Hardner 2004), large early tree size, and high yield performance of '695' and 'A268' cutting rootstocks. Conversely, 'NG8' and '849' cuttings were among the smallest trees in the nursery and also performed poorly for yield. This finding may be useful for early screening of rootstock cultivars in future research projects.

7.0 Oil Development in Kernel of Wild Germplasm

Oil content of macadamia kernel has been shown to be related to eating quality (Ripperton, et al., 1938). Early research demonstrated that kernel with oil content below 72% were more variable on roasting with a hard texture unsuitable for consumption. This work also demonstrated that this oil content coincided with a specific gravity of 1.0. Thus floatation became a commercial practice to identify kernel of lower commercial value, although subsequent work demonstrated that the relationship between oil content and specific gravity was not consistent among commercial germplasm (Hardner et al. 2009).

In commercial cultivars of *Macadamia integrifolia*, oil accumulation typically commences between 90 and 120 days post-anthesis and continues until 190 to 215 days depending on variety and location (Baigent, 1983; Jones, 1937; McConchie, et al., 1996). All studies to date have been conducted on commercial varieties of the one macadamia species: *M. integrifolia*, the most commonly cultivated species. *M. tetraphylla* also produces edible nuts, and the remaining two macadamia species, *M. ternifolia* and *M. jansenii*, while not producing edible nuts, are of interest for inclusion into the macadamia breeding program for their high kernel recovery and excellent kernel appearance.

The objective of this study was to use oil content analysis to evaluate the oil development of nuts and maximum oil content of three wild macadamia species, *M. integrifolia*, *M. tetraphylla* and *M. ternifolia*, as well as *M. integrifolia* × *M. ternifolia* and *M. integrifolia* × M. *jansenii* hybrids, and three industry cultivars. This will allow us to identify differences that may need to be accounted for in future germplasm trial experiments.

7.1 Methods

The wild trees used in this study were grown from cuttings taken from wild populations, planted into an ex-situ germplasm conservation trial at the Centre for Tropical Horticulture, Alstonville, NSW (Hardner et al. 2004). Twenty-one genotypes were selected from wild populations spanning the geographic distributions of *M. integrifolia*, *M. tetraphylla* and *M. ternifolia* (Table 7.1). Two hybrids of cultivar '660' (*M. integrifolia*) x *M. jansenii*, and three of '660' x *M. ternifolia* were also selected for evaluation from an adjacent breeding trial, along with three macadamia cultivars: 'A4', '842' and 'Daddow'. Replicate trees were included for seven of the genotypes (Table 7.1).

Starting in mid-December and continuing every four weeks until mid-May, each of the 30 trees were harvested, for a total of six harvests. Twenty nuts of average size were randomly picked from the tree canopy at each harvest and dried down to 1% moisture content using the methodologies outlined in Chapter 5. Nuts were stored in sealed plastic bags containing silica gel at 4°C until assessed.

Maturity of nuts was determined by oil content analysis, using methodology developed by Tim O'Hare (pers. comm.), modified from the methods of Kannamkumarath, et al. (2002) and Moodley, et al. (2007). For each tree and harvest a sample of ten sound kernel were ground in a coffee blender and in liquid nitrogen with a mortar and pestle until a smooth paste was formed.

The water content of each sample was assessed by drying approximately two grams of the kernel paste in an oven at 90˚C for 24 hours and recording the weight before and after. Total moisture content, including both oil and water, was then determined using the following protocol: Approximately two grams of ground sample was weighed and placed into a 50 mL Falcon tube with 10 mL of solvent (2 parts chloroform to 1 part methanol). Samples were placed on a shaker for 15 minutes at 180 rpm, before being filtered through a Büchner funnel under vacuum. Samples were rinsed in an additional 10 mL of solvent before vacuuming again until dry. The filter paper and kernel sample were carefully removed and placed into an oven for 15 minutes at 40˚C until completely dry. The final sample weight was recorded, and the moisture content (oil and water) determined by subtracting the final sample weight from the original weight. The oil content was then calculated by subtracting the amount of water estimated from the first step.

Two traits were calculated for each tree from the oil content data for analysis: (i) time of nut maturity; and (ii) the maximum percentage oil content. Dues to fluctuations and plateaus in oil content across harvests for individual trees, the time of nut maturity for each tree was calculated as the harvest where 90% of maximum oil content was first

reached, rather than the harvest at which maximum oil content was reached. Both traits were analysed using Analysis of Variance with species as a factor.

Table 7.1. Tree genotypes used in this study by species/type, including geographic location of the original wild populations and number of replicate clonal trees.

Species/type	Tree ID	Population location	Replicate trees
M. integrifolia	2-5	Bauple	1
	9-3	Mary River	1
	23-3	Mt Cotton	1
	60-3	Numinbah Valley	2
	103-1	Villeneuve	2
M. ternifolia	51-4	Draper	2
	72-2	Burpengary	1
	72-3	Burpengary	1
	88-1	Woodford	1
	88-3	Woodford	2
M. tetraphylla	39-1	Uki	2
	42-5	South Ballina	2
	81-1	Couchy Creek	1
	96-4	Mullumbimby	1
	110-2	Dorroughby	1
M. integrifolia x M. jansenii	660 x Jans-1	N/A	1
	660 x Jans-2	N/A	1
M. integrifolia x M. ternifolia	660 x Tern-1	N/A	1
	660 x Tern-2	N/A	1
	660 x Tern-3	N/A	1
Cultivar	842	N/A	1
	A4	N/A	1
	Daddow	N/A	2

7.2 Results and Discussion

Maturity time differed significantly with species (P=0.025). *M. integrifolia* and *M. tetraphylla* both matured significantly later than *M. ternifolia*, with maturity times averaging around February compared with late December for *M. ternifolia* (Figures 7.1 and 7.2). The hybrids and cultivars were not significantly different to any of the other species. *M. ternifolia* flowers two to four weeks before *M. integrifolia* and *M. tetraphylla*, and thus may begin and finish oil accumulation earlier.

Many trees also displayed a decrease in oil content around April and May (Figure 7.2). This coincided with an increased incidence of very immature nuts in the samples, presumably as a result of a late flowering flush. McConchie, et al. (1996) studied oil accumulation in cultivar 'A16', assessed via NIR. Their results suggested that nuts of this variety first exceeded 72% oil content between February and March, and reached maximum in April at 79%. This is somewhat later than the mean time to nut maturity observed here.

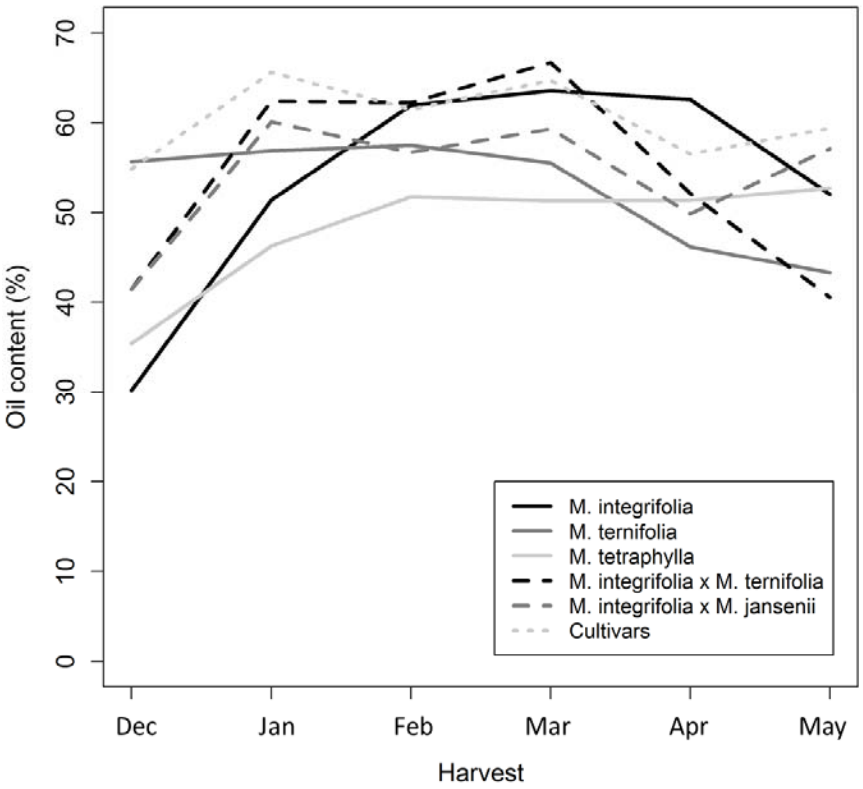

Figure 7.1. Mean percentage oil content over time.

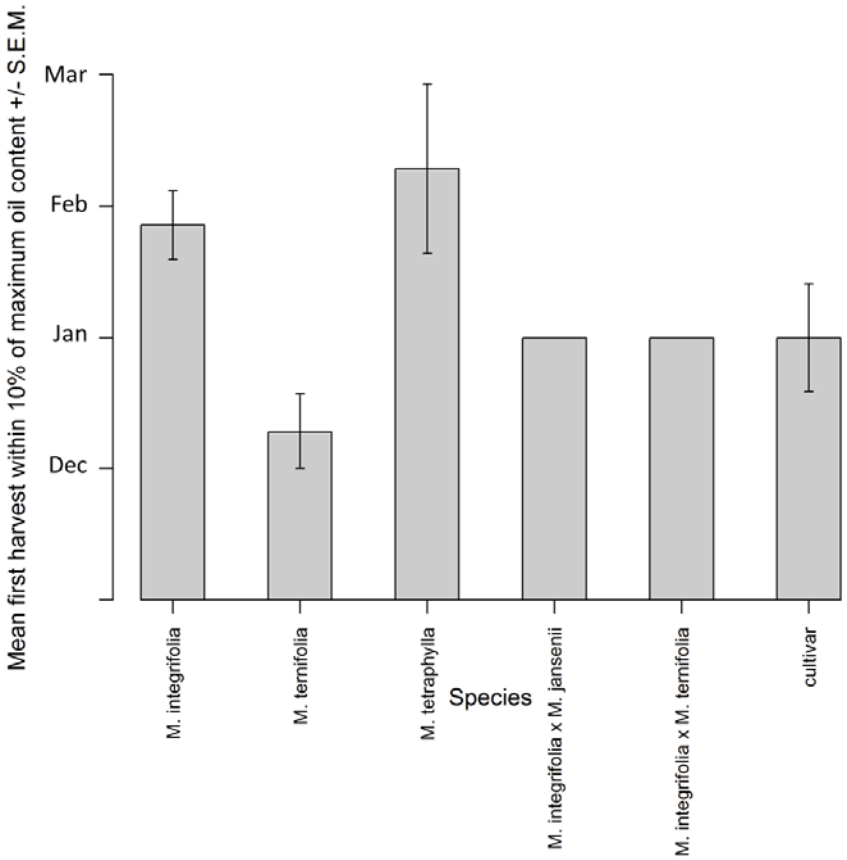

Figure 7.2. Mean harvest for each species at which 90% of maximum oil content was first achieved.

No significant difference in maximum oil content was observed across species (Figure 7.3). Maximum oil content ranged from 34% (tree 88-3, *M. ternifolia*) to 77%, (tree 60-3, *M. integrifolia*) with a mean of 63%. The oil contents in this study were generally very low, compared with previous studies and the industry first grade kernel standard of 72% oil. Only five of the 30 trees reached oil content above 70%, and only two equal to or above 72%. The industry cultivars included in this study were also observed to have low maximum oil contents (between 63 and 72%), suggesting that the generally low oil contents observed in this study may be an artefact of the oil extraction protocol used, or due to the combination of multiple nuts per sample, allowing for the inclusion of less mature nuts. Maguire, et al. (2004) used a similar protocol on store-purchased macadamia nuts, and observed oil content of 59.2 ± 1.5, much lower than grade 1 requirements. This suggests that the protocol used may be at least partially responsible for the low figures observed here.

Large differences were also observed between replicate trees, in particular for genotype 88-3, which had maximum values of 34% and 67% for its two replicates. Environment may therefore have a larger influence on oil accumulation than previously assumed.

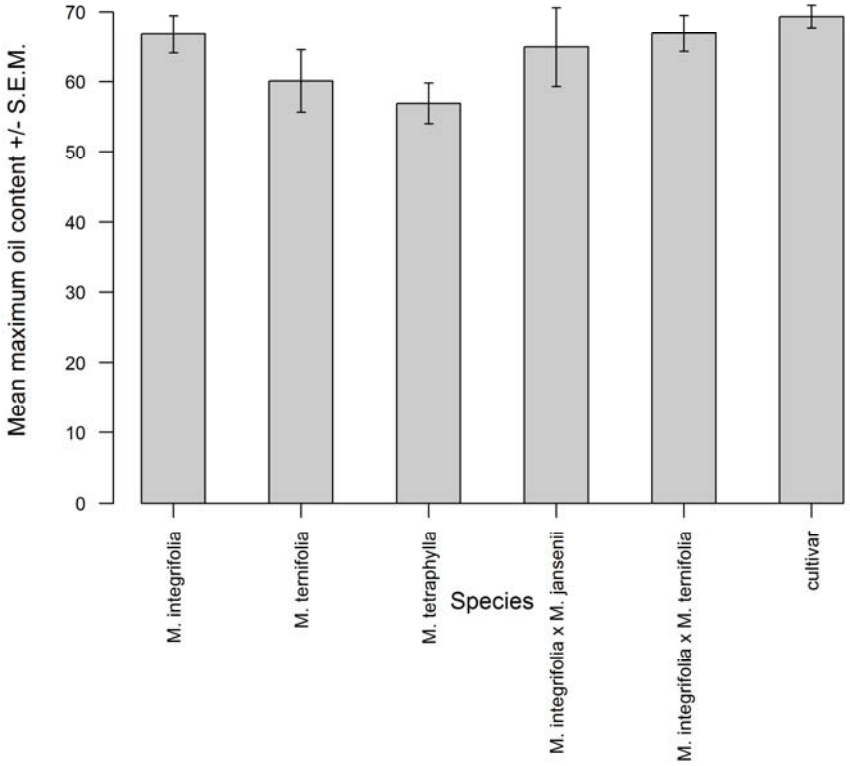

Figure 7.3. Mean maximum oil content.

7.3 Conclusions

Wild *M. integrifolia* and *M. tetraphylla* trees both matured later than wild *M. ternifolia*, with hybrids and cultivars being intermediate. In contrast, little difference between species was observed for maximum oil content, however large differences were present between individual trees. This experiment allowed us to identify individual wild trees with high levels of oil, and individuals with early and late oil accumulation phenotypes.

8.0 Non-RVT Cultivar Trials

Two cultivar trials were established external to the Regional Variety Trial project, with the aim of evaluating cultivar performance in growing areas beyond the traditional range used by the macadamia industry (Hardner and McConchie, 2009). The trials were located at Emerald in Queensland and Pretty Gully (Figure 8.1) in New South Wales.

Figure 8.1. Macadamia cultivar trial Pretty Gully.

8.1 Methods

Trials were designed as incomplete randomised blocks, and detailed trial information is presented in Table 8.1. The cultivars assessed at each trial is included in Table 8.2. Only four cultivars were in common between the two trials: '741', '814', '849' and 'A38'.

Table 8.1. Trial properties, including year of planting, number of experimental trees, number of cultivars assessed, and X (row) and Y (space) trial dimensions.

Trial	Year planted	No. Trees	No. Cultivars	No. Rows	No. Spaces	Tree spacing (m)
Pretty Gully	2001	91	21	7	22	8 x 4
Emerald	2003	59	12	1	62	10 x 4

Table 8.2. Cultivars assessed in each trial.

Cultivar	Pretty Gully	Emerald	Country of Origin	Breeding Program
Keauhou; HAES 246		✓	Hawaii	HAES
Ikaika; HAES 333	✓		Hawaii	HAES
Kau; HAES 344		✓	Hawaii	HAES
Mauka; HAES 741	✓	✓	Hawaii	HAES
HAES 804	✓		Hawaii	HAES
HAES 814	✓	✓	Hawaii	HAES
HAES 816		✓	Hawaii	HAES
HAES 842		✓	Hawaii	HAES
HAES 849	✓	✓	Hawaii	HAES
HAES 856	✓		Hawaii	HAES
1/40	✓		Australia	I. McConachie, DPI
2/48	✓		Australia	I. McConachie, DPI
4/7	✓		Australia	I. McConachie, DPI
2/12	✓		Australia	I. McConachie, DPI
A199	✓		Australia	HVP
A203		✓	Australia	HVP
A268		✓	Australia	HVP
A38	✓	✓	Australia	HVP
A4		✓	Australia	HVP
A9/9	✓		Unknown	Unknown
Beaumont	✓		Australia	NSW Dept. of Agriculture
D4; Renown	✓		Australia	N. Greber
Daddow		✓	Australia	N. Greber
NG18	✓		Australia	N. Greber
NG29	✓		Australia	N. Greber
NG35	✓		Australia	N. Greber
NG8	✓		Australia	N. Greber
Own Venture	✓		Australia	N. Greber
Yonik	✓		Israel	Unknown

HAES = Hawaii Agricultural Experiment Station; DPI = Queensland Department of Primary Industries; HVP = Hidden Valley Plantations.

Yield data was collected in 2007 and 2012 at Emerald (trial ages 4 and 9, respectively), and in 2006, 2007, 2009, 2010 and 2011 at Pretty Gully (ages 5, 6, 8, 9, and 10). Total nut in shell (NIS) mass at 1% moisture content was collected for each tree. Yield at age nine has been analysed for each trial.

In 2013, total kernel recovery (TKR) was determined for 25 nuts from each experimental tree in both trials, from the 2011 Pretty Gully harvest and 2012 Emerald harvest. Individual nuts were cracked and total weight and kernel weight determined. TKR was calculated as kernel mass / total NIS mass.

Data was analysed separately for each trial using Unbalanced Analysis of Variance in GenStat (Fifteenth Edition), including Replicate as a blocking term. Fisher's Least Significant Difference Test was conducted to determine significant differences between cultivars. An analysis combining the two trials was not conducted as it would be limited by the small number of cultivars in common between trials.

8.2 Results

8.2.1 Yield

There were significant differences for yield among the cultivars at Pretty Gully (P<0.001) and Emerald (P=0.02). Predicted mean values are presented in Figures 8.2 and 8.3. Cultivars 'NG18' and '4/7' produced the highest yields at Pretty Gully, with predicted yield of 13.5 and 10.5 kg per tree at age 9, respectively (Figure 8.2). Differentiation between cultivar yields at Emerald was lower than at Pretty Gully. Cultivars '344' and '842' ranked the highest, however did not produce significantly greater yields than five other cultivars (Figure 8.3).

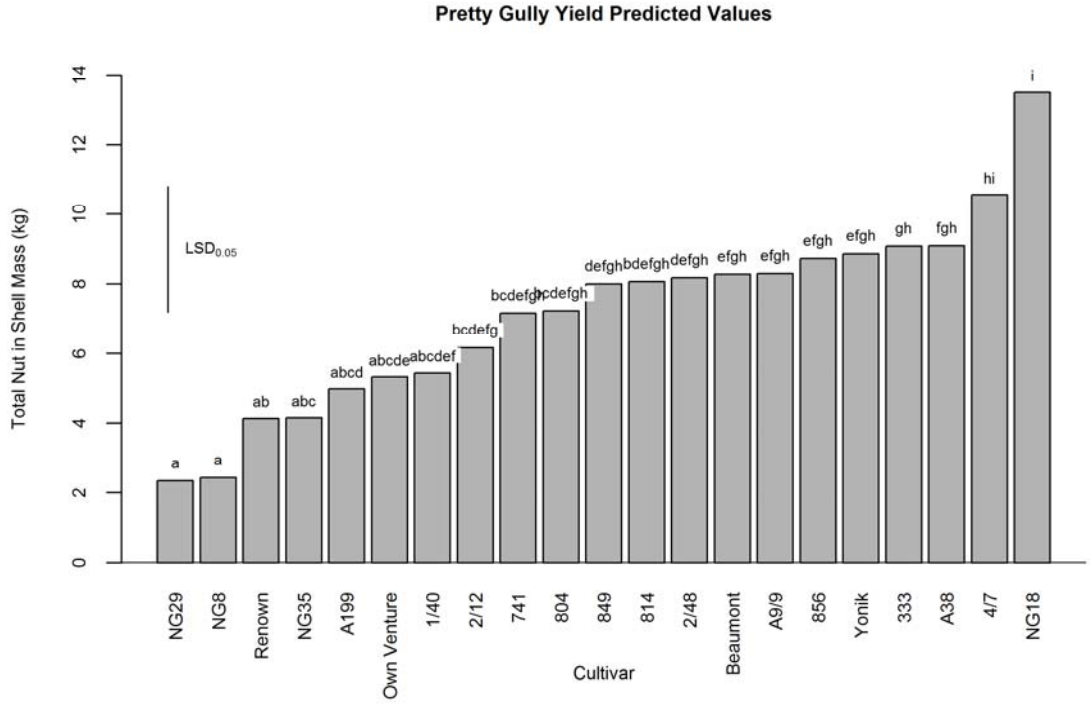

Figure 8.2. Predicted mean values and least significant differences for yield at Pretty Gully.

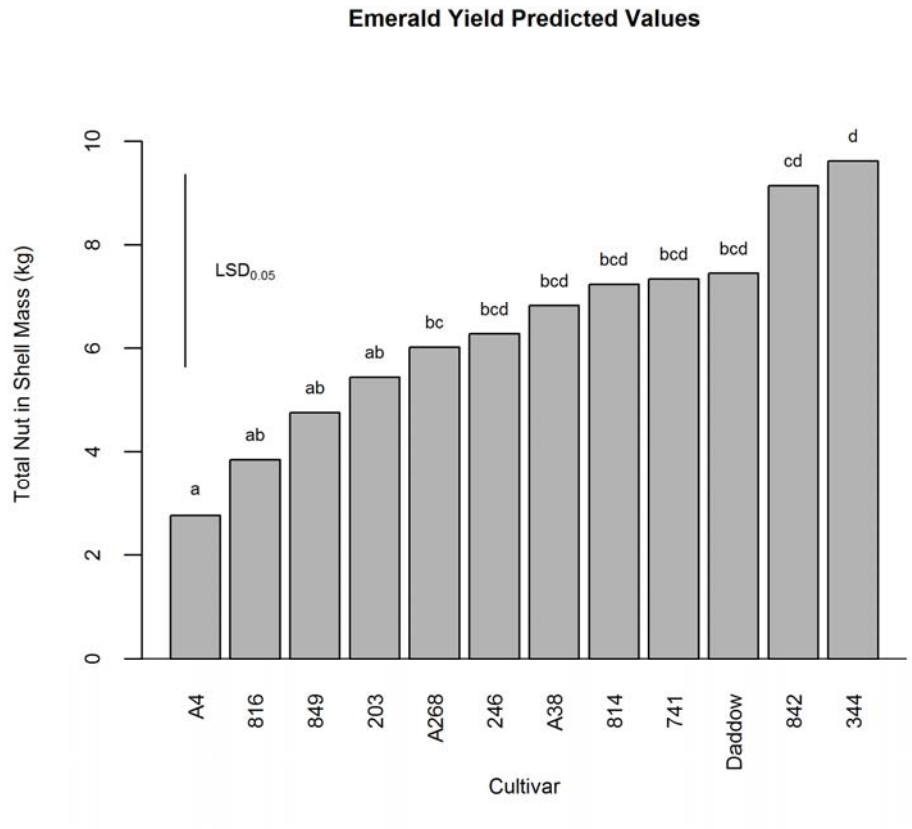

Figure 8.3. Predicted mean values and least significant differences for yield at Emerald.

8.2.2 Kernel recovery

There were significant differences for kernel recovery among the cultivars at both trials ($P<0.001$). Cultivars 'NG29' and '804' produced the highest TKR at Pretty Gully, at 48% and 46% respectively (Figure 8.4). At Emerald, cultivars '849', 'A4' and '816' performed the best, with kernel recoveries of 44%, 43% and 40% (Figure 8.5). Cultivar '849' ranked highly at both sites.

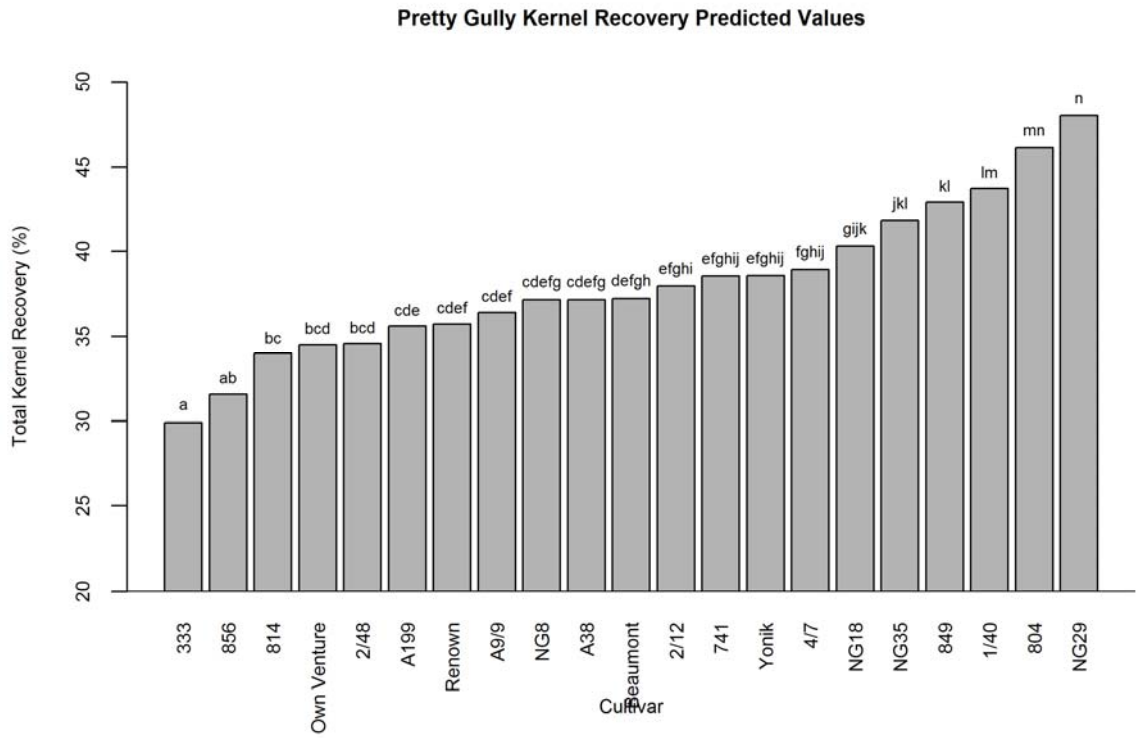

Figure 8.4. Predicted mean values and least significant differences for total kernel recovery at Pretty Gully.

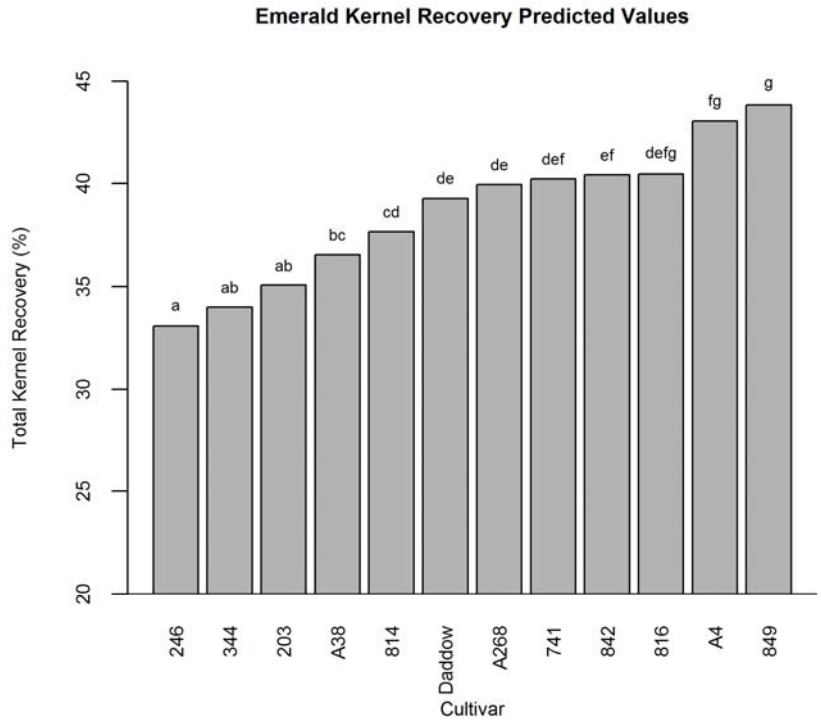

Figure 8.5. Predicted mean values and least significant differences for total kernel recovery at Emerald.

8.3 Discussion

Initial analyses of yield and total kernel recovery at the two cultivar trials show significant differences between cultivar performance. Ranking of the four cultivars in common between the trials was similar between trials for both traits, suggesting that GxE (genotype by environment) effects may not be significant. Given the small number of cultivars in common, however, this result should be treated with caution.

This trial has shown that macadamias can be grown successfully at Emerald and Pretty Gully. Total kernel yields of 1.70 t/ha were obtained from 'NG18' at Pretty Gully and 0.82 t/ha obtained from 344 at Emerald. These total kernel yields are 61% and 4% greater respectively than the industry total kernel yield average (estimated from average saleable kernel yield and average percentage reject kernel) of 0.66 t/ha in 2011 and 0.79 in 2012 (Mulo et al. 2013). Average kernel yields across all tested cultivars were 0.85 t/ha at Pretty Gully and 0.62 t/ha at Emerald. These are 23% greater and 27% lower than industry averages for those years, respectively.

The remoteness of the trial sites and lack of common cultivars in the two trials reduced the usefulness of this experiment. The RVT3 trials that are currently in progress at the remote sites of Emerald and Mackay in Queensland and Macksville in NSW have rendered this experiment obsolete and will provide definitive information about GxE and remote site performance.

9.0 Database Management

The Macadamia Breeding Program database system was created for the purpose of efficiently storing the large quantities of data generated by the program and to allow easy data retrieval. Two databases are currently in use by the program. Data from all trials established in previous projects (B1.1, B1.2, cultivar, germplasm and rootstock trials) are stored in a single Microsoft Access database. Due to the large size of this database and the substantial slow-down in functioning that resulted, a second database of the same format was created for all second-generation trial data (B2.1 and B2.2). This report describes the structure of the databases and summarises the stored data.

9.1 Database structure

The data in databases are contained in a series of tables, structured in a way that optimises space (i.e. computer memory) efficiency and ease of importation. Several queries have also been written for the databases, with the purpose of combining, filtering, and checking the data. These tables and queries are described in more detail below.

9.1.1 Tables

The tables in the databases may be categorised according to their function as either:

a. data tables, containing data from the field trials;
b. reference tables, containing explanations of codes or terms used in the data; or
c. utility tables, which have utility roles such as being used as part of the data import process, or to easily access the data for export.

A list of the tables, along with their class and description are presented in Table 9.1 below. Explanations of the headers within each table are included in Appendix 7. In all tables except *tbl_AllTreeData*, descriptions of column header meanings also show at the bottom left of the screen when columns are clicked on in Access.

Table 9.1. List of tables in the database in alphabetical order, including class and description.

Table	Class	Description
tbl_AllTreeData	Utility	Created by qry_AllTreeData_Create_4. Combines the majority of the information stored in the separate tables below into one table that can be easily filtered and exported for analysis.
tbl_AnnualAssessDate_import	Data	Date of measurement for all collected tree data.
tbl_AnnualTreeData_import	Data	All collected data.
tbl_AnnualTreeExpstat_import	Data	Annual Tree_id, Tree_pdate and Expstat data.
tbl_B1-1 2006 snsk data	Data	B1.1 nut and kernel assessment data from 2006. This can't be combined with tbl_AnnualTreeData_import as each row in this data is a single nut, instead of a single tree.
tbl_B1-1 Rankings	Data	List of the top 40 selected B1.1 trees.
tbl_B1-1 TreeAnalstat	Data	Overall analysis status code for B1.1 trial data. 0=analysed, 1=not analysed.
tbl_ExpstatCodes	Reference	Expstat codes and their meanings.
tbl_GapCalculator_Output	Utility	Output from the Gap Calculator program. Delete existing data in here before appending new data!
tbl_Tree_idData	Data	Tree_id data. Includes family data (for breeding trials) rootstock and scion cultivars (for rootstock trials) and germplasm site IDs and species (for germplasm trials).
tbl_TrialData	Data	Trial-level data.
tbl_TrialDesign	Data	Details of trial design (e.g. replicate, block, spacing) and barcodes. Gap_calc_trial is a trial code shared by physically adjacent trials so that gaps can be properly estimated by

Table	Class	Description
		the Gap Calculator program.
tbl_VariableList	Reference	List of variables and their meanings.

9.1.2 Queries

The queries in the databases are described in Table 9.2. The queries named *qry_AllTreeData_Create_** generate the table *tbl_AllTreeData*. This query deletes the existing *tbl_AllTreeData* and recreates it using the data contained in *tbl_AnnualTreeData_import* and *tbl_AnnualTreeExpstat_import*. It is run every time that new data is added to a database to ensure that *tbl_AllTreeData* remains up-to-date.

Table 9.2. Names and descriptions of queries in the database.

Query	Description
qry_AllTreeData_Create_1	Step 1 in creating tbl_AllTreeData. Generates a short-list of trials and years for which data was collected.
qry_AllTreeData_Create_2	Step 2 in creating tbl_AllTreeData. Combines years of data collection with acol and arow so that acol & arow can be properly incorporated into the annual tree data.
qry_AllTreeData_Create_3	Step 3 in creating tbl_AllTreeData. Combines the majority of trial and tree data into a single table.
qry_AllTreeData_Create_4	Final step in creating tbl_AllTreeData. Adds family data to qry_AllTreeData_Create_3 and makes tbl_AllTreeData.
qry_Elite_Selections_Data	tbl_AllTreeData filtered for the top 40 selected B1.1 trees.
qry_GapCalculator_InputFileCreate	Creates the input file for the Gap Calculator program. Export as a CSV file.
qry_GapCalculator_OutputAppend	Formats the data in tbl_GapCalculator_Output and appends to tbl_AnnualTreeData_import.
qry_VariableList_Check	Check to see if all imported variables are in tbl_VariableList. If there are blank cells at the top then those variables need to be manually added to tbl_VariableList.

9.2 Data summary

The two databases contain a total of 960,723 records (Table 9.3). Prior to entry into the database, all data is rigorously checked for mistakes using a structured protocol. Data is checked and entered as soon as possible following collection. This ensures high data quality and that all data in the database is the latest and final version.

Table 9.3. Number of records for trial and trial type in the databases.

Trial type	Trial	Records	Trial type totals
B1.1	BQBRS97	123,936	
B1.1	BQBRS98	261,477	
B1.1	BTFRS98	217,727	603,140
B1.2	BALLO02	13,332	
B1.2	BAMAM02	12,810	
B1.2	BAMAM03	14,700	
B1.2	BBAFF02	14,337	
B1.2	BBAFF03	19,278	
B1.2	BDUNO00	19,987	
B1.2	BDUNO03	12,191	
B1.2	BEGYM01	13,728	
B1.2	BHINK00	16,058	
B1.2	BNEWR02	15,600	
B1.2	BQBRS01	40,800	
B1.2	BQBRS03	13,208	
B1.2	BYAND00	13,566	
B1.2	BYAND02	10,902	230,497
B2.1	BNAMB11	4,930	4,930
B2.2	BQBRS12	1,647	
B2.2	BQBRS13	2,817	
B2.2	BQBRS14	664	5,128
Cultivar	CEMER03	1,550	
Cultivar	CPRET01	7,084	8,634
Germplasm	GTFRS00	24,276	
Germplasm	GTIAR01	24,300	48,576
Rootstock	RBAFF02	14,127	
Rootstock	RNEWR02	13,772	
Rootstock	RQBRS02	16,830	
Rootstock	RWOLL02	15,089	59,818
Grand total			960,723

9.3 Conclusions

The data management and database systems used by the breeding program are designed to optimise data quality and accessibility. Self-checking data entry forms, rigorous post-collection data checking, and efficient storage and retrieval processes ensure the best possible results.

10.0 Husk Spot Resistance Screening

Husk spot is a major cause of immature nut drop in macadamias. It is caused by the fungus *Pseudocercospora macadamiae*. Industry stakeholders voted tolerance or resistance to husk spot as the second top priority within the breeding program during industry consultation at the start of this project.

10.1 Methods

10.1.1 Field evaluation of husk spot

The 17 elite selections from the B1.1 progeny planted at the Bundaberg Research Facility (BRF) were evaluated over two seasons for husk spot severity and incidence. The methods used included assessment of proportion of fruit with husk spot lesions, proportion of fruit that abscised with husk spot lesions, average number of lesions per abscised fruit and sticktight incidence. Sticktights are dessicated nut-in-husk that does not drop but remains in the tree after maturity. Sticktight nuts are a major source of husk spot infection.

The prevalence of sticktights was assessed using ordinal rating scale based on the number and distribution of sticktights in the tree canopy:

0 = clean and no sticktight
1 = <5 pieces of sticktights (scanty)
2 = 5-10 sticktights pieces in the canopy
3 = >10 sticktights pieces well distributed within canopy
4 = few (<5) clusters of sticktights and several single pieces scattered in the canopy
5 = several (>5) clusters of sticktights distributed throughout the canopy

Husk spot severity was measured as the proportion of total nuts under the tree canopy with visible husk spot symptoms.

10.1.2 Assessment of husk spot resistance indicators

Potential indicators of husk spot resistance were evaluated based on the husk spot infection through the fruit stomata, since pathogen entry into host tissue is a critical first step in the infection process of husk spot. The relationship between stomatal abundance in the epidermis of the fruit and leaf was explored in order to screen and select appropriate germplasm early in the breeding cycle.

10.2 Results and discussion

10.2.1 Field evaluation of husk spot

Significant variations were observed among the macadamia genotypes for disease intensity variables and prevalence of sticktights. A range of prevalence of sticktights ratings (0-5) exist in the macadamia genotypes populations (Figure 10.1). The results showed that the observed prevalence of sticktights was near normal distribution (Kurtosis = -0.87 ±0.45 standard error), and most genotypes had low to average sticktight ratings (0 – 2).

Nuts of about 45% of macadamia genotypes abscised readily with less than five lesions. This indicates that these genotypes were intolerant to husk spot infection. Only 10% of the genotypes were able to withstand high number of lesion before abscission. These were categorised as tolerant to infection (Figure 10.2).

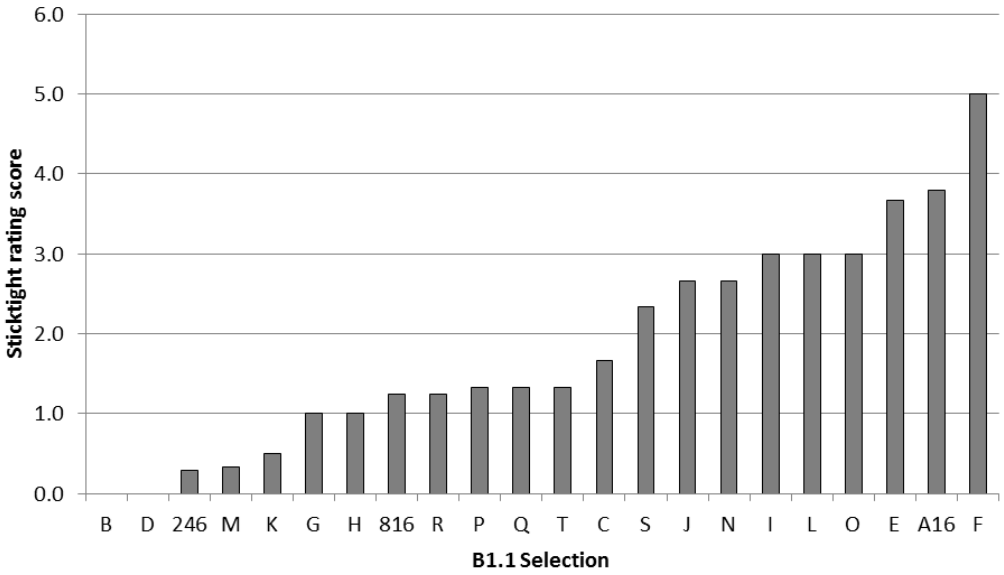

Figure 10.1. Sticktight rating score (0-5) for B1.1 elite selections and three cultivars planted at Bundaberg Research Facility.

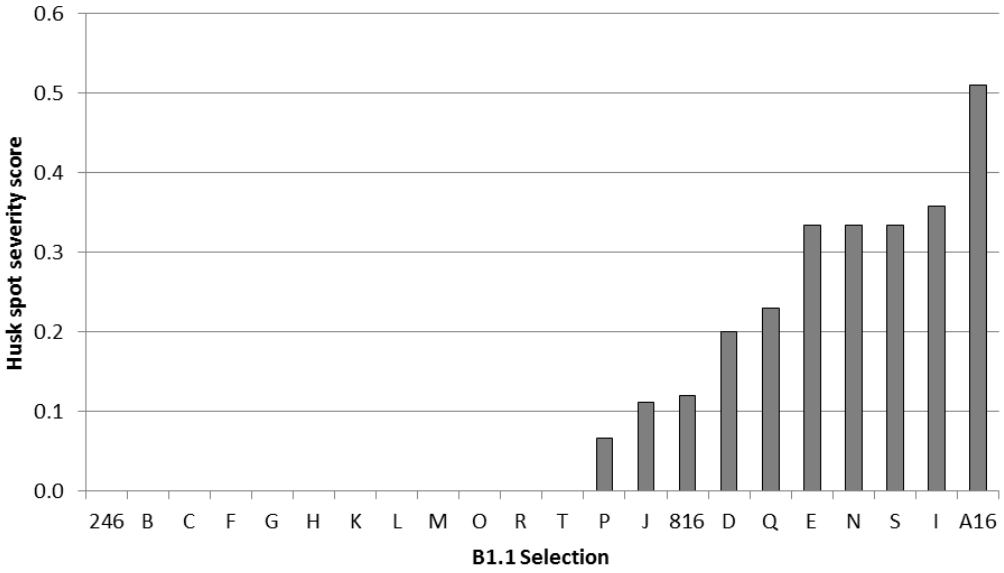

Figure 10.2. Husk spot severity for B1.1 elite selections and three cultivars planted at Bundaberg Research Facility.

10.2.2 Assessment of husk spot resistance indicators

Cultivars '660', 'A4' and '741' had the least fruit stomatal abundance out of the 21 macadamia genotypes including the elite candidates sampled. Lines I and G had the least fruit stomatal abundance among the elite candidates.

Results showed that there is a significant ($P < 0.001$) interaction between the genotypes and leaf stomatal abundance. Lines F, O and N had the least leaf stomatal abundance among the elite candidates, and were significantly different from other genotypes. Lines T and L had the highest leaf stomatal abundance.

Results showed a significant relationship between fruit stomatal abundance and disease intensity including the lesion number, disease incidence and severity. This suggests that fruit stomatal abundance is a useful trait to predict genotype resistance-susceptibility to husk spot. It appears

resistant genotypes have less stomata per unit area than susceptible genotypes, suggesting that low stomatal abundance plays important roles in providing defence against pathogens that enter via stomata. Relationship between fruit and leaf stomata abundance of all the genotypes tested was explored with a simple linear regression which accounted for about 48% (P<0.01) of variances observed (Figure 10.3).

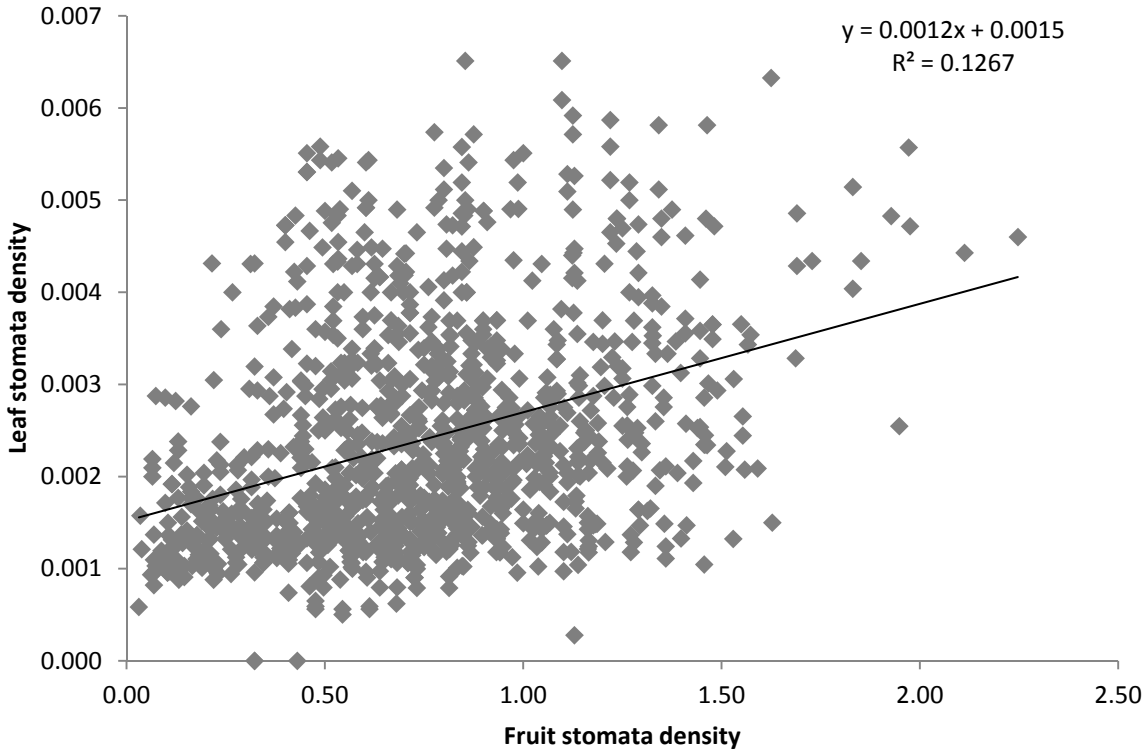

Figure 10.3. Relationship between fruit and leaf stomatal abundance in macadamia genotypes.

10.2.3 Recommendations on the potential for further pre-breeding research aimed at improving resistance in new cultivars in the medium to long term.

This study has identified phenotypic traits useful as selection tools in the screening and selection for husk spot resistant cultivars in breeding programs. In addition to pre-breeding selection of parents with desirable horticultural traits, the inclusion of characters such as low stomatal abundance and freedom from sticktights would provide useful as tools for disease resistance screening. This may lead to improved resistance in new macadamia cultivars and contribute to a cost-effective sustainable macadamia disease management strategy.

We have identified that both fruit and leaf stomatal abundance vary significantly between the macadamia genotypes. The combined analysis suggests that fruit stomatal abundance may be predicted from leaf stomatal abundance.

Since the relationship using matured leaf obtained from trees in the orchards explained approximately 48% of the variation, further studies should evaluate the relationship using leaves from plantlets of known parents growing under similar conditions. We recommend that future studies should also evaluate rapid pathogenicity assay on macadamia seedlings or plantlets for husk spot resistance, and examine other putative indicators including biochemical indicators of husk spot resistance.

11.0 Fruit Spotting Bug Resistance Screening

A perennial insect threat to macadamia is the fruit spotting bug (FSB) *Amblypelta nitida* Stål (Hemiptera: Coreidae) (Brimblecombe 1948, Ironside 1981, Treverrow 1983, Gallagher *et al.* 2003, O'Hare *et al.* 2004). It is the pest that will be a constant threat to the quality of the nut crop throughout the growing season if cover spraying with insecticides becomes more difficult (Fay 2002).

In South East Queensland, the conspecies *Amblypelta lutescens lutescens* (banana spotting bug, BSB) is also common on many fruit crops including macadamia (Ironside 1981, Donaldson 1983, Waite *et al.* 1993, Huwer 1996, Waite *et al.* 2000). This insect will also attack the shoot growth as well as the fruit on many crops.

Our task as part of the macadamia breeding project has been to evaluate the germplasm orchards at Alstonville and Tiaro to establish if there is germplasm that shows resistance to attack, and conversely, to establish if there are genotypes more prone to heavy attack by FSB and BSB. The following is a summary of the experimental work on this topic. The full report is included as Appendix 8.

11.1 Methods

FSB and BSB incidence were measured at the ex-situ germplasm blocks at Alstonville and Tiaro. On the northern side of each tree (sunny aspect), we recorded if the tree was flowering, setting nut, carrying old nut, or dead. Numbers of adults and nymphs, which were visible on the fruit in the lower 3m of the canopy were recorded. This process was repeated 5 times over the season beginning early spring, then late spring, early summer, late summer and autumn to detect where the population started, and where it spread to. Analysis was made of FSB incidence data at the individual tree level, the germplasm site level and the grouping of the germplasm sites to plant species. Incidence data is reported as percentage of trees infested at each time.

The damage levels were determined by harvesting nuts under and on trees during March for the earlier maturing germplasm and in June for the later maturing ones each season.

The Tiaro gemplasm trial was not sprayed with insecticides. The Alstonville germplasm trial was sprayed with the insecticide beta-cyfluthrin (Bulldock®) in the 2009-10, 2010-11 and 2013-14 seasons and was left unsprayed in the 2011-12 and 2012-13 seasons. The trees were examined within 3 days of each spray application to make sure the spray was effective (no bugs were found). The crop was harvested in March and May/June and damage levels determined.

11.2 Results

Incidence of FSB was high for *M. ternifolia* compared to *M. tetraphylla* and *M. integrifolia* (Figure 11.1). The highest incidence was on 9 December 2010 when 40% of *M. ternifolia* trees were recorded with FSB infestation evidence. This compares with 4.2% and 0.6% incidence on *M. tetraphylla* and *M. integrifolia*, respectively on the same date (Figure 11.1).

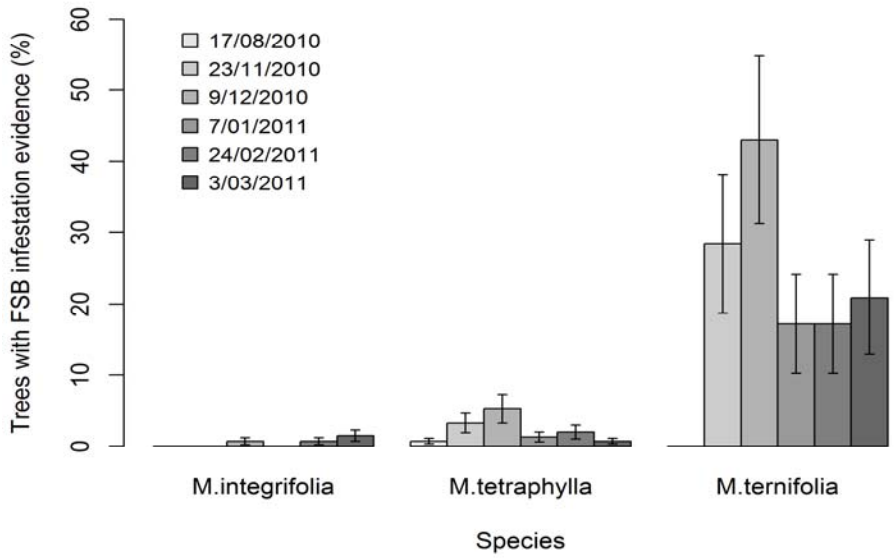

Figure 11.1. Incidence of *Amblypelta nitida* (FSB) at Alstonville from July 2010 to March 2011, on *Macadamia ternifolia* (n=30), M. tetraphylla (n=188) and *M. integrifolia* (n=162).

The macadamia crop is vulnerable to invasion from FSB for long periods of a season and a means of identifying the flights into this and many other crops is an important step forward in managing the insect. The FSB preference for *M. ternifolia* (Figure 11.2) is an important finding in this project and augments the period of FSB activity found on *Murraya paniculata* hedges since 2003 perfectly (Huwer *et al.* 2006, 2011).

Examining the kernel damage (Figure 11.3) samples, FSB have shown a consistent preference for *M. tetraphylla* germplasm over *M. ternifolia* and *M. integrifolia*. At the unsprayed Tiaro trial in 2012 the 48% damaged nut on 9 *M. tetraphylla* trees was significantly greater than the 22% damaged nut on 83 *M. integrifolia* trees. No nuts were set on the *M. ternifolia* trees. At the sprayed Alstonville trial in 2014 there was 21% damaged nut on *M. tetraphylla* which was significantly higher than the 9% and 6% damage on *M. ternifolia* and *M. integrifolia*.

A 

B 

Figure 11.2. (A) Adult *Amblypelta nitida* (FSB) mating on *Macadamia ternifolia* nut at Alstonville January 2012. (B) *Amblypelta nitida* nymph feeding on *Macadamia ternifolia* at Alstonville November 2011.

Figure 11.3. Fruitspotting bug damage on macadamia kernel.

It is unlikely that any macadamia germplasm is immune to attack by FSB during the early part of the growing season, hence the commercial reliance on sprays in spring. The mechanism by which FSB feed is such that they could penetrate nutshell to a depth of 6-7mm (Miles & Taylor 1994, Maddox *et al.* 2012), which is far thicker than any commercial macadamia shell known. The critical question is determining if FSB are coming back into the orchard during summer after the early season spraying had finished.

11.3 Conclusions

The early flowering, terminal bearing trees of the *M. ternifolia* populations 71, 72 and 73 appeared to be highly attractive to FSB and the adults kept coming back to these genotypes. It is currently unknown whether it is the tree itself (i.e. flower) that attracts FSB back to the tree, or the bug (i.e. chemical produced by the bug while feeding on the nuts or chemical on fed nut). The *M. ternifolia* preference is probably most influenced by the timing of the nutset and florescence. *M. ternifolia* tend to be already set nut at the time when the rest of the macadamia genotypes are still flowering. This gives the overwintering FSB adults their first chance to breed in the crop. Damaged fruit hanging in the plant may be attractive in their own right. We have taken this principle to the field with the hedge concept and have had some success (Huwer & Maddox current work MT 10049).

Genotypes of *M. integrifolia* were identified with little or no damage at both Tiaro and Alstonville. A *M. integrifolia* genotype collected from Villeneuve in Queensland recorded no nut damage at Alstonville from 2010 to 2014. It should be kept in mind that the overall attractiveness of specific genotypes is relative to what genotypes are present in the orchard. Least susceptible genotypes may still be attacked by FSB if that is the only food source around (Drew, 2005). With regards to macadamias, all genotypes are susceptible during the nut development phase. It is important to find out what triggers FSB to re-infest the orchard.

12.0 Breeding Strategy Comparison

Four breeding strategies were compared in terms of the time to commercial deployment of elite selections, cost of breeding and expected rates of genetic gain determined by stochastic modelling. A tandem selection strategy, which selected for kernel recovery in a seedling trial and nut-in-shell yield in a clonal trial, produced the highest gain to cost ratio but was limited in the number of genotypes assessed in the clonal trial. The full assessment and progeny cull strategies, which selected for nut-in-shell yield and kernel recovery in both a seedling and clonal trial were similar in terms of gain per unit cost and a cloned seedling strategy was intermediate in value. The following chapter was presented at the 25th International Horticulture Congress (Topp et al., 2012).

12.1 Methods

12.1.1 Description of breeding strategies

The four breeding strategies (Table 12.1) are described in the following section.

Full Assessment (FA). This consists of a seedling progeny trial followed by a candidate cultivar trial and is similar to the strategy used in previous Australian macadamia breeding (McConchie, 2008; Hardner et al., 2009).

The seedling progeny trial involves controlled pollination of 30 parents to produce 60 families each with 20 progeny thus providing a base population of 1,200 hybrid seedlings. Plants are established in complete blocks with single tree plots at spacings of 4 m x 6 m. The seedlings are surrounded by guard trees and managed to optimize tree growth. Tree size is assessed in year 7 when the seedlings are 5 years old. Tree yields are measured from year 5 to year 9. Kernel recovery and percentage of whole kernels are assessed in year 9. Twenty candidate cultivars are selected from the 1,200 seedlings using an economically weighted selection index (Hardner et al., 2006) for yield, kernel recovery, percentage wholes and canopy width.

The candidate cultivar trial consists of 6 replicates of 20 candidate cultivars that are propagated onto commercial rootstock. Trees are planted in single tree plots at 4 m x 6 m spacing with guards surrounding the trial. Trees are assessed in a similar manner to the progeny trial for yield, but for eight years from year 14 to 21. This is when the trees are from 3 to 10 years old. In year 21, the trees are also evaluated for kernel recovery, percentage whole kernels and canopy width. The top five cultivars are identified using an economically weighted selection index of clonal values as shown above.

Progeny Culling (PC). This strategy is similar to the full assessment strategy in that there is a seedling progeny trial followed by a candidate cultivar trial. It differs from the full assessment strategy in that there is culling of the seedlings as the trial progresses. No trees are physically removed but fewer are assessed as the high yielding trees are identified. All 1,200 seedlings are assessed for yield in years 5 to 7. The top yielding 600 trees are assessed in year 8 and only the top yielding 300 trees are assessed in year 9. Kernel recovery, percentage whole kernels and canopy width are assessed in year 9. The 20 candidate cultivars are selected and the candidate cultivar trial is conducted as described above.

Tandem Selection (TS). This strategy has a seedling progeny trial that finishes two years earlier than the previous two strategies. A base population of 1,200 seedlings is produced and planted out. The trees are assessed for kernel recovery, percentage whole kernels and canopy width at year 7. Twenty candidate cultivars are selected from the 1,200 seedlings using an economically weighted selection index for kernel recovery, percentage wholes and canopy width. The candidate cultivar trial is planted, assessed and selected as described for the full assessment strategy.

Cloned Seedling (CS). This strategy commences with the creation of 200 hybrid seedlings from controlled pollinations of 20 parents producing 40 families with 5 seedlings per family. The seedlings

are grown for two years then budwood is taken from each seedling to propagate 6 ramets by grafting onto commercial rootstock. A candidate cultivar trial of 200 genotypes x 6 replicates is planted in single tree plots at 4 m x 6 m spacing with guard trees surrounding the trial. Trees are maintained, assessed and selected as described in the candidate cultivar trial of the full assessment strategy.

Table 12.1. Major activities and tree numbers for the four breeding strategies. Italics in shaded regions represent seedling progeny trials and non-italics in shaded regions represent candidate cultivar trials.

Year	Full assessment	Progeny cull	Tandem selection	Cloned seedling
1	Cross parents Grow seedlings	Cross parents Grow seedlings	Cross parents Grow seedlings	Cross parents Grow seedlings
2	Plant SPT	Plant SPT	Plant SPT	Grow seedlings
3	Trial maintenance	Trial maintenance	Trial maintenance	Propagate CCT
4	Trial maintenance	Trial maintenance	Trial maintenance	Plant CCT
5	Assess 1200 for Y	Assess 1200 for Y	Trial maintenance	Trial maintenance
6	Assess 1200 for Y	Assess 1200 for Y	Trial maintenance	Trial maintenance
7	Assess 1200 for Y+CW	Assess 1200 for Y+CW	Assess 1200 for KR+PW+CW	Assess 1200 for Y
8	Assess 1200 for Y	Assess 600 for Y	Propagate CCT	Assess 1200 for Y
9	Assess 1200 for Y+KR+PW	Assess 300 for Y+KR+PW	Plant CCT	Assess 1200 for Y
10	Propagate CCT	Propagate CCT	Trial maintenance	Assess 1200 for Y
11	Plant CCT	Plant CCT	Trial maintenance	Assess 1200 for Y
12	Trial maintenance	Trial maintenance	Assess 120 for Y	Assess 1200 for Y
13	Trial maintenance	Trial maintenance	Assess 120 for Y	Assess 1200 for Y
14	Assess 120 for Y	Assess 120 for Y	Assess 120 for Y	Assess 1200 for KR+PW+CW
15	Assess 120 for Y	Assess 120 for Y	Assess 120 for Y	Release
16	Assess 120 for Y	Assess 120 for Y	Assess 120 for Y	
17	Assess 120 for Y	Assess 120 for Y	Assess 120 for Y	
18	Assess 120 for Y	Assess 120 for Y	Assess 120 for Y	
19	Assess 120 for Y	Assess 120 for Y	Assess 120 for Y+KR+PW+CW	
20	Assess 120 for Y	Assess 120 for Y	Release	
21	Assess 120 for Y+KR+PW+CW	Assess 120 for Y+KR+PW+CW		
22	Release	Release		

Abbreviations: CCT candidate cultivar trial; CW canopy width; KR kernel recovery; PW percent whole kernels; SPT seedling progeny trial; Y individual tree yield.

12.1.2 Simulation of breeding populations

Each breeding strategy was compared via a simulation study using genetic architecture for the traits derived from published genetic parameters. Phenotypic and genetic values for yield, kernel recovery, percentage whole kernels and canopy width were generated stochastically from a multivariate normal distribution using environmental and genetic parameters from Hardner et al. (2001, 2002) (Table 12.2). The broad sense heritabilities were converted to narrow sense heritabilities assuming that 50% of the total genetic variance was additive. A linear mixed model was fitted to the multi-trait data and genetic variances and covariances for each strategy were estimated using residual maximum likelihood (REML), (Patterson and Thompson, 1971) using the software package ASREML-R, (Butler *et al.,* 2009). Best linear unbiased predictions (BLUPs) of clonal values for nut-in-shell yield, kernel recovery, percentage of whole kernels and canopy width were calculated for each genotype and combined in an economically weighted selection index (Hardner et al., 2006). The expected genetic gain for each breeding strategy was calculated as the mean of 50 simulated populations using the selection index values of the top five genotypes. The simulation study was implemented in the R software environment (R Development Core Team, 2008).

Table 12.2. Estimates of broad-sense heritability and genetic correlations for the traits Y (cumulative nut-in-shell yield to age 10), KR (kernel recovery), PW (percentage of whole kernels) and CW (canopy width at age 10). Estimates are from Hardner et al. (2001, 2002). These estimates were used in constructing the simulated seedling progeny and candidate cultivar populations for breeding strategy comparisons.

	Y	KR	PW	CW
Y		-0.37	-0.04	0.07
KR			0.10	-0.30
PW				0.18
Heritability	0.14	0.63	0.31	0.28

12.1.3 Calculation of breeding strategy costs

Costs of conducting the breeding strategies were calculated using orchard establishment, maintenance and tree assessment costs obtained from field trial and industry records (O'Hare et al., 2004). Costs were standardised across years by use of net present value using a 10% compound rate.

12.2 Results and discussion

This study developed a structured stochastic model for simulating gains from alternative breeding strategies. The model involved prediction of breeding values using a relationship matrix formed from a known pedigree, multi-stage selection, and multi-trait selection using a selection index formed from the sum of the product of economic weight and genetic value for each trait. Nut-in-shell yield, kernel recovery and canopy width are assessed to allow calculation of kernel yield per ha which is a primary determinant of profitability. Percentage of whole kernels is included as future returns may include this trait.

12.2.1 Comparison of the four strategies

Three of the strategies required two stages of field trials. Firstly, a seedling progeny trial (SPT) where initial selection was made on single seedling plants of each genotype. Secondly, a candidate cultivar trial (CCT) where cultivar selection was made on clonally replicated genotypes. The CS strategy required only one field trial, a CCT that was propagated directly from the seedling nursery.

The length of a breeding cycle from cross pollination to the release of new cultivars varied among the strategies (Table 12.1). The FA and PC strategies were the longest and required 22 years. The TS strategy was two years shorter due to a reduction in the length of its SPT. The CS strategy which required only one field trial was the most rapid strategy and was completed in 15 years.

The annual cost of the four strategies was compounded to time of release to compare cost at a common time. FA was most expensive at $1.545M to produce five new cultivars in 22 years (Table 12.3). PC was less expensive than FA due to the reduced number of seedling progeny that were assessed in years 8 and 9 (Table 12.1). Although seedling assessment was reduced in PC, all seedlings were left in the trial in order to provide even competition throughout the trial and hence the SPT maintenance costs of PC and FA were identical. The TS strategy was the least costly at $0.795M (Table 12.1) due to elimination of yield evaluation in the SPT stage.

A major expense for each strategy was the assessment of tree yield. This involves two or three harvests, at four to six week intervals of the nuts that have fallen from each tree. Nuts are collected using a hand-pushed finger-wheel harvester then de-husked, weighed for wet nut-in-shell yield, dried to 1.5% moisture content and then weighed to obtain dry nut-in-shell yield.

Methods to reduce the cost of assessing yield will be important in improving the efficiency of breeding. In some experiments in Bundaberg a small machine harvester is being trialled to increase the speed of harvesting and to reduce the labour costs. The shed and laboratory stages of drying and weighing will still need to be performed. Other options that may be explored include the use of

visual evaluation of yield by estimation of nut numbers on the ground or the use of X-ray techniques to count the number of nuts per tree (R.A. Stephenson, pers. comm.).

Table 12.3. Comparison of the four breeding strategies for cycle length, cost of breeding and gain from breeding expressed in Net Present Value (NPV), and ratio of gain to cost of breeding.

Strategy	Cycle length (years)	Cost of breeding 5 released cultivars (NPV)	Gain (±SD) per tree from commercial production for 20 years (NPV)	Ratio of gain to cost of breeding (x 100,000)
Full assessment	22	$1,545,922	$88 (23)	5.7
Progeny cull	22	$1,284,101	$75 (26)	5.9
Tandem	20	$795,508	$86 (21)	10.8
Cloned seedling	15	$986,075	$67 (24)	6.8

Gain from each breeding strategy was measured in NPV dollars as the profit from commercial production of the top five selected cultivars over 20 years expressed on a per tree basis. This was calculated using a selection index that used economic weights developed by Hardner et al (2006) to account for the impact of the independent variation in each trait on costs and returns for production and processing of macadamias. FA produced the greatest gain at $88 per tree and CS produced the least gain at $67 per tree (Table 12.3).

12.2.2 Rationale and possible variations of the strategies

The FA strategy was used in the previous work to produce 20 elite selections that are predicted to improve profitability by 30% compared to industry standards (McConchie, 2008). It was therefore the starting point for our comparisons.

PC is a variant of FA, whereby the cost of assessment is reduced by progressively reducing the number of seedlings that are assessed for yield as more information about the high-yielding genotypes is obtained. Gain from the PC strategy was slightly less than for FA, possibly due to the reduced data that was available for BLUP estimation of the clonal values used for selection.

TS was included in the comparison because it allows for selection of the higher heritability traits when there is only a single seedling tree of each genotype in the progeny trial. Selection for the low heritability trait, yield, is delayed until there is replication in the CCT. There is high selection pressure for kernel recovery in the SPT, with 20 genotypes selected from 1,200 seedlings. Problems with this strategy are the small number of genotypes for nut-in-shell yield selection and the impact of the negative correlation between nut-in-shell yield and kernel recovery. Increasing the number of genotypes at this stage may lead to greater gains. Despite these flaws, the TS strategy produced the highest ratio of gain to cost. The SPT of this strategy terminates when trees are five years old. It should be possible to increase seedling planting density and reduce costs and expand the number of genotypes evaluated in the CCT within a similar budget. Another modification of the TS strategy, which we plan to model in future work, is to select earlier for kernel recovery, in year 5 or 4. This would reduce the cost of the strategy and apply indirect selection on precocity.

CS was included because by eliminating the SPT it reduces the time to complete one cycle of breeding. We included six replicates of 200 genotypes in the trial so that it was a similar size to the other three strategies and provided clonal data that was of similar accuracy and because this is the number of replicates required for Australian Plant Breeders Rights testing. While replication of genotypes undertaken in this strategy is expected to increase gain by increasing selection accuracy, gain is compromised by the lower selection intensity (five cultivars from 200 genotypes) compared with the other strategies (five cultivars from 1,200 genotypes). Another loss in efficiency of this

strategy is the waste of resources in the replication of untested, non-elite gentoypes. Further research may include optimising the number of genotypes and replication.

The simulation model used in this study for predicting gains from four breeding strategies has provided a useful method to identify directions for our future breeding. Further work to refine this model and undertake sensitivity analysis of predicted response to alternative assumptions is planned.

13.0 Visual Estimation of Yield

Currently yield evaluation, by finger-wheel and hand-harvesting of the total crop, in the macadamia seedling progeny trials is a major cost. The objective of this study is to compare alternative methods of yield assessment in terms of accuracy and cost.

Different methods of indirect assessment of yield were trialled prior to nut drop, and also at each harvest time. The three methods used prior to nut drop are termed the 'in tree' methods and these were based on a score, a scaled estimate of nut number and a count method. Similarly, three methods were used to estimate yield after nut drop for each of the four harvests and these were also based on a score, an estimate of nut number and a count method based on a quadrat. The four individual measures were totalled across harvest times.

A literature review on visual estimation of yield was prepared for MS106 and has been included here in Appendix 9.

13.1 Methods

We selected a total of 60 trees that were located in the BRS rootstock trial and in the BRS B1.2 progeny. They were chosen to represent a range from low to high yields (based on previous yield data). One tree had died prior to evaluation so only 59 trees were evaluated.

The trees were harvested four times during the 2013 season and weights recorded to obtain actual harvest data. The standard method was to use a finger-wheel and hand-harvesting to collect all the nuts under each tree. These collections for each tree were counted and used to record **total nut number (tnn)**. A sample of these nuts were de-husked, dried and weighed to give an average nut weight. Total nut number was multiplied by average nut weight to give a **dry nut in shell mass (tnm)**.

A number of methods of indirect yield assessment were also undertaken on these 60 trees. The methods assessed prior to harvest while nuts were still in the tree included:

1. **In tree score** - In February, prior to nut drop, each tree was scored by 3 assessors on a 0-9 scale for yield.
2. **In tree number (estimate)** - In February, prior to nut drop, 3 assessors counted the number of nuts in one segment of the tree and multiplied by the number of segments to give an estimate of total nut number. This estimate was multiplied by average nut weight to calculate **In tree mass (estimate)**.
3. **In tree number (count)** - In February, prior to nut drop, 3 assessors counted the number of nuts in each tree using a hand held clicker. This count estimate was multiplied by average nut mass to calculate **In tree mass (count)**.

Three methods were used after nut drop and repeated at each of four harvests during the season. These methods were:

4. **Total score** - At each of 4 harvests the number of nuts that had fallen on the ground was scored by 3 assessors on a 0-9 scale for yield and summed to give a total score.
5. **Total number (estimate)** - At each of 4 harvests 3 assessors counted the number of nuts in one segment of the tree and multiplied by the number of segments to give an estimate of total number. This estimate was multiplied by the average nut weight to give **Total mass (estimate)**.
6. **Total number (quadrat)** - At each of 4 harvests a 50cm x 50cm quadrangle (Figure 13.1) was randomly placed on the ground in the SE, NE, NW and SW sectors and the number of nuts counted. This was completed by one assessor. This count was multiplied by the average nut weight to calculate **Total mass (quadrat).**

The measurements given in bold form the variables used in the following statistical analysis.

Figure 13.1. Metal 50cm x 50cm square used for estimation of nut number on the ground prior to harvest.

13.1.1 Statistical analysis

A linear model was fitted to total nut number (tnn) for each of the indirect yield assessment methods of in tree score, in tree number (estimate), in tree number (count), total score, total number (estimate) and total number (quadrat).

A linear model was also fitted to total nut mass (tnm) for each of the indirect yield assessment methods of in tree score, in tree mass (estimate), in tree mass (count), total score, total mass (estimate) and total mass (quadrat).

Average nut weight and Canopy area were included in the model to test for significant improvements in explaining variation in total nut mass. An overall effect for differences between assessors was also included in the model, as well as terms to test for the interaction of assessor with the indirect assessment method.

The models were assessed in terms of the percentage variance explained (R^2 value) and prediction errors for an individual observation predicted from the linear model.

13.2 Results and discussion

In general, the indirect methods measured through the duration of the harvest times (total score, total estimate and total count) explained a greater proportion of the variance in total nut number ranging from 79-84% than the in tree methods which ranged from 58-63% (Table 13.1). This was also consistent for total nut mass. For this trait, the in tree score methods alone explained from 53-66% variance, while the total harvest methods explained 66-79% (Table 13.3).

The relationships were improved by including information on average nut weight and canopy area of the tree. For total nut number, average nut weight provided little additional information, but

adjustments for canopy area of the tree improved the % variance explained by 9-15%. For total nut mass, the addition of the measured information on both average nut weight and canopy area of the tree improved this relationship by approximately 16-20%. For models including these additional traits, the in tree score methods explained from 73-80% variance, while the total harvest methods explained 82-85% (Table 13.3).

The effect of assessor was significant for both the in tree score and the total estimate measures, and so separate models were required for each assessor for these methods. Results for other methods could be pooled across assessors (Tables 13.1 and 13.3).

The prediction errors give a similar comparison between the in tree and total harvest methods (Tables 13.2 and 13.4). Note that separate models are used for each assessor where significant. In general, the total harvest methods give lower prediction errors than the in tree methods for both total nut number (Table 13.2) and total nut mass (Table 13.4).

Table 13.1. A summary of results for six indirect methods of assessing total nut number in explaining the variation in measured total nut number.

Total nut number	**% variance explained**				**Significance of assessor**
Indirect assessment method	**Overall**	**Assessor1**	**Assessor2**	**Assessor3**	
In tree score	58	60	55	60	*
+ average nut weight	59	60	55	62	*
+ canopy area	69	65	75	67	*
In tree number (estimate)	65	79	64	55	ns
+ average nut weight	65	79	63	54	ns
+ canopy area	77	82	76	74	ns
In tree number (count)	63	58	82	55	ns
+ average nut weight	64	59	84	52	ns
+ canopy area	78	79	88	68	ns
Total score	79	76	81	82	ns
+ average nut weight	80	76	81	82	ns
+ canopy area	83	80	87	83	ns
Total number (estimate)	82	83	79	83	**
+ average nut weight	82	83	79	83	**
+ canopy area	86	87	86	85	**
Total number (quadrat)		84			

Table 13.2. Average prediction errors for six indirect methods of assessing total nut number in predicting total nut number.

Total nut number	**Average prediction error (nut number)**			
Indirect assessment method	**Overall[#]**	**Assessor1**	**Assessor2**	**Assessor3**
In tree score	-	786	813	807
In tree number (estimate)	738			
In tree number (count)	994			
Total score	614			
Total number (estimate)	-	575	582	538
Total number (quadrat)	513			

[#] Overall prediction error is only given when the effect of assessor is not significant.

Table 13.3. Variation in total nut mass explained by each of six indirect methods of assessing yield. Significance of including terms for average nut weight and canopy area is also given.

Total nut mass	**% variance explained**				**Significantly different**
Indirect assessment method	**Overall**	**Assessor1**	**Assessor2**	**Assessor3**	**regressions**
In tree score	53	57	45	61	*
+ average nut weight	61	66	55	65	*
+ canopy area	73	71	77	71	*
In tree mass (estimate)	65	81	61	53	ns
+ average nut weight	ns	ns	65	ns	ns
+ canopy area	79	86	79	75	ns
In tree mass (count)	66	62	85	54	ns
+ average nut weight	ns	ns	88	ns	ns
+ canopy area	80	83	91	67	ns
Total score	66	70	67	75	ns
+ average nut weight	75	77	80	82	ns
+ canopy area	82	82	88	84	ns
Total mass (estimate)	79	80	78	80	*
+ average nut weight	80	ns	ns	ns	*
+ canopy area	85	85	86	84	*

Table 13.4. Average prediction errors for six indirect methods of assessing yield when predicting total nut mass.

Total nut mass	**Average prediction error (total nut mass (g))**			
Indirect assessment method	**Overall[#]**	**Assessor1**	**Assessor2**	**Assessor3**
In tree score	-	4030	3629	4135
In tree mass (estimate)	3279			
In tree mass (count)	4323			
Total score	3057			
Total mass (estimate)	-	3436	2884	3224

[#] Overall prediction error is only given when the effect of assessor is not significant.

13.2.1 Time taken for indirect assessment

A further consideration in method comparison is the time taken for each indirect assessment method. A summary of these times for each of the five indirect methods are given for each assessor in Figure 13.2. These results are further summarised based on average time taken for each assessor to make the measurement on a tree in Table 13.5.

In general the scoring methods are the quickest to perform, with the in tree score being faster than the total score. The in tree estimate was the next most time efficient method, with the total estimate and the quadrat method taking much longer than all. All indirect methods took much less time than the current harvest method which required over 10 minutes per tree, as opposed to the fastest method of in tree scoring which took 31 seconds per tree (on average, across assessors).

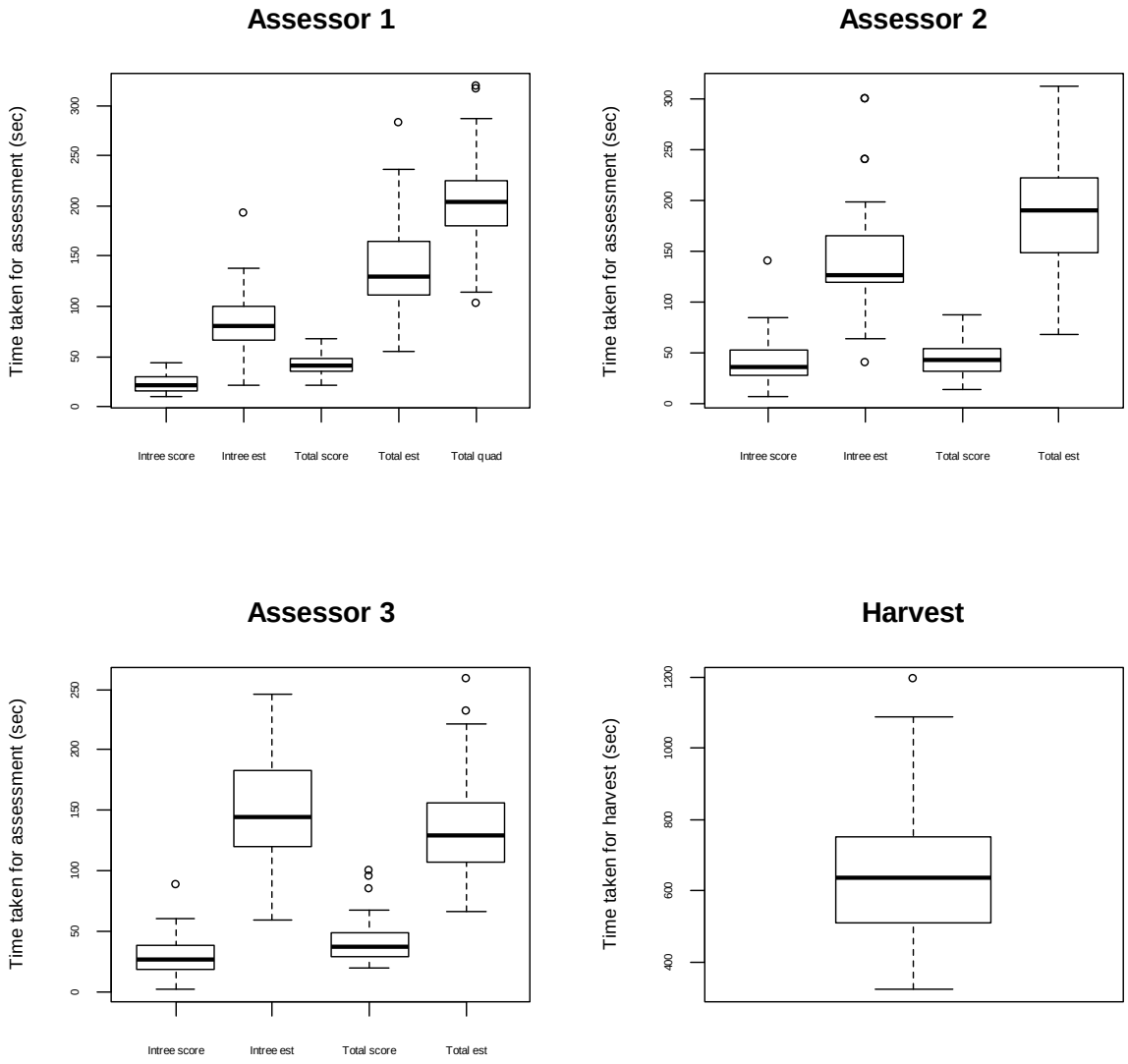

Figure 13.2. Summary statistics given as box plots for the time taken for each indirect yield assessment method for three assessors.

Table 13.5. Average time (seconds) taken for each indirect scoring method for assessing total nut number.

	Assessor		
Scoring method	**1**	**2**	**3**
Intree score	23	41	29
Intree estimate	84	143	152
Total score	43	45	42
Total estimate	139	188	136
Total quadrat	206		

13.2.2 Use of the in tree score method as a selection tool

The methods of scoring yield on a 0-9 scale are highly desirable in terms of time efficiency. Furthermore, the in tree scoring method has advantages over the total measure as a one-off visit prior to harvest with nil risk of information loss during the harvest process. While the in tree score method explains the lowest % variance of total nut mass relative to other indirect measures, and has one of the highest prediction errors in this relationship there is still potential in using it for selection of trees with superior yield within a breeding program. The following section documents the impact of using in tree score as a selection tool in the breeding program.

Analysis of this pilot study indicated that assessors tend to have a different underlying scoring scale for an indirect measure of total nut number. As a result, individual relationships between total nut number (and mass) were developed for each assessor. However, it is reassuring that there was reasonably strong correlation between the scores for each assessor (Table 13.6), even though the relationship with total nut mass was different for each assessor.

The use of the in tree score as a selection tool for total nut mass is now considered. To represent the selection process in a breeding program, we select the top 10% of trees (six in total for this study) using each measurement method of in tree score and total nut mass. Figures 13.3, 13.4 and 13.5 show the relationship between total nut mass and the in tree score for each assessor, where each tree is represented by a red dot on the graph. The selection of trees due to the total nut mass harvest data would take all trees (red dots) above the solid horizontal green line, and the selection of trees due to the in tree score method would take all trees to the right of the solid vertical green line. The intersection of the solid green lines defines four quadrants on the graph. The upper right quadrant contains trees which would be selected using either method. The bottom left quadrant contains trees which would be discarded by both selection measures. The loss of potentially high yielding trees through use of the in tree score selection is shown by the number of trees (red dots) in the upper left quadrant defined by the intersecting solid green lines. These trees are high yielding as measured by total nut mass, but have not been scored as highly by the in tree score method. Finally, the number of trees in the lower right quadrant are those lower yielding trees selected by using the in tree score method.

A second set of dotted green lines have been included to show a selection intensity of 20%. By relaxing the proportion of individuals selected there is a lesser chance of missing the top yielding trees.

If the realised error rate is too high, the method can be used for a preliminary selection of trees for intensive harvesting using established methods for total nut number and mass.

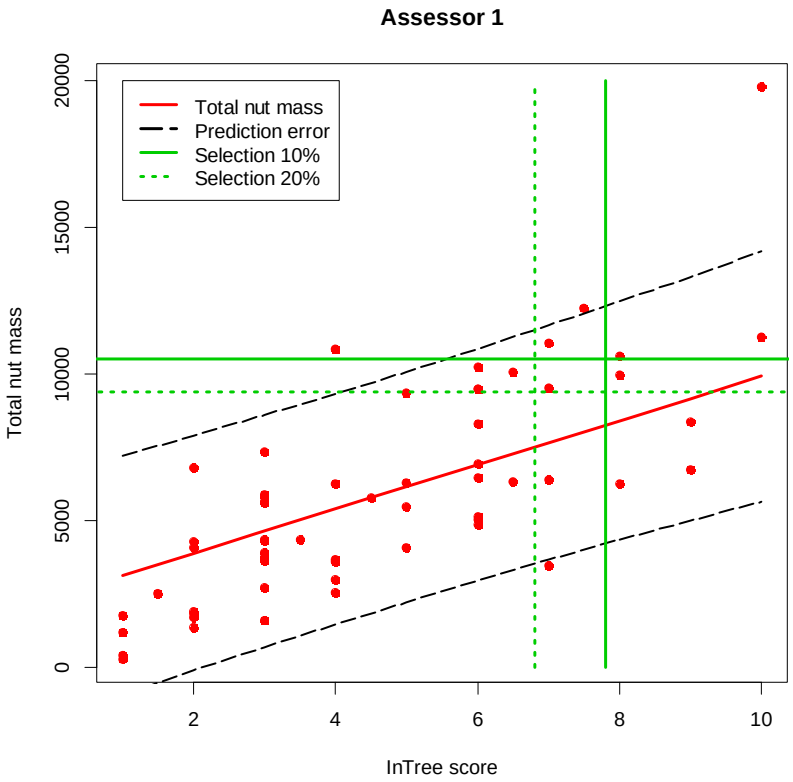

Figure 13.3. Regression of total nut mass against in tree score with prediction errors (black dotted lines) for individual tree yield from the regression equation – Assessor 1.

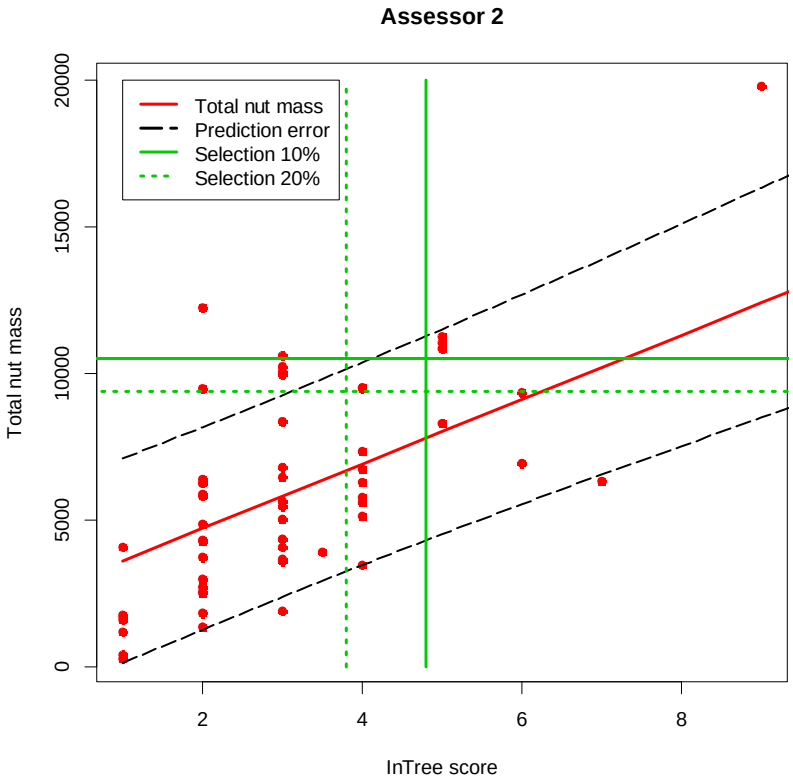

Figure 13.4. Regression of total nut mass against in tree score with prediction errors (black dotted lines) for individual tree yield from the regression equation – Assessor 2.

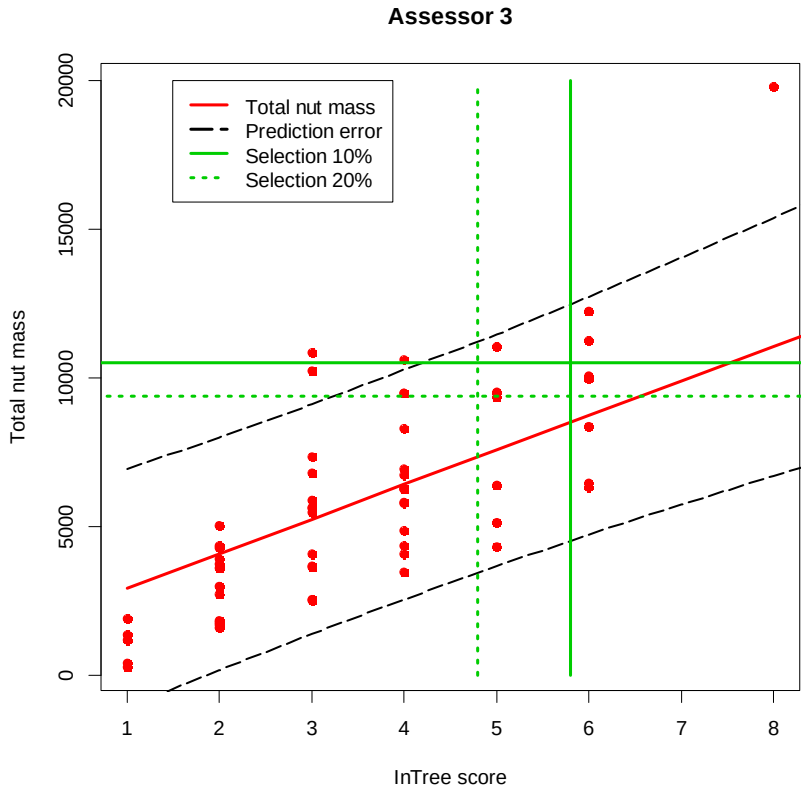

Figure 13.5. Regression of total nut mass against in tree score with prediction errors (black dotted lines) for individual tree yield from the regression equation – Assessor 3.

13.2.3 Consistency of the scoring method

There was substantial variability between assessors for the 1-10 in tree score method. Assessors 1 and 3 were relatively similar with a correlation of 0.80 (Table 13.6). Assessor 2 differed from assessors 1 and 3 with correlations of 0.54 and 0.57 respectively.

Table 13.6. Correlation between assessors of the 1-10 rating scale for the in tree score method.

Correlation	Assessor 1	Assessor 2	Assessor 3
Assessor 1			
Assessor 2	0.54		
Assessor 3	0.80	0.57	

The in tree scores of assessors 1 and 3 increased approximately linearly with increasing total nut mass (Figure 13.6). Assessor 2 poorly allocated high scores, tending to overestimate the score compared to total nut mass.

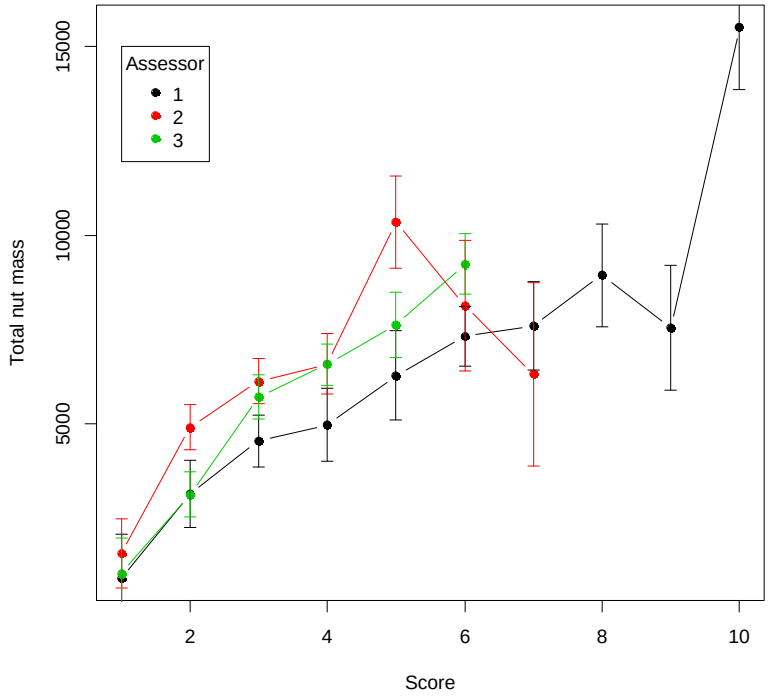

Figure 13.6. Mean and standard error of total nut mass for each category in the 1-10 rating scale for in tree score. Lines are shown to demonstrate change in mean across the rating scale for each assessor (categories with only 1 tree have been excluded from the graph).

13.3 Conclusions

Visual evaluation of yield has the potential to significantly reduce assessment time and costs. We propose to increase population sizes but this will only be possible if we have a less expensive yield evaluation system.

The in tree scoring method has advantages over the other systems we tested because it is completed with one evaluation of the tree in February before nut drop has occurred. It was the quickest method of assessment. It has the additional advantage that as a one-off visit prior to harvest there is no risk of information loss during the harvest process through accidental harvest by co-operating growers.

The in tree score method explained the lowest % variance of total nut mass relative to other indirect measures, and has one of the highest prediction errors in this relationship. However when combined with correlated information on average nut mass and tree size it explained 69% of the variance. We consider that it has merit as a method that will allow culling of the low yielding selections in a large segregating seedling population. In the final years of assessment the few remaining elite selections could be hand harvested to provide final yield data.

14.0 Forum on Regional Variety Trial Design

14.1 Methods

On Thursday the 8th November macadamia researchers, industry representatives, growers and specific experts were invited to attend and contribute to the 2012 Regional Variety Trial (RVT) Workshop. The workshop was designed to examine the technical issues of RVTs. The goal of the workshop was to create some new design principles for future RVT projects. A full report on the meeting by Robbie Commens is included as Appendix 10. The following is an abridged version of that report.

14.2 Results and discussion

At the conclusion of the RVT workshop all attendees voted on what they believed to be the most important points raised during the day. These were:

1) Communication. There is a need to improve communication with and between growers that are currently involved in RVTs. An annual grower RVT forum would be an ideal communication platform.

2) Create an improved RVT design that delivers a greater balance between the level of detailed information obtained from the RVTs and the cost of the RVTs.
    i. Further investigation into the viability of whole-row trial design is required taking into account parameters to a relevant enable statistical analysis.
    ii. RVTs with whole-row plots deliver the opportunity to move towards mechanical harvesting.

3) Increase grower involvement in future RVTs to reduce running costs. Grower run RVTs will greatly reduce maintenance costs however the following challenges need to be addressed:
    i. Consistent management practices (fertiliser, canopy management, irrigation/water) across the different sites.
    ii. The data that growers record. A template needs to be created for growers to utilise.
    iii. An individual needs to be made responsible to coordinate RVT growers.
    iv. Microclimate information needs to be captured at each site.

4) Mechanical harvesting in RVTs required.
    i. The cost of hand harvesting is not sustainable into the future.
    ii. There is a need to move to mechanical harvesting within RVTs to reduce costs of hand harvesting and ensure ongoing harvesting ability as suitable consistent labour is difficult to find.
    iii. New RVT design should accommodate currently available mechanical harvesting equipment.

5) Investigate the opportunities for topworking within, or in association with, RVTs to save resources and time.

6) RVTs need to capture and deliver information that adopters are seeking.
    i. The data analysis needs to be sufficient enough to enable a statistical analysis and a confident recommendation to be made in the most economical manner possible.
    ii. Investigate the value of incorporating a 'ranking' of varieties by growers and researchers.

7) Investigate opportunities for a tiered or staged RVT system, similar to the citrus RVT program of the following tiers:
    i. Experimental – undertaken by growers in commercial orchards with grower contributions.
    ii. Semi-commercial – grower sites but with a greater level of data collected.
    iii. Commercial – varieties ready for release.

Based on the information obtained from the RVT workshop the macadamia industry, through MIVIC, we will work towards creating an RVT program that has a more suitable balance between statistical relevance of the data and cost to the industry. The workshop delivered an excellent platform for leading growers, researchers and experts to discuss the challenges of the current system, work through potential solutions and introduce new ideas into the RVT program.

As a result of the workshop, the future of the macadamia industries RVT program will most likely incorporate:

1) A greater level of grower involvement;
2) a more economical balance between gathering statistically relevant data and the cost of maintaining the RVTs; and
3) mechanical harvesting systems within the RVTs.

15.0 Precocity Trial

Macadamia trees in commercial orchards grow to a height of 12-15 m and may take 15 years to attain peak production. The large size and slow maturity of the trees pose problems for breeding and selection. Topp et. al, (2012) proposed a two-stage (tandem) breeding strategy that finishes two to three years earlier than the strategy currently employed by the Macadamia Breeding Program. Trees are planted at higher densities and assessed for tree height, canopy width, early bearing and total kernel recovery until age four or five. The best genotypes from this strategy are then incorporated into a cultivar trial and assessed for all traits of interest, including yield. This strategy allows for selection of the higher heritability traits when there is only a single seedling tree of each genotype in the progeny trial. Selection for the low heritability trait, yield, is delayed until there is replication in the cultivar trial.

The precocity trial (BNAM11) was designed to test the tandem breeding strategy outlined by Topp et. al, (2012). The establishment and current status of this trial is described here.

15.1 Methods

15.1.1 Parental selection

All offspring were open-pollinated, collected in 2009. Maternal parents were selected to include highly precocious genotypes, as well as a number of the B1.1 generation elite selections. Cuttings from the maternal genotypes were propagated by Hidden Valley Plantations, for use as standards in the trial. The propagation protocol used is described in Chapter 3.

15.1.2 Seed germination

Seeds were planted into sand in square native propagation tubes (50mm x 125mm deep) and placed in a shadehouse with 30% shade. Following germination, seedlings were fertilised with Osmocote Plus Native 8-9 month slow release fertilizer.

15.1.3 Planting

The precocity trial was planted at the Maroochy Research Facility in November 2011 at tree spacings of 1 × 4 m. Trial design incorporated the open-pollinated seedlings and maternal genotypes in an incomplete block design. Prior to planting, soil tests and improvement were conducted, rows were mounded and irrigation installed.

A total of 986 trees were planted in the trial. 720 seedlings from 32 maternal parents were planted (Table 15.1), with 23 seedlings per family on average. 139 standard trees from 29 maternal genotypes were also planted, plus 127 buffer trees. Trees were fertilised, irrigated and managed for insect and pest control as per commercial practices (O'Hare et al., 2004). To allow for more effective weed spraying, pruning was conducted to remove branches below 50cm where tree height allowed. In order to more accurately determine tree size and habit no other form pruning was undertaken.

Table 15.1. Counts of progeny in each of the open-pollinated families in the precocity trial. OP = open pollinated.

Family	Count of progeny
246 X OP	26
344 X OP	25
788 X OP	26
791 X OP	25
814 X OP	25
A268 X OP	25
A376 X OP	25
A38 X OP	25
A4 X OP	24
A538 X OP	25
BAMAM02-6-3 X OP	26
BQBRS97-2-46 X OP	22
BQBRS97-6-16 X OP	25
BQBRS98-10-111 X OP	26
BQBRS98-10-93 X OP	11
BQBRS98-11-35 X OP	23
BQBRS98-11-80 X OP	6
BQBRS98-13-115 X OP	15
BQBRS98-14-25 X OP	21
BQBRS98-14-93 X OP	18
BQBRS98-15-37 X OP	21
BQBRS98-16-41 X OP	26
BQBRS98-6-73 X OP	24
BQBRS98-6-79 X OP	25
BQBRS98-7-109 X OP	23
BQBRS98-7-74 X OP	17
BQBRS98-8-87 X OP	16
BQBRS98-9-72 X OP	26
D4 X OP	23
Daddow X OP	25
M141 X OP	25
Macadamia jansenii (point) X OP	25

15.2 Results

The precocity trial is currently three years old. Measurements have been collected for tree health, yield, tree height and canopy width across multiple years (Table 15.2). No trees produced nuts in 2012, 2013 or 2014. A small number of trees currently appear likely to produce nuts in 2015. Large variations in tree size are present, ranging from 0.2 to 4.4 m in height.

Table 15.2. Data collection by year.

Year	Tree health	Yield	Tree height	Tree canopy width
2012	✓	✓		
2013	✓	✓	✓	
2014	✓	✓	✓	✓

15.3 Conclusions

The precocity trial established in this project will allow assessment of the tandem selection strategy aimed to reduce breeding time and increase gain per unit cost. The trial is estimated to continue for one to two more years, dependant on yield patterns.

16.0 Molecular Markers in Breeding

Various experiments were conducted on trees from the breeding program using molecular genetics tools. Three primary areas of research were undertaken:

(1) confirming phenotypic cultivar identification in the rootstock trials;
(2) fingerprinting B1.1 elite selections for paternity analysis and quality control;
(3) testing the effectiveness of controlled cross-pollinations;

These are discussed in detail below.

16.1 Quality Control of Rootstock Trials

In 2002 and 2003 five trials were established to investigate rootstock effects for macadamias (see Chapter 6). Some issues with tree identification were later discovered at the Newrybar rootstock trial. Phenotypic examination of the trees suggested that the originally recorded scion cultivars were incorrect for a number of trees. All trees were phenotypically assessed, and differences to original cultivar IDs recorded. In order to verify phenotyping, DNA of 26 trees with potentially incorrect cultivar IDs was extracted and genotyped by DArT markers at the Diversity Arrays Technology Pty Limited (Canberra, Australia; http://www.triticarte.com.au). These results are displayed in Table 16.1.

Of the 26 trees genotyped, 21 matched the cultivar IDs determined phenotypically. Most mismatches between original and genotypic IDs were due to the 842 tree used to source most of the propagation material for that cultivar having been overgrown by its H2 seedling rootstock. Similarly, three other mismatches (RNEWR02-8-35, RNEWR02-9-7 and RNEWR02-12-42) also appear to be the result of rootstocks overgrowing scions following planting in the trial. These issues highlight the utmost importance of: (i) cultivar verification of source trees for propagation material; (ii) correct labelling of propagation material and nursery trees; (iii) correct allocation of trees to planting spaces within trials; and (iv) high levels of trial maintenance to prevent overgrowing by rootstocks.

Finally, the relatively high match rate between phenotypes and genotypes provided confidence that phenotyping alone could be used to verify cultivar ID at all other trials. DNA fingerprinting currently remains too expensive for widespread use within the Macadamia Breeding Program, however as demonstrated here is a highly valuable tool for smaller-scale investigations.

Table 16.1 Newrybar scion phenotypic and genetic cultivar identification, and genetic distances to the tested cultivars. Smallest genetic distances are in bold print.

Field position	Original rootstock ID	Original scion ID	Phenotypic scion ID	Genetic scion ID	P & G Match	Genetic distance												
						A203	A268	H2	344	660	695	741	781	791	814	816	842	849
RNEWR02-10-11	A16	842	H2 progeny	H2 progeny	Yes	34	34.2	**20.6**	31.3	31.6	39	31.1	27.4	33.4	28.2	25.6	30.2	28.1
RNEWR02-10-27	246	842	H2 progeny	H2 progeny	Yes	34	34.1	**20.6**	31.4	31.7	39	31.2	27.4	33.4	28.3	25.8	30.3	28.2
RNEWR02-10-43	695	A16	A203	Unknown	No	30.3	31	34.3	30.3	33.3	20.7	32.8	29.7	32.1	30	29.6	28.6	29.6
RNEWR02-11-17	A16	842	H2 progeny	H2 progeny	Yes	33.2	33.3	**19.9**	30.8	31.3	38.2	30.7	26.8	32.7	27.7	25.1	29.2	27.5
RNEWR02-11-21	842	842	H2 progeny	H2 progeny	Yes	33.9	33.8	**20**	30.8	31.3	38.3	30.8	26.8	32.7	27.8	25.2	29.7	27.8
RNEWR02-11-3	695	A268	344	344	Yes	27.9	30.2	33.8	**0.1**	14.6	34.1	13.9	21.3	29.4	22	23.6	21.4	21.1
RNEWR02-11-31	NG8	842	H2 progeny	H2 progeny	Yes	33.6	33.7	**19.9**	31	31.3	38	30.8	27.1	32.8	27.9	25.1	29.6	27.8
RNEWR02-11-39	NG8	842	H2 progeny	H2 progeny	Yes	33.6	33.4	**19.7**	30.7	31.3	37.6	30.8	26.7	32.4	27.5	25	29.3	27.4
RNEWR02-11-42	816	842	Unknown	H2 progeny	No	27.7	28	**20.7**	25.7	27	34.5	26.8	21.8	31.5	24	26.7	21	22.2
RNEWR02-12-14	A268	695	344	344	Yes	27.8	30.1	33.6	**0**	14.6	34.1	13.9	21.5	29.4	22	23.8	21.5	21.1
RNEWR02-12-15	842	842	H2 progeny	H2 progeny	Yes	33.7	33.8	**19.8**	31	31.4	38	30.9	26.7	32.7	28	25.2	29.6	27.6
RNEWR02-12-42	842	A268	814	842	No	22.6	23.5	32.8	20.8	24.5	31.9	23.8	18.5	28	19.6	22.8	**0.1**	15.5
RNEWR02-13-3	246	842	H2 progeny	H2 progeny	Yes	34	34	**20.3**	31.3	31.5	38.8	31	27.2	33.2	28	25.5	30.1	28
RNEWR02-13-34	A16	842	H2 progeny	H2 progeny	Yes	33.6	33.8	**20.4**	31	31.3	38.6	30.9	27	33.1	27.9	25.5	29.7	27.7
RNEWR02-13-8	842	842	H2 progeny	H2 progeny	Yes	32	32.6	**19.6**	28.2	28.8	38.7	28.1	24	31.8	22	23	27.2	24.7
RNEWR02-8-13	246	842	H2 progeny	H2 progeny	Yes	33.9	34	**20.3**	31.1	31.5	38.6	31	27.1	33.2	28.1	25.6	30	27.9
RNEWR02-8-35	816	842	H2 progeny	816	No	28.8	27.4	33.5	23.2	24	35.3	23.4	19.4	31.3	20.9	**0**	22.4	18.2
RNEWR02-8-45	344	816	814	814	Yes	27.7	28.7	34.7	22.1	23.3	35.2	22.9	17.7	29.7	**0.2**	22	20.4	18.5
RNEWR02-8-6	816	842	695	695	Yes	33.8	30.7	33.1	34.7	35.6	**0.2**	35.4	37	31.9	35.7	36	34.2	36.4
RNEWR02-8-7	D4	842	H2 progeny	H2 progeny	Yes	34	34.1	**20.6**	31.3	31.6	38.9	31.1	27.4	33.4	28.2	25.7	30.2	28.1
RNEWR02-9-14	842	842	H2 progeny	H2 progeny	Yes	33.7	33.8	**20.2**	31	31.3	38.6	30.8	27	33.2	27.9	25.5	29.8	27.7
RNEWR02-9-23	814	NG8	A268	A268	Yes	20.6	**0**	36.4	29.8	30.8	29.5	30.5	24.7	30.3	28.3	27.7	23.8	24.9
RNEWR02-9-27	NG8	842	H2 progeny	H2 progeny	Yes	34	34.2	**20.7**	31.4	31.6	38.9	31.1	27.4	33.5	28.3	25.7	30.3	28.1
RNEWR02-9-35	842	842	H2 progeny	H2 progeny	Yes	33.9	34.1	**20.6**	31.3	31.5	38.9	31.1	27.3	33.4	28.2	25.6	30.2	28
RNEWR02-9-36	741	814	695	695	Yes	34	30.9	33.2	34.7	35.6	**0.1**	35.5	37.2	32	35.7	36.1	34.4	36.5
RNEWR02-9-7	H2	A268	A203	H2 progeny	No	24.3	24.9	**19.5**	27.2	29.2	35.4	29	23.6	30.1	25.5	26.1	22.7	23.2

16.2 Fingerprinting B1.1 Elite Selections

Fingerprinting of the B1.1 elite selections was undertaken with simple sequence repeats (SSR) and DArT markers. The different experiments undertaken for each of these are outlined below.

16.2.1 SSR Paternity Analysis

The objective of this project was to apply recently described SSR markers to paternity testing of offspring from known mother trees the Childers Regional Variety Trial (RVT). Better understanding of pollen movement within the RVTs will assist in nut collection planning for the new project MC14000, where polycross populations will be created using the elite first generation AMS genotypes as parents. Microsatellites are the marker of choice for most parentage studies because of their co-dominant inheritance and high variability.

A two-phase approach to paternity testing was undertaken. The objective of Phase 1 was to assess the suitability of the new SSR markers for paternity testing, and the results from this phase are provided here. The paternity analysis itself will be undertaken in Phase 2, and will be presented at a later date. This report on the SSR fingerprinting of the elite RVT selections was prepared by Cathy Nock from Southern Cross University School of Plant Science.

Materials and Methods

Leaf material was collected from all known potential parents within and bordering the Childers orchard. Sampling included replicates (two individual trees of each selection and cultivar) to assess the accuracy of allele scoring. In total, 32 individuals were sampled. Leaf tissue was dried with silica before sending to Southern Cross Plant Science, Southern Cross University, Lismore NSW where it was stored at room temperature prior to extraction.

Total DNA was extracted using a Qiagen DNeasy Plant Mini kit (Qiagen) according to manufacturer's protocols. Final DNA concentrations ranged from 28.5 to 366.9 ng/µl and were normalized to 4ng/ µl prior to PCR. The forward primer of each of 12 *Macadamia integrifolia* microsatellite primer pairs (Nock *et al.* in press) was fluorescently labelled on the 5' end and the following PCR protocol was used: in 20 µL reaction volumes containing approximately 20 ng DNA template, 0.5 U Platinum *Taq* (Life Technologies), 2 µL Platinum *Taq* PCR buffer, 0.1 mM dNTPs, 2 mM $MgCl_2$, 0.2 µM of each primer, and sterile water to 20 µL. Thermal cycling was conducted in a GeneAmp PCR System 9700 (Life Technologies) with the following conditions: initial denaturation at 94ºC for 2 min; followed by 35 cycles of 94ºC for 10 s, annealing T_a (Table 16.2) for 10 s, extension at 70ºC for 1 m; followed by final extension at 70ºC for 5 min.

Genotypic data were collected using an ABI PRISM 3730 Genetic Analyser (Applied Biosystems Inc.). Allele size was scored in reference to ABI PRISM GS (Liz) internal size standards using the program Geneious, version 6.1.6 (Biomatters Ltd). Genetic diversity parameters, principle coordinates analysis and estimates probabilities of identity and exclusion were calculated using Genalex v6.5 (Peakall and Smouse 2012). The program ML-NullFreq was used to test for null alleles as they can interfere with parentage identification (Kalinowski and Taper 2006).

Results

The 12 SSR loci amplified in all 62 individuals. Variable peak heights resulted in allele scoring errors in four individuals at locus Mac003. This locus was excluded from further analyses. Mean observed and expected heterozygosity were 0.60 and 0.60 respectively. A total of 50 alleles were detected with an average of 4.55 alleles per locus (Table 16.2).

Table 16.2. Genetic diversity statistics for 11 SSR loci in the parent population

Locus	Na	I	Ho	He
Mac005	5	1.21	0.84	0.66
Mac008	3	0.16	0.03	0.06
Mac012	5	1.13	0.34	0.62
Mac004	5	1.40	0.25	0.73
Mac002	4	1.20	0.66	0.67
Mac011	5	1.49	0.94	0.76
Mac009	2	0.08	0.03	0.03
Mac001	5	1.40	0.78	0.73
Mac007	5	1.45	0.81	0.72
Mac010	5	1.50	0.97	0.76
Mac006	6	1.72	0.97	0.81
Mean	4.55	1.16	0.60	0.60

Na = number of alleles; I = Shannon's information index; Ho = observed heterozygosity; He = expected heterozygosity

Identical multi-locus genotypes (22 of 22 alleles shared) were obtained for replicate trees of 30 selections/cultivars for 11 SSR loci. Identical genotypes were also shared by CH52_7/CH53_2 and CH51_9/52_1. Probability of identity (PI) provides an estimate of the average probability that two unrelated individuals drawn from the same population will have the same multi-locus genotype. PI for the dataset and 11 loci was 5.2×10^{-9}. Probability of identity of siblings (PIsib) is the probability that full siblings will share the same multi-locus genotype. PIsib for the dataset was 3.8×10^{-4}, suggesting that these trees are clones.

In total, 31 genotypes were observed. One pair of replicate samples, CH48_12 and CH_52_2, had distinct genotypes indicating that these trees are not clones. In addition, the genotypes of CH48_12 and CH_52_2 were distinct from all others, so sampling error is an unlikely explanation for the result.

Multi-locus allelic profiles and heterozygosity for each distinct genotype are provided in Table 16.3. Genetic distances among distinct genotypes are illustrated in a principal coordinates plot, Figure 16.1. Null alleles were predicted at 2 SSR loci, Mac004 and Mac0012. Following removal of the these loci, probability of identity, PI was 2.0×10^{-7} and probability of identity of siblings, PIsib was 1.9×10^{-3} for the reduced set of 9 SSR loci.

Probability of exclusion with one-parent known was estimated to determine the number of loci needed to confidently exclude potential pollen source in future assessments of paternity among seedlings of elite selections where identity of the mother tree is known. The combined probability of exclusion for paternal assignment with one-parent known was 96% for 7 loci, increasing to 99% with 9 SSR loci, Figure 16.2.

Table 16.3. Heterozygosity and multi-locus allelic profiles for 31 distinct genotypes.

Variety	Rep 1 tree	Rep 2 tree	Ho	Allelic profile
A	46_4	51_1	0.545	337343388388309309235235289289184188243243417419367383260270326332
B	49_15	52_14	0.727	337343388388311311235239289295184192243243413415381383268270326353
C	53_11	51_8	0.727	331343388388309321237237295297180192243243417419373389270282326328
D	47_9	50_1	0.636	334337388388313313235235283295180184243243413415373381268298324353
E	52_3	47_3	0.545	331343388388309309223223283283180192243243413419367373270282328330
F	52_6	51_12	0.818	331337396398309313239239289295184192243243413415373381270282326328
G	47_10	51_7	0.364	337343388388313313223223289289180180243243413413373383270298324326
H	51_15	46_6	0.636	331337388388309311237237289289184192243243417419367373260270326332
I	50_13	49_3	0.636	337343388388309309237237289295186192243243417419383389282298324328
J	50_15	49_13	0.545	331343388388309309235235289289184192243243417419367383260298324332
K	47_6	53_14	0.273	337343388388313313223223283289180180243243413413373373270270326330
L	52_2		0.545	337337388388313313223223289295180188243243413415381383268298324326
M	51_2	53_7	0.636	343343388388313321235237283297180186243243415417373373270298324326
N	52_10	53_1	0.636	331343388388309313237237283283180192243243413419367373260270330332
O	51_10	49_5	0.455	331337388388309309235235289289184188243243417419373373270282326328
P	50_7	48_8	0.727	331343388388309321235237289289180192243243417419367383260298324332
Q	51_6	46_2	0.545	331343388388309309223223283295180192243243413413373389270282328330
R	46_3	53_12	0.545	337343388388313313223223289295180184243243413413381383268298326330
S	53_3	50_8	0.636	331343388388309309239239295297186192243243417419373389270282326328
T	53_5	50_14	0.727	337343388388313313235237289295180184243243415417373381268270326353
246	51_13	53_9	0.636	337343388388313313223223283289180188243243413415373383270298324330
344	52_8	50_2	0.182	337337388388313313239239289289186192243243419419383383270298324324
741	51_3	47_12	0.727	337343388388309309237239283289188192243243415419373383270298324326
816	50_12	47_4	0.727	337343388388313317235239289289184192243243411415373383270298324326
A16	50_6	49_14	0.545	331337388388309309235235295295188192243243413419367389260282328332
A376	51_5	52_15	0.727	328334388388309313233235283289184192243243413417373373282298328330
A422	51_9	52_1	0.545	337343388388309309235235283295180188243243413413373389260270330332
A447	52_4	47_15	0.636	337343388388309313223223283289180188243243413415373373270282328330
A538	53_15	50_9	0.818	337343388388309313235235283295180188243246413419373381282298328332
Daddow	53_0	45_13	0.636	337337388388311311235237289295180184243243413417373381268270326353
Unknown variety	48_12		0.636	337337388388309313237237283295180192243243415419373389282298324328
Mean			0.602	

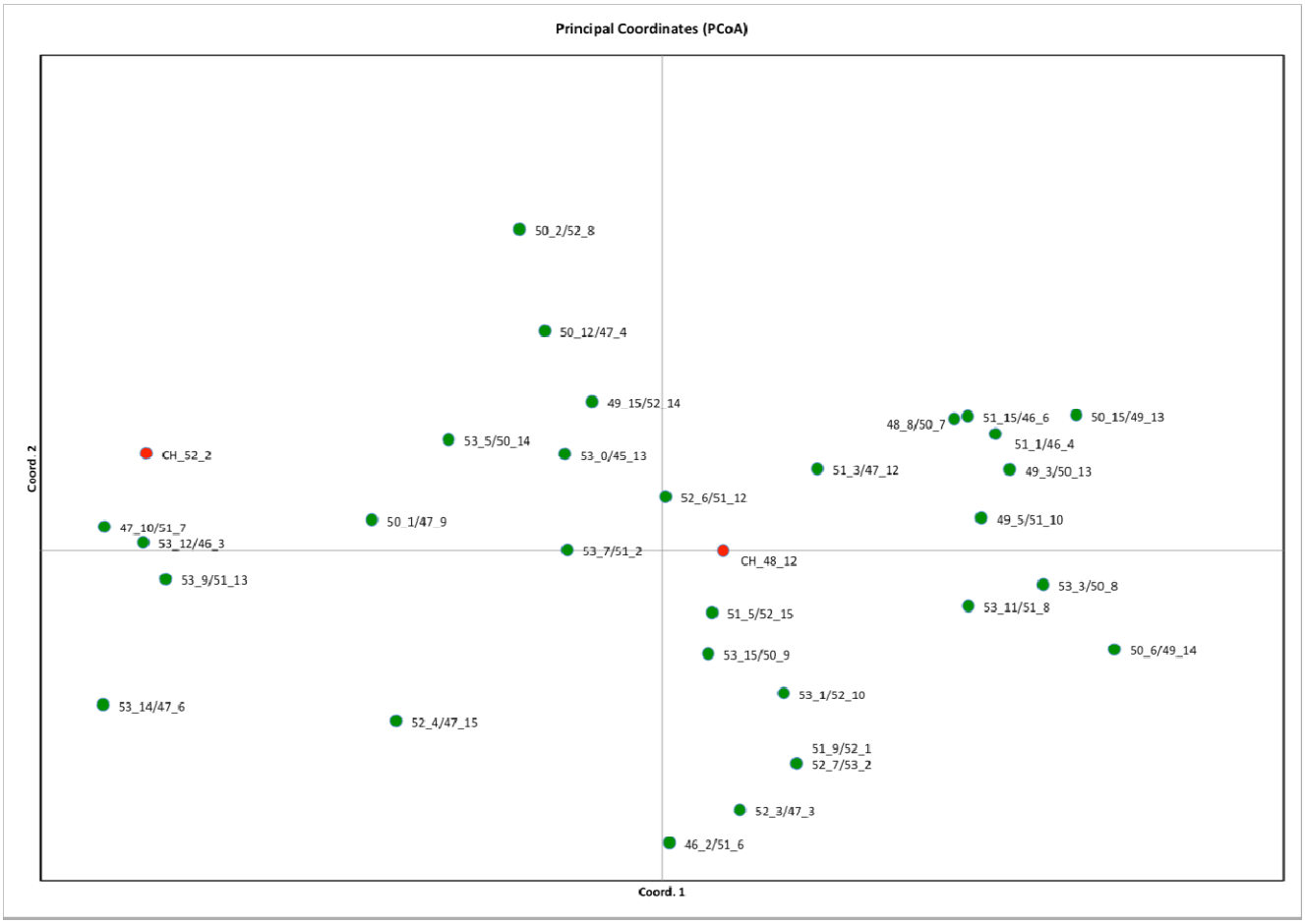

Figure 16.1. Principal coordinates plot based on genetic distance among 31 multi-locus genotypes. First and second coordinates explain 19.98 and 15.68% of the variation respectively. Red dots represent replicate samples with distinct genotype.

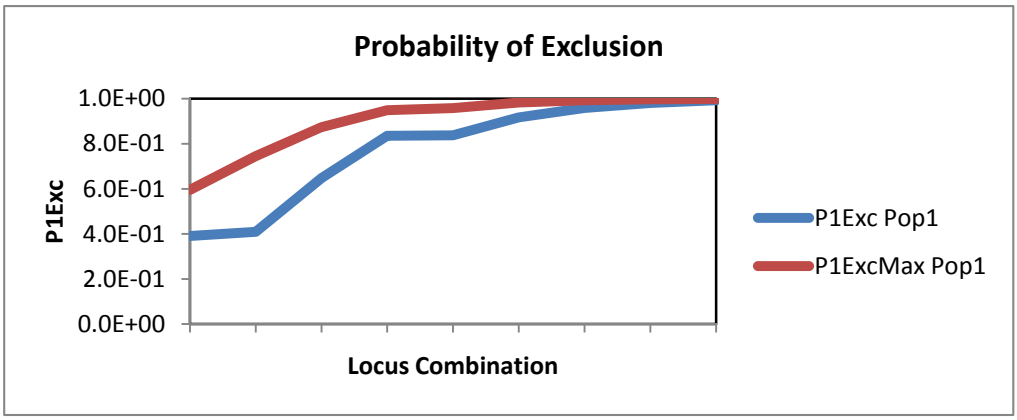

Figure 16.2. Probability of paternal exclusion with one-parent known for increasing combinations of SSR loci from 2 to 9.

Conclusions

The tested SSR markers amplified in all individuals of the parental population. Among replicate samples of 31 selections/cultivars, two with identical genotypes were identified, Samples 'A403' (52_7/53_2) and 'A422' (51_9/52_1). Phenotypically all four of these trees match 'A422', and so the error has been attributed to mislabelling of the 'A403' trees prior to planting in the trial.

The pair of replicates for variety 'L' (52_2 and 48_12) produced distinct genotypes. Phenotypic examination of the trees suggested that the 48_12 was incorrectly allocated to variety 'L'. This tree

was included as a potential parent in the paternity analysis, however its true identity could not be determined.

Results of multi-locus genotyping of the parental population during Phase 1 suggest the tested SSR markers have high statistical power for paternal assignment of progeny. The combined probability of paternal exclusion with one-parent known is 96% for 7 loci, increasing to 99% with 9 SSR loci.

The SSR genetic profiles developed in this study have since been successfully applied to verifying B1.1 selections from RVT grower trials. The tested trees are being used as a source of propagation material in preparation for the release of the new cultivars in 2017. Through the application of genetic markers, a mistake in the map at one of the RVT grower trials was identified and successfully resolved.

16.2.2 DArT B1.1 Fingerprinting

Genetic distances between B1.1 elite elections and a selection of cultivars was determined using the DArT markers described in section 16.1. It was observed that the B1.1 selections are genetically diverse (Figure 16.3), with clusters of half-sib and full-sib individuals.

Selections G and I were observed to be genetically identical, as were N and R. This was not observed to be the case in the SSR study. The source material for this DArT analysis were grafted trees removed from an early RVT trial, and given the above findings it is likely that mislabelling of the trees occurred at some point. Based on the SSR analysis it was determined that G and R had the incorrect genotypes, and were subsequently removed from Figure 16.3.

Cultivars 'HAES 660' and 'HAES 741' were found to be genetically identical. This has been observed in previous studies using SSRs, despite the cultivars being considered phenotypically distinct by some in the industry.

16.2.3 Conclusions

Both SSR and DArT markers have been shown to be useful in identifying, and describing relationships, between individuals of *Macadamia*. The application of these tools has detected mistakes in tree records, and has provided confidence in tree identification for industry propagation.

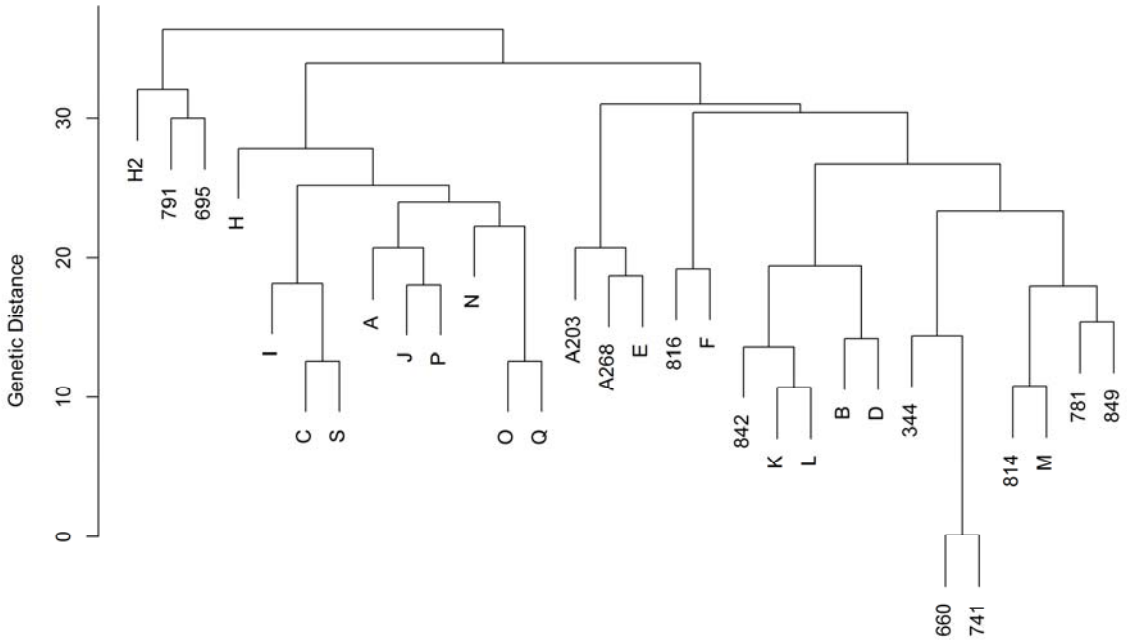

Figure 16.3. Dendrogram of genetic distances between B1.1 elite selections and a selection of cultivars.

16.3 Testing Cross Pollination System

Most progeny within the breeding program to date have been produced via controlled cross pollinations. These were performed by hand, using the method described in Chapter 3. Macadamia cultivars are typically mostly self-incompatible, and so emasculation of flowers prior to pollination is not undertaken. Some self-compatibility has been observed, however, and so there is a chance of some progeny being selfs of the recorded female parent. There is also a possibility of accidental pollen contamination which may result in differences between the recorded male parent of a progeny, and the actual parent.

In 2013, DArT DNA fingerprinting was employed on a sample of cross-pollinated progeny to determine if the genetically determined parentage matched the recorded parentage. A number of B1.2 progeny were examined, as well as nine B1.1 interspecific hybrids. All putative parents were genotyped, with the exception of cultivar 'NG7' where leaf material was unable to be obtained.

16.3.1 Parentage Testing of B1.2 Progeny

A total of 125 B1.2 progeny were genotyped and genetic distances used to determine most likely parents among those tested. Genetically determined parents matched recorded parents for 94% of the progeny (Table 16.4). Four progeny were mismatched for both female and male parents, suggesting an issue with tree labelling or record-keeping rather than cross-pollination. Two progeny were mismatched for the female parent (Table 16.5), although this is uncertain as the second most likely candidate parent was the same as the recorded parent. Finally, two progeny (1% of all progeny tested) were mismatched for the male parent. This result suggests that pollen contamination during cross-pollination is rare, and that the parentage of most progeny is likely to be accurate.

Table 16.4 Summary of matches and mismatches between recorded and genetically identified parents.

Recorded vs. genetic parents	Count of progeny	% of total
Match	118	94
Complete mismatch	4	3
Partial mismatch (female parent)	2	2
Partial mismatch (male parent)	1	1
Total	125	

Table 16.5 Progeny with complete or partial mismatches with recorded parents. Genetically identified parents, where they differ from recorded parent, are in parentheses.

Progeny ID	Female parent	Male parent	Status
BQBRS03-35-14	741 (781)	NG29 (A4)	Complete mismatch
BQBRS03-35-11	NG18 (804)	Beaumont (333)	Complete mismatch
BAMAM03-10-25	Renown (333)	741 (781)	Complete mismatch
BQBRS01-5-16	Yonik (741 or 660)	NG8 (333)	Complete mismatch
BAMAM03-9-2	Renown (A4*)	741	Partial mismatch
BQBRS03-37-8	Renown (A4*)	741	Partial mismatch
BAMAM03-10-4	781	A4 (804)	Partial mismatch

* However next closest match was 'Renown'.

A second consideration in this investigation was the percentage of unwanted selfed progeny (where the female parent was also the pollen parent). This had the potential to occur as the florets were not emasculated prior to cross-pollination, due to most cultivars being largely self-incompatible. Of the progeny tested, 15 had the self-fertile cultivar '741' as a female parent. One of the progeny from these crosses was a complete mismatch for both female and male parents (Table 16.4), however all

remaining '741' progeny were identified as being correctly-allocated outcrosses. These results suggest that self-fertility of female parents is not likely to be a concern for crossing. Even in self-fertile varieties, outcrossed pollen may be likely to outcompete self pollen when both are present on a stigma, or selfed nutlets may be preferentially aborted.

16.3.2 Resolving B1.1 Hybrid Status

Interspecific crosses conducted as part of the B1.1 progeny were observed to possess a lower parental match rate than that observed with the B1.2 progeny. Two of the nine progeny tested appeared to be selfs for the mother cultivar '660', rather than interspecific crosses (Figure 16.4, progeny '11-1' and '9-20'). The remaining progeny were all correctly identified as hybrids between '660' and either *M. jansenii* (progeny '47-16', '48-8', '23-9' and '34-11') or *M. ternifolia* (progeny '37-1', '15-1' and '23-4').

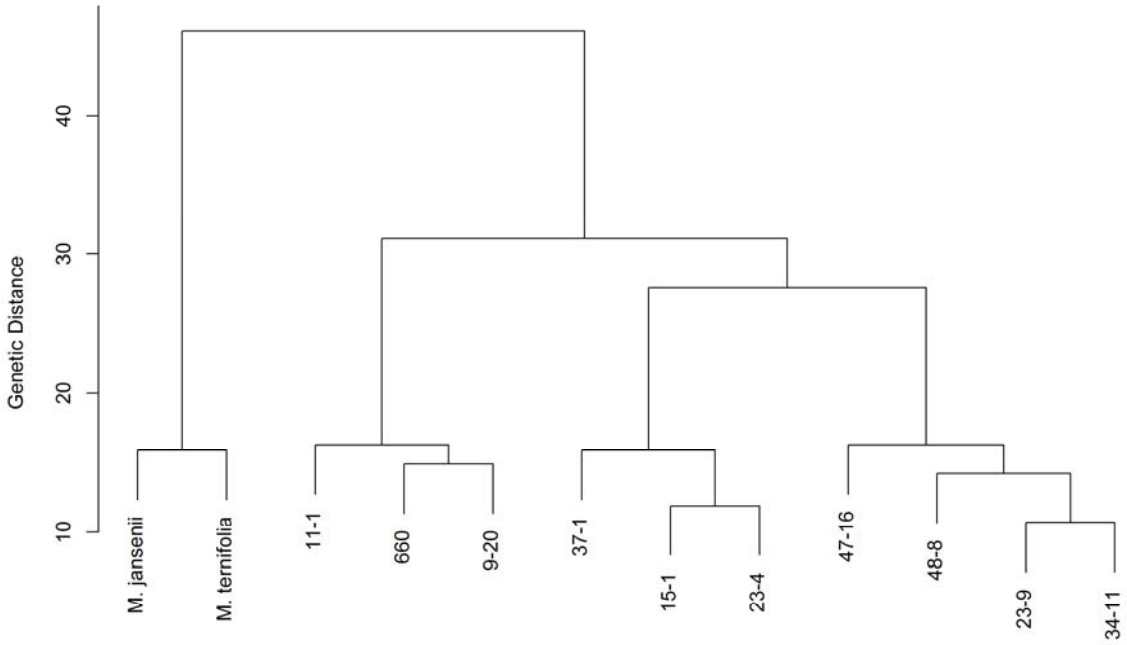

Figure 16.4. Dendrogram of genetic distances between interspecific parents and progeny.

16.3.3 Conclusions

The results of the parentage tests conducted suggest that pollen contamination during cross-pollination is low, at 1.3%. Selfing was only detected in interspecific crosses. It should be noted that the female parent in the interspecific crosses, 'HAES 660', has been found to possess low levels of self-compatibility (Sedgley, Bell, et al., 1990), and is almost genetically identical, although not phenotypically identical, to the self-compatible cultivar 'HAES 741'. The amount of selfing observed from the inter-specific crosses may therefore be inflated due to female parent choice.

17.0 Recommendations

1. *Continue genetic improvement through breeding to provide more profitable cultivars that allow the Australian industry to withstand future competition.*

Breeding is a foundation investment on which the Australian industry can build its competitive advantage. Competition from overseas macadamia supply and other nut crops will continue to increase. The industry will require new cultivars with improved profitability to address this competition.

We recommend continued breeding that concentrates on increasing the number of progeny, reducing the evaluation costs, continues the use of quantitative genetics methodology and explores the use of molecular methods such as genomic selection to reduce breeding time.

In 24 years from 1994 to 2008 a total of 3,572 seedling progeny trees were produced, planted and evaluated. In five years from 2009 to 2014 we have created another 3,555 progeny seedlings. It is important to increase the number of seedling trees in the second generation of breeding to increase the probability of obtaining elite individuals with high levels of desirable traits. We recommend increasing the second generation population size to 10,000 trees.

2. *Test the 23 elite selections from MC09021 in randomised, replicated trials that are supplemented with semi-commercial grower plantings.*

We identified 23 elite selections from the B1.2 population as part of MC09021. The 20 elite selections with highest selection index value had a 39% increase in NIS yield compared to clonally propagated varieties. However, the seedling selection data is based on single seedling trees planted at only one location. These results need to be confirmed and the very best of the elite candidates be selected in randomised, replicated, regional trials.

An industry workshop to identify the best approach for future regional variety tests (RVTs) was conducted as part of our project and the results are presented in chapter 14 of this report. The recommendations were that future RVTs should incorporate a greater level of grower involvement, balance the need for gathering statistically relevant data with cost of evaluation and investigate mechanical harvesting systems.

The new HIA co-investment system should be explored as a possible method of assisting in funding future RVTs. If the RVTs are structured so that they provide outcomes that are valuable across other perennial horticultural tree crops this may be possible.

3. *Trial 'Beaumont' cutting rootstocks with certain caveats and investigate rootstock breeding as a method of increasing productivity*

'Beaumont' cutting rootstock produced 13% higher cumulative NIS yield to age 12 than 'H2' seedling rootstock. We suggest trial of 'Beaumont' rather than full scale adoption because of limitations in the current trial design and lack of testing in a wide range of soils and environments. The RVT3 project has both 'H2' seedling and 'Beaumont' cutting used on 30 scion varieties at nine locations and will provide a more robust comparison of the two rootstocks. Full recommendations will be possible at the conclusion of RVT3 in 2017.

Scion cultivar accounted for more of the variation in yield and tree growth than did rootstock (see chapter 6 for details). Thus, focussing breeding efforts on producing superior scion genotypes is warranted. However, rootstock cultivar did account for between 19-41% of NIS yield variability and is therefore a source of further genetic gain which should be considered by industry.

The incomplete and unbalanced design of the current trial resulted in many of the rootstock types not being included. For example, 'H2' and 'D4' were included as seedling but not as cutting

rootstocks. We recommend that any future rootstock trials use a balanced design and include the highest yielding cultivars identified in this project as both cuttings and seedlings.

4. *Continue a program of active communication and extension with industry stakeholders*

Industry's large investment in breeding and regional variety trials coupled with the long wait for the release of the first new cultivars add increased importance to the role of communication with stakeholders. This will be particularly important in enabling stakeholders to make the best decisions about the adoption of superior cultivars released from the breeding program.

Continued feedback from industry by consultation is also required to guide input on the direction of the breeding program. We recommend that an industry steering committee such as MIVIC (Macadamia Industry Varietal Improvement Committee) be used for this purpose.

5. *Reduce the cost of yield evaluation*

Currently all breeding trials are harvested by hand or using hand operated harvesters. Each tree is harvested separately and resulting nuts are bagged, labelled and then processed. This is a major cost not only for the breeding trials but also for regional variety trials and for many other field trials. We have examined methods of visual estimation of yield that will reduce this cost. There are other possible methods of obtaining yield data without hand harvesting. We recommend that these types of systems, including small trial-sized mechanical harvesters, be developed. These could be used throughout the research community and significantly reduce research costs.

6. *Continue and expand the ex-situ germplasm trials*

The ex-situ germplasm trials at Tiaro and Alstonville are valuable resources for the macadamia industry. They should be maintained and the possibility of developing a new trial should be investigated.

A new trial should include the selections in the current trial but also be expanded to include new accessions that are now threatened in the wild. Additionally the new germplasm trial should be designed so that it includes commercial cultivars. Evaluation of the wild accessions and comparison of their attributes with commercial cultivars is necessary to determine the relative value of the traits in the wild accessions. This is only possible if both wild and domesticated accessions are included in the trial design.

18.0 Extension Communication

18.1 Meetings and field days

Nine industry consultation meetings were organised by Paul O'Hare during 2010. An outline of the new breeding project was presented and feedback was obtained on industry's priority issues for breeding. The meetings were as follows:
Macadamia marketing meeting, Brisbane Technology Park, 7 June 2010
Bundaberg MacGroup meeting, Bundaberg Enterprise Centre, 20 July 2010
Gympie MacGroup meeting, Hinkler Park, Mt Bauple, 21 July 2010
Glasshouse Mountains MacGroup meeting, Doug Benjafield's property, Peachester 22 July 2010
Dunoon MacGroup meeting, Dunoon Hall, 2 August 2010
Bangalow MacGroup meeting, Bangalow Scout Hall, 3 August 2010
Alstonville MacGroup meeting, Alstonville CTH, 4 August 2010
Nambucca MacGroup meeting, Macksville Services Club, 5 August 2010
Macadamia pest consultants meeting, Brisbane Technology Park, 28 July 2010

Two face to face project steering MIVIC (Macadamia Industry Varietal Improvement Committee) meetings were conducted on 23 March and 27 October. A further five phone/web conferences were conducted during 2010.

A grower field walk of the Amamoor progeny field trial was conducted in February 2011.

A MIVIC meeting was conducted in March 2011 and another is scheduled for 6th September 2011.

Members of MIVIC were invited to the Newrybar progeny trial site in 2011 to inspect the elite seedlings that have been selected for further evaluation and have been propagated.

B.Topp attended the Macadamia Conservation Council (MCC) meeting in Brisbane on 15 December 2011.

B.Topp and P.O'Hare attended a phone MIVIC project steering meeting on 2 February 2012.

R. Broadley and B. Topp organised a macadamia forum at the Annual Research Meeting of QAAFI (Queensland Alliance for Agriculture and Food Innovation) in July 2012. Forum was attended by about 30 researchers.

B. Topp attended the MIVIC meeting on 22 August 2012.

B. Topp and J. Neal attended, as committee members, two meetings of the MCC in August and October 2012.

A public field walk at the Tiaro germplasm trial was attended by about 50 members of the public (cyclists on the Cycle Queensland tour) in September 2012.

Two field walks at Bundaberg (February 2013) and Amamoor (April 2013) were held in conjunction with Macgroup meetings. The field walks were attended by about 70 growers in total. Growers were shown elite selections from the breeding and evaluated these selections for yield, quality and tree characteristics.

B. Topp attended MIVIC steering committee meetings held by phone in August 2012 and 22 April 2013.

An industry forum on regional variety trial design was conducted at Maroochy Research Station on 4 March 2013.

A mid project meeting of all 12 team members was held at Maroochy Research Station on 6-7 March 2013.

The breeding team provided representation and input at the AMS Canopy Management Workshop on 30 April and 1 May 2013.

Breeding team representatives helped to organise and participated in a Supplementary RVT grower meeting, 27 November 2013.

B. Topp and J. Neal attended an MCC meeting on 24 January 2014.

A project report was presented at the project steering meeting of MIVIC on 13 February 2014.

J. Neal attended an MCC meeting on 4 July 2014.

B. Topp and J. Neal organised a steering committee meeting 10 September 2014 to decide on the shortlist of B1.2 progeny to be tested further in the next round of RVT trials.

18.2 Conference and industry presentations

Topp, B.L., Hardner, C.M. and Kelly, A.M. Strategies for breeding macadamias in Australia. International Horticulture Congress, Lisbon, August 2010.

Topp, B.L. Review of the MC09021 breeding project. Australian Macadamia Society Researchers Forum, Nambour, October 2010.

Topp, B. and Neal, J. Macadamia Breeding and Conservation. Australian Macadamia Society Researchers Forum, Wollongbar, October 2011.

Topp, B. and Neal, J. Observations from a breeding perspective. Australian Macadamia Society Two-Tonne Task Force Meeting, Nambour, March 2012.

Topp, B. Overview of macadamia crop improvement research. Macadamia forum at the Annual Research Meeting of QAAFI (Queensland Alliance for Agriculture and Food Innovation), Brisbane, July 2012.

Topp, B. Genetic Improvement Projects in Macadamia. International Macadamia Symposium, Brisbane, September 2012.

Neal, J. Performance of Macadamia Rootstocks. International Macadamia Symposium, Brisbane, September 2012.

Topp, B., Neal, J., Hardner, C., O'Hare, P., Kelly, A., Russell, D. and Daley, R. Poster Presentation: Macadamia Breeding and Conservation. International Macadamia Symposium, Brisbane, September 2012.

Neal, J., Topp, B., Hardner, C., Kelly, A., Russell, D. and Daley, R. Poster Presentation: Macadamia Breeding Analysis and Selection. International Macadamia Symposium, Brisbane, September 2012.

Neal, J. Performance of Macadamia Rootstocks. Australian Nut Industry Research Forum, Brisbane, September 2012.

Neal, J. Macadamia Breeding and Conservation Program. Australian Macadamia Society Consultant's Forum, Brisbane, July 2013.

Neal, J. Impressions of the Macadamia Industry in Xishuangbanna, China. Australian Macadamia Society Consultant's Forum, Brisbane, July 2013.

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Neal, J.M., Russell, D.M., Giles, J. and Topp, B.L. (Submitted 2014) Assessing nut germination protocols for macadamia cultivar 'Beaumont'. Acta Horticulturae

Russell, D.M., Neal, J.M., Mayer, R. Bell, D. and Topp, B.L. (Submitted 2014) Variation of cutting rooting ability in cultivated and wild species of *Macadamia*. Acta Horticulturae

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19.0 IP Commercialisation

The seedling progenies created in this project are a major source of future IP. In total 3,965 progeny trees were created and field planted during the project. A further 1,000 (approx.) hand pollinated hybrid seeds and seedlings have been created which are scheduled for field planting in 2015 and 2016. Collectively this second generation of breeding material is described as the B2.1 population with "B" referring to breeding, "2" signifying the second generation and "0.1" indicating it is the first population of the second generation. These trees have been planted at DAF research facilities at Bundaberg (BRF) and Nambour (MRF). No IP agreement has been created other than the HIA contract.

The B1.2 population (second population of the first generation of breeding) was created by CSIRO. It was planted from 2000 to 2003 and consisted of 2,500 progeny trees. The trees were planted at eight locations (see MC02054 final report for details of locations). In our current trial we have been completing the evaluations of these B1.2 trials.

The following activities relate to the IP management of B1.2 populations:

- The progeny trials at BRF and grower properties at Yandina and Newrybar have been removed. Elite selections were propagated prior to trial removal. The elite selections have been planted at BRF and MRF.

- The IP of the remaining 5 progeny trials has been protected using a non-propagation agreement between DAF and the owner of the trial site. These agreements allow bulk harvest and sale of nuts but prohibit propagation of the germplasm.

- During MC09021 we have evaluated the B1.2 populations and selected a total of 23 elite genotypes. These have been propagated and planted at BRF and MRF. From this group of 23 we will select new cultivars for commercialisation and industry release. Representatives from AMS, DAF, QAAFI, HIA, NSW DPI, processors and nurseries were invited to a selection meeting at Nambour in September 2014 were the final 23 elite genotypes were selected.

- An RVT forum was held in March 2013 to formulate plans for the future testing of the B1.2 elites. A summary of the participants, discussions and recommendations is included in chapter 14 of this report.

- The HIA project MC09017 "Supplementary grower trial of elite macadamia selections" was negotiated by the grower Lindsay Bryen (the contact for property owner AS&FJ Bogg) and CSIRO prior to commencement of our MC09021 project. MC09017 is testing 15 elite B1.1 and B1.2 selections at a grower trial in Queensland. DAF has arranged an MTA from 2011 to 2021 to protect the IP in this trial.

The first cultivars from the HIA-AMS breeding program are due for release in 2017. They will be released from the HIA project MC11001 "Macadamia Regional Variety Trials - Series 3 Phase 2". This trial is testing 20 HIA-AMS selections from the B1.1 breeding population at 9 locations. DAF is managing all IP from the breeding and RVT projects. DAF has been negotiating with stakeholders HIA, AMS and UQ to develop a commercialisation plan and to define cultivar release time lines. In broad outline the commercialisation process will involve a publicly advertised call for expression of interest (EOI) to commercialise the new cultivars. A commercialisation partner will be selected by a panel of stakeholder representatives who will evaluate the EOI responses according to set selection criteria. The selected commercialisation partner and DAF will then negotiate the final commercialisation agreement.

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22.0 Appendices

22.1 Appendix 1 – Breeding objectives. Detailed results from each MIVIC meeting.

Location	Topic	Specific topic	Votes	Total votes	% of votes
Bundaberg	AVG	AVG resistance (5 votes – 4 growers, 1 agribusiness)	5	64	7.8
Pest consultants	AVG	AVG resistance (1 vote)	1	60	1.7
Alstonville	Consumer preferred	Good kernel colour (1 vote)	1	113	0.9
Bangalow	Consumer preferred	Top tasting kernel (4 votes)	4	75	5.3
Bundaberg	Consumer preferred	Consistent premium kernel that meets consumer needs (14 votes – 10 grower, 4 agribusiness)	14	64	21.9
Bundaberg	Consumer preferred	Range of varieties to suit different end uses (3 votes – 3 growers)	3	64	4.7
Bundaberg	Consumer preferred	Whatever comes off the tree is marketable (1 vote – 1 grower)	1	64	1.6
Dunoon	Consumer preferred	High quality kernel (good eating qualities for taste and texture) (5 votes)	5	48	10.4
Dunoon	Consumer preferred	No kernel discolouration (1 vote)	1	48	2.1
Glasshouse	Consumer preferred	Consumer acceptance (5 votes)	5	36	13.9
Gympie	Consumer preferred	More whole kernel (1 vote – 1 grower)	1	38	2.6
Marketing	Consumer preferred	Taste (6 votes)	6	21	28.6
Marketing	Consumer preferred	Colour consistency (3 votes)	3	21	14.3
Marketing	Consumer preferred	Consistent crunchy texture (2 votes)	2	21	9.5
Marketing	Consumer preferred	Less defects – especially taste traits (2 votes)	2	21	9.5
Marketing	Consumer preferred	Maintain consistent taste (1 vote)	1	21	4.8
Nambucca	Consumer preferred	Superior eating quality for taste and texture (1 grower vote)	1	48	2.1
Nambucca	Consumer preferred	18 -22 mm kernel size (1 agribusiness vote)	1	48	2.1
Pest consultants	Consumer preferred	Higher beneficial oil content (1 vote)	1	60	1.7
Marketing	Cost of production	Cost of production (1 vote)	1	21	4.8
Bangalow	Flowering extended	Extended flowering (1 vote)	1	75	1.3
Nambucca	Flowering young wood	Ability to bear on young wood e.g. 1 year old (1 grower vote and 1 agribusiness)	2	48	4.2
Pest consultants	Flowering young wood	Cropping on 1 year old wood (2 votes)	2	60	3.3
Pest consultants	Flowering young wood	Terminal bearing (1 vote)	1	60	1.7
Glasshouse	Less leaf drop	Lower leaf drop at harvest (1 vote)	1	36	2.8
Marketing	Low reject levels	Level of unsaleable kernel (1 vote)	1	21	4.8
Glasshouse	Nut drop earlier	Early season harvest (1 vote)	1	36	2.8
Pest consultants	Nut drop earlier	Early season maturing (2 votes)	2	60	3.3

Location	Topic	Specific topic	Votes	Total votes	% of votes
Alstonville	Nut drop later	Nut drop later in the year – out of the wet season (1 vote)	1	113	0.9
Glasshouse	Nut drop later	Not early season harvest (1 vote)	1	36	2.8
Alstonville	Nut drop shorter	Short nut drop period (3 votes)	3	113	2.7
Bangalow	Nut drop shorter	Short drop pattern (1 vote)	1	75	1.3
Bundaberg	Nut drop shorter	Short harvest period (1 vote – 1 grower)	1	64	1.6
Gympie	Nut drop shorter	Short drop pattern (1 vote – 1 grower)	1	38	2.6
Nambucca	Nut drop shorter	Short nut drop period (1 grower vote)	1	48	2.1
Alstonville	Post harvest quality	Good shelf life (3 votes)	3	113	2.7
Alstonville	Post harvest quality	Good keeping quality of nut on the ground (2 votes)	2	113	1.8
Alstonville	Post harvest quality	No open micropyles or smaller micropyles (1 vote)	1	113	0.9
Bundaberg	Post harvest quality	Long shelf life (2 votes – 2 agribusiness)	2	64	3.1
Dunoon	Post harvest quality	Increased level of oil (1 vote)	1	48	2.1
Gympie	Post harvest quality	Kernel quality – improved shelf life (pv resistant) (1 vote – 1 grower)	1	38	2.6
Marketing	Post harvest quality	Shelf life (5 votes)	5	21	23.8
Nambucca	Post harvest quality	Good shelf life (1 agribusiness vote)	1	48	2.1
Bangalow	Propagation easy	Ease of graftability (3 votes)	3	75	4.0
Nambucca	Propagation easy	Compatibility with existing rootstocks (2 grower votes)	2	48	4.2
Nambucca	Propagation easy	Ability to rework existing trees (1 grower vote)	1	48	2.1
Gympie	Quality consistent	Consistent quality from year to year (3 votes – 3 agribusiness)	3	38	7.9
Alstonville	Resistance fruit spotting bug	Fruit spotting bug resistance	12	113	10.6
Bangalow	Resistance fruit spotting bug	Fruit spotting bug resistance (5 votes)	5	75	6.7
Bundaberg	Resistance fruit spotting bug	Fruit spotting bug resistance	6	64	9.4
Dunoon	Resistance fruit spotting bug	Fruit spotting bug resistance (5 votes)	5	48	10.4
Gympie	Resistance fruit spotting bug	Fruit spottingbug resistance (3 votes – 3 grower)	3	38	7.9
Nambucca	Resistance fruit spotting bug	Fruit spotting bug resistance (3 grower votes)	3	48	6.3
Pest consultants	Resistance fruit spotting bug	Fruit spotting bug resistance (8 votes)	8	60	13.3
Alstonville	Resistance husk spot	Husk spot resistance (12 votes)	12	113	10.6
Bangalow	Resistance husk spot	Husk spot resistance (6 votes)	6	75	8.0
Bundaberg	Resistance husk spot	Husk spot resistance (2 votes – 1 grower, 1 agribusiness)	2	64	3.1
Dunoon	Resistance husk spot	Husk spot resistance (6 votes)	6	48	12.5
Glasshouse	Resistance husk spot	Husk spot resistance (9 votes)	9	36	25.0
Gympie	Resistance husk spot	Husk spot resistance (6 votes – 6 grower)	6	38	15.8
Nambucca	Resistance husk spot	Husk spot resistance (8 grower votes)	8	48	16.7
Pest consultants	Resistance husk spot	Husk spot resistance (10 votes)	10	60	16.7
Alstonville	Resistance other abiotic factors	Adaptable to extreme weather conditions (4 votes)	4	113	3.5
Bangalow	Resistance other abiotic factors	Tolerant of hot, dry conditions (esp during flowering) (2 votes)	2	75	2.7

Location	Topic	Specific topic	Votes	Total votes	% of votes
Bundaberg	Resistance other abiotic factors	Tolerance to wet areas (2 votes – 2 growers)	2	64	3.1
Dunoon	Resistance other abiotic factors	Adaptability to climate change (5 votes)	5	48	10.4
Gympie	Resistance other abiotic factors	Bigger nuts under moisture stress (1 vote – 1 grower)	1	38	2.6
Pest consultants	Resistance other abiotic factors	Herbicide tolerant (1 vote)	1	60	1.7
Alstonville	Resistance other biotic factors	Lace bug resistance (4 votes)	4	113	3.5
Alstonville	Resistance other biotic factors	Rat resistance (2 votes)	2	113	1.8
Bangalow	Resistance other biotic factors	Nutborer resistance (3 votes)	3	75	4.0
Bangalow	Resistance other biotic factors	Lace bug resistance (2 votes)	2	75	2.7
Bangalow	Resistance other biotic factors	Rat damage resistance (1 vote)	1	75	1.3
Bundaberg	Resistance other biotic factors	Nutborer resistant (1 vote – 1 grower)	1	64	1.6
Dunoon	Resistance other biotic factors	Lace bug resistance (2 votes)	2	48	4.2
Glasshouse	Resistance other biotic factors	Resistance to branch girdlers (1 vote)	1	36	2.8
Nambucca	Resistance other biotic factors	Nutborer resistance (1 grower vote)	1	48	2.1
Pest consultants	Resistance other biotic factors	Harder shell (1 vote)	1	60	1.7
Alstonville	Resistance to drought	Adaptable to heat and less water (2 votes)	2	113	1.8
Bundaberg	Resistance to drought	Drought tolerant (1 vote – 1 grower)	1	64	1.6
Dunoon	Resistance to drought	Drought tolerance (1 vote)	1	48	2.1
Glasshouse	Resistance to drought	Drought resistance (2 votes)	2	36	5.6
Gympie	Resistance to drought	Drought tolerant (1 vote – 1 grower)	1	38	2.6
Pest consultants	Resistance to drought	Drought tolerance (2 votes)	2	60	3.3
Alstonville	Resistance to wind	Wind resistance (1 vote)	1	113	0.9
Bangalow	Resistance to wind	Wind tolerance (2 votes)	2	75	2.7
Bundaberg	Resistance to wind	Wind resistance (1 vote – 1 grower)	1	64	1.6
Glasshouse	Resistance to wind	Resistant to wind damage (1 vote)	1	36	2.8
Bangalow	Resistance trunk canker	Trunk canker resistance (2 votes)	2	75	2.7
Glasshouse	Resistance trunk canker	Resistance to trunk canker (4 votes)	4	36	11.1
Gympie	Resistance trunk canker	Trunk canker resistance (2 votes – 2 grower)	2	38	5.3
Nambucca	Resistance trunk canker	Trunk canker resistance (1 grower vote)	1	48	2.1
Pest consultants	Resistance trunk canker	Trunk canker resistant (3 votes)	3	60	5.0
Alstonville	Rootstock	Deep rooted tree for soil erosion (3 votes)	3	113	2.7
Bangalow	Rootstock	Rootstock to match scion growth (1 vote)	1	75	1.3
Gympie	Rootstock	Stronger, more reliable rootstock (1 vote – 1 grower)	1	38	2.6
Bundaberg	Self pollinating	Self pollinating (2 votes – 1 grower, 1 agribusiness)	2	64	3.1
Nambucca	Self pollinating	Self pollination (2 grower votes)	2	48	4.2
Pest consultants	Self pollinating	Self pollinating (2 votes)	2	60	3.3
Alstonville	Sticktights	No sticktights – clean dropping (6 votes)	6	113	5.3

Location	Topic	Specific topic	Votes	Total votes	% of votes
Bangalow	Sticktights	No sticktights (1 vote)	1	75	1.3
Dunoon	Sticktights	No sticktights (1 vote)	1	48	2.1
Gympie	Sticktights	Eliminate sticktights (1 vote – 1 growers)	1	38	2.6
Nambucca	Sticktights	No sticktights (5 grower votes)	5	48	10.4
Pest consultants	Sticktights	Clean dropping trees – no sticktights (4 votes)	4	60	6.7
Alstonville	Tree structure	Good tree structure, strong central leader (7 votes)	7	113	6.2
Alstonville	Tree structure	Flowering on younger wood (e.g. 1 year old wood) (4 votes)	4	113	3.5
Bangalow	Tree structure	Open tree structure (2 votes)	2	75	2.7
Bundaberg	Tree structure	Amenable to pruning (1 vote – 1 grower)	1	64	1.6
Bundaberg	Tree structure	Flower on 1 year old wood (1 vote – 1 agribusiness)	1	64	1.6
Dunoon	Tree structure	Open canopy for spray penetration (1 vote)	1	48	2.1
Gympie	Tree structure	Open canopy (3 votes – 3 grower)	3	38	7.9
Nambucca	Tree structure	Well structured centre leader tree (1 grower vote)	1	48	2.1
Pest consultants	Tree structure	Open canopy in trees (2 votes)	2	60	3.3
Pest consultants	Tree structure	Tree structure so that water does not funnel down the trunk (1 vote)	1	60	1.7
Alstonville	Trees smaller	Small trees (9 votes)	9	113	8.0
Bangalow	Trees smaller	Dwarf trees (6 votes)	6	75	8.0
Bundaberg	Trees smaller	Compact dwarf tree (2 votes – 1 grower, 1 agribusiness)	2	64	3.1
Dunoon	Trees smaller	Smaller trees (7 votes)	7	48	14.6
Glasshouse	Trees smaller	Dwarf/compact trees (1 vote)	1	36	2.8
Gympie	Trees smaller	More compact dwarf tree (2 votes – 2 grower)	2	38	5.3
Nambucca	Trees smaller	Dwarf trees (2 grower votes)	2	48	4.2
Pest consultants	Trees smaller	Smaller trees (8 votes)	8	60	13.3
Dunoon	Variety adaptation	Different varieties for different areas (2 votes)	2	48	4.2
Alstonville	Yield consistent	Consistent yield from year to year (9 votes)	9	113	8.0
Bangalow	Yield consistent	Consistent yield across seasons (3 votes)	3	75	4.0
Bundaberg	Yield consistent	Consistent yield from year to year (7 votes – 6 growers, 1 agribusiness)	7	64	10.9
Nambucca	Yield consistent	Consistent yield from year to year (6 grower votes and 2 agribusiness)	8	48	16.7
Alstonville	Yield high	High yield per hectare (17 votes)	17	113	15.0
Bangalow	Yield high	High yield of premium kernel per square metre of canopy area (15 votes)	15	75	20.0
Bangalow	Yield high	High yield per hectare (12 votes)	12	75	16.0
Bangalow	Yield high	Longevity of tree yield (1 vote)	1	75	1.3
Bundaberg	Yield high	High yield per cubic metre of canopy / per hectare (11 votes – 8 growers, 3 agribusiness)	11	64	17.2
Dunoon	Yield high	Increased yield per canopy volume (4 votes)	4	48	8.3
Dunoon	Yield high	Longevity in peak production (2 votes)	2	48	4.2
Glasshouse	Yield high	High kernel yield per hectare (10 votes)	10	36	27.8

Location	Topic	Specific topic	Votes	Total votes	% of votes
Gympie	Yield high	High kernel yield per hectare (8 votes – 3 grower, 5 agribusiness)	8	38	21.1
Gympie	Yield high	Improved yield per cubic metre of canopy (3 votes – 2 grower, 1 agribusiness)	3	38	7.9
Nambucca	Yield high	High yield per hectare (5 grower votes and 1 agribusiness)	6	48	12.5
Pest consultants	Yield high	High yield of premium kernel per hectare (7 votes)	7	60	11.7
Pest consultants	Yield high	High yield per canopy volume (4 votes)	4	60	6.7
Alstonville	Yield high through kernel recovery	High kernel recovery (10 votes)	10	113	8.8
Dunoon	Yield high through kernel recovery	Increased kernel recovery (4 votes)	4	48	8.3
Nambucca	Yield high through kernel recovery	High kernel recovery (1 grower vote)	1	48	2.1
Bangalow	Yield precocity	Precocity (early bearing) (2 votes)	2	75	2.7
Bundaberg	Yield precocity	Early bearing (1 vote – 1 grower)	1	64	1.6
Dunoon	Yield precocity	Early bearing (precocious) (1 vote)	1	48	2.1
Gympie	Yield precocity	Earlier bearing (precocious) (1 vote – 1 grower)	1	38	2.6
Nambucca	Yield precocity	High early yield – precocious (1 grower vote)	1	48	2.1

22.2 Appendix 2 – Progeny seedling production. Counts of standards included in each trial year for B2.1 progeny trials.

Yes = final numbers are still unknown. * Excluding 2014 crosses.

Genotype	BNAMB11	BQBRS12	BQBRS13	BQBRS14	BQBRS15	2014 crosses	Total*
A268			5	4	3	yes	12
A376	6						6
A38	7						7
A4	5	2	3	3		yes	13
A538	6						6
BALLO02-6-17					2	yes	2
BALLO02-6-60					2	yes	2
BALLO02-6-76					2	yes	2
BAMAM02-2-3					2		2
BAMAM02-6-3	7	3	4	3		yes	17
BBAFF02-4-32				3	3		6
BBAFF03-15-24					2		2
BBAFF03-15-8				3	2		5
BBAFF03-21-29				3	3		6
Beaumont					2	yes	2
BEGYM01-12-3				3			3
BEGYM01-19-4				3			3
BHINK00-1-208					2	yes	2
BHINK00-1-54				3	3	yes	6
BHINK00-1-55				3	2	yes	5
BNEWR02-4-2				3			3
BQBRS01-12-12					3	yes	3
BQBRS01-20-4				3		yes	3
BQBRS03-11-19					1		1
BQBRS03-11-6			4	3			7
BQBRS03-12-12					2	yes	2
BQBRS03-12-4			4	4			8
BQBRS03-34-10					2	yes	2
BQBRS03-37-8					3	yes	3
BQBRS97-2-46	6	2	1				9
BQBRS97-6-16	3						3
BQBRS98-10-101		3			3		6
BQBRS98-10-111	6						6
BQBRS98-10-93	5	3	5	3		yes	16
BQBRS98-11-35	6						6
BQBRS98-11-80	7	2	5				14
BQBRS98-13-115	5						5
BQBRS98-14-25	6	3					9
BQBRS98-14-93	6	4	4	3	3	yes	20
BQBRS98-15-37	3						3
BQBRS98-16-37			4		3		7
BQBRS98-16-41	1	3	4	3		yes	11

BQBRS98-4-73			4		3		7
BQBRS98-5-74			4				4
BQBRS98-6-79	6	2	5	3	3	yes	19
BQBRS98-7-109	6						6
BQBRS98-8-87	6	4	5				15
BQBRS98-9-72	3						3
BTFRS98-11-1				3	2		5
BTFRS98-37-1				3	2		5
D4; Renown	6						6
Daddow	5	2	4		3	yes	14
Fuji; HAES 791	3	2	1				6
HAES 788	1						1
HAES 814	1						1
HAES 849		3					3
Ian McConachie dwarf		3			2	yes	5
Kau; HAES 344	3	2					5
Keauhou; HAES 246	2						2
M141	6						6
Macadamia jansenii (MRF)		2		3			5
Macadamia jansenii (point)	6						6
Macadamia ternifolia (MRF)		2		3			5
Mauka; HAES 741			3	6	3	yes	12
Release					2	yes	2

22.3 Appendix 3 – Progeny Seedling Production. Counts of progeny per family included in each trial/cross year.

OP = open-pollinated; * Non-germinated nut count.

Family	BNAMB11	BQBRS12	BQBRS13	BQBRS14	BQBRS15	2014 crosses*	Total
246 X OP	26						26
344 X 849			4				4
344 X A4			1				1
344 X Daddow			20				20
344 X Macadamia jansenii (MRF)		5					5
344 X OP	25						25
741 X 741			49		35	14	98
741 X NG18						166	166
788 X OP	26						26
791 X 849		14	89				103
791 X BAMAM02-6-3		1					1
791 X BQBRS98-14-93		42					42
791 X BQBRS98-16-37			7				7
791 X Daddow		54	18				72
791 X OP	25						25
814 X OP	25						25
849 X 791		8	52				60
849 X A4		3					3
849 X Daddow		11	12				23
849 X Ian McConachie dwarf		5					5
A268 X 741				89			89
A268 X A268				1			1
A268 X BQBRS98-14-93			13				13
A268 X BQBRS98-16-37			13				13
A268 X BTFRS98-11-1				2			2
A268 X BTFRS98-37-1				2			2
A268 X GTIAR01-14-16				19	18		37
A268 X GTIAR01-7-11				30	18		48
A268 X Macadamia jansenii (MRF)				1	1		2
A268 X Macadamia ternifolia (MRF)				1	3		4
A268 X OP	25						25
A376 X OP			61				61
A376 X OP	25						25
A38 X OP	25						25
A4 X 791		12	26				38
A4 X 849			3				3
A4 X Daddow		21	6				27
A4 X OP	24						24
A538 X OP	25						25
BALLO02-6-17 X BALLO02-6-60						16	16
BALLO02-6-17 X BALLO02-6-76					26		26
BALLO02-6-17 X BQBRS01-12-12					93		93

Family	BNAMB11	BQBRS12	BQBRS13	BQBRS14	BQBRS15	2014 crosses*	Total
BALLO02-6-60 X BQBRS01-12-12					25		25
BAMAM01-6-21 X BAMAM01-2-3					37		37
BAMAM02-6-3 X A4		11					11
BAMAM02-6-3 X BQBRS01-12-12					1		1
BAMAM02-6-3 X BQBRS98-16-37					3		3
BAMAM02-6-3 X OP	26	6					32
BBAFF02-4-32 X BEGYM01-19-4				1			1
BBAFF03-15-24 X BQBRS01-12-12					1		1
BBAFF03-15-8 X BBAFF03-21-29				3			3
BBAFF03-15-8 X BHINK00-1-55				10			10
BBAFF03-15-8 X BQBRS01-20-4				7			7
BBAFF03-21-29 X BBAFF03-15-8				18			18
BBAFF03-21-29 X BHINK00-1-55				11			11
BBAFF03-21-29 X BQBRS01-20-4				4			4
Beaumont X Release					19	32	51
BEGYM01-19-4 X BNEWR02-4-2				2			2
BHINK00-1-208 X BALLO02-6-60					27		27
BHINK00-1-208 X BALLO02-6-76					32	22	54
BHINK00-1-208 X BBAFF03-15-24					13		13
BHINK00-1-208 X BHINK00-1-208					3		3
BHINK00-1-54 X BEGYM01-19-4				7			7
BHINK00-1-54 X BQBRS01-20-4				6		25	31
BHINK00-1-55 X BBAFF03-21-29				20			20
BHINK00-1-55 X BQBRS01-20-4				8		30	38
BQBRS01-20-4 X BBAFF03-15-8				7			7
BQBRS01-20-4 X BBAFF03-21-29				47			47
BQBRS01-20-4 X BEGYM01-12-3				35			35
BHINK00-1-208 X BQBRS01-12-12					54		54
BQBRS01-20-4 X BHINK00-1-55				35			35
BQBRS03-11-6 X A268			44				44
BQBRS03-11-6 X BQBRS03-12-4			54	1			55
BQBRS03-12-12 X 741					22		22
BQBRS03-12-4 X A268			13				13
BHINK00-1-54 X BBAFF03-15-8				40			40
BQBRS03-34-10 X 741					45		45
BHINK00-1-54 X BBAFF03-21-29				7			7
BQBRS03-37-8 X 741					4		4
BHINK00-1-54 X BEGYM01-12-3				16			16
BQBRS01-15-8 X BQBRS01-15-8					9		9
BQBRS97-2-46 X BQBRS98-10-93			45				45
BQBRS97-2-46 X BQBRS98-11-80			2				2
BQBRS01-20-4 X BHINK00-1-54				21			21
BQBRS97-2-46 X BQBRS98-16-41		45					45
BQBRS97-2-46 X BQBRS98-7-74		15					15
BQBRS03-12-12 X BQBRS03-12-12					3		3
BQBRS03-12-13 X BQBRS03-12-13					2		2
BQBRS03-13-19 X NG7						22	22

Family	BNAMB11	BQBRS12	BQBRS13	BQBRS14	BQBRS15	2014 crosses*	Total
BQBRS03-34-10 X BBAFF02-21-5					48		48
BQBRS03-34-10 X BQBRS03-34-10					1		1
BQBRS03-37-8 X BQBRS03-12-12						7	7
BQBRS03-37-8 X BQBRS03-34-10					13	5	18
BQBRS97-2-46 X BQBRS98-14-25		35					35
BQBRS97-2-46 X BQBRS98-8-87			7				7
BQBRS97-2-46 X OP	22						22
BQBRS97-6-16 X OP	25						25
BQBRS98-10-101 X BQBRS98-14-25			29				29
BQBRS98-10-101 X BQBRS98-14-25		18					18
BQBRS98-10-101 X BQBRS98-16-41			6				6
BQBRS98-10-101 X BQBRS98-6-79		5					5
BQBRS98-10-111 X OP	26						26
BQBRS98-10-93 X OP	11						11
BQBRS98-11-35 X OP	23						23
BQBRS98-11-80 X BQBRS98-8-87		12					12
BQBRS98-11-80 X OP	6						6
BQBRS98-13-115 X OP	15						15
BQBRS98-14-25 X 791			58				58
BQBRS98-14-25 X OP	21						21
BQBRS98-14-93 X OP	18						18
BQBRS98-15-37 X OP	21						21
BQBRS98-16-37 X BQBRS98-14-25			6				6
BQBRS98-16-37 X BQBRS98-8-87			2				2
BQBRS98-16-41 X BQBRS97-2-46			7				7
BQBRS98-16-41 X BQBRS98-14-25			20				20
BQBRS98-16-41 X OP	26						26
BQBRS98-4-73 X BQBRS98-10-93			4				4
BQBRS98-4-73 X BQBRS98-16-37			12				12
BQBRS98-5-74 X BQBRS98-8-87			5				5
BQBRS98-6-73 X OP	24						24
BQBRS98-6-79 X BQBRS98-10-93			5				5
BQBRS98-6-79 X BQBRS98-16-37			1				1
BQBRS98-6-79 X OP	25						25
BQBRS98-7-109 X OP	23						23
BQBRS98-7-74 X BQBRS98-6-79		7					7
BQBRS98-7-74 X OP	17						17
BQBRS98-8-87 X BQBRS98-14-25		5					5
BQBRS98-8-87 X BQBRS98-6-79		5					5
BQBRS98-8-87 X OP	16						16
BQBRS98-9-72 X OP	26						26
BRS98-14-93-1 X OP		9					9
BRS98-2-46-1 X OP		10					10
BRS98-8-87-1 X OP		9					9
BTFRS98-37-1 X A268				8			8
BTFRS98-37-1 X A4				11			11
BTFRS98-37-1 X BAMAM02-6-3				2			2

Family	BNAMB11	BQBRS12	BQBRS13	BQBRS14	BQBRS15	2014 crosses*	Total
BTFRS98-37-1 X BTFRS98-37-1				5			5
D4 X OP	23						23
Daddow X 344			11				11
Daddow X 791		15	35				50
Daddow X 849			29				29
Daddow X OP	25						25
Ian McConachie dwarf X 849		9					9
Ian McConachie dwarf X BAMAM02-6-3		2					2
Ian McConachie dwarf X BQBRS98-14-93		15					15
Ian McConachie dwarf X BQBRS98-16-41		5					5
M141 X OP	25						25
Macadamia jansenii (MRF) X Macadamia ternifolia (MRF)		9					9
Macadamia jansenii (point) X OP	25						25
Macadamia ternifolia (MRF) X 344		9					9
NG18 X 741						18	18
NG7 X 816						14	14
NG7 X BALLO02-6-60						22	22
NG7 X BALLO02-6-76						4	4
NG7 X BQBRS01-12-12						25	25

22.4 Appendix 4 - Arboreta establishment. Counts of trees planted in the arboreta at Bundaberg (BRF) and Nambour (MRF).

Genotype / Cross	Description	BRF low density	MRF low density	MRF high density	Total
A376	Cultivar		2		2
A538	Cultivar		2		2
D3	Cultivar		2		2
D4/Renown	Cultivar		1		1
BQBRS97-2-46	B1.1 selection	2			2
BQBRS97-6-16	B1.1 selection	1			1
BQBRS98-10-111	B1.1 selection	2			2
BQBRS98-10-93	B1.1 selection	2			2
BQBRS98-11-35	B1.1 selection	1			1
BQBRS98-11-80	B1.1 selection	2			2
BQBRS98-13-115	B1.1 selection	1			1
BQBRS98-14-25	B1.1 selection	2			2
BQBRS98-14-93	B1.1 selection	2			2
BQBRS98-15-37	B1.1 selection	1			1
BQBRS98-16-41	B1.1 selection	2			2
BQBRS98-4-97	B1.1 selection	1			1
BQBRS98-6-73	B1.1 selection	1			1
BQBRS98-6-79	B1.1 selection	2			2
BQBRS98-7-109	B1.1 selection	1			1
BQBRS98-7-74	B1.1 selection	1			1
BQBRS98-8-87	B1.1 selection	2			2
BQBRS98-9-72	B1.1 selection	1			1
BTFRS98-43-23	B1.1 selection	2			2
BTFRS98-44-15	B1.1 selection	2			2
BTFRS98-9-22	B1.1 selection	2			2
BAMAM02-7-23	Amamoor B1.2 selection			2	2
BDUNO00-10-13	Dunoon B1.2 selection			2	2
BDUNO00-10-6	Dunoon B1.2 selection	2		2	4
BDUNO00-11-10	Dunoon B1.2 selection	2			2
BDUNO00-11-7	Dunoon B1.2 selection			2	2
BDUNO00-16-2	Dunoon B1.2 selection			2	2
BDUNO00-2-19	Dunoon B1.2 selection			2	2
BDUNO00-4-4	Dunoon B1.2 selection			2	2
BNEWR02-3-5	Newrybar B1.2 selection			2	2
BNEWR02-4-2	Newrybar B1.2 selection			2	2
BNEWR02-5-16	Newrybar B1.2 selection			2	2
BNEWR02-5-28	Newrybar B1.2 selection	2			2
BNEWR02-5-8	Newrybar B1.2 selection	2			2
BNEWR02-6-27	Newrybar B1.2 selection	2			2
BNEWR02-6-33	Newrybar B1.2 selection			1	1
BNEWR02-7-10	Newrybar B1.2 selection			2	2

Genotype / Cross	Description	BRF low density	MRF low density	MRF high density	Total
BNEWR02-7-3	Newrybar B1.2 selection	2			2
BYAND00-16-11	Yandina B1.2 selection	2		2	4
BYAND00-16-14	Yandina B1.2 selection			2	2
BYAND00-8-10	Yandina B1.2 selection	2		2	4
BYAND00-8-19	Yandina B1.2 selection			2	2
BYAND00-8-5	Yandina B1.2 selection			2	2
BYAND00-9-16	Yandina B1.2 selection			2	2
BYAND00-9-17	Yandina B1.2 selection			2	2
BYAND00-9-7	Yandina B1.2 selection	2		2	4
BYAND02-2-14	Yandina B1.2 selection			2	2
BYAND02-3-14	Yandina B1.2 selection	2		2	4
BYAND02-3-16	Yandina B1.2 selection			1	1
BYAND02-3-19	Yandina B1.2 selection			2	2
BYAND02-3-20	Yandina B1.2 selection	2		2	4
BYAND02-3-27	Yandina B1.2 selection			2	2
BYAND02-6-5	Yandina B1.2 selection			2	2
BYAND02-7-8	Yandina B1.2 selection			2	2
BBAFF02-4-26	Lindsay B1.2 selection	2	1		3
BBAFF03-15-32	Lindsay B1.2 selection	2	1		3
BBAFF03-17-7	Lindsay B1.2 selection	2			2
BHINK00-1-35	Lindsay B1.2 selection	1			1
BHINK00-1-54	Lindsay B1.2 selection	2			2
BHINK00-1-55	Lindsay B1.2 selection	1			1
BQBRS01-12-7	Lindsay B1.2 selection	1			1
BQBRS01-20-2	Lindsay B1.2 selection	1			1
BQBRS01-20-4	Lindsay B1.2 selection	2			2
BQBRS01-2-15	Lindsay B1.2 selection	1			1
BAMAM02-6-3	Putative dwarf	2	2		4
BQBRS01-12-4	Putative dwarf		2		2
BQBRS01-13-4	Putative dwarf	2			2
BQBRS03-11-6	Putative dwarf		2		2
BQBRS03-12-6	Putative dwarf	2			2
BQBRS98-13-18	Putative dwarf	2	2		4
BQBRS98-5-75	Putative dwarf	2	2		4
Ian McConachie dwarf	Putative dwarf	2	2		4
BAMAM02-6-3 x BQBRS98-16-37	Dwarf cross		4		4
Variegated seedling	Variegated seedling		1		1
GTIAR01-3-7	Wild M. integrifolia		2		2
GTIAR01-4-17	Wild M. integrifolia		2		2
GTIAR01-3-10 (IMC#4)	Wild M. jansenii		2		2
GTIAR01-6-7 (IMC#1)	Wild M. jansenii		1		1
M. Jansenii IMC#1	Wild M. jansenii		1		1
MRF M. jansenii	Wild M. jansenii		1		1
GTFRS00-1-22	Wild M. ternifolia		2		2
GTIAR01-17-2	Wild M. ternifolia		2		2
MRF M. ternifolia	Wild M. ternifolia		1		1

Genotype / Cross	Description	BRF low density	MRF low density	MRF high density	Total
GTFRS00-5-30	Wild M. tetraphylla		2		2
GTFRS00-8-22	Wild M. tetraphylla		2		2
344 x M. jansenii	Species hybrids			2	2
344 x M. ternifolia	Species hybrids			7	7
M. jansenii x M. ternifolia	Species hybrids			17	17
M. ternifolia x 344	Species hybrids			17	17
M. ternifolia x M. jansenii	Species hybrids			40	40
BAMAM03-8-18_OP_10	741 selfing experiment progeny			1	1
BAMAM03-8-18_OP_11	741 selfing experiment progeny			1	1
BAMAM03-8-18_OP_3	741 selfing experiment progeny			1	1
BAMAM03-8-18_OP_5	741 selfing experiment progeny			1	1
BAMAM03-8-18_OP_8	741 selfing experiment progeny			1	1
BAMAM03-8-18_Self_1	741 selfing experiment progeny			1	1
BQBRS01-12-16_OP_1	741 selfing experiment progeny			1	1
BQBRS01-12-16_OP_4	741 selfing experiment progeny			1	1
BQBRS01-12-16_OP_5	741 selfing experiment progeny			1	1
BQBRS01-12-16_OP_6	741 selfing experiment progeny			1	1
BQBRS01-12-16_OP_8	741 selfing experiment progeny			1	1
BQBRS01-12-16_self?_1	741 selfing experiment progeny			1	1
BQBRS01-12-16_self?_2	741 selfing experiment progeny			1	1
BQBRS01-12-16_self?_3	741 selfing experiment progeny			1	1
BQBRS01-12-16_self?_4	741 selfing experiment progeny			1	1
BQBRS01-12-16_Self_2	741 selfing experiment progeny			1	1
BQBRS01-12-16_Self_3	741 selfing experiment progeny			1	1
BQBRS01-12-16_Self_4	741 selfing experiment progeny			1	1
BQBRS01-12-16_Self_5	741 selfing experiment progeny			1	1
BQBRS01-12-16_Self_6	741 selfing experiment progeny			1	1
RQBRS02-6-6_OP_19	741 selfing experiment progeny			1	1
RQBRS02-6-6_OP_27	741 selfing experiment progeny			1	1
RQBRS02-6-6_OP_4	741 selfing experiment progeny			1	1
RQBRS02-6-6_Self_11	741 selfing experiment progeny			1	1
RQBRS02-6-6_Self_12	741 selfing experiment progeny			1	1
RQBRS02-6-6_Self_13	741 selfing experiment progeny			1	1
RQBRS02-6-6_Self_14	741 selfing experiment progeny			1	1
RQBRS02-6-6_Self_15	741 selfing experiment progeny			1	1
RQBRS02-6-6_Self_16	741 selfing experiment progeny			1	1
RQBRS02-6-6_Self_2	741 selfing experiment progeny			1	1
RQBRS02-6-6_Self_3	741 selfing experiment progeny			1	1
RQBRS02-6-6_Self_5	741 selfing experiment progeny			1	1
RQBRS02-6-6_Self_9	741 selfing experiment progeny			1	1
RQBRS02-6-8_OP_15	741 selfing experiment progeny			1	1
RQBRS02-6-8_OP_26	741 selfing experiment progeny			1	1
RQBRS02-6-8_OP_3	741 selfing experiment progeny			1	1
RQBRS02-6-8_Self_1	741 selfing experiment progeny			1	1
RQBRS02-6-8_Self_10	741 selfing experiment progeny			1	1
RQBRS02-6-8_Self_11	741 selfing experiment progeny			1	1

Genotype / Cross	Description	BRF low density	MRF low density	MRF high density	Total
RQBRS02-6-8_Self_12	741 selfing experiment progeny			1	1
RQBRS02-6-8_Self_13	741 selfing experiment progeny			1	1
RQBRS02-6-8_Self_14	741 selfing experiment progeny			1	1
RQBRS02-6-8_Self_15	741 selfing experiment progeny			1	1
RQBRS02-6-8_Self_16	741 selfing experiment progeny			1	1
RQBRS02-6-8_Self_18	741 selfing experiment progeny			1	1
RQBRS02-6-8_Self_19	741 selfing experiment progeny			1	1
RQBRS02-6-8_Self_2	741 selfing experiment progeny			1	1
RQBRS02-6-8_Self_22	741 selfing experiment progeny			1	1
RQBRS02-6-8_Self_23	741 selfing experiment progeny			1	1
RQBRS02-6-8_Self_5	741 selfing experiment progeny			1	1
RQBRS02-6-8_Self_6	741 selfing experiment progeny			1	1
RQBRS02-6-8_Self_8	741 selfing experiment progeny			1	1
RQBRS02-6-8_Self_9	741 selfing experiment progeny			1	1
RQBRS02-8-3_OP_16	741 selfing experiment progeny			1	1
RQBRS02-8-3_OP_22	741 selfing experiment progeny			1	1
RQBRS02-8-3_OP_25	741 selfing experiment progeny			1	1
RQBRS02-8-3_OP_27	741 selfing experiment progeny			1	1
RQBRS02-8-3_OP_4	741 selfing experiment progeny			1	1
RQBRS02-8-3_Self_10	741 selfing experiment progeny			1	1
RQBRS02-8-3_Self_11	741 selfing experiment progeny			1	1
RQBRS02-8-3_Self_12	741 selfing experiment progeny			1	1
RQBRS02-8-3_Self_13	741 selfing experiment progeny			1	1
RQBRS02-8-3_Self_14	741 selfing experiment progeny			1	1
RQBRS02-8-3_Self_15	741 selfing experiment progeny			1	1
RQBRS02-8-3_Self_18	741 selfing experiment progeny			1	1
RQBRS02-8-3_Self_20	741 selfing experiment progeny			1	1
RQBRS02-8-3_Self_21	741 selfing experiment progeny			1	1
RQBRS02-8-3_Self_24	741 selfing experiment progeny			1	1
RQBRS02-8-3_Self_28	741 selfing experiment progeny			1	1
RQBRS02-8-3_Self_4	741 selfing experiment progeny			1	1
RQBRS02-8-3_Self_5	741 selfing experiment progeny			1	1
RQBRS02-8-3_Self_7	741 selfing experiment progeny			1	1
RQBRS02-8-3_Self_8	741 selfing experiment progeny			1	1
RQBRS02-8-3_Self_9	741 selfing experiment progeny			1	1
RQBRS02-8-4_OP_12	741 selfing experiment progeny			1	1
RQBRS02-8-4_OP_13	741 selfing experiment progeny			1	1
RQBRS02-8-4_OP_23	741 selfing experiment progeny			1	1
RQBRS02-8-4_Self_1	741 selfing experiment progeny			1	1
RQBRS02-8-4_Self_12	741 selfing experiment progeny			1	1
RQBRS02-8-4_Self_15	741 selfing experiment progeny			1	1
RQBRS02-8-4_Self_16	741 selfing experiment progeny			1	1
RQBRS02-8-4_Self_17	741 selfing experiment progeny			1	1
RQBRS02-8-4_Self_18	741 selfing experiment progeny			1	1
RQBRS02-8-4_Self_19	741 selfing experiment progeny			1	1
RQBRS02-8-4_Self_2	741 selfing experiment progeny			1	1

Genotype / Cross	Description	BRF low density	MRF low density	MRF high density	Total
RQBRS02-8-4_Self_20	741 selfing experiment progeny			1	1
RQBRS02-8-4_Self_21	741 selfing experiment progeny			1	1
RQBRS02-8-4_Self_22	741 selfing experiment progeny			1	1
RQBRS02-8-4_Self_23	741 selfing experiment progeny			1	1
RQBRS02-8-4_Self_24	741 selfing experiment progeny			1	1
RQBRS02-8-4_Self_26	741 selfing experiment progeny			1	1
RQBRS02-8-4_Self_3	741 selfing experiment progeny			1	1
RQBRS02-8-4_Self_4	741 selfing experiment progeny			1	1
RQBRS02-8-4_Self_5	741 selfing experiment progeny			1	1
RQBRS02-8-4_Self_6	741 selfing experiment progeny			1	1
RQBRS02-8-4_Self_7	741 selfing experiment progeny			1	1
RQBRS02-8-4_Self_8	741 selfing experiment progeny			1	1
Grand Total		82	44	234	360

22.5 Appendix 5 – B1.2 Progeny evaluation. An example of the data sheets used to shortlist selections.

2-3

Female parent: NG7 Male parent: 816

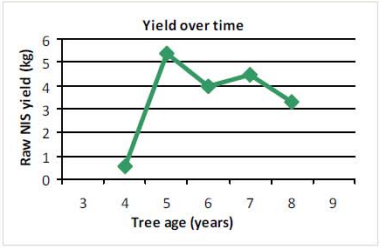
Genetic strengths:	Raw data strengths:	Negatives:
Excellent H, NIS & kernel yield, & yield efficiency. Very good TKR.	Very good TKR & standardised kernel yield	High ST & % rejects in 2012 & 2014. 2013 kernel quality is better.

Genetic data

Index (H) rank:	3	Index (H):	0.92
Kernel yield to age 8 rank:	2	Kernel yield to age 8:	11.6 kg
NIS yield to age 8 rank:	6	NIS yieldto age 8:	26.2 kg
TKR 2010 rank:	37	TKR 2010:	44.1 %
Yield efficiency rank:	7	Yield efficiency:	0.07 kg/m3
		Canopy volume:	164 m3

Raw data

Kernel yield to age 8 rank:	341	Kernel yield to age 8:	7.7 kg
NIS yield to age 8 rank:	488	NIS yieldto age 8:	17.2 kg
TKR 2010 rank:	92	TKR 2010:	45 %
Yield efficiency rank:	468		
		Canopy volume:	173 m3



2-3

Tree assessment 2013-2014

Relative tree size:	Medium - Large	Phytophthora:	None	Notes:	Somewhat sick, sparse leaves but recovering? ST could be problematic.
Branch angle:	Intermediate	Husk spot:	None		
Foliage density:	Open	Out of season flowers:	None		
Healthy:	No - dead twigs	Wind damage:	None		
Stick-tights:	Medium - High - husk				

Nut and kernel traits

TKR 2010:	45 %			Av. kernel wt 2012:	1.9 g	Wholes 2012:	42.8 %	Twins 2012:	0 %
TKR 2012:	41.1 %	SKR 2012:	34.2 %	Av. kernel wt 2013:	2.2 g	Wholes 2013:	75 %	Twins 2013:	0 %
TKR 2013:	44.8 %	SKR 2013:	44.5 %			Wholes 2014:	54 %		
TKR 2014	42.9 %	SKR 2014:	38.6 %	Av. NIS wt 2014:	5.9 g				
				Av. NIS diameter 2014:	2.3 cm			Taste:	acceptable

Kernel assessment (% of total kernel weight)

2012		2013		2014 - Suncoast Gold	
Premium:	56.4 %	Premium:	38.9 %	Premium:	74.6 %
Commercial:	26.8 %	Commercial:	56.6 %	Commercial:	15.4 %
Reject:	13.50 %	Reject:	4 %	Reject:	10 %
Premium wholes:	19.00 %	Premium wholes:	38.9 %	Genetic reject:	4.9 %
Premium halves/pieces:	37.30 %	Premium halves/pieces:	0 %	Environmental reject:	5.1 %
Commercial wholes:	14.60 %	Commercial wholes:	33.7 %		
Commercial halves/pieces:	12.20 %	Commercial halves/pieces:	22.9 %		

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Kernel assessment (% of total kernel weight). Genetic traits in grey. C=commercial grade, R=reject grade

2012

Mould R:	5.45 %	Concaved halves C:	5.17 %	Disc. tops C:	3.86 %	Grey disc. C:	0 %	Shrivelled C:	12.02 %
Insect damage R:	2.96 %	Concaved halves R:	0 %	Disc. tops R:	0 %	Grey disc. R:	0 %	Shrivelled R:	1.03 %
Internal disc. R:	0 %	Pitted centres C:	0 %	Basal disc. C:	9.85 %	Streaks/lines C:	0 %	Open micropile R:	0 %
Immature C:	3.39 %	Pitted centres R:	0 %	Basal disc. R:	6.79 %	Streaks/lines R:	0 %	Pink staining R:	0 %
Immature R:	0 %	Suture lines C:	0 %	Discoloured rings C:	0 %	Spots C:	0 %	Adhered skin C:	0 %
		Suture lines R:	0 %	Discoloured rings R:	0 %	Spots R:	0 %	Adhered skin R:	0 %
		Disc. crest C:	2.29 %	Green/blue disc. C:	0 %	Shell Marks C:	0 %	Hail damage C:	0 %
		Disc. crest R:	0 %	Green/blue disc. R:	0 %	Shell Marks R:	0 %	Hail damage R:	0 %

2013

Mould R:	0 %	Concaved halves C:	0 %	Disc. tops C:	0 %	Grey disc. C:	0 %	Shrivelled C:	21.14 %
Insect damage R:	4 %	Concaved halves R:	0 %	Disc. tops R:	0 %	Grey disc. R:	0 %	Shrivelled R:	0 %
Internal disc. R:	0 %	Pitted centres C:	0 %	Basal disc. C:	0 %	Streaks/lines C:	0 %	Open micropile R:	0 %
Immature C:	0 %	Pitted centres R:	0 %	Basal disc. R:	0 %	Streaks/lines R:	0 %	Pink staining R:	0 %
Immature R:	0 %	Suture lines C:	20.57 %	Disc. rings C:	0 %	Spots C:	0 %	Adhered skin C:	0 %
		Suture lines R:	0 %	Disc. rings R:	0 %	Spots R:	0 %	Adhered skin R:	0 %
		Disc. crest C:	0 %	Green/blue disc. C:	0 %	Shell Marks C:	0 %	Hail damage C:	0 %
		Disc. crest R:	0 %	Green/blue disc. R:	0 %	Shell Marks R:	0 %	Hail damage R:	0 %

2014 - Suncoast Gold

Mould R:	0 %	Internal disc. R:	0 %	Discolouration C:	2.56 %
Insect damage R:	0.23 %	Immature C:	9.56 %	Discolouration R:	4.9 %
Insect Type:	MNB	Immature R:	4.9 %	Disc. crest C:	3.26 %
				Disc. crest R:	0 %

22.6 Appendix 6 – B1.2 Progeny evaluation. Selection summary sheet and description.

22.6.1 Description of Summary Spread Sheet

You have received a bound booklet by post which contains the detailed information on the elite selections. To assist you in processing this information we have attached a summary spread sheet with this email. This spread sheet summarises important data for the top selections.

There are 43 selections each with a unique tree identification (Tree ID) listed in the first column of the spread sheet. The selections with a green colour are the best and are the ones we will discuss in detail on Wednesday. The ones in yellow are less desirable and the ones in orange are the least desirable on the list.

We have presented the data as raw values and genetic predictions. Raw values are the statistically un-touched values that we measure. Genetic predictions are obtained using quantitative genetic and statistical analysis. Genetic predictions adjust the value depending on location and performance of each tree's relatives. For all the traits each tree is ranked from 1 (the best) to 2,518 (the worst). So you are presented with 4 values for each trait as follows:

- the ranking of the genetic prediction.
- the ranking of the raw value
- the genetic prediction
- the actual raw value

Blue is used to highlight the genetic predictions that are in the top 30 for each trait. Red is used to highlight raw data values that are in the top 30 for each trait.

The following information is summarised on the spread sheet:

- Page no. The page in your bound booklet where you can find detailed information on the selection.
- Index (H) is the selection index which combines NIS yield, kernel recovery and tree growth into a single economically weighted value. The higher the index value the better the selection.
- Kernel yield is the sum of the kg of kernel per tree to age 8. The figure is obtained from NIS yield and total kernel recovery.
- NIS yield is the kg of nut-in-shell per tree to age 8.
- TKR is the total kernel recovery expressed as a percentage.
- Yield efficiency is the kernel yield per cubic metre of tree volume.

The final two columns of the spread sheet are for the verdict and any notes you may wish to make.

22.6.2 Summary Spread Sheet

		Genetic predictions - RANK					Raw data - RANK				Genetic predictions - VALUES					Raw data - VALUES			
Tree ID	Page no.	INDEX (H)	Kernel yield	NIS yield	TKR 2010	Yield effic.	Kernel yield	NIS yield	TKR 2010	Yield effic.	INDEX (H)	Kernel yield (kg to age 8)	NIS yield (kg to age 8)	TKR 2010 (%)	Yield effic. (kg/m^3)	Kernel yield (kg to age 8)	NIS yield (kg to age 8)	TKR 2010 (%)	Yield effic. (kg/m^3)
B6-5	170	1	1	1	54	1	1045	1127	261	787	1.06	12.8	29.3	43.7	0.08	2.8	6.8	42	0.03
B8-10	154	2	3	3	200	39	986	1008	857	1118	0.99	11.2	27.2	41	0.06	3.2	8.6	37	0.01
B2-3	14	3	2	6	37	7	341	488	92	468	0.92	11.6	26.2	44.1	0.07	7.7	17.2	45	0.04
B6-76	6	4	5	2	1587	15	30	16	1100	129	0.83	9.8	27.6	35.4	0.06	14.7	42	35	0.07
B7-23	26	6	4	9	22	2	281	468	21	194	0.82	10.9	24	45.3	0.07	8.5	17.7	48	0.06
B11-17	70	7	11	4	1717	70	229	139	1394	959	0.79	9.3	26.6	34.9	0.05	9.2	27.8	33	0.02
B9-28	30	8	16	7	1489	282	90	45	1388	922	0.75	8.8	24.6	35.7	0.04	12	36.4	33	0.02
B4-32	46	9	6	21	27	63	129	254	30	284	0.72	9.7	21.7	44.8	0.05	10.9	23.1	47	0.05
B3-19	162	10	19	8	1496	68	1144	1119	1317	1061	0.71	8.7	24.4	35.6	0.05	2.3	6.9	34	0.02
B3-14	158	11	12	12	444	62	1149	1170	730	895	0.71	9.2	23.4	39.3	0.05	2.3	6	38	0.02
B12-12	126	13	14	13	335	8	15	21	203	8	0.67	9.1	22.9	39.9	0.07	16.8	40	42	0.14
B2-13	42	14	10	26	46	55	79	163	53	178	0.67	9.3	21.2	43.9	0.05	12.2	26.6	46	0.06
B10-16	122	16	15	10	1033	19	12	10	492	4	0.64	8.8	23.8	37	0.06	17	43.7	39	0.16
B6-21	22	18	22	11	1369	3	556	503	1132	585	0.64	8.5	23.4	36.1	0.07	5.9	17	35	0.03
B6-27	106	22	13	80	1	20	44	192	2	41	0.61	9.2	18.8	48.7	0.06	13.8	25.5	54	0.1
B20-4	138	24	27	29	466	114	34	59	205	81	0.61	8.3	21	39.3	0.05	14.4	34.3	42	0.08
B1-54	82	26	26	42	240	145	2	3	77	42	0.6	8.3	20.4	40.6	0.05	24.1	53.7	45	0.1
B1-56	90	40	69	113	222	626	1	2	76	37	0.54	7.4	18.1	40.8	0.03	24.5	54.6	45	0.1
B29-3	142	45	59	30	1498	87	22	15	784	78	0.52	7.5	21	35.6	0.05	15.7	42.3	37	0.08
B15-8	50	73	81	54	1024	98	4	5	629	256	0.47	7.3	19.6	37.1	0.05	20.2	53.3	38	0.06
B36-2	146	148	145	97	1450	96	5	1	1099	115	0.38	6.6	18.5	35.8	0.05	19.9	56.8	35	0.07
B17-16	134	474	318	492	210	52	308	446	90	2	0.21	5.8	14.1	40.9	0.05	8.2	18.2	45	0.17
B1-55	86	507	433	1020	11	144	96	271	6	5	0.2	5.4	11.6	46.1	0.05	11.8	22.6	52	0.15
B2-19	62	5	7	5	1633	64			1627		0.82	9.4	26.6	35.2	0.05			31	

Tree ID	Page no.	Genetic predictions - RANK					Raw data - RANK				Genetic predictions - VALUES					Raw data - VALUES			
		INDEX (H)	Kernel yield	NIS yield	TKR 2010	Yield effic.	Kernel yield	NIS yield	TKR 2010	Yield effic.	INDEX (H)	Kernel yield (kg to age 8)	NIS yield (kg to age 8)	TKR 2010 (%)	Yield effic. (kg/m^3)	Kernel yield (kg to age 8)	NIS yield (kg to age 8)	TKR 2010 (%)	Yield effic. (kg/m^3)
B10-24	34	19	17	25	211	73	399	465	325	774	0.63	8.7	21.4	40.9	0.05	7.2	17.7	41	0.03
B1-119	94	21	31	19	1016	154	31	31	634	295	0.62	8.1	22	37.1	0.05	14.7	38.7	38	0.05
B3-4	18	25	25	24	582	43	471	473	670	630	0.6	8.3	21.5	38.7	0.05	6.7	17.6	38	0.03
B5-89	2	27	32	43	282	211	41	78	161	308	0.59	8.1	20.2	40.4	0.04	13.9	32.3	43	0.05
B1-18	10	29	20	52	44	25	390	537	94	449	0.58	8.7	19.8	43.9	0.06	7.3	16.3	45	0.04
B10-6	66	42	83	16	2286	83	85	49	1246	753	0.53	7.2	22.2	32.4	0.05	12.2	35.7	34	0.03
B1-210	98	49	51	62	413	97	7	19	112	85	0.51	7.6	19.3	39.5	0.05	17.8	40.5	44	0.08
B5-16	118	143	102	89	985	23	21	17	495	3	0.38	6.9	18.7	37.2	0.06	15.8	40.6	39	0.16
B37-2	150	233	278	380	396	1109	3	6	111	228	0.31	5.9	14.9	39.5	0.03	23.2	52.8	44	0.06
B4-2	102	15	8	39	17	21	103	241	13	67	0.64	9.4	20.6	45.6	0.06	11.6	23.6	49	0.09
B3-20	166	17	29	18	971	185	1022	1033	1018	1088	0.64	8.2	22	37.3	0.04	3	8.3	36	0.02
B7-10	110	20	33	14	1430	202	167	99	1251	548	0.62	8.1	22.6	35.8	0.04	10.2	30.1	34	0.04
B11-6	38	23	21	36	195	131	287	364	320	704	0.61	8.5	20.7	41	0.05	8.3	20.3	41	0.03
B19-4	74	30	43	20	1452	156	904	943	569	56	0.58	7.8	21.9	35.8	0.05	3.7	9.4	39	0.09
B14-6	130	33	38	27	931	129	46	61	394	29	0.56	7.9	21.2	37.5	0.05	13.6	34.1	40	0.11
B1-7	78	70	112	107	791	537	6	9	630	174	0.47	6.9	18.2	37.9	0.03	18.4	48.3	38	0.06
B15-32	54	103	134	313	73	701	8	25	78	689	0.43	6.7	15.6	43	0.03	17.8	39.5	45	0.03
B16-25	58	120	131	55	1846	146	17	8	1244	151	0.41	6.7	19.5	34.4	0.05	16.6	48.8	34	0.07
B5-2	114	421	498	1268	2	846	57	219	3	110	0.23	5.2	10.8	48.2	0.03	13	24.6	53	0.07

22.7 Appendix 7 – Database management. Column header descriptions for tables in the databases.

22.7.1 tbl_AllTreeData

Created by *qry_AllTreeData_Create_4*. Combines the majority of the information stored in the separate tables below into one table that can be easily filtered and exported for analysis.

Column header	Description
Trial_type	trial type. B1.1 = First iteration breeding trial, B1.2 = second iteration breeding trial, B2.1 = precocity trial, B2.2 = second-generation breeding trial, C = cultivar trial, G = germplasm trial, R = rootstock trial.
State	Australian state where trial is located
Region	Region where trial is located (BUND=Bundaberg, NNSW=Northern NSW, SEQ=South-East Queensland)
Site	trial location
Series	grouping factor for B1.1 trial planting year (2000 and 2001 grouped). Used for analysis.
Trial	trial ID code
Trial_pyear	year of trial planting
Fcol	column number used by field staff - is not necessarily indicative of real placement of trees in grid plan
Frow	row number used by field staff - is not necessarily indicative of real placement of trees in grid plan
Acol	column number used in analysis - indicates real placement of trees in grid plan
Arow	row number used in analysis - indicates real placement of trees in grid plan
Barcode	barcode text
Rep	experimental replicate reference
Block	experimental block reference
Col_width	distance between columns (m)
Row_width	distance between rows (m)
Tree_pdate	planting date
Tree_id	unique (or close to) tree id number
Prop_group	code for plant type. Group 1=clones, 2=seedlings, 3=selfs, 4=wild germplasm, 5=trees from rootstock trials
Parent1	cultivar ID of parent 1. Should always be the lower cultivar ID number of the two parents.
Parent2	cultivar ID of parent 2. Should always be the higher cultivar ID number of the two parents.
Family	numerical ID for family
Female	cultivar ID of female parent
Male	cultivar ID of male parent
Assess_year	year of data collection
Age	age of trial in years from planting date (planting_year - year)
Expstat	experimental status code. See tbl_ExpstatCodes for meanings.
Var_id	variable name
Var_class	overall variable grouping
y	value associated with each variable
comments	field comments
issues	issues raised in data checking process

Column header	Description
solution	resolution of issues discovered in data checking process

22.7.2 tbl_AnnualAssessDate_import

Date of measurement for all collected tree data.

Column header	Description
Trial	trial ID code
Assess_year	year of data collection
Var_id	variable name
Assess_date	measurement date

22.7.3 tbl_AnnualTreeData_import

All annual tree data (minus progid, pdate & expstat) as imported.

Column header	Description
Assess_year	year of data collection
Trial	trial ID code
Fcol	column number used by field staff - is not necessarily indicative of real placement of trees in grid plan
Frow	row number used by field staff - is not necessarily indicative of real placement of trees in grid plan
comments	field comments
issues	issues raised in data checking process
solution	resolution of issues discovered in data checking process
Var_id	variable names
y	value associated with each variable

22.7.4 tbl_AnnualTreeExpstat_import

Annual tree_id, pdate and expstat data.

Column header	Description
Assess_year	year of data collection
Trial	trial ID code
Fcol	column number used by field staff - is not necessarily indicative of real placement of trees in grid plan
Frow	row number used by field staff - is not necessarily indicative of real placement of trees in grid plan
Tree_id	unique (or close to) tree id number
Tree_pdate	planting date
Expstat	experimental status code. See tbl_ExpstatCodes for meanings.

22.7.5 tbl_B1-1 2006 snsk data

B1.1 nut and kernel assessment data from 2006. This can't be combined with *tbl_AnnualTreeData_import* as each row in this dataset is a single nut, instead of a single tree.

Column header	Description
Assess_year	year of data collection
Trial	trial ID code
Acol	column number used in analysis - indicates real placement of trees in grid plan
Arow	row number used in analysis - indicates real placement of trees in grid plan
skdatstat	data status code
assessor	assessor identification (1= Darren Morrow, 2=Rod Daley)
week	the week when assessment was conducted
day	the day when assessment was conducted
order	the order in which the samples were assessed on each day
nutid	the number of the individual nut or kernel from a sample
ss1inis	presence or absence of insect damage to the nut-in-shell
ss1ings	presence or absence of germination on the nut-in-shell
ss1inos	presence or absence of an open micropile on the nut-in-shell
ss1inm	individual nut-in-shell mass
ss1iktm	individual kernel mass
ss1ikwsN	whether a kernel was retrieved as a whole or halves after hand cracking the nut-in-shell (assessed by Tim Kowitz). whole =1; halves = 2
ss1ikwsK	whether a kernel was assessed as a whole or a half by kernel assessor. whole =1; halves = 2
ss1ikms	severity rating for mould contamination on the kernel
ss1ikis	severity rating for insect damage on the kernel
ss1ihkbs	severity rating for internal browning on the inner surface of the kernel half
ss1ihkps	severity rating for pitted centre on the inner surface of the kernel half
ss1ikbd	severity rating for discolouration on the base of the kernel
ss1ikdr	severity rating for discoloured rings on the kernel
ss1iksk	severity rating for shrivelling of the kernel
ss1iwksl	severity rating for suture lines on the base of the kernel (wholes only)
ss1iwkdc	severity rating for discoloured crest of the kernel
sncomm	general comments relating to the assessment of the nut-in-shell
skcomm	general comments relating to the assessment of the kernel
skissues	kernel assessment issues
sksolution	kernel assessment solutions

22.7.6 tbl_B1-1 Rankings

List of the top 40 selected B1.1 trees.

Column header	Description
Rank	rank of tree as calculated by Craig Hardner
Tree_id	progeny id - unique tree identifier
Trial	trial ID code
Acol	column number used in analysis - indicates real placement of trees in grid plan

Column header	Description
Arow	row number used in analysis - indicates real placement of trees in grid plan

22.7.7 tbl_B1-1 TreeAnalstat

Overall analysis status code for trial data.

Column header	Description
Tree_id	progeny id - unique tree identifier
Trial	trial ID code
Acol	column number used in analysis - indicates real placement of trees in grid plan
Arow	row number used in analysis - indicates real placement of trees in grid plan
Analstat	analysis status of tree. 0 = to be included in analyses, 1 = not included in analyses.

22.7.8 tbl_ExpstatCodes

Expstat codes and their meanings.

Column header	Description
Expstat	experimental status code
Tree_type	intended purpose/type of tree
Tree_status	tree condition
Notes	clarification of expstat code use in several cases

22.7.9 tbl_GapCalculator_Output

Output from the Gap Calculator program. Delete existing data in here before appending new data!

Column header	Description
Assess_year	year of data collection
Trial	trial ID code
Fcol	column number used by field staff - is not necessarily indicative of real placement of trees in grid plan
Frow	row number used by field staff - is not necessarily indicative of real placement of trees in grid plan
Var_id	variable names
y	value associated with each variable

22.7.10 tbl_Tree_idData

Tree ID data. Includes family data (for breeding trials) rootstock and scion cultivars (for rootstock trials) and germplasm site IDs and species (for germplasm trials).

Column header	Description
Tree_id	unique (or close to) tree id number
Prop_group	code for plant type. Group 1=clones, 2=seedlings, 3=selfs, 4=wild germplasm, 5=trees from rootstock trials
CV_name	cultivar name
Germplasm_siteno	ID code for wild germplasm population/site
Germplasm_treeno	numeric species ID
Species_code	numeric code for macadamia species present in population/site
Species_desc	numeric code for macadamia species present in population/site
Rootstock_treeno	numeric id of rootstock tree (unique only within rootstock type)
Rootstock_tmtno	numeric id for genetic identity of plant (treatment): type (2 digits, 11=cutting, 22=seedling) rootstock (3 digits) scion (3 digits)
Rootstock_type	whether the rootstock is a cutting or seedling
Rootstock_cv	cultivar ID of rootstock
Scion_cv	cultivar ID of scion (0 = own roots)
Progeny_cross_year	year that cross was performed
Parent1	cultivar ID of parent 1. Should always be the lower cultivar ID number of the two parents.
Parent2	cultivar ID of parent 2. Should always be the higher cultivar ID number of the two parents.
Family	numerical ID for family
Female	cultivar ID of female parent
Male	cultivar ID of male parent
Comments	comments

22.7.11 tbl_TrialData

Trial-level data.

Column header	Description
Trial	trial ID code
State	Australian state where trial is located
Site	trial location
Series	grouping factor for B1.1 trial planting year (2000 and 2001 grouped). Used for analysis.
Trial_pyear	year of trial planting
Trial_type	trial type. B1.1 = First iteration breeding trial, B1.2 = second iteration breeding trial, C = cultivar trial, G = germplasm trial, R = rootstock trial.

22.7.12 tbl_TrialDesign

Details of trial design (e.g. rep, block, spacing) and barcodes. Gap_calc_trial is a trial code shared by physically adjacent trials so that gaps can be properly estimated by the Gap Calculator program.

Column header	Description
Trial	trial ID code
Acol	column number used in analysis - indicates real placement of trees in grid plan
Arow	row number used in analysis - indicates real placement of trees in grid plan
Fcol	column number used by field staff - is not necessarily indicative of real placement of trees in grid plan
Frow	row number used by field staff - is not necessarily indicative of real placement of trees in grid plan
Block	experimental block reference
Rep	experimental replicate reference
Col_width	distance between columns (m)
Row_width	distance between rows (m)
Barcode	barcode text
Gap_calc_trial	trial code used by the Gap Calculator program.

22.7.13 tbl_VariableList

List of variables and their meanings.

Column header	Description
Var_class	overall variable grouping
Var_id	variable names
Meaning	description of variables

22.8 Appendix 8 – Evaluation of Macadamia Germplasm Resistance to *Amblypelta sp.* Attack in NSW and Queensland

Craig Maddox[1], Ruth Huwer[1], Ian Purdue[1], David Robertson[1] and Stephen Morris[1]

[1] NSW DPI, Wollongbar Primary Industries Institute, 1243 Bruxner Highway, Wollongbar, NSW 2477

22.8.1 Introduction

The establishment of the germplasm blocks at the Tiaro (QLD) and Alstonville (NSW) sites was a visionary act, as an off shoot of the macadamia breeding programme giving the macadamia industry a chance to exploit variations within the genome that will enable the local industry to keep improving the local product quality. These sites have allowed us to get data on factors as diverse as nut flavour, (which will only be briefly mentioned here) and how pest insect resistance can vary across the entire macadamia genome (McConchie *et al.* 1999, originally planted in 2001 by Hardener, McConchie CSIRO, project managed by Bruce Topp DAAFQ and QAFFI since 2009).

A perennial insect threat to the macadamia crop in New South Wales is the fruitspotting bug (FSB) *Amblypelta nitida* Stål (Hemiptera: Coreidae)(Brimblecombe 1948, Ironside 1981, Treverrow 1983, Gallagher *et al.* 2003, O'Hare *et al.* 2004). It is the pest that will be a constant threat to the quality of the nut crop throughout the growing season if cover spraying with insecticides becomes more difficult (Fay 2002). Our task as part of the macadamia breeding project has been to evaluate the germplasm orchard at the Centre for Tropical Horticulture (CTH) Alstonville to establish if there are germplasm sites that show resistance to attack, and conversely, to establish if there are genotypes more prone to heavy attack by FSB. The genetic makeup of such plants could then be examined to see which traits are linked to either FSB resistance or susceptibility and incorporated into the overall strategy of the breeding project. The pest is known for its wide host range (Waite & Huwer 1998), "hotspot" behaviour as well as varietal preferences (Waite *et al.* 2000, Huwer & Maddox 2004, Waite 2004, Huwer *et al.* 2006, Danne *et al.* 2014), and as such, spatial analysis of the block is required to determine if there are key areas where the population will establish first. There is also a significant climatic and spray management influence on the activity of the pest (Huwer *et al.* 2006, 2011) which has to be taken into account before we comment on overall germplasm preferences.

In South East Queensland, the conspecies *Amblypelta lutescens lutescens* (banana spotting bug, BSB) is also common on many fruit crops including macadamia (Ironside 1981, Donaldson 1983, Waite *et al.* 1993, Huwer 1996, Waite *et al.* 2000). This insect will also attack the shoot growth as well as the fruit on many crops. The damage to the actual macadamia crop is three fold. *Amblypelta sp.* present at flowering and nut set in spring tends to cause nut drop thus impacting on final yield. Nut damaged by *Amblypelta sp.* in December/ January tends to stay in the tree and is associated with the classic deep, wide, necrotic lesions in the kernel and shell damage which can be sorted out by the grower before delivery to the processor (see Figure 22.8.3). Damage occurring in late January until harvest is often not detected until the processors crack open the nut. The damage is not as deep (similar to *Nezara viridula* damage) and no visible trace is left on the shell. This is the damage that growers are often penalised for, the damage that needs to be minimised, and why the Horticulture Innovation Australia (HIA) project MT10049 - (A multi targeted approach to fruitspotting bug management) needs to find an effective monitoring tool for late season activity. For many of the high kernel recovery varieties (eg. cv 849) and the A series, feeding is possible through the shell (Huwer & Maddox 2004, Maddox *et al.* 2012). The risk of late *Amblypelta sp.* feeding is compounded by the presence of earlier damage (from this study and MT10049 work on avocado and guava) and the tendency for poorly set trees to flower out of season in February and May. This results in an unseasonal food source, which enables breeding of the *Amblypelta sp.* into autumn and leads to higher overwintering populations, as most growers are not spraying the crop until after harvest in August/ September.

The incidence of FSB in most orchards is usually measured by counting the proportion of damaged nut among the freshly dropped green nut under a tree (Ironside 1988, O'Hare *et al.* 2004). This is not an effective method of estimating the presence of FSB later in the season when nut drop ceases or when the shell is too hard to cut. Visible *Amblypelta sp.* population estimates can be done easily on crops where the fruit is terminal bearing (eg. mango, lychee), however most macadamia plants do not bear fruit this way. Mature trees (6-10m high) will require an elevated work platform (i.e. Afron®) for this task to see the fruit in the upper canopy where the FSB reside. The germplasm blocks are still young enough to see a large proportion of the nutset from the ground. FSB are by nature elusive and cryptic, and visual counts of bugs present are always an underestimate. Work on *Murraya paniculata* (L.) Jack (Rutaceae) hedges with this insect has dramatically improved our capacity to see the pest (Huwer *et al.* 2006 and 2011) and become more familiar with its seasonal movement and generation times (see Figure 22.8.7 for FSB catch 2009-2014 at CTH). By monitoring during the most suitable weather conditions (calm, sunny weather) during the sampling period we can give ourselves the best chance to see the insects, enable the team to build up accurate seasonal distribution maps.

There are two null hypotheses that we made for the study,
1) That FSB is equally likely to be found on any particular tree in the orchard and
2) That FSB damage is equally likely to be expressed on all kernels in the orchard when fed on by the bugs.

Unfortunately the impact of another Hemipteran pest, *Ulonemia decoris* (Macadamia lace bug) made it difficult to quantify exactly how much nut drop may have been lost to FSB at CTH Alstonville. The lace bug has had a major impact on nut set across northern NSW macadamia growing areas, with up to 90% reductions measured in crop on some varieties from 2008 onwards. It was remedied by a change in spray timing in that area from 2013 (Maddox *et al.* 2009, 2010, Huwer *et al.* 2011, Commens 2014, and Bright 2014). In particular, during seasons where FSB movement within the CTH block was monitored without the impact of spraying (2012-2013), we found that without managing lace bug, there was no crop. Fortunately at Tiaro in 2012 we were able to view the crop unsprayed, and without significant lace bug activity and this does give an indicator of the full impact of the *Amblypelta* spp on production. Endosulfan use was banned in Australia in October 2012, and this study is in some ways hard evidence of how important that chemical was to the growing of macadamia and many other crops in Australia that face this type of pest (Maddox *et al.* 2014).

22.8.2 Methods

CTH Alstonville planting: Incidence studies and nut harvest 2010-2014.

22.8.2.1 Plot Design
The trees are arranged in an 'L' shaped block as shown in Figure 22.8.1. Germplasm sites are interspersed throughout with varying numbers of trees representing each variety as shown in Table 22.8.1 and Figures 22.8.1 and 22.8.2. The identity of genomes of trees has been reviewed and corresponding labels on the trial maps updated since the 2010/2011 reports. There are also some changes in the level of varietal replication compared to the previous reports as a result of cyclonic weather on 4 separate occasions during the study. Not all trees were harvested in every season, and the 2012, 2013 seasons yields were particularly sparse for most germplasm sites (Figures 22.8.1 & Table 22.8.1).

The following approach was used to measure FSB incidence for each plant in the CTH germplasm block (n=615 trees). On the northern side of each tree (sunny aspect), we recorded if the tree was flowering, setting nut, carrying old nut, or dead. Numbers of FSB adults and nymphs, which were visible on the fruit in the lower 3m of the canopy were recorded (Figure 22.8.3). This process was repeated 5 times over the season beginning early spring, then late spring, early summer, late summer and autumn to detect where the population started, and where it spread to.

The damage levels were determined by harvesting nuts under and on trees during March for the earlier maturing germplasm and in June for the later maturing ones each season. For each tree with

sufficient crop, 10-30 nuts per tree were collected into mesh bags (labelled by row, tree number, and germplasm code). These were dehusked within 2 days, dried down to 1.5% moisture content (2 days at 38°C, 2 days at 45°C, 2days at 57°C), weighed, then cracked out and examined for kernel damage (AMS kernel assessment guide 2001 onwards). The kernel recovery figure is the proportion of kernel over the total dry nut in shell weight, FSB damage is the number of kernel halves removed because of FSB damage over the total number of kernel halves examined as a percentage (Figure 22.8.3). The figure for percent FSB damage was used to rank the germplasm in terms of visible damage in the samples. It does not include the early nut drop effect and as such would not reflect the total impact on yield for each germplasm site.

From our previous work, it is unlikely that any macadamia germplasm is immune to attack by FSB during the early part of the growing season, hence the reliance on sprays in spring. The mechanism by which they feed is such that they could penetrate nutshell to a depth of 6-7mm (Miles & Taylor 1994, Maddox *et al.* 2012), which is far thicker than any commercial macadamia shell known. The critical question is determining if FSB are coming back into the orchard during summer after the early season spraying had finished. Prior to the withdrawal of endosulfan, a typical grower would apply 2 sprays with a mixture of Endosulfan® (1.5ml/L)+Spin® (0.5ml/L) at nut set (September) then 4 weeks later. With this in mind, we adjusted the second seasons' (2011) sampling and spraying to address the issue of reinfestation of the orchard. Insecticide was applied at the site with a Tornado air blast spray unit using beta-cyfluthrin (Bulldock®) at the rate of 5L per tree and a mixture of 0.5ml/L. In the first season (2010) it was applied once in early December after the early spray only, in the second season (2011) it was applied twice once in late December and again in late January without the early spraying. The trees were examined within 3 days of each spray application to make sure the spray was effective (no bugs were found), and then after 3 weeks to see where the adults returned to and if that was related to any particular genome.

During the 2012 and 2013 seasons, no spraying was conducted to test if the result from 2011 was repeatable and attempt to find the most attractive genotype to the bugs, as this other extreme of the data is also of use for monitoring purposes. In 2014, macadamia lace bug treatments were applied in early spring to ensure a nutset, followed by sprays in December 2013 and January 2014. The crop was harvested in March and May/June 2014 and damage levels determined.

Tiaro Queensland Germplasm site: Incidence studies 2011-2012 and harvest 2012.
The same techniques as used in CTH Alstonville were applied to the Tiaro site for bug incidence. Bugs were observed while walking past each tree on fruit in the lower canopy. Where possible these were collected and recorded for sex and life stage on the trial plot map. Both *A. nitida* and *A. lutescens* will inhabit the orchards in this part of the macadamia growing regions. We visited the orchard 27/9/11, 22/11/11, 8/2/12, 28/8/12, and 6/11/12 and spent around 2 hours walking through the planting each time. Virtually all bugs collected were *A. lutescens* from this site on each occasion we visited but as all bugs were not caught, it is difficult to say with any certainty that no *A. nitida* adults were present (Figure 22.8.6). Recent work (in 2014) has shown *A. nitida* to be active throughout Bundaberg macadamia farms and on avocado orchards at Childers and Goodwood, so it is highly likely both species will frequent the Tiaro site when conditions are right. Harvesting of all the dropped nut under each tree at 1-2 month intervals, tagging, dehusking and storing of the samples was conducted by Dougal Russell (QDAFF April 2012 – October 2012). These were processed at the end of December 2014 by taking sub samples of 30 nuts from each of the labelled bags, re-drying them and examining the cracked nut for bug damage and weighing the total nut sampled under each tree at each harvest. The aim of this study is to investigate what pattern or preferences exist in the spotting bug activity on the wild macadamia germplasm at this site, and how it compares with activity at the CTH Alstonville germplasm where only *Amblypelta nitida* is active on the trees.

The Tiaro germplasm orchard contains a large number of macadamia genotypes each represented by one to sixteen trees and arranged in a space defined by 18 rows of 30 tree positions (Figure 22.8.2 and Table 22.8.5). A number of the trees bore significantly less fruit than the Alstonville site, cyclonic damage occurred in late January 2013, and very dry conditions with irrigation failures has also lead to significant tree death. There are also some management issues in the plot and no insecticide spraying

is possible at this stage. This has left the orchard open to some flower caterpillar issues and a little macadamia lace bug activity which has reduced nutset.

22.8.2.2 Data Analysis
Analysis was made of the *Amblypelta sp*. incidence data at the individual tree level, the germplasm site level and the grouping of the germplasm sites to plant species following the coding provided by the Plant Breeding group (Hardener, Neal, Topp pers. comm.). Incidence data is reported as percentage of trees infested at each time.

The proportion of FSB damaged kernel pieces was modelled as a response to germplasm site with adjustment for spatial location and seasons. Spatial effects were estimated by inclusion of an underlying FSB incidence probability function based on row and tree position. Seasonal effects were estimated as random deviations about an overall average FSB damage probability. The model could be described as a generalised linear mixed model of the log-odds of FSB damage as a response to fixed germplasm site effects and random effects associated with row, tree and season.

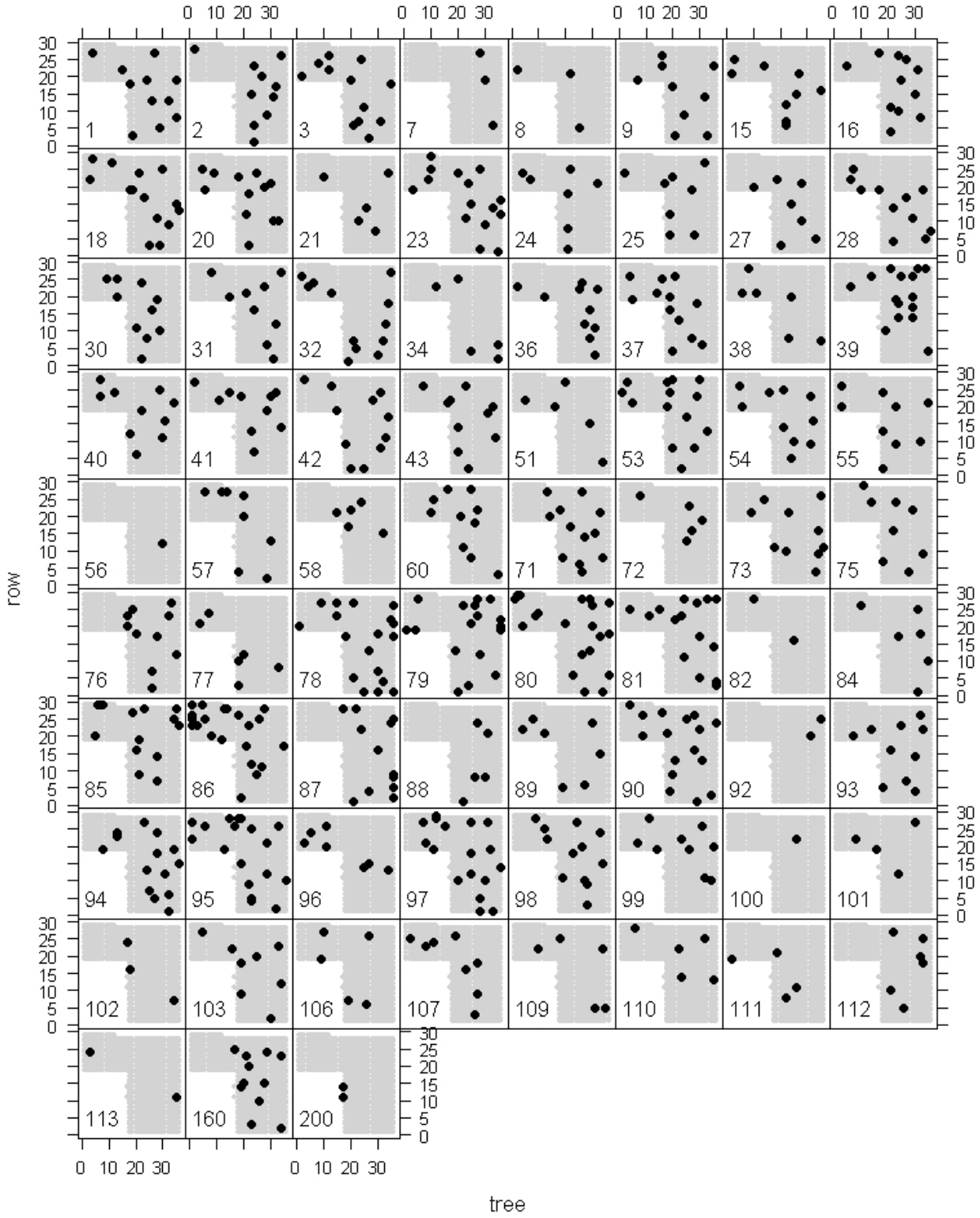

Figure 22.8.1. Each pane within the plot shows the recorded position of each tree of each germplasm site in the CTH Alstonville block. Plantings in row 1 start at tree position 18, from row 18 on trees are found from positions 1-36 giving the "L" shaped block originally 720 trees currently only 600+ left.

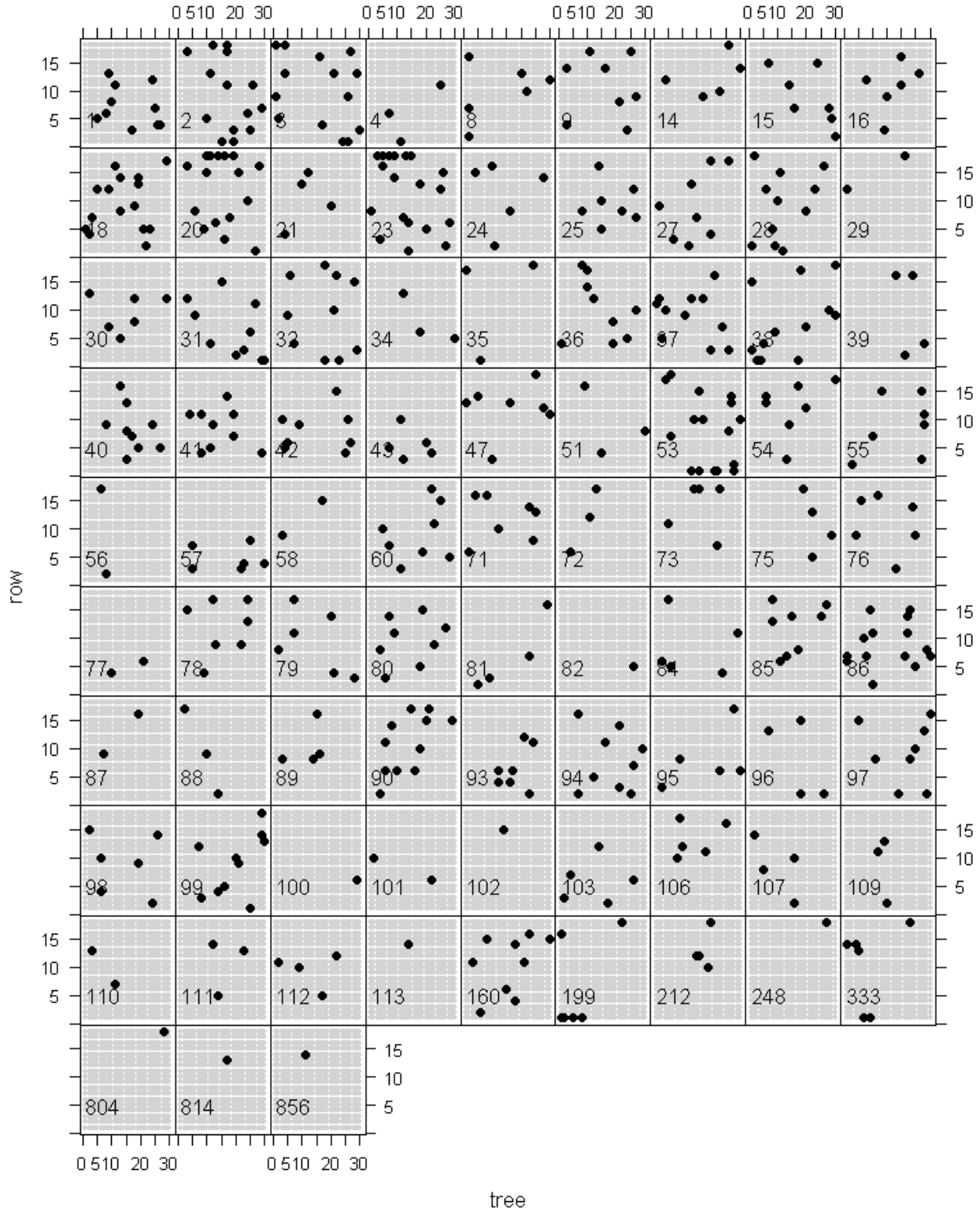

Figure 22.8.2. Each pane within the plot shows the recorded position of each tree of each germplasm site in the Tiaro block. Block is planted as a rectangle, originally 540 trees currently there are only 430 trees remaining.

Nuts harvested from the germplasm trial were evaluated for *Amblypelta sp.* damage in the kernel and visible shell damage over 5 seasons; 2010 - 2014. The genotype, season and location of each tree in the orchard are expected to be indicators for damage levels. Actual yields per tree were only collected at the Tiaro site in 2012, giving us an indication of which germplasm had still carried nuts despite *Amblypelta sp.* activity. The proportion of trees that were sampled of a given germplasm at CTH does give a similar indication, but only where the lace bug effects were masked by spraying (i.e. seasons 2010, 2011& 2014).

We are developing a tree ranking system for *Amblypelta sp*. resistance based on the data recovered so far. We have listed the top 5 germplasm sites based on crop carried (kg/tree/harvest) with consistently low damage (D< 0.1 or 10%) each season studied. Those germplasm that have been consistently low at CTH Alstonville and not harvested at Tiaro are also noted.

A  B 

C 

Figure 22.8.3. (A) Adult *Amblypelta nitida* (FSB) mating on *Macadamia ternifolia* nut at Alstonville CTH Germplasm site January 2012. (B) *Amblypelta nitida* nymph feeding on *Macadamia ternifolia* at CTH Alstonville in November 2011. (C) How fruitspotting bug damage looks on macadamia kernel depending on which part of the season it is inflicted upon the nut (A4 variety in this case) damage level is the number of FSB rejected half kernels / total number of half kernels sampled as a percentage.

Table 22.8.1. Number of trees harvested each season for each germplasm site at CTH Alstonville. Highlighted are the most resistant germplasm sites

Germplasm Site	2010*	2011*	2012	2013*	2014	Trees remaining	% Harvested
0	10	7	1	2	5	5	100
1	6	1	0	0	8	11	73
2	4	1	0	0	8	10	80
3	7	2	0	0	7	8	88
7	0	0	0	0	2	2	100
8	0	0	0	0	3	3	100
9	5	2	0	0	7	9	78
15	5	3	0	0	6	7	86
16	6	5	0	0	8	8	100
18	10	9	0	0	13	13	100
20	5	2	1	1	10	10	100
21	0	1	1	1	4	4	100
23	8	3	0	0	14	15	93
24	3	1	0	0	6	7	86
25	4	2	0	0	7	7	100
27	3	6	0	0	6	6	100
28	5	2	0	0	10	10	100
30	0	0	0	0	6	9	67
31	3	1	0	0	6	8	75
32	6	3	0	0	8	9	89
34	5	1	0	0	6	6	100
36	2	2	0	0	9	10	90
37	11	3	0	0	12	12	100
38	5	2	0	0	4	6	67
39	9	1	0	0	11	11	100
40	5	1	0	0	5	8	63
41	6	5	0	0	9	11	82
42	3	2	0	0	7	9	78
43	4	3	0	0	10	10	100
51	0	4	4	4	4	6	67
53	4	3	0	0	8	9	89
54	2	3	0	0	4	4	100
55	3	3	0	0	6	7	86
56	1	0	0	0	1	1	100
57	4	2	0	0	5	5	100
58	2	0	0	0	3	3	100
60	8	3	0	0	11	11	100
71	2	4	5	5	7	7	100
72	1	5	5	5	3	5	60
73	4	4	4	5	5	7	71
75	2	0	0	0	5	7	71

* Tree loss due to storm activity

Table 22.8.1. continued.

Germplasm Site	2010*	2011*	2012	2013*	2014	Trees remaining	% Harvested
76	5	5	0	0	10	10	100
77	7	3	0	0	9	9	100
78	12	3	0	0	14	14	100
79	10	7	0	0	12	14	86
80	11	2	0	0	16	17	94
81	9	2	0	0	11	14	79
82	3	1	0	0	3	3	100
84	2	0	0	0	3	3	100
85	6	5	0	0	12	13	92
86	11	4	0	0	17	19	89
87	3	4	0	0	9	10	90
88	3	5	4	4	7	7	100
89	1	2	0	0	3	3	100
90	9	7	0	0	14	15	93
93	2	0	0	0	6	6	100
94	4	1	0	0	10	12	83
95	5	4	0	0	11	15	73
96	2	1	0	0	4	5	80
97	8	5	0	0	12	13	92
98	3	2	0	0	7	9	78
99	3	1	0	0	10	10	100
100	1	0	0	0	2	2	100
101	3	1	1	1	5	5	100
102	1	0	0	0	1	2	50
103	6	2	0	0	8	8	100
106	5	2	0	0	6	6	100
107	2	4	0	0	6	6	100
109	4	3	0	0	4	5	80
110	3	3	0	0	5	5	100
111	1	0	0	0	4	5	80
112	3	5	0	0	6	6	100
113	1	2	0	0	2	2	100
160	5	1	0	0	7	9	78
200	0	0	2	2	1	2	50

* Tree loss due to storm activity

22.8.3 Results

22.8.3.1 CTH Alstonville

Incidence and damage patterns were strongly influenced by areas where spraying could not be applied (rows 25-29 border residential houses at CTH, Figure 22.8.4). Individual trees within the plot however did show much higher tendencies to be colonised by *Amblypelta nitida* (FSB). Recolonization was witnessed in 2011 (Table 22.8.2, Figure 22.8.4), and 12 tress within the block of 600+ trees, carried 80% of all the FSB seen that year (Table 22.8.2). Those trees were all *Macadamia ternifolia* species, and FSB were more likely to be seen on the *M. ternifolia* trees than they were on *M. tetraphylla* or *M. integrifolia* species (Figures 22.8.5 and 22.8.6, Table 22.8.2). Importantly, the FSB presence on some of those *M. ternifolia* is generally earlier than on the other genotypes (flowering is normally 1 month in front of the main crop), and this has implications for monitoring the pest generally if the cropping is consistent (Figure 22.8.6).

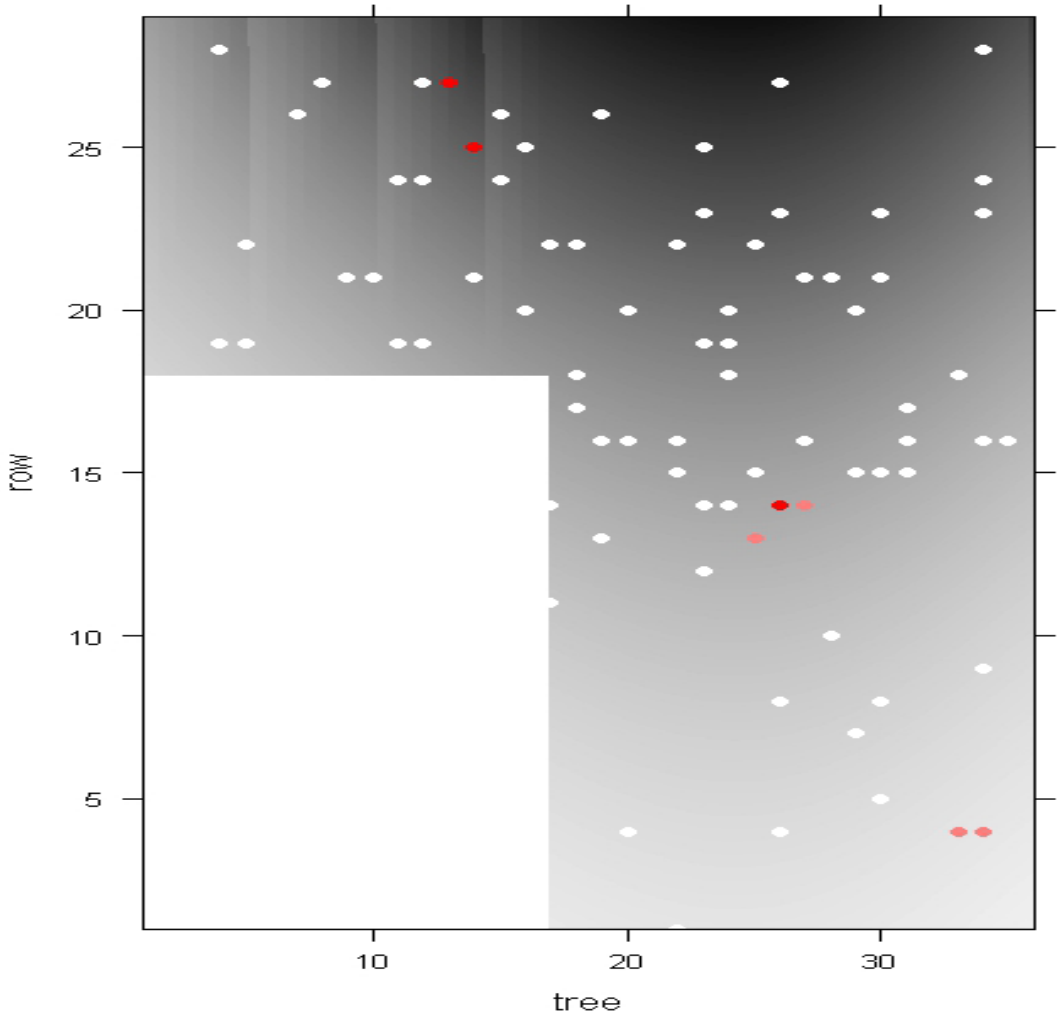

Figure 22.8.4. Spatial distribution of FSB damage rates ranging from 2% (light areas) to 22% (dark areas). Dots show location of total observed live FSB adults or nymphs aggregated over the five seasons and coloured according to arbitrary totals of 1-17 bodies (white), 17-33 bodies (mid red all *M. ternifolia* type) and 33-49 bodies (full red all *M. ternifolia* type).

Table 22.8.2. Incidence of *Amblypelta nitida* (FSB) at the CTH Alstonville Macadamia germplasm site when surveyed from July 2010 to March 2011, showing the reinfestation back onto *Macadamia ternifolia* (* 3 weeks after beta-cyfluthrin sprayed trees in grey highlight) and the lower incidence on the *Macadamia integrifolia* types.

		Macadamia species						
date surveyed		**M. tetraphylla**	**M. integrifolia**	**M ternifolia**	**Hybrid**	**Uncertain**	**Planted seedling**	**Totals**
17/08/2010	FSB total	1						1
	trees with bugs	1						1
	trees	188	162	30	51	130	57	618
23/11/2010	FSB total	5		16	2			23
	trees with bugs	5		8	1			14
	trees	188	162	30	51	130	57	618
9/12/2010	FSB total	11	2	50	7	8	3	81
	trees with bugs	8	1	12	3	6	3	33
	trees	188	162	30	51	130	57	618
7/01/2011*	FSB total	2		22		1		25
	trees with bugs	2		5		1		8
	trees	188	162	30	51	130	57	618
24/02/2011*	FSB total	3	3	32		3		41
	trees with bugs	3	1	5		2		11
	trees	194	171	30	45	122	53	615
3/03/2011	FSB total	2	9	22	1	1		35
	trees with bugs	1	2	6	1	1		11
	trees	194	171	30	45	122	53	615
	% total trees with FSB	4.2	1.2	40	5.9	4.6	5.3	
9/12/2010	% FSB seen on that type	13.5	2.5	62	8.6	9.8	3.7	
7/01/2011*	% FSB seen on that type	8		88		4		
24/02/2011*	% FSB seen on that type	7.3	7.3	78		7.3		

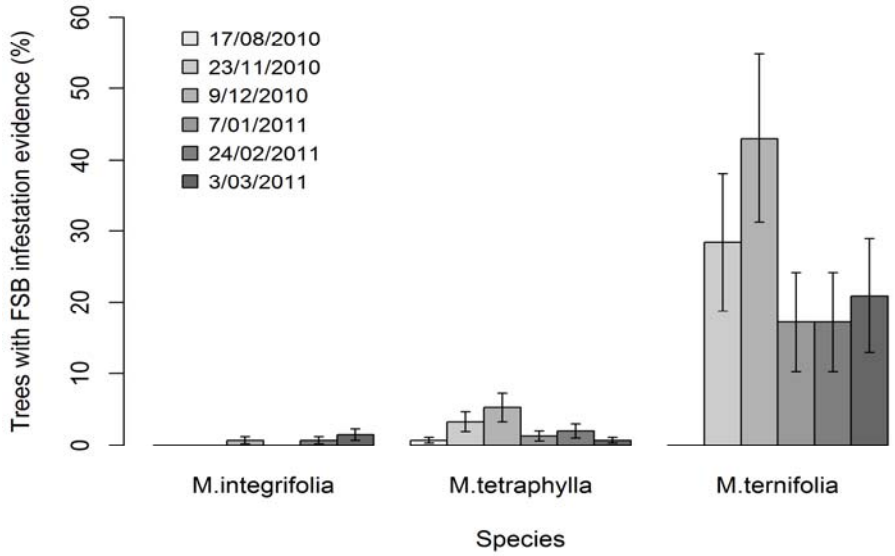

Figure 22.8.5. Incidence of *Amblypelta nitida* (FSB) at the CTH Alstonville *Macadamia* germplasm site from July 2010 to March 2011, showing the higher likelihood of detection on *Macadamia ternifolia* trees and the lower incidence on the *Macadamia integrifolia* types.

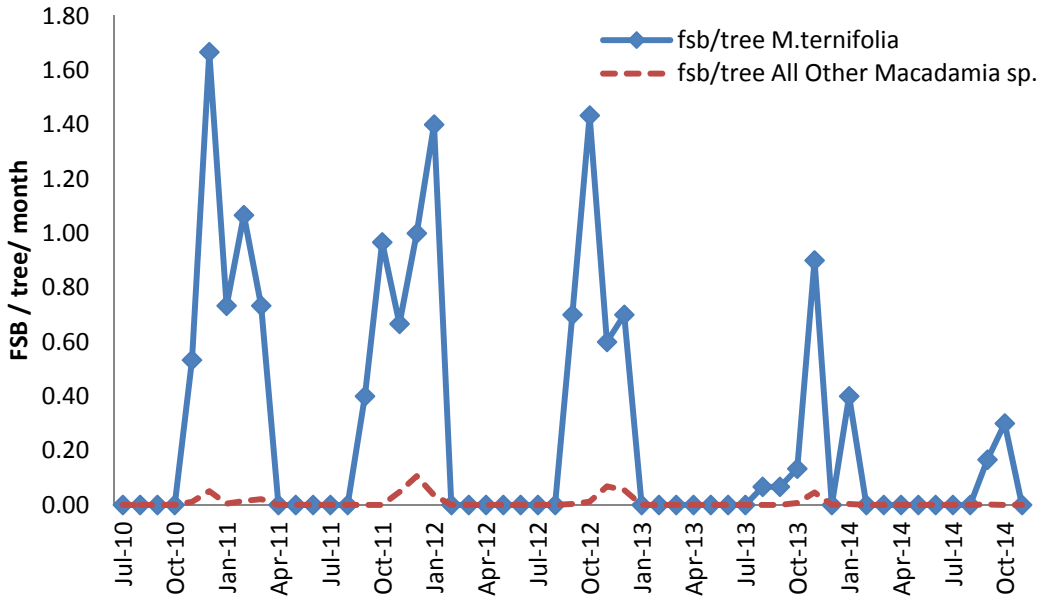

Figure 22.8.6. The incidence of *Amblypelta nitida* (FSB) at the CTH Alstonville *Macadamia* germplasm site from July 2010 to November 2014. Cumulative monthly counts on the *Macadamia ternifolia* (n=30 trees) compared to all other genotypes in the plot (n=550 trees) are earlier and higher (per tree) each season.

The macadamia crop is vulnerable to invasion from FSB for long periods of a season and a means of identifying the flights into this and many other crops is an important step forward in managing the insect. The FSB preference for *Macadamia ternifolia* is an important finding in this project and augments the period of FSB activity found on *Murraya paniculata* hedges since 2003 perfectly (Huwer *et al.* 2006, 2011). The weekly monitoring of FSB at CTH Alstonville on the trap hedges and the entomology macadamia orchard activity levels at the Alstonville site are shown in monthly summary

form (Figure 22.8.8). FSB population levels have been increasing in activity since the beginning of the work in 2009, especially in winter/spring over the last 3 years, and without the spraying damage would also increase (Figure 22.8.8).

Examining the kernel damage samples, FSB have shown a consistent preference for the *M. ternifolia* and *M. tetraphylla* germplasm over the *M. integrifolia* types (Table 22.8.4). In season 2009/10 we did not sample often enough to check where the initial infestation pressure was, (sampled 24/6/09 & 19/11/2009) and were unaware of the effect until the next year. Seasons 2012 and 2013 confirmed the incidence pattern when the orchard was unsprayed (Table 22.8.4) and season 2014 shows what the damage and incidence looks like when normal spraying is conducted (Table 22.8.4). Under a managed system in 2014 the *M. tetraphylla* is still significantly higher than the *M. integrifolia* germplasm and the *M. ternifolia* still has more FSB visible.

It is tempting to suggest the spray management changes were the major factor causing the seasonal effects, which were very strong with damaged kernel rates of 24% (se=6%) in 2012 and 2013. Rates in the other seasons were 5 - 7% (se=1 - 2%). Strong spatial effects were detected as damage rates increased with row number from about 2% at the low row numbers to about 20% at the high end, and with rates declining with trees further away from the centre of the top rows. Predicted FSB damage rates for each germplasm site were adjusted for these effects accordingly so that, for example, germplasm harvested in 2012, 2013 and with trees near the top of the orchard have an estimated damage rate lower than what was observed. These estimates are included in Table 22.8.10 with approximate standard errors that include variability due to seasons and overlaid on the observed damage rates over all seasons.

The most resistant macadamia germplasm sites at CTH are shown in Table 22.8.10 with only site 102 showing no damage to kernels over the study period. This germplasm site is only represented by 2 trees and only harvested twice during the entire study. Those germplasm sites which had crop damage always below 10% are the ones of most interest regarding FSB resistance (Table 22.8.10). It is also important to realise that the expressed damage value is only the level of damage occurring to the final crop after the period when FSB would normally cause nut abscission. Higher damage could also be a reflection of a trees' tendency to hang on to nut more readily despite the damage to the kernel, and that type of trait has caused problems with "sticktight" varieties before (eg A16, A38 and huskspot disease). Earlier seasons data collected (2010 and 2011) included a rating of trees with visible sticktights, and the green nut in husk was also scored for visible huskspot lesions during the harvest and kernel assessment (Table 22.8.3). The incidence of huskspot on the most FSB resistant germplasm sites is shown in Table 22.8.3 and only site 56 appears to be huskspot free as well.

Table 22.8.3. Trees with huskspot infected nuts / total trees harvested at CTH Alstonville in years 2010 and 2011. Proportion of trees carrying sticktights in the field examinations in spring at CTH in 2009 and at Tiaro in 2011.

Trial plot	**CTH**	**CTH**	**CTH**	**Tiaro**
Year	**2010**	**2011**	**2009**	**2011**
Evaluation	Husk Harvest	Husk Harvest	Sticktights Field	Sticktights Field
Germplasm site				
23	2/8	-	7/15	12/17
103	1/6	-	5/9	3/5
75	0/2	-	2/8	1/4
3	0/7	-	5/12	9/14
27	1/3	1/1	3/7	5/8
2	0/4	-	6/10	8/14
102	0/1	-	0/2	1/1
7	-	-	1/1	-
94	0/4	-	6/14	2/9
54	1/2	1/2	4/10	3/7
56	0/1	-	0/2	0/2
15	2/5	-	3/9	4/7
109	0/4	-	2/5	0/3
18	1/10	0/1	8/13	11/13

A  B 

Figure 22.8.7. (A) Distinct markings of *Amblypelta lutescens* nymphs feeding on *Murraya paniculata* berries from Nambour area in Queensland. (B) Mating behaviour of the two different *Amblypelta* species, *A. nitida* above and *A. lutescens* below males are the smaller individuals.

Table 22.8.4. The average level of *Amblypelta sp* damaged nut (100%=1.0) within the various macadamia species nut samples at each harvest for the germplasm blocks at CTH Alstonville NSW and Tiaro QLD. Data is the average across all samples harvested during each season with standard error, number followed by different letters are significantly different at 5% level z test. Trees are the number of that species harvested (H) and bugs are the total *Amblypelta sp.* seen on all trees of that species during the growing season.

Species groupings	**CTH 2010**	**CTH 2011**	**CTH 2012****	**Tiaro 2012 423 trees**	**CTH 2013****	**CTH 2014**
Macadamia tetraphylla 178 trees	0.17 [a] +/- 0.02 (100 H 34 bugs)	0.10 [b] +/- 0.02 (42 H 28 bugs)	(0 H 40 bugs)	0.48 [a] +/- 0.05 (111 tot 9 H 76 bugs)	(0 H 14 bugs)	0.21 [a] +/- 0.02 (158 H 11 bugs)
Macadamia integrifolia 163 trees	0.04 [b] +/- 0.01 (93 H 8 bugs)	0.07 [b] +/- 0.01 (52 H 14 bugs)	(0 H 13 bugs)	0.22 [b] +/- 0.01 (143 tot 83 H 67 bugs)	(0 H 0 bugs)	0.06 [b] +/- 0.01 (150 H 4 bugs)
Macadamia ternifolia 29 trees	0.04 [b] +/- 0.02 (6 H 0 bugs)	0.32 [a] +/- 0.04 (25 H 136 bugs)	0.28 [ab] +/- 0.04 (24 H 72 bugs)	(9 tot 0 H 0 bugs)	0.36 [a] +/- 0.05 (26 trees 86 bugs)	0.09 [b] +/- 0.04 (24 H 22 bugs)
M. ternifolia hybrids 48 trees	0. 07 [b] +/- 0.02 (26 H 4 bugs)	0.10 [b] +/- 0.05 (11 H 12 bugs)	0.37 [ab] +/- 0.04 (11 H 6 bugs)	(57tot 0 H 18 bugs)	0.06 [b] +/- 0.05 (11 trees 12 bugs)	0.10 [b] +/- 0.02 (43 H 12 bugs)
M. jansenii X M. ternifolia 2 trees			0.71 [a] +/- 0.29 (2 H 17 bugs)		0.26 [a] +/- 0.12 (2 H 3 bugs)	0.0 (1 H 0 bugs)
Management	Conventional spray timing	Sprayed Dec Jan only	Unsprayed	Unsprayed	Unsprayed	Conventional spray timing

** Block was heavily impacted by *Ulonemia decoris* (Macadamia lace bug) without spraying at nutset/ pre-flowering virtually no nut set was the result. In 2014 the block was managed with a September spray (conventional) and most trees cropped well. Tiaro showed the impact of *Amblypelta lutescens lutescens* rather than *A. nitida*, incidence and damage followed same pattern with the preference for *M. tetraphylla* over *M. integrifolia* germplasm but no *M. ternifiolia* nut was recovered.

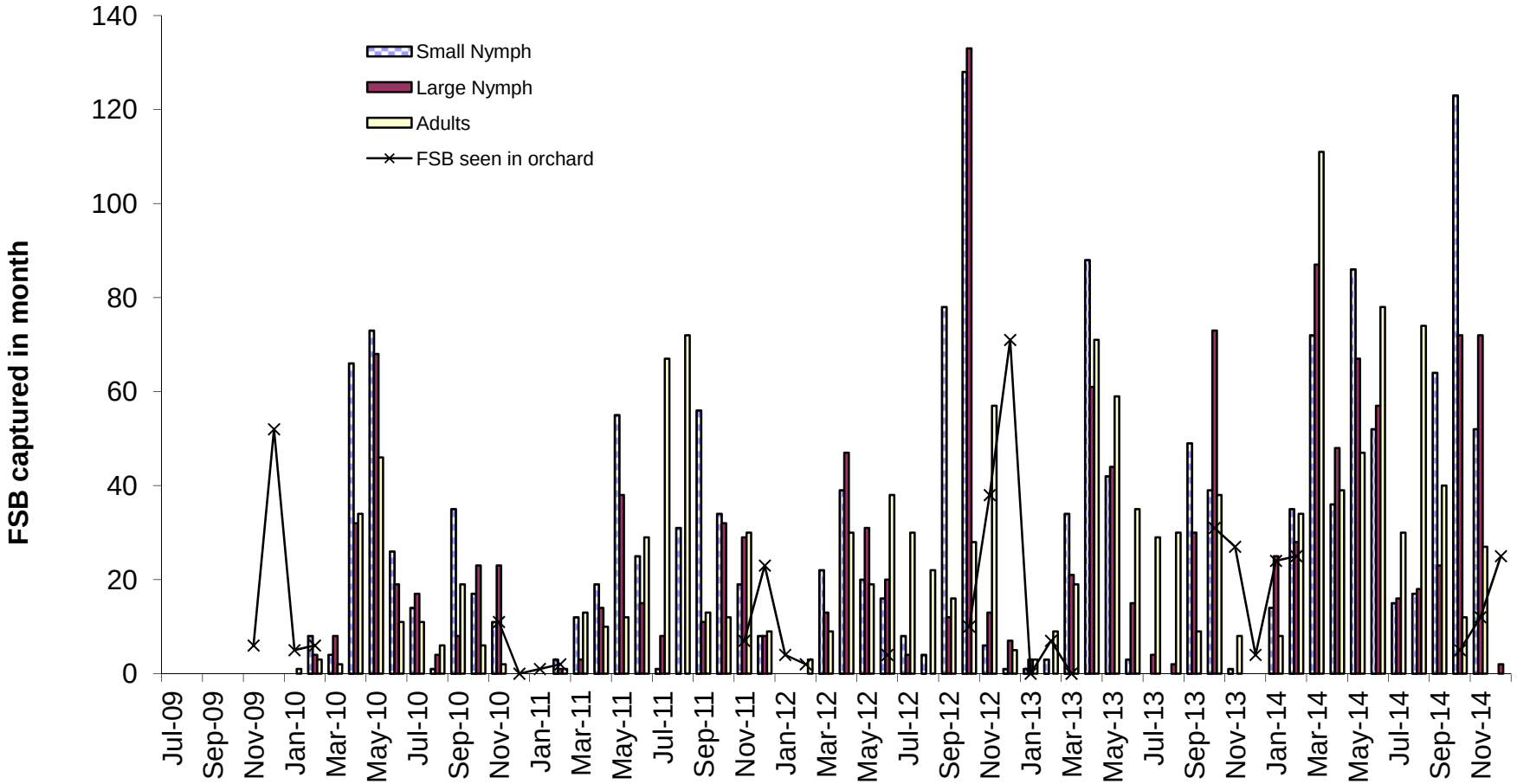

Figure 22.8.8. Monthly *Amblypelta nitida* (FSB) captured on *Murraya paniculata* plants (n=39) between July 2009 and December 2014 compared to the level of live FSB seen in the Entomology macadamia orchard at Centre for Tropical Horticulture Alstonville NSW. Increasing activity is being detected throughout winter months leading to higher spring populations

22.8.3.2 Tiaro

Historically we know that point samples when observations are made do not give the entire picture for *Amblypelta sp.* damage. FSB damage and incidence trials at CTH Alstonville 2001-2014 (Huwer *et al.* 2006, 2011) have recorded FSB damage >70% on some trees. Other sites like the Maroochy research variety block (Nambour QLD) where a single *Macadamia ternifolia* tree on the corner of that plot can carry damage levels >90% but only showing the odd nymph of both species when visited during the season, yet if visited at the "right time" it would be swarming with bugs. From the field map of the site with the overlay of *Amblypelta sp.* incidence during the 2011-2012 periods, it is clear that the bugs were present across the block and those collected were exclusively *A. lutecsens* (Table 22.8.5 and Figure 22.8.7). Highest *Amblypelta lutescens* populations were found on the *Macadamia tetraphylla* plants from germplasm sites **85** and **60** (Tables 22.8.5, 22.8.8 & 22.8.9). The levels were also higher than average on the *Macadamia integrifolia* germplasm site **28**. Incidence rankings were based on the germplasm with bug pressure per tree greater than the block average rate of 0.5/tree (Table 22.8.9). This is different to the observed preference for *Macadamia ternifolia* shown by *A. nitida* at the Alstonville CTH germplasm site.

From the kernel damage data comparisons at an overall macadamia species level FSB showed a significant preference for feeding on the *M. tetraphylla* (mean=48%) over the *M. integrifolia* (mean=22%) which is similar to that shown at CTH Alstonville (Table 22.8.4). When looked at spatially (Tables 22.8.5, 22.8.6 and 22.8.7), incidence alone is not a good guide to damage. Trees with the highest incidence do not always carry high damage and those with the highest damage levels in the kernel have often not had any bugs seen on them at the times we sampled (Tables 22.8.5, 22.8.6 & 22.8.7). The crop yields at the tree level also give an indication of how much activity occurred on that germplasm before nut shedding stopped and some high cropping trees in areas where the bugs were active show some resistance (Table 22.8.7). Many sites failed to produce any crop at this site and were not able to be directly compared to those harvested at CTH Alstonville (Tables 22.8.7 and 22.8.10).

The only germplasm sites to show little or no activity (<10%damage and some crop left) through that high pressure season are **23, 75, 103, 3, 27 and 101** (all from *Macadamia integrifolia* group see Table 22.8.9 and 22.8.10). Germplasm site **2** carried the most nuts per tree per harvest (2kg), but was also carrying *Amblypelta sp.* damage at the 30% level. Conversely germplasm site **96** had clean nut from the Tiaro site (9%) but carried virtually no crop i.e. 60gms/tree/harvest (Table 22.8.10).

22.8.3.3 Overall

The overall ranking of germplasm resistance to *Amblypelta sp*. attack has the following order **23, 103, 75, 3, and 27** with little separating the top 2 germplasm based on crop loads and kernel damage (Table 22.8.10).

The negative correlation between how far north (northings) a germplasm site was collected from and FSB damage was stronger for those measured at CTH Alstonville as opposed to the measured damage at Tiaro (Figures 22.8.9, 22.8.10). This supports the feeding preference detected for *M. tetraphylla* species which dominate the germplasm sites collected in the southern end and also the main distribution area for *Amblypelta nitida.* Germplasm sites from the *M. integrifolia* group are roughly half as likely to carry bug damage through to harvest as those with more *M. tetraphylla* traits.

Without *M. ternifolia* nut to compare from Tiaro, we cannot confirm how well the monitoring effect works for *A. lutescens* areas. We have however observed high numbers of both *Amblypelta* species on a *M. ternifolia* tree which can have damage as high as 90% on the Maroochy Research station at Nambour in Queensland in 2013 and again in early 2015. The tree also belongs to the 71, 72, 73 germplasm site plants which are the 7 obvious monitoring plants found at the CTH Alstonville site.

22.8.4 Conclusions

Ranking the different germplasm sites in the two early seasons did not give conclusive results. In the season 2009/2010 recurrent late damage was not reflected. There was no more damage after the

spray application in December 2009. In the following season (2010/2011) however, there was continual re-infestation, despite two spray applications, which were both effective. The critical damage period appears to be throughout December and January, and on some thinner shelled varieties such as A4 and 849, right through to maturity. This is compounded by the fact that after January, FSB damaged nuts don't dehisce from the tree and can't be monitored (Huwer & Maddox 2004).

Were there germplasm sites with significant levels of resistance from the two germplasm sites?
Yes: germplasm sites **23, 103, 3, 27, and 75** showed some level of resistance to *Amblypelta sp*. attack.

Were there trees / species which are significantly more attractive and if so, at what phenological stages are they most attractive?
The early flowering, terminal bearing trees of the M. ternifolia genotypes 71, 72 and 73 appeared to be highly attractive in the season 2010/2011. These trees did not rank highly with regards to their attractiveness in season 2009/2010. They also did not bear a lot of nuts in the first season of the study, which made for no kernel assessment.

FSB adults certainly kept coming back to the trees of genotypes *M. ternifolia* 71, 72 and 73. The question that now needs to be answered is, whether it is the actual tree (i.e. flower) that attracts FSB back to the tree, or is it the bug itself (i.e. chemical produced by the bug while feeding on the nuts or chemical on fed nut itself). The *M. ternifolia* preference is probably most influenced by the timing of the nutset and florescence. *M. ternifolia* tend to be already set when the rest of the macadamia genotypes are still flowering. This gives the overwintering *A. nitida* adults their first chance to breed in the crop. The other aggregation traits around early fruiting would compound this. The post spraying observations in 2010/11 where the *A. nitida* came back on to *M. ternifolia* nut in January and February after beta- cyfluthrin spray applications does beg the question that the damaged fruit hanging in the plant are somehow attractive in their own right. We have taken that principle to the field with the hedge concept and have had some success (Huwer & Maddox current work MT 10049).

It also has to be kept in mind that the overall, attractiveness of specific genotypes are relative to what is in the orchard. Least susceptible genotypes will still be attacked by FSB, if that is the only food source around (Drew, 2005). With regards to macadamias, all genotypes are susceptible during the nut development phase. It is important to find out what triggers FSB to re-infest the orchard.

The period of attack is slightly different for both bug species and the *A. lutescens* activity may indeed follow the shoot growth period whereas the *A. nitida* activity is stimulated whenever florescence is found on the plant. *A. nitida* is dominant in NSW and is more active through winter than first thought. Spring management appears to offer the best defence against this pest. *A. lutescens* has re-appeared in SE QLD after a relatively long absence (started mid October 2014 Nambour area – not seen after March 2014). In the Nambour region the population has re-established on passionfruit initially before attacking re-growth on custard apple and other crops, the *A. nitida* in the area have moved to the fruit on *Murraya panniculata*, macadamia , mango, avocado (Paxton crop hedge data and orchard collections August 2014-November 2014).

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These results could not have been obtained without the help of the following people. Special thanks to Alister Janetzki, and Magda Verbeek (the harvest and kernel recovery team at CTH Alstonville) for their commitment to the task and practical help along the way. It was a big effort on quite a few wet days and an extra level of work in the germplasm areas above and beyond the normal entomology trial work. Thanks also to Tina Robertson, and Carly Maddox for their help with the later harvests, kernel recovery and data. Thanks to Mathew Stewart and Geoff Quinn for the orchard mowing, spraying, maintenance and orchard repair tasks. During the 2009-2014 period, there was more cyclonic activity than usual, 5 major clean-up events have occurred and Mark Hickey and Jeremy Bright assisted with our tree repair operations. The Horticulture unit was moved to Wollongbar during this time making that process more difficult. Thanks also to Dougal Russell, Jodi Neal, Rod Daley and

Bruce Topp for the harvesting at Tiaro and access to the samples for the 2012 season damage analysis.

Table 22.8.5. Tiaro site map showing germplasm plantings originally 540 trees planted, currently down to 423 with losses to *Phytophthora sp.* and storms. Overlayed is the distribution of the *Amblypelta* species found during the 5 observation visits from September 2011-November 2012.

Row	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	Total
Tree																			FSB
1	199	28	38	36	18	86	86		3		37							3	2
2	199	8		18		71	8	79	27	101		37	30	107	98	8	88	28	1
3	38	55	95	9	37	84	18	89	58	42	160	31	110	9	78	20	2	23	2
4	38	90	23	21	42		103	80	76	37	41		3		24		53	3	6
5	199	81	57	38	1	42	57	107	32	60	73	18			97	23	84	23	3
6	35	160	80	98	84	90	53	20	31	98	90	28	54	54	76	32	56	53	6
7	333	94	27	32	43	4	60		87	86	79		96	80	15	94		23	7
8	199	56	99	41	28	1	86	25	40	106		16	85	90	160	71		36	21
9		28	81	78	20	38	30	95		112	80		1	23	86	51	106		9
10		86		77	2	90	55			28	86	106	21	36	20	24		20	5
11		24	60	31	41	85	110	97	37	43		72	2		28	18	9	20	7
12	28	27	43	93	94	93			41		109	36	34		21	76	78	2	3
13	53		54		30	20	85	18	78			37	27		55	40		23	5
14	23	88	16	99		23		89	54	53	15	103	109		102	25		20	7
15	2	109	40	51	25	160				25			40		31	89	90	23	6
16	53	107	20	93		90	15	24	89	107	94			9	53	3		20	3
17	38	103	1	3	112	93	40	85		53	2	37		41	58			2	2
18	32	96	76	160	80	34	20	30	18	90	106	30	23	160	96	39	38	32	11
19	2	97	2	36	40	60	41	36	98		41		18	18	80	87	75	20	4
20		31	37	27	23	43	38	28	21	99	16	54		79	90	16	27		10
21	53	39	94	79	18	77	86		99	32	160		3	94		37	90		7
22	53	18	57	43	75	101		25	78	8	86	112	75	86		32	60		3
23	32	93	31		18	95		97			60	28	111	71	86	160			23
24	3	98	9	84	36	2	37	71	40	20	93	1	78	76	15	39	78		12
25		94	2		86	31	1	57	76	97		23		85	60		9		5
26	3	96	37	1	82	103	94	53	3	42	2	25	16	98			27		13
27	20	23			40	42	25		9	36	31	80	53	53			3		4
28	53	53	79	39	60	23	15		55		55		97		32		95		9
29	31		32	41	15	100		86	75	94	84	30	3	99	90		18		7
30	31		3	57	34	95	86		38	53									6
FSB	6	3	9	16	25	16	26	10	12	6	13	10	19	4	7	5	11	11	209

FSB
1
2
2<x<6
8
15

Table 22.8.6. Tiaro site map showing the spatial distribution and average % kernel loss to *Amblypelta* species in the harvest nut samples. Some plants were only harvested once; some were harvested 6 times between April and October 2012.

	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18
1								0.0					56.1	20.6	25.0	50.6	63.5	
2							16.1		16.9	13.7						42.1		
3	21.3	1.6	30.0	16.1					50.0					29.2			23.3	
4	13.1	15.8	2.7				12.6	52.0	11.1				4.9				42.4	
5			8.3		22.7		43.1											
6						25.8	15.3				32.1	10.0		0.0				
7	70.0		6.3											52.7				
8					55.6			50.4										
9		17.0											51.3	7.9				
10							82.4			14.3								
11													5.4		66.6		39.2	
12		4.8														51.5		
13				46.1									12.4		20.8			
14						22.2				7.5		13.8				20.8		
15			60.3		56.8					35.7							15.7	
16														11.9		1.3		
17		3.7	9.7	5.4			100.0			14.4	35.1							
18										20.6								
19						37.8												
20								71.1							27.5			
21						32.5							1.7				11.0	
22			7.1		10.2	10.1		31.0		25.0							37.3	
23												69.5						20.8
24			6.0			28.9			100.0			43.3						
25							38.8	12.1	100.0						48.6		25.1	
26		9.1		3.9	25.0	18.6		0.0	10.3		67.8	45.0					2.1	
27		8.9					68.2		44.1				29.8	2.6			20.0	
28						8.5			34.4		71.5							
29									7.1				2.2					
30																		

Table 22.8.7. Tiaro site map showing the spatial distribution and the final crop load (gms) for the trees harvested in the germplasm plantings. Some plants were only harvested once; some were harvested 6 times between April and October 2012.

	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18
1								4.5					291.9	215.2	845.7	1092.2	353.7	
2							73.7		321.2	846.2						536.2		
3	656.3	1092.2	285.9	1117.9					30.6					2132.2			1702.3	
4	1451.0	707.2	4087.0				1705.2	155.5	36.5				1986.7				768.5	
5			1385.2		479.5		170.5											
6						1177.8	413.9				1151.7	1505.3		140.8				
7	27.6		664.4											110.5				
8					560.2			113.5										
9		2477.3											109.5	1119.9				
10							388.0			61.5								
11													2267.4		477.9		224.7	
12		2172.4														590.9		
13				295.9									402.7		108.0			
14						982.7				1632.4		2047.5				254.5		
15			671.9		388.1					47.4							424.8	
16														1607.5		1430.5		
17		3204.1	3597.6	1112.8			17.7			706.6	109.7							
18										163.7								
19						369.6												
20								710.7							124.0			
21						353.5							2256.8				408.9	
22			294.3		868.6	1146.0		324.6		25.2							401.3	
23												421.2						80.2
24			971.9			96.4			24.5			44.7						
25							100.2	1612.4	30.6						410.5		736.3	
26		61.4		1856.9	35.8	192.3		1352.3	185.8		3881.2	253.9					848.3	
27		1879.0					43.8		132.8				649.3	484.5			23.9	
28						911.0			432.0		306.6							
29									1418.4				639.2					
30																		

Table 22.8.8. Sex and Age distribution (male + female + nymphs= total BSB) of the *Amblypelta* species (*A. lutescens* BSB mainly) found during the 5 observation visits from September 2011-November 2012 at Tiaro site separated by the dominant species code for each germplasm site. A total of 209 bugs were found over 423 trees overall and the % of catch refers to each visit and the proportion of bugs on that species type.

Macadamia Species	**Date (total bugs)**	**27/9/2011 (30)**	**22/11/2011 (93)**	**8/2/2012 (59)**	**28/8/2012 (0)**	**6/11/2012 (27)**
Type 1 *M.tetraphylla*	Trees	111	111	111	111	111
	Male BSB	4		1		
	Female BSB	4	12	6		3
	Total BSB	10	50	10	Nil	6
	% of catch	33%	54%	17%		22%
Type 2 *M.integrifolia*	Trees	143	143	143	143	143
	Male BSB	4		10		4
	Female BSB	8	3	11		4
	Total BSB	12	18	27	Nil	10
	% of catch	40%	19%	46%		37%
Type 3 *M.ternifolia*	Trees	5	5	5	5	5
	Male BSB					
	Female BSB					
	Total BSB	Nil	Nil	Nil	Nil	Nil
	% of catch					
Type 4 *M.ternifolia* hybrid	Trees	48	48	48	48	48
	Male BSB	2		2		0
	Female BSB	2	3	1		1
	Total BSB	4	10	3	Nil	1
	% of catch	13%	11%	5%		4%
Type 5 Unknown	Trees	27	27	27	27	27
	Male BSB			2		
	Female BSB	2	4			1
	Total BSB	2	9	3	Nil	3
	% of catch	7%	10%	5%		11%
Type 6 Planted seedlings	Trees	89	89	89	89	89
	Male BSB	1		6		1
	Female BSB	1	4	7		5
	Total BSB	2	6	16	Nil	7
	% of catch	7%	6%	27%		26%

Table 22.8.9. Ranking of Macadamia germplasm sites based on the incidence of *Amblypelta* species (*A. lutescens* BSB mainly) found during the 5 observation visits from September 2011-November 2012 at the Tiaro site separated by the dominant species at each germplasm site.

Species	Site	No. trees	BSB	Bugs/tree
M. tetraphylla	85	5	21	4.2
	60	8	18	2.3
	110	1	1	1.0
	79	5	4	0.8
	41	7	5	0.7
	80	7	5	0.7
	84	5	3	0.6
	40	8	4	0.5
	96	4	2	0.5
	37	11	5	0.5
	95	5	2	0.4
	94	9	3	0.3
	39	4	1	0.3
	93	6	1	0.2
	98	6	1	0.2
	38	7	0	0.0
	42	5	0	0.0
	81	2	0	0.0
	99	5	0	0.0
	111	1	0	0.0
	333	2	0	0.0
	Overall	**111**	**76**	**0.7**
M. integrifolia	28	10	12	1.2
	57	5	5	1.0
	54	4	3	0.8
	90	11	8	0.7
	3	14	9	0.6
	55	5	3	0.6
	53	16	8	0.5
	77	2	1	0.5
	103	4	2	0.5
	27	6	2	0.3
	1	7	2	0.3
	9	7	2	0.3
	25	7	2	0.3
	23	15	4	0.3
	75	4	1	0.3
	2	12	2	0.2
	76	6	1	0.2
	8	4	0	0.0
	56	1	0	0.0
	58	2	0	0.0
	82	1	0	0.0
	101	2	0	0.0
	Overall	**143**	**67**	**0.5**
M. ternifolia	51	2	0	0.0
	72	1	0	0.0
	88	2	0	0.0
	Overall	**5**	**0**	**0.0**

Table 22.8.10. Comparison of germplasm site by average *Amblypelta nitida* (FSB) damage at harvest. Mean and standard error (se) for years 2011 and 2014 at CTH Alstonville, the entire 2010- 2014 period at CTH and then for Tiaro Queensland in 2012 where *Amblypelta lutescens* (BSB) was the primary pest species. Highlighted strips are most resistant germplasm sites.

	CTH						Tiaro 2012					
	2011		2014		2010-14							
Site	Raw FSB damage	S.E.	Raw FSB damage	S.E.	FSB damage rate	S.E.	Raw BSB damage rate	S.E.	Crop/tree/ harvest kgs	No. trees	No. trees harvested	Ranking 1=most resistant
84			0.27	0.11	0.38	0.16						
73	0.7	0.14	0.07	0.03	0.21	0.07						
72	0.56	0.13	0.01	0	0.16	0.06						
82	0.48	0.25	0.06	0.03	0.17	0.08	0.35	0.35	0.04	1	1	
40	0.46	0.24	0.07	0.03	0.22	0.09	0.74	0.14	0.36	9	2	
71	0.43	0.13	0	0	0.1	0.04						
107	0.4	0.13	0.12	0.04	0.37	0.12						
160	0.39	0.21	0.13	0.04	0.36	0.12						
21	0.38	0.13	0.02	0.01	0.11	0.05						
51	0.31	0.11	0.11	0.05	0.23	0.08						
39	0.28	0.17	0.1	0.03	0.23	0.08						
34	0.27	0.17	0.02	0.01	0.08	0.04						
79	0.26	0.09	0.18	0.05	0.28	0.09						
78	0.24	0.12	0.11	0.03	0.2	0.07						
42	0.23	0.12	0.13	0.04	0.23	0.09						
1	0.23	0.15	0.05	0.02	0.13	0.05	0.29	0.08	0.87	9	5	
106	0.22	0.11	0.05	0.02	0.17	0.07						
86	0.21	0.08	0.08	0.02	0.17	0.06						
36	0.21	0.11	0.04	0.01	0.1	0.04						
97	0.21	0.08	0.11	0.03	0.16	0.06						
76	0.2	0.08	0.04	0.01	0.12	0.05	0.53	0.22	0.33	6	2	
20	0.18	0.1	0.03	0.01	0.07	0.03						
41	0.16	0.07	0.09	0.03	0.21	0.08						
60	0.15	0.07	0.06	0.02	0.13	0.05	0.41	0.09	0.39	8	3	
90	0.15	0.06	0.03	0.01	0.09	0.03	0.21	0.05	0.59	11	7	
110	0.14	0.07	0.13	0.05	0.28	0.1						
98	0.14	0.08	0.17	0.05	0.38	0.13						

Site	CTH						Tiaro 2012					
	2011		2014		2010-14							
	Raw FSB damage	S.E.	Raw FSB damage	S.E.	FSB damage rate	S.E.	Raw BSB damage rate	S.E.	Crop/tree/ harvest kgs	No. trees	No. trees harvested	Ranking 1=most resistant
89	0.13	0.07	0.06	0.03	0.15	0.07						
25	0.13	0.07	0.04	0.02	0.1	0.04	0.41	0.08	0.24	7	6	
32	0.11	0.06	0.05	0.02	0.09	0.04						
38	0.1	0.05	0	0	0.08	0.04						
18	0.09	0.03	0.05	0.01	0.09	0.03						
85	0.08	0.04	0.06	0.02	0.14	0.05						
95	0.08	0.04	0.1	0.03	0.19	0.07	0.3	0.17	0.29	5	1	
80	0.08	0.05	0.1	0.03	0.22	0.07	0.52	0.07	0.13	8	2	
16	0.08	0.03	0.13	0.04	0.19	0.07						
81	0.07	0.04	0.08	0.02	0.24	0.08						
99	0.07	0.05	0.2	0.06	0.34	0.12						
53	0.07	0.03	0.04	0.02	0.05	0.02	0.17	0.07	0.86	16	7	
3	0.06	0.04	0.02	0.01	0.04	0.02	0.05	0.02	1.09	14	7	4
27	0.06	0.02	0.01	0.01	0.04	0.02	0.08	0.04	0.88	8	5	5
112	0.06	0.03	0.07	0.03	0.14	0.06						
88	0.06	0.03	0.03	0.02	0.11	0.04						
43	0.06	0.04	0.11	0.04	0.22	0.08						
77	0.06	0.03	0.01	0.01	0.06	0.03	0.33	0.34	0.35	2	1	
2	0.06	0.04	0.07	0.02	0.13	0.05	0.3	0.12	2.01	14	4	Most nut
96	0.05	0.04	0.15	0.06	0.18	0.08	0.09	-	0.06	4	1	
109	0.05	0.03	0.02	0.01	0.05	0.03						
9	0.05	0.03	0.01	0.01	0.05	0.02	0.21	0.06	0.99	8	7	
57	0.05	0.02	0.03	0.01	0.07	0.03	0.15	0.08	1.15	6	3	
15	0.05	0.02	0.02	0.01	0.06	0.02						
55	0.04	0.02	0.04	0.02	0.09	0.04	0.43	0.18	0.58	7	4	
87	0.04	0.02	0.07	0.03	0.12	0.05						
113	0.04	0.03	0.09	0.05	0.12	0.06						
103	0.04	0.02	0.02	0.01	0.05	0.02	0.11	0.04	1.79	5	4	2
8	0.03	0.02	0.03	0.02	0.07	0.04	0.31	0.34	0.21	4	3	
23	0.02	0.01	0.02	0.01	0.05	0.02	0.1	0.02	1.8	16	5	1

Site	CTH						Tiaro 2012					
	2011		2014		2010-14							
	Raw FSB damage	S.E.	Raw FSB damage	S.E.	FSB damage rate	S.E.	Raw BSB damage rate	S.E.	Crop/tree/ harvest kgs	No. trees	No. trees harvested	Ranking 1=most resistant
37	0.02	0.01	0.1	0.03	0.18	0.06						
31	0	0	0.13	0.04	0.16	0.07						
24	0	0	0.05	0.02	0.1	0.05						
54	0	0	0.05	0.02	0.05	0.02						
7	0	0	0	0	0.01	0.01						
28	0	0	0.03	0.01	0.05	0.02	0.41	0.11	0.89	11	7	
30	0	0	0.02	0.01	0.05	0.03						
94	0	0	0.07	0.02	0.08	0.04						
0			0.08	0.05	0.09	0.04						
58			0.03	0.02	0.04	0.02	0.5	0	0.03	2	1	
75			0.01	0.01	0.02	0.01	0.09	0.05	1.14	4	2	3
92			0.15	0.08	0.15	0.08						
93			0.15	0.05	0.25	0.1						
100			0.1	0.05	0.26	0.14						
101			0.05	0.02	0.1	0.04	0.12	0.04	1	2	2	
102			0	0	0	0						
111			0.09	0.04	0.16	0.08						
200			0	0	0.3	0.14						

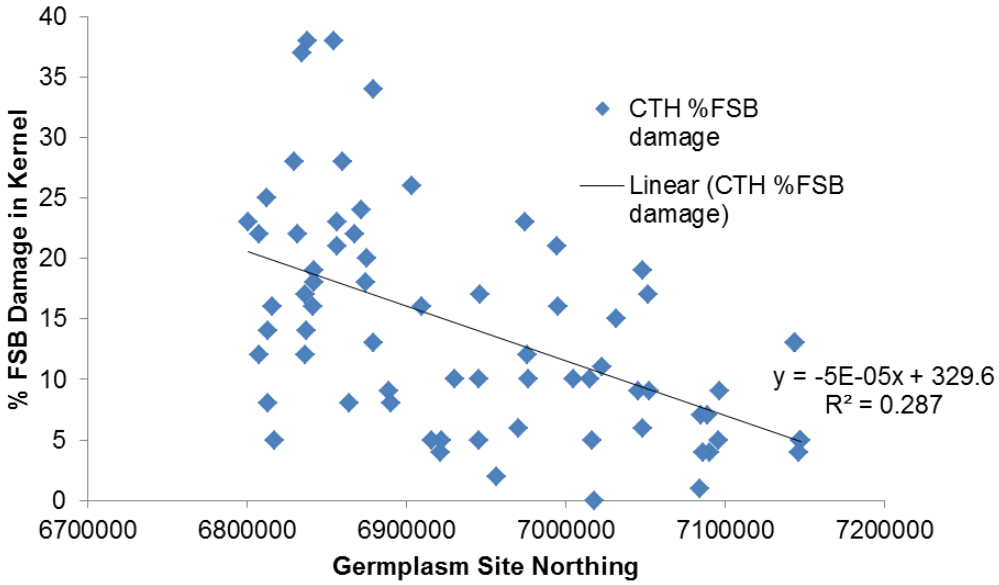

Figure 22.8.9. Plot of original collection site northings verses the level of FSB damage found in the kernels for nut collected at CTH Alstonville in 2014.

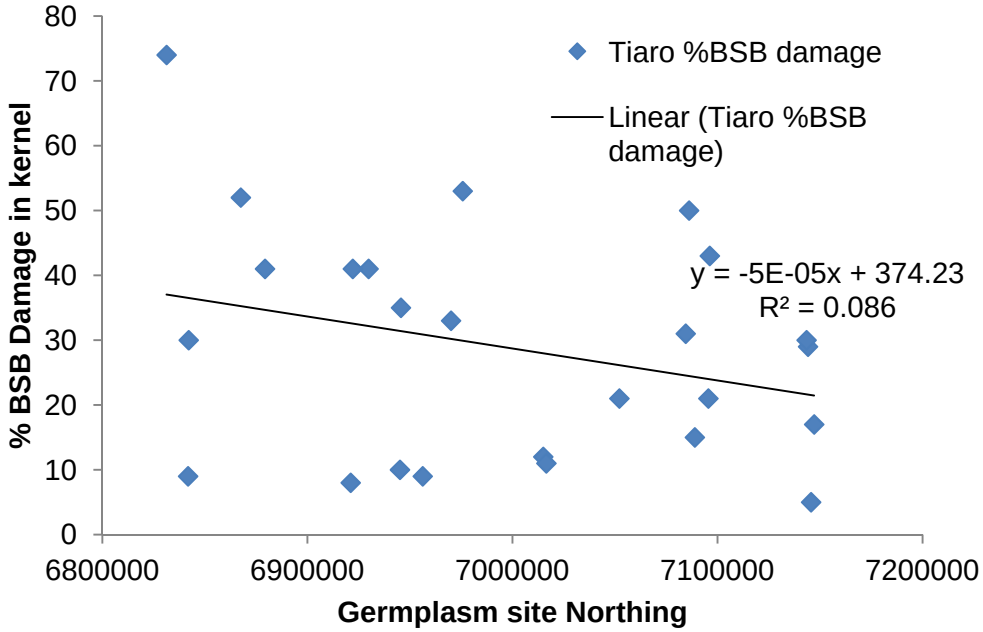

Figure 22.8.10. Plot of original collection site northings versus the level of BSB damage found in the kernels for nut collected at Tiaro Queensland in 2012.

22.9 Appendix 9 – Visual estimation of yield. Literature review on indirect methods of yield assessment in macadamia.

Individual tree yield is a major trait for selection and management in macadamia. The conventional method of assessing individual tree yield is manually harvesting all nuts from the ground underneath the tree, with three harvests per fruiting season and a final strip of all nuts remaining in the tree at the end of the season. This method provides an accurate measure of individual tree yield, however is time consuming and expensive in terms of labour requirements. Three alternative and potentially cheaper indirect methods for estimating individual tree yield are available. These are visual assessment, sub-sampling, and mechanised harvesting. This review briefly covers some of ways that these approaches have been applied in other crops, and work that has been conducted to date in applying these to macadamia.

22.9.1 Visual Assessment

The most common practise for visual assessment of yield across crops is to score crop load based on fruit/grain number. No examples were found where the mass of the crop was estimated. In macadamia, Bell (1983) described a scoring system based on nut count relative to the common cultivar HAES 246 (Table 22.9.1).

Table 22.9.1. Yield rating system developed by Bell (1983). A rating of "0" is equivalent to the yield of cultivar HAES 246 within the same trial.

Rating	Criteria
+2	Very high yield
+1	High yield
0	Average
-1	Below average
-2	Low yield

Rating systems have similarly been used in many other crops, including walnut (Hansche et al., 1972a; Hansche et al., 1972b; McGranahan and Forde, 1985; Radicati et al., 1990), hazelnut (Fischbach et al., 2010) and cashew (Foord et al.). These systems are typically based on scales from "no yield" or "very light yield" through to "very high yield". This style of rating system suffers, however, from the lack of a standard such as that applied by Bell (1983), which makes across-trial comparisons difficult.

In apple, a computerised vision-based system has been developed to assess yield (Stajnko et al., 2009). Visual recognition software was used to process images from a charge-coupled device (CCD) camera and identify individual apples. The number, diameter and texture of fruit was automatically calculated by the software.

22.9.2 Sub-Sampling

Sub-sampling is one of the more common approaches to indirect yield estimation, particularly in grains. Hardner (2005) examined sub-sampling of macadamia nuts from a five year old progeny trial. Sub-sampling of nuts was undertaken from a 60 cm strip of ground, perpendicular to the row direction, near the base of tree. There was a strong correlation between the sub-sampled yield and the individual harvest whole tree yield ($r^2=0.87$). On average, 33% of whole tree yield was captured in the sub-sample, however this proportion varied between 0.06 and 0.75.

In the Georgia pecan breeding program the entire harvest is collected when the trees are young. On older trees they use a pie shaped wedge placed under the tree. This wedge is sized so that you are getting 1/100 of the area under the tree. It is placed once on each side of the tree to collect the nuts in the wedge. The nuts are collected and multiply the weight by 25 to estimate the total yield. An alternative to collecting the nuts in the pie wedge is to count them and then record the number; then use the avg. nut weight to calculate from a random 50 nut sample in order to determine the yield. This is all possible because the tree is mechanically shaken to get all the harvest at one time (Patrick Conner, pers. comm.).

For crops such as grains, corn, soybean and grapes, sub-sampling is more commonly used to estimate yield per hectare (e.g. The Department of Agriculture and Food, 2000; Dunn and Martin, 1998; Lee and Herbek, 2005). Most procedures recommend using a sample area of 1/1000 acre, and rely upon estimating multiple yield components such as number of seeds and weight per seed (The Department of Agriculture and Food, 2000; Lee and Herbek, 2005; Wiebold, 2012). It is important to recognise that in approaches relying on multiple yield estimators, small errors made at several steps of total yield calculation can result in large errors in the final estimation (Wiebold, 2012).

22.9.3 Mechanised Harvesting

In macadamia, progress has been made on estimating individual tree yield using mechanical harvesters (Billingsley and Dunn, 2005; Dunn et al., 2006). A working prototype harvester has been developed that uses tree-identification software and GPS to determine tractor location within orchards, and using image recognition software, counts nuts per tree as they are harvested. Using this approach, tractor and nut locations can be accurately determined to 12 mm, with a potential cost of 61% of manual harvesting, including transportation between locations, maintenance and depreciation (Hardner, 2005).

22.9.4 Discussion

Of the three indirect methods for assessing individual tree yield, visual assessment has the potential to be the most inexpensive. A rating system such as that developed by Bell (1983) is the simplest option, however it relies heavily on the ability of the assessor to accurately calibrate against the standard. In addition, it has been suggested that approaches such as these tend to be more successful at eliminating low yielding trees, rather than selecting high yielding trees (Hardner, 2005; Jensen, 1988).

A computerised vision-based system such as that developed for apples (Stajnko et al., 2009) has many advantages including potential accuracy and lower costs than manual harvesting. Implementation for macadamia would be difficult, however, primarily due to the density of the macadamia canopy and the location of many nuts inside the canopy. This would likely result in nuts being obscured from the camera, consequently resulting in reduced recorded yield.

Sub-sampling of individual tree yield has the potential for significantly higher accuracy than rating systems. The system examined by Hardner (2005) for macadamia, where sub-samples are taken from a 60cm strip close to the tree trunk, shows promise for wider application. Testing is still required, however, on a range of sites, tree ages, and band widths before it could be implemented. The amount of time saved using this approach would also need to be assessed, as harvesting time may not differ significantly from whole-tree harvesting.

Of the indirect approaches to individual tree yield assessment, mechanised harvesting has the highest potential accuracy. Cost has also been estimated at 61% of manual harvesting, making this method highly attractive. If available, it is recommended that the acquisition of a commercial unit be investigated.

22.9.5 References

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22.10 Appendix 10 – Forum on RVT Design. Regional Variety Trials Workshop Summary Report by Robbie Commens.

22.10.1 Introduction

On Thursday the 8th November macadamia researchers, industry representatives, growers and specific experts were invited to attend and contribute to the 2012 Regional Variety Trial (RVT) Workshop. The workshop was designed to examine the technical issues of RVTs. It was made clear to all attendees that is was unlikely that all of the issues will be resolved in a single session, rather the workshop would be the starting point of a design process to be undertaken over the next twelve months. The goal of the workshop was to create some new design principles for future RVT projects, meeting the following criteria:

- Cost effective, allowing an affordable ongoing RVT program.
- Accurate, measuring the genetic value of selections with an acceptable degree of certainty.
- Robust, allowing trials to remain viable in the event that some related blocks are abandoned.
- Efficient, creating minimal disruption to the farming operations of grower co-operators.
- Flexible, allowing grower/co-operators to get involved with the RVT program at a time and level that suits them.

22.10.2 Structure of the meeting

The structure of the meeting was designed to encourage open discussions about the key issues involved with establishing and maintaining regional variety trials as well as the relevance of the data produced for grower adoption. Each attendee was provided with a note book and was asked to write down the three 3 key points that they took away from each presentation. This enabled the AMS to have a greater understanding of the different perspectives that were being seen throughout the meeting to effectively capture the "true" key issues.

At the conclusion of this meeting the attendees were asked to vote on what they believed the "Top Seven Key Issues" were. This information would then be summarised within this report to aid in the Macadamia Industry Varietal Improvement Committee (MIVIC) making informed decisions for improvement of the RVT program in the future. The top seven key issues are outlined in the next section followed by a summary of all of the points documented during each different presentation at the workshop.

The AMS greatly appreciated the individual contributions to this meeting. All attendees volunteered their time free of charge to this meeting to help improve the entire Australian macadamia industry.

Figure 22.10.1. Growers, researchers and industry representatives at the RVT workshop on the 8th November 2012.

22.10.3 Top Seven Opportunities to Improve the Macadamia Industry Regional Variety Trials

At the conclusion of the RVT workshop all attendees voted on what they believed to be the most important points raised during the day. They are listed below.

Key issues:

1) Communication.

    i. There is a need to improve communication with and between growers that are currently involved in RVTs. An annual grower RVT forum would be an ideal communication platform.

2) Create an improved RVT design that delivers a greater balance between the level of detailed information obtained from the RVTs and the cost of the RVTs.

    i. Further investigation into the viability of whole-row trial design is required taking into account parameters to a relevant enable statistical analysis.

    ii. RVTs with whole-row plots deliver the opportunity to move towards mechanical harvesting.

3) Increase grower involvement in future RVTs to reduce running costs.

    i. Grower run RVTs will greatly reduce maintenance costs however the following challenges need to be addressed –

        a. Consistent management practices (fertiliser, canopy management, irrigation/water) across the different sites.

        b. The data that growers record. A template needs to be created for growers to utilise.

        c. An individual needs to be made responsible to coordinate RVT growers.

        d. Microclimate information needs to be captured at each site.

4) Mechanical harvesting in RVTs required.

    i. The cost of hand harvesting is not sustainable into the future.

    ii. There is a need to move to mechanical harvesting within RVTs to reduce costs of hand harvesting and ensure ongoing harvesting ability as suitable consistent labour is difficult to find.

    iii. New RVT design should accommodate currently available mechanical harvesting equipment.

5) Investigate the opportunities for topworking within, or in association with, RVTs to save resources and time.

6) RVTs need to capture and deliver information that adopters are seeking.

    i. The data analysis needs to be sufficient enough to enable a statistical analysis and a confident recommendation to be made in the most economical manner possible.

    ii. Investigate the value of incorporating a 'ranking' of varieties by growers and researchers.

7) Investigate opportunities for a tiered or staged RVT system, similar to the citrus RVT program of the following tiers:

    iii. Experimental – undertaken by growers in commercial orchards with grower contributions

    iv. Semi-commercial – grower sites but with a greater level of data collected

    v. Commercial – varieties ready for release

22.10.4 Points from Presentations

Presentations were delivered by 8 different individuals. The AMS captured and summarized the information that was recorded by all attendees at the 2012 RVT Workshop as written in their notebooks. Accordingly, the points from each of the presentations are listed below.

Speaker	Three Key Points	Additional Comments from notebooks of attendees
Bruce Topp	Cost of harvesting within RVTs is very high (hand harvesting) Time involved with RVTs is very high, with a long lead time prior to commercial harvest RVTs cover a large regional area (from Nambucca to Mackay).	
David Bell	RVTs are very expensive, hand harvesting being a major cost. Opposite profitability curve to commercial farm. As trees get older RVTs get more expensive. Major cost within RVTs is harvesting by hand Opportunity to increase growers involvement with RVTs by incorporating grower RVTs.	Need to attract growers by giving the participating growers preference to the new varieties. Also the potential for a tax deduction for the participating growers as it is an R&D investment.
Russ Stephenson	Sufficient replications are required to make data valid. Moving to whole-row RVT design will require greater number of replicates to ensure validity of data obtained. Careful selection of grower co-operators is required, as it is a time consuming task. Recommend appointing a single grower RVT project coordinator.	Moving to grower run RVTs will potentially increase the amount of budding wood available in the future when the new varieties are released. There is no point in having RVTs unless the data and the results provide clear, meaningful guidance to growers on the best variety to plant. Grower trials and collecting data, there is a need for consistency in the data collection. Potential ways of reducing costs: 1. quality assessments 2. precision harvesters; 3. modification of system, leaf sorter, rake material.
Craig Hardner and Alison Kelly Craig Hardner and Alison Kelly cont.	Need to find a balance between accuracy and cost. If we skimp on the investment in the RVT we will reduce the value of investment in breeding program. Two key questions need to be answered – a. What sort of information do adopters need? b. How much confidence do adopters need? RVTs need to focus on delivering the information that growers need. Need to ask ourselves the question; How specific does the data need to be for grower confidence and adoption?	Currently there is no consideration for cultural practices. Some varieties need cultural practices to make them high yield. Potential for saving costs: 1. reduce the number of replications 2. harvest every 2nd year 3. utilise whole-row trials rather than within row trials.

Speaker	Three Key Points	Additional Comments from notebooks of attendees
		RVT trials - set up a rating score, say 1-5 for major attributes and update that each year. Publicise well and present hard copy at MacGroups. Overlay averages with replication data. Increase accuracy by averaging across replicates in one trial. Some of the best varieties in the world require specific cultural practices to make them high yielding e.g. Pruning, growth regulators, girdling etc Environmental interaction - Mapping micro climates. Each RVT site must have a basic weather station situated at or close to the site. We will never otherwise understand what turns trees/production on and off
Lindsay Bryen	Mechanised harvesting required. An individual needs to take responsibility for the data collection from grower trials, the first step is to determine what will be systematically collected - criteria to be established. Consistent management of trees required, fertiliser, water, canopy management. How?	Need professional support for growers involved in RVTs for recording and reporting service. There is much more to selection than yield! Potential to use small harvesters in the whole-row trial plots prior to rest of block being commercially harvested.
Wayne Parr Wayne Parr cont.	The citrus industry utilises stages of RVTs (tiers) that are all planted at the same time are as listed but managed differently – experimental - undertaken by commercial growers, semi-commercial - grower sites, but rigorous data collection, Commercial - released to growers Select varieties based on; microclimate, longitude, latitude, rainfall, humidity. Potential for topworking trees to reduce time involved in RVTs.	Cross pollination differences between whole-row plots (similar to commercial plantings) and within row plots (that are NOT similar to commercial plantings) needs to factored in. Management control of trees would need to be consistent and would be difficult for growers to 'accept' and do Grower involvement is realistic and is done successfully in the citrus industry. VC with grower costs of establishing land. ($15,00/ha = $25,000 matched through HAL). However, a HAL VC is not guaranteed Within row plots sound to be more suitable. Mechanical harvesting is required for cost effectiveness. What's in it for the growers? Professional assistance, shared workload, the most current R&D info.

Speaker	Three Key Points	Additional Comments from notebooks of attendees
Kim Wilson	Connectivity between sites required for data analysis. Utilise a combination of growers and researchers to gauge/assess the amount of crop within a tree. Use five people to view the site separately (and on their own), average the results and then revisit any BIG differences. This discriminates low yield variations. A 20% increase in yield validates orchard replacement with new varieties (Chris Searle).	

22.10.5 Additional issues highlighted at the RVT Workshop

A wealth of information was presented and shared at the RVT Workshop, the additional points that were raised during the meeting but were not ranked within the top 7 key issues are listed below -

1) The size of the RVT and breeding program needs to be evaluated
    a) Limited by resources
    b) Limited by grower sentiment
    c) Limited by regional locations
    d) Need to factor in the cost benefit ratio
2) Need to learn and implement/document cultural practices that improve specific varieties
3) Use historical RVT data to aid in future decisions
    a) Objective process
4) RVT and breeding program need to have a clear end focus/objective
    a) There is a risk of losing commercial value by releasing cultivars without thorough testing and by not involving growers in RVTs
5) Continue to focus on introducing new varieties into program
6) Focus on making developments in RVT harvesting methods
7) Appreciate the value of the current RVT system/program
    a) Compared to previous variety information this structured and staged program provides much greater value to growers
8) Understand the level of confidence (accuracy) required to make informed recommendations to industry
    a) For both researchers and growers
        i. Is the level of accuracy the same for both parties?
9) There is a risk of early adoption and poorer varieties being implemented
10) Provide an option for existing growers to upgrade some of their orchard to the new varieties to encourage growers sentiment and industry adoption / communication

22.10.6 The Next Step

Within the Macadamia Industry Varietal Improvement Committee (MIVIC) an RVT design group will be established. This group of people will be tasked with developing and recommending improvements for the RVT projects into the future. The 3 key areas that this group will focus on, as a result of the issues highlighted within the workshop, are:

1) Improving the communication between growers that are currently involved in RVTs.

    a. Coordinate a meeting or forum with growers that are involved within the RVTs to enable sharing of ideas and information

2) Recommending improved RVT designs that deliver a greater balance between the level of detailed information obtained from the RVTs and the cost of the RVTs. Further investigation into the viability of whole-row trial design and the potential for topworking is required.

3) Investigate opportunities for RVTs to incorporate a greater level of grower involvement

22.10.7 Conclusion

Based on the information obtained from the RVT workshop the macadamia industry, through MIVIC, will work towards creating an RVT program that has a more suitable balance between statistical relevance of the data and cost to the industry. The workshop delivered an excellent platform for leading growers, researchers and experts to discuss the challenges of the current system, work through potential solutions and introduce new ideas into the RVT program.

As a result of the workshop, the future of the macadamia industries RVT program will most likely incorporate:

1) A greater level of grower involvement

2) A more economical balance between gathering statistically relevant data and the cost of maintaining the RVTs

3) Mechanical harvesting systems within the RVTs.

22.10.7 Acknowledgements

The AMS would like to thank all of the people that were involved in the RVT workshop. Their investment of time, energy and ideas will pay dividends into the macadamia industry well into the future.