Macadamia improvement and conservation

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CSIRO Plant Industry

Project Number: MC02054

MC02054

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Final Report

Macadamia improvement and conservation

MC 02054
(November 2008)

Compiled by Dr C. McConchie

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AUSTRALIAN MACADAMIAS®

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CHAPTER 1 – Project Details

Project Title:	Macadamia improvement and conservation
HAL Project Number:	MC 02054

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Purpose of the report:
The main objectives of the project were to investigate:

1. Develop improved cultivars for the Australian Industry.
2. Identify elite rootstocks for the Australian industry.
3. Conserve native germplasm for future use in the breeding program.
4. Develop economic models for the evaluation of genetic material and critical cost sources in macadamia production and processing
5. Develop efficient assessment methods for selection and management
6. Optimise genetic improvement methods for macadamia

Funding sources:	HAL, AMS, CSIRO and collaborating Growers
Date of report:	November 2008

CHAPTER 2 – Summaries

Media Summary

The Macadamia improvement and conservation program has had major impact on the management of genetic resources for the Australian industry. This project has selected 20 candidate cultivars that are predicted to increase the profitability of the Australian industry by 30%. A discounted cash flow model of macadamia production and processing has been developed and used to identify selections with the suite of characteristics that had the greatest economic impact. Much of this gain will be delivered to the grower, as the major trait influencing selection is yield, although selections are also on average, smaller trees, and have higher kernel recovery, percentage of whole kernels and kernel quality. The field performance and commercial kernel quality were evaluated by a selection committee to identify any candidates that did not meet industry standards. Improved knowledge on the performance of different rootstocks in the nursery and early orchard production has prompted the adoption of 'Beaumont' as an alternative to 'H2' for propagation of the new selections for RVT testing. To support these decisions improved kernel assessment methods have been developed that allow selection to target kernel traits so that kernel quality can be maintained and improved in future selections. To assist in cultivar identification and protect the industries research investment a new suite of DNA markers have been developed. Further gains are anticipated since monitoring of the 2nd series of crosses that were established across 12 sites over three growing regions to better sample the range of environmental variation for macadamia production has been maintained. The wild germplasm collections at Tairo and Alstonville have been maintained to support the future incorporation of this material into the improvement program to deliver new cultivars with novel characteristics for transformational change to the industry.

Technical Summary

The Macadamia improvement and conservation program has had major impact on the management of genetic resources for the Australian industry. This project has selected 20 candidate cultivars that are predicted to increase the profitability of the Australian

industry by 30%. A discounted cash flow model of macadamia production and processing has been developed and used to identify selections with the suite of characteristics that had the greatest economic impact. The value weights and economic weights derived in this study assume a linear relationship between the change in the trait and the effect on the profitability. Much of this gain will be delivered to the grower. Seven traits measured on the candidate cultivars were used in the selection index, the important determinates of profitability were average rate of yield increase, canopy width at 10 years and total kernel recovery. There was little gain in assessing cultivars for percentage of marketable whole kernels, and average grade of whole and half kernels under the current assumptions and unless there were massive changes to the relationship between these traits and raw kernel price.

One of the major challenges for the project has been the development of research tools for selection for kernel quality. It is a basic requirement that traits must be able to be measured for them to be changed. It was found that commercial kernel assessment while able to accurately value kernel did not consistently identify the same defect. This was because kernels with multiple deficiencies are only put in one category in commercial kernel assessment, making it difficult to identify the underlying biological control of quality. Secondly, because kernel assessment is subjective results differed significantly among assessors.

In 2004 then again 2006, the use of modified sensory evaluation techniques for visual kernel assessment was evaluated. In its final form this method employed 2 assessors to evaluate on a continuous scale the severity of five attributes of kernel quality (basal discolouration, discoloured rings, shrivelled kernel, discoloured crest and suture lines) for replicate kernel from the same individual. Key to this approach was a strong experimental design that enabled the effects of day, order of presentation and assessor to be controlled so that the genetic performance of individuals could be predicted more accurately. This work indicated that only basal discolouration and shrivelled kernel were under genetic control.

The role of rootstocks in macadamia performance has also been investigated in 15 cultivars propagated as seedling rootstocks and 12 of these cultivars were also

propagated as cuttings and as clonal rootstocks. The 12 cultivars were budded onto the established clonal and seedling rootstocks in an incomplete design. Analyses have shown there were significant differences among rootstocks for germination, strike of cuttings, growth of rootstock, and budding success, but there was little correlation among cultivars for these traits. Successful plants were used to establish a field trial in 2002-3 across 4 sites to evaluate field performance. There were no significant differences in the mean tree size or yield due to the type of rootstock but highly significant differences between cultivars within a type of rootstock. The highest yielding trees derived from cuttings were Beaumont and A268. The same two cultivars also had the highest yield when used as a clonal rootstock. The highest yielding seedling rootstock was A16 that was slightly higher than A268. While these two cultivars had the highest yields as seedling rootstocks they were not significantly higher than a range of other cultivars that included Beaumont. Beaumont and A268 appear to have superior performance as cuttings and clonal rootstocks because of their superior strike rate, growth budding success and early yields. Beaumont also has high germination and budding success as a seedling.

Thirty-three microsatellite loci were isolated for the commercial macadamia cultivars. Genotyping across a test panel of 43 commercial cultivars, revealed five monomorphic loci and significant linkage disequilibrium in ten pairwise comparisons, including two pairs of loci identified from the same clone sequence. These markers were then applied to identify cultivars, as a foundation for the development and implementation of marker-facilitated selection and breeding programs. To facilitate this goal, specific emphasis was placed on verification of reliability and accuracy of genotyping procedures and quantitative analysis of data. The 33 microsatellite loci represent a significant tool for genome mapping and population genetic studies.

Trial maintenance and input have been documented and relevant performance data collected. Database management has been improved so that data can be reconciled year of collection facilitating annual reporting.

CHAPTER 3
Recommendations

Recommendation 1: Confirm economic gains of candidate cultivars

The candidate cultivars potentially offer a 30% increase in profitability over existing commercial cultivars. This needs to be confirmed in regional variety trials. A parallel system for controlled grower evaluation to identify suitable agronomic practices to manage these cultivars should be supported

Recommendation 2: Maintain existing Rootstock trial sites

Beaumont and A268 are proving to have superior performance as rootstocks both in the nursery and during early orchard establishment. Longer term performance is required as early performing trees as evidence of sustain performance is required. Since Beaumont was a smaller tree than A268 and had good budding success wider adoption could be considered to expand the diversity of rootstocks used in the Australian Industry.

Recommendation 3: Revised economic model and improve estimates of precocity

The analysis of the regional trial data identified the average rate of yield increase, canopy width at 10 years and kernel recovery as the important determinates of profitability for use in the selection index. While precocity, that had relatively high value and economic weights contributed little. This was attributed to the lack of genetic variation for this trait. The dismissal of precocity reflects the desk-top approach to these analyses since the commencement of cropping by individual trees was not monitored precisely. Decisions on when to harvest trials has been done on a site basis and only commenced once there was sufficient crop at a site to justify collection. This led to cropping in the regional variety trials appearing to commence in year 4 or 5 and in the current project in year 5. The adoption of this artificial estimate of precocity referred to as average year of first crop has wider implications as it is used as the intercept to estimate the average rate of yield increase. Trees coming late into production but yielding heavily are favored by this approach while trees that yield earlier than 5 years were potentially overlooked. The economic model needs revision in these areas and investigation of a more accurate estimate of average year of first crop developed that compensates for harvests only commencing in year 5.

Recommendation 4: Reduce the cost of estimating tree yields.

Major costs in operating the improvement program have been the harvesting, processing and kernel assessment. The adoption of an intelligent harvesting system for estimating yield was planned at the commencement of the project. While there have been major advances in this area and there are promising leads to resolve the remaining issues considerable development is still required to make this a reality. The development of such a system should remain a priority for reducing the cost of monitoring yield in orchard based trials such as the improvement program.

Recommendation 5: Develop objective kernel assessment.

Considerable advances have also been made in developing objective measures of kernel quality using trained assessors. This has taken a process that was obviously confounded to a stage where the levels of several kernel defects are able to be measured. However many challenges still remain including the development of quality standards to allow comparisons over years, standardising assessor training, optimising sampling and even having enough assessors to eliminate biases.

Recommendation 6: Investigate development of specific challenges to detect inherent kernel faults.

Despite these considerable inputs into measuring kernel quality they had little impact in the selection of candidate cultivars indicating little advance has been in this area. This may be due to nuts be handled by best practice preventing the detection of inherent faults. An alternate approach that could be considered it the development specific challenges to test for susceptibility to the expression of defects under controlled conditions.

Recommendation 7: Incorporate experimental rigor into alternative methods for kernel assessment.

There are currently a number of commercial projects to develop image analysis for kernel quality assessment. While these have the potential for application in the improvement program they will also face the same potentially confounding effects and biases. The adoption of the rigorous experimental design used in the development

of the continuous scale for trait assessment using trained assessors may assist in ensuring the utility of these systems.

Recommendation 8: Review fruit and kernel assessment protocols.

The elimination of candidate cultivars at the final field inspection due to twin seeded fruit indicates that there needs to be a review of nut and fruit assessment protocol. This trait was considered as unacceptable by the growers and had not been previously measured or considered. A possible option is to monitor losses during dehusking when small and odd shaped nuts are eliminated.

Recommendation 9: Investigate other breeding strategies.

The current project at various stages employed 5 consulting biometricians, an agricultural economist and several senior technicians/post doctoral graduates. This was largely due to the complexity of the design used to address the multiple traits that potentially contributed to the selection index. The resulting trials, while elegant in concept, have diminished in value due to tree deaths and withdrawal of collaborators. An alternative approach may be incorporate pre-breeding cycle for highly heritable and cheap to measure traits. This could simplify and increase the robustness of the more expansive and expensive long term trials.

Recommendation 10: Standardise database

Considerable efforts have been made to improve data management and ensure the maintenance of sample and tree identity. This has been extended to the transfer of candidate material for the next series of regional variety trials. Consideration should be made in the development of future databases to ensure compatibility access for collaborators.

Recommendation 11: Expand, renovate and utilise the germplasm collections

The germplasm collections are now in their prime for incorporation into the improvement program with most trees flowering and cropping. These collections are under threat from restructuring of the NSW DPI and attrition of trees from specific collection sites. In some cases the original collection sites of this material have been cleared. These collections are of international significance, they are now too old to transplant, will take several years to re-establish through propagation and

establishment at another site if it were available. Protection of the existing collections needs to be a priority. The collections need to be renovated to protect vulnerable collection sites (detailed in MIVIC minutes) and representatives of *Macadamia jansenii* included in the collections. Consideration should be given to develop a further enduring replicate of the collection. Characterisation of these trees also needs to be completed as a means of identifying traits of interest.

Recommendation 12: Model inter-tree competition for yield estimates

A mistake was made early in the design of the 1.1 progeny trials in attempting to simultaneously perform genetic evaluation and investigate the effects of different tree spacing. This was investigated because there was little or no information on the appropriate spacing to use and it we wanted to evaluate as many candidate trees as possible. Even under uniform tree spacing, inter-family competition following canopy closure may bias estimates of genetic parameters and predictions of individual merit, particularly when progenies are planted in single-tree plots. This problem is further complicated when tree spacing varies within the trial and between trials. An analysis was carried out where the effects of the two levels of spacing on the mean and variance were accounted for. In this sense, a factor with two levels was fitted in the fixed part of the model, and the random and residual effects were defined by considering the same trait measured in the two levels of spacing as two different traits, and thus heterogeneous variances were incorporated in the appropriate matrices. The estimated genetic correlation across the two levels of spacing was high, indicating that parental ranking was not significantly affected by the spacing treatment. However, a further refinement would have been to model competition at the individual tree level, so that the accounting for missing trees could have been taken into consideration.

Recommendation: 13. Investigate the effects of hedging and pruning on yield

Further information on the effects of hedging and pruning are needed. In the economic model developed in this project it is assumed that trees maintain production after hedging but there is very limited data to support this conclusion. Since all progeny trials were hedged at different times in the year, at different ages and varying severity this has potential important implications for candidate rankings. It shoul be

noted that this is not an issue for the 1.2 progeny populations as they have not been hedged and are planted at wide spacings

CHAPTER 4
Management Overview: Field Trials

Introduction

This report summarises field trial management activities for the 'Macadamia Improvement and Conservation' project (MC02054), between July 2003 and June 2008. This project manages the breeding trials (1.1 and 1.2), rootstock trials, cultivar trials and ex-situ germplasm conservation trials. The two main activities have been: data collection, and trial maintenance.

There are two types of **breeding trial, 1.1 and 1.2.** The 1.1 generation progeny are the result of pollen crosses (using 12 parents) undertaken in 1994 and 1995. These hybrid seedlings were planted into field trials in 1997 and 1998. Twenty selections of elite performing progeny from this generation were made in 2007. These selections have recently been planted into RVT3 trials in QLD and NSW by QLD Department of Primary Industry and Fisheries, in collaboration with CSIRO staff. In addition, elite parents are being considered from 1.1 to be used as parents in generation 2.1 of a rolling front breeding program. Generation 1.2 were planted into field trials between 2000 and 2003. These trees were produced from pollen crosses (using 40 parents) between 1997 and 2000. Data is currently being collected from these trials. This population will be used to identify elite parents (for 2.1 generation) and cultivars. In addition, these trials are used to estimate genetic parameters for important breeding traits. (Hardner et al, 2002). The 1.2 generation work is ongoing.

Ex-situ germplasm trials at Tiaro and Alstonville contain cuttings from 356 individuals collected from 76 wild populations located in NSW and QLD (Hardner et al, 2004). The 3 species collected *M. integrifolia*, *M. tetraphylla*, and *M. turnifolia* are classified as either vulnerable or endangered. (Commonwealth EPBC Act 1999). The purpose of these trials are to (i) enhance the competitiveness of the Australian macadamia industry by using novel germplasm in future breeding, and (ii) conserve a unique component of Australian biodiversity. (Hardner et al., 2004). This work is

ongoing, and we are looking forward to incorporating some *M. jansenii* selections into the germplasm trials in autumn 2009.

The **cultivar trials** are designed to investigate *genotype x environment* interactions, and evaluate the suitability of these cultivars in less traditional growing areas. In addition data can be collected for those cultivars used as parents in the breeding trials. (Hardner et al., 2003). The preliminary performance of the cultivar trial planted at Emerald has already attracted inquiries from the financial investment sector after CSIRO articles in the AMS bulletin. This work is ongoing until 2011.

The aims of the **rootstock trials** are to:

(i) identify elite rootstocks for the Australian macadamia industry;
(ii) quantify the importance of rootstock effects for macadamia production;
(iii) quantify the importance of rootstock-scion interactions in macadamia;
(iv) quantify the differences between own-rooted, grafted seedling, or clonal rootstock; and
(v) develop early screening methods for elite rootstock selection.
(Hardner 2004)
As part of the Pacific highway upgrade the rootstock work is likely to experience disruption at the Newrybar site. Plans to upgrade the highway will lead to the removal of the trial to make way for north and south bound lanes. A time frame has not yet been established by the RTA. CSIRO remains in contact with the RTA in regards to construction timeframes and compensation. This work is ongoing.

Field Trial Management

CSIRO manage 27 field trials across three growing regions. Below is a summary of these sites. Tables 4.1.1 and 4.1.2 represent how the trials are split between the Brisbane and Bundaberg management units.

Sites Managed from Brisbane

Site ID	Location	Region	Trial Type	Year Planted	No. of Trees Planted
BTFRS98	**Alstonville**	**Northern Rivers NSW**	**Breeding 1.1**	**1998**	1119
GTFRS00	**Alstonville**	**Northern Rivers NSW**	**Germplasm**	**2000**	623
BNEWR02	**Newrybar**	**Northern Rivers NSW**	**Breeding 1.2**	**2002**	192
RNEWR02	**Newrybar**	**Northern Rivers NSW**	**Rootstock**	**2002**	256
BDUNO00	**Dunoon**	**Northern Rivers NSW**	**Breeding 1.2**	**2000**	210
BDUN003	**Dunoon**	**Northern Rivers NSW**	**Breeding 1.2**	**2003**	128
RWOLL02	**Wollongbar**	**Northern Rivers NSW**	**Rootstock**	**2002**	128
CPRET01	**Pretty Gully**	**Northern Rivers NSW**	**Cultivar**	**2001**	138
BYAND01	**Yandina**	**Sunshine Coast Hinterland**	**Breeding 1.2**	**2001**	237
BYAND02	**Yandina**	**Sunshine Coast Hinterland**	**Breeding 1.2**	**2002**	237
BMACL02	**Maclean**	**North Coast NSW**	**Breeding 1.2**	**2002**	260
BMACL03	**Maclean**	**North Coast NSW**	**Breeding 1.2**	**2003**	139
RMACL02	**Maclean**	**North Coast NSW**	**Rootstock**	**2002**	155

TABLE 4. 1.1 (NB. Maclean trial site discontinued as of 2006)

Sites Managed from Bundaberg					
Site ID	Location	Region	Trial Type	Year Planted	**No. of Trees Planted**
BQBRS97	**Kalkie**	**Bundaberg**	**Breeding 1.1**	**1997**	556
BQBRS98	**Kalkie**	**Bundaberg**	**Breeding 1.1**	**1998**	1249
BQBRS01	**Kalkie**	**Bundaberg**	**Breeding 1.2**	**2001**	544
BQBRS03	**Kalkie**	**Bundaberg**	**Breeding 1.2**	**2003**	188
RQBRS02	**Kalkie**	**Bundaberg**	**Rootstock**	**2002**	334
BEGYM01	**Gympie**	**Cooloola**	**Breeding 1.2**	**2001**	275
BBAFF02	**Baffle Creek**	**Wide Bay**	**Breeding 1.2**	**2002**	192
BBAFF03	**Baffle Creek**	**Wide Bay**	**Breeding 1.2**	**2003**	256
RBAFF02	**Baffle Creek**	**Wide Bay**	**Rootstock**	**2002**	260
BHINK00	**Welcome Creek**	**Bundaberg**	**Breeding 1.2**	**2000**	206
BAMAM02	**Amamoor**	**Sunshine Coast Hinterland**	**Breeding 1.2**	**2002**	196
BAMAM03	**Amamoor**	**Sunshine Coast Hinterland**	**Breeding 1.2**	**2003**	140
BALLO02	**Alloway**	**Bundaberg**	**Breeding 1.2**	**2002**	196
GTIAR00	**Tiaro**	**Wide Bay**	**Germplasm**	**2000**	448
CEMER01	**Emerald**	**Central West**	**Cultivar**	**2001**	62

TABLE 4.1.2

Each site has current testing contracts. The length of each contract varies between trial type:

- Rootstock trials – 14 years.
- 1.2 generation breeding trials – 10 years.
- Germplasm trials – 20 years.
- Cultivar trials – 10 years.

The starting date for each trial is generally the same as the year of planting. The testing agreements are currently being renegotiated for 1.1 breeding trials at the Bundaberg Research Station (QLD DPI&F). The intention at this site is to convert the land to an arboretum, allowing CSIRO and other stakeholders to consolidate 'elite cultivars' as a propagation resource. The contract for the 1.1 breeding trial at the

Centre for Tropical Horticulture was varied (detail in 'field trial summaries') in September 2007, and will be valid until December 31st 2008.

Horticultural Management

CSIRO staff work with collaborators to manage the horticultural performance of field trials. In general, mechanical canopy management, fertilising, pest and disease monitoring, and chemical applications are carried out by collaborators. CSIRO give direction for the strategies involved for each of these activities. Planning meetings each spring (between CSIRO staff and collaborator) were used to plan the following season's maintenance activities for each site. Each trial is managed to: i) maximise its production capacity using a balanced approach: and ii) minimise bias that would be created by various management treatments. Standards and record keeping standards have been developed for the activities below:

- Canopy management
- Nutrition management
- Integrated pest and disease management
- Irrigation (not available at all sites)

Canopy Management

Two strategies have been developed. The method implemented, is dependent on the type of trial. The breeding, rootstock, and cultivar trials are pruned 'minimally'. While the germplasm trials are pruned using a 'comprehensive' method.

Mechanical hedging is undertaken to maintain vehicle access on all trial types. Hedging is undertaken in year 6 at the earliest in trials where the row spacing is 4 meters. The preference when hedging is to take off small amounts of canopy at each prune, at an annual or biannual period to minimise yield loss. Pruning is carried out in June so as to minimise the effect on the following crop. Trees are pruned to the standard of - no lateral shoots below 1meter.

Minimal pruning method - The aim with these trials is to minimise yield reduction by only skirting trees to a standard height. This strategy also minimise's variation created by the subjectivity of staff deciding which limbs to prune.

Comprehensive - The second strategy used is implemented on germplasm trials. In these trials more pruning is done in the canopy. The trees are pruned to a central leader where possible. The techniques used to do this are, window pruning, and removing tight crutch angles. The aim with these trees is to preserve the genetic resource. Pruning can aid in this by minimising wind blow and maximise spray penetration. We also find that by managing the size of the canopy we can reduce the number of trees blown over in wet and windy conditions. This aspect is particularly important as we are dealing with clonal cuttings in these trials.

Nutrition Management

Fertiliser programs are based on a combination of industry standard recommendations (tree age based), periodic soil test results (biannually), and visual assessment of tree nutrient requirement indicators. There may be some fertiliser differences between trials. These differences are a result of several factors that differ between trial sites. Factors that are taken into consideration when developing fertiliser program's, include:

- Soil type and health.
- Irrigation capabilities or rainfall (amount and timing).
- Length of growing season.
- Yields.

Fertiliser applications are targeted during spring and autumn. However in some seasons, due to limiting factors such as rain fall, applications were delayed into summer and winter.

Due to the genetic variation in each of the trials, leaf tests are not used as a tool in nutritional management.

Pest and Disease Management

Collaborators carry out the pest and disease monitoring and any subsequent management actions (insecticides etc). CSIRO assist in developing management programs. The trials located on the Bundaberg Research Station have been crop monitored by 'Crop Tech' since 2004. A sub contractor was used to monitor crops at the Centre for Tropical Horticulture (Alstonville) and the Dunoon progeny trials in 2005/06 and 2006/07 seasons.

CSIRO maintains regular contact with collaborators during the critical crop monitoring period. CSIRO have taken over the monitoring and control actions at some sites where the collaborators are not able to carry out this function.

We use an integrated approach to pest and disease management, which means we do experience pest and disease damage within trial sites to varying levels. In general this damage is on par with seasonal fluctuations experienced across different regions and different varieties. An integrated approach allows us to i) integrate with the management strategies of the majority of our collaborators, and ii) be responsible users of agricultural chemicals in and around the communities where we carry out research. The second of these points is particularly important to us and our collaborators at sites where urban development can be exposed. Our integrated approach uses methods of minimising chemical use. These include one or a combination of: beneficial pest promotion, use of biological control options, pest and disease monitoring, low toxicity chemicals.

Irrigation

All sites were irrigated during establishment. Trial sites at Kalkie, Baffle Creek, Welcome Creek, Amamoor, Tiaro and Emerald continue to be irrigated throughout

the year. Irrigations are scheduled based on visual soil conditions, crop stage, and climatic conditions. Soil moisture monitoring technology is an option for the future, which may assist in irrigation scheduling.

Field Trial Summary

This section contains management summaries for each trial.

Progeny Trials

The aim of the 1.1 generation progeny during the 5 year phase, 2003 to 2008 was to (i) maintain productive trees, and (ii) select elite cultivars using yield, kernel assessment and height and width assessment, data. Some Trial information prior to 2003 is contained the final reports for MC602 (1999) and MC9904 (2002).

SITE: Alstonville (Centre for Tropical Horticulture)

TRIAL CODE: BTFRS98

COLLABORATOR: NSW DPI

Key Point Summary:

- 3 progeny identified as elite cultivars and incorporated into RVT3 field trials.
- Testing agreement extended to 31st of December 2008.
- The 2004/05 yield data was affected by flooding. The third round pick up was lost.
- Generally healthy trees with no significant site issues.
- Hedged in spring 2003, and winter 2006.

Trial maintenance and data collection activities are summarised for each year, in Table 4.2.1.

A variation to the original testing agreement was negotiated in late 2007, between CSIRO and NSW DPI. This was done in recognition that data collection had ceased at this trial. However there would be a number of trees - clonal parents, and pollen parents needed to maintained on site until this material could be consolidated into an arboretum. The changes to the testing agreement are summarised below;

- The trial "completion date" was extended to the 31st of December 2008.
- NSW DPI will remove every second row to improve machinery access and reduce costs. NSW DPI will not remove trees that are nominated by CSIRO. These trees have been identified as resources for future research.
- Pro-rata trial payments for the 2007-2008 financial year, and the period from 1st of July 2008 to 31st of December 2008, have been replaced by a standard trial payment.

	SEASON				
ACTIVITY	**2003/04**	**2004/05**	**2005/06**	**2006/07**	**2007/08**
Weed management	✓	✓	✓	✓	✓
Biological pest management	✓	✓	✓	✓	✓
Chemical pest /disease management	✖	✓	✓	✓	✓
Fertiliser applied	✓	✓	✓	✓	✖
Soil test	✓	✖	✓	✓	✖
Skirted	✖	✓	✓	✖	✖
Hedged	✖	✖	✓	✓	✖
Ethrel applied	✓	✓	✖	✖	✖
Experimental harvest	✓	✓	✓	✓	✖
Kernel assessment (previous season nuts)	✓	✓	✓	✓	✖
Height and width measurements	✓	✓	✓	✖	✖
Irrigated	✖	✖	✖	✖	✖

Table 4.2.1

SITE: Kalkie (Bundaberg Research Station)

TRIAL CODE: BQBRS97 and BQBRS98

COLLABORATOR: QLD DPI&F

Key Point Summary:

- 16 progeny identified as elite cultivars and incorporated into RVT3 field trials.
- Planned creation of a national macadamia arboretum on the site of BQBRS97.
- Generally healthy trees with no significant site issues.
- Hedged for the first time in winter 2006.
- This trial has been irrigated using 8L/hr single line drippers. Spacing is 5x2 and 5x4.

Trial maintenance and data collection activities are summarised for each year, in Table 4.2.2.

	SEASON				
ACTIVITY	**2003/04**	**2004/05**	**2005/06**	**2006/07**	**2007/08**
Weed management	✓	✓	✓	✓	✓
Biological pest management	✖	✖	✖	✖	✖
Chemical pest /disease management	✓	✓	✓	✓	✓
Fertiliser applied	✓	✓	✓	✓	✖
Soil test	✖	✖	✓	✓	✖
Skirted	✖	✓	✖	✖	✖
Hedged	✖	✖	✖	✓	✖
Ethrel applied	✖	✓	✖	✖	✓
Experimental harvest	✓	✓	✓	✓	✖
Kernel assessment (previous season nuts)	✖	✓	✓	✖	✖
Height and width measurements	✓	✓	✓	✖	✖
Irrigated	✓	✓	✓	✓	✓

Table 4.2.2

SITE: Acacia Ridge

TRIAL CODE: BACAC97

COLLABORATOR: NSW State Forests

Key Point Summary:

- No data has been collected, or maintenance carried out since 2003. (Direction from project leader)
- An audit of trees in September 2007 showed only 31 trees remaining.

1.2 Progeny Trials

SITE: Newrybar

TRIAL CODE: BNEWR02

COLLABORATOR: Newrybar Macadamia Partnership

Key Point Summary:

- The property this trial is located has been resumed for the Pacific Highway upgrade. There has been no date provided by the RTA as to when the work will begin.
- Canker has been an ongoing challenge at this site. Approximately 32% of the experimental trees have either died or show symptoms of canker. Phosphoric foliar sprays, composted manure applications, white washing trunks, extensive staking of trees, and Ridomil granular fungicide have all been used to try and manage canker. The canker pressure comes from several environmental pressures, which cannot be manipulated directly.
- The majority of this trial was replanted at the current site after being planted at anther farm in Knockrow.

Trial maintenance and data collection activities are summarised for each year, in Table 4.3.1.

	SEASON				
ACTIVITY	**2003/04**	**2004/05**	**2005/06**	**2006/07**	**2007/08**
Weed management	✓	✓	✓	✓	✓
Biological pest management	✗	✗	✓	✓	✓
Chemical pest /disease management	✗	✗	✓	✓	✓
Fertiliser applied	✓	✓	✓	✓	✓
Soil test	✗	✗	✓	✓	✗
Skirted	✗	✗	✓	✓	✓
Hedged	✗	✗	✗	✗	✗
Ethrel applied	✗	✗	✗	✗	✗
Experimental harvest	✗	✗	✓	✓	✓
Kernel assessment (previous season nuts)	✗	✗	✗	✗	✓
Height and width measurements	✓	✓	✓	✗	✓
Irrigated	✗	✗	✗	✗	✗

Table 4.3.1

SITE: Dunoon

TRIAL CODE: BDUNO00 AND BDUNO02

COLLABORATOR: Tony and Lyn Rowlands

Key Point Summary:

- Good performing site with low mortality. Very good trial to model 'on-farm' style trial collaborations.
- Suffered from minor hail damage, and high winds during the summer 2008.
- Very vigorous site.

Trial maintenance and data collection activities are summarised for each year, in Table 4.3.2.

	SEASON				
ACTIVITY	**2003/04**	**2004/05**	**2005/06**	**2006/07**	**2007/08**
Weed management	✓	✓	✓	✓	✓
Biological pest management	✖	✖	✖	✓	✓
Chemical pest /disease management	✓*	✓	✓	✓	✓
Fertiliser applied	✓	✓	✓	✓	✓
Soil test	✖	✖	✓	✓	✖
Skirted	✖	✖	✓	✓	✓
Hedged	✖	✖	✖	✖	✖
Ethrel applied	✖	✖	✖	✖	✓
Experimental harvest	✖	✖	✓	✓	✓
Kernel assessment (previous season nuts)	✖	✖	✖	✖	✓
Height and width measurements	✓	✓	✓	✖	✓
Irrigated	✖	✖	✖	✖	✖

Table 4.3.2 (* - BDUNO00 only)

SITE: Yandina

TRIAL CODE: BYAND01 AND BYAND02

COLLABORATOR: Lesley and Stuart Grace

Key Point Summary:

- Collaborators release from contracted obligations in early 2006. CSIRO increased inputs including cost of using subcontractors to slash and apply herbicides.
- Subcontractors who have to appropriate equipment to apply pesticides to the foliage of trees are very difficult to employ due to the location of the trial. The pest and disease management has had a strong focus on biological methods.

- Dry conditions prior to 2005 significantly stunted the vegetative growth of the trees in both trials. The trees were mulched in early 2005 and water was trucked in for one irrigation event. Rainfall has improved since.
- General health of the trees has improved over the past 3 seasons.

Trial maintenance and data collection activities are sumarised for each year, in Table 4.3.3.

	SEASON				
ACTIVITY	2003/04	2004/05	2005/06	2006/07	2007/08
Weed management	✓	✓	✓	✓	✓
Biological pest management	✘	✘	✘	✘	✓
Chemical pest /disease management	✘	✘	✘	✓	✘
Fertiliser applied	?	✓	✓	✓	✓
Soil test	✘	✘	✓	✓	✘
Skirted	✘	✘	✘	✓	✓
Hedged	✘	✘	✘	✘	✘
Ethrel applied	✘	✘	✘	✘	✘
Experimental harvest	✘	✘	✘	✓	✓
Kernel assessment (previous season nuts)	✘	✘	✘	✘	✘
Height and width measurements	✓	✓	✓	✘	✓
Irrigated	✘	✓	✘	✘	✘

Table 4.3.3

SITE: Maclean

TRIAL CODE: BMACL02 AND BMACL03

COLLABORATOR: Tynwood Farms

Key Point Summary:

- This trial was removed in February 2007 after the new owner decided his priorities did not include the continuation of the trials.

SITE: Kalkie (Bundaberg Research Station)

TRIAL CODE: BQBRS01 AND BQBRS03

COLLABORATOR: QLD DPI&F

Key Point Summary:

- The site suffers from poor drainage. Consequently trunk canker and phytophora root rot are an ongoing problem. Remedial action has been carried out since 2004. A quote for subsurface drainage was considered, however this action was not pursued.
- Sprinkler irrigation used.

Trial maintenance and data collection activities are summarised for each year, in Table 4.3.4.

	SEASON				
ACTIVITY	**2003/04**	**2004/05**	**2005/06**	**2006/07**	**2007/08**
Weed management	✘	✓	✓	✓	✓
Biological pest management	✘	✘	✘	✘	✘
Chemical pest /disease management	✘	✓	✓	✓	✓
Fertiliser applied	✘	✓	✓	✓	✓
Soil test	✘	✘	✓	✘	✓
Skirted	✘	✓	✓	✓	✓
Hedged	✘	✘	✘	✓	✓
Ethrel applied	✘	✘	✘	✘	✓
Experimental harvest	✓	✓	✓	✓	✓
Kernel assessment (previous season nuts)	✘	✘	✓	✓	✘
Height and width	✓	✓	✓	✘	✓

measurements					
Irrigated	✓	✓	✓	✓	✓

Table 4.3.4

SITE: Gympie

TRIAL CODE: BEGYM01

COLLABORATOR: Sue and Gary Kelly

Key Point Summary:

- This site has experienced a low rainfall period prior to 2005. A drip line irrigation system was installed in 2005 to make use of limited water onsite.
- Management of the site, weed spraying and fertilizer applications have been taken over by CSIRO personnel since 2006.
- Generally in good health.

EGYM01 2004

EGYM01 2007

Trial maintenance and data collection activities are summarised for each year, in Table 4.3.5.

	SEASON				
ACTIVITY	**2003/04**	**2004/05**	**2005/06**	**2006/07**	**2007/08**
Weed management	✘	✘	✓	✓	✓
Biological pest management	✘	✘	✘	✘	✘
Chemical pest /disease management	✘	✓	✓	✓	✓
Fertiliser applied	✘	✓	✓	✓	✓
Soil test	✘	✘	✓	✓	✘
Skirted	✘	✘	✘	✘	✘
Hedged	✘	✘	✘	✘	✘
Ethrel applied	✘	✘	✘	✘	✘
Experimental harvest	✘	✓	✓	✓	✓
Kernel assessment (previous season nuts)	✘	✘	✘	✘	✘
Height and width measurements	✓	✓	✓	✘	✓
Irrigated	✘	✘	✓	✓	✓

Table 4.3.5

SITE: Baffle Creek

TRIAL CODE: BBAFF02 AND BBAFF03

COLLABORATOR: Grant Rural Industries

Key Point Summary:

- In 2004 CSIRO started monthly visits to this site to assist with day to day management of the trial.
- Weeds have been an issue especially climbing Sirartro, but supplementary ROUNDUP sprays by CSIRO have now fixed this.
- The site is close to the coast and wind damage in the early years was bad enough that many trees needed to be staked and skirted to improve access for equipment.

- This area seems to be prone rat damage especially in thin shelled varieties. Supplementary baiting by CSIRO commenced in 2006.
- Vigorous growing trees.
- Sprinklers are used to irrigate this site.

Trial maintenance and data collection activities are summarised for each year, in Table 4.3.6.

	SEASON				
ACTIVITY	**2003/04**	**2004/05**	**2005/06**	**2006/07**	**2007/08**
Weed management	✓	✓	✓	✓	✓
Biological pest management	✗	✗	✗	✗	✗
Chemical pest /disease management	✗	✓	✓	✓	✓
Fertiliser applied	✗	✓	✓	✓	✓
Soil test	✗	✗	✓	✗	✓
Skirted	✗	✓	✓	✓	✓
Hedged	✗	✗	✗	✗	✗
Ethrel applied	✗	✗	✗	✗	✗
Experimental harvest	✓	✓	✓	✓	✓
Kernel assessment (previous season nuts)	✗	✗	✗	✗	✗
Height and width measurements	✓	✓	✓	✗	✓
Irrigated	✓	✓	✓	✓	✓

Table 4.3.6

SITE: Welcome Creek

TRIAL CODE: BHINK00

COLLABORATOR: Hinkler Park Plantations

Key Point Summary:

- Good yielding trial in good health.
- Sprinkler irrigation used.
- The tree row has been mounded.

Trial maintenance and data collection activities are summarised for each year, in Table 4.3.7.

	SEASON				
ACTIVITY	**2003/04**	**2004/05**	**2005/06**	**2006/07**	**2007/08**
Weed management	✓	✓	✓	✓	✓
Biological pest management	✗	✗	✗	✗	✗
Chemical pest /disease management	✓	✓	✓	✓	✓
Fertiliser applied	✓	✓	✓	✓	✓
Soil test	✗	✗	✓	✓	✗
Skirted	✗	✓	✓	✓	✓
Hedged	✗	✗	✗	✗	✗
Ethrel applied	✗	✗	✗	✗	✗
Experimental harvest	✓	✓	✓	✓	✓
Kernel assessment (previous season nuts)	✗	✗	✗	✗	✗
Height and width measurements	✓	✓	✓	✗	✓
Irrigated	✓	✓	✓	✓	✓

Table 4.3.7

SITE: Amamoor

TRIAL CODE: BAMAM02 AND BAMAM03

COLLABORATOR: L.R and J.C Gain

Key Point Summary:

- Fertiliser applied monthly.
- Limited irrigation water available from Amamoor Creek. This trial was established using under tree drippers and has now changed over to sprinklers.
- The cooperator is focused on an outcome for the Breeding Trial - applying extra resources and time to the site.

BAMAM02 2004

BAMAM02 2007

Trial maintenance and data collection activities are summarised for each year, in Table 4.3.8.

	SEASON				
ACTIVITY	**2003/04**	**2004/05**	**2005/06**	**2006/07**	**2007/08**
Weed management	✓	✓	✓	✓	✓
Biological pest management	✗	✗	✗	✗	✗
Chemical pest /disease management	✗	✗	✓	✓	✓
Fertiliser applied	✓	✓	✓	✓	✓
Soil test	✗	✗	✓	✓	✗
Skirted	✗	✗	✗	✓	✓
Hedged	✗	✗	✗	✗	✗
Ethrel applied	✗	✗	✗	✗	✓
Experimental harvest	✓	✓	✓	✓	✓
Kernel assessment (previous season nuts)	✗	✗	✗	✗	✗
Height and width measurements	✓	✓	✓	✗	✓
Irrigated	✓	✓	✓	✓	✓

Table 4.3.8

SITE: Alloway

TRIAL CODE: BALLO02

COLLABORATOR: Richard Peterson

Key Point Summary:

- Wasps tried in 2007 with little success. Program now focusing on chemical options.
- Irrigated using trickle tape.
- Trees in good health.
- The tree row has been mounded.

Trial maintenance and data collection activities are summarised for each year, in Table 4.3.9.

	SEASON				
ACTIVITY	2003/04	2004/05	2005/06	2006/07	2007/08
Weed management	✓	✓	✓	✓	✓
Biological pest management	✗	✗	✗	✗	✗
Chemical pest /disease management	✗	✓	✓	✓	✓
Fertiliser applied	✓	✓	✓	✓	✓
Soil test	✗	✗	✓	✓	✗
Skirted	✗	✓	✓	✓	✓
Hedged	✗	✗	✗	✗	✗
Ethrel applied	✗	✗	✗	✗	✓
Experimental harvest	✗	✓	✓	✓	✓
Kernel assessment (previous season nuts)	✗	✗	✗	✗	✗
Height and width measurements	✓	✓	✓	✗	✓
Irrigated	✓	✓	✓	✓	✓

Table 4.3.9

Rootstock Trials

SITE: Newrybar

TRIAL CODE: RNEWR02

COLLABORATOR: Newrybar Macadamia Partnership

Key Point Summary:

- The property this trial is located has been resumed for the Pacific Highway upgrade. There has been no date provided by the RTA as to when the work will begin.
- Canker has been an ongoing challenge at this site. Approximately 23% of the experimental trees have either died or show symptoms of canker. Phosphoric

foliar sprays, composted manure applications, white washing trunks, extensive staking of trees, and Ridomil granular fungicide have all been used to try and manage canker. The canker pressure comes from several environmental pressures, which cannot be manipulated directly.

- The majority of this trial was replanted at the current site after being planted at another farm in Knockrow.
- In early 2008 a phenotype audit was carried out. This discovered that combinations involving 842 as a grafted scion were in fact what are believed to be a H2 variation.
- Weed management strategy varied in late 2007 to combat erosion issues.

Trial maintenance and data collection activities are summarised for each year, in Table 4.4.1.

	SEASON				
ACTIVITY	2003/04	2004/05	2005/06	2006/07	2007/08
Weed management	✓	✓	✓	✓	✓
Biological pest management	✗	✗	✓	✓	✓
Chemical pest /disease management	✗	✗	✓	✓	✓
Fertiliser applied	✓	✓	✓	✓	✓
Soil test	✗	✗	✓	✓	✗
Skirted	✗	✗	✓	✓	✓
Hedged	✗	✗	✗	✗	✗
Ethrel applied	✗	✗	✗	✗	✗
Experimental harvest	✗	✗	✓	✓	✓
Kernel assessment (previous season nuts)	✗	✗	✗	✗	✗
Height and width measurements	✓	✓	✓	✗	✓
Irrigated	✗	✗	✗	✗	✗

Table 4.4.1

SITE: Wollongbar

TRIAL CODE: RWOLL02

COLLABORATOR: TAFE NSW

Key Point Summary:

- In early 2008 a phenotype audit was carried out. This discovered that combinations involving 842 as a grafted scion were in fact what are believed to be a H2 variation.
- This trial was mulched with material sourced from the previous orchard planted on that site. The trees here gained significant benefit from soil moisture retention during the early years of establishment.
- Very low mortality at this site.
- Very little chemical use at this site as a result of promoting inter-row sward as a repository for beneficial insects. This strategy has had a particularly good effect on minimising issues with twig girdler and leaf minor.

Trial maintenance and data collection activities are summarised for each year, in Table 4.4.2.

	SEASON				
ACTIVITY	2003/04	2004/05	2005/06	2006/07	**2007/08**
Weed management	✓	✓	✓	✓	✓
Biological pest management	✓	✓	✓	✓	✓
Chemical pest /disease management	✘	✘	✘	✘	✘
Fertiliser applied	✘	✓	✓	✓	✓
Soil test	✘	✘	✓	✓	✘
Skirted	✘	✘	✓	✓	✓
Hedged	✘	✘	✘	✘	✘
Ethrel applied	✘	✘	✘	✘	✘
Experimental harvest	✘	✘	✓	✓	✓
Kernel assessment (previous season nuts)	✘	✘	✘	✘	✘
Height and width measurements	✓	✓	✓	✘	✓
Irrigated	✘	✘	✘	✘	✘

Table 4.4.2

SITE: Maclean

TRIAL CODE: RMACL02

COLLABORATOR: Tynwood Farms

Key Point Summary:

- This trial was removed in February 2007 after the new owner decided his priorities did not include the continuation of the trials.

SITE: Kalkie (Bundaberg Research Station)

TRIAL CODE: RQBRS02

COLLABORATOR: QLD DPI&F

Key Point Summary:

- Suffered during establishment from drought and hard compacted soils. The soil has had high Mg/Ca ratio. Yearly applications of gypsum 2T/ha since 2005 and lime 3T/ha one application 2006 have improved water penetration and root growth.
- Giant bana grass inter-row windbreaks removed in 2005.
- All buffer trees planted in 2006 (autumn).
- Some canker issues that are managed using ridomil applications.

Trial maintenance and data collection activities are summarised for each year, in Table 4.4.3.

	SEASON				
ACTIVITY	2003/04	2004/05	2005/06	2006/07	2007/08
Weed management	✓	✓	✓	✓	✓
Biological pest management	✗	✗	✗	✗	✗
Chemical pest /disease management	✗	✓	✓	✓	✓
Fertiliser applied	✗	✓	✓	✓	✓
Soil test	✗	✗	✓	✓	✗
Skirted	✗	✓	✓	✓	✓
Hedged	✗	✗	✗	✗	✗
Ethrel applied	✗	✗	✗	✗	✓
Experimental harvest	✓	✓	✓	✓	✓
Kernel assessment (previous season nuts)	✗	✗	✗	✗	✗
Height and width measurements	✓	✓	✓	✗	✓
Irrigated	✓	✓	✓	✓	✓

Table 4.4.3

SITE: Baffle Creek

TRIAL CODE: RBAFF02

COLLABORATOR: Grant Rural Industries

Key Point Summary:

- In 2004 CSIRO started monthly visits to this site to assist with day to day management of the trial.
- Weeds have been an issue especially climbing Sirartro, but supplementary ROUNDUP sprays by CSIRO have now fixed this.
- The site is close to the coast and wind damage in the early years was bad enough that many trees needed to be staked and skirted to improve access for equipment.
- This area seems to be prone rat damage especially in thin shelled varieties. Supplementary baiting by CSIRO commenced in 2006.

- Vigorous growing trees.
- Sprinklers are used to irrigate this site.

Trial maintenance and data collection activities are summarised for each year, in Table 4.4.4.

RBAFF02 2007

RBAFF02 2006

	SEASON				
ACTIVITY	2003/04	2004/05	2005/06	2006/07	2007/08
Weed management	✓	✓	✓	✓	✓
Biological pest management	✗	✗	✗	✗	✗
Chemical pest /disease management	✗	✓	✓	✓	✓
Fertiliser applied	✗	✓	✓	✓	✓
Soil test	✗	✗	✓	✓	✗
Skirted	✗	✓	✓	✓	✓
Hedged	✗	✗	✗	✗	✗
Ethrel applied	✗	✗	✗	✗	✗
Experimental harvest	✓	✓	✓	✓	✓
Kernel assessment (previous season nuts)	✗	✗	✗	✗	✗
Height and width measurements	✗	✓	✓	✗	✓
Irrigated	✗	✗	✗	✗	✗

Table 4.4.4

Germplasm Trials

SITE: Alstonville (Centre for Tropical Horticulture)

TRIAL CODE: GTFRS01

COLLABORATOR: NSW DPI

Key Point Summary:

- The site has been prone to blow over'. A comprehensive pruning strategy is starting to reduce the incidence of wind damage. All trees are to be topped back to 4m, hedged, and undergo annual structural pruning.
- Generally a vigorous and healthy site.
- Tetraphylla genotypes have been prone to twig girdler attack due to their very small petioles.

Trial maintenance and data collection activities are summarised for each year, in Table 4.5.1.

	SEASON				
ACTIVITY	2003/04	2004/05	2005/06	2006/07	2007/08
Weed management	✓	✓	✓	✓	✓
Biological pest management	✓	✓	✓	✓	✓
Chemical pest /disease management	✘	✘	✘	✓	✓
Fertiliser applied	✓	✓	✓	✓	✓
Soil test	✘	✘	✓	✓	✘
Skirted	✘	✘	✓	✓	✓
Hedged	✘	✘	✘	✓	✓
Ethrel applied	✘	✘	✘	✘	✘
Experimental harvest	✘	✘	✓	✓	✘
Kernel assessment (previous season nuts)	✘	✘	✘	✘	✘
Height and width measurements	✓	✓	✓	✘	✓
Irrigated	✘	✘	✘	✘	✘

Table 4.5.1

SITE: Tiaro

TRIAL CODE: GTIAR00

COLLABORATOR: Fraser Coast Regional Council

Key Point Summary:

- Originally managed by council staff but now wholly by CSIRO from Bundaberg, except for slashing.
- The original irrigation system was modified in 2005 using drippers on a system controlled by battery operated solenoid valves. This allowed irrigation events to be programmed by CSIRO staff.
- Approximately 10% of the trees have died or show symptoms of canker. Concentrated efforts on amelioration over the last 3 seasons has helped alleviate the issue.

Trial maintenance and data collection activities are summarised for each year, in Table 4.5.2.

GTIARO01 August 2008

	SEASON				
ACTIVITY	2003/04	2004/05	2005/06	2006/07	2007/08
Weed management	✘	✓	✓	✓	✓
Biological pest management	✘	✘	✘	✘	✘
Chemical pest /disease management	✘	✓	✓	✓	✓
Fertiliser applied	✓	✓	✓	✓	✓
Soil test	✘	✘	✓	✓	✘
Skirted	✘	✓	✓	✓	✓
Hedged	✘	✘	✘	✘	✘
Ethrel applied	✘	✘	✘	✘	✘
Experimental harvest	✘	✓	✓	✓	✓
Kernel assessment (previous season nuts)	✘	✘	✘	✘	✘
Height and width measurements	✓	✓	✓	✘	✓
Irrigated	✓	✓	✓	✓	✓

Table 4.5.2

SITE: Burpengary (Caboolture Region Environmental Education Centre)

TRIAL CODE: GBURP01

COLLABORATOR: Moreton Bay Regional Council

Key Point Summary:

- CSIRO staff have had no formal involvement in this trial during the life of this project. This was a management decision made after severe frost damage that occurred in 2001.
- Day to day operations of the planting have been carried out and managed by CREEC staff. Funding for an operational budget is provided by the local council.
- Recent meetings between CREEC and CSIRO are looking at including the Burpengary site in a replanting program for the *ex-situ* germplasm trials.
- The health status of the trees at this site means it is not suitable for use as a source for data collection. However it is a valuable education resource. Several hundred school children tour the site each year and it is featured in the annual 'Sustainable Living Expo'. Four thousand visitors were expected for the 2008 expo.

Cultivar Trials

SITE: Pretty Gully

TRIAL CODE: CPRET01

COLLABORATOR: Ian Colditz

Key Point Summary:

- Dry conditions prior to 2005 significantly stunted the vegetative growth of the trees in both trials. The trees were mulched in early 2005 and water was trucked in for one irrigation event. Rainfall has improved since.
- This site is remote with no permanent resident. Timing for herbicide sprays and fertiliser applications may have been optimized if it were more accessible.
- The trial has benefited from recent good rainfall, and is in good health.

Trial maintenance and data collection activities are summarised for each year, in Table 4.6.1.

	SEASON				
ACTIVITY	2003/04	2004/05	2005/06	2006/07	2007/08
Weed management	✓	✓	✓	✓	✓
Biological pest management	✘	✘	✘	✘	✘
Chemical pest /disease management	✘	✘	✘	✘	✘
Fertiliser applied	✓	✓	✓	✓	✓
Soil test	✘	✘	✓	✓	✘
Skirted	✘	✘	✓	✓	✓
Hedged	✘	✘	✘	✘	✘
Ethrel applied	✘	✘	✘	✘	✘
Experimental harvest	✘	✘	✓	✓	✓
Kernel assessment (previous season nuts)	✘	✘	✘	✘	✘
Height and width measurements	✓	✓	✓	✘	✓

Table 4.6.1

SITE: Emerald

TRIAL CODE: CEMER01

COLLABORATOR: Waterways Pastoral Company

Key Point Summary:

- Collaborators are responsible for day to day management. CSIRO Visit biannually for yield assessment.
- Higher average summer temps than Bundy and Alstonville.
- Drip irrigation.
- Trees are vigorous and in good health. There were few pest issues during establishment.

Trial maintenance and data collection activities are summarised for each year, in Table 4.6.2.

	SEASON				
ACTIVITY	2003/04	2004/05	2005/06	2006/07	2007/08
Weed management	✓	✓	✓	✓	✓
Biological pest management	✘	✘	✘	✘	✘
Chemical pest /disease management	✓	✓	✓	✓	✓
Fertiliser applied	✓	✓	✓	✓	✓
Soil test	?	?	✓	?	?
Skirted	✘	✘	✘	✓	✘
Hedged	✘	✘	✘	✘	✘
Ethrel applied	✘	✘	✘	✘	✘
Experimental harvest	✘	✘	✓	✘	✘
Kernel assessment (previous season nuts)	✘	✘	✘	✘	✘
Height and width measurements	✘	✘	✘	✘	✘

Table 4.6.2

References

Hardner C, McConchie C, Boyton S, Campbell G, Maddox C, Mayers P, Greaves B. 2004. Improvement of macadadamia through breeding. HAL August 2004 final report.

Hardner C, Pisanu P, Boyton S. 2004. National macadamia germplasm conservation program. HAL March 2004 final report.

Hardner C. 2004. Rootstock evaluation for the Australian macadamia industry - stage II. HAL March 2004 final report.

Chapter 5

The effects of rootstock on propagation success and early field performance.

Abstract

A major project was initiated in 1998 by CSIRO, Horticulture Australia Limited and the Australian Macadamia society to evaluate alternative rootstocks for the Australian macadamia industry. Rootstocks can have significant effects on growth and production in other tree crops but information on rootstocks effects in macadamia is lacking. Nuts were collected from 15 cultivars to propagate seedling rootstocks and 12 of these cultivars were also propagated as cuttings and as clonal rootstocks. The 12 cultivars where budded onto the established clonal and seedling rootstocks in an incomplete design. There were significant differences among rootstocks for germination, strike of cuttings, growth of rootstock, and budding success, but there were little correlation among cultivars for these traits. Successful plants were used to establish a field trial across 4 sites to evaluate field performance. Results from early growth of field trials and yields are presented.

Introduction

Rootstocks are commonly used in macadamia production 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. Since the early 1990's, the majority of orchards in Australia have been established using seedling rootstocks derived from open pollinated seed collected from the cultivar H2. It has been reported that this cultivar is favoured because it has a broad stem that is considered an advantage for grafting at a younger age (Stephenson 1990).

Own-rooted cuttings have also been used for propagation of selected cultivars (Cormack and Bate 1976); (Bell 1996) and clonal rootstocks of Beaumont are used in the South Africa (Bell 1996). Clonal propagation of the rootstock may produce: (i) increase in uniformity in scion; (ii) greater control of genetic variation, and (iii) a reduction or avoidance of juvenility (Howard 1987). However, some clonal rootstocks propagated by cuttings may be more susceptible to wind throw compared

to seedling rootstocks (Trochoulias 1992), supporting commercial experience that own-rooted cuttings of some cultivars tend to be more prone to wind throw.

Individual rootstocks genotype have profound effects on many scion characteristics in Apple (Ferree and Carlson 1987). Different clonal rootstocks genotypes are used to alleviate unfavourable soil and climate conditions; affect resistance to root and scion disease; increase precocity; and affect tree size. However, the use of individual rootstock genotypes is not common in nut crops. In almonds, seedling rootstocks only derived from almond, peach, hybrid or plum crosses using a variety of parents are used for uniformity and vigour of the rootstock; to increase productivity in high calcareous soils with limited irrigation; and to manage pests and diseases (Kester and Grasselly 1987). In pecans, it has been difficult to demonstrate rootstocks have any major effect on growth, vigour or yield (Hanna 1987).

Little information is available to support the choice of rootstock in macadamia, although there have been calls for rootstock improvement for dwarfing rootstocks to be a major component of breeding programs (Huett 2004). In a macadamia rootstock trial in Australia, yield per m^2 of projected canopy area (yield efficiency) was greater for Gower seedling rootstocks compared to clonal rootstocks from the same cultivar, but there was no difference between seedling or clonal rootstocks derived from the cultivar 246 (Trochoulias 1992). Differences between rootstock cultivars were inconsistent between years. No significant effect of rootstock was demonstrated for kernel recovery or kernel sensory characteristics. However, this study only examined two cultivars as rootstocks and these are not currently used as rootstocks.

The current study was initiated with the Australian macadamia industry to identify (i) elite propagation methods, (ii) elite rootstock genotypes; (iii) the influence of rootstock on a range of production and kernel characteristics; and (iv) opportunities for early rootstock evaluation methods.

Material and Methods

Genetic design

Twelve selected cultivars (246, 344, 742, 781, 814, 816, 842, 849, A16, A268, Beaumont, and X8) were identified for evaluation as scions, own rooted cuttings, seedling rootstocks and clonal rootstocks. An additional 3 cultivars (H2, D4 and A38) were used as seedling rootstocks. These cultivars were chosen to represent a range of

genetic material based on DNA profiles (Peace 2003). Each of the selected cultivars was budded onto 5 clonal rootstocks, and 6 seedling rootstocks in a circular design.

Nursery

Seedling rootstocks were propagated from open-pollinated seed collected from 15 seedling rootstock cultivars in Autumn 1999. Seed was sown in August 1999 after being held at 4^{o}C since collection. Germinating seeds were potted up into forest pots and repotted into large 7 L plastic bags in Spring 2000. Sowing success of seedlings was assessed as the proportion of sown seed that were repotted into the large plastic bags. Following repotting, seedlings were held in a shade house until budding.

Own rooted cuttings and clonal rootstocks were propagated by collecting 400 shoots bearing four-five leaves of newly flushing apical material early in the morning from mature trees of the 12 selected cultivars in March 1999. Shoots were prepared for setting by striping the leaves to the youngest whorl and shortening the leaves to the next whorl by cutting squarely across the leaf. The base of the cutting was dipped in Clonex hormone treatment prior to setting in forestry tubes containing a 1:1:1 mix of decomposed granite: polystyrene prills: coconut peat. Forestry tubes were held in a side vented plastic house with misting controlled by level of leaf wetness. Cuttings were held in the misting house until roots appeared from the base of the pots. Most of the cuttings were repotted in Spring 2000 (18 months after setting) into 7 L bags containing a mix of 1:1:1 composted hardwood sawdust: pine-bark: river sand. Repotted plants were held in a shade house until budding. Strike rate of cuttings was assessed by the proportion of set cuttings that were repotted.

Growth of rootstocks was measured as the height above the potting mix of plants 1 year after potting up. Scions were propagated by budding in Summer of 2001/02 following procedures detailed in Bell(Bell 1998). Budding success was measured as the proportion of budded scions producing elongating buds within 4 weeks after budding.

Field trials

Field trials were established at 5 locations (Baffle Creek, Bundaberg in Qld, Maclean, Newrybar and Wollongbar in NSW) across 2 growing regions in late 2002 and early 2003 with plants produced in the nursery. Due to variable strike rate, sowing success, or budding success there were variable numbers of plants for the different rootstock:scion combinations. At Wollongbar (NNSW), a single replicate of 128 plants were established. Two replications were established at the 2 Bundaberg sites,

Baffle Creek and Bundaberg Horticultural Research Station, and at Newrybar (NNSW). The property in Maclean was sold after the establishment of the trial and the new owner choose not to continue to be involved in the project and the trees were removed and destroyed.

Height of plants was assessed at planting and annually 2 years after planting. Height of plants at planting was used as a covariate in the analysis of height 2 years after planting to remove variations due to initial height. Nut-in-shell yields were collected for NSW sites in years 2006 to 2008 while yields were only collected in the Queensland sites for 2007 and 2008.

Statistical analysis of early orchard yields was performed using Residual Maximum Likelihood (REML) in the GenStat (2008). The random model was trial site/(row + column). The fixed terms in the model were type, rootstock and scion. The model included interaction terms between these three factors, but due to a very patchy three way table of observations it was only realistic to consider the possibility of two factor interactions.

The yield data was square root transformed prior to analysis in order to stabilize the variance. The height and width variables did not require any transformation. The canopy volume was calculated based on the formula used by McFadyen et al., (2006) except the skirting height of 0.5 m was subtracted from the tree height

Canopy volume = π * (tree height-0.5) *width across row* width along row/ 6.

It was necessary to take a log transformation of the canopy volume prior to analysis (one was added to the volume prior to taking logs to avoid the problems of zeros).There was little evidence to support the existence of any interactions between pairs of factors for any of the variables analysed. The main effects of rootstock and scion were highly significant ($P<0.001$) for all five variables, however the effect of type was only significant for height.

Results

Propagation

Timetable

This trial took 3 years to produce the seedling, clonal and own-rooted cuttings. There was 1 year between sowing and repotting for the seedling rootstocks and between set and repotting for the cuttings used as own-rooted cuttings and clonal rootstocks. It then took 1 ¼ years from repotting until budding and 9 months for establishment of viable plants ready for planting. Cuttings took 2 years for full growth.

Seedlings

Average sowing success of the seeds was 41% and ranged from 15% for A268 to 69% for seed collected from 849 (Table 5.1). In this study, the sowing success of H2 was moderate (32%), compared to seeds from the other cultivars examined.

The average height of seedlings 1 year after potting up (18 months after sowing) was 60cm. Beaumont produced the most vigorous seedlings (80cm), followed by A268 and H2 (Table 5.1). Seedlings from 849 and 246 were the slowest growing (45 cm after 12 months). The sowing success and vigour of seedlings for different cultivars was not significantly correlated (0.16).

Cuttings

Mean strike rate of cuttings was 57%. Strike rate of cultivars ranged from 23% for 849 to 80% for 695. The strike rate of the cuttings collected from a cultivar was not significantly correlated with the sowing success of the cultivar (-0.35).

Average height of cuttings 1 year after potting up was 62 cm. A268 and 695 produced the most vigorous cuttings (Table 5.1). There was a moderate significant correlation (0.56, p=0.03) between the nursery vigour of cuttings from a cultivar and the strike rate of a cultivar. Nursery vigour of a cuttings was also correlated with the nursery vigour of seedlings from the same cultivar (0.63, p=0.01)

Budding success

The budding success was only 22%. There were significant differences between rootstocks but the effect of scion was about 3 times larger (Table 5.2). However, this is confounded with day of budding, as all scions of a particular cultivar were budded on the same day. There was no significant effect of rootstock vigour or type (seedling v clonal) on budding success. Budding success of scion cultivar was not dependant on the rootstock cultivar. A268 was clearly the most successful scion for budding

(51%) and the poorest budding was for 842 (2%) (Table 5.1). Budding success was highest for rootstocks of 695, A268 and 246, but lowest for A16 and 741.

Field growth and early yields

Plants propagated as seedling rootstocks were significantly taller at planting (1.2m) than plants propagated as clonal rootstocks or own-rooted cuttings (1.0m). Planting height was significantly different among scion cultivars in all 3 propagation types, but there was no significant effect of rootstock cultivar on height at planting. There was a very significant correlation of height at planting among scions cultivars propagated as seedling rootstocks or clonal rootstocks (0.72). However the correlation was not significant between the height at planting for cultivars propagated as own rooted cuttings or as seedling (0.2) or clonal rootstocks (0.4).

The height after 2 years growth was significantly lower for own-rooted cuttings (1.8m) than for plants propagated as seedling (2.0m) or clonal rootstocks (2.0m) (Figure 5.1.). There was no significant difference in growth between locations at Bundaberg and in NNSW but there were very significant differences in growth between trials within each region.

Also after 2 years, when the plants from the different propagation types were analysed separately, there was no significant effect of rootstock cultivar on growth of plants propagated as seedling or clonal rootstocks. Scion was only significant for plants propagated as seeding rootstocks or own-rooted cuttings. Differences between the growth of cultivar scions to 2 years propagated on seedling rootstocks was not significantly correlated with differences in cultivars propagated as own-rooted cuttings (Figure 5.2.)

In reviewing the performance of plantings in 2008 it was evident that the majority of trees that were supplied as 842 had a different phenotype and appeared to have affinity to H2 as the nut-in-shell had the characteristic depression caused by the infertile ovule. Similarly there were several trees that were supplied as A268 but physically resembled A203. These trees have been identified and removed from the analyses. The loss of the Maclean site that had some unique scion and rootstock combinations and tree deaths at the other sites has made the matrix of rootstock-scion combinations more incomplete than originally intended (Table 5.3.). However the design was sufficiently robust to cope with these contingencies and significant and economically important responses are being observed. A comparison of the

percentage of the difference types of rootstock that were alive, sick or had died are shown in Figure 3. While there were no statistical differences detected a marginally higher proportion of clonal rootstock had died than the seedling or cuttings. The number trees of each rootstock type being monitored in 2008 is shown in Table 5.4.

There were no significant differences in mean tree volume due to type of rootstock after 6 years. However there were significant differences between cultivars within a rootstock type. After 6 years for cutting derived trees A268 followed by 842 and 781 had the largest canopy volume with the smallest tree being 814 (Figure 5.4A.). The trees with the largest canopy volume with clonally derived rootstocks came from 695, A268 and 741 with the smallest being on 344 (Figure 5.4B.). The largest trees seedling rootstocks were on D4, H2 and A268 rootstock but they were not significantly different to 695 (Figure 5.4C).

Data for 2008 was analysed separately but gave similar outputs to the combined yields of 2007 and 2008. There was also no significant difference in the mean tree yield due to the type rootstock but highly significant difference between cultivars within a type of rootstock. The highest yielding trees derived from cuttings were 695 and A268 (Fig 5.5A). The same two cultivars also had the highest yield when used as a clonal rootstock (Fig 5.5B). The highest yielding seedling rootstock was A16 that was slightly higher than A268 (Fig 5.5C). While these two cultivars had the highest yields as seedling rootstocks they were not significantly higher than a range of other cultivars that included 695.

Discussion

Nursery growth

This study has demonstrated that significant variation may exist between rootstock cultivars for important traits influencing nursery production. Strike rate of cultivars ranged from 23% to 80%, sowing success rate from 15% to 69%, average height in the nursery 1 year after repotting from 42cm to 75cm, and budding success from 2% to 51%.

Sowing success in this trial is effectively the same as germination percentage as few plants died between germination and repotting. The moderate sowing success of H2 is somewhat lower than industry expectations (K. Wilson pers. comm.). Different nursery conditions and interaction with other cultivar characteristics (e.g. nut size,

(1997) may increase overall germination percentage or change the relative response of cultivars.

The relative high sowing success of 849 corresponds to industry experience with processing of nuts from this cultivar of relatively high frequencies of reject kernel due to germination. Further trials are required to confirm this link.

The results from this study also appear to correspond to anecdotal evidence that the strike rate of Hawaiian selections is lower than that of Australian selections (Bell 1996), and that the strike rate of Beaumont is high (Cruz-Castillo *et al.* 2000). However, it is possible the conditions used for striking cuttings in this study may not optimal for the striking of Hawaiian genotypes and that strike rate could differ under alternative conditions.

The results from this study suggest that budding success is strongly determined by the cultivar of the scion compared to the cultivar of the rootstock. However, as scion and budding day were confounded by the operations (i.e. all rootstocks for a given scion were budded on the same day) it is impossible to determine the more important factor.

The lack of a relationship between plant height and budding success suggests that smaller rootstocks could be used for propagation of macadamia. This could reduce propagation costs as plants could be held for a shorter time in the nursery. While budding was used to propagate the scions in this study, it is likely that grafting may be less sensitive to environmental conditions than budding and therefore open up greater flexibility for propagation methods. It is recommended that budding in summer should be avoided (Bell 1998).

The length of time taken to propagate the plants in this project is considerably longer than commercial practice. Commercial production of material is generally achieved in 2 years with H2 seedling rootstocks; however, greater production times were allowed in this project given the variability of rootstock material.

Field growth

The absence of a significant effect of rootstock on young plant growth suggests that further monitoring is required to determine if characteristics expressed at a later age are influenced by differences in rootstocks. It could not be determined if the previously reported differences in tree yield per square metre of projected canopy area among rootstocks (Trochoulias 1992) were due to an effect on yield per tree or differences in growth of canopy area.

Selection of rootstocks

Currently the information on the effects of rootstocks is insufficient to support selection among cultivars for effects on productivity. The variability in propagation success between propagation type and rootstock cultivar suggest choice of propagation will affect propagation costs. Information on these costs and the repeatability of the results in this study in other nursery and under different conditions is required to choose between the rootstocks examined in this paper.

In this study, Beaumont was the best performed seedling rootstocks as germination percentage; growth and budding success were high. Although A268 is favourable for growth and budding success, the sowing success of seed from this cultivar was not high. On the other hand, sowing success and growth were high for D4, budding success was low as a rootstock cultivar. Interestingly, the sowing success and budding success of H2 rootstocks was relatively poor compared to the other cultivars examined in this study.

Beaumont and A268 were the best performing clonal rootstocks due to superior strike rate, growth and rootstock budding success. The superior performance of Beaumont is consistent with its use as a clonal rootstock by the South African industry.

Selection of rootstock needs to also consider the impact of different rootstocks on orchard production as well as nursery economics. At present there is little evidence to indicate that alternative rootstock cultivars can be used to manipulate tree structure. The genetic diversity of the rootstocks in this study ranged from *M. tetraphylla* X *M. integrifolia* hybrids to *M. integrifolia* cultivars. Possibly a wider genetic range may be required to find major rootstock effects if they exist.

The analyses of early tree yields are based on the rootstock type and the cultivar or seed source used and treat the scions as fixed effects. Further analyses that treat the scions as a random effect are planned. The precision of these investigations is limited by the incomplete design and continuing loss of trees. The imminent loss of the Newrybar site due to road construction will eliminate over 25% of the trial trees and will have a major impact on the implications that can be drawn from these combined trials. Despite this 695 and A268 appear to have superior performance as cuttings and clonal rootstocks because superior strike rate, growth, budding success and early yields.

References

Bell, H. F. D. (1996) The propagation of macadamia from cuttings. In 'Challenges for Horticulture in the Tropics. Proceedings of the 3rd Australian Society of Horticultural Science Conference'. (Eds R. A. Stephenson and C. W. Winks.) pp. 223-228. (Australian Society for Horticultural Science, Sydney).

Bell, H. F. D. (1998). Punch budding in New South Wales. What is the problem? *Australian Macadamia Society News Bulletin* 24, 73-76.

Cormack, D. B. and Bate, G. C. (1976). Seasonal changes in carbohydrate levels and rooting efficiency of Macadamia [integrifolia]. *Acta Horticulturae* **57**, 21-28.

Cruz-Castillo, J. G., Nicolas-Cruz, M., and Rogel-Castellanos, I. (2000). Macadamia Propagation by Grafting and Stem Cuttings. *Revista Chapingo. Serie Horticultura* **6**, 97-100.

Ferree, D. C. and Carlson, R. F. (1987). Apple Rootstocks. In 'Rootstocks for Fruit Crops'. (Eds R. C. Rom and R. F. Carlson.) pp. 107-144 (Wiley).

GenStat (2008). 11th edition, VSN International, Hemel Hempstead, U.K.

Hanna, J. D. (1987). Pecan Rootstocks. In 'Rootstocks for Fruit Crops'. (Eds R. C. Rom and R. F. Carlson.) pp. 401-410. (Wiley).

Hartmann, H. T., Kerster, D. E., Davies, F. T., and Geneve, R. L. (1997). 'Plant Propagation.' (Prentice-Hall Inc: Upper Saddle River, US.).

Howard, B. H. (1987). Propagation. In 'Rootstocks for Fruit Crops. (Eds R. C. Rom and R. F. Carlson.) pp. 29-78. (Wiley).

Huett, D. O. (2004) Macadamia physiology review: canopy light response study and literature review. *Australian Journal of Agricultural Research* **55**:609-624.

Kester, D. E. and Grasselly, C. (1987). Almond rootstocks. In 'Rootstocks for Fruit Crops. (Eds R. C. Rom and R. F. Carlson.) pp. 265-294.

McFadyen, L.M. Morris, S. G., McConchie, C. A. and Oldham, M. A. (2005). Effect of hedging and tree removal on productivity of crowding macadamia orchards. Australian Journal of Experimental Agriculture 45: 725-730.

Peace, C. P. (2003) Genetic characterisation of Macadamia with DNA markers. PhD thesis University of Queensland, St. Lucia, Qld. 259 pp.

Stephenson, R. A. (1990). Macadamia nut. In 'Fruits: tropical and subtropical'. (Eds T. K. Bose and S. K. Mitra.) pp. 490-521. (Naya Prokash: Calcutta.).

Trochoulias, T. (1992). Rootstock type affects macadamia performance. *Acta Horticulturae*. 296:147-152.

Table 5.1. Means for 15 cultivars for seedling sowing success, and nursery growth, cutting strike success and nursery growth, and budding success as scion or as rootstock.

Cultivar	Seedlings		Cuttings		Budding %	
	Sowing (%)	Height (cm)	Strike %	Height (cm)	Scion	Rootstock
246	22	49.2	59	56.1	12	21
344	42	52.1	54	47.6	6	14
741	60	61.3	61	44.0	6	5
781	34	51.6	55	54.6	13	12
814	32	56.6	68	53.0	23	10
816	35	60.2	34	53.0	6	20
842	47	51.1	40	51.7	2	6
849	69	49.1	23	45.5	6	15
A16	51	62.7	61	48.5	11	4
A268	15	65.1	76	71.7	51	23
BMT	57	81.1	80	69.4	8	34
X8	40	58.9	70	48.1	29	12
H2	30	64.7				11
D4	67	68.2				8
A38	21	62.3				9

Table 5.2. Significance of rootstock effects

	Propagation type		
Effect	**Seedling**	**Clonal**	**Cutting**
Region	ns	ns	ns
Farm	***	***	*
Index	ns	ns	-
H0	***	***	***
Scion	***	ns	***
Rootstock	ns	ns	-
Scion.Rootstock	ns	ns	-
Variance	0.11	0.13	0.08

Table 5.3. Number of healthy trees of each of the rootstock scion combination across all sites in 2008

Rootstock Type	*Rootstock*	*X8*	*A16*	*246*	*A268*	*344*	*695*	*741*	*781*	*814*	*816*	*842*	*849*	*Grand Total*
Clonal	X8	1		1							1		1	**4**
	246			7							4	1	4	**16**
	A268		4		10	8	6			10				**38**
	344	1	2	1		1					3			**8**
	695	11	7			12	13			17				**60**
	741					2		1		2				**5**
	781				4			5	2	5				**16**
	814	3		3		1				5				**12**
	816							2	1		3	1	1	**8**
	842				4			3	2			1	1	**11**
	849				1		3	3					2	**9**
Total		16	13	12	19	24	22	14	5	39	11	3	9	**187**
Cutting	X8	17												**17**
	A16		15											**15**
	246			11										**11**
	268				6									**6**
	344					9								**9**
	695						13							**13**
	741							17						**17**
	781								4					**4**
	814									14				**14**
	816										2			**2**
	842											6		**6**
	849												4	**4**
Total		17	15	11	6	9	13	17	4	14	2	6	4	**118**

Seedling	H2				9		6			4				**19**
	D4							4	5				2	**11**
	X8	11		8							5		5	**29**
	A16	3	2	5							2			**12**
	A38	7	2	4							6			**19**
	246			3					4		1	2	2	**12**
	A268		1		4		3			7				**15**
	344	2	1	2		3					3			**11**
	695	12	4			6	12			9				**43**
	741				5	2	4	4		11				**26**
	781				3		2	7	3	8				**23**
	814	2		1		3				2				**8**
	816							2	2		3	1	6	**14**
	842				3			3	5				2	**13**
	849				8		1	4	2				3	**18**
Total		**37**	**10**	**23**	**32**	**14**	**28**	**24**	**21**	**41**	**20**	**3**	**20**	273
Grand Total		70	38	46	57	47	63	55	30	94	33	12	33	578

Trial	*clonal*	*cutting*	*seedling*	*Grand Total*
RBAFF02	49	38	72	**159**
RNEWR02	59	28	78	**165**
RQBRS02	43	32	76	**151**
RWOLL02	36	20	47	**103**
Grand Total	**187**	**118**	**273**	**578**

Table 5.4. Numbers of trees of each type of rootstock either clonal, cutting or seedling remaining at each of the sites in 2008

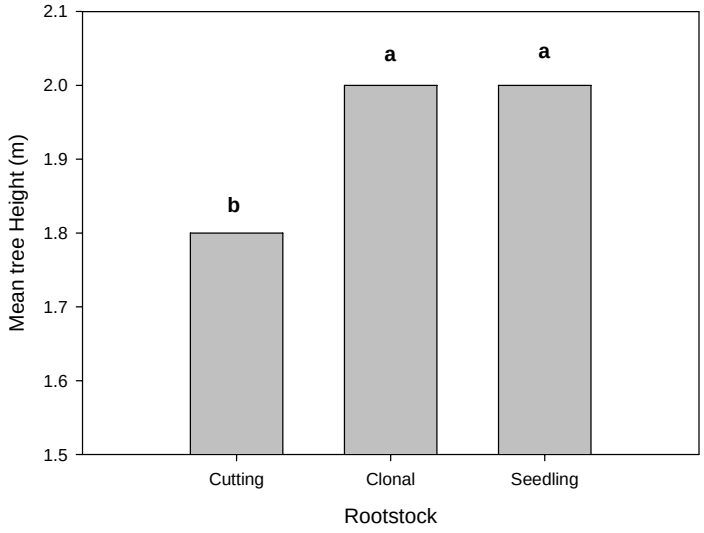


Figure 5.1. Mean height trees propagated using different rootstocks after 2 years. Different letters significant differences P<0.05.

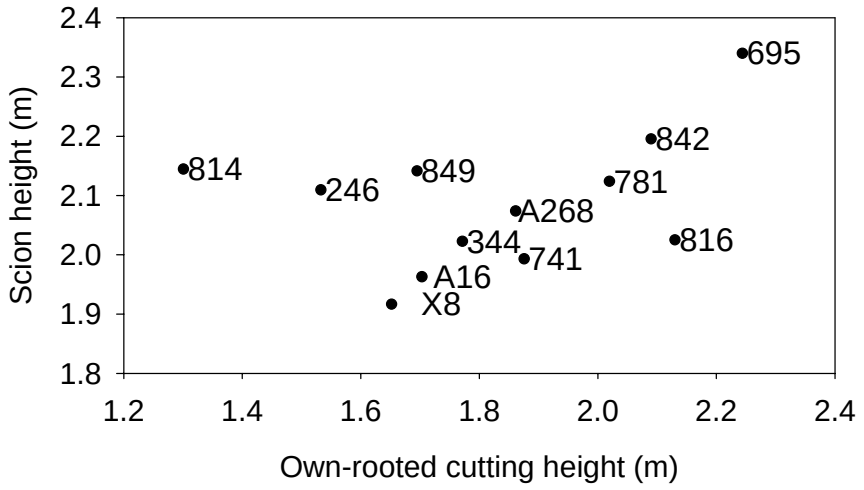


Figure 5.2. Height of cultivars propagated on seedling rootstocks against height of cultivars as own-rooted cuttings at 2 years of age.

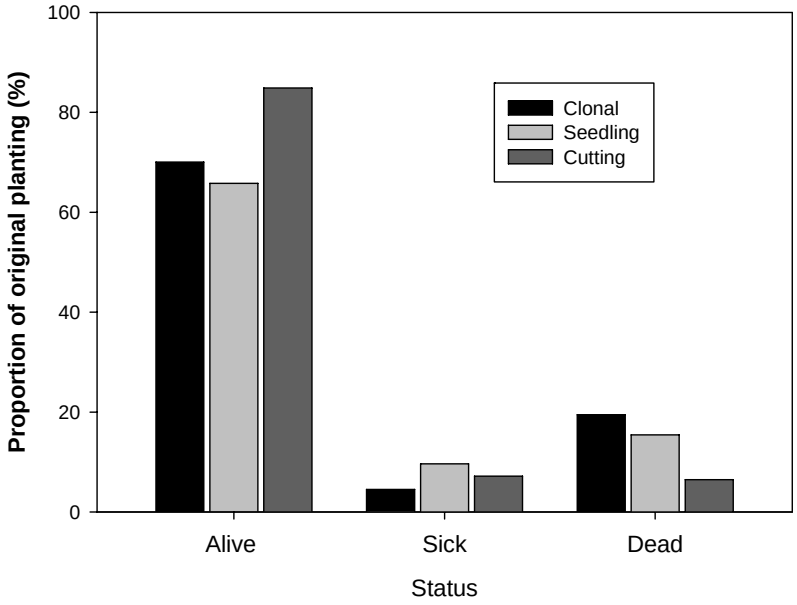
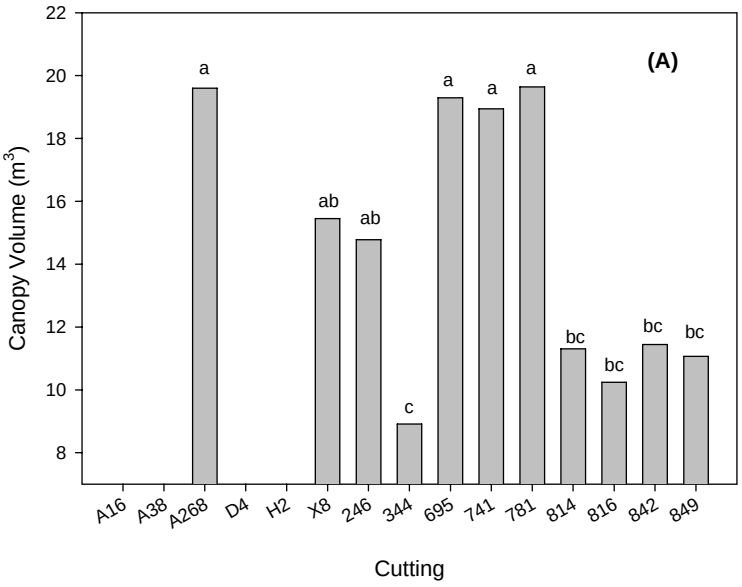
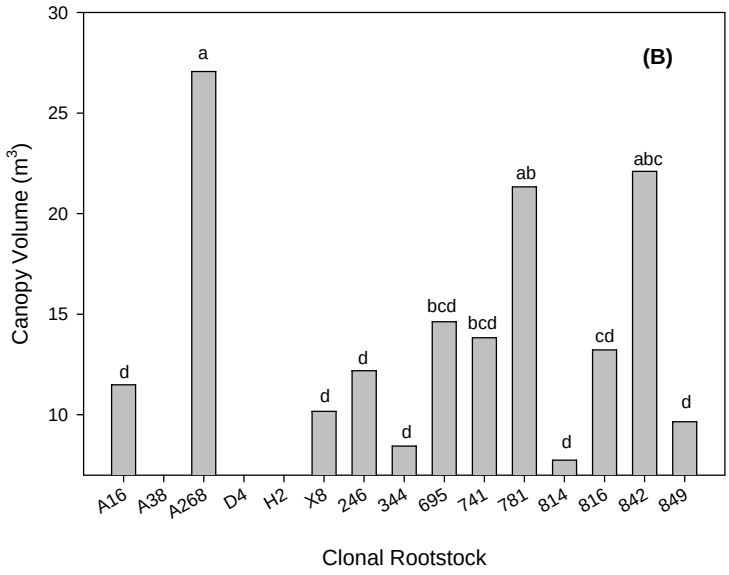
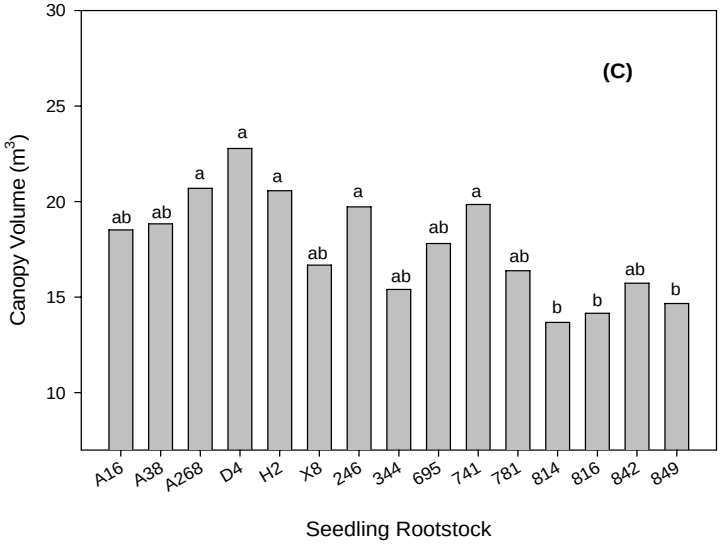


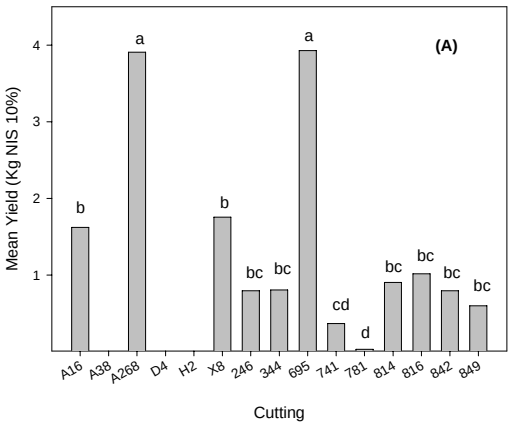
Figure 5.3. Proportion of each type of rootstock that was still alive, sick or had died 8 years after planting cross all trial sites.

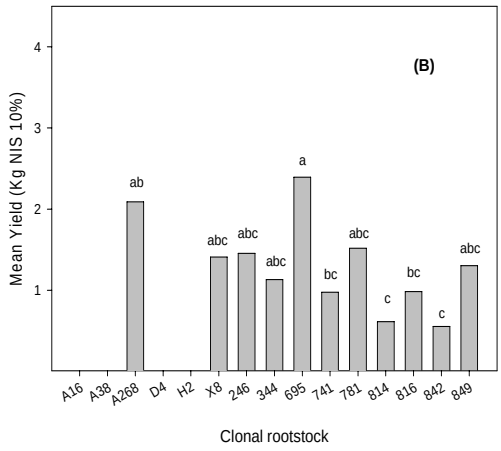


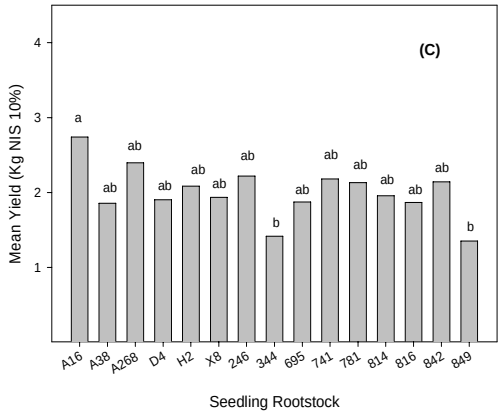




Figures 5.4 A-C. Comparison of mean tree volume for Cuttings (A), Clonal rootstocks (B) and seedling rootstocks (C) derived from the cultivars indicated on the axis.







Figures 5.5. A-C. Comparison of mean yield for Cuttings (A), Clonal rootstocks (B) and seedling rootstocks (C) derived from the cultivars indicated on the axis.

Chapter 6
An economic model of macadamia production and processing for the calculation of economic weights for selection and breeding

Abstract

Macadamias are a valuable horticultural tree crop of sub-tropical Australia. The profitability of macadamia production and processing can be increased through use of superior cultivars. Selection among candidate genotypes requires consideration of many traits that may impact on profitability. In this study, economic weights for use in a conventional selection index are developed and applied to objectively rank cultivars. This is an objective, robust and transparent approach that quantifies which traits are most important for selection. Further research is needed to included other traits considered important by the industry and validate underlying assumptions

Introduction

Macadamias are an important and expanding perennial tree crop in Australia (Mason and McConachie, 1994). Establishment of macadamia orchards requires a large initial investment of capital, which, after several years of zero or low yields, is expected to provide a high return on investment as the orchard matures. Assuming that managerial practices are adequate, the profitability of the enterprise can be raised through the selection of cultivars that are superior in traits that impact the economics of the production system.

There are many traits of interest for improvement in macadamia Hardner and McConchie, 1999). When multiple traits are considered for selection, some method must be employed to combine information of these traits across candidates. The most efficient approach is to apply a selection index, where the basis of selection is a single index value for each candidate that is calculated as a linear function of the genetic value of the candidate for each of the different traits of interest weighted by the importance of the trait (Smith 1936, Hazel 1943). This approach is objective, transparent and offers the opportunity to test the sensitivity of selection to alternative weights (Hazel and Lush 1942). Commonly, the weights used in the selection index

are derived by modelling the impact of an independent change in the trait on the economics of the production system (Ponzoni and Newman, 1989). The aim of this study is to derive economic weights for important traits in macadamia and apply these to selection of candidate cultivars.

The macadamia production system can be broken into 4 sectors: orchard production, processing of nut-in-shell (NIS), wholesale and consumption. Orchards may vary in size from small part-time enterprises of less than 20 hectares to large commercial operations more than 100 hectares. After site preparation, orchards are generally planted with elite cultivars that have been vegetatively propagated, usually by grafting onto seedling rootstock (Nagao and Hirae 1992). Planting densities may range from 200 to 500 trees per hectare (Stephenson 1990), (Nagao and Hirae 1992), (Mayer et al., 2006). Fertiliser, herbicide, and slashing and mulching operations commence in year one. Trees start to produce nuts between 3 and 6 years of age, with yields increasing as the orchard develops (Stephenson, 1990), (Nagao and Hirae 1992), (Mayer et al., 2006). Insect pest and disease management programs usually also begin at this age. Nut-in-husk (*NIH*) is mechanically harvested from the ground after which the husk is removed and the remaining nut-in-shell (*NIS*) dried to approximately 10% moisture content. As trees increase in size with age, the canopy closes and skirting and hedging are required to manage the canopy and maintain orchard productivity (Stephenson and Trochulias 1994), (McFadyne et al 2004), (Huett, 2004). NIS is sorted to remove nuts containing unacceptable kernel (e.g. insect damage, mould and kernels that have started to germinate), and the remaining NIS is sold to processors.

At the processing factory nuts are received, dried to 1.5 % kernel moisture content and then crack them to extract the raw kernel (Mason and McConachie, 1994). Raw kernel is mechanically and manually sorted to remove shell and unacceptable or lower quality kernel (e.g. insect damage, immature, germinating kernel, discoloured). Lower quality kernel is sold for oil extraction or as commercial grade depending on the severity of the kernel disorder. The remaining premium kernel is sorted into styles for sale based on proportion of whole kernel and kernel size range. Sorted kernel is then packaged and stored prior to distribution. Kernel may be used for roasted snack products, chocolate enrobed confectionary, ingredient in ice cream or bakery, as cooking oil or for cosmetics (Stephenson 2005), (Cavaletto, 1981).

Economic models of macadamia production have previously been developed (Reilly and Bevan 1995), (Keeler and Fukunaga 1968), (Scott and Marutani 1982), however, these do not extend past the farm gate and are not sufficiently flexible to enable economic weights to be calculated. The model presented in this paper has been developed to examine the impact of 10 important traits: tree height, canopy width per tree, age of first crop, early yield per tree development, total kernel recovery, proportion of kernel that is un-commercial, proportion of commercial kernel that is whole and average diameter of commercial whole kernels. These traits were selected from a set of 35 potential selectable characteristics which were initially modelled for their potential impact on profitability, their ease of assessment, and their overall suitability towards breeding for improvement of macadamia nut production.

Materials and Methods

Definition of selection objective

The selection objective was defined as the maximisation of profitability across the production and processing sectors of macadamia from plantation establishment to sale of raw kernel. This can formally be described as an index value calculated for the jth selection candidate:

$$H_j = w_1 * g_{1j} + w_2 * g_{2g} + \ldots + w_i * g_{ij}$$

where g_{ij} is the genetic value of the i^{th} trait for the j^{th} genotype expressed as a deviation from its population mean and w_i is the economic weight for the i^{th} trait.

Overview of production system

The production system was defined as on-farm production of NIS through to processing and sale of raw kernel. Costs were assumed to be incurred in growing the NIS and processing the NIS to raw kernel, and income was derived from selling the raw kernel. The price that the grower receives from the processors for NIS was not included in the economic model as this is cancelled out by the cost to the grower of purchasing the raw material. In the long term, changes that affect the cost of production in one sector would likely be passed onto to the other sector.

A spreadsheet model was developed to account for all costs and income in growing and processing and link biological traits to this production system model. The model

was developed for current cost structures and kernel prices for a 100 ha farm in northern NSW and a single factory where data was obtained. Changes in these characteristics or other management scenarios need to be evaluated before the results can be generalised across the macadamia industry.

Definition of biological traits

Economic weights were developed for 11 traits that impact on the cost of production and processing, and the value of raw kernel (Table 6.1). These traits were selected on the basis of both industry perceptions of importance and the ability to quantify the relationship between tree trait and the production system.

Tree height at planting (h_0; m) was set at 1.2m with height at 10 years (h_{10}; m) set to 6.4m. It was assumed that annual increase in height between planting and year five was four times that between years six and 15 after planting. Annual rate of growth in height after year 15 was assumed to be zero.

Canopy width was modelled assuming 0 m at planting, and a linear increase between planting and tree size at 5 years of age (cw_5; m) with a base value of 3.6 m. It was assumed canopy width would increase at a constant rate until canopy width was controlled by hedging operations undertaken to maintain a 2m distance between canopies across a planting row.

Yield was modelled by a the parameters age of first crop and cumulative yield to eight years using a function developed from general yield models presented in Mayer et al (2006). Base values for age of first crop (afc) and cumulative yield to year 8 ($CumY_8$) were 4 years and 12.5 kg (at 1.5 % moisture content, MC), respectively. It was assumed yield would increase linearly from age of first crop to 8 years of age at a rate calculated as:

$$\frac{CumY_8}{\sum_{1}^{8-afc} n}.$$

Hedging to control canopy size was assumed to lead to a reduction in the rate of yield increase, with the rate halved from 4 years after the application of hedging operations and to be reduced to zero by 9 years after hedging. Mass of nuts remaining in the tree at the beginning of September was used to assess mass of late dropping nuts ($ldnm_7$)

and calculate percentage of late dropping nuts that was assumed to be constant throughout the orchard.

Percentage of NIS at 1.5 % moisture content that was reject due to visible insect damage to kernel, double nuts, open micropyle (*prn*) and old nuts suggesting the presence of mouldy kernel was assumed to be 2%. Total kernel recovery (*tkr*; kg kernel/kg NIS) is the percentage mass of the remaining NIS that is kernel and was set to 33%.

The base value for the percentage of marketable whole kernels (*pmwk*; kg kernel/kg marketable kernel) was set to 49%. Average size grade of marketable whole kernels (*agmw*) was defined as the dimension of a square hole in a sieve that a kernel would not pass through with assumed base values of 18.4 mm. Average size grade of marketable half kernels (*agmh*) was defined as the dimension of a square hole in a sieve that a half kernel would not through with assumed base value of 15mm. Constant values for kernel recovery, percentage of marketable whole kernels and kernel size were used across all years, as these traits do not exhibit a trend with age (Hardner et al., 2001).

Farm production costs

Farm characteristics

The production model was for a farm established on gentle grazing land in northern NSW comprising an orchard area of 100 hectares (T_a = 100) with an additional 10 hectares for farm infrastructure (e.g. roads, fences, sheds). The planning period was 20 years. Tree spacing was set at 7m across rows and 4 m between trees along rows (357 trees/ha).

Costs

Independent inputs were defined as those not directly influenced by the levels of target traits and included: (i) land, (ii) machinery; (iii) infrastructure; (iv) fixed labour, (v) general fixed costs, (vi) site preparation, (vii) planting, (viii) irrigation, (ix) herbicide, (x) fertiliser, (xi) foliar spray, (xii) slashing and (xiii) mulching. Costs modelled as being directly affected by variation in biological traits included application of (xiv) pesticides and (xv) ethephon, (xvi) harvesting, (xvii) on-farm sorting, (xviii) canopy management, and (xiv) off-farm transport.

The cost of land was included in the model to account for the opportunity cost of owning the land for macadamia farming using a purchase price of $5,000 per hectare, which is common for the Bundaberg growing area in Australia. The machinery and infrastructure required for a 100 hectare orchard are outlined in Table 6. 2. The large 100HP cab tractor was required for operating the air blast sprayer and the large harvester. The smaller 70HP orchard tractor was used to operate the slasher, fertiliser spreader, small harvester, the mulcher and a tipper trailer during harvesting. An additional 70HP orchard tractor was purchased in year four to meet the increasing machinery requirements of the orchard. A small second-hand tractor was required for herbicide applications, using a small under tree boom, and a second tipping trailer.

It was assumed that all 100 hectares are established in the same year to simplify the accounting of cost and income streams. It was assumed that the cost of preparing grazing land for planting was $1,000 per hectare. The assumed permanent labour requirements were a full-time manager ($40,000 per annum) and a full-time mechanic ($30,000 per annum). Annual costs included $7,000 for repairs and maintenance of farm machinery (excluding the tractors where this was included in the hourly operating charge) and infrastructure; $5,000 for fuel and oil (for all farm machinery

except tractors); $7,000 for electricity for dryers, de-husker, elevators and other equipment; $10,000 for general rates, fees, and expenses; and $1,000 from year three for rat control.

Cost of dependent inputs

Dependent inputs were defined as those that are directly influenced by the biological traits of interest and included: (i) tree planting; (ii) fertiliser; (iii) foliar sprays; (iv) pesticides; (v) herbicides; (vii) slashing; (viii) mulching; (ix) canopy management; and (x) harvesting. In general, costs for these inputs were expressed per tree with total farm values determined by multiplying per-tree values by planting density and orchard area.

The annual cost per tree of the i^{th} dependent input in year t (Vc_{it}) was modelled as:

$$Vc_{it} = (Mc_{it} + Ac_{it}) * N_{it}$$

where Mc_{it} was the cost of the material required per application, Ac_{it} was the cost of applying the input per application and N_{it} was the number of applications of the input per year. The cost per tree of the material for the i^{th} input was modelled as:

$$Mc_{it} = R_{it} * Uc_{it}$$

where R_{it} was the application rate per tree (units/tree) of the input in year t, and Uc_i was the unit cost of the material ($/unit). The cost per tree in year t of applying the i^{th} input was modelled as:

$$Ac_{it} = P_{it} * \frac{cw_{10}}{Ts_{it}} * \frac{1}{1000} * \{Tc_{it} + Oc_{it}\}$$

where P_{it} was the number of times a tractor must pass along each planting row per application in year t, Ts_{it} was the tractor speed for the operation (km per hour) in the t^{th} year, Tc_{it} was cost per hour of the tractor used to apply the input ($ per hour) in the t^{th} year, and Oc_{it} was the cost of the operator ($22). A summary schedule of the values of variables used to calculate the per tree costs of different inputs is presented in Table 6.2. The operating cost per hour (including fuel, lubricants and repairs, but excluding labour and depreciation) for the tractors used on the model farm was assumed to be $15 for a 100HP tractor, $12 for a 70HP tractor, and $10 for a small second hand tractor.

The cost of tree planting (including purchase and labour) was set at $15 per tree. It was assumed that 2% of the trees would die per annum in the first 4 years and require replanting at the same cost.

It was assumed that a complete fertiliser would be used for all the major nutrient requirements (O'Hare et al., 1995). Fertiliser application rates (kg/tree) increased with the age of the orchard to accommodate a general increase in tree size (0.1 in year one, 0.2 in year two, 0.4 in year three, 0.5 in year four, 0.7 in year five, 0.9 in year six and 1.2 from year seven). Essential micro-nutrients and trace elements were applied as foliar sprays (Table 6.3) from year four with no application cost as it was assumed they would be combined with pesticides.

The model included preventive pesticide applications from year four. Endosulfan™ was applied twice a year to control flower caterpillar and fruit spotting bug while beta-cyfluthrin™ was used twice for macadamia nut-borer (O'Hare et al., 1995). The annual application rate per tree of these chemicals was determined by the canopy volume of the tree, which was calculated from tree height and canopy assuming a cylindrical shape approximated the canopy form.

The major fungal diseases of macadamia include blossom blight and husk spot (O'Hare et al. 1995). The model assumed carbendazim™ was used for the control of blossom blight, while copper oxychloride was used for husk spot. It was assumed there was no cost of applying these chemicals because they could be mixed with either of the two insecticide sprays.

The model assumed weeds under the tree canopy were controlled using a herbicide sprayed in a one metre strip each side of the tree at a rate of three litre/ha. Slashing was undertaken to control grass and weeds within the inter-row area. To account for a decrease in weed vigour with age, eight herbicide applications were undertaken in years one to three, seven in years four to six, six in years seven to nine, and five thereafter. For the same reason, the model assumed that the number of slashing applications per year would decrease and tractor speeds increase with orchard age (Table 6.4). Mulching of grass slashings, leaf drop and nut husks from the inter-row area to under the tree canopy was undertaken from year four to control weeds,

decrease soil temperature fluctuation, add organic matter, and reduce erosion (O'Hare *et al*. 1995).

The model included skirting and hedging at later ages to manage canopy size for orchard access and reduce conditions favourable for fungal diseases (Huett, 2004). It was assumed these operations were undertaken by external contractors with costs per tree ($Ac_{skirt+hedge,t}$) determined as:

$$Ac_{skirt+hedge,t} = \left(\left[P_{skirt+hedge,t} * \frac{cw_{10}}{1000} * \frac{Rate_{skirt+hedge,t}}{Ts_{skirt+hedge,t}} \right] + \$removal \right) * N_{skirt+hedge,t}$$

where $Rate_{skirt+hedge,t}$ was the hourly rate of contract skirting and hedging (assumed \$100), *$removal* was the additional machinery cost per tree of removing the debris (assumed \$0.30), and the other variables were as defined above. Operations did not start until year six with skirting and hedging carried out in alternative years. Tractor speed was assumed to decrease from 3.5 km/h in years six and seven to 3.0 km/h in years eight and nine, 2.5 km/h in years ten and eleven, and 2.0 km/h thereafter, as the orchard aged.

Harvesting was undertaken from the first year of yield with a large and a small tractor drawn harvester. The small harvester was not required during the first harvest of the season, but followed the large harvester for the remaining harvests. The number of harvests per year increased from three in years four and five, to four in years six and seven, and five thereafter. It was assumed tractor speed was unrelated to yield per tree (Table 6.2). Harvesting costs were not modelled as being dependant on tree yield.

On-farm transport of harvested NIS was limited by the assumed capacity of the tractor-drawn trailer (*Trc*, 800kg/load) and the assumed tractor speed (*Ts*, 5 km/hour). Transport costs were incurred for use of the tractor (*Tc*, \$10/hour) and the cost of a casual tractor operator (*Oc*, \$22/hour). The cost of on-farm transport of NIS per tree in a given year (*Tonfarmtransport*) was thus estimated as the total harvested NIS (*HNIS*) divided by the trailer capacity (*Trc*, 800 kg) to give number of trailer trips per tree, multiplied by the assumed average distance travelled per trip ([km/trip], 0.5km) and divided by the assumed tractor speed to give transport time) multiplied by the

transport cost rate (being tractor rate plus operator rate), with the addition of assumed turnaround time ([turnaround], 15 minutes) multiplied by operator rate:

$$Tonfarmtransport = \frac{HNIS}{Trc} * \left[\frac{[km/trip]}{Ts} * (Tc_{it} + Oc_{it}) + [turnaroundtime] * Oc_{it} \right]$$

It was assumed causal labour ($15/hour) was used for dehusking and on-farm removal of reject NIS. It was assumed that causal labour could dehusk and remove rejects from 150kg of NIS per hour per person, but that the maximum reject removal rate was 4kg/hour/person. Thus, for NIS reject rates under 2.7% the dehusking and sorting rate was assumed to be 150kg of NIS per hour, but for greater reject proportions the sorting rate was estimated as 4kg/hour/person divided by percentage of reject NIS (*prn)*. The cost of on-farm dehusking and sorting per kilogram of NIS was calculated as the casual labour rate divided by the estimated dehusking and sorting rate.

Transport of harvested and sorted NIS from the farm to the processing facility (per tree in a given year, *NIStransportcosts*) was estimated assuming a per-tonne cost of transport ([transportrate], $10 per tonne) multiplied by the total mass of on-farm-sorted NIS per tree (*SNIS*), with an adjustment for the assumed actual moisture content of the sorted NIS (*MCNIS*, 15%, being different to the 3% used in all calculations of NIS yield as the standard for NIS purchase):

$$NIStransport\,costs = SNIS * \frac{(1-3\%)}{(1-MCNIS)} * \frac{[transportrate]}{1000}$$

Processing costs

The model of processing green NIS to marketable raw kernel was based on the cost structure of a processor buying green NIS from many growers. All costs were treated as variable so that total processing costs were derived from the cost per kilogram multiplied by the amount of material processed.

Capital and operating costs were expressed per kg of delivered NIS at 1.5%MC for receival of NIS ($0.014, $0.009), drying ($0.022, $0.060), and cracking ($0.018, $0.180). This does not allow storage and drying costs to vary with moisture content, although this is possible. Total kernel recovery (*trk*) was used to convert delivered NIS to total kernel mass after cracking. The capital cost of sorting was assumed to be $0.10 per kg of total kernel. Operating costs for sorting were derived from data supplied by an anonymous processor. Percentage of reject kernel (*prk*) was used to

calculate total mass of reject kernel and total mass of marketable kernel. The estimated fixed operation cost per kg of total kernel sorted was $0.22 with a variable cost of $3.25 per kg of reject kernel removed. Assumed operating (and capital) costs per kg of marketable kernel were $0.45 (and $0.004) for packing, $0.03 ($0.00) for quality assurance, and $0.17 ($0.00) for internal transport. A figure of $0.08 was allowed for capital overheads.

Increasing either percentage of marketable whole kernels (*pmwk*), average grade of marketable whole kernel (*agmw*) or average grade of marketable half kernel (*agmh*) were expected to reduce processor kernel sorting costs as in each case the number of kernel "pieces" per kg of kernel to be sorted would be decreased and sorting costs are related to the number of pieces sorted.

Increasing the proportion of marketable whole kernels by 10% (*pmwk*, on a mass basis, from 0.40 to 0.44) was assumed to reduce the mass-fraction of half kernels (from 0.57 to 0.53, maintaining the mass-fraction of undersize pieces at 0.03), resulting in an estimated net reduction in the number of pieces to be sorted per kilogram of sound kernel by 2.6%. The cost of sorting was assumed to be reduced in direct proportion with the number of pieces to be sorted (i.e. by 2.6% for a 10% increase in the fraction of whole kernel).

Increasing average marketable whole kernel grade (*agmw*) was assumed to increase average piece volume by the cube of the increase (ratio) in size (grade), thus reducing the total number of pieces per kilogram and associated sorting cost by the inverse of the increased piece size weighted by the fraction of whole kernel:

$$\Delta\text{sortcost} = \left[\frac{1}{\left(1 + \frac{\Delta agmw}{\overline{agmw}}\right)^3} - 1 \right] pmwk$$

Increasing average grade marketable half kernel (*agmh*) was similarly assumed to increase sorting cost with weighting by the fraction of half kernel being (1 - *pmwk*).

Value of marketable raw kernel

Total kernel value (TKV) was derived from 2003 raw kernel prices that were defined by percentage of whole kernel and kernel size (Table 6.5). The percentage of kernel within a certain style from a particular consignment was estimated from percentage of marketable whole kernels (*pmwk*), average and variance in grade of marketable whole kernel (*agmw*, σ^2_{gmw} assumed to be 1.44), mean and variance of grade of marketable half kernel (*agmh*, σ^2_{gmh} assumed to be 2.56), and the 2003 prices for the defined styles (detailed in Appendix 6.1). Percentage of marketable half kernels (*phk*) was derived from percentage of marketable whole kernels by assuming the proportion of pieces was constant at 0.03.

Estimating enterprise profitability

Enterprise profitability was summarised as Profitability index (*PI*), being the ratio of profit (discounted income minus discounted costs) to discounted costs:

$$PI = \frac{PV_I - PV_C}{PV_C}$$

where PV_I is the present value of all future income from establishment to year 20 discounted to the time of orchard establishment:

$$PV_I = \sum_{y=0}^{20} \frac{I_y}{(1+d)^y}$$

I_y is the income occurring in year y, and d is the annual inflation-free discount rate (assumed to be 0.08, or 8%).

The present value of all future costs PV_C, was calculated similarly.

Derivation of value weight and economic weights

A value weight was derived for each trait as the effect on profitability of an independent increase in the trait mean by 10%. Although this calculation does not consider how easily the trait can be changed through selection (i.e. the extent of genetic variation), it is a useful comparative measure of the value of changing different traits, as it is not a function of the scale of measurement of the trait. The economic weight for a trait was calculated as the effect of an independent increase of

one unit on the overall profitability index of the production system and were calculated by multiplying the value weight by 10 and dividing by the average of the trait. Note, economic weights and value weights were calculated for a positive change in each trait, thus a negative value indicates a negative effect on the profitability of the enterprise by an increase in the level of the trait. Economic weights were also expressed relative to 1% point of total kernel recovery, by dividing the respective weight by the economic weight of total kernel recovery.

Evaluation of cultivars

Derived economic weights were used to evaluate 40 cultivars from a regional variety trial for a number of the traits examined in this paper (Hardner et al., 2006). Best linear unbiased predictions (BLUP) of cultivar deviations were only available for precocity (*afc*), rate of linear increase in yield between precocity and 10 years of age (ary_{10}), tree canopy diameter (cw_{10}), total kernel recovery (*tkr*), percentage of marketable whole kernel (*pmwk*), and average grade of marketable whole and half kernel (*agmw*, *agmh*). The standard deviation of the cultivar BLUP deviations by economic weight for the i^{th} trait ($w_i * g_{i,j}$) was calculated to quantify the influence of each trait on the overall selection index value.

Sensitivity analysis

A sensitivity analysis was undertaken to examine the impact of variation in some of the assumptions of the economic model on the ranking of cultivars by calculating the correlation between index values for the 40 cultivars estimated using the economic weights derived using the assumptions in the base model and index values estimated when input assumptions were varied by plus and minus 20%: (i) land costs, (ii) other production costs; (iii) processing costs, and (iv) kernel prices.

Results

Model overview

Over a 20-year planning horizon the base model for the 100 ha farm produced 1,674 tonnes of NIS at 1.5%MC. After processing, this produced 1,372 tonnes of kernels of which 0.5% were style 0, 18% were style 1, 25% were style S, 46% were style 4 large, 8% were style 4 and 3% were pieces. The total net present value for cost of

production and processing the NIS produced by the model farm was $ 6,129,612 (82% production costs, 18 % processing costs). The largest production cost component was the cost of land (38%), followed by fixed costs (20%, including the costs of a manager and mechanic), then establishment cost (9%) (Fig. 6.1). The major processing cost components were cracking (30% of processing costs), packing (22%) and sorting (20%) (Fig. 6.2). The total present value of the kernel produced by the model of production and processing was $7,358,378, giving a net present value of $1,228,765 and a profitability index of 0.2005.

Impact of trait changes on economic model

There was no effect on land rent, total capital and total fixed costs from changes to any biological traits examined in this study. An increase in cw_{10} reduced all production costs, except foliar spray costs (which increased slightly), mainly due to reduced tractor operation costs in lower density orchards. An independent decrease in plant density also reduces NIS production and hence reduces total processing costs and total kernel value. Only affected total pesticide costs and total height at 10 years affected pesticide and foliar spray costs were affected by a change to h_0. Increasing *afc* without changing ary_{10}, reduced maximum yield at age 10 and thereafter, thus reducing total farm yield. Farm production costs were unchanged except for small reductions in on-farm sorting and transport costs, reduced processing costs and total kernel value due to reduced productivity. Increasing ary_{10} resulted in a small increase in farm sorting and transport costs, and large increases in processing costs and total kernel value due to an increase in overall productivity. Farm sorting costs increased with increasing percentage of reject NIS (*prn*), but processing costs and total kernel value decreased due to decrease in the absolute amount of nuts and kernel produced and processed by the enterprise. Production, NIS receival, drying and cracking costs were not affected by changing *tkr*, *prk*, *pmwk*, *agmw* or *agmh*. Increasing *tkr* increased total sorting costs, but reduced total packing, internal factory transport quality assurance and other overhead costs, and increased total kernel value. Increasing *pmw*, *agmw* and *agmh* decreased sorting costs as there were less pieces to sort, but did not affect total packing, internal factory transport quality assurance and other overhead costs. Total kernel value increased with the increase in *pmwk* as

wholes have a higher value than half kernels. Total kernel value decreased slightly with an increase in *agmw* but increased with increasing *agmh*.

Relationship between kernel price and kernel size

The relationships between kernel price and *agmw* and *agmh* were not linear (Figs 6.3a and 6.3b). Raw kernel price increased with an independent increase in average marketable whole kernel size grade from 13 to 15.8mm, then decreased between 15.8 and 19mm, and then slowly increases again after 19mm. This pattern was more pronounced at higher values for proportion of marketable whole kernel. Raw kernel price was less responsive to changes in average grade of marketable half kernel. The relationship between raw kernel price and proportion of marketable whole kernel was generally linear.

Economic weights

Economic weights for 11 production traits are presented in Table 6.1. The effect of increasing kernel recovery (*tkr*)by 1% point on the profitability index was equivalent to the effect on the profitability of the enterprise of increasing rate of yield increase (ary_{10}) by 0.06 kg/year, decreasing the percentage of reject NIS (*prn*)by 1.4%, decreasing the percentage of reject kernel (*prk*) by 2%, increasing the percentage of whole kernel (*pmwk*)by 11%, decreasing the average grade of marketable whole kernel (*agmw*) by 12mm and increasing the average grade of marketable half kernel (*agmh*) by 7mm.

Results from selection

Based on the traits included in the selection objective, the top 5 cultivars were 849 Own venture, 814, A4 and 804 (Table 6.5). 849 is predicted to raise the profitability index of macadamia by 0.32, or 60%, over the average of the 40 cultivars evaluated in this study. If the top 5 cultivars were planted in equal proportions, the profitability index would be increased by 0.25, or 22

The highest ranked cultivar, 849, had the 4th lowest average year of initial yield, was 16th for rate of yield increase, the 21st largest canopy width, the 3rd highest kernel recovery, the 5th highest percentage of marketable whole kernel, and the 6th largest grade of marketable whole and half kernel. The cultivar with the lowest average year

of initial yield was Own Venture which ranked 2nd overall. 344 had the highest average rate of yield but ranked 15th. A4 (ranked 4th) had the smallest canopy diameter and highest average grade of marketable whole and half kernels. X7 (ranked 7th) had the highest total kernel recovery, and 835 (ranked 23rd) the highest percentage of marketable whole kernel. The correlation with an index that did not include canopy width was 0.77 with the top cultivars being 849, 804, Own Venture, 842 (ranked 8th when canopy width was included) and 814. A4 was ranked 16th when canopy width was excluded from the index.

The magnitude of the standard deviation of the blup deviation of an individual traits multiplied by the economic weight was similar for ary_{10} (0.13), cw_{10} (0.11) and *tkr* (0.12). The standard deviation was lower for year of *afc* (0.02), *pmwk* (0.02), *agmw* (0.002) and *agmh* (0.003). The ranking of cultivars was unaffected by changes in land costs, other production costs, processing costs of kernel prices by 20% as the correlation between the index under the different scenarios was greater than 0.99 in all cases.

Discussion

This study has successfully developed a model of economics of macadamia production and processing that extends to the factor dispatch gate that was linked with variation of 11 biological traits. This model was developed with considerable input from members of the Australian macadamia industry. All major sources of income and costs for the production of raw kernel were identified with a level of detail that allows assumptions to be easily checked and modified. The model suggests that over the long term macadamia production and processing is profitable (PI=0.20). However, the distribution of this profit between the growing and processing sectors is outside the scope of this paper. This model also does not include individual taxation situations which should be considered if used to evaluate investment options.

The largest sources of costs of production were sources were land, fixed results from this analysis indicate that land is the largest costs source for the production of macadamias is land. In this study, the cost of land has been treated as a land rental as this spreads the cost of land over the planning period rather than a large initial cost

that must be carried through the production life of the orchard. This approach, however, is not dissimilar to buying land with borrowed money and paying interest over the life of the planning period. Including the cost of land also accounts for the opportunity cost of investment in macadamia for growers that own land outright.

Both capital and operating costs were treated as variables in the analysis of the costs of processing in this study. The results of productivity increases due to breeding and deployment of advanced cultivars will not be felt by a processor for 10-20 years. If productivities are increased through breeding and the total orchard estate size remains unchanged (or increases), more processor capacity would be required, but it could be installed as required by the time the productivity increases are felt. Current processing plants have capacities ranging from 6,000 to 15,000 tonnes of NIS per annum and are thus likely operating at levels where plant capital costs per kilogram of nut processed are relatively independent of plant capacity. Further research needs to undertaken to examine more closely the relationship between variability in biological traits and the costs of macadamia processing. For example, there are likely to be differences in the efficiency of removing different classes of reject kernel though mechanical or labour sorting.

The impact of variations in biological traits on the economics of macadamia production and processing was compared in this study using the profitability index of the entire enterprise. Interpretations of these results depend on this perspective. For example, an independent increase in average rate of yield increases processing costs, as there were more nuts and kernels to process, but overall profitability increased, as processing cost per kg of marketable kernel were lower.

The profitability index was used to quantify changes in profitability rather than *Net Present Value* (*NPV*, total discounted profit). *NPV* should only be used if the total investment remains unchanged (Anthony and Reece 1989), however, in the model presented in the study, changes in traits influence total enterprise costs differentially. For example, increasing ary_{10} increases the production of *NIS* per hectare and will thus require an increase in processing capacity per hectare, and if processing is profitable, will cause an apparent increased in profit which is due simply to rescaling the processing component of the production system. Smith et al., (1986) argue that

gains from rescaling should not be considered as true gain due to genetic improvement, as the gain could have been achieved increasing the size of the business in the absence of any genetic improvement.

The effects of tree size on the profitability of macadamia production and processing was modelled in this study by linking planting density to canopy width at 10 years of age and assuming that trees would maintain a constant production after canopy management of trees were commenced. However, there is no strong data to support this assumption other than the observation that there is only a slight decline in production in very crowd older orchards and in proportion to the level of hedging (McFadyen et al., 2004). Further information is needed to understand the impacts of canopy closure and canopy management on this model.

Manipulating total kernel recovery, canopy width and average rate of yield increase through breeding or management is likely to have the largest impact on profitability. There seems little evidence to suggest that manipulating percentage of reject NIS, percentage of reject kernel, or average grade of marketable whole and half kernel will have much of an impact on the profitability of macadamia production under current cost and pricing structures. However, these are only approximate indicators and do not reflect how easily a change of 10% can be achieved. For example, it has been suggested that percentage of reject kernel could be high as 40% in some cultivars (Jones, 2002), which is over a 1000% increase in this trait with an effect of decreasing PI by 0.56 and would make the enterprise unprofitable.

The value weights and economic weights derived in this study assume a linear relationship between the change in the trait and the effect on the profitability index. The relationship between raw kernel price and average grade of whole and half kernel indicate this is not the case in reality. However, cultivar means for average grade of marketable whole kernel ranges from 15.2 to 18.3 and the relationship with raw kernel price over this range is consistently negative. Several authors (Gibson and Kennedy 1990; Weller 1994; Greaves et al., 1997) have shown that linear approximations to nonlinear relationship are close to optimum.

This is the first study to report economic weights and their application to selection for a horticultural tree crop. Economic weights are important for the evaluation of genotypes that differ in performance across a number of traits (Cotterill and Dean 1990) and predicting response to selection (Finney 1963). Economic weights are important for objective, repeatable and transparent selection decisions.

The value of a trait for selection is determined not only by the value of a unit change in the trait, but also the extent of genetic variation (Cotterill and Dean 1990). For example, precocity did not contribute greatly to the selection index compared to average rate of yield increase and canopy width even though precocity had relatively high value and economic weights. This is the performance data used is based on the regional trial data that indicated little genetic variation for precocity (Hardner et al 2006). This information is confounded by pragmatic decisions by those running these trials on when to harvest. This appears to be based on there being sufficient production to justify collection but the threshold for this is unclear. Hardner et al., (2006) reported a very large interaction between cultivar and site for precocity. The experimental design used in this study make it impossible to separate variation among sites within a growing region from variation among growing regions or between cultivars.

Based on the traits evaluated in this study, significant gains in profitability can be made by selection of superior cultivars. Although there is large standard errors associated with each BLUP predicted value of each trait for each cultivar (Hardner et al. 2006), selection of multiple cultivars will decrease the risk of deploying genotypes with overestimated performance, and not selecting truly superior cultivars

The cultivars that ranked high in this study have been adopted to some extent by the Australian macadamia industry. The trials that were used to estimate the cultivar BLUP deviations used in this study were established in 1986 (Hardner et al., 2006) and results were released from year 8 after establishment. A selection of cultivars began to be planted in the industry and anecdotal knowledge has accumulated since then (O'Hare et al., 2005), although no further quantitative information has been published. The highest ranked cultivar in this study, 849, has been found to suffer from on-tree germination that would increase percentage reject NIS and percentage

reject kernel. Own Venture and 804 are not on the list of widely planted cultivars. There are reports that H814 has very high (40%) levels of kernel immaturity (low oil content leading to undesirable texture and appearance) and A4 suffers from poor kernel appearance and may require more fertiliser inputs. 344 and 246 are still widely planted even though they are ranked 15th and 14th when canopy width is considered and 14th and 10th when canopy width is not considered. The selection index calculated with canopy width excluded assumes all cultivars are planted at 7x5m and are hedged at year 10 irrespective of tree size.

The very high correlation among the selection index value of individual cultivars under the different sensitivity scenarios indicates that the selection of cultivars is robust to variations in the assumptions of the economic model. Similar results have been reported in animal (Smith, 1983).

Of the seven traits measured on the candidate cultivars and used in the selection index, the important determinates of profitability were average rate of yield increase (ary_{10}), canopy width at 10 years (cw_{10}) and total kernel recovery (tkr). These need to further validated to understand the implications for planting distance, canopy management and the importance of precocity outside the constraints imposed by the manner past trials harvested and monitored. The results from this study also suggest there is little gain in assessing cultivars for percentage of marketable whole kernels (pmwk), and average grade of whole (agmw) and half (agmh) kernels under the current assumptions and unless there were massive changes to the relationship between these traits and raw kernel price. Percentage reject NIS and percentage reject kernels were not included in the selection index because they were not measured in the original trial.

Further work on economic weights in macadamia is needed to evaluate the economic value of other important traits and to examine how the economic weights vary with changes to the assumptions of the model. The model presented in this paper provides a strong framework for undertaking this research. Other traits that may be important are: tree structure as it can affect tree longevity; drop pattern as it may affect harvest costs; pest and disease susceptibility; sticktights as these may harbour disease; sources

of reject NIS and kernel; and kernel quality which may affect kernel price (Hardner and McConchie 1998)

These weights were developed for production and processing structure current for 2003 -2005. These will only be relevant for selection from any new regional trials for deployment provided production methods do not change greatly by the time trees begin productive output. However, the outcomes to the industry of selection from a breeding trial of candidate cultivars to be included in a second stage regional variety trial may not be felt for 15-20 years after the decision has been made. As kernel supply increases it is likely markets will become more discriminating for quality that may be defined by visual appearance, shelf life or other sensory traits that affect consumer preference. However, lack of knowledge of the extent of genetic variation for these traits, primarily due to a lack of objective, repeatable and quantitative assessment methods, have not allowed their inclusion in the economic model and hence the selection index.

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References

Borralho, N.M.G., Cotterill, P.P. and P.J. Kanowski, 1993. Breeding objectives for pulp production of *Eucalyptus globulus* under different industrial cost structures. Canadian Journal of Forest Research 23:648-656.

Chilsolm, A.H. and J.L. Dillon, 1968. Discounting and other interest rate procedures in farm management. University of New England Press, Armidale, Australia.

Cotterill, P.P. and C.A Dean, 1990. Successful tree breeding with index selection. CSIRO Publications, Melbourne Australia.

Greaves, B.L., Borralho, N.M.G. and C.A. Raymond, 1997. Assumptions underlying the use of economic weights – are they valid in breeding for eucalypt kraft pulp? Forest Genetics 4:35-42.

Hardner, C.M., Winks, C., Stephenson, R.A. and E.C. Gallagher, 2001. Genetic parameters for nut and kernel traits in macadamia. Euphytica. 117:151-161

Hardner, C. Greaves, B Coverdale, C. and Wegner, M. 2006. Application of economic modelling to support selection decisions in macadamia. In 'Breeding for Success: Diversity in Action' C.F. Mercer (ed.) Proceedings of the 13th Australasian Plant Breeding Conference, Christchurch, New Zealand 18-21st April 2006 pp. 426-431.

Hazel, L. N. (1943). "The genetic basis for constructing selection indexes." Genetics 28: 476-490.

Hinton, A.W., 1996. Growing macadamia nuts on the Atherton Tableland – An Economic Perspective. Queensland Department of Primary Industries, Choices Seminar Series.

Huett, D.O., 2004. Macadamia physiology review: a canopy light response study and literature review. Australian Journal of Agricultural Research 55:609-624.

Ito, P.J., Hamilton, R.A. and H.H. Hirae, 1983. Yield and quality of five major *Macadamia* cultivars in Hawaii. Tropical Agriculture 60:64-65.

Mason, R.L. & I. McConachie, 1994. A hard nut to crack. A review of the Australian macadamia nut industry. Food Australia 46:466-471.

McConchie, C.A., McFadyen, L.M., Meyers, N.M. and D. Huett, 1999. Canopy management for consistent yield in macadamia. Final Report; Project MCC508, Horticultural Research and Development Corporation, Canberra, Australia.

O'Hare, P., Loebel, R. and I. Skinner, 1995. Growing macadamias. Queensland Department of Primary Industries, Queensland.

Ponzoni, R. W. and S. Newman 1989. "Developing breeding objectives for Australian beef cattle production." Animal Production 49: 35-47.

Reilly, T., and P. Bevan, 1995. Profitability of macadamia growing. Australian Macadamia Society New Bulletin, November 1995. Australia Macadamia Society, Lismore.

Stephenson, R.A., Gallagher, E.C., Winks, C.W., and D. Firth, 1995. The breeding, selection, and development of new macadamia cultivars for the Australian industry. Final Report Project MC014, Horticultural Research and Development Corporation, Canberra, Australia.

Stephenson, R.A., Gallagher, E.C. and V.J. Doogan, 1997. Leaf nitrogen as a guide for fertilising macadamia. Australian Journal of Experimental Agriculture 37:599-604.

Trochoulias, T. & G.G. Johns, 1992. Poor response of macadamia (*Macadamia integrifolia* Maiden and Betche) to irrigation in a high rainfall area of sub-tropical Australia. Australian Journal of Experimental Agriculture 32:507-512.

Appendix 6.1. Derivation of relationship between kernel price and average kernel size and proportion of whole and half kernels

The proportion of marketable kernel mass in styles 0, 1, and S was estimated as the proportion of whole and half kernel in each style, i.e.:

$p_t = pw_t + ph_t$

where t = style 0, 1 or S,

$$pw_t = pw.\Pr\left(\left(\frac{\max d_t - dw}{\sqrt{\sigma_{dw}^2}}\right) < U < \left(\frac{\min d_y - dw}{\sqrt{\sigma_{dw}^2}}\right)\right)$$

$$ph_t = ph.\Pr\left(\left(\frac{\max d_t - dh}{\sqrt{\sigma_{dh}^2}}\right) < U < \left(\frac{\min d_t - dh}{\sqrt{\sigma_{dh}^2}}\right)\right)$$

$$\text{if } ph.\Pr\left(\left(\frac{\max d_t - dh}{\sqrt{\sigma_{dh}^2}}\right) < U < \left(\frac{\min d_t - dh}{\sqrt{\sigma_{dh}^2}}\right)\right) \le \left(\frac{1 - \min pw_t}{\min pw_t}\right) * pw_t$$

$$\text{else } ph_t = \left(\frac{1 - \min pw_t}{\min pw_t}\right) * pw_t$$

and maxd_t is the maximum diameter of kernels for style t, mind_t is the minimum diameter of kernels in style t, and U is the standard normal probability distribution.

No style 3 or 4 kernel will be produced under this pricing system because all whole kernels will be allocated to style S as the price is higher for this style.

The proportion of kernel in style 4L is estimated from the proportion of half kernel that could have fitted into styles 0, 1 and S but was in excess of needs as defined by $\left(\frac{1 - \min pw_t}{\min pw_t}\right) * pw_t$.

The proportion of kernel that is in styles 4 and 4s is estimated as:

$$p_t = pw.\Pr\left(\left(\frac{\max d_t - dw}{\sqrt{\sigma_{dw}^2}}\right) < U < \left(\frac{\min d_t - dw}{\sqrt{\sigma_{dw}^2}}\right)\right)$$

$$+ ph.\Pr\left(\left(\frac{\max d_t - dh}{\sqrt{\sigma_{dh}^2}}\right) < U < \left(\frac{\min d_t - dh}{\sqrt{\sigma_{dh}^2}}\right)\right)$$

.

Trait	***Symbol***	***Units***	***base value***	v_y	w_y	$\frac{w_{tkr}}{w_y}$
				Δ PI / Δ 10%	Δ PI/ Δ unit	
Height at planting	h_0	m	1.2	0.0000	0.0003	**102.57**
Height at 10 years	h_{10}	m	6.4	-0.0034	-0.0053	**-6.07**
Canopy width at 10 years	cw_5	m	5	-0.1026	-0.2412	**-0.13**
Age of first crop	*afc*	year	4	-0.0771	-0.1927	**-0.17**
Rate of yield increase	ary_{10}	kg/year	1.7	0.0911	0.5356	**0.06**
Proportion of reject NIS	*prn*	kg NIS/kg NIS	3 %	-0.0071	-0.0235	**-1.38**
Total kernel recovery	*tkr*	kg kernel/kg NIS	33 %	0.1067	0.0323	**1.00**
Proportion of reject kernel	*prk*	kg kernel/kg kernel	3 %	-0.0045	-0.0151	**-2.14**
Proportion of marketable whole kernel	*pmwk*	kg kernel/kg kernel	40 %	0.0117	0.0029	**11.08**
Average grade marketable whole kernel	*agmw*	mm	18	-0.0044	-0.0026	**-12.33**
Average grade marketable half kernel	*agmh*	mm	15		0.004	

Table 6.1. Description of target traits linked to the economic model, including base value, economic weight for the traits calculated as the difference in profitability due to an independent unit change in the level of the trait, and relative economic weight calculated in comparison to the economic weight for kernel recovery

Item	*Total cost*	*Life*
		(years)
Machinery		
Year 0		
70HP 4x4 tractor	$ 45 000	**15**
Small tractor (2nd hand)	$ 8 000	**10**
Utility	$ 15 000	**10**
Slasher (3.6m)	$ 12 000	**20**
Tipping trailer (2)	$ 10 000	**10**
Herbicide applicator	$ 4 000	**20**
Fertiliser spreader	$ 10 000	**20**
Year 4		
Machinery:		
70HP orchard tractor	$ 45 000	**15**
100HP orchard tractor	$ 65 000	**15**
Mulcher	$ 9 000	**20**
Small harvester (nut-naber)	$ 25 000	**20**
Large harvester (Macmaster finger wheel)	$ 100 000	**20**
Air blast sprayer	$ 20 000	**15**
Infrastructure		
Year 0		
Shed	$ 25 000	> **20**
Sundry tools	$ 5 000	**5**
Year 4		
Dehusking plant (inc. dehusker, 2*sorting tables, 2*water sorter, hopper, 3*elevators, tromel)	$ 34 000	**20**
Power (3 phase)	$ 20 000	
6* Silos 40 (inc. fan, elevator etc.)	$ 150 000	**20**
Installation	$ 30 675	

Table 6.2. Machinery and infrastructure requirements for model farm

Input		*unit*	*unit/tree*	*$ unit*	*P*	*Tractor type*	*Tractor speed*	*N*	*notes*
Fertiliser		kg	see Table 3	$ 0.47	2	70HP	3.0	3	
Foliar spray	zinc heptahydrate	g	3.7	$ 0.90	-	-	-	1	**1**
	solubor	g	3.7	$ 3.00	-	-	-	2	**1**
Pesticide	Endosulfan	ml	0.16*V	$ 8.40	2	100HP	1.8	2	**2**
	beta-cyflurin	ml	0.04*V	$ 35.00	2	100HP	1.8	3	**2**
	carbendazim	ml	0.015*V	$ 12.80	-	-	-	1.5	**1,2**
	copper oxychloride	ml	0.03*V	$ 3.90	-	-	-	3	**1,2**
Herbicide	glyphosate	L	0.5/Nt	$ 7.00	2	small	3.0	see text	
Slashing		-	-	-	see Table 4	70HP	see Table 4	see Table 4	
Mulching		-	-	-	2	70HP	3.0	4	
Skirting		-	-	-	2	-	see text	1	**3**
Harvesting	Nut naber	-	-	-	2	70HP	2.5	see text	
	Mac master	-	-	-	**2**	**100HP**	**2.5**	**see text**	

Notes : 1. Combined with insecticides for application.

2. V = canopy volume (m^3).

3. contractors employed for application.

Table 6.3. Summary of costs per tree for dependent inputs in the model farm. Shown for each input are: units of the input, application rate (unit/tree), cost per unit; number of tractor passes per row (P), tractor type, tractor speed (km/hr), and number of applications per year (N).

Age (years)	*Applications per year*	*Passes per row*	*Tractor speed (km per hour)*
1-4	6	3	2.5
5	6	2	3.5
6	5	2	3.5
7	4	1	3.5
8+	3	1	3.5

Table 6.4. Number of slashing applications per year, passes per row and tractor speed for slashing by orchard age.

Style	*max d (mm)*	*min d (mm)*	*max % w*	*min % w*	*$/kg*
S0		20	100	95	$ 15.50
S1	20	17	100	95	$ 15.00
SS	17	13	100	90	$ 15.50
S2	17	13	90	50	$ 13.50
S3	17	13	30	15	$ 13.00
S4L		13	100	0	$ 12.50
S4	13	9	100	0	$ 12.50
S4s	9	7	100	0	$ 12.50
pieces	-	-	-	-	$ 13.50

Table 6.5 Definition of kernel styles and wholesale price per kg

Cultivar	*afc*	ary_{10}	cw_{10}	*tkr*	*pmwk*	*agmw*	*agmh*	*H*
849	-0.1	0.1	-0.1	6.21	8.98	0.9	0.9	0.32
Own Venture	-0.1	0.3	-0.2	-0.14	7.06	1.1	1.1	0.28
814	-0.1	0.4	-0.2	-0.04	-10.06	-0.7	-0.7	0.23
A4	-0.1	-0.5	-0.8	8.03	0.08	1.4	1.4	0.22
804	0.2	0.2	0.2	4.39	1.04	0.2	0.2	0.18
X18	-0.1	0.1	0.0	2.14	13.35	-0.3	-0.3	0.16
X7	-0.1	-0.7	-0.5	10.43	11.77	-0.7	-0.7	0.15
842	0.0	0.2	0.2	1.91	6.24	-0.4	-0.4	0.15
Daddow	0.0	0.3	-0.2	-0.51	-4.43	-0.3	-0.2	0.15
A16	0.1	0.0	-0.3	2.63	4.51	0.2	0.2	0.15
816	0.0	-0.1	0.0	4.91	4.38	1.3	1.2	0.13
741	0.1	0.2	0.0	0.82	-3.88	0.2	0.1	0.11
783	0.1	0.0	-0.2	1.04	7.47	0.1	0.1	0.07
660	-0.1	0.0	-0.1	0.41	-1.69	-0.6	-0.6	0.07
344	0.0	0.4	0.1	-4.13	-5.94	-0.3	-0.3	0.05
705	0.0	-0.1	-0.5	-1.56	-1.87	0.4	0.3	0.05
762	0.3	0.3	-0.2	-6.05	19.37	-0.3	-0.3	0.03
772	-0.1	0.0	-0.4	-2.64	6.51	0.3	0.3	0.03
815	0.0	-0.1	-0.1	0.80	6.36	0.3	0.3	0.03
X4	-0.1	-0.4	-0.2	3.88	-1.42	0.2	0.2	-0.01
X8	0.1	-0.2	-0.6	0.76	-13.60	0.5	0.5	-0.01
795	0.3	-0.2	-0.8	-1.52	-1.97	0.6	0.6	-0.01
835	0.0	0.2	0.5	-1.25	21.43	-0.3	-0.3	-0.01
791	-0.1	-0.3	-0.7	-0.60	-6.35	0.0	0.0	-0.03
294	0.0	0.0	0.0	-1.01	0.08	0.3	0.3	-0.04
X13	0.2	0.2	0.2	-2.22	1.87	-0.7	-0.7	-0.04
797	-0.1	0.1	0.0	-2.07	-10.72	-1.1	-1.1	-0.04
NRG43	-0.1	0.0	-0.1	-2.70	-1.33	0.0	0.0	-0.05
794	0.0	0.2	0.1	-2.32	-15.10	-1.1	-1.1	-0.06
246	-0.1	0.3	0.7	-2.84	-2.15	-0.1	-0.1	-0.06
781	0.0	0.3	1.0	-0.07	4.74	0.5	0.5	-0.08
836	0.0	-0.2	-0.1	-1.21	-0.92	-0.5	-0.5	-0.09
789	0.0	-0.1	0.4	1.64	-5.25	-0.6	-0.6	-0.12
828	0.0	-0.1	0.8	1.97	-1.82	0.2	0.2	-0.18
800	0.0	0.1	0.7	-1.82	-5.03	0.2	0.2	-0.21
837	-0.1	-0.1	0.6	0.75	-16.20	1.2	1.1	-0.22
807	0.1	-0.2	0.2	-1.46	-9.21	-0.6	-0.5	-0.25
Seedling51	0.0	-0.4	-0.2	-2.80	-1.15	1.1	1.0	-0.27
Release	0.0	-0.2	-0.2	-6.89	-3.61	-0.9	-0.9	-0.29
790	0.1	0.1	0.7	-6.84	-1.56	-1.5	-1.5	-0.40

Table 6.6. Best linear predicted means for precocity (*afc*), rate of yield increase between precocity and year 10 ($ary_{p:10}$), canopy width at year 10 (cw_{10}), total kernel recovery (*tkr*), proportion of marketable whole kernel (*pmwk*), average size grade of marketable whole kernel (*agmw*), and average size grade of marketable half kernel (*agmh*), and index value calculated using economic weights derived from base economic model for 40 cultivars evaluated over 2 sites.

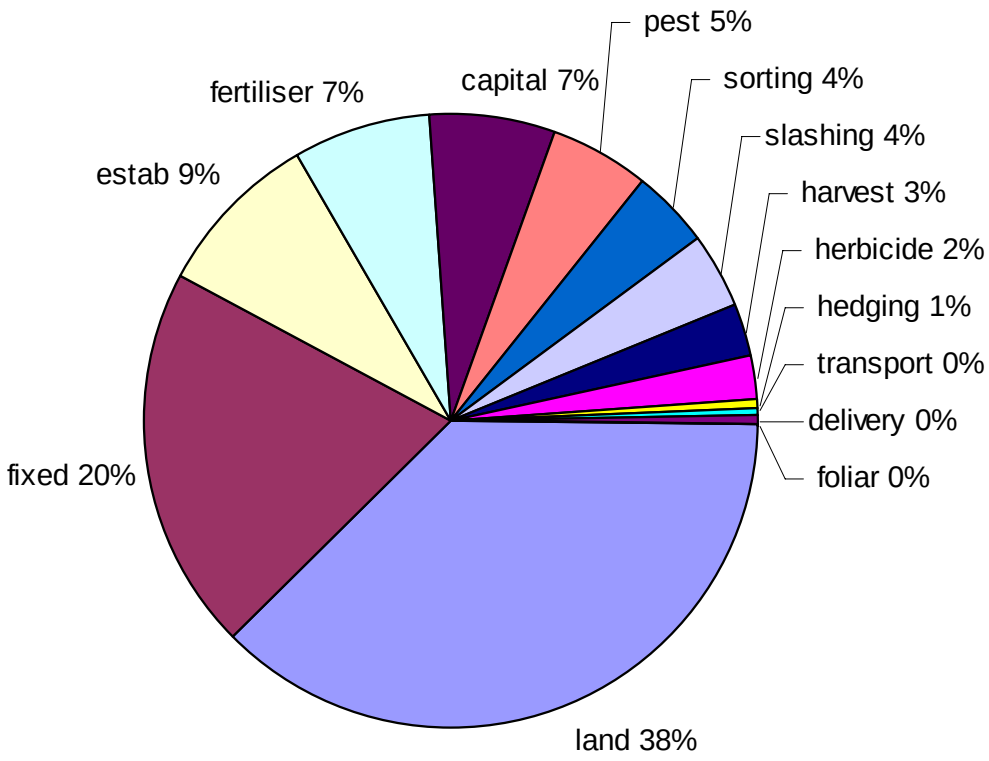


Figure 6.1. Proportion of net present value of production costs for different sources.

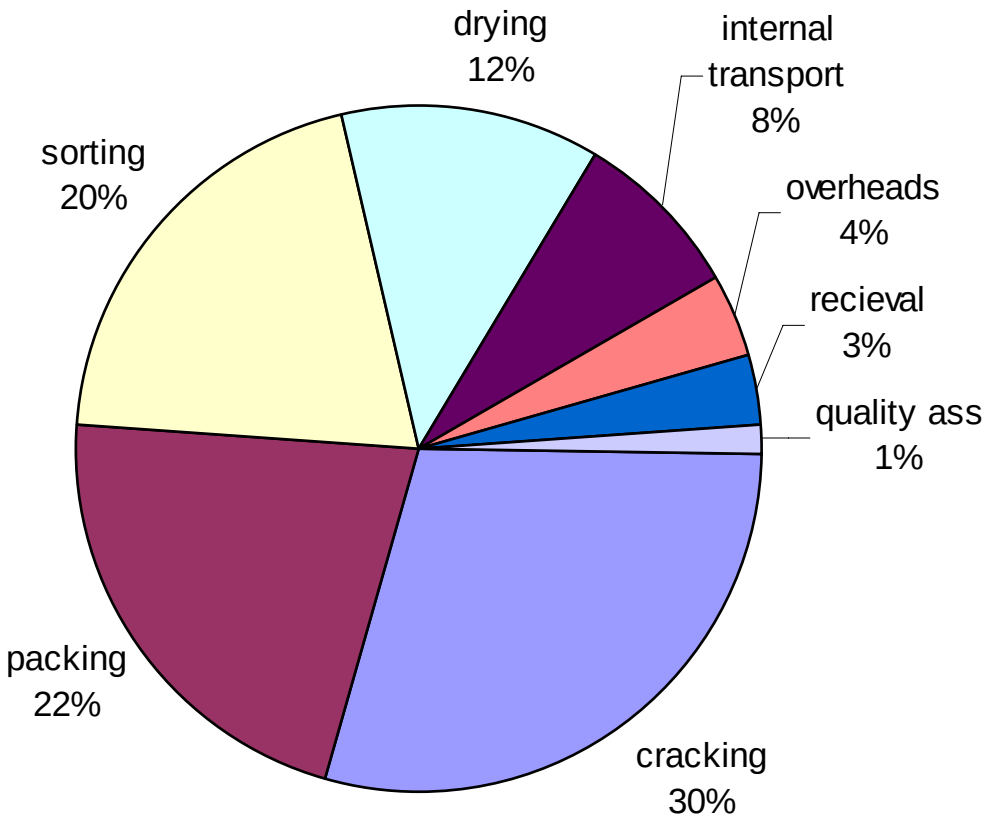


Figure 6.2. Proportion of net present value of processing costs for different sources

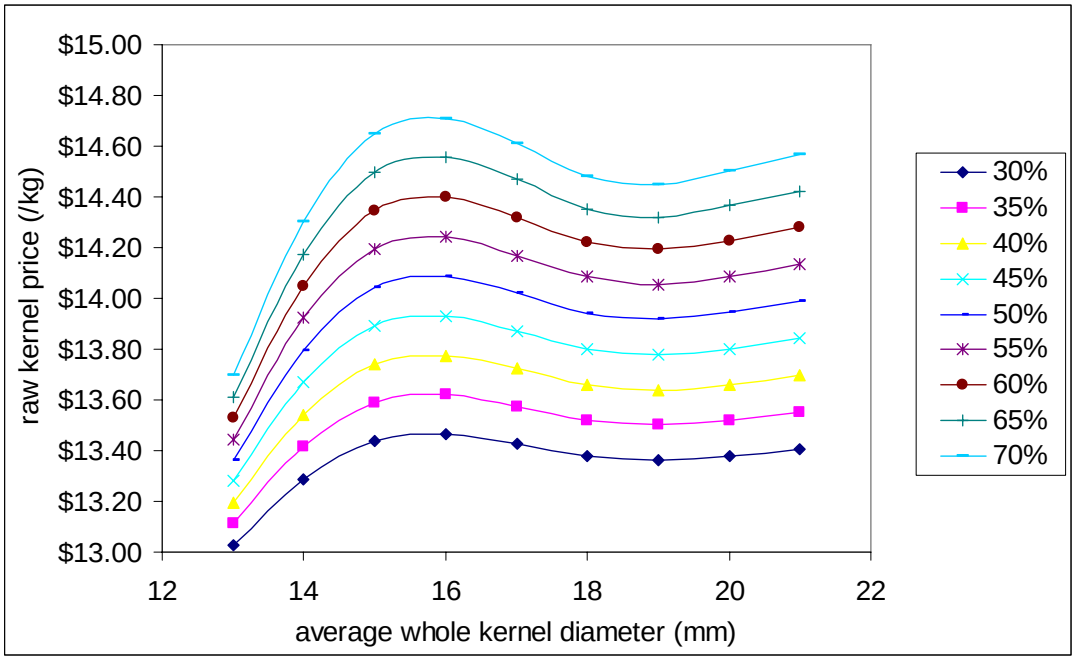


Figure 6.3a Relationship between average whole kernel size grade and raw kernel price for different levels of proportion marketable whole kernel

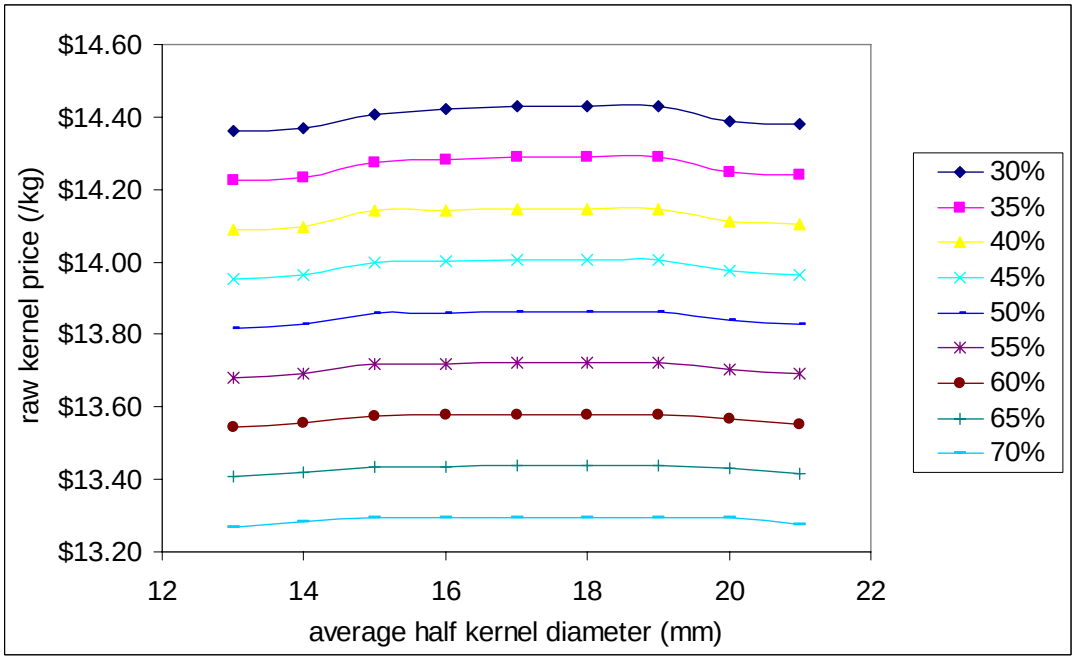


Figure 6.3b Relationship between raw kernel price and average half kernel size grade for different levels of proportion marketable whole kernel (currently proportion marketable half kernel)

CHAPTER 7
Objective assessment and valuation of raw macadamia kernel quality

Abstract

A sensory panel has been used to assess a selection of defects included in the Australian macadamia industry product description manual and to grade kernel from 8 commercial cultivars. Selected samples of the kernel used in these investigations were also examined by processors to determine the effects of the severity of a disorder on kernel value. The panel initially developed a continuous consensus scale of defect severity for the 7 disorders using a biased selection of kernel enriched for these defects. An assessment for overall kernel colour was also developed. Whole and half kernel from 8 cultivars were then examined by members of a trained sensory panel for 7 kernel defects and overall kernel colour and significant differences were found between cultivars for all disorders. This panel also assessed a prepared sample containing all levels of defect proposed in the industry product description manual and generally found a linear relationship between the panel continuous scale and industry rankings. This indicated that the assessors classified the disorders in the same manners as the industry though this relationship was unreliable in some of the higher severity levels of some defects The most notable exception was shrivelled kernel suggesting a new class of defect may need to be developed. An instrumental based measurement was made of each kernel for comparison with panel assessments. A sample of the graded kernel were then examined by commercial processors for an individual kernel value.

Significant difference were found between cultivars for Basal discolouration, discoursed rings, shrivelled kernel, overall colour and internal discolouration. There were no significant differences among cultivars for suture lines, discoloured crests and pitted centres. There were no significant main effects of kernel type (whole or half), but there was significant interactions between assessor and type for discoloured rings and overall colour suggesting kernel type affected how assessors scored the disorders. The major effect on kernel market value shown in the analysis of variance of the biased disorders samples was level the of disorder).There were also significance differences between regions of processor for the value of kernel for discoloured rings,

discoloured crests and pitted centres. Significant interactions between processors and levels for discoloured rings, internal discolouration, suture lines, discoloured crests and pitted centres suggesting some processors valued kernel differently for these traits. Plots of kernel value against panel score demonstrated a decrease in kernel value with an increase in panel score for all traits except shrivelled kernel. Implications of these results for kernel assessment and application in the improvement program are discussed.

Introduction

Kernel assessment has been used in macadamias to quantify responses to agronomic treatments (Stephenson et al., 2002, 2003), identify elite cultivars (Leverington., 1962; Stephenson et al., 1995; Hardner et al., 2002) and measure kernel quality as part of commercial transaction between growers and processors (Mason 1982, Atkinson 1991, Evans and Hofman, 2006). The criteria used to perform these activities have gradually been refined as knowledge of the product has developed. The initial criteria used by Leverington (1962) to select cultivars for Australian conditions were based on criteria previous used Ripperton et al. (1938) in Hawaii and included oil content based on specific gravity, nut shape and roasting while mould and insect damage were considered agronomic problems. The need for product specifications resulted in the inclusion further descriptions of kernel shape, evidence of germination, discoloured bases (Mason, 1982). The industry develop drying and sampling protocols and further expanded the criteria for unsound to include shrivelled, a range of form of kernel discolouration and rancidity (Atkinson, 1991; O'Hare et al., 1995).Continuing concern about commercial assessment procedures resulted in a commissioned review of the process that made a series of recommendations and identified the need for automated sampling, a general increase in sample size for assessment and a training and accreditation (Mengerson, 2000). The publication of Evans and Hofman (2006) addressed many of these concerns and provided a basis for training assessment staff. This work also introduced the concept of severity levels for the kernel defects that has not been universally been adopted across the Australian industry.

This system for commercial evaluation of kernel quality sorts individual kernels into one of 43 possible disorder categories (No disorder present or 3 levels of 14 possible disorders) (Table 7.1). However, this protocol does not enable assessment of individual traits or produce results that are highly repeatable, the use of a 4 point scale is not highly accurate unless a large number of kernel are used.

Application of these methods in the current improvement program have demonstrated the large bias that occurs when commercial kernel assessment methods are followed which place each kernel in a single disorder category, irrespective of the level of other disorders. The main bias that arises is due to assessors choosing priorities for the disorders. This means that all disorders should be assessed independently of other disorders

Kernel quality is has been identified as a priority for selection in the breeding program. Repeatable and accurate assessment of component traits that affect commercial kernel quality are required to maximise gain from breeding.

This study was undertaken to

(i) develop a continuos scale for assessment of 7 disorders affecting commercial kernel quality by a trained sensory panel,

(ii) evaluate this scale against a range of kernels,

(iii) investigate how colour meter readings predict trained panel assessments, and

(iv) determine how the level of a disorder changes the commercial value of a kernel.

Materials and Methods

In discussion with the industry committee for varietal improvement (MIVIC) 7 commercially important kernel traits were selected for evaluation that were considered to be under genetic control. These were (i) Basal discolouration (BD), (ii) Discoloured rings (DR), (iii) Shrivelled kernel (SK), (iv) Internal discolouration (ID), (v) Discoloured crest (DC), (vi) Suture lines (SL), and (vii) Pitted centres (PC).

A sample containing kernels with a range of severities for each disorder was used by the trained panel to develop a consensus continuous scale for each industry defined disorder and overall colour (OC). A panel of 8, experienced assessors (screened and trained in line with international guidelines), underwent 5 training days to familiarise them with the traits under investigation, to standardise the protocol for assessment and to align the industry terms with what they perceived to be the key differences for each attribute

Since some severity levels were rare a biased set of 24 kernel was created for each of the 7 kernel disorders (168 kernel in total) to evaluate the performance of the trained

panel. For each disorder, a sample of kernel was sorted into the 4 levels by a leading industry kernel assessor, irrespective of the presence of any other disorder. Six kernels were then selected from the kernel of each level for each disorder. Three whole and 3 half kernels were selected for the disorders BD, DR, SK and ID. Only whole kernels were selected for DC and SL and only half kernel were selected for PC. A third set of 120 kernel was created by randomly selecting 8 whole kernel and 7 half kernel from 8 cultivars (A16, A38, 246, 344, 741, 816, 842, 849) randomly selected from a sample of kernel for each cultivar that had been collected from a QDPI&F regional variety trial in Bundaberg, Queensland and pooled across the 4 replicates of individual trees of each cultivar in the 4 replicated blocks at this site.

The reflective colour of the surface of the raw kernel was measured using a Minolta® Chroma Meter CR-300 (Minolta, Japan). The instrument is a tristimulus colorimeter which measures four specific wavelengths in the visible range, specified by the Commission Internationale de l'Esclairage (CIE). Tristimulus values are the amounts of the three primary colours (blue, green and red) that specify a colour stimulus, thereby creating a three-dimensional value for colour. The L^*, a^*, and b^* values are the three dimensions where L^* (light-dark spectrum) is the lightness variable, a^* (red-green spectrum) and b^* values (blue-yellow spectrum) are the chromaticity coordinates. A single measure was taken on the top and bottom of each cotyledon, so that there were only 4 measurements for whole kernel and 2 measurements for half kernel.

Three high resolution digital images were taken of each kernel. These were taken from the top (crest), base (rounded) and the side showing the junction of the cotyledons.

Each member of the trained panel assessed each kernel in the evaluation sample for each of the 7 disorders and overall colour over 4 evaluation days. On each day the panel visually assessed 8 trays of 9 kernels (randomly divided between trait standards and cultivar kernels)

The evaluation sample of 288 kernels was restructured into 6 collections of 48 kernel. Each collection was surveyed by 6 processors to allocate a value to the kernel by classifying the kernel in terms of commercial category. Some processors used premium, commercial, oil stock and reject, and some used only sound (premium) and

unsound (reject). A value of $15 per kg was allocated to kernel classified as premium, $13 for commercial, $3 for oil stock, and 0$ for reject.

Statistical design and analysis

Statistical analyses were carried out using non-orthogonal analysis of variance to incorporate all the blocking factors included in the experimental design and interactions between assessors and families. The statistical package GenStat (2008) was used for all analyses.

Results

The trained panel assessment of the set 24 kernel for each kernel sample was analysed separately. Early in the analysis it became evident that one assessor was unable to classify a large number of kernels for some most of the disorders. This assessor was excluded from further analysis. Only half kernel was examined for internal discolouration as preliminary analyses indicated that there was a large difference between the assessment of this disorder on whole and half kernels.

Effects of assessor and disorder level

The analysis of variance of the panel scores for the disorders (Table 7.2) shows that there were significant effects of severity level on panel scores for all disorders. These differences were generally large except for SK confirming that the panel could detect differences between kernels for the different disorders. The effect of type (whole or half kernel) was only significant for BD but there were significant interactions with assessor for both BD and SK. This suggests that assessors may assess disorders differently depending on whether they are examining a whole or half kernel. However the variance ratio was much lower than the main effect of level suggesting the interaction may not confound greatly the assessment of these disorders. There was also a large effect of assessors on panel score for SK but the variance ratio for assessor was larger than the effect of level suggesting differences between assessors had a greater influence on panel score than differences among kernels. There was a significant interaction between level and assessor for BD, DR, and DC but again the variance ration for this effect was much lower than the main effect of level.

The panel score increased with industry disorder level for BD, DC and PC (Figs 7.1-7.3). The panel were unable to distinguish industry level 1and 2 for DR (Fig 7.4), and

levels 2 and 3 for ID and SL (Figs 7.5and 7.6). This generally indicates that the assessors were classifying the disorders in the same manners as the industry though this relationship was unreliable in some of the higher severity levels of some defects. The measure for SK differed from all the other disorders in that the panel score did not incrementally increase with the industry assessment (Fig.7.7) and appeared to reduce from levels 2-3. The contradiction between panel score and SK level suggests that the assessors were not assessing the disorder that the industry described as shrivelled in the same manner as the industry.

Cultivar differences and panel assessment

Cultivar means were predicted using 8 whole and 7 half kernel for BD, DR and SK and OC, only for the 8 whole kernel for DC and SL, and 7 half kernel for PC and ID. All kernels were collected from a single site within a single year and therefore cannot be used to predict industry trends. The average of adjacent levels (ie. 0/1; 1/2; 2/3) were used to estimate the value of the boundary between disorder levels defined by the industry

The analysis of variance of the panel scores for the 8 cultivars (Table 7.3) shows that significant difference between cultivars in DB, DR, SK, OC and ID. There were no significant differences among cultivars for SL, DC and PC. There were no significant main effects of kernel type (whole or half), but there was significant interactions between assessor and type for DR and OC suggesting kernel type affected how assessors scored the disorders. The greatest cultivar separation occurred with means for BD (Fig. 7.8) and similar numbers of groupings for DR, ID and OC (Figs 7.9-7.11). The separation for SK was only into two groups (Fig 7.12). All cultivar means for DR, SK and ID were in the area where the disorder would be not scored by the industry (level 0) while the mean value for DB would cause the kernel to be commercial or reject for some of the cultivars.

The major effect on kernel market value shown in the analysis of variance of the biased disorders samples was level the of disorder (Table 7.4).There were also significance differences between regions of processor (QLD vs NNSW) for the value of kernel for DR, DC and PC indicating kernels with these disorders were less valuable to processors in one region. There were small but significant differences between in kernel value for the interaction between region and processor for the value of kernel with ID and SL but the variance ratios indicated that this was not a large as

the main effect of level. There was a significant interaction between processors and levels for DR, ID, SL, DC, and PC suggesting some processors valued kernel differently for these traits. Plots of kernel value against panel score (Figs 7.13- 7.18) demonstrated a decrease in kernel value with an increase in panel score for all traits except SK (Fig 7.19). It appears this relationship could be approximated by a linear function but the implications on the precision of these estimates need further investigation.

The only significant effect on market value of kernels in the cultivar sample was the region of the processor (Table 7.5.). Processors in one region valuing the sample as $13.65 /kg and while processors in the other region valuing the kernel as $14.34 /kg. There were no significant differences in value detected among cultivars

There were large significant effects of level of disorder on L^* value for BD, DR, and ID, and a^* for DC (Table 7.6). There were no strong effects of level of b^* for any disorder. There were non-linear incremental relationships between L^* values and panel scores for BD and DR (Figs 7.20 & 7.21), although the L^* values may be useful at setting decision points for internal discolouration (Fig.7.22). There were significant differences in all L^*, a^* and b^* values among the cultivars but most of the effect was due to the different surfaces being measured (Table 7.7.). There was a small but significant interaction between surface and cultivar.

Discussion

These results indicate that a trained panel was able to assess most disorders in the same manner as a leading industry assessor and detect differences between cultivars using a restricted sample of kernel. Based on these results combined with commercial valuations relationships between kernel value and panel score, and level of disorder have been developed. This information could be further developed to evaluate the economic impact when used for selection within the macadamia improvement program but will need to be supplemented by information about the distribution of these defects in experimental populations. The exception to these generalisations in the commercial cultivars examined was shrivelled kernel where further refinement of the descriptors and possibly the development of a new category of defect maybe required.

The aim of the assessment protocol being studied was to predict the trait, in this case a kernel defect in a cultivar defined by its mean and variance. This differs from the

current commercial categorical system that aims to estimate the proportion of a consignment based on a sample in each category from which an overall consignment value is determined.

Theoretically analyses using continuous scales are more powerful for detecting real differences between treatments compared to differences that are due to random variation not related to the treatments. Statistical methodology is well developed for handling continuous data. While statistical methodology exists for analysis of categorical data, it is not as flexible more kernels would be assessed to detect significant differences or the same level of accuracy. A categorical scale may appear more appealing for assessment than a continuous scale as the categories may be conceptually easier to define. Also estimation of a category may be faster than estimation of a point on a scale.

As there are fewer points on a categorical scale compared to a continuous scale, it would be expected more kernel are required for assessment using a categorical scale to achieve the same level of accuracy. However in the current study mid points of the relationship between the panel score and the industry levels appear close to linear for industry level 0-2 for most defects. This suggests there is little difference in between the categorical and continuous scale. The exception was SK. It is suggested that a new disorder be developed, deformed kernel, to accommodate the kernel appearance not covered by shrivelled kernel for which the panel used plump grape to raisin as anchors to describe this kernel appearance. Reduced oil content is thought to be the major cause of shrivelled and deformed kernels in macadamia. However, it has also been suggested that mildly shrivelled kernel may be due imprinting by the internal surface of the shell. While low oil content kernel is thought to be hard and unacceptable to consumers the surface defects due to imprinting may not be perceived by consumers if kernel is used in applications where the surface is obscured.

The variation among assessors demonstrated in this study for the different disorders indicates that more than one assessor is required to accurately estimate the population average for a disorder. The experience has also demonstrated that some assessors are not as accurate as other assessors. Preliminary analyses not reported here indicated that results from the panel scores from 5 assessors were as accurate as from the 7 included in the full analysis. However for the efficient implementation of this protocol

the number of assessors and the training requirements for these assessors needs to be defined. The ability of assessors to repeatability give the same score for the same disorder in a specific kernel has not been examined in these investigations and would provide a measure of the accuracy of assessors. The significant effect in the order that kernel were examined for BD suggests that assessment of this defect could be less accurate than others.

Use of Wholes and halves

Even before commencing this study it was evident that SL and DC could only be assessed on whole kernel as these defects occur at the junction of the two cotyledons that make up the kernel. Our results indicate that this is not universal amongst assessors as some individuals were able to detect severe levels of these disorders on half kernels. This was not the case for pitted centres (PC) that can only be assessed on half kernels.

This study has demonstrated that the assessment of ID differs between whole and half kernels. This may be caused by the severity of the discolouration, proximity to the surface of the kernel and opacity of the kernel. The severity of discolouration is influence by the drying regime (McConchie and Macpherson, 2008) and could affected by the level of reactive components that produce this response in the kernel. It is suggested that only half kernel should be used to assess ID as the assessment on whole kernel is likely to under estimate the level of ID.

The lack of a significant interaction between level and kernel type whole or half for DR suggests that either kernel type can be used for the assessment of this trait. Similarly, there is a significant interaction between level and kernel type for BD, the size of this effect relative to the main effect of level suggests that that either kernel type could be used for assessment of this trait. If only whole kernel is used the estimation of the extent of BD can be expected to be higher.

Cultivar samples

Estimates of cultivar means in this study are from a limited number of kernels, collected from a small number of replicates at a single site, and processed using industry protocols that would not be expected in commercially batches of kernels. The ranking of cultivars in this study may not reflect industry performance and the results were not intended for use in selection of cultivars for commercial use.

The kernels of the cultivar sample appear to be of high quality with very low levels of disorder detected. In addition, the distribution of the disorders has been approximated by a normal distribution which does not account for a long tail. The analyses would underestimate the extent of severe occurrences of the disorders.

While significant differences among cultivars were found, particularly for DR and BD, more than the 15 kernel may be required to detect smaller differences between genotypes and quantify the level of rare kernels exhibiting extreme defects. It may be that the differences between cultivars would be better assessed by monitoring responses to challenges that could be associated with mishandling of nut-in-shell as has been suggested for brown centred kernel (Le Lagadec pers comm). Further investigations are required to determine whether there is a genetic component associated kernel damage resulting from poor management.

This study has developed a relationship between current industry kernel value and panel score, and level of disorder, for all disorders except SK. This could be used to evaluate the economic impact of selecting amongst genotypes for differences in kernel disorders for the kernel cost structure that existed in 2006. While the value of kernel has changed dramatically since this investigation these relationships may provide a baseline for modifying the relationship between kernel value and disorders affecting kernel quality.

Analysing the individual colour components suggests that L^*, a^* and b^* were unable to provide an absolute value useful for assessing kernels for the disorders examined here, except for ID. Although there are significant differences among cultivars for L^*, a^* and b^*, these value could be combined in a similar manner as (McConchie, 2006) to monitoring the responses to roasting macadamia kernel. The Chroma meter only samples a limited area and methods that analyse images of the entire kernel in a resembling visual inspection such as being developed by Bell (2006) appear to have promise. Chroma meter values of the kernel surface appeared to be a good predictor of the severity of internal discolouration. Further study is required to evaluate the accuracy and sample number needed for implementing colour measurements of the internal surface of half kernels to predict the severity of this disorder within a sample. In a similar way that kernel colour measurement can be simplified it maybe possible to perform a multi-trait analyses to determine the main disorders driving variation in kernel value so that resources are directed to making key assessments.

A primary determinant for sampling design whether for commercial assessment or genetic improvement is knowledge of the distribution of the defect in the population. Quantitative information is not available for macadamia; however, industry experience suggests that most traits have a skewed distribution with rare extreme values. In this case, approximation to a continuous normal distribution will underestimate the frequency of extreme expressions of a disorder.

The assessment system studied in the current investigations used for a limited number of kernel defects in commercial cultivars from a single site in one year. There are several other defects that remain to be considered including streaks and lines, mould or pink staining, insect damage, open micropiles, adhered skin, and rancidity (Evans and Hofman, 2006). However development of improved cultivars will in part be dependent the degree to which these defect are under genetic control.

There would appear to be several alternatives for the assessment scales: (i) continuous scale of panel scores; (ii) continuous scale from 0 to 3 with anchors defined by the margins between the current industry defined levels; (iii) multiple categorical scale based on levels defined by the industry (Current AMS system); or (iv) threshold score such as a colour measure, particular for ID. An evaluation of alternative assessment scales using information collected on distribution of the disorder, strength of genetic control and relationship between kernel value and severity of disorder, needs to be undertaken to balance accuracy, assessment cost and value of changing a trait. Even with these an optimised assessment system there may be the need for the development of a robust reference system to ensure that results in one year are comparable in subsequent seasons.

References

Atkinson, B. 1991. Recommended standards for sampling and nut-in-shell evaluation. Australian Macadamia Society News Bulletin 17(6):46-52

Bell, D. 2006. Breeding for kernel quality. Proceedings of the Australian Macadamia Society conference 2006. p101-106.

Evans, R. and Hofman, P. 2005. Product description manual Australian macadamia kernel. Australian Macadamia Society, Lismore. pp 41.

GenStat (2008). 11th edition, VSN International, Hemel Hempstead, U.K.

Hardner, C., Winks,C., Stephenson, R. and Gallagher, E. 2001.Genetic parameters for nut and kernel traits in macadamia. Euphytica 117: 151–161.

Hardner, C. M. Winks, C.W. Stephenson, R. A. Gallagher, E. G. and McConchie, C. A. 2002. Genetic parameters for yield in macadamia. Euphytica 225: 255-264.

Leverington, R.E. 1962. Evaluation of macadamia varieties for processing. Queensland Journal of Agricultural Science. 19: 33-46

Mason, R.L. 1982. Recommendations for the commercial processing of macadamia nuts. Australian Macadamia Society News Bulletin 9(11): 4-6.

Mengerson, K. 2000. Statistical review of nut in shell sampling and kernel assessment procedures. Proceedings Australian Macadamia conference 2000. Pp 30-33.

McConchie, C. A. 2006 Control of after roast darkening in macadamia MC01008 Horticulture Australia Final Report pp.288.

McConchie, C and Macpherson, K. 2008 After roast darkening does not require roasting. AMS News Bulletin 35 (2) 46-48.

O'Hare, P., Loebel, R. and I. Skinner, 1995. Growing macadamias. Queensland Department of Primary Industries, Queensland.

Ripperton, J. C. Moltzau, R.H. Edwards, D. W. (1938) Methods for evaluating the macadamia for commercial use and the variation occurring among seedlings in Hawaii. Washington DC. US Department of Agriculture Hawaii Agricultural Experimental Station Bulletin 79.

Stephenson, R.A., Gallagher, E.C.,Winks, C.W. and Firth, D. (1995).The Breeding, Selection, and Development of New Macadamia Cultivars for the Australian Industry. Final Report Project MC014. Horticultural Research and Development Corporation, Canberra, Australia

Stephenson, R. A. Gallagher, E. C. and Gogel, B. J. 2002. Macadamia nut size and maturity influenced by lime and nitrogen applications. Australian Journal of Agricultural Research 53: 677-680.

Stephenson, R.A. Gallagher, E.C. and Doogan, V.J. 2003. Macadamia responses to mild water stress at different phonological stages. Australian Journal of Agricultural research 54: 67-75.

Disorder	***Severity 1***	***Severity 2***	***Severity 3***
Shrivelled	Premium	Commercial	**Reject**
Basal Discolouration	Premium	Commercial	**Reject**
Suture lines	Premium	Commercial	**Reject**
Discoloured crest	Premium	Commercial	**Reject**
Discoloured rings	Premium	Commercial	**Reject**
Adhered Skin	Commercial	Commercial	**Reject**
Streaks and Lines Shell marks	Commercial	Commercial	**Reject**
Pitted Centre	Commercial	Reject	**Reject**
Open Micropile	Reject	Reject	**Reject**
Mould	Reject	Reject	**Reject**
Internal Discolouration	Reject	Reject	**Reject**
Pink staining	Reject	Reject	**Reject**
Insect	Reject	Reject	**Reject**
Rancidity	**Reject**	**Reject**	**Reject**

Table 7.1. The proposed classification kernel quality of Evans and Hofman (2005) matched to the processor grades. Note some processors do not have a commercial grade and the kernel in these categories are treated as reject. Kernel with 0 level of defect is premium for all disorders.

Table 7.2. Analysis of variance of panel score for 7 kernel disorders. BDw&h = Basal Discolouration whole and half kernel, DRw&h= Discoloured Rings whole and half kernel, SKw&h= Shrivelled whole and half Kernel, SL w= Suture Lines in whole kernel, DC w= Discoloured Crest in whole kernel, IDh = Internal Discolouration in half kernel, PCh= Pitted Centres in half kernel. F= F prob, df= degrees of freedom, vr= variance ration and Sig = Level of Significance

Source	***BDw&h***				***DRw&h***			***SKw&h***			***SLw***				***DCw***			***IDh***			***PCh***	
	d.f.	vr	F		vr	F		vr	F		d.f.	vr	sig		vr	sig		vr	sig		vr	**sig**
Kernel stratum																						
Day	3	1.8	0.192		0.6	0.603		0.1	0.951		3	0.9	0.477		0.3	0.796		0.9	0.477		0.3	**0.831**
day.session	4	0.8	0.540		0.1	0.967		0.3	0.867		4	0.5	0.715		0.2	0.942		0.5	0.715		0.1	**0.995**
Order	3	8.4	0.001		0.1	0.936		0.1	0.930		3	2.4	0.144		0.3	0.805		2.4	0.144		0.0	**0.993**
Type	1	16.2	0.001		0.5	0.495		2.0	0.174													
Level	3	152.0	0.000		32.1	0.000		8.5	0.001		3	32.8	0.000		20.5	0.000		32.8	0.000		11.7	**0.000**
type.level	3	11.2	0.000		2.2	0.129		1.4	0.271													
Kernel	16										8											
Assessor	6	22.3	0.000		7.2	0.000		16.1	0.000		6	5.3	0.000		8.5	0.000		5.3	0.000		12.1	**0.000**
type.assessor	6	3.9	0.002		1.6	0.153		3.1	0.008													
level.assessor	18	3.0	0.000		3.7	0.000		1.2	0.274		18	1.8	0.072		1.7	0.046		1.8	0.072		1.0	**0.451**
order.assessor	18	0.8	0.716		2.7	0.001		1.0	0.464													
Residual	86										38											

Table 7.3. Analysis of variance of panel score for 8 cultivar samples. BD = Basal Discolouration whole and half kernel, DR= Discoloured rings whole and half kernel, SK= shrivelled whole and half kernel, OC= overall colour in whole and half kernel SL = suture lines in whole kernel, DC= Discoloured crest in whole kernel, ID = internal discolouration in half kernel, PC= pitted centres in half kernel. F= F prob, df= degrees of freedom, vr= variance ration and Sig = Level of Significance

Source	w&h	BD		DR		SK		OC		w	SL		w	DC		h	ID		h	PC	
	df	vr	F	vr	F	vr	F	vr	F	df	vr	sig		vr	sig	df	vr	sig		vr	sig
kernel stratum																					
day	3	0.4	0.777	0.6	0.622	1.3	0.265	0.5	0.657	3	0.2	0.903		3.4	0.034	3	0.6	0.630		0.9	0.483
day.session	4	0.2	0.957	0.1	0.983	2.0	0.107	0.1	0.988	4	0.2	0.956		0.3	0.900	4	0.7	0.606		0.2	0.914
order	3	0.1	0.978	0.2	0.882	0.3	0.830	0.4	0.781	3	0.1	0.967		0.3	0.846	3	0.9	0.446		1.0	0.432
tray	31	0.9	0.653	1.3	0.211	1.4	0.100	0.9	0.615	31	1.1	0.421		1.7	0.079	31	2.0	0.058		1.6	0.152
cv	7	3.9	0.001	5.9	0.000	3.1	0.007	3.4	0.003	7	1.7	0.147		2.2	0.070	7	3.6	0.012		1.5	0.238
type	1	0.4	0.553	0.6	0.443	3.1	0.081	0.2	0.654												
cv.type	7	1.3	0.286	1.0	0.435	1.3	0.244	0.3	0.958												
kernel	74									25						19					
kernel.assessor stratum																					
assessor	6	104.9	0.000	83.0	0.000	186.2	0.000	105.3	0.000	6	14.4	0.000		40.0	0.000	6	42.5	0.000		13.5	0.000
type.assessor	6	1.4	0.202	3.0	0.007	2.5	0.021	3.5	0.002												
cv.assessor	42	1.5	0.030	1.3	0.088	2.0	0.000	1.9	0.001	42	2.0	0.001		1.4	0.057	42	1.1	0.389		0.8	0.773
order.assessor	18	1.0	0.492	1.0	0.516	1.0	0.457	1.1	0.366	18	1.6	0.050		1.4	0.151	18	2.0	0.011		0.6	0.869
residual	637									298						268					

Table 7.4. Analysis of variance of market value for 7 kernel disorders. BD = Basal Discolouration, DR= Discoloured Rings l, SK= Shrivelled Kernel, ID = Internal Discolouration, SL = Suture Lines, DC= Discoloured Crest, PC= Pitted Centres. F= F prob, df= degrees of freedom, vr= variance ration and Sig = Level of Significance

		BD		DR		SK		ID			SL		DC		PC	
Source	df	vr	sig	vr	sig	vr	sig	vr	sig	df	vr	sig	vr	sig	vr	sig
Kernel stratum																
type	1	0.5	0.478	0.4	0.524	1.9	0.197	0.2	0.644							
type.collection	5	1.5	0.271	0.4	0.870	0.8	0.554	1.0	0.460	6	0.8	0.564	1.0	0.442	1.1	0.419
level	3	27.7	0.000	14.9	0.000	29.8	0.000	260.2	0.000	3	31.2	0.000	35.1	0.000	8.4	0.002
type.level	3	2.4	0.121	2.3	0.130	2.0	0.167	0.6	0.636							
Kernel	12									15						
Kernel.processor stratum																
Order	5	2.1	0.070	1.2	0.340	1.6	0.159	0.4	0.860	5	0.6	0.706	0.9	0.502	0.7	0.616
Region	1	0.0	0.887	9.3	0.003	3.0	0.090	0.0	0.917	1	0.4	0.521	6.8	0.011	21.1	0.000
Region.processor	3	2.4	0.071	1.1	0.341	2.3	0.084	5.9	0.001	3	3.5	0.019	2.2	0.097	1.1	0.364
Type.processor	4	3.8	0.007	1.2	0.329	0.5	0.727	0.9	0.463							
Level.processor	12	1.5	0.130	3.6	0.000	1.2	0.320	2.4	0.010	12	2.1	0.025	4.7	0.000	3.0	0.002
Order.processor	15	0.6	0.844	0.8	0.713	1.0	0.449	1.4	0.170	19	0.9	0.613	1.1	0.326	2.0	0.018
Residual	75									75						

Table 7.5. Analysis of variance of market value for 8 cultivar samples.

Source	*df*	*ms*	*vr*	*sig*
Kernel stratum				
Type	1	23.2	2.07	**0.153**
Type.collection	12	15.1	1.35	**0.205**
Cv	7	15.8	1.41	**0.210**
Type.cv	7	14.8	1.32	**0.249**
Kernel	94	11.2		
Kernel.processor stratum				
Order	5	1.8	0.64	**0.667**
Region	1	78.7	28.11	**0.000**
Region.processor	3	0.6	0.21	**0.887**
Type.processor	4	6.4	2.29	**0.059**
Cv.processor	28	2.1	0.75	**0.821**
Order.processor	19	1.2	0.43	**0.984**
Residual	**518**	**2.8**		

Table 7.6 Analysis of variance of CIE *L**, *a** and *b** values for 7 kernel disorders. BD = Basal Discolouration, DR= Discoloured Rings l, SK= Shrivelled Kernel, ID = Internal Discolouration, SL = Suture Lines, DC= Discoloured Crest, PC= Pitted Centres

Source	***df***	L*		a*		b*	
		vr	sig	vr	sig	vr	**sig**
BD							
kernel stratum							
type	1	0.5	ns	0.2	ns	0.2	**ns**
level	3	66.7	***	5.6	**	1.1	**ns**
type.level	3	10.7	***	0.5	ns	1.1	**ns**
residual	16						
kernel.surface stratum							
surface	1	154.6	***	68.3	***	103.9	*******
surface.level	3	13.3	***	4.8	*	2.8	**ns**
surface.type	1	6.6	*	0.0	ns	1.4	**ns**
residual	19					1.1	
DR							
kernel stratum							
type	1	1.1	ns	5.0	*	0.0	**ns**
level	3	12.5	***	5.6	**	0.5	**ns**
type.level	3	0.9	ns	4.1	*	0.8	**ns**
residual	16						
kernel.surface stratum							
surface	1	59.4	***	93.0	***	126.0	*******
surface.level	3	1.7	ns	1.6	ns	0.6	**ns**
surface.type	1	1.0	ns	0.2	ns	0.0	**ns**
residual	19						
SK							
kernel stratum							
type	1	4.0	ns	1.1	ns	0.4	**ns**
level	3	4.8	*	4.7	*	5.7	******
type.level	3	1.5	ns	0.9	ns	0.4	**ns**
residual	16						
kernel.surface stratum							
surface	1	23.3	***	23.0	***	87.4	*******
surface.level	3	0.4	ns	1.7	ns	1.2	**ns**
surface.type	1	1.2	ns	0.5	ns	0.9	**ns**
residual	19						
ID							
kernel stratum							
type	1	0.2	ns	4.6	*	0.1	**ns**
level	3	23.3	***	6.4	**	1.3	**ns**
type.level	3	1.9	ns	1.2	ns	0.5	**ns**
residual	16	2.0		2.4			
kernel.surface stratum							
surface	1	6.9	*	0.3	ns	35.6	*******
surface.level	3	5.0	*	5.0	*	7.1	******
surface.type	1	0.0	ns	0.0	ns	0.0	**ns**
residual	**19**						

Table 7. 6 (cont)

Source	***df***	L*		a*		b*	
		vr	sig	vr	sig	vr	**sig**
SL							
kernel stratum							
level	3	3.7	*	3.3	*	1.0	**ns**
residual	20	1.9		1.4		1.1	
kernel.surface stratum							
surface	1	65.3	***	141.6	***	89.1	***
surface.level	3	2.8	ns	4.1	*	0.7	**ns**
residual	20						
DC							
kernel stratum							
level	3	3.5	*	7.6	***	3.2	*
residual	20	4.1		1.7		1.4	
kernel.surface stratum							
surface	1	32.7	***	36.4	***	60.7	***
surface.level	3	0.5	ns	0.2	ns	1.4	**ns**
residual	20	4.8		4.2			
PC							
kernel stratum							
level	3	0.8	ns	1.7	ns	1.6	**ns**
residual	20	3.2		4.3		3.6	
kernel.surface stratum							
surface	1	37.5	***	37.0	***	89.2	***
surface.level	3	0.5	ns	1.7	ns	1.2	**ns**
residual	**20**						

Table 7.7 Analysis of variance of *L**, *a** and *b** values for cultivar sample

Source	***df***	L*		a*		b*	
		vr	sig	vr	sig	vr	**sig**
kernel stratum							
type	1	0.3	ns	0.3	ns	8.7	**
cv	7	8.2	***	4.9	***	11.2	***
type.cv	7	0.8	ns	0.2	ns	3.0	**
residual	104	1.5		1.7		1.6	
kernel.surface stratum							
surface	1	403.1	***	769.6	***	766.1	***
surface.cv	7	4.5	***	6.2	***	7.2	***
surface.type	1	0.0	ns	0.0	ns	0.7	**ns**
residual	**128**						

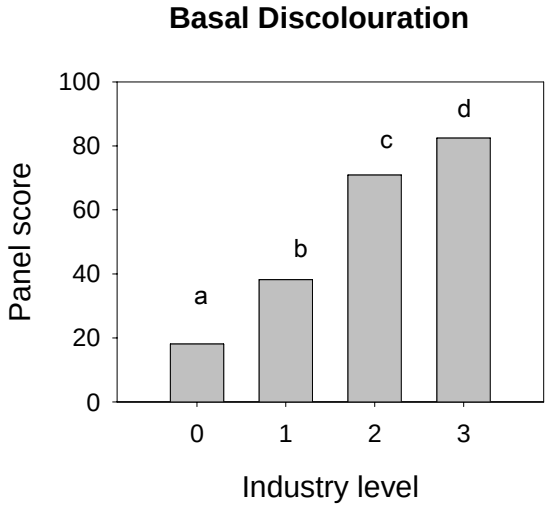


Figure 7.1. Average panel score for the 4 industry defined levels of basal discolouration. Columns with different letters are significantly different, P>0.05

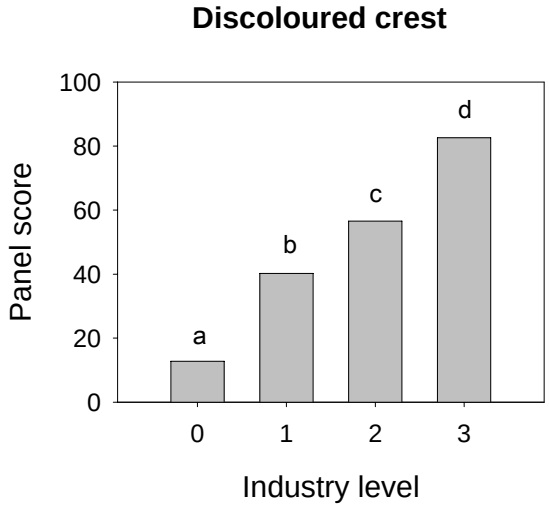


Figure 7.2. Average panel score for the 4 industry defined levels of discoloured crest. Columns with different letters are significantly different, P>0.05

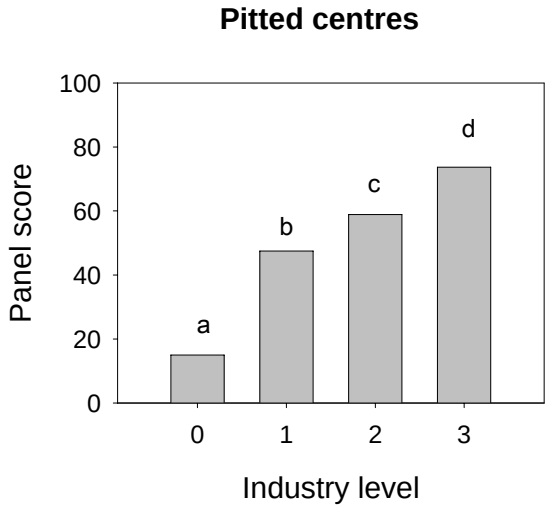


Figure 7.3. Average panel score for the 4 industry defined levels of pitted centres. Columns with different letters are significantly different, P>0.05

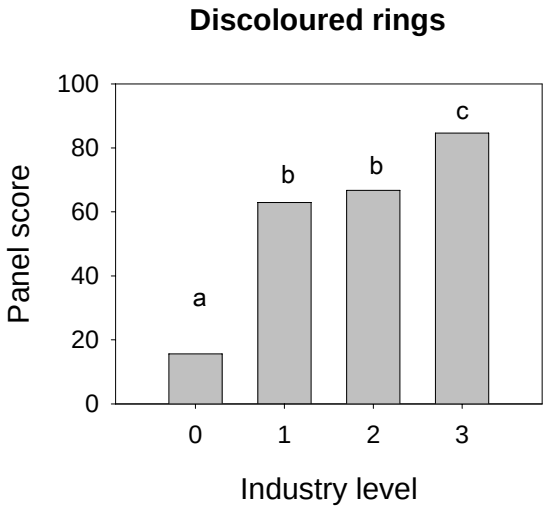


Figure 7.4. Average panel score for the 4 industry defined levels of discoloured rings. Columns with different letters are significantly different, P>0.05

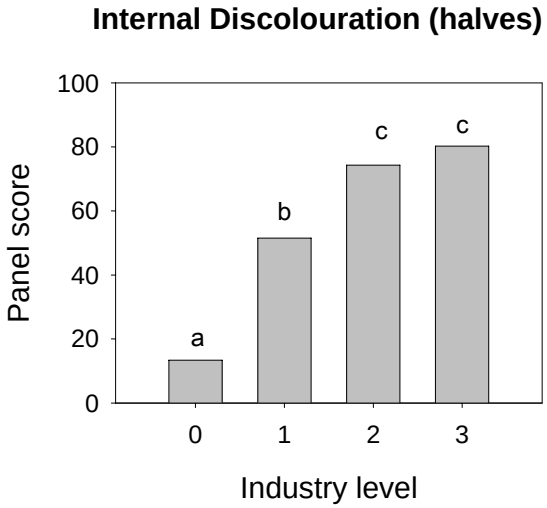


Figure 7.5. Average panel score for the 4 industry defined levels of internal discolouration. Columns with different letters are significantly different, P>0.05

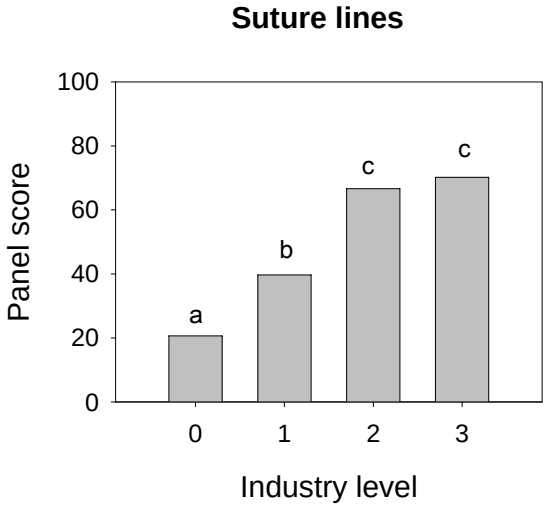


Figure 7.6. Average panel score for the 4 industry defined levels of suture lines. Columns with different letters are significantly different, P>0.05

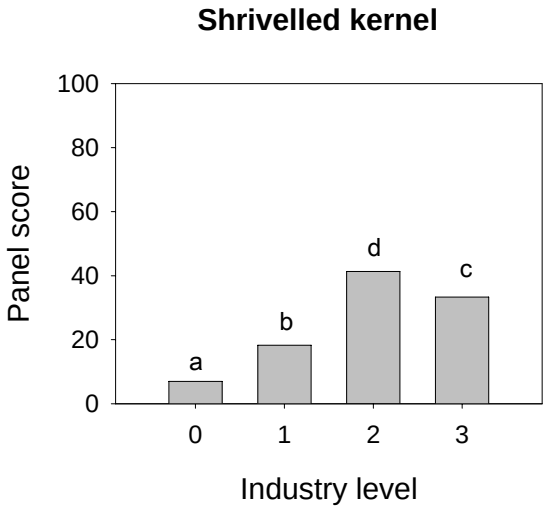


Figure 7.7. Average panel score for the 4 industry defined levels of shivelled kernel. Columns with different letters are significantly different, P>0.05

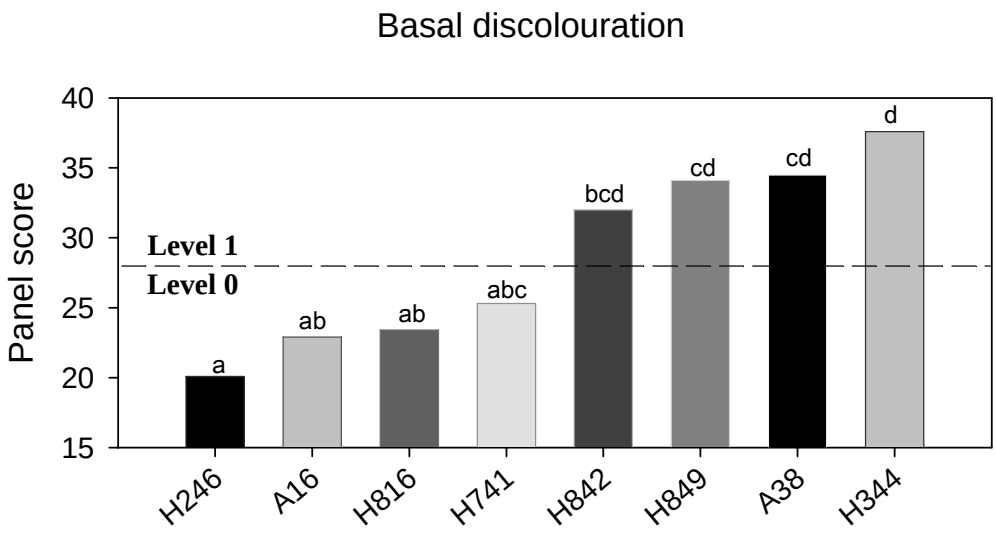


Figure 7.8. Cultivar means for basal discolouration. Commercial threshold for level 0 and 1 indicated. Means with different letters are significantly different, P>0.05

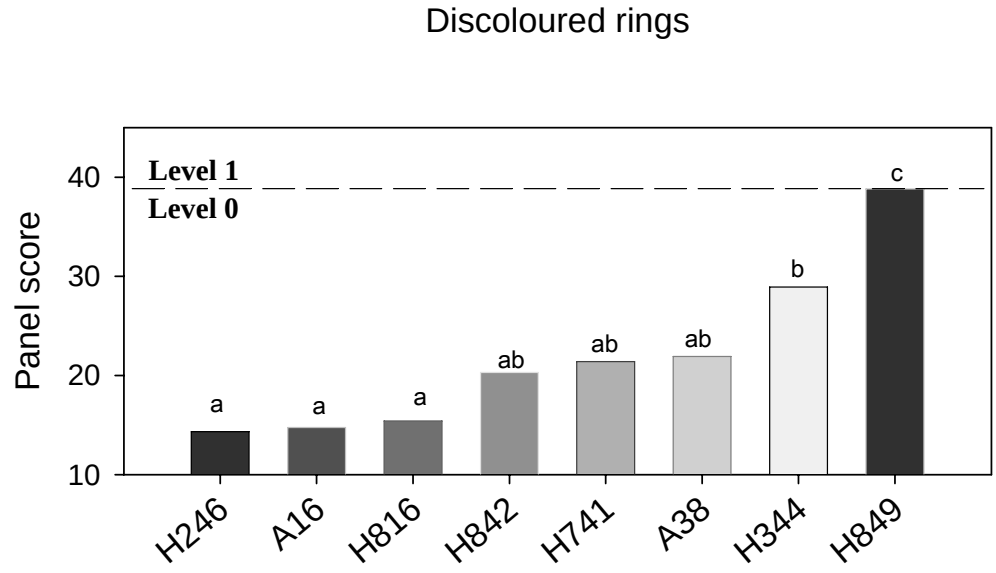


Figure 7.9. Cultivar means for discoloured rings. Commercial threshold for level 0 and 1 indicated. Means with different letters are significantly different, P>0.05
7.1

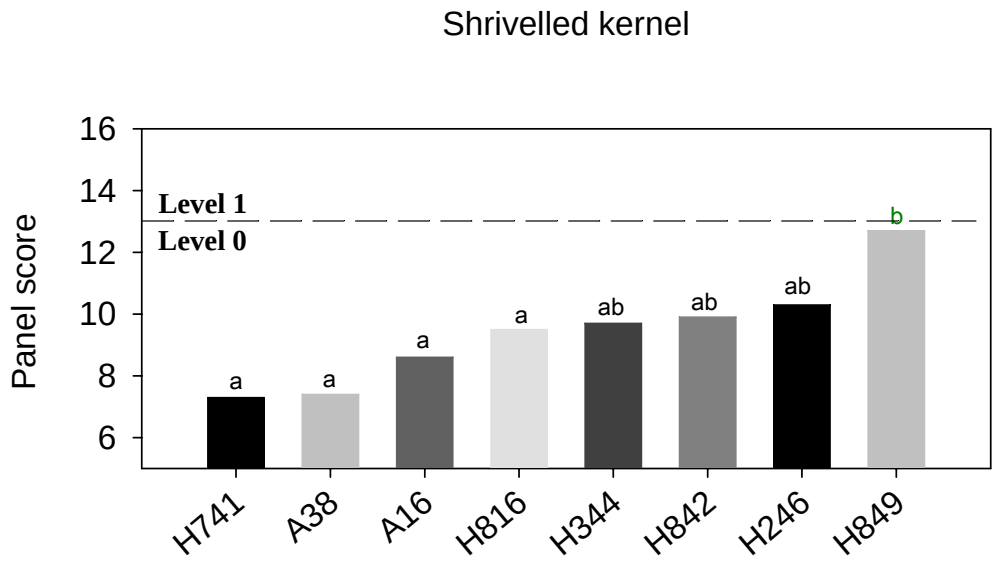


Figure 7.10. Cultivar averages for shrivelled kernel. Commercial threshold for level 0 and 1 indicated. Means with different letters are significantly different, P>0.05
7.1

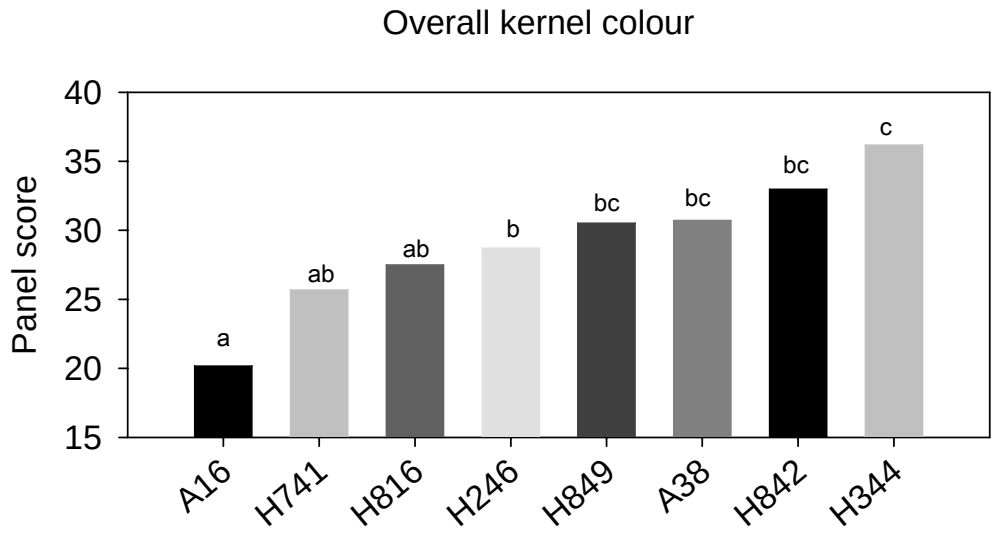


Figure 7.11. Cultivar means for overall kernel colour. Means with different letters are significantly different, P>0.05
7.1

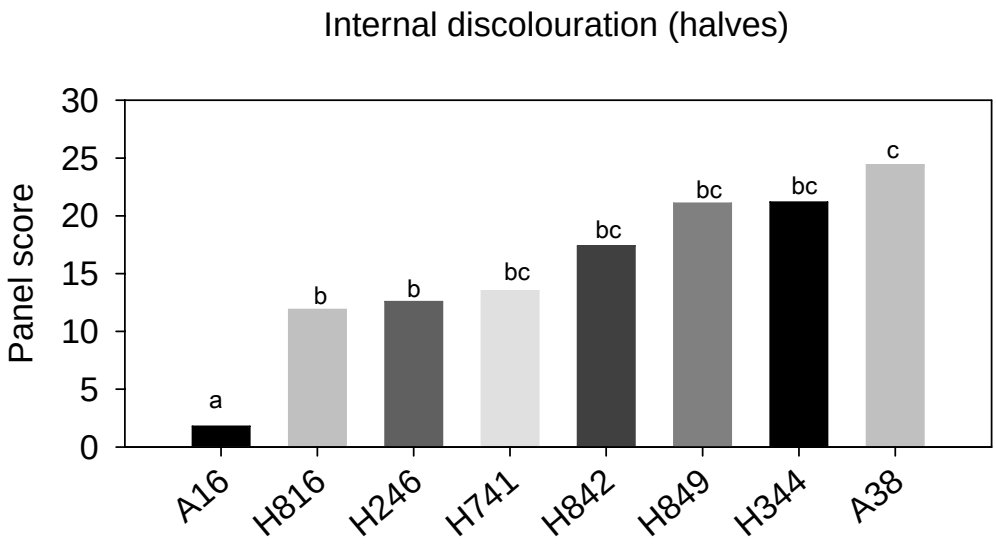


Figure 7.12. Cultivar means for internal discolouration. Means with different letters are significantly different, P>0.05

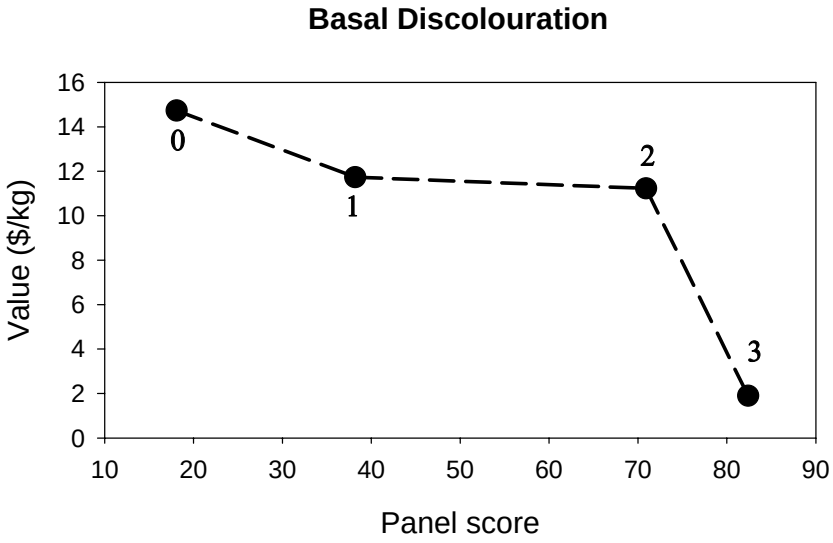
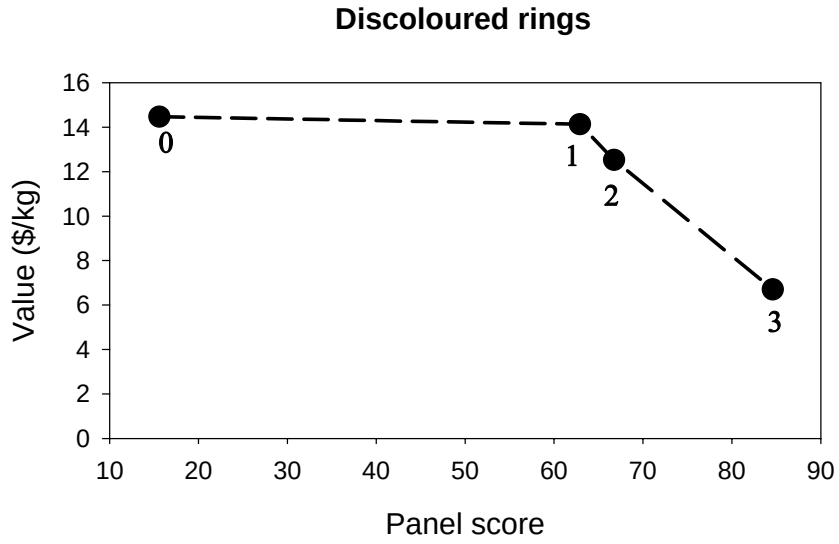


Figure 7.13 Relationship between mean panel score and mean kernel value for basal discolouration. Numbers indicate industry level of defect.



0

Figure 7.14. Relationship between mean panel score and mean kernel value for discoloured rings. Numbers indicate industry level of defect.

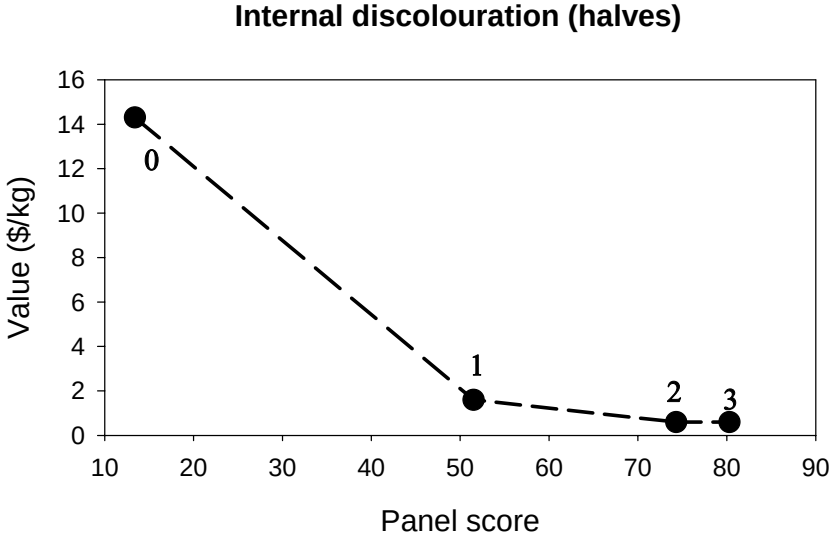


Figure 7.15. Relationship between mean panel score and mean kernel value for internal discolouration. Numbers indicate industry level of defect.

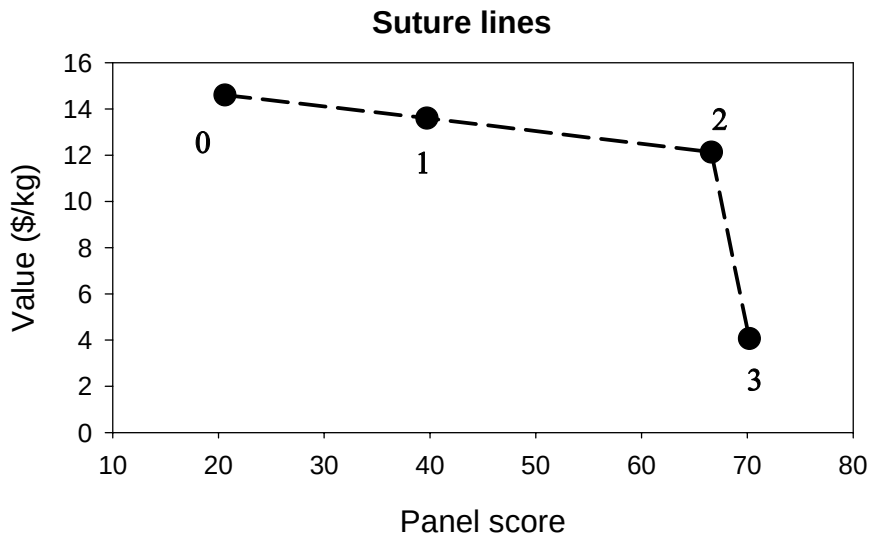


Figure 7.16. Relationship between mean panel score and mean kernel value for suture lines. Numbers indicate industry level of defect.

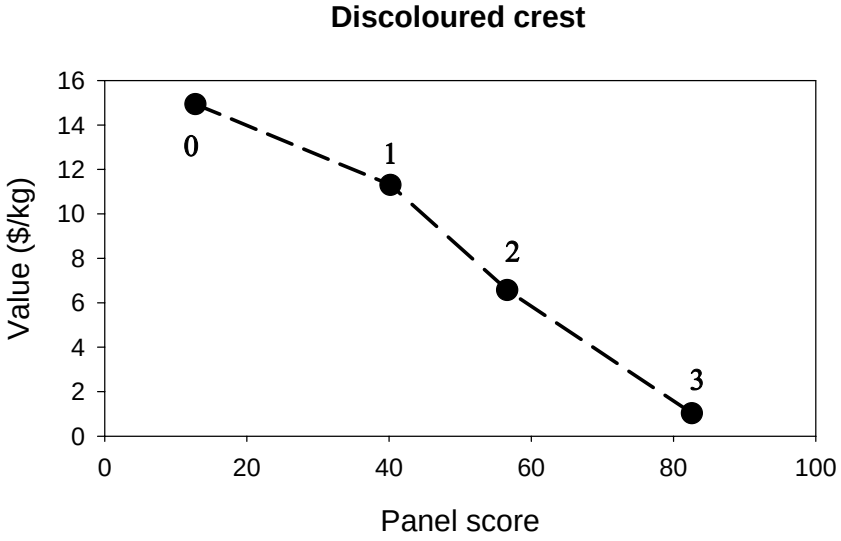


Figure 7.17 Relationship between mean panel score and mean kernel value for discoloured crest. Numbers indicate industry level of defect

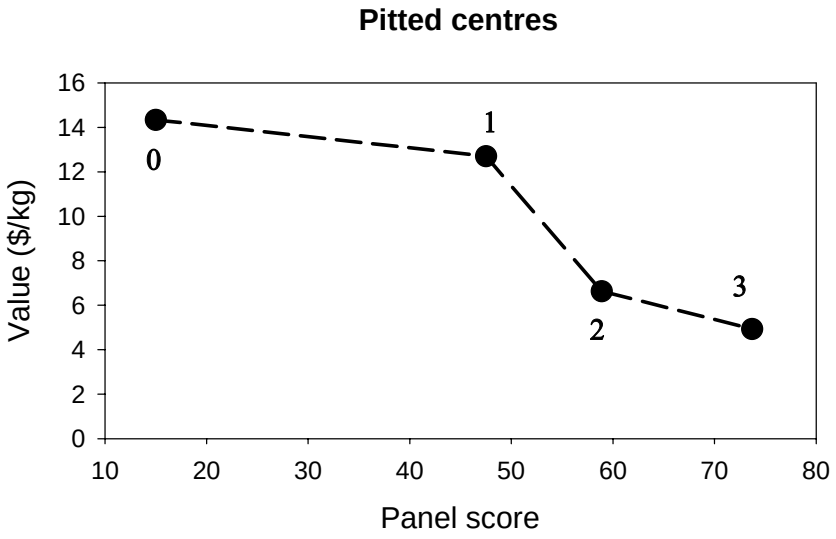


Figure 7.18. Relationship between mean panel score and mean kernel value for pitted centres. Numbers indicate industry level of defect

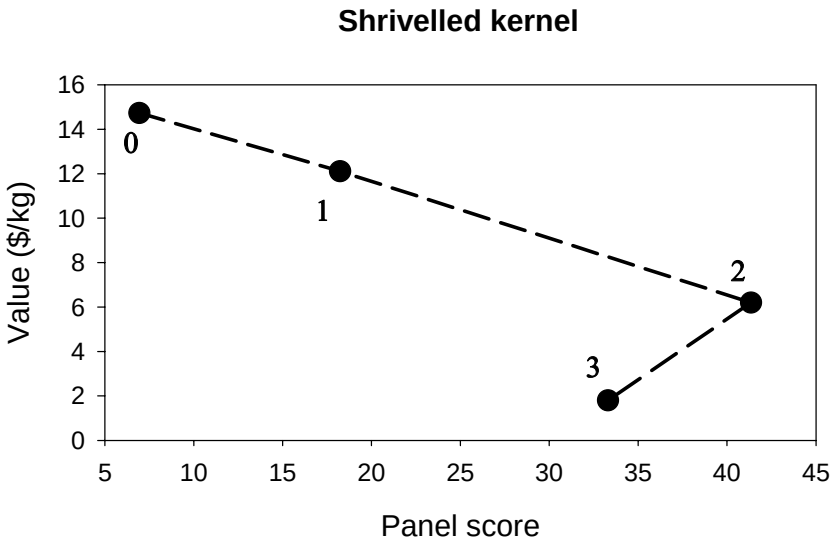


Figure 7.19. Relationship between mean panel score and mean kernel value for shrivelled kernel. Numbers indicate industry level of defect

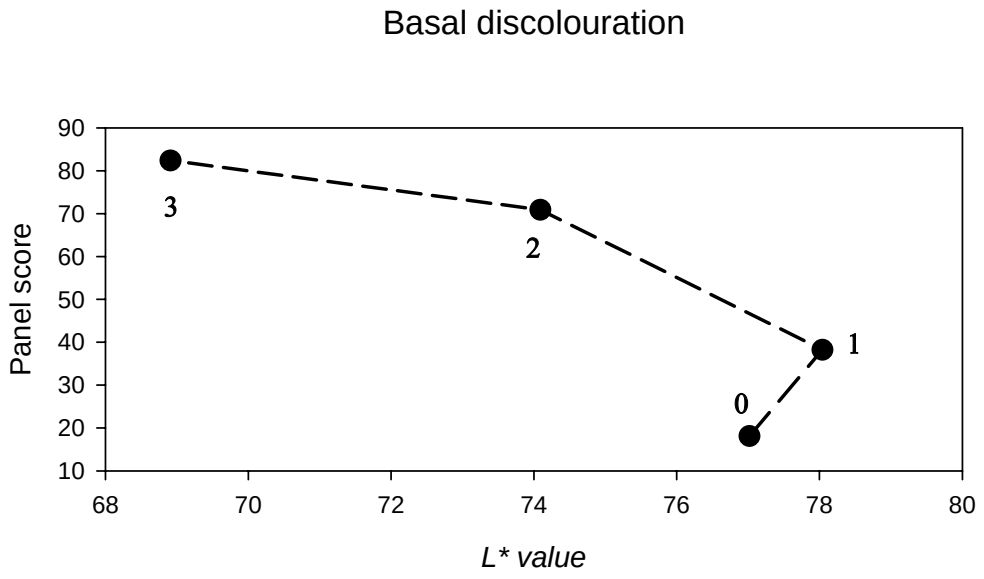


Figure 7.20. Relationship between mean *L** value and panel score for basal discolouration. Numbers indicate industry level of defect

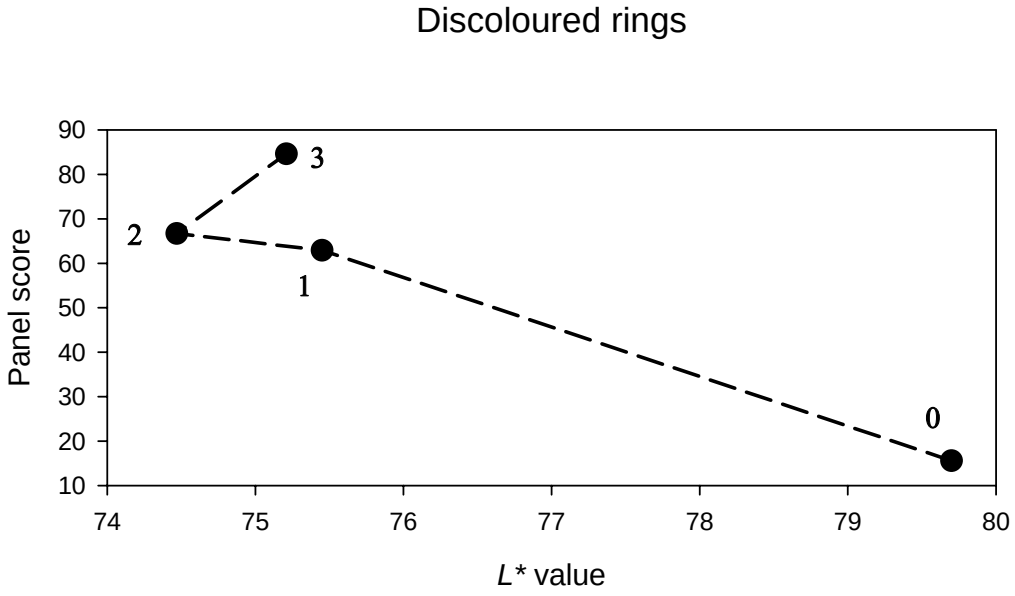


Figure 7.21. Relationship between mean *L** value and panel score for discoloured rings. Numbers indicate industry level of defect

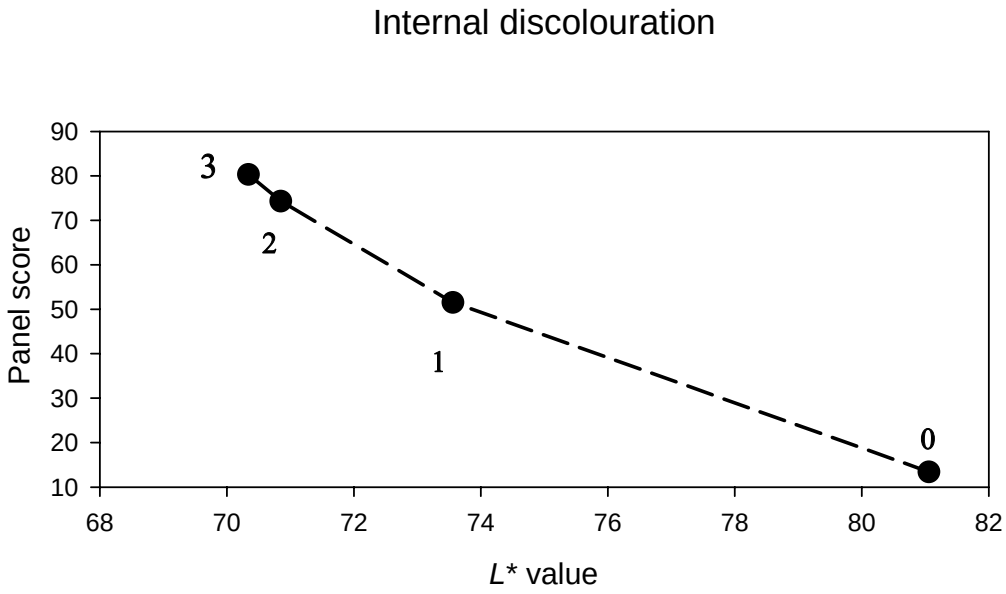


Figure 7.22. Relationship between mean *L** value and panel score for internal discolouration. Numbers indicate industry level of defect

Chapter 8
Assessment of nut and kernel quality for selection of candidate cultivars

Abstract

The genetic value of macadamia progeny for five kernel traits basal discolouration, discoloured rings, shrivelled kernel, suture lines and discoloured crest has been developed. To assessors each examed0 individual nuts from 10 members of progeny families generated in 6and 11 way diallel crosses grown at 2 sites at 2 planting densities. In these plantings there were replicates of 10 of the parental trees that were also included in the analysis. These assessments were performed over a 5 week period with each assessor examining 24 nuts each day for the 5 kernel defects. The defects were assessed on semi-continuous scale from 0 to 3 at 0.25 intervals. There were significant affects of site, assessor and the week in which the assessment was performed for all kernel defects. High density planting in the NSW site also was associated with an elevation of basal discolouration. There was only a significant effect of the order that the assessment was done during the day on shrivelled kernel. There were no significant differences between any of the cultivars, nor between the mean of cultivars and seedlings for any of the kernel defects. The difference between assessors can in part be explained by the differences in the shelling methods that changed amount of whole and half kernels being examined by each assessor and may have eliminated some shriveled kernel due to crushing. The lack of difference between any of the cultivars for any of these defects suggests there may be minimal gain to be had through selecting for these traits.

Introduction

In order to have genetic gain in macadamia kernel quality it needs to be able to be measured. and heritable. Currently kernel quality is monitored within the industry for use in grower payments on delivery to processors and ultimately for setting product specifications. This information is also used as feedback for growers to change on-farm practices that may be supplemented by monitoring of kernel rejected during post-harvest sorting (O'Hare et al., 2005). There has been progress towards instrumental measurement of kernel traits focused mainly on forms of decolouration

(Bell, 2006) but a comprehensive system that includes the more visually cryptic disorders such shrivelled kernel is still required. The spectral properties indicative of kernel defects have been exploited to segregate kernel types in other nut industries such as almonds (Pearson 1999) and macadamia kernel have been found to have similar spectral signals (Guthrie et al., 2004 , McConchie, 2006). Many of the major macadamia processors use automated colour sorters to remove reject kernel and shell during processing based on shade comparison that may include wavelengths in the visual or ultra violet spectrum. Ultimately the kernel quality for all these processes in macadamia relies on visual inspection.

Several methods for assessing macadamia kernel quality have already been investigated that have contributed to the incremental development of the assessment processes. The initial trials used research staff implementing published industry assessment methods. In this system the kernels were assessed against 14 possible defects on accept or reject basis. To ensure the assessments were related to commercial practice this was repeated in 2004 with the research staff located within 2 commercial laboratories. While comparable levels of reject kernel were obtained from the two labs the basis for rejection were very different with the one lab having high levels of shrivelled kernel while in the other basal discolouration was more prevalent. These biases may have reflected regional concerns. In 2005 a new product description manual was developed (Evans and Hofman, 2005) that proposed 4 levels of defect severity. While this represented a potential improvement in the precision of quantifying defects over a binomial accept/reject system it was also evident that the kernel quality measurement was confounded because a reject kernel was still allocated to single classification but may display several defects at different levels of severity. This meant that the absolute level of any defect was not being assessed unless all kernels were assessed for each defect independently. The implication from this was that in order to measure kernel quality in candidate progeny a representative sample of kernel from each tree had to be separately assessed for each of the 14 kernel quality parameters.

This led to the collaborative project between Food Science Australia, ANU and CSIRO Plant Industry to investigate the use of a trained panel to develop consensus based continuous scales to evaluate independently seven of the kernel defects and a measure of overall kernel colour. While this work generally showed that all current defects could successfully be ranked to reflect industry standards there some severity

levels that were not separated. The most important of these was the distinction shrivelled kernel at severity 1 and 2 that represents the cut-off between commercial and reject kernel. It was concluded that there needed to be a distinction made between grossly shrivelled and kernel with a rough basal surface.

Improving macadamia kernel quality has been proposed as a strategy for differentiating Australian macadamia kernel from international competitors. Development of cultivars that produce high quality kernel is one option to achieve this aim.

There are many potential issues that need to be clarified to enable the incorporation of kernel traits into the assessment of candidate cultivars. The strength of genetic control of these traits is unknown. There is also limited data on the distribution of the severity levels among and within samples to develop sample designs. These traits are visually assessed, but the traits are not strictly defined, so the repeatability of assessments and among assessors is unknown. There is the additional problem that resources to measure these traits are finite and to assess nut traits for the entire progeny population is prohibitive. An alternative strategy to be evaluated in the current study to estimate the genetic values based on estimates of subsamples of progeny families.

The aim of this process is to predict total genetic values of progeny for selected kernel traits that may be under genetic control for incorporation into the selection indices used to identify candidate macadamia cultivars. These candidate trees will then be established in 2nd stage clonally replicated trials and the best ultimately released to industry.

Materials and Methods

For this investigation there were 1518 individual progeny available for selection planted in trials at two sites, Bundaberg and Alstonville (1 and 2). Within these trials there were 205 grafted plants of 10 of the parental genotypes, giving a total number of 1723 study individuals (Table 8.1). The progeny were derived from crossings undertaken in 1993 using a 6 diallel and in 1994 in a 11 parent diallel. Five of the parents used in 1993 were also used in the 1994 crossings. The progeny were planted across 2 sites separated by approximately 700 km. The trial at Bundaberg was planted in 3 blocks and the trial at Alstonville was planted in 2 blocks. Trees within these

blocks were planted at 5 m between rows and either 2 m (high density) or 4 m (low density) within rows. The layout of high and low density plantings differed between sites with alternating rows of high and low density plantings in Bundaberg while in Alstonville there were 3 or 4 rows at least planted at the different densities (Figures 8.1 and 8.2)

Nuts that were old, damaged by rats, dehusking or insects, had an open micropile which may be an entry point for spoilage, and showed signs of germination are considered unacceptable for processing. Total kernel recovery measures the amount of kernel recovered from nuts. Kernels with any observable mould, insect damage, adhered skin, streaks and lines or internal discolouration are considered unacceptable for human consumption but may be used for oil. Kernels with specific levels of other disorders including basal discolouration, discoloured rings, shrivelled, suture lines or discoloured crest may be rejected, used as commercial grade kernel or used as premium product. Grades of kernel that are not rejected are defined by the percentage of whole kernels and the size range of the kernel pieces.

Based on previous experience the cost of assessing the entire population was considered prohibitive. In response, a strategy was developed to sub-sample the population to estimate genetic parameters for the kernel traits, and predicted genetic values at the family level and at the individual level for individuals sampled.

Some traits were assumed to not be under genetic control and some were only assessed as presence or absence. Traits where the range of the severity was assessed were: basal discolouration, discoloured rings, shrivelled kernel, suture lines and discoloured crest.

However, because little is know about the underlying distribution of kernel quality traits, and visual assessment by trained human operators is open to variation, a reference standard was developed for each quality trait.

Samples of 100 nuts were collected from all 1723 individuals. Ten individual progeny were randomly selected from each family (55x10=550 progeny) and 5 grafted plants for each of 10 parents were also selected (5x10=50 grafted parents). Attempts were made to evenly spread these selections across the trials and replicates. These were used as study individuals for assessment of individual kernel traits. This gave a total of 600 study individuals. This represents 36 % of the progeny (550/1518) and 24 %

of the grafted parents (50/205). Individuals from each family were selected from each block in proportion to their representation in the block relative to the total number in the family.

The 100 nut samples from each of the 600 study individuals were harvested from the nominated tree. Where there were insufficient nuts remaining on the tree, collections were supplemented by ground harvested nuts. All nuts were then dehusked through a commercial dehusker and dried to 3.5% nut-in-shell moisture content using sequentially 2 days at 35, 45 and 60^{o}C. Nuts that were old (black or grey), or damaged by dehusker or rats were removed irrespective of presence or absence of other nut traits as it was assumed these losses do not have a strong genetic component. From the remaining nuts 2 sub-samples of 10 nuts were selected and individually packaged and labelled in sealed foil bags and kept at 4^{o}C until analysed. Prior to analysis they were removed from storage and allowed to come to room temperature to avoid condensation

Excess nuts harvested from trees that were not required for nut and kernel assessment without any visual defect (old nut, damage from rats or dehusker, germination, insect damage, open micropile) were retained to create the reference standards. A composite 200 nut sample derived from several trees were shelled and the kernel graded by both assessors and consensus reached for each defect. The severity levels were referenced to the Industry kernel description manual (Evans and Hoffman, 2003). After 3 weeks the assessors met to confirm that assessment criteria had not drifted.

Assessment of individual nuts

The nut was then scored for presence or absence of insect damage, germination, or open-micropile. Insect damage was defined as damage to the shell that exposes the kernel. Open-micropile was considered to be an opening at the micropile that exposed the kernel. A nut was recorded as germinated when the radical was visible, or the cotyledons were obviously separated, or the crest of the kernel was clearly visible and discoloured. Nuts that had multiple types of reject character were recorded under each of the defects. Nuts with external shell damage were not scored for any other trait and kernel were not examined.

Assessment of kernel mass and kernel status

All remaining nuts were manually cracked. Nuts for assessor 1, were shelled using modified pliers that were individually adjusted to the dimensions of each nut while nuts for assessor 2 used a TJ's Nut cracker (Moore Qld). The total mass of the nut-in-shell and extracted kernel were recorded. The extracted kernel status was recorded as either a whole kernel if at least 7/8 intact, or otherwise as a half. The cracking and weighing of nuts was done by a separate person who recorded whether the extracted kernel was whole or a half. The kernel were then individually labelled and bagged prior ready for assessment.

Assessment for presence/absence of kernel spoilage

Kernel assessors were located in a room with minimal visual distractions at benches illuminated with a warm florescent light. Each individual kernel will be assessed for presence or absence of mould and insect damage. Defects including internal browning, pitted centres, adhered skin or streaks and lines were ignored. Severity of basal discolouration (BD), discoloured rings (DR), shrivelled kernel (SK) were scored on all kernel. Only whole kernels were assessed for severity of suture lines (SL) and discoloured crest (DC). Descriptions of these disorders are shown in Table 8.2a and the levels of severity Table 8.2b. These descriptions were based on the descriptions by Evans and Hoffman (2005). All kernel disorders severities were scored on a semi-continuous scale from 0 to 3 in increments of 0.25 making a total of 13 possible states for each disorder. When a disorder could not be assessed such as when another disorder was dominant and obscured another disorder the affected disorder was scored as missing data. This was also invoked when kernel were damaged or destroyed when having the shell removed.

Statistical design and analysis

Each kernel assessor examined a package of 10 kernels from each study individual. The order of presentation of the packages to each assessor was determined using a experiment design generated by CycDesigN (Whitaker et al., 2006). The experimental design took into account the order of families and parents presented to asessors over a five week period, five days per week with 24 individual packages being evaluated by each assessor each day.

Statistical analyses were carried out using non-orthogonal analysis of variance to incorporate all the blocking factors included in the experimental design and interactions between assessors and families. The statistical package GenStat (2008) was used for all analyses.

Results

These analyses have produced estimates of the genetic value of progeny families based on examination of kernel from subsampled progeny across different sites and planting densities.

There were significant effects of site on all kernel defects measured except discoloured rings (Table 8.3). Generally kernel from the Alstonville site had a higher score for the defects that were significantly different than kernel from Bundaberg. The only exception was for discoloured crest which was significantly higher in Bundaberg. There was a highly significant effect of planting density on the level of basal discolouration (Table 8.4). This appears to have been largely due to the interaction of planting density and site on discoloured bases where levels were elevated in high density plantings at Alstonville but were otherwise comparable (Table 8.5). There were no other effects of planting density or planting density site interactions for any of the other kernel defects

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There were significant differences between assessors for discoloured bases, shrivelled kernel suture lines and discoloured crests but there were no differences and discoloured rings (Table 8.6). There were large differences between the number of whole and half kernel examined by each of the assessors. Assessor 1 viewed more than a thousand whole kernel more than assessor 2 (Table 8.7). Assessor 2 also had many more missing assessments for shrivelled kernel rating for kernel where a weight was recorded. Many of these kernel had records for basal discolouration and discoloured rings but appear to have been damaged during kernel extraction as subsequent assessments such as the level of shrivelling were recorded as missing.

There were no significant differences between any of the cultivars for any of the defects measured (Table 8.8). Similarly seedlings had marginally higher scores for all disorders than cultivars but none of these were statistically significant (Table 8.9).

There was a significant difference in severity scores for all defects over the 5 weeks (Table 8.10). Basal discolouration and shrivelled kernel generally increased over the 5 weeks. While discoloured rings, discoloured crest and suture lines tended to decrease.

Most of the defects were not affected by the order in which the kernel were assessed through out a day except for shrivelled kernel. The scores for shrivelled kernel appeared to have two peaks one around middle of the day and the other towards the end of the day. (Figure 8.3).

The correlation matrix between these showed no significant relationship between any of the disorders (Table 8.11).

Discussion

These analyses have produced estimates of kernel quality parameters for progeny families that are able to be incorporated into selection indices to rank progeny. While In developing these family estimates significant site effects were identified for all kernel characters measured with the severity generally being higher in the southern site, Alstonville. The exception was discoloured crest, a defect that has been thought to be associated with germination. Little is known about the environmental cues associated with germination in macadamia seed. Further investigation to confirm the association between the discoloured crest and germination are needed and whether this is promoted in a warm climate as would be expected in Bundaberg compared to Alstonville. If this were the case it may elevate the importance of this trait in selecting trees to grow in warmer regions.

The detection of any kernel disorder associated with an agronomic decision has rarely been demonstrated in macadamia. Examples include reduction in first grade kernel assessed by floatation of kernel from trees subjected to water stress (Stephenson et al., 2003) or different fertiliser treatments (Stephenson et al., 2002). The floatation test was developed in Hawaii (Ripperton et al., 1938) and confirmed in Australia (Mason and Wills, 1983) where it was found that roasting and eating quality was associated with kernel oil content above ~72% oil at which point kernel had a specific gravity of 1.0. Subsequent studies have shown that in some cultivars this critical oil content can be achieved when only 70% of the mature kernel weight is attained and the kernel is still immature (McConchie et al., 1996) indicating that it is weakly associated with kernel maturity. The floatation test is not used as a measure of kernel quality in Australia and has been replaced by shrivelled appearance. Increases in other individual kernel disorders in raw kernel such as mould discoloured kernel, germination, and brown centres (Mason et al., 1998) onion rings (synonymous with discoloured rings) (Raspel et al., 2001) have been reported to be due to post-harvest

treatments of nut-in-shell after harvest. In the current study all nuts were tree harvested and processed using recommended drying and storage protocols to minimise the risk of additional damage.

The lack of any difference between the commercial cultivars in the level of any kernel defect suggests there may be minimal gain to be achieved for these kernel traits or the sampling was inadequate or the screening protocol avoids conditions that would be likely to reveal these differences. These nuts were harvested from the tree where as commercially nuts are harvested from the ground which if delayed and the soil is wet will result in kernel deterioration (Liang et al., 1996). These conditions are difficult to replicate and will interact with other environmental factors such as temperature. However a systematic protocol that enhances the development of these traits maybe a more appropriate method to ensure susceptible candidates are eliminated from the breeding population.

The significant elevation of basal discolouration in high density plantings in Alstonville is one of the few examples where differences in kernel quality due to an agronomic decision have been observed in macadamia. The lack of any effects of planting density on kernel quality in Bundaberg may reflect the different layout of the high and low density plantings at the two sites. Only in the high density planting in Alstonville did neighbouring trees occupy all 8 possible positions around a tree. In Bundaberg high density plantings there were either four trees missing on the diagonal or 2 within a row, while in the low density planting there were two trees missing within the column. This was an idealised situation because trees died or were missing that made the inter tree competition difficult to quantify/categorise.

The previous investigation with Food Science Australia had indicated there were significant differences between assessors. Results from the current investigations confirm these results with only DR not showing a significant difference in assessment between the two assessors used in these investigations. The assessor giving the most severe ranking differed between defect with assessor 1 being more severe on SK and SL while assessor 2 was more severe on BD and DC. The current investigations differ from the previous investigations with Food Science Australia in that the nuts were shelled in the process of assessment while they were supplied shelled in the previous work. While both assessors were highly experienced, and it was assumed that this would minimise the influence of assessor, the method of shelling the nuts appears to

have confounded the results. The person cracking the nuts for assessor 1 used a modified pair of pliers with a sharpened cutting edge that were individually adjusted to the dimensions of the nut being cracked that minimised the separation of the kernel. In contrast the person shelling the kernel for assessor 2 used a geared cam that compressed a broad area of shell and crushed kernel or caused the individual cotyledons to separate. More than 10% of the kernel examined by assessor 2 had no measure of shrivelling that was more than double the level of assessor 1. The separating the kernel from a whole to a half had previously been shown to affect kernel assessment study with Food Science Australia study and may have contributed to the difference is evaluations made be the two assessors in the current investigations. These analyses would have been improved by having a standardised nut cracking procedure.

Suggests that they are under independent genetic control or expression has different causes. It supports the need for each of the disorders to be assessed independently to accurately measure kernel quality.

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References

Evans, R. and Hofman, P. 2005. Product description manual Australian macadamia kernel. Australian Macadamia Society, Lismore. pp 41.

Bell, D. 2006. Breeding for kernel quality. Proceedings of the Australian Macadamia Society conference 2006. p101-106.

GenStat (2008). 11th edition, VSN International, Hemel Hempstead, U.K.

Guthrie, J. Greensill, C., Bowden, R. and Walsh, K. 2004. Assessment of quality defects in macadamia kernels using NIR spectroscopy. Australian Journal of Agriculture Research 56: 471- 476.

Liang, T. Meng, Q. and Ji, F. 1996. Prediction of nut spoilage for harvest decision making. Journal of Agricultural Engineering Research 63: 237-242.

Mason, R. L. and Wills, R.B.H. 1983. Evaluation of the use of specific gravity as an objective index of the quality of Australian macadamia nuts. Food Technology in Australia. 35: 245- 248.

Mason, R.L. Kowitz, T. Bowden, R.A. and Isaacs, A.R. 1998. Storage of macadamia nut-in-shell Final Report MC 607.

McConchie, C.A. Meyers, N.M. Anderson, K. Vivian-Smith, A. O'Brien, S. and Richards, S.1996. Development and maturation of macadamia nuts in Australia. *In* Challenges for Horticulture in the tropics edited by R. Stephenson and C. Winks Proceedings of the 3rd Australian Society for Horticultural Science and first Australian Macadamia Research Conference Gold Coast Australia. Pp. 234- 238.

McConchie, C. A. 2006 Control of after roast darkening in macadamia MC01008 Horticulture Australia Final Report pp.288.

Pearson, T. C. 1999. Use of near infrared transmittance to automatically detect to almonds with concealed damage. Lebensm. –Wiss u Technologie 32: 73-78.

Raspel, J. Bradley, D. Wilkie, J.D. Hardner, C. Forrester, R and McConchie, C. A. 2001. Effects of temperature and time on kernel quality after wet nut-in-shell storage. Proceedings Australian Macadamia Society 2001 conference. p 49.

Ripperton, J. C. Moltzau, R.H. Edwards, D. W. Methods for evaluating the macadamia for commercial use and the variation occurring among seedlings in Hawaii. Washington DC. US Department of Agriculture Hawaii Agricultural Experimental Station Bulletin 79.

Stephenson, R.A. Gallagher, E.C. and Doogan, V.J. 2003. Macadamia responses to mild water stress at different phonological stages. Australian Journal of Agricultural Research 54: 67-75.

Stephenson, R. A. Gallagher, E. C. and Gogel, B. J. 2002. Macadamia nut size and maturity influenced by lime and nitrogen applications. Australian Journal of Agricultural Research 53: 677-680.

Strochen, B. 1986. Contributions to the biology of useful plants:. 4. Anatomical studies of fruit development and fruit classification of macadamia nut (*Macadamia integrifolia* Maiden and Betche). Angewardte Botanik 60: 239-247.

Whitaker, D., Williams, E.R. and John, J.A. (2006). CycDesigN 3.0. A package for the computer generation of experimental designs. CycSoftware Ltd., Hamilton, N.Z.

	A4	*A16*	*246*	*344*	*660*	*781*	*814*	*816*	*842*	*849*	*Parents*
OV	23	21	19				17	17			**17**
Daddow	28	36	35	34	20	35	15	27	36	32	**19**
A4		24	30	19	34	27	28	42	17	37	**42**
A16			57	21	20	41	48	48	37	21	**19**
246				12	14	14	33	45	9	22	**22**
344					12	12	12	15	22	23	
660						19	27	32	26	22	
781							17	16	14	20	**19**
814								52	14	5	**9**
816									25	23	**20**
842										15	**19**
849											**19**

Table 8.1. Overview of numbers of progeny and grafted trees of parents

Disorder	***Description***
Insect damage	**Characterised by depressions or lesions on the surface of the kernel. This may have resembled a fissure or cleft and may have been bordered by brown or flaky scar tissue and be accompanied by webbing and excreta.**
Mould	**Discolouration of the kernel and may have occurred on the surface or in the kernel proper. It sometimes smudges when touched and had a range of characteristic colours from orange, red, brown, green, through to blue, dark purple, black or white.**
Basal Discolouration	**Staining that encompassed the entire rounded base of the kernel. The colours ranged through brown, grey green to blue and contrasted with the creamy kernel colour.**
Shrivelled Kernel	**Kernel that has a shrunken or wrinkled appearance. The kernel was most readily identified by a wrinkled base. This may also be evident as a depression or concave base with a pinched of flattened appearance. Kernel may have been hard with a glossy appearance. In half kernel the flat face was cupped or concave**
Suture line	**Darkened lines on the base of the kernel where the halves of the kernel meet. The severity of the disorder is related the darkness and width of the line.**
Discoloured Crest .	**Changes in the crest region as a result of germination. The discolouration varied from light colour change through to a dark brown. If it has a green colour it was considered to be mould.**

Table8 2a. Description of disorders as applied in the current investigations

Disorder	*Severity*	*Description*
For all Disorders	0	**Kernel without disorders**
Insect damage	1	**Present**
Mould	1	**Present**
Basal Discoloration	1	**Light colouration**
	2	**Medium Colouration**
	3	**Dark colouration**
Discoloured Crest	1	**Small coloured spots, light coloured peak**
	2	**Dark colour along peak**
	3	**Very Distinct colour**
Suture lines	1	**Light colour**
	2	**Medium Colour**
	3	**Dark spreading line**
Shrivelled	1	**Slight wrinkled or flattened base**
	2	**Moderately wrinkled base**
	3	**Shrunken and deformed kernel**

Table 8.2b Description of the severity descriptions as applied in the current investigations

	Site	*Bundaberg*	*Alstonville*	*SED*	*F pr*
Disorder	Basal Discolouration	1.244	1.338	0.028	**<0.001**
	Discoloured rings	0.3807	0.5512	0.018	**0.062**
	Shrivelled	1.446	1.598	0.023	**<0.001**
	Suture lines	0.3190	0.3550	0.019	**0.016**
	Discoloured crest	**0.2617**	**0.2127**	**0.014**	**<0.001**

Table 8.3. Comparison of mean severity for the five disorders for the two sites Bundaberg and Alstonville.

Planting Density		*5 x 2*	*5 x 4*	*SED*	*F pr*
Disorder	Basal Discolouration	1.334	1.247	0.032	**<0.001**
	Discoloured rings	0.4712	0.4607	0.021	**ns**
	Shrivelled	1.534	1.510	0.026	**ns**
	Suture lines	0.3391	0.3349	0.021	**ns**
	Discoloured crest	**0.2414**	**0.2330**	**0.016**	**ns**

Table 8.4. Comparison of mean severity for the five disorders for the high (5x2m) and low density (5x4m) plantings.

	Planting density (m)	***5x 2***	***5x 4***
Site	Bundaberg	1.240	**1.247**
	Alstonville	**1.428**	**1.247**

Table 8.5. Means for site.planting density for basal discolouration. There were elevated levels in high density plantings at Alstonville F pr <0.001

Assessor		***1***	***2***	***SED***	***F pr***
Disorder	Basal Discolouration	1.234	1.348	0.016	**<0.001**
	Discoloured rings	0.4739	0.4581	0.009	**ns**
	Shrivelled	1.721	1.347	0.019	**<0.001**
	Suture lines	0.4282	0.3915	0.016	**<0.001**
	Discoloured crest	**0.1955**	**0.3672**	**0.055**	**<0.001**

Table 8.6. Comparison of mean severity given by two assessors for the five disorders investigated.

Assessor	***wholes***	***halves***	***missing***	***total***
1	3680	2131	88	**5899**
2	**2598**	**3019**	**293**	**5810**

Table 8.7. Counts of the number of whole and half kernel examined by each assessor. Missing is the count of kernel for which no record of whole or half was made.

Cultivar		*Own Venture*	*Daddow*	*A4*	*A16*	*246*	*781*	*814*	*816*	*842*	*849*	*F pr*
Disorder	Basal discolouration	1.284	1.195	1.384	1.137	1.499	1.173	1.021	1.480	1.198	1.545*	**ns**
	Discoloured Rings	0.347	0.5130	0.4334	0.5163	0.4895	0.6028*	0.4773	0.3419	0.5241	0.5779	**ns**
	Shrivelled	1.721	1.303	1.431	1.543	1.726*	1.281	1.451	1.377	1.662	1.525	**ns**
	Suture lines	0.3610	0.3345	0.4617	0.5361*	0.3065	0.2675	0.1927	0.3139	0.3426	0.2770	**ns**
	Discoloured crest	**0.3173***	**0.1939**	**0.2804**	**0.2622**	**0.2592**	**0.1268**	**0.2018**	**0.2310**	**0.1902**	**0.1819**	**ns**

Table 8.8. Comparison of mean kernel severity measures for 10 parental cultivars for the five disorders investigated.

Plant Type		*Cultivar*	*Seedling*	*SED*	*F pr*
Disorder	Basal Discolouration	1.142	1.313	0.285	**ns**
	Discoloured rings	0.3239	0.3780	0.165	**ns**
	Shrivelled	1.507	1.561	0.234	**ns**
	Suture lines	0.3652	0.3866	0.149	**ns**
	Discoloured crest	**0.2789**	**0.2838**	**0.036**	**ns**

Table 8.9. Comparison of mean severity for cultivars and seedlings for the five disorders investigated.

		Week					***SED***			
		1	2	3	4	5	Average	Max	Min	**F pr**
Disorder	Basal Discolouration	1.187	1.180	1.374	1.318	1.395	0.027	0.027	0.027	**<0.001**
	Discoloured Rings	0.5573	0.4869	0.4271	0.4218	0.4368	0.016	0.016	0.016	**<0.001**
	Shrivelled	1.464	1.480	1.538	1.555	1.572	0.027	0.027	0.027	**<0.001**
	Suture Lines	0.4330	0.3797	0.3050	0.2855	0.2819	0.023	0.023	0.022	**<0.001**
	Discoloured crest	**0.2927**	**0.2473**	**0.2098**	**0.2228**	**0.2134**	**0.017**	**0.017**	**0.017**	**<0.001**

Table 8.10. Comparison of mean severity for cultivars and seedlings for the five disorders investigated.

Disorder	*Basal discolouration*	*Discoloured Rings*	*Shrivelled*	*Suture lines*	*Discoloured crest*
Basal discolouration	1.000				
Discoloured Rings	0.273	1.000			
Shrivelled	0.164	0.060	1.000		
Suture lines	0.140	0.165	0.110	1.000	
Discoloured crest	**0.130**	**0.024**	**-0.010**	**0.095**	**1.000**

Table 8.11. Correlation matrix between measured kernel disorders

Table 8.12. Mean family values for the disorders

	Disorder				
Family	**Basal discolouration**	**Discoloured Rings**	**Shrivelled**	**Suture lines**	Discoloured crest
0	1.167	0.3683	1.357	0.3749	**0.2533**
Daddow x A4	1.167	0.3683	1.357	0.3749	**0.2533**
Daddow x A16	1.182	0.3959	1.624	0.5043	**0.3612**
Daddow x 246	1.246	0.3783	1.298	0.3498	**0.2295**
Daddow x 344	1.379	0.4628	1.456	0.2851	**0.1808**
Daddow x 660	1.220	0.6106	1.378	0.3269	**0.1483**
Daddow x 781	1.232	0.4434	1.340	0.3089	**0.2049**
Daddow x 814	1.091	0.4503	1.515	0.2601	**0.1675**
Daddow x 816	1.154	0.3210	1.242	0.2768	**0.2286**
Daddow x 842	1.202	0.4548	1.358	0.2845	**0.2044**
Daddow x 849	1.168	0.4042	1.431	0.3736	**0.2255**
A4 x A16	1.203	0.4692	1.640	0.3697	**0.2738**
A4 x 246	1.343	0.4289	1.609	0.3772	**0.3277**
A4 x 344	1.387	0.3997	1.671	0.4035	**0.2175**
A4 x 660	1.271	0.4143	1.578	0.2938	**0.2228**
A4 x 781	1.191	0.3805	1.500	0.3957	**0.2716**
A4 x 814	1.085	0.420	1.527	0.3030	**0.3075**
A4 x 816	1.124	0.3379	1.444	0.2731	**0.2054**
A4 x 842	1.181	0.4969	1.603	0.4447	**0.2556**
A4 x 849	1.503	0.4643	1.642	0.3900	**0.3047**
A16 x 246	1.221	0.3292	1.521	0.4589	**0.3529**
A16 x 344	1.226	0.4771	1.568	0.2513	**0.2659**
A16 x 660	1.290	0.4334	1.537	0.4971	**0.3962**
A16 x 781	1.110	0.4603	1.446	0.4437	**0.2105**
A16 x 814	1.168	0.5162	1.632	0.4056	**0.3153**
A16 x 816	1.123	0.3677	1.514	0.3739	**0.2301**
A16 x 842	1.232	0.4491	1.509	0.3984	**0.2956**
A16 x 849	1.159	0.3534	1.503	0.3853	**0.2281**

	Disorder				
246 x 344	1.430	0.5066	1.557	0.3328	**0.2269**
246 x 660	1.269	0.6845	1.377	0.2114	**0.2053**
246 x 781	1.338	0.4724	1.526	0.3037	**0.2479**
246 x 814	1.122	0.4158	1.798	0.3284	**0.2193**
246 x 816	1.227	0.4165	1.693	0.3032	**0.2409**
246 x 842	1.659	0.6757	1.806	0.2571	**0.2229**
246 x 849	1.551	0.6709	1.685	0.3639	**0.2034**
344 x 660	1.605	0.5912	1.493	0.3000	**0.2517**
344 x 781	1.533	0.5477	1.546	0.2503	**0.2252**
344 x 814	1.278	0.4428	1.529	0.2732	**0.1839**
344 x 816	1.533	0.4328	1.508	0.2888	**0.1939**
344 x 842	1.471	0.4427	1.513	0.2882	**0.1962**
344 x 849	1.732	0.4817	1.679	0.4186	**0.1818**
660 x 781	1.338	0.5679	1.389	0.2549	**0.2823**
660 x 814	1.488	0.6881	1.499	0.3336	**0.1877**
660 x 816	1.413	0.4153	1.417	0.2278	**0.2288**
660 x 842	1.477	0.5756	1.468	0.3947	**0.2815**
660 x 849	1.543	0.6372	1.457	0.4045	**0.1822**
781 x 814	1.018	0.5159	1.538	0.2886	**0.1722**
781 x 816	1.280	0.4560	1.548	0.2822	**0.2527**
781 x 842	1.254	0.5492	1.345	0.3593	**0.1697**
781 x 849	1.263	0.4856	1.480	0.4207	**0.2581**
814 x 816	1.157	0.3856	1.449	0.2587	**0.1667**
814 x 842	1.235	0.4594	1.769	0.3682	**0.2363**
814 x 849	1.029	0.3559	1.682	0.2135	**0.3115**
816 x 842	1.204	0.3621	1.489	0.3624	**0.1998**
816 x 849	1.356	0.4190	1.605	0.2676	**0.2222**
842 x 849	1.457	0.6016	1.584	0.3229	**0.1938**
SE Average	0.1529	0.09968	0.1237	0.09329	**0.06712**
SE Maximum	0.2058	0.1342	0.1663	0.1262	**0.09220**
SE Minimum	0.1490	0.09715	0.1204	0.08696	**0.6197**
Average Variance	**0.02347**	**0.009971**	**0.01536**	**0.00874 1**	**0.004528**

X	X	X	X	**X**
O	X	O	X	**O**
X	X	X	X	**X**
O	X	O	X	**O**
X	X	X	X	**X**
O	X	O	X	**O**
X	X	X	X	**X**
O	**X**	**O**	**X**	**O**

Figure 8.1. Schematic diagram of the layout of high density and low density plantings at Bundaberg trial site. Depicted are alternating columns of trees planted at 5 m between rows and either 2 or 4 m within a row. X = planted tree, O= Gap.

X	X	X	X	X	X	X	**X**
X	X	X	O	O	O	X	**X**
X	X	X	X	X	X	X	**X**
X	X	X	O	O	O	X	**X**
X	X	X	X	X	X	X	**X**
X	X	X	O	O	O	X	**X**
X	X	X	X	X	X	X	**X**
X	**X**	**X**	**O**	**O**	**O**	**X**	**X**

Figure 8.2. Schematic diagram of the layout of high density and low density plantings at Alstonville trial site. Depicted are blocks of trees planted at 5 m between rows and either 2 or 4 m within a row. X = planted tree, O= Gap.

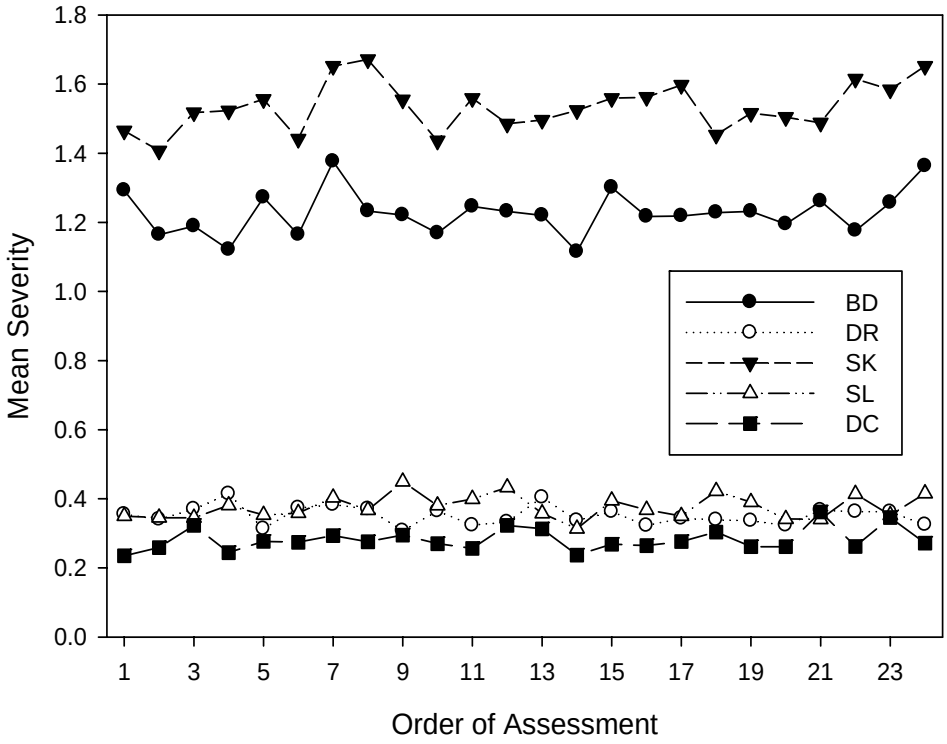

Figure 8.3. The effect of assessment order on assessment of disorder severity. BD basal discolouration, DR discoloured rings, SK shrivelled kernel, SL suture lines and DC discoloured crest. LSD = 0.107 for SK P>0.01

Chapter 9.

Resolving Issues of Identity and Relatedness in Macadamia using Microsatellite Markers

Abstract

Macadamia is a clonally propagated nut crop, derived from two species, *Macadamia integrifolia* and *M. tetraphylla*, and their hybrids. The aim of this study was to test the utility of recently developed microsatellite markers in resolving identity and relatedness in 32 commercial cultivars (1-6 trees per cultivar) collected from two field sites. The cumulative probability of identity (PI_{ave}) generated using ten markers was 1.04×10^{-6}, indicative of strong discriminatory power, despite significant genetic structure resulting from selection/inbreeding/founder effects ($F_{IS} = 0.130$; $\Gamma_{IS} = 0.202$; $F_{ST} = 0.301$; $\Gamma_{ST} = 0.715$). Detection of variation among replicate samples for several cultivars led to the identification of mislabeling in the field. Following adjustment for this mislabeling, replicated samples (n = 2-6) were assigned to the nominal group ($R \geq 1.00$, $p \leq 0.001$) in 15 of 30 cultivars represented by multiple samples. A further 12 cultivars showed variation between replicated samples involving one or two loci; consistent with an overall genotyping error rate of ~3% (calculated through replicated genotyping of multiple single-leaf DNA extractions). Replicated samples of three cultivars showed more substantial variation (>2 haplotypes, involving variation at >2 loci), probably resulting from unclarified misidentification in the field.

Introduction

Macadamia is a clonally propagated nut crop, derived from two species, *Macadamia integrifolia* and *M. tetraphylla* (Johnson 1954; Smith 1956) and their hybrids (Storey and Saleeb 1970; Hardner et al. 2000). Both *Macadamia integrifolia* and *M. tetraphylla* also occur in fragmented natural populations (Gross 1995), as do the remaining five species in the genus (*M. ternifolia, M. grandis*, *M. whelanii, M. claudiensis* and *M. jansenii*), none of which are grown commercially (Gross 1995).

Previous molecular marker research in macadamia has focused on isozymes (Vithanage and Winks 1992; Aradhya *et al.* 1998), RAPD (random amplified polymorphic DNA), and RAF (randomly amplified DNA fingerprinting) marker

systems (Peace *et al.* 2003). RAF and RAPD markers however, are not ideal, for a variety of reasons. In particular, these dominant markers are easily used to establish that two individuals are genetically distinct, but allow only crude estimations of the extent of genetic divergence that are not amenable to statistical analysis (Kirst et al. 2005).

Locus-specific co-dominant markers such as microsatellites offer significant advantages, although they do have a number of potential drawbacks including difficulty and expense associated with development of loci, potential for null alleles, allelic dropout (inconsistent selective amplification of alleles in heterozygotes) and incomplete understanding of the selective and mutational processes that influence the origin and evolution of loci, which affects validity of statistical analyses (Hedrick 1999; Chenuil 2006; Selkoe & Toonen 2006). These drawbacks also apply to other marker systems however, and one of the key advantages of microsatellites is that datasets can be tested for deviation from underlying assumptions of analytical procedures.

Application of microsatellites in crop species has provided substantial benefits in genome mapping and QTL identification (Darvasi & Pisante-Shalom 2002), but studies of relatedness within and among the same, or very closely related species, can be problematic. Resolution of individual identity is one fundamental issue. This is usually assessed through calculation of the probability that a given multilocus genotype will be observed more than once in a given population (Smouse & Chevillon 1998; Graham, Curran & Weir 2000). This probability is determined by the frequencies of individual alleles within the population under study (Danforth & Freeman-Gallant 1996; Waits, Luikart & Taberlet 2001).

In groups of cultivars produced through generations of selection and inbreeding using a relatively small number of parental genotypes however, allele frequencies do not conform to common underlying assumptions such as Hardy-Weinberg equilibrium, random mating and independence of loci (Waits et al. 2001). Fortunately, Ayres & Overall (2004) have developed a method for calculation of probability of identity (PI_{ave}) in structured groups and, where some pedigree information is available, this is easily implemented in crop species.

Problems arising from repeated selection and inbreeding pose a more significant obstacle in resolution of relatedness among cultivars. Studies of crop species are generally concerned with recently diverged (≤100-200 years) entities; making

hypervariable markers (e.g. microsatellites and AFLPs) more appropriate for resolving relationships than more slowly evolving markers (e.g. organelle gene sequencing).

Most published studies of relatedness in cultivated species use relatively simple measures of similarity/difference based on the extent of allele-sharing, usually with some adjustment for allele frequencies (e.g. Aradhya et al. 2003; Cavagnero et al. 2006; George et al. 2006; Ghislain et al. 2006; Mariac et al. 2006). It is important to note however, that the unique properties of hypervariable markers require unique analytical frameworks (Hedrick 1999; Selkoe & Toonen 2006).

Paetkau et al. (1997) demonstrated that metrics developed specifically to accommodate known and/or theoretical models of microsatellite mutation provide poor separation of sister taxa, largely because of their higher intrinsic variance. Takezaki & Nei (1996) also tested the performance of a range of distance measures making use of different microsatellite mutation models in reconstructing known phylogenies. Their results demonstrated that an early Euclidean distance measure, Cavalli-Sforza and Edward's (1967) chord distance (D_C), and a modification of this (D_A; Nei, Maruyama and Wu 1983), perform better in resolving questions of population divergence, but for deeper phylogenetic questions, measures that are less affected by variation within populations, such as Nei's standard D_S and Goldstein's $(\delta\mu)^2$ (Goldstein et al., 1995) are more appropriate.

Nei's D_s has been used in previous studies of relatedness between macadamia cultivars (Peace 2004). As this study used randomly amplified dominant (RAF) markers however, the validity of any metric based on assumptions regarding allele frequencies, particularly in the absence of any preliminary testing for linkage disequilibrium between markers, is questionable. Destro-Bisol, Spedini and Pascali (2000) have also pointed out that metrics of this nature assume that drift is the primary differentiating agent, but in horticultural/agricultural breeding, differentiation is driven almost exclusively by selection, with compounding effects associated with relatively small numbers of parental genotypes.

To determine the most appropriate distance metric for studies of relatedness within breeding programmes requires recognition that most metrics have been developed for use in studies of natural populations where the emphasis is on elucidation of the evolutionary history of the group/s of interest (Destro-Bisol et al. 2000; Nagamine &

Higuchi 2001). The aim of this study was to employ recently developed microsatellite markers (Schmidt, Scott & Lowe 2006) (see Appendix 9.1) in resolution of identity and relatedness in macadamia cultivars, using a replicated, regression-based distance metric developed for studies of proximal relatedness between individuals (Queller & Goodnight 1989).

Materials and Method

Cultivar Samples and DNA Extraction

Leaf tissue was collected from 32 commercial cultivars (Table 9.2) and a common rootstock cultivar (H2) growing in field trials planted at Clune and The New South Wales Centre for Tropical Horticulture (CTH), northern New South Wales (McConchie et al. 1999; Hardner et al. 2000). Both sites were established for assessment of selections and informally serve as bud wood sources for the Australian Macadamia industry. For comparative purposes, four *M. jansenii* specimens (three wild specimens, plus two grown at the CTH site) were also included in the study, along with a single specimen of unknown identity (labelled '*Species*') from the CTH site (Table 9.2).

DNA extractions were performed on freeze-dried, ground leaf tissue with CTAB buffer (2% CTAB, 100 mM Tris, 20 mM EDTA, 1.4 M NaCl, pH 8.0) lysis and deproteination via chloroform:isoamylalcohol purification, as per Schmidt *et al.* (2004). Aliquots of initial extractions were diluted in water to a final concentration of ~25 ng/μL prior to PCR.

Initial DNA extractions and PCR amplifications were performed on ground tissue from multiple leaves. Following detection of variation between specimens of a given cultivar in early phases of the study however, trees from Clunes were resampled in a double-blind trial with DNA extractions were repeated using single leaves for 44 trees (21 cultivars). A further 33 required pooling of extractions from multiple leaves to yield sufficient DNA for further genotyping.

Microsatellite Genotyping

Samples were genotyped at eleven loci, using nine primer sets (*Min*μ*S0001A, Min*μ*S0002, Min*μ*S0003, Min*μ*S0004, Min*μ*S0005, Min*μ*S0007, Min*μ*S0016, Min*μ*S0048, Min*μ*S0050*) described in Schmidt et al. (2006) and a previously

unpublished $(A)_m/(GA)_n$ repeat marker (*Minμ S0074*) amplified using the forward and reverse primers MinμS0074F (5' AAA AGT GGT GGG TCG GTA TC 3') and MinμS0074R (5' GGA TCC ATA TCC ACC AAA CC 3').

Genotyping was performed using either radioactive or fluorescent labelling. Radio-labelled PCR products were generated in 7.0 μL reactions containing 0.2 m**M** low C dNTPs (0.2 mM dATP, 0.2 mM dTTP, 0.2 mM dGTP and 0.02 mM dCTP), 0.10 μ**M** of each primer, 1 x PCR buffer, 0.1 unit of *Taq* DNA polymerase (Fisher Biotech) and ~25 pg of genomic DNA and fragments were visualised via electrophoresis through denaturing 5% polyacrylamide gels (6 M urea, 5% acrylamide:bisacrylamide 19:1, 1 x TBE). Alleles were scored to 1 bp accuracy with reference to a pUC18 sequence ladder.

Fluorescently-labelled PCR products were generated in 12 μL reactions containing 0.2 mM dNTPs (0.2 mM dATP, 0.2 mM dTTP, 0.2 mM dGTP and 0.2 mM dCTP), 0.006 μM of M13-tagged forward primer, 0.094 μM of dye-labelled M13 forward primer (reference), 0.10 μM of reverse primer, 1 x PCR buffer, 0.1 unit of *Taq* DNA polymerase (Fisher Biotech) and ~25 pg of genomic DNA, resolved on a Beckman *CEQ™8000*.

PCR was performed in a Perkin-Elmer *GeneAmp 2700/9700* thermocycler, using a basic amplification cycle (15s at 94°C, 40 cycles of 94°C for 15s, 50-60°C for 15s, 72°C for 20s), with modifications to annealing temperature as per Schmidt et al. (2006) for loci *Minμ S0001A-Minμ S0050*. Annealing temperature for *Minμ S00074*was 50°C.

To minimise mis-scoring, all samples were genotyped from a minimum of two independent PCR reactions/locus. Genotyping error rates for individual loci were calculated as the proportion of genotypes from the initial trial (when sample ID was known) that disagreed with genotypes from the double-blind replication (n ranging from 25-33 for individual loci).

Data Analysis

Descriptive Statistics

Polymorphic information content (PIC) and null allele frequency estimates were calculated for each locus using submodules of *CERVUS version 2.1* (Marshall et al.

1998). Observed (H_O) and expected (H_E) heterozygosity and linkage disequilibrium were assessed using *GENEPOP on the web* (http://wbiomed.curtin.edu.au/genepop/; Raymond & Rousset 1995a), employing the Markov-Chain method for loci with greater than five alleles and Fisher's exact method for loci with less than five alleles (Raymond & Rousset 1995b). Since the test panel of individuals used to calculate these descriptive parameters are not from a single natural population however, deviation from Hardy-Weinberg equilibrium is expected and results of tests such as null allele frequencies should be interpreted with caution.

Average probability of identity (PI_{ave}) values were calculated using *API-CALC 1.0* (Ayres & Overall 2004), with adjustment for F_{IS} and F_{ST} and estimated proportions of related individuals and modification of the dataset to include only one individual for each unique haplotype. Estimators of F_{IS} and F_{ST} (Weir & Cockerham 1984) were calculated using *GENEPOP on the web*. Estimated proportions of parent-offspring (0.08), full sibling (0.00), half-sibling (0.20) and cousin (0.05) relationships were made with reference to the current breeding database (C. McConchie, CSIRO Plant Industry).

Inbreeding and Coancestry

As a general investigation of inbreeding and co-ancestry among cultivars, inbreeding (F_{IS}) and co-ancestry (F_{ST}) coefficients (Wright 1965) were calculated using *GENEPOP on the Web* (http://wbiomed.curtin.edu.au/genepop/). For the purposes of these analyses, each cultivar was treated as a distinct sub-population. In addition to standard F-statistics, the parallel Γ_{IS} and Γ_{ST} parameters, which incorporate a stepwise mutation model considered more appropriate for microsatellite markers (Slatkin 1995), were also calculated using *GENEPOP on the Web.*

In interpretation of results, we follow the convention that negative values of F_{IS}/Γ_{IS} indicate heterozygote excess suggestive of outbreeding and positive values indicate heterozygote deficiency, indicative of inbreeding. Similarly, F_{ST}/Γ_{ST} is interpreted as a measure of the reduction of heterozygosity in sub-populations as a result of drift, where the minimum value of 0 indicates no subdivision and the maximum value of 1 indicates complete isolation of sub-populations (values up to 0.05 are considered negligible, while values above 0.25 are interpreted as indicating strong differentiation).

Relatedness

Relatedness between individual specimens, and groups of cultivars, was evaluated through calculation of unbiased *R*-values, using *Relatedness Version 5.0* software (Queller & Goodnight 1989), which implements the formula:

$$R = \left[\sum_x \quad \sum_k \quad \sum_l \left(P_y - \quad P^* \right)\right] \div \left[\sum_x \quad \sum_k \quad \sum_l \left(P_x - \quad P^* \right)\right]$$

Where: x indexes individuals in the data set, k indexes loci and l indexes allelic position. P_x is the frequency (within the current x individual) of the allele found at locus k, allelic positon 1; P_y is the frequency of that same allele in the set of pairwise comparisons and P^* is the frequency of the allele in the population at large. Standard errors of R were obtained by jacknifing across loci. To maximise accuracy in calculation of relatedness among replicated samples, all samples missing data at more than two loci were deleted prior to calculation of relatedness.

Further analysis of structure and relatedness was undertaken using *Kinship Version 1.2* (Queller & Goodnight 1989; Queller et al., 1993). The likelihood calculation module of the *Kinship 1.2* program was used to test for pedigree relationships between individuals in the data set corresponding to $R = 1.00$, $R = 0.50$, $R = 0.25$ and $R = 0.125$ ($p \leq 0.001$).

As *Kinship 1.2* cannot distinguish between relationships with similar *R*-values (e.g. mother-young and maternal half-siblings), kinship tests were primarily undertaken to identify closely related individuals/cultivars and potential mislabelling of specimens, while more distant relationships were examined as a means of identifying individuals sharing common ancestry.

The method of testing is based upon calculation of probabilities of allele-sharing, using the sampled allele frequencies as a reference point. Pairwise comparisons are then conducted for all individuals in the data set and relationships are marked as significant at the $p<0.05$, $p<0.01$ and $p<0.001$ levels.

To provide a visual summary of relatedness between cultivars, the matrix of average *R*-values were assembled into a phylogeny using the neighbour-joining algorithm implemented in *MEGA version 3.1* (Kumar, Tamura and Nei 2004).

Results

Descriptive Statistics

Primer sets for both *MinμS0016* and *MinμS0048* amplified multiple loci (Schmidt et al. 2006) Appendix 9.1, (Table 9.1). In the case of *MinμS0048*, the second locus was monomorphic (Fig. 9.1) and was not included in any further analysis. The two independent loci amplified by the *MinμS0016* primers are designated *MinμS0016a* and *MinμS0016b*.

Due to detection of an unacceptably high (0.13) error rate for locus *MinuS0050*, this locus was removed prior to further analysis. Error rates for the remaining loci ranged from 0.023 (*MinuS0048*) to 0.051 (*MinuS0001A*), with an average of 0.037 (Table 9.3). Visual examination of the data indicated that the majority of incidences of mistyping involved scoring of individuals as heterozygotes in one instance, and homozygotes in another, rather than shifts from one allele to another.

Initial detection of variation among replicate trees for several cultivars (246, 344, 741, 762, 772, 783, 791 and A16) led to re-examination of planting records. This allowed identification of seven cases (1 x 246, 1 x 344, 1 x 741, 1 x 762, 3 x A16) where the original tree had died and been replaced with an unknown variety (not otherwise included in the investigation). These trees were retained in the overall dataset, but not included in calculation of relatedness within cultivar samples.

A further six trees (1 x 762, 1 x 772, 1 x 783, 1 x 791, 2 x 816) showed significant variation among replicates (≥ 2 haplotypes with variation at ≥ 3 loci). Where the variation was distinct from other replicates for that cultivar, these were also excluded from calculation of relatedness.

A number of other cultivars showed variation between replicates involving one or two loci/samples. Where sample size was insufficient to determine the correct cultivar haplotype (660, 741, 781, 783, 791, 816, 828, 849, A16, Yonik), the variant trees were retained for calculation of relatedness.

Observed hetozygosity ranged from 0.100 (*MinμS0002*) to 0.953 (*MinμS0007*), with an average of 0.421 (Table 9.3). Expected heterozygosity ranged from 0.252 (*MinμS0003*) to 0.776 (*MinμS0007*), with an average of 0.550 (Table 9.2). PIC values ranged from 0.236 (*MinμS0003*) to 0.746 (*MinμS0007*) and averaged 0.483

(Table 9.2). No pairwise comparisons of loci revealed significant linkage disequilibrium.

The cumulative PI_{ave} across all 10 loci was 1.03×10^{-6}. Observed single-locus values for individual loci ranged from 0.056 (*MinμS0007*) to 0.804 (*MinμS0016b*) (Table 2).

Inbreeding and Co-ancestry

Both standard and microsatellite specific F-statistics indicated substantial subdivision among cultivars (Table 9.3). The extent of inbreeding indicated by the microsatellite-specific measure ($\Gamma_{IS} = 0.202$) was however, substantially greater than that indicated by the standard measure ($F_{IS} = 0.130$).

A similar pattern was observed in relation to subdivision (Table 9.3), with the microsatellite specific measure ($\Gamma_{ST} = 0.715$) indicating a greater extent of subdivision than the standard measure ($F_{ST} = 0.301$).

Relatedness

Average relatedness among all samples included in the analysis was 0.004 ± 0.36. No samples of known identity showed 100% identity ($R = 1.00$, $p \leq 0.001$) with other samples of known identity.

Of 30 cultivars represented by multiple samples, 15 (246, 333, 344 772, 794, 804, 814, 842, A38, A4, A99, Beaumont, D4, N635, X29) showed 100% identity ($R = 1.00$, $p \leq 0.001$) in all replicated samples (Table 1). A further 12 (660, 762, 781, 783, 791, 816, 828, 835, 849, Daddow, NRG43, Own Venture) showed variation consistent with the presence of missing data for some samples/loci and an overall genotyping error rate of ~3%, generating R-values ranging from 0.85 ± 0.11 to 0.97 ± 0.04 (Table 9.2). Replicated samples of three cultivars (741, A16, Yonik) showed variation greater (≥ 2 haplotypes, with variation across ≥2 loci) than could be attributed to these causes (Table 9.1).

Significant familial ($R = 0.50$, $R = 0.25$, $R = 0.125$; $p \leq 0.001$) relationships were detected between a number of cultivars (Table 1). Despite considerable genetic variation between replicated samples of A16, the known sibling relationship with A4 (Hardner et al. 2000) was confirmed for one A16 individual (Table 9.2).

Neighbour-joining clustering identified two major clades, representing derivations from 246 and *Renown*/*Own Choic*e (Fig. 9.2).

Discussion

Early attempts to isolate and characterize microsatellite loci for macadamia generated very small numbers of sequence-tagged loci, with limited polymorphism and reliability (Vithanage et al., 1999; Peace et al., 2004). The development of a new suite of microsatellite loci suitable for studies of macadamia genetics was however, recently reported by Schmidt et al. (2006). This paper reports on application of these markers in identifying cultivars, as a foundation for the development and implementation of marker-facilitated selection and breeding programs. To facilitate this goal, specific emphasis has been placed on verification of reliability and accuracy of genotyping procedures and quantitative analysis of data.

At <5%, genotyping error rates for microsatellite markers in macadamia are comparable to those reported in other studies of crop varieties (Schnell, Brown & Olano 2006; Zhang et al., 2006) and the procedures used in this study allowed identification and removal of one problematic locus. When trees were accurately identified in the field, no samples showed 100% identity ($R = 1.00$, $p \leq 0.001$) with other samples of known identity, indicating that the ten loci employed are sufficient to establish identity. This is confirmed by the very low adjusted PI_{ave} value (1.04×10^{-6}), which demonstrates that the markers have considerable discriminatory power even in the presence of significant genetic structure resulting from founder effects/selection/inbreeding.

When genotyping error rates (~3%) are taken into consideration, resolution of identity was possible at the $p = 0.001$ level for 27 of 32 cultivars. Of the remaining five cultivars, two were represented by single samples only (precluding comparisons between replicates), while three showed more substantial variation between replicates (Table 9.2). As macadamia is clonally propagated, there are a limited number of explanations for this variation. The lack of similar results in previous studies (Aradhya et al., 1998; Peace 2004) is not problematic as these are all based on single samples for individual cultivars.

The most likely explanation for the unexpected variation is misidentification/mislabelling of samples. The double-blind sampling procedure helps to control for this during genotyping and in fact demonstrates the utility of the markers in detecting substitution of cultivars in cases where dead trees (246, 333, 741,

762, 772, A16) had been replaced with unidentified cultivars. The additional variation may therefore be the result of unrecorded substitutions.

It is however, also possible that biological factors have contributed. Studies of natural populations of clonal plants suggest that variation accumulates over time. When studying clonal populations of trembling aspen (*Populus tremuloides*), Wyman, Bruneau and Tremblay (2003) found that morphological assessments of clonal structure greatly underestimated clonal diversity and application of four microsatellite loci revealed twice as many haplotypes as expected. A similar study of genetic diversity and ploidy levels of 10 populations of *Ranunculus carpaticola* in central Slovakia found that marker resolution was a significant factor. In this case, addition of two microsatellite markers to an AFLP data set substantially increased the number of clonal lineages detected (Paun et al., 2006).

It is also interesting to note that macadamia is a grafted crop. There has been little or no study of the genetic and epigenetic effects of combining genotypically distinct entities in this manner. It is possible that grafting generates chimaeric plants, as has been detected in wine grapes (Regner, Hack & Santiago 2006). At this stage however, misidentification of samples is a more likely explanation.

Despite the variation within cultivars, analysis of relatedness and structure indicates a relatively robust clade comprised of lines derived from the early Australian varieties, Renown and Own Choice (Fig. 9.2). The relatedness measure used to classify individuals and their relationships is conceptually similar to other similarity indices used in studies of cultivar diversity, but has the additional advantage of being weighted for allele frequency. This is of particular importance when repeated selection, inbreeding and founder effects generate deviation from assumptions underlying analytical methodology. Like most metrics, the performance of R depends on a range of factors, including the number of loci and alleles, the shape of the allele frequency distribution, (Queller and Goodnight 1989; Ritland 1996; Lynch and Ritland 1999; Csilléry et al. 2006) and sampling variance (Van de Casteele et al. 2001). The utility of R as a distance metric in studies of cultivar relatedness is demonstrated in this case by 'correct' classification of replicates to the $p \leq 0.001$ level for 27 cultivars, detection of known pedigree relationships and the lack of any significant cases of misidentification of correctly labeled samples ($R = 1.00$, $p \leq 0.001$).

Overall, the results illustrate the relative power of microsatellites in resolution of proximal relationships among individuals. Not only do microsatellites detect variation not revealed with dominant (p/a), or even allozyme, markers by encoding more information per unit marker**,** the information that they provide is more reliable because locus-specific primers reduce technical issues associated with generation of data and estimation of genotyping error and the information that they provide can be analysed in rich theoretical/statistical frameworks that are unparalleled for dominant markers. In relation to macadamia in particular, the detection of variation among replicate cultivar samples, and subsequent confirmation of misidentification in the field, highlights the importance of establishing and monitoring sample identity throughout development and implementation of marker assisted selection for commercial and research programs.

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References

Aradhya MK, Yee LK, Zee FT, Manshardt RM (1998) Genetic variability in Macadamia. Genet Resources Crop Evol 45:19-32

Aradhya MK, Dangl GS, Prins BH, Boursiquot J-M, Walker MA, Meredith CP, Simon CJ (2003) Genetic structure and differentiation in cultivated

grape, *Vitis vinifera* L. Genetical Res Camb 81:179-192

Ayres KL, Overall ADJ (2004) api-calc 1.0: a computer program for calculating the average probability of identity allowing for substructure, inbreeding and the presence of close relatives. Mol Ecol Notes 4:315-318

Cavagnaro PF, Cavagnaro JB, Lemes JL, Masuelli RW, Passera CB (2006) Genetic diversity among varieties of the native forage grass *Trichloris crinita* based on AFLP

markers, morphological characters, and quantitative agronomic traits. Genome 49:906-918

Cavalli-Sforza LL, Edwards AWF (1967) Phylogenetic analysis: Models and Estimation Procedures. Evolution 21:550-570

Chenuil A (2006) Choosing the right molecular genetic markers for studying biodiversity: from molecular evolution to practical aspects. Genetica 127:101-120

Csilléry K, Johnson T, Beraldi D, Clutton-Brock T, Coltman D, Hansson B, Spong G, Pemberton JM (2006) Performance of Marker-Based Relatedness Estimators in Natural Populations of Outbred Vertebrates. Genetics 173:2091-2101

Danforth BN, Freeman-Gallant CR (1996) DNA fingerprinting data and the problem of non-independence among pairwise coparisons. Mol Ecol 5:221-227

Darvasi A, Pisante-Shalom, A. (2002) Complexities in the genetic dissection of quantitative trait loci. Trends Genet 18:489-491

Destro-Bisol G, Spedini G, Pascali VL (2000) Application of different genetic distance methods to microsatellite data. Hum Genet 106:130-132

George J, Dobrowolski MP, van Zijll de Jong E, Cogan NOI, Smith KF, Forster JW (2006) Assessment of genetic diversity in cultivars of white clover (*Trifolium repens* L.) detected by SSR polymorphisms. Genome 49:919-930

Ghislain M, Andrade D, Rodríguez F, Hijmans RJ, Spooner DM (2006) Genetic analysis of the cultivated potato *Solanum tuberosum* L. Phureja Group using RAPDs and nuclear SSRs. Theor Appl Genet 113:1515-1527

Goldstein DB, Linares AR, Cavalli-Sforza LL, Feldman MW (1995) An evaluation of genetic distances for use with microsatellite loci. Genetics 139:463-471

Graham J, Curran J, Weir BS (2000) Conditional genotypic probabilities for microsatellite loci. Genetics 155:1973-1980

Gross CL (1995) Macadamia. Flora of Australia 16:419-425

Hardner C, McConchie CA, Vivian-Smith A, Boyton S (2000) Hybrids in macadamia improvement. H. S. Dungey, M. J. Dieters, and D. G. Nikles, eds. Hybrid Breeding and Genetics of Forest Trees: Proceedings of the QFRI/CRC-SPF Symposium:472-476.

Hedrick PW (1999) Perspective: Highly variable loci and their interpretation in evolution and conservation. Evolution 53:313-318

Johnson LAS (1954) *Macadamia ternifolia* F. Muell. and a related new species. Proc Linn Soc NSW 79:15-18

Kirst M, Cordeiro CM, Rezende GDSP, Grattapaglia D (2005) Power of Microsatellite Markers for Fingerprinting and Parentage Analysis in *Eucalyptus grandis* Breeding Populations. J Hered 96:161-166

Kumar S, Tamura K, Nei M (2004) MEGA3: Integrated software for Molecular Evolutionary Genetics Analysis and sequence alignment. Brief Bioinform 5:150-163

Lynch M, Ritland K (1999) Estimation of Pairwise Relatedness With Molecular Markers. Genetics 152:1753-1766.

Mariac C, Robert T, Allinne C, Remigereau MS, Luxereau A, Tidjani M, Seyni O, Bezancon G, Pham JL, Sarr A (2006) Genetic diversity and gene flow among pearl millet crop/weed complex: a case study. Theor Appl Genet 113:1003-1014

Marshall TC, Slate R, Kruuk LEB, Pemberton JM (1998) Statistical confidence for likelihood-based paternity inference in natural populations. Mol Ecol 7:639-635

McConchie CA, Hardner C, Vithanage B, Campbell A, Mayers P (1999) Macadamia improvement by breeding MC96002. Horticultural Research & Development Corporation, Gordon, N.S.W.

Nagamine Y, Higuchi M (2001) Genetic distance and classification of domestic animals using genetic markers. J Anim Breed Genet 118:101-109

Nei M, Maruyama T, Wu C-I (1983) Models of evolution of reproductive isolation. Genetics 103:557-579

Paetkau D, Waits LP, Clarkson PL, Craighead L, Strobeck C (1997) An Empirical Evaluation of Genetic Distance Statistics Using Microsatellite Data From Bear (Ursidae) Populations. Genetics 147:1943-1957

Paun O, Greilhuber J, Temsch EM, Hörandl, E (2006) Patterns, sources and ecological implications of clonal diversity in apomictic *Ranunculus carpaticola* (*Ranunculus auricomus* complex, Ranunculaceae). Mol Ecol 15:897-910.

Peace CP (2004) Genetic Caharcterisation of Macadamia with DNA Markers. PhD thesis, University of Queensland, Brisbane.

Peace CP, Vithanage V, Turnbull CGN, Carroll BJ (2003) A genetic map of macadamia based on randomly amplified DNA fingerprinting (RAF) markers. Euphytica 134:17-26

Peace CP, Vithanage V, Neal J, Turnbull CGN, Carroll BJ (2004) A comparison of molecular markers for genetic analysis of macadamia. J Hort Sci Biotech 79:965-970

Queller DC, Goodnight KF (1989) Estimating relatedness using genetic markers. Evolution 43:258-275

Queller DC, Strassman JE, Hughes CR (1993) Microsatellites and kinship. Trends Ecol Evol 8:285-288

Ritland K (1996) Estimators for pairwise relatedness and individual inbreeding coefficients. Genetical Research 67:175-185.

Raymond M, Rousset F (1995a) GENEPOP (Version 1.2): Population genetics software for exact tests and ecumenicism. J Heredity 86:248-249

Raymond M, Rousset F (1995b) An exact test for population differentiation. Evolution 49:1280-1283

Regner F, Hack R, Santiago JL. 2006. Highly variable *Vitis* microsatellite loci for the identification of Pinot Noir clones. Vitis 2:85-91.

Schmidt AL, Scott L, Lowe AJ (2006) Isolation and characterization of microsatellite loci from Macadamia. Mol Ecol Notes 6:1060-1063

Schmidt AL, Gale KR, Ellis MH, Giffard PM (2004) Sequence variation at a microsatellite locus (XGWM261) in hexaploid wheat (*Triticum aestivum*) varieties. Euphytica 135:239-246

Schnell R, Brown J, Olano C (2006) Mango Genetic Diversity Analysis and Pedigree Inferences for Florida Cultivars Using Microsatellite Markers. J Amer Soci Hort Sci 131:214-224

Selkoe KA, Toonen RJ (2006) Microsatellites for ecologists: a practical guide to using and evaluating microsatellite markers. Ecol Lett 9:615-629

Slatkin M (1995) A measure of population subdivision based on microsatellite allele frequencies. Genetics 139:457-462

Smith LS (1956) New species of and notes on Queensland plants. Proc Roy Soc Qld 67:29-40

Smouse PE, Chevillon C (1998) Analytical aspects of population-specific DNA fingerprinting for individuals. J Hered 89:143-150

Takezaki N, Nei M (1996) Genetic distances and reconstruction of phylogenetic trees from microsatellite data. Genetics 144:389-399

Van De Casteele T, Galbusera P, Matthysen, E (2001) A comparison of microsatellite-based pairwise relatedness estimators. Mol Ecol 10:1539-1549.

Vithanage V, Winks CW (1992) Isozymes as genetic markers for Macadamia. Scientia Hort 49: 103-115

Vithanage V, Peace C, Thomas MR, Anderson KL (1999) Development and utility of molecular markers of macadamia. In McConchie CA, Hardner C, Vithanage B, Campbell A, Mayers P (1999) Macadamia improvement by breeding MC96002. Horticultural Research & Development Corporation, Gordon, N.S.W.

Waits LP, Luikart G, Taberlet P (2001) Estimating the probability of identity among genotypes in natural populations: cautions and guidelines. Mol Ecol 10:249-256

Weir BS, Cockerham CC (1984) Estimating F-statistics for the analysis of population structure. Evolution 38:1358-1370

Wright S (1965) The interpretation of population structure by F-statistics with special regard to systems of mating. Evolution 19:395-420

Wyman J, Bruneau A, Tremblay MF. 2003. Microsatellite analysis of genetic diversity in four populations of *Populus tremuloides* in Quebec. Canadian Journal of Botany 81:360-367.

Zhang D, Mischke S, Goenaga R, Hemeida AA, Saunders JA (2006) Accuracy and Reliability of High-Throughput Microsatellite Genotyping for *Cacao* Clone Identification. Crop Sci 46:2084-2092

Thirty-three microsatellite loci were isolated for *Macadamia integrifolia* (Schmidt et al 2006).
Table 9.1: *Macadamia* microsatellite loci

Appendix 9.1

Annealing temperature (T_a), product size (in bp) and Genbank Accession numbers are also given for each locus. Loci with two T_a were amplified using five cycles at the lower T_a, followed by 35 cycles at the higher T_a. Primer sets for *MinμS00016* and *MinμS00044* amplify duplicated loci.

Locus ID	*Forward primer*	*Reverse Primer*	*Repeat Motif*	*Product Size*	*T_a*	*Genbank Accession Number*
MinμS0001a	TAGCACCCATGACTGAATTCC	CCCTCTTATTGTTGCAGCTC	$CTC(CT)_2CCCTTCCCTCTTC$	197-310	52	**DQ412959**
MinμS0001b	CTAGCATAAGGCCATTGAGC	CCCTCTTATTGTTGCAGCTC	$(AGG)_2GAG(GGA)_2(GA)_2GGA$	108-158	50	**DQ412959**
MinμS0002	AGTGGAGAAGTGACTTGCAC	ACAAAGATGGCAATGCGAGG	$(CA)_6$	132-232	50	**DQ412964**
MinμS0003	TGGCAAGGGTTGTAGCACCAG	AGGGTGACCAACGGTTGATG	$(CA)_4TAT(CA)_{20}$	122-134	53	**DQ412962**
MinμS0004	GTGGACAGTACGAGATATCAAATG	TTGAGGAATGAGAGGGCAAGG	$(GT)_{16}$	112-158	53	**DQ412963**
MinμS0005	GCTTCAAAGACGGACGATCC	GAGGTATGTGTAATTCTCTCC	$(GA)5(AGA)_4$	172-200	50	**DQ412970**
MinμS0007	CTGATTTATGATGGTAAAGGAC	GGTGAATCAAAGATTAGACAAC	$(GA)_{11}$	112-+172	50	**DQ412971**
MinμS0008	GAACTCTAGAAGTCGAAGCAC	ACCTCTCCACCATGCATTAC	$(GAA)_4ATTACAGAAGA$	184	50	**DQ412966**
MinμS0012	GGGAGTGGATGTAGATGAAG	AAAGTTTCGTTGGGGGTAGG	CAGATATGAAGAGAAGAGAG	72-124	50	**DQ412968**
MinμS0013	CTATAACAGATATTACACCCTC	CGAATCAATGTCGCGATACC	$(CA)_4CCCCC$	144-178	48, 50	**DQ412967**
MinμS0014	GGTATCGCGACATTGATTCG	AGAGGGGGTGATACTGATTC	$A(T)_9(TATTTT)_2TATTT$	140-149	53	**DQ412967**
MinμS0015	TGGCTATGCTTTGATCCCTTG	GACTACTGCTTTGGGTGGTA	$AA(AG)_2(GA)_3$	150	50	**DQ412969**
MinμS0016	AGCAGGTAGAGAAGGCAATC	AACCAACCCGTACAGAACCC	$(GA)_4GCGTTGAA(GA)_3AGAAGAGGA$	146-176, 182-208	50	**DQ412969**
MinμS0017	ACTTAAATGAAGTTTCAGCTAGCCCC	GACTTATACCTCAAAAATAAGAGGTCC	$(C)_{13}(A)_{10}$	88-134	52	**DQ412972**
MinμS0020	CACACCACAGACCCCCCA	TCCTCCGATAAGCAAGAGCA	$(CCA)_8(CCTCCA)_2$	75-103	48, 50	**DQ412973**
MinμS0021	ACCATTCCGACATTGACAGG	GTTGACAAATGGAAATCCATC	$CCTTCATTC(CTTT)_2CT$	100	50	**DQ412946**
MinμS0025	CACGGCATAGCAGGCACAGA	TGATCCTTCAGCTTACCTCC	$(GCA)_{44}$	202-270	53	**DQ412965**
MinμS0029	AGTTGCATTCACAGGCTCAC	CGCGTGATGTATATGATCCAG	$(GA)_{27}$	74-122	46, 50	**DQ412955**
MinμS0030	GCAAGAGCACAATCATCTCATAC	TTCGACTGTCAACCACACCAG	$(GA)_{18}$	106-185	48, 53	**DQ412954**
MinμS0032	GCGTAAGCAAGAGCACAC	TAAGGAAAGATCGCGACCAC	$(CAG)_{24}$	189-192	50	**DQ412948**
MinμS0033	GTCCACGCGTAAGCAAGAGC	CGCTCATATTGGAGATGGTG	$(CAC)_{12}(CAT)_6$	182-246	50, 55	**DQ412956**
MinμS0037	TGTTGTAGAACGGGGTTCAC	TGTTTTCTCAGTCGCGATGG	$(GGT)_6GTT(GGT)_7$	124-146	46, 50	**DQ412953**
MinμS0038	CAGCAGCAGCAACAACCACCTG	AAACTCAATACCGAGGAAGC	$(ACC)_2(ACA)_2(GCA)_{11}$	101-116	50	**DQ412952**
MinμS0040	TAAGCAAGAGCACAGGGCAG	ACTCATAAGAGACCACGCCC	$TG(GGT)_{14}$	143-199	53	**DQ412950**
MinμS0044	AAAGCACACACCAGATGTGG	GAGCGGGAATCCAAAAGATG	$(GGT)_6$	187-286	46, 50	**DQ412957**
MinμS0047	GGAGAAAGGATGGAGATGTG	TCTGGTTCGGAGAAGTCTAC	$(GGT)_5$	140-170	48	**DQ412951**
MinμS0048	GAGCACAAGCGGATACATTC	AGAGCACAGGTGACAATAGG	CATCAACCAAACACA	168-195	50	**DQ412947**
MinμS0049	ATGGACTTGAAGTCTGCAGC	TAAGCAAGAGCACAGAGCAG	$GAAA(GAA)_2(AGG)_2AGAAG$	226	50	**DQ412949**
MinμS0050	GAGCACAATTGCATCAGCATC	TGGAGGGTACAGGTATAGAC	$(GA)_7$	102-122	50	**DQ412961**
MinμS0052	GAGTGCTTGTCGACGAATTC	CAGGCCCATCTTGTATACTG	$(CT)_4CC(CT)_{13}$	120-258	48, 50	**DQ412958**
MinμS0053	**TTAGTCCACGCGTAAGCAAC**	**GGGGGTGGAATATACTTTCC**	**$(AG)_6$**	**83**	**50**	**DQ412960**

Table 9.2: Relatedness Testing. Relatedness was tested through calculation of average pairwise R-values (Average R). The number of haplotypes (represented as A, B, C, D) detected within each sample (n) is recorded, along with the number of loci at which variation was detected. The number of pairwise comparisons within each sample (nc) varied with sample size (n). The number of pairwise comparisons within each sample that showed 100% identity (R = 1.00, $p < 0.001$) is shown as In Group R = 1.00. Proximal relatedness between cultivars was investigated through testing for R= 0.50, R = 0.25 and R= 0.125 ($p < 0.001$) relationships.

Cultivar	***N***	***Haplotypes***	***No. Var. 4.1***	***4.1***	***Nc***	***In Group R=1.00***	***R=0.50***	***R=0.25***	***R=0.125***
246	5	5 x A	0	1.00±0.00	10	10	842		
333	2	2 x A	0	1.00	1	1	835		
344	3	3 x A	0	1.00±0.00	3	3		H2	
660	2	2 x A, 1 x B	1	0.85±0.11	2	1	741, 762		
741	2	1 x A, 1 x B	3	0.29±0.03	1	0		H2, 660	
762	4	4 x A	0	0.95±0.03	6	1	660, 772, H2	828	
772	4	4x A	0	1.00±0.00	6	6	828, A199	A16	**762**
781	6	4 x A, 2 x B	1	0.83±0.16	15	7	783, 794, 814, 849		
783	5	3 x A, 2 x B	1	0.74±0.17	10	2	781, 804, 828		
791	4	2 x A, 1 x B	2	1.00±0.12	6	1			
794	5	5 x A	0	1.00±0.00	15	15	814, 781		
804	5	5 x A	0	1.00±0.00	15	15	783, 849, A99		
814	5	5 x A	0	1.00±0.00	15	15	781, 794		
816	3	3 x A	-	0.74±0.18	3	1	835, 842		
828	5	3 x A, 1 x B, 1 x C	2	0.80±0.15	10	3	762, 772, 783		
835	5	5 x A	0	0.97±0.04	10	3	333, 816		
842	6	6 x A	-	1.00±0.00	15	15	246, 816		
849	3	2 x A, 1 x B	1	0.82±0.13	3	1	849, 781, 804		
856	1	1 x A	-	-	0	-			**A4**
A4	4	4 x A	0	1.00±0.00	6	6	A16, D4		
A16	3	1 x A, 1 x B, 1 x C	7	0.23±0.03	3	0	A4, A199, 772, D4	NRG43	
A38	2	2 x A	0	1.00	1	1		D4	
A99	2	2 x A	0	1.00	1	1	804		
A199	1	1 x A	-	-	1	1	A16, 772		
Beaumont	2	1 x A, 1 x B	1	1.00±0.00	1	1			
D4	2	2 x A	0	1.00	1	1	Daddow, A16		
Daddow	5	5 x A	0	0.94±0.05	10	3	D4	NRG43	
H2 rootstock	3	1 x A, 1 x B, 1 x C	5	0.34±0.27	3	0		344, 741, 762	
M. jansenii	4	1 x A, 1 x B, 1 x C, 1 x D	9	0.15±0.21	6	0			
N635	2	2 x A	0	1.00	1	1	NRG43		
NRG43	2	1 x A, 1 x B	1	0.92	1	0	N635		
Own Venture	5	4 x A, 1 x B	1	0.95±0.07	10	6			
X29	2	2 x A	0	1.00	1	1			
Yonik	**2**	**1 x A, 1 x B**	**4**	**0.67**	**1**	**0**			

Table 9.3: Locus summary Information. Allele number (No. Alleles), observed (H_O) and expected (H_E) heterozygosity, polymorphic information content (PIC) and null allele frequency estimates (Null) are shown for all loci along with estimated genotyping error rates (Error) and observed single-locus probability of identity (PI_{ave}). In addition to standard estimates of F_{IS} and F_{ST}, Γ_{IS} and Γ_{IT} equivalents were calculated under a model incorporating stepwise mutation. Note that null allele frequency estimates in particular are unlikely to be inaccurate as cultivar samples do not represent a natural population in Hardy-Weinberg equilibrium. Entries in bold type in the final row are mean values, except in the case of PI_{ave}, where the entry is the cumulative probability of identity across all ten loci. Data for locus *MinμS0050* are not presented because this locus showed an unacceptably high rate of genotyping error.

Locus	*No. Alleles*	H_O	H_E	*PIC*	F_{IS}	Γ_{IS}	F_{ST}	Γ_{ST}	*Null*	PI_{ave}	*Error*
MinμS0001A	8	0.183	0.739	0.708	0.348	-0.405	0.629	0.747	0.611	0.132	**0.051**
MinμS0002	4	0.100	0.615	0.542	0.745	0.625	0.475	0.729	0.716	0.305	**0.032**
MinμS0003	4	0.276	0.252	0.236	0.251	-0.571	0.107	0.304	-0.050	0.398	**0.026**
MinμS0004	17	0.289	0.628	0.605	0.406	0.164	0.388	0.372	0.373	0.172	**0.046**
MinμS0005	4	0.103	0.315	0.287	0.656	0.028	0.367	0.980	0.499	0.462	**0.040**
MinμS0007	15	0.824	0.776	0.746	-0.144	-0.635	0.244	0.310	-0.031	0.056	**0.029**
MinμS0016a	6	0.315	0.693	0.633	0.403	0.610	0.386	0.778	0.373	0.166	**0.038**
MinμS0016b	3	0.953	0.506	0.381	-0.348	-0.879	-0.048	0.004	-0.309	0.804	**0.043**
MinμS0048	3	0.750	0.475	0.361	-0.394	-0.863	0.080	0.154	-0.227	0.435	**0.023**
MinμS0074	2	0.419	0.499	0.373	0.164	-0.364	0.242	0.391	0.085	0.254	**0.037**
Average	7	0.421	0.550	0.483	0.130	0.202	0.301	0.715	0.204	1×10^{-6}	0.037

Figure 9.1: Autoradiographs of Microsatellite Loci. Note that primers for both MinμS0016 (panel g) and MinμS0048 (panel h) amplified multiple loci. In the case of MinμS0048, the second locus (upper band) was monomorphic and was not included in further analysis.

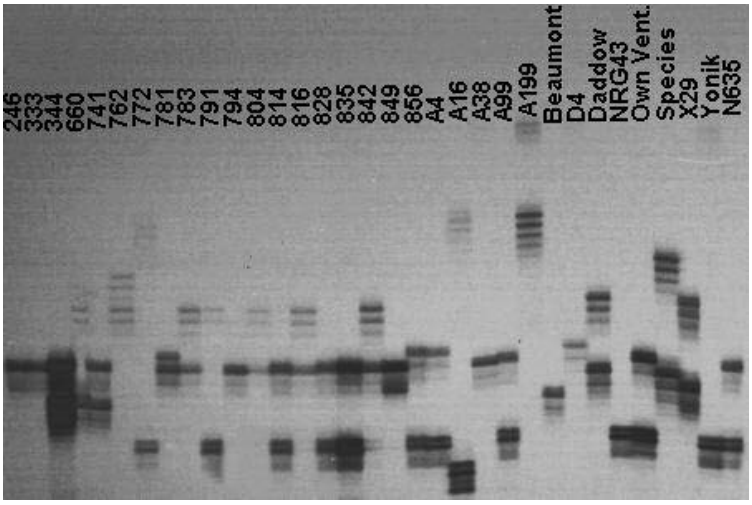
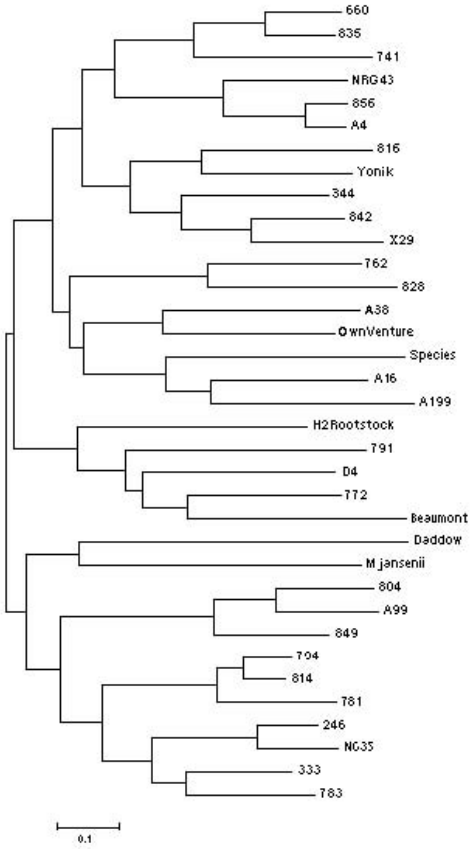


Figure 9.2: Intercultivar Relatedness.

Neighbour-joining summary of intercultivar relatedness, based on R-values calculated from genotyping at ten microsatellite loci. Bootstrap values are not presented because R-values are jacknifed across loci during calculation.



	Female Parent	Female Species	Source	Aus. Growing Era
660		integrifolia	Hawaii (Lawai)	1970-1980
835	246	integrifolia	Hawaii	>1980
741		integrifolia	Hawaii (Lawai)	1970-1980
NRG43			Australia	
856	685		Hawaii	
A4	Renown	Hybrid	Australia (Amamoor)	>1980
816	666	integrifolia	Hawaii	>1980
Yonik			Israel	
344	Kau	integrifolia		
842		integrifolia	Hawaii	>1980
X29	Greber	Hybrid	Australia (Beerwah)	>1980
762		integrifolia	Hawaii	>1980
828	246	integrifolia	Hawaii	>1980
A38	Own Choice	integrifolia	Australia (Beerwah)	>1980
OwnVenture	Own Choice	integrifolia	Australia (Beerwah)	1970-1980
Species				
A16	Renown	Hybrid	Australia (Amamoor)	>1980
A199	Renown	Hybrid	Australia (Amamoor)	>1980
H2Rootstock		integrifolia	Australia	<1970
791		integrifolia	Hawaii	>1980
D4		integrifolia	Thailand	
772	Own Choice	integrifolia	Hawaii	>1980
Beaumont		tetraphylla	Australia	>1980
Daddow		integrifolia	Australia	<1970
M jansenii			Australia	
804	246	integrifolia	Hawaii	>1980
A99			Israel	
849		integrifolia	Hawaii	>1980
704		integrifolia	Hawaii	>1980
814		integrifolia	Hawaii (Honokaa)	>1980
781		integrifolia	Hawaii	>1980
246		integrifolia	Hawaii (Kona)	<1970
NG35				
333		integrifolia	Hawaii (Mt Tantalus)	1970-1980
783	246	integrifolia	Hawaii	>1980

Chapter 10
Prediction of individual Genetic Value from B1.1 progeny trials

Introduction

Three progeny trials were established at 2 sites for the selection of candidate cultivars based on an index called Total Genetic Value (TGV). The index incorporates all traits of interest. However, selection gain is limited to a combination of the variation in those traits for which heritable variation was detected with the relative economic weighting of that trait. The net effect being that only two traits had a large effect on the TGV. These traits were canopy width at year 5 and modelled yield to year 8. Because of the way yield to year 8 was modelled, there are some unfortunate negative correlations between this trait and age at first crop, total kernel recovery, canopy width at year 5 and weight of sound kernel. Selected candidate cultivars therefore tend to be somewhat slower to come into crop but have very high yields in later years.

Method

Trials

Location

Two trials (BQBRS97, BQBRS98) were established in Bundaberg and a single trial in NNSW (BTFRS98), to evaluate progeny for selection as candidate cultivars in replicated regional variety trials and to identify elite parents of a new breeding generation.

Pedigree

94 Progeny

The progenies evaluated in BQBTRS97 were from crossings made prior to the commencement of the improvement program in September 1993 and germinated in 1994 (94 progeny). This progeny was produced by crossing 6 parents (246, 814, 816, A16, A4 and Own Venture) in a near complete diallel (selfs excluded) (Table 10.1) .

95 progeny

The progeny evaluated in trials BQBRS98 and BTFRS98 were from crossings made in September 1994 and germinated in 1995 (95 progeny). This progeny was produced by crossing in a complete diallel 5 of the parents used to produce 94progeny (246, 814, 816, A16 and A4) with 6 other parents (344, 660, 781, 842, 849, Daddow) (Table 10.2).

Other genetic entries

The 3 trials also contained other genetic entries. The plans are in Appendix A. Numbers less than 1000 are commercial cultivars and were planted within the experimental blocks. There are also progeny with prefixes 92, 93, 96 and 98. These are from nuts sown in 1992, 1993, 1996 and 1998 respectively. These were included in the trial in buffers or as replants within the experimental blocks to maintain the intended competition between trees. These progeny and progeny arising from selfing were not included in the analyses. The activities monitoring performance are shown in

Table 10.3. BQBRS97

The 3 year old seedling trees used in this trial were derived from the parental combinations listed in Table 10.1 and planted in the field in March 1997 with 345 plants in 25 incomplete blocks of 15 single tree plots. All planting rows are 5 m apart, and plants are at 2 m apart along the planting row. In addition to the seedling progenies, a replicate of one of the parents, A4, is planted throughout the trial. The trial is surrounded by at least 1 row of buffer trees (Appendix A). Details of replants and harvests are reported in milestones and final reports for earlier stages of the project. Major interventions included structural pruning to reduce wind damage in year 3 (2000) and the trial was mechanically hedged in July 2003 after the year 6 harvest. Ethephon was used to promote nut drop in 2003- 2005, years 6-8. Flowering was noted in year 3. This is the only trial in which year 4 yields were collected and were derived from nut counts due to high levels of insect and fungal damage. Flowering was widespread in year 4 leading to the harvest in 2002, year 5. Due to trees inter-growing in year 8, 2005, only the nuts lying in a 60 cm strip centred on the trunk but perpendicular were harvested at each of the harvests. Twenty trees were randomly selected and had the inner band and outer nuts harvested. The mean of the

ratio of the outer nut mass/ inner mass for those twenty trees at each harvest was calculated and used to covert the inner harvest for all the other trial trees for that harvest. However if the inner zone had no crop this value was not used in developing the harvest mean. The overall yield for 2005 was determined by summing the derived values for each harvests with the crop stripped from each tree.

Table 10. 1 Parental combination, family size planted in BQRS97

cross_year	parent1	parent2	family	BQBRS97
1994	OV	A4	94057	23
1994	OV	A16	94051	21
1994	OV	246	94008	19
1994	OV	814	94034	17
1994	OV	816	94045	17
1994	A4	A16	94052	13
1994	A4	246	94007	16
1994	A4	814	94033	17
1994	A4	816	94044	17
1994	A16	246	94006	17
1994	A16	814	94028	16
1994	A16	816	94043	18
1994	246	814	94004	16
1994	246	816	94005	18
1994	814	816	94029	18
Total				**263**

BQBRS98

The year old seedling trees were derived from the parental combinations listed in Table 10.2 and established with 875 plants in 22 blocks (Appendix B). Blocks were aligned along planting (and irrigation) lines. Blocks ranged in size from 55 plants to 25 plants.

Two planting densities were established. All planting rows are 5 m apart. 10 blocks were established with plants planted at a density of 2 m along the planting row and 10 blocks were planted with plants 4m along the planting row to evaluate the correlation between performances in the different densities. In general the trees planted at different densities were in alternating rows. Replicates of 10 cultivars used as parents (246, 781, 814, 816, 842, 849, A4, A16, Daddow and Own Venture) grafted on to H2 seedling rootstock, are planted throughout the trial. The trial was surrounded by at least 1 row of buffer trees. Details of replants and harvests are reported in milestones and final reports for earlier stages of the project. There was storm damage in year 4 and trees were hedged after harvesting in 2003, year 5. Only a vertical cut was made

on the trees in this trial. The rows that were hedged were 1&2, 5&6, 9&10, 13&14 and 17. In 2006 trees that were not hedged in 2005 were hedged. Ethephon was used in 2006 and 2007 to assist nut fal

Table 10. 2 Parental combinations, family size planted in BQRS98 and BTFRS98

cross_year	parent1	parent2	family	BQBRS98	BTFRS98
1995	Daddow	A4	95137	14	14
1995	Daddow	A16	95131	20	16
1995	Daddow	246	95001	16	19
1995	Daddow	344	95025	19	15
1995	Daddow	660	95048	12	8
1995	Daddow	781	95066	15	20
1995	Daddow	814	95087	11	4
1995	Daddow	816	95103	14	13
1995	Daddow	842	95115	13	23
1995	Daddow	849	95123	16	16
1995	A4	A16	95132	5	6
1995	A4	246	95003	10	4
1995	A4	344	95026	19	0
1995	A4	660	95049	19	15
1995	A4	781	95067	10	17
1995	A4	814	95094	9	2
1995	A4	816	95104	8	17
1995	A4	842	95118	12	5
1995	A4	849	95124	21	16
1995	A16	246	95004	25	15
1995	A16	344	95027	12	10
1995	A16	660	95051	14	6
1995	A16	781	95068	20	21
1995	A16	814	95092	17	15
1995	A16	816	95107	13	17
1995	A16	842	95116	20	17
1995	A16	849	95126	12	9
1995	246	344	95035	8	4
1995	246	660	95009	9	6
1995	246	781	95008	6	8

Table 10. 2 (continued) Parental combinations, family size planted in BQRS98 and BTFRS98

cross_year	parent1	parent2	family	BQBRS98	BTFRS98
1995	246	814	95007	9	8
1995	246	816	95012	13	15
1995	246	842	95006	6	3
1995	246	849	95005	8	14
1995	344	660	95031	4	8
1995	344	781	95030	8	4
1995	344	814	95024	10	2
1995	344	816	95029	10	5
1995	344	842	95033	11	11

cross_year	parent1	parent2	family	BQBRS98	BTFRS98
1995	344	849	95028	13	10
1995	660	781	95052	15	4
1995	660	814	95050	17	10
1995	660	816	95054	13	19
1995	660	842	95046	15	11
1995	660	849	95045	9	13
1995	781	814	95071	9	8
1995	781	816	95073	10	6
1995	781	842	95070	11	3
1995	781	849	95069	9	11
1995	814	816	95089	19	14
1995	814	842	95090	11	3
1995	814	849	95091	5	0
1995	816	842	95102	8	17
1995	816	849	95105	19	4
1995	842	849	95114	10	5
Total				**691**	**566**

BTFRS98

The three year old seedlings were derived from the parental combinations listed in Table 10.2 and established with 725 plants in 29 blocks (Appendix B). Blocks were designed to compartmentalise site variation. Blocks ranged in size from 9 plants to 28 plants. Again two planting densities were used but the low and high density plantings were planted as blocks and not alternating rows. All planting rows are 5 m apart. 10 blocks were established with plants planted at a density of 2 m along the planting row and 10 blocks were planted with plants 4 m along the planting row to evaluate the correlation between performances in the different densities. In addition to the seedling progenies, replicates of some 10 cultivars used as parents (246, 781, 814, 816, 842, 849, A4, A16, Daddow and Own Venture) grafted on to H2 seedling rootstock, were planted throughout the trial. The trial is surrounded by at least 1 row of buffer trees. Details of replants and harvests are reported in milestones and final reports for earlier stages of the project. Flowering was observed in year 4 and the first harvest made in year 5. The trial was severely hedged after harvest in year 6. Ethephon was used to assist nut fall in 2007 year 7 and 8 but the crop was lost due to a severe rain storm displacing the crop.

Plans for the three trials can be found in Appendix A. The numbers in the cells are the blocks that progeny were planted in. Cells numbered 99 and 95 are buffer trees or replants replacing trees that died. A cell missing a number indicates that either no tree

was planted or the tree died after replanting ceased. Cells outlined indicate trees from which nuts were collected in 2006 for use in kernel assessments. The shaded cells are candidate trees with their ranking in the top 40 bracketed.

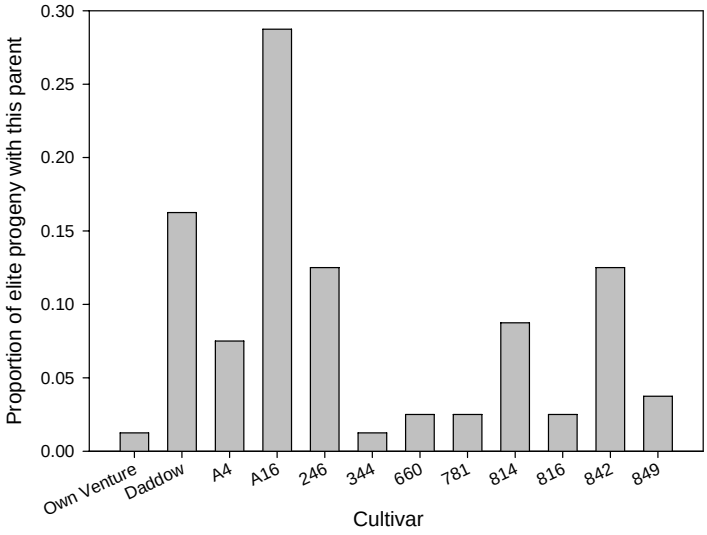


Figure 10.1. Proportion of the top 40 ranked candidate cultivars with a specific cultivar as parent.

Table 10.3. The calendar year and the measurement or activity undertaken at that site. CW= canopy width, TNM= Total nut mass, Ethephon = Application ethephon to promote nut drop, Hedging= Mechanical hedging to allow access of spray equipment.

Calendar Year	Number of Years After planting BQBRS 97	Measurements made BQBRS97	Number of Years After planting BQBRS 98	Measurements made BQRS98	Number of Years After planting TFRS98	Measurements made TFRS98
2000	3	CW3				
2001	4	CW4 TNM4	3	CW3	3	CW3
2002	5	CW5 TNM5	4	CW4	4	CW4
2003	6	CW6 TNM6 Hedging	5	CW5 TNM5 Hedging	5	CW5 TNM5
2004	7	TNM 7 Ethephon	6	TNM6 Hedging	6	CW6 TNM6 Hedging
2005	8	CW8 TNM8[a] Ethephon	7	CW7 TNM 7 Ethephon	7	CW7 Ethephon
2006			8	CW8 TNM8	8	CW8 TNM8

Data analysis

Canopy width

Canopy width was analysed only for year 5 since dimensions were not measured at BQRS98 in year 6 and there was extensive inter tree growth in year 7 and 8. In addition hedging was not uniformly performed across sites in later years.

Density

To adjust for planting density each tree was coded as being planted in a low or high density row or a low or high density column. This made 4 possible planting densities. Allowance was made for trees hedged in year 5 at BQRS98. However, there were on occasion missing trees which were not adjusted for in the analysis of neighbouring trees.

Yield

Total tree yield at BQBRS97 at age 5 (y5_t1, 2002) was assessed by ground harvest and stripping the tree in May. Yield at 6 (y6_t1, 2003) was assessed by ground

harvest and stripping the tree in June. Yield at age 7 (y7_t1, 2004) was assessed by 4 ground harvests (mid-April, late May, mid-July, late August) and a final strip harvest (late August). Prior to the late May harvest ethephon was used to stimulate nut fall. Yield at year 8 (y8_t1) was assessed using a strip sub sample of the inner 60 cm perpendicular to the rows (sy8_t1, 2005) and randomly sampling trees to calculate a ratio to convert strip yield to total yield.

Total tree yield at BQBRS98 at age 5 (y5_t2, 2003) was assessed by ground harvest and stripping the tree in June. Yield at age 6 (y6_t2, 2004) was assessed by ground and strip harvest of the tree in May. Yield at age 7 (y7_t2, 2005) was assessed by 4 ground harvests (mid-April, late May, mid-July, late August) and a final strip harvest (late August). Yield at age 8 (y8_t2, 2006) was assessed by 4 ground harvests (mid-April, late May, mid-July, late August) and a final strip harvest (late August).

Total tree yield at BTFRS98 at age 6 (y6_t3, 2004) was assessed by ground harvest in April and May and strip harvest in May. At age 7, yield minus harvest 3 (mid-July) was assessed, as a storm washed away the 3rd harvest just prior to harvest. This was the sum of a ground harvest in mid April, a ground harvest in late May, a ground harvest of the nut fall between mid-July and late August, and the strip of the tree at late August (y7-h3_t3).

To estimate correlation between yield at age 7 minus harvest 3 and total yield at age 7 at BQBRS97 and BQBRS98, yield minus harvest 3 was calculated for these trials (y7-h3_t12).

For each trait for each tree, variances were partitioned into a mean, block, density, density x replicate, density x tree, density x family and density x residual variances. In instances where hedging interfered with nut yield, effects of density and hedging were also modelled. The linear effect of trial was also removed.

Cumulative yield to year 8 (sumy8), was estimated from age at first crop (afc) and genetic values of yield at year 6, year 7 and year 8 summed. Genetic values were estimated for each tree for the full suite of traits. The genetic values for each trait were then multiplied by their economic weight and a total genetic value (TGV) calculated. This is expressed with the average of the whole population being zero. The relative effects of the selection index are presented in Figure 10.2.

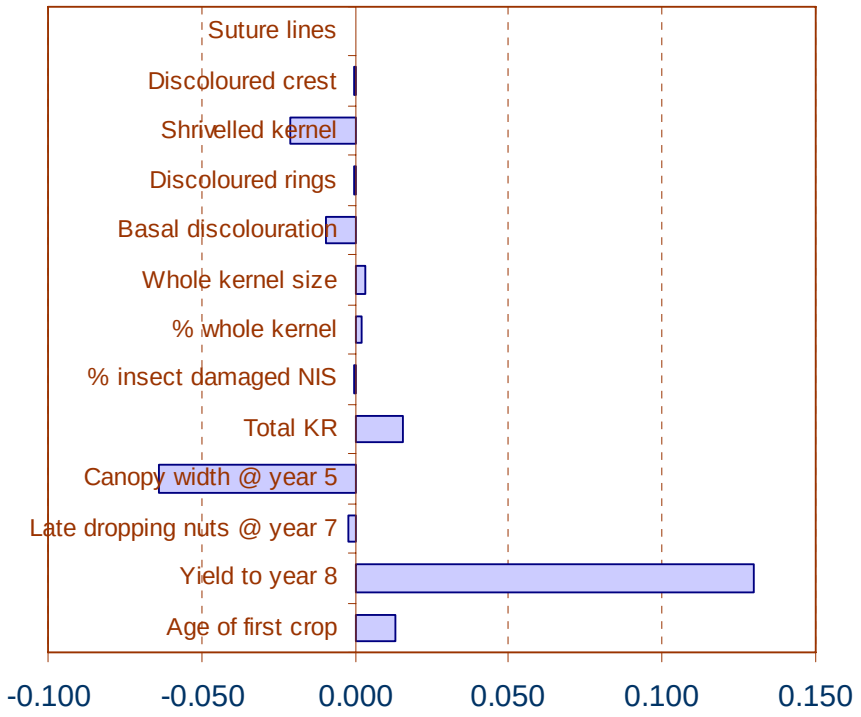


Figure 10.2. Relative importance of characteristics used in selecting new candidate cultivars. The bars represent the weighting of each of the characters in the selection process.

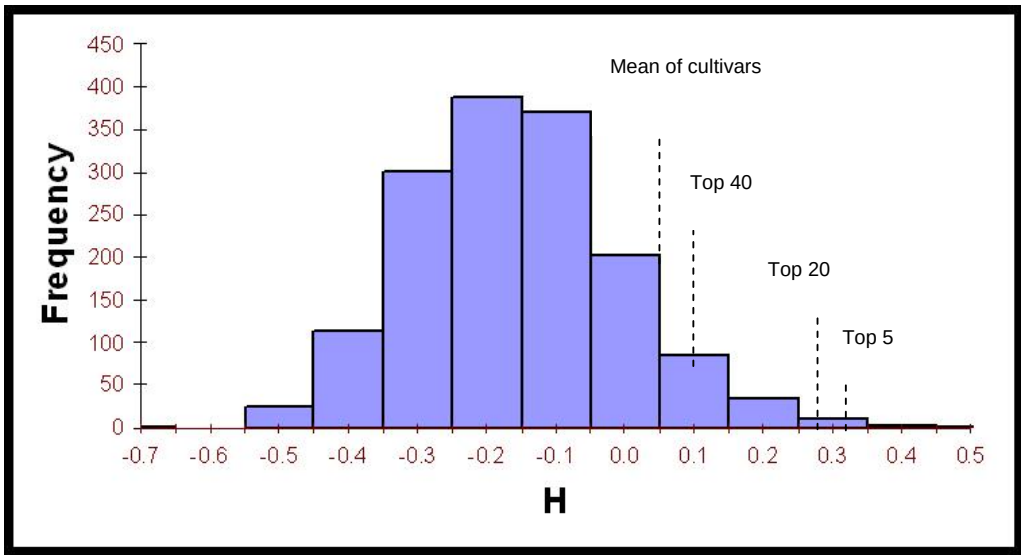


Figure 10.3. Relative economic merit as measured by economic value (H) of progeny from breeding population 1.1.

A list of the top 40 selected candidate trees is presented in Table 10.5. The relative economic merit of clones and selected progeny can be seen in Figure 10.3. The mean

of the top 20 candidates are predicted to increase profitablility of the Australian industry by 30% compared to the mean of the current cultivars.

The parents A16, Daddow, 842, and 246 are very highly represented in the candidates. Compared with the average of the progeny as a whole, the candidates were generally somewhat slower to age at first crop, they had much higher total yield to year 8, they had higher late dropping nuts, generally larger canopy, generally lower kernel recovery, much lower frequency of whole kernel and about the same rate of kernel defects.

Table 10.4a Characteristics and value of the top ranked 40 candidate cultivars (candidate 1-20)

Selection Ranking	Tree Number	Parent 1	Parent 2	Average age at first crop	Total yield to age 8 (Kg)	Mass of late dropping nuts at age 7(kg)	Canopy width at age 5 (m)	Total kernel recovery	% kernel with insect damage	Average basal discolouration score	Average discoloured rings score	Average shrivelled kernel score	Average discoloured crest score	Average suture lines score	% whole kernel	Whole kernel size (mm)	H
	mean			5	12.5	0.023	3.6	38.2	0.009	1.0	0.3	1.4	0.2	0.4	49.2	18.4	
	w ($/unit)			0.065	0.031	-0.104	-0.143	0.011	-0.360	-0.080	-0.004	-0.255	-0.007	-0.001	0.001	0.006	
1	9401672	A16	246	5.39	25.0	0.04	3.8	36.50	0.009	0.90	0.34	1.52	0.26	0.50	36.8	19.1	0.35
2	9504161	A4	816	4.99	19.7	0.03	3.1	39.95	0.008	0.92	0.25	1.37	0.22	0.46	40.7	18.4	0.32
3	9505869	Daddow	246	5.31	23.4	0.02	4.0	36.05	0.007	0.91	0.34	1.37	0.21	0.44	34.3	18.1	0.28
4	9503532	Daddow	A16	5.32	23.8	0.05	4.2	36.26	0.009	0.76	0.29	1.37	0.23	0.50	40.6	18.0	0.28
5	9501923	A16	814	5.35	23.4	0.05	3.9	35.89	0.011	0.72	0.31	1.49	0.23	0.47	40.5	17.4	0.27
6	9502422	A16	781	5.30	24.7	0.07	4.6	37.75	0.010	0.81	0.34	1.39	0.23	0.49	32.5	19.4	0.26
7	9504143	Daddow	842	5.74	18.6	0.01	3.7	35.86	0.008	0.83	0.33	1.30	0.19	0.45	37.2	17.8	0.23
8	9503361	Daddow	842	5.46	19.8	0.04	3.7	35.46	0.008	0.89	0.35	1.35	0.19	0.45	37.8	17.7	0.22
9	9505654	A16	781	5.53	21.4	0.07	4.1	35.80	0.010	0.80	0.34	1.44	0.23	0.49	40.8	18.6	0.21
10	9502502	Daddow	849	5.49	20.8	0.02	4.3	37.66	0.008	0.97	0.35	1.36	0.19	0.45	38.7	18.4	0.20
11	9501661	A4	842	5.03	23.1	0.03	4.4	37.70	0.008	0.87	0.34	1.49	0.22	0.49	40.6	18.1	0.19
12	9500812	Daddow	A16	5.43	17.4	0.07	3.5	35.72	0.009	0.76	0.29	1.37	0.23	0.50	40.5	18.2	0.19
13	9504117	A16	842	5.54	21.0	0.05	4.1	35.82	0.009	0.82	0.33	1.53	0.24	0.50	41.2	18.2	0.18
14	9503978	660	842	5.45	20.2	0.02	4.1	36.38	0.009	1.07	0.47	1.40	0.20	0.44	39.8	17.5	0.17
15	9505963	Daddow	A4	5.36	18.0	0.03	4.1	37.20	0.008	0.75	0.26	1.28	0.22	0.47	39.9	18.8	0.17
16	9504239	Daddow	246	5.42	18.8	0.02	4.0	36.51	0.007	0.91	0.34	1.37	0.21	0.44	38.7	18.1	0.16
17	9506018	A16	814	5.42	19.7	0.05	4.0	36.31	0.011	0.72	0.31	1.49	0.23	0.47	40.5	17.6	0.16
18	9502719	A16	814	5.34	21.4	0.05	4.4	36.13	0.011	0.72	0.31	1.49	0.23	0.47	39.6	18.0	0.15
19	9503824	A16	246	5.22	21.2	0.03	4.3	36.32	0.009	0.90	0.34	1.52	0.26	0.50	36.6	18.9	0.14
20	9503308	Daddow	842	5.46	18.4	0.05	4.1	35.99	0.008	0.88	0.32	1.36	0.19	0.45	39.7	17.9	0.14

Table 10.4b. Characteristics and value of the top ranked 40 candidate cultivars (candidate 21-40)

Selection Ranking	Tree Number	Parent 1	Parent 2	Average age at first crop	Total yield to age 8 (Kg)	Mass of late dropping nuts at age 7(kg)	Canopy width at age 5 (m)	Total kernel recovery	% kernel with insect damage	Average basal discolouration score	Average discoloured rings score	Average shrivelled kernel score	Average discoloured crest score	Average suture lines score	% whole kernel	Whole kernel size (mm)	H
	mean			5	12.5	0.023	3.6	38.2	0.009	1.0	0.3	1.4	0.2	0.4	49.2	18.4	
	w ($/unit)			0.065	0.031	-0.104	-0.143	0.011	-0.360	-0.080	-0.004	-0.255	-0.007	-0.001	0.001	0.006	
21	9400523	A16	814	5.46	16.2	0.06	3.5	35.81	0.011	0.72	0.31	1.49	0.23	0.47	38.6	18.8	0.13
22	9500892	A16	660	5.47	16.8	0.02	3.8	38.09	0.010	0.88	0.44	1.44	0.26	0.49	38.2	18.1	0.13
23	9504551	Daddow	849	5.45	16.8	0.02	4.1	38.52	0.008	0.89	0.33	1.35	0.19	0.45	39.4	18.5	0.12
24	9503631	Daddow	A16	5.45	17.0	0.03	3.9	36.73	0.009	0.70	0.25	1.41	0.25	0.51	39.9	18.2	0.12
25	9505835	Daddow	246	5.44	18.8	0.01	4.2	34.73	0.007	0.91	0.34	1.37	0.21	0.44	39.6	18.1	0.12
26	9505781	Daddow	16	5.53	16.0	0.07	3.7	36.60	0.009	0.74	0.29	1.42	0.22	0.51	40.2	18.2	0.12
27	9503991	A16	842	5.30	18.0	0.04	3.9	37.09	0.009	0.87	0.34	1.48	0.23	0.51	40.4	18.3	0.12
28	9503607	Daddow	849	5.32	16.5	0.02	3.9	37.81	0.008	0.93	0.34	1.35	0.19	0.45	39.1	18.6	0.12
29	9502946	Daddow	A4	5.07	16.1	0.02	3.8	37.83	0.008	0.92	0.32	1.30	0.22	0.48	39.6	18.6	0.12
30	9500589	Daddow	660	5.47	17.1	0.02	4.1	36.45	0.009	0.88	0.42	1.28	0.19	0.44	37.1	17.5	0.11
31	9500628	A16	814	5.54	18.6	0.17	4.1	36.23	0.011	0.77	0.31	1.48	0.24	0.47	41.8	17.7	0.11
32	9503017	Daddow	A4	5.37	15.8	0.03	4.0	38.45	0.008	0.84	0.27	1.33	0.22	0.48	38.2	18.8	0.11
33	9505965	Daddow	A4	5.02	19.0	0.04	4.4	37.09	0.008	0.84	0.27	1.33	0.22	0.48	37.2	18.4	0.11
34	9505657	A16	781	5.37	17.9	0.05	4.0	33.69	0.010	0.74	0.32	1.37	0.22	0.49	39.7	18.5	0.11
35	9400242	A16	246	5.39	17.9	0.02	3.8	34.92	0.008	0.90	0.34	1.52	0.26	0.50	36.6	18.7	0.11
36	9400426	A16	814	5.46	17.2	0.05	3.8	36.60	0.012	0.72	0.31	1.49	0.23	0.47	39.3	17.2	0.11
37	9501162	A4	849	5.05	18.3	0.03	4.0	39.30	0.008	1.04	0.33	1.50	0.22	0.48	41.0	18.8	0.11
38	9503476	A16	814	5.51	15.8	0.04	3.6	36.47	0.011	0.72	0.31	1.49	0.23	0.47	40.2	17.8	0.11
39	9501698	Daddow	246	5.18	20.2	0.01	4.4	35.30	0.007	1.01	0.32	1.39	0.21	0.45	35.4	18.1	0.10
40	9503883	Daddow	246	5.34	18.0	0.02	4.1	36.31	0.007	0.91	0.34	1.37	0.21	0.44	34.3	18.0	0.10

Clonal trees were also represented in the data analysis. The results for these varieties prepared in the same way as for the candidates is presented in Table 10.6. Generally the clones were somewhat slower to come into crop, about the same as the progeny average for yield to year 8, canopy size, kernel recovery and kernel defects.

Table 10.5 Characteristics and value of the commercial cultivars analysed as for the candidate cultivars

Trait	Age of first crop	total yield to age 8	canopy width at age 5	Mass of late dropping nuts	Total kernel recovery	% kernel with insect damage	% whole kernel	Kernel size	H	Ranking
tree	(year)	(kg)	(m)	(kg)	(%)	(%)	(%)	mm		
mean	5	12.5	3.6	0.023	38.2	0.9	49.2	18.4		
816	5.47	13.4	3.5	0.0101	41.23	0.9	57.2	19.3	0.11	30
A4	5.37	14.2	3.7	0.0108	42.48	2.0	47.3	18.8	0.10	46
Daddow	5.66	14.3	3.5	0.0189	35.24	0.5	40.3	18.0	0.07	75
814	5.70	12.8	3.5	0.0350	36.42	0.9	44.0	17.6	0.04	114
849	5.50	12.7	3.7	0.0232	42.33	0.9	51.5	18.8	0.04	115
Own Venture	5.64	12.8	3.5	0.0633	36.38	0.7	50.4	18.7	0.04	120
A16	5.46	12.3	3.7	0.0254	40.20	1.2	46.6	18.9	0.03	122
842	5.53	12.5	3.4	0.0288	34.51	0.8	46.8	17.5	0.00	204
246	5.52	12.9	3.9	0.0191	35.43	0.8	52.8	18.2	-0.05	356
781	5.77	10.1	3.7	0.0621	37.49	0.5	55.0	18.1	-0.06	405

Subsequent to the selection of the candidate varieties, concern has been expressed about the apparent drift to later age at first crop in the progeny. For this reason, a table 10.6 is presented of the frequency of cropping at each site for year five of the trials. Generally, the Bundaberg sites cropped much earlier than the Alstonville site and progeny on average cropped somewhat earlier than clones. Yield was collected in year 4 at BQBRS97 when more than 25% of the trees cropped suggesting that some level of flowering occurred in year 2. It is not known with any certainty what the age at first crop truly is. Comparisons with clones is also somewhat confounded due to small numbers of clones used and lack of orthogonal trial design and use of rootstocks.

Table 10.6 Summary of the number of progeny and cultivar trees cropping in year 5. Note that at BQBRS97 all 25 cultivars were A4. At the other sites there were 10 cultivars planted. BTFRS98 has much lower levels of flowering but especially in the commercial cultivars. Growth was greater in the BQRS sites as they were in Queensland while TFRS was in NSW.

trial	Planting density	Number of progeny	number cropping	proportion
BQBRS97	2	261	252	0.97
BQBRS98	2	483	271	0.56
BQBRS98	4	208	117	0.56
BTFRS98	2	347	89	0.26
BTFRS98	4	220	75	0.34
trial	Planting density	Number of Clonal trees	number cropping	proportion
BQBRS97	2	25	25	1.00
BQBRS98	2	65	42	0.65
BQBRS98	4	12	11	0.92
BTFRS98	2	68	3	0.04
BTFRS98	4	36	0	0.00

Subsequent to the selection of candidate varieties, concern was raised as to whether selection of candidate trees was biased upward by the absence of adjacent trees due to death as this was not included in the first round of analysis. Considering there were fewer than half the number of trees planted at low densities they are over represented in the top ranked selections. Generally, the candidate trees were selected from within either dense or sparse planting and had normal populations (for the density) of trees surrounding them. In the instance of candidates ranked 10, 14, 18, 20 and 31 some unaccounted for upward bias in yield traits may have occurred due to trees missing from the competition matrix.

Table 10.7. The ranked candidate cultivars showing whether they were planted in high density 2X5 or low density 4x5 plantings. The trees missing of the potential 8 around the specific candidate tree are recorded. There is potential 2 within a row, 2 within a column and 4 on the diagonal. The orientation of the rows is also indicated.

Ranking	Density	Row	Column	Diagonal	Orientation	Ranking	Density	Row	Column	Diagonal	Orientation
1	High	0	0	0	N-S	21	High	0	0	0	N-S
2	Low	2	0	0	N-S	22	High	0	0	4	N-S
3	Low	2	0	0	N-S	23	High	0	0	4	N-S
4	Low	2	0	0	N-S	24	High	0	0	0	NE-SW
5	Low	2	0	0	N-S	25	Low	2	0	2	E-W
6	Low	2	0	0	N-S	26	High	0	2	0	N-S
7	High	1	2	0	N-S	27	Low	2	0	2	N-S
8	High	0	0	4	N-S	28	Low	2	0	0	N-S

Ranking	Density	Row	Column	Diagonal	Orientation	Ranking	Density	Row	Column	Diagonal	Orientation
9	Low	2	0	4	E-W	29	Low	2	0	0	N-S
10	Low	2	0	3	NE-SW	30	High	0	2	0	N-S
11	High	0	0	0	NE-SW	31	High	0	0	3	NE-SW
12	High	0	0	4	N-S	32	High	0	0	2	N-S
13	Low	2	0	0	N-S	33	Low	0	2	2	NE-SW
14	Low	2	1	1	N-S	34	Low	2	0	4	E-W
15	High	0	2	0	N-S	35	High	0	0	0	N-S
16	High	0	0	2	E-W	36	High	0	0	0	N-S
17	High	0	2	0	N-S	37	Low	2	0	0	N-S
18	Low	2	1	0	N-S	38	High	0	2	0	N-S
19	Low	2	0	0	N-S	39	Low	2	0	0	N-S
20	High	0	1	4	N-S	40	Low	2	0	0	N-S

Quite strong genetic correlations occurred for a number of traits. The most important of these is the negative correlations (Table 10.8) between age at first crop and cumulative yield to year eight (-0.44), total kernel recovery (-0.37), canopy width at year 5 (-0.54) and sound kernel (-0.42) and positive correlations between yield to year eight and late nut drop (0.39) and canopy width at year five (0.56). It would therefore appear that trees with a high genetic value for yield to year eight also had substantially delayed age at first crop and much larger canopy size.

Table 10.8 Correlation between traits used in selection. afc8= age of first crop, sumy8= cumulative yield to year 8, tkr=total kernel recovery, cw5=canopy width at year 5, pwkm=percentage whole kernel, ks= sound kernel

	sumy8	lnd7	tkr	cw5	pwkm	ks
afc	-0.44	-0.25	-0.37	-0.54	0.02	-0.42
sumy8		0.39	-0.07	0.56	-0.18	0.16
lnd7			0.02	0.32	-0.14	0.21
tkr				0.16	0.23	0.48
cw5					-0.12	0.27
pwkm						-0.19

Discussion

Across sites effects

The trial design takes into account differential site effects and adjusts for these effects in a linear fashion. A key assumption being that there is no significant genotype by environment (G X E) interaction. In effect any G X E will be partitioned into error in this analysis lowering the heritability of the trait. The plan being that G X E interaction would be thoroughly evaluated in a subsequent regional variety trial that would have a balanced orthogonal design and biological replication at each site.

Plant density effects

Effects of density on traits was partitioned, again using a linear effects model. Attributes for which there was a statistically significant effect of density, such as yield, were estimated as two different traits for each individual and estimated using the family structure and correlation with yield in the other density. Estimates of genetic variance for yield on a family basis in sparse spacing and the correlations between spacings approached 1 (Appendix A). Therefore family effects at the different spacings were essentially similar. However, variance for yield traits in dense plantings was substantially smaller than in sparse planting and for this reason sparse planted trees are represented at a much higher frequency in candidate cultivars than dense. This is simply a function of greater detectable genetic variance (and therefore higher heritability) in sparse planting.

Hedging effects

A challenging component of the analysis of yield was to account for the effect of hedging on the estimation of individual genetic value. This is because hedging was applied unevenly across the trials. As the design was not sufficiently balanced, there was insufficient information to estimate parameters or there were singularities that within the matrix which precluded estimation of variance components. However, it was critical to the estimation of genetic values that an adjustment be made and estimates derived. Whilst, it is believed that a reasonable estimate of various yield traits was made hedging may have been a cause of additional variance being attributed to error and consequent lowering of genetic variances. It is not possible to validate this component of work as the data is insufficiently balanced to permit a valid comparison.

Clonal vrs seedling comparisons

Clones were grafted onto rootstocks and were 1 year younger than the seedlings. Whilst seedlings were treated separately from clones in the analysis, an assumption that there was no difference in seedling versus grafted clones was made. This assumption needs to be validated.

Precocity effects

The negative correlations surrounding age to first crop and other traits are a concern in this analysis. It is not known whether these correlations are a true genetic correlation or an artefact of the model used to derive yield traits since afc was mostly not measured directly. It is possible that trees that produce high early crop were subsequently disadvantaged in this selection due to their allocation of early resources to nuts rather than to production of canopy. The effect of early cropping on lifelong production of an orchard, and if increasing density is a strategy to compensate for lower canopy size in precocious trees needs to be evaluated. The genetic model from which age at first crop was estimated and the economic model weighting for the trait should be validated if possible.

Modelling quality

Genetic component of kernel recovery and a range of kernel quality measures were estimated indirectly via family effects (see chapter 8). Generally quality attributes had little effect on the selection process since the modelled genetic effects had near zero variance, that is heritability of the modelled genetic effects was small and effectively of no consequence as a result. However, for known high heritability traits like kernel recovery (See Appendix C for data), it is surprising and perhaps alarming that the modelled genetic values had near zero variance and are only weekly correlated with actual measures of the trait made by processors ($r^2 = 0.25$), or made within the project ($r^2 = 0.41$) whereas comparison of processor and within project assessments made on different nuts collected in different years was good ($r^2 = 0.53$). It would appear that the process by which genetic values for hitherto high heritability traits such as kernel recovery were modelled needs to be examined and validated because it has proven to be a constraint to genetic improvement.

Appendices

Appendix A Block layout of BQBRS97 and BQBRS98.

North to the top of the page

	Column																
Sp	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17
1	99	99	99	99	99	99	99	99	99		99			99			99
2	99	1	2	3	4	5	6	7	99		27		29		31		99
3	99	1	2	3	4	5	6	7	99	26	27	28	29	30	31	32	99
4	99	1	2	3	4	5	6	7	99		27		29		31		99
5	99	1	2	3	4	5	6	7	99	26	27	28	29	30	31	32	99
6	99	1	2	3	4	5	6	7	99		27		29		31		99
7	99	1	2	3	4	5	6(35)	7	99	26	27	28	29	30	31		99
8	99	1	2	3	4	5	6	7	99		27		29		95		99
9	99	1	2	3	4	5	6	7	99	26	27	28	29		31	32	99
10	99	1	2	3	4	5	6	7	99		27		95				99
11	99	1	2	3	4	5	6	7	99	26	27	95	29	30		32	99
12	99	1	2	3	4	5	6	7	99		27		29		99		99
13	99	1	2	3	4	5	6	99	99	26	27	28(39)	29	30	31	32	99
14	99	1	2	3	4	5	6	0	99		27		29		31		99
15	99	1	2	3	4	5	6	99	99	26	27	28	29	30	31	32	99
16	99	8	9	10	11	12(21)	13	14	99		27		29		31		99
17	99	8	9	10	11	12	13	14	99	26	27	28	29	30	31	32	99
18	99	8	9	10	11	95	13	14	99		27		29		31		99
19	99	8	9	10	11	12	13	14	99	26	27	28	29	95	31	32	99
20	99	8	9	10	11	12	13	14	99		27		29		31		99
21	99	8	9	10	11	12	13	14	99	26	27	28	29		31	95	99
22	99	8	9	10	11	12	13	14	99		27		95		31		99
23	99	8	9	10	11	12	13	14	99	26	27	28	29	30	31	32	99
24	99	8	9	10	11	12	13	14	99		27		99		31		99
25	99	8	9	10	11	12	13	14	99	26	27	28		30 (18)	95	32	99
26	99	8	9	10	11	12	13	14	99		27		99		95		99
27	99	8	9	10	11	12	13	14	99	26	27	28	29	30	31	32	99
28	99	8	9	10	11	12	13	14	99		27		95		31		99
29	99	8	9	10	11	12	13	14	99	26	27	95	29	30	31		99

30	99	15	16	17	18	19	20	21	99		27		29		31		99
31	99	15	16	17	18	19	20	21	99	26	27	28	29	30	31	32	99
32	99	15	16	17	18	19	20	21	99		27		29		31		99
33	99	15	16	17	18	19	20	21	99	26	27	28	29	30(40)	31	32	99
34	99	15	16	17	18	19	20	21	99		27		29		31		99
35	99	15	16	17	18	19	20	21	99	95	27(23)	28	29	30	31	32	99
36	99	15	16	17	18	19	20	95	99		27		29		31		99
37	99	15	16	17	18	19	20	21(36)	99	26	27	28	29	30	31(12)	32(27)	99
38	99	15	16	17	18	99	20	21	99		27		29		31		99
39	99	15	16	17	18	0	20	21	99	26	27	28	29	30	31	32	99
40	99	15	16	17	18	99	20	21	99		27		29		31		99
41	99	15	16	17	18	19	20	21	99	26	27	28	29	30	31	32(4)	99
42	99	15	16	17	18	19	20	21	99		95		29		31		99
43	99	15	16	17	18	19	20	21	99	26	27	28	29	30	31	32	99
44	99	22	23	24	25	99	99	99	99		95		29		31		99
45	99	22	23	24	25	99	99	99	99	26	27	28	29	30	31	32	99
46	99	22(1)	23	24	25	99	99	99	99		95		29		31		99
47	99	22	23	24	25	99	99	99	99	26	27	95	29	30	95		99
48	99	22	23	24	25	99	99	99	99		27		29		31		99
49	99	22	23	24	25	99	99	99	99	26	27	28	29	30	31	32	99
50	99	22	23	24	25	99	99	99	99								99
51	99	22	23	24	25	99	99	99	99	26	27	28	29	30	31	32	99
52	99	22	23	24	25	99	99	99	99		27		29		31		99
53	99	22	23	24	25	99	99	99	99	99	95	99	29	99	95	99	99
54	99	22	23	24	25	99	99	99	99		27		29		31		99
55	99	95	23	24	25	99	99	99	99	99	27	99	95	99	31	99	99
56	99	22	23	24	25	99	99	99	99		99		99		99		99
57	99	22	23	24	25	99	99	99	99	99	99	99	99	99	99	99	99
58	99	99	99	99	99	99	99	99	99	99	99	99		99	99	99	99
59	99	99	99	99	99	99	99	99	99	99	99	99	99	99	99	99	99
60	99	99	99	99	99	99	99	99	99	99	99	99	99	99		99	99
61	99	99	99	99	99	99	99	99	99	99	99	99	99	99		99	99
62	99	99	99	99	99	99	99	99	99	99	99			99	99	99	99
63	ROAD																

64						ROAD										
65																
66	99	33	34		99		38		40		42		44	46	47	
67	99	33	34	35	0		38	39	40	41	42	43	95	46	47	
68	99	33	34		99		38		40		42		44	46	47	
69		33	34	35	36	99	38	39	40	41	42	43	44	46	47	
70	99	33	34				38		40		42		44	46	47	
71	99	33	34	35	36	37	38	39	40	41	42	43	44	46	47	
72	99	33	34		36		38		99(7)		95		44	46	95	99
73	99	33	95	35	36	37(13)	38	39		41	42	43	44	46	47	
74	99	33	34		36		38(15)		99		42		44	46	95	
75		33	34	35	36	37	38	39	40(22)	41	42	43	44	46	47	
76	99	33	34		36		38		40		42		44	46	47	
77	99	33	34	35	36	37	38	39	40	41	42	43	44	46	47	
78	99	33	34		36		38		40		42		44	46	47	
79	99	33	34	35	36	37(5)	38	39	40	41	42	95	44	46	95	
80	99	33	34		36		38		40		42(17)		44	46	47	
81	99	33	34	35	36	37	38	39	40	41	42	43(37)	44	46	47	
82	99	95	34		36		38		40		95		44	95	47	
83	99	33	34	35	36	37	38	39	40	41	42	95	44	46	47	99
84	99	33	34		36		95		40		42		44	46	47	
85	99		34	35	36	37	38	39	40	41	42	43	95	46	47	
86	99	33	34		36		38		40		42		44	46	47	
87	99	33	34	35	95	37	38	39(2)	95	41	42	43	44	46	47	
88	99	33	34		36		38		40		42		44	46	47	
89	99	33	34	35	36	37	38	39	40	95	42	43	44	46	47	
90	99	33	34		36		38		40		42		44	46	47	
91		33	34	35	36	37	38	39	40	41	42	43	44	46	47	
92	99	33	34		36		38		40(30)		42		44	46	47	
93	99	99	34	35	36	37	38	39	40	41(6)	42	43	44	46	95	99
94	99		34		36		38		40		42		44	99	99	
95	99	99	95	35	36	37	38	39	40	95	42	43	44			
96	99	33	34		36		38		40		42		44	99	99	
97		33	34	35	36(26)	37	38	39	40	41	42	43(28)	44	46	47	
98	99	33	34		36		38		40		42		44	46	47	
99	99	33	34	35	36	37	38	39	40	41	42	43	44	46	47	
100		33	34		36		38				42		44	46	47	
101	99	33	34	35	36	37	38	39		41(14)	95	95	44	46	47	99
102	99	33	34		36		38		99		42		44	46	47	
103	99		34	35	36	37	38	39	40	41	42	95	44	46	47	
104	99	33	34		36		38		40		42		44	46	47	99
105	99	95	34	35	36	37	38	39	40	41(29)	42	43	44	46	47	
106	99	33	34		36		38		40		42		44	46	47	
107	99	95	34	35	95	95	38	39	40	41	42	43	95	46	47	
108	99	33	95		36		38		40		42		44	46	47	
109	99	33	34	35	36	37	38(8)	39	40	41	42	43	44	46	47	99
110		99	99		36		38		40		42		44	46	47	
111				35	36	37	38	39	40	41(19)	42	99	44	46	47	
112					36		38		40		42		44	46	47	
113				35	36	37	38		40	95	42		44	46	47	
114					36		38		40		42		44	46	47	99
115				35	36	37	38	39	40	41	42	99	44(20)	46	47	
116					36		38		40		42		44	46	47	
117					36	99	38	99	40	99	42	99	44	46(32)	47	99
118					36		38		40				44(38)	46	47	
119					36	99	38	99	40	99	42	99	44	46	47	
120					99		99		99		99	99	44	46	47	
121					99	99	99	95	99	99	99	99	99	95	47	
122			99		99	99	99	99	99	99	99		99	46	95	

123			99		99	99	99	99	99	99	99	99	99	99	99	
124			99		99	99	99				99	99	99	99	99	
125											99	99	99	99	99	
126											99	99	99	99	99	
127											99	99	99	99	99	
128											99		99	99	99	
129											99	99	99	99	99	
130													99	99	99	
131													99	99	99	
132													99	99	95	
133														99	99	
134														99	99	
135															95	
136																
137																

Appendix B Trial Map of BTFRS98

	Column																						
Space	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23
1	99	99	99	99	99	99	99	99	99	99	99	99	99	99	99	99	99	99	99	99	99	99	99
2	99	48	48	48				52	99	52				61	61	61				66	66	66	
3	99	48	48		50	50		52	52	52	56	56	56	61	61	61	64(33)	64	64	66	66	66	99
4	99	48	48	48				52	52	52				61	61	61				66	66	66	99
5	99	48	48	48	50	50	50	52	52	52	56	56	56	61	61	61	64	64	64	66	66	66	99
6	99	48	48	48				52	52	52				61	61	61				66	66	66	99
7	99	48	48	48	50	50	50	52		52	56	56	56	61	61	61	64	64	64	66	66	66	99
8	99	48	48	48				52	52	52				61	61	61				66	66	99	99
9	99	48	48	48	50		50	52(31)	52	52	56	56	56	61	61	61	64	64	64	66	66	66	99
10	99	48(24)	48	48				52	52	52				61	61	61				66	66	66	99
11	99	99	49	99	50	50	50		53	53		56	56	62	62	62	64	64	64	99	99	99	99
12	99	49	49	49						53				62	62	62							99
13	99	49	49	49	50	50	50	53	53	53	56	99	56	62	62	62	64	64	64		67	67	99
14		99	49	49					53	99				62	62	62							
15		99	49	49	50	99	50	53	53	53	56	0	56	62	62	62	64	64	64	99	67	67	99
16		99	49	49				53	53	53				62	62	62							
17		99	49	49	50		50	53	53	53	56	99	56	62	99	62	64	64	64	67	67	67	99
18			49	49				53	53	53				62	62	62							
19			99	49	50	99	50	53	53	53	56	99	56	62	62	62	64	64	64	67	67	67	99
20			99					99		99				99		99							
21			99	49	99	99	99				99	99	99	76	63	63	99	99	99	67	67	67	
22				49	51	51	51	54	54(10)	54	57	57	57	76	63	63	65	65	65				
23				99	51	51	51				57	57	57	76	63	63	65	65	99	67	67	67	99
24				99	51	51	51	54	54	54	57	57	57	76	63	63	65	65	65				
25				99	51	51	51				57	57	57	76	63	63	65	65	99	67	67	67	99
26					51	51	51	54	54	54	57	57	57	76	63	63	65	65	65				
27					99	51	51				57	57	57	76	63	63	65	65	99	67	67	67	99
28					99	51	51	54	54	54	57	57	57	76	99	63(11)	65	65	65				
29					99	51	51				57	57	57	99	0	99	65	65	99	67	67	67	99
30						51	51	54	54	54	57	57	57	76	99		65	65	65				
31						99	51				99	99	99	76	63	99	99	99	99	99	99	99	
32						99	51	54	54	54	58	58	99	99	63	99	99	99	99	99	99		
33							51				58	58		76	63	99	99	99		99			
34							99	54	54	54	58	58	99	76	60	99	99	99	99	99			
35							99				58	58	58	76	60	99	99	99					
36							99	54	54	54	58	58	58	99	60	99							
37							99				58	58	58	76	60	99							
38								54	54	54	58	58	58	76	60	99							

	Column																						
39											58	58	58	76	60	99							
40			**BTFRS98**					99	99	99	59	59	99	99	99	99							
41								99	55	55	59	59	60	60	99								
42									55	55	59	59	60	60	99								
43									55	55	59	59	60	60	99								
44									99	55	59	59	60	60	99								
45									99	55	59	59	60	99	99								
46										55	59	59	99	99									
47										55	59	59	99	99									
48										99	59	99	99	99									
49											99	99	99	99									
50											99	99	99	99									
51												99											

Map of BTFRS (cont)

	Column																		
Space	31	32	33	34	35	36	37	38	39	40	41	42	43	44	45	46	47	48
1	99	99	99	99	99	99	99	99	99	99	99	99	99	99	99	99	99	99
2	99	68	68	68				72	72	72					99	99	99	
3	99	68	68	68	70	70	70	72	72	72	74	74	75	75	99	99	99	99
4	99	68	68	68				72	72	72					99	99	99	99
5	99	68	68	68	70	70	70	72(16)	72	72	74	74	75	75	99	99	99	99
6	99	68	68	68				72	72	72					99	99	99	99
7	99	68	68	68	70	70	70	72	72	72	74	74	75		99	99	99	99
8	99	68	68	68				72	72	72					99	99	99	99
9	99	68	68	68	70	70	70	72	72	72	74	74	75	75	99	99	99	99
10	99	68	68	68				72	72	72					99	99	99	99
11	99	99	99	99	70	70	70	99	99	99	74	74	75	75	99	99	99	99
12	99														99	99	99	99
13	99	69	69	69	70	70	70	73	99	73	74	74	75	75(25)	99	99	99	99
14	99														99	99	99	99
15	99	69		69	70	70	70	73	73	73	74	74	75	75	99	99	99	99
16	99														99	99	99	99
17	99	69	69	69	70	70	70	73	73	73	74	74(34)	75	75	99	99	99	99
18	99														99	99	99	99
19	99	69	69	69	70	70	70	73	73	73	74	74	75	75	99	99	99	99
20	99														99	99	99	99
21	99	69	69	69	99	99	99	73	73	73	74	74	75	75	99	99	99	99
22	99				71	71	71								99	99	99	99
23	99	69	69	69	71	71	71	73	73	73	74	74	75(9)	75	99	99	99	99
24	99				71	71	71								99	99	99	99
25	99	69	69	69	71	71	71	73	73	73	74	74	75	75	99	99	99	99
26	99				71	71	71								99	99	99	99
27	99	69	69	69	71	71	71	73	73	73	74	74	75	75	99	99	99	99
28	99				71	71	71								99	99	99	99
29	99	69	69	69	71	71	71	73	99	73	74	99	75	99	99	99	99	99
30	99				71	71	71								99	99	99	
31	99	99	99	99	99	99	99	99	99	99	99	99	99	99	99	99	99	99
32	99	99	99	99	99	99	99	99	99	99	99	99	99	99	99	99	99	99
33	99	99	99	99	99	99	99	99	99	99	99	99	99	99	99	99	99	99
34	99	99	99	99	99	99	99	99	99	99	99	99	99	99	99	99	99	99

Appendix C. Processors' assessment of candidate cultivars and review of selections.

Introduction

Nuts were collected from each of the 40 selected candidate cultivars and three standard cultivars (344, 246, 741) to permit commercial and internal review of quality. Information collected was then considered in a meeting of processors and industry nominees to finalise the selection of the 20 candidate cultivars to be included in new regional variety plantings.

Material and Methods

A list of the 40 candidate cultivars was supplied to members of MIVIC in March 2007. Each of the candidate trees were harvested in late March with the aim of obtaining at least 8 kg of wNIS from each tree. Any nuts on the ground were initially harvested into a separate bag and were only used if there were insufficient nuts to make up the required commercial sample. Where possible, fruit were randomly collected from the entire tree canopy. Selected trees of commercial cultivars (246 and 741) were harvested from BTFRS98 and BQBRS98 and 344 from the Wolvi Regional Variety Trial. Fruit were then mechanically dehusked nuts on the day they were harvested and transported to Maroochy Research Station where they were dried following the industry standard 2 days at 35^{o}C, 45^{o}C and 60^{o}C. When there was a delay in transporting nuts to Maroochy then the dehusked were kept in a forced oven at ambient temperature until delivered. Included with these assessments were nuts of candidate trees from the Hidden Valley selections and Ian McConachie's property at Wolvi. After drying, nuts were divided into samples, labelled with confidential codes by QDPI&F and delivered to one of three commercial processors. Limited numbers of replicated tree samples were included in the batches to examine the repeatability of the assessments. The nuts were assessed using the industry severity levels described in Evans and Hofman (2005) using the classification set out in Table 10.9. A 200g sample of sound kernel from each tree was oil roasted at 125^{o}C for 12 minutes and assessed for colour change and then taste tested.

The results of the commercial assessments were compiled and tabulated by Dr Russ Stephenson individually, datasheets for each of the candidate trees were prepared.

Similar information was also compiled for the Hidden Valley selections and Ian McConachie's nominated cultivars and commercial cultivars.

Information from each of the breeding programs was included on the QDPI&F datasheets presented to the committee. While the information from Hidden Valley and Ian McConachie's program was based of data for 2005-6 and 2006-7 the information for the Improvement program cultivars were the corrected yields for years 5 to 8 and kernel data used for ranking the candidate cultivars.

The committee inspected the trees at Alstonville, Hidden Valley Plantation Beerwah, Wolvi and Bundaberg. A summary of the tree and cropping characteristics noted are shown in Table 10.10. After inspecting the trees a round table discussion was held at each of the sites about the kernel. At these meetings the sorted kernel samples from the processors were inspected. To finalise the decisions further discussions were held in Bundaberg. While the discussions were about all candidate trees from the different programs only the information relevant to the Industry cultivars is discussed here.

Results and Discussion

A total of nine candidate cultivars were eliminated from consideration for incorporation into the third cycle of regional variety trials based on these inspections (Table 10.11). The major reasons based on the field inspections were the occurrence of twins, stick tights and out of season flowering. When any of the 5 top ranked industry selections were found to have evidence of stick-tights or twins this was disregarded because of its high ranking. Twins were not detected previously because no information about nut-in-husk was collected during harvests and the dehuskers used in the program either eliminate twins during dehusking or allowed twin nuts to fall through the 18 mm gaps between bars on inspection table after dehusking. The observation of stick-tights was complimented by the information in the minor variations in the proportion of the crop falling late. The occurrence of out of season flowering had also not been incorporated into the economic model. Candidate cultivars were also rejected because of the elevated levels of adhered skin, internal discolouration, exposed kernel due to cracks in the shell and high levels of commercial kernel. Much of this could be explained by the incomplete drying of the kernel prior to distribution. Two of the processors reported that they had to re-dry

kernel before they could assess or roast kernel. This indicates that the nut-in-shell was exposed to high temperatures while still moist. The nut-in-shell should achieve safe moisture contents at 45^{o}C and to still be moist after 2 days at 55^{o}C indicates drying problems. The material supplied from TFRS and BQBRS were less affected than samples from Hidden Valley and Wolvi because samples were largely dried before shipment. Use of shared drying conditions with nuts from the other sites may have also resulted in kernel damage due to rehydration. At the joint meeting defects such as adhered skin were attributed to immaturity however raw kernel showing these defects were invariably associated with elevated roast defects indicative of poor drying procedures. It was also evident there were large differences between the modelled kernel recovery used in selection, the measures determine by the commercial processors and those that were recorded at different stage of semi annual assessments (Table 10.12). These assessment methods differed in the way samples were taken, the size of the sample, and cracking methods. The effects of the various transformations and use of family means on yield are shown in Tables 10.13a and 10.13b.

Acknowledgements

The project was extremely grateful for time and critical input made by the processors and growers in the final selection of cultivars.

Table 10.9. Template of data sheet used by processors to record nut and kernel assessment

Processor			
Coded sample Number			
Original weight (g)			
Shell weight (g)			
Total weight of Kernel			
Severity) kernel			
Disorder	Severity 1	Severity 2	Severity 3
Suture line (g)			
Discoloured crest (g)			
Basal Discolouration (g)			
Discoloured rings (g)			
Shrivelled kernel (g)			
Pitted centres (g)			
Open micropile (g)			
Insect damage (g)			
Internal discolouration (g)			
Mould			
Streaks lines shell marks			
Adhered skin			
Others/Rancidity			
Wholes			
% wholes			
Premium severity 0 (%)			

Premium severity 1 (%)			
Commercial (%)			
Reject Genetic (%)			
Reject Environmental (%)			
Total Reject (%)			
Sound Kernel recovery (%)			
Unsound Kernel recovery (%)			
Total Kernel Recovery (%)			
Comments			
Overall colour			
NIS Appearance			
Kernel Appearance			
Roasting Test (%)	No Change	Minor Change	Major change
Taste Test	Good	Average	Poor

Observation			
Size	Large	Medium	Small
Structure	Upright	Spreading	
Canopy	Dense	Open	
Fungal infection on branches	Present	Absent	
Embedded Bark	Present	Absent	
Out of season flowering	Present	Absent	
Stick-tights	Present	Absent	
Twins	Present	Absent	

Table 10.10. Summary of the observations and categories used by the panel to assess candidate trees under orchard conditions

Ranking	Trial	Column	Row	Problems noted by the committee
11	TFRS98	16	28	Fungal infection on branches, 2-3 leaders, poor kernel recovery, high level of suture lines
14	BQBRS98	10	101	Cracks in shell that exposed kernel, High proportion of twins, off season flowering
16	TFRS98	38	5	Very dense canopy resembling 344, discoloured rings, splits in shell, high levels of commercial
17	BQBRS98	11	80	Stick-tights, strange kernel appearance, high levels of internal discolouration and minor
22	BQBRS98	9	75	Internal discolouration
24	TFRS98	2	10	Upright tree adhered skin, major change after roasting, shrivelled kernel
26	BQBRS98	5	96	Dense, upright tree Late nut drop stick-tights
27	BQBRS98	16	37	Basal discolouration, Out of season flowering
28	BQBRS98	12	97	Twins

Table 10.11 Summary of defects leading to the exclusion of candidate trees based on tree structure, fruit and kernel characteristics of candidate cultivars noted during the MIVIC assessment

Ranking	Identity	Mean Processor	Modelled	Measured			Mean 2003-6
				2003	2004	2006	
1	BRS97 C2R46	43.2	37	35	38.1		36.6
2	BRS98 C8R87	48.3	40	47.1		49.1	48.1
3	BRS98 C14R93	44.0	36	39.2	40.6		39.9
4	BRS98 C16R41	40.1	36	34.9	35.7		35.3
5	BRS98 C6R79	38.0	36	33.5	35		34.3
6	BRS98 C10R93	47.6	38	39.7	43.1		41.4
7	BRS98 C9R72	35.8	36			36.7	36.7
8	BRS98 C7R109	37.3	35	30.5	33.8		32.2
9	TFRS98 C43R23	31.1	36		38.1	34.3	36.2
10	TFRS98 C9R22	35.9	38	30.9	34.1		32.5
11	TFRS98 C16R28	33.7	38	34.5	34.5	38	35.7
12	BRS98 C15R37	36.0	36	41.3	37.9		39.6
13	BRS98 C6R73	32.8	36		31.1	33.9	32.5
14	BRS98 C10R101	38.9	36	35.7	36.1		35.9
15	BRS98 C7R74	39.8	37	33.5	36.4	41.2	37.0
16	TFRS98 C38R5	41.3	37	34.2	41.6		37.9
17	BRS98 C11R80	38.0	36	34.5	35.2		34.9
18	BRS98 C14R25	41.8	36	33.4	36.6		35.0
19	BRS98 C10R111	35.8	36	30.3	36.4		33.4
20	BRS98 C13R115	40.3	36	37.1	36.9	43.2	39.1
21	BRS98 C6R16	37.0	36	30.5	35.5		33.0
22	BRS98 C9R75	45.9	38		40.7	47.2	44.0
23	BRS98 C11R35	45.6	39	41.1	40.5	46.7	42.8
24	TFRS98 C2R10	38.2	37			36.2	36.2
25	TFRS98 C44R15	37.9	35	34	33.9		34.0
26	BRS98 C5R96	33.6	37		37	40.5	38.8
27	BRS98 C16R37	40.6	37	33.6	32		32.8
28	BRS98 C12R97	41.7	38	39	38.2	42.7	40.0
29	BRS98 C10R105	40.6	38	39.9	39.1	43.1	40.7
30	BRS98 C9R92	42.9	36	34.6	37.9	44	38.8
31	TFRS98 R8C9	44.7	36			39.5	39.5
32	BRS98 C15R117	43.8	38	44.3	42.8		43.6
33	TFRS98 C17R3	39.6	37	29.1	39.8		34.5
34	TFRS98 C42R17	32.9	34	28	30.5	35.6	31.4
35	BRS97 C7R8	32.7	35	28	28.9		28.5
36	BRS97 C8R37	36.4	37	37.4	35.9		36.7
37	BRS98 C12R81	39.3	39	35.2	35		35.1
38	BRS98 C13R118	39.8	36				
39	BRS98 C12R13	37.9	35	35.6	34	38.1	35.9
40	BRS98 C14R33	44.0	36	36.6	39.3		38.0

Table 10.12. Total kernel recovery of the ranked candidate cultivars. The mean processor is based mean of the 3 commercial processors. The modelled value is as used in the development of the rankings. The actual value measured for the years that kernel were assessed are shown and the mean of these values calculated. Note no measure of kernel recovery was made for candidate 38 and the method of processing nut-in-shell to determine kernel recovery differed between years

Table 10.13a

Rank	Identity	Trial	Row	Column	Canopy width @ Age 5 (m)	Modelled Age of first crop	Modelled Yield (kg)					Measured Total Yield (kg)	Modelled Kernel recovery	% Whole kernel	Ave whole diameter (mm)
							Age 5	Age 6	Age 7	Age 8	Total				
Mean Commercial Cultivars					3.6	5.3	0.1	1.1	5.3	6.0	12.5	B97= 7.5 T98= 4.4 B98=11.1	38	49	18.4
1	9401672	B97	2	46	3.8	5.4	0.3	0.8	14.8	9.2	25	24.0	37	37	19.1
2	9504161	B98	8	87	3.1	5.0	0.4	2.7	8.1	8.6	19.7	26.7	40	41	18.4
3	9505869	B98	14	93	4.0	5.3	0.2	1.02	11.6	10.3	23.4	40.7	36	34	18.1
4	9503532	B98	16	41	4.2	5.3	0.2	1.3	11.8	10.4	23.8	38.4	36	41	18.0
5	9501923	B98	6	79	3.9	5.3	0.2	1.0	10.5	11.8	23.4	34.1	36	41	17.4
6	9502422	B98	10	93	4.6	5.3	0.2	1.3	13.7	9.6	24.7	36.2	38	33	19.4
7	9504143	B98	9	72	3.7	5.7	0.1	0.2	3.0	15.2	18.6	15.5	36	37	17.8
8	9503361	B98	7	109	3.7	5.5	0.2	0.6	9.0	9.9	19.8	22.6	35	38	17.7
9	9505654	T98	43	23	4.1	5.5	0.1	1.7	8.8	10.8	21.4	35.6	36	41	18.6
10	9502502	T98	9	22	4.3	5.5	0.1	1.1	7.4	12.2	20.8	40.4	38	39	18.4

11	9501661	T98	16	28	4.4	5.0	0.3	3.4	11.0	8.4	23.1	23.7	38	41	18.1
12	9500812	B98	15	37	3.5	5.4	0.2	0.6	8.4	8.2	17.4	20.6	36	41	18.2
13	9504117	B98	6	73	4.1	5.5	0.1	0.5	11.5	9.0	21.0	25.3	36	41	18.2
14	9503978	B98	10	101	4.1	5.5	0.2	1.2	10.3	8.6	20.2	37.2	36	40	17.5
15	9505963	B98	7	74	4.1	5.4	0.4	0.9	6.7	10.1	18.0	26.0	37	40	18.8
16	9504239	T98	38	5	4.0	5.4	0.2	0.7	11.4	6.5	18.8	11.5	37	39	18.1
17	9506018	B98	11	80	4.0	5.4	0.2	0.8	9.5	9.1	19.7	29.4	36	41	17.6
18	9502719	B98	14	25	4.4	5.3	0.2	1.1	10.7	9.4	21.4	36.8	36	40	18.0
19	9503824	B98	10	111	4.3	5.2	0.3	0.7	10.8	9.5	21.2	27.3	36	37	18.9
20	9503308	B98	13	115	4.1	5.5	0.2	0.7	9.6	7.9	18.4	22.4	36	40	17.9

Table 10.13a and b. Modelled performance of top 40 ranked candidate cultivars indicating the site they originated from, the site average yield and the actual yield for those specific trees.

Rank	Identity	Trial	Row	Column	Canopy width @ Age 5 (m)	Modelled Age of first crop	Modelled Yield (kg)					Measured Total Yield (kg)	Modelled Kernel recovery	% Whole kernel	Ave whole diameter (mm)
							Age 5	Age 6	Age 7	Age 8	Total				
Mean Commercial Cultivars					3.6	5.3	0.1	1.1	5.3	6.0	12.5	B97= 7.5 T98= 4.4 B98=11.1	38	49	18.4
21	9400523	B97	6	16	3.5	5.5	0.1	0.6	9.1	6.3	16.2	8.5	36	39	18.8
22	9500892	B98	9	75	3.8	5.5	0.1	0.5	7.1	9.0	16.8	19.6	38	38	18.1
23	9504551	B98	11	35	4.1	5.4	0.1	0.6	6.5	9.5	16.8	22.6	39	39	18.5
24	9503631	T98	2	10	3.9	5.5	0.2	0.6	10.8	5.3	17.0	11.1	37	40	18.2
25	9505835	T98	44	15	4.2	5.4	0.2	0.8	7.4	10.4	18.8	27.8	35	40	18.1
26	9505781	B98	5	96	3.7	5.5	0.2	0.6	7.8	7.5	16.0	14.8	37	40	18.2
27	9503991	B98	16	37	3.9	5.3	0.3	1.2	7.8	8.8	18.0	33.5	37	40	18.2
28	9503607	B98	12	97	3.9	5.3	0.1	1.2	9.2	6.1	16.5	21.9	38	39	18.6
29	9502946	B98	10	105	3.8	5.1	0.4	1.6	6.6	7.5	16.1	27.8	38	40	18.6

Rank	Identity	Trial	Row	Column	Canopy width @ Age 5 (m)	Modelled Age of first crop	Modelled Yield (kg)					Measured Total Yield (kg)	Modelled Kernel recovery	% Whole kernel	Ave whole diameter (mm)
							Age 5	Age 6	Age 7	Age 8	Total				
30	9500589	B98	9	92	4.1	5.5	0.1	0.6	6.8	9.6	17.1	20.4	36	37	17.5
31	9500628	T98	8	9	4.1	5.5	0.2	0.9	13.9	3.6	18.6	9.0	36	42	17.7
32	9503017	B98	15	117	4.0	5.4	0.3	0.9	7.2	7.4	15.8	27.7	38	38	18.8
33	9505965	T98	17	3	4.4	5.0	0.4	2.8	7.3	8.6	19.0	22.8	37	37	18.4
34	9505657	T98	42	17	4.0	5.4	0.1	0.7	5.8	11.3	17.9	24.7	34	40	18.5
35	9400242	B97	7	8	3.8	5.4	0.3	0.7	8.2	8.8	17.9	7.0	35	37	18.7
36	9400426	B97	8	37	3.8	5.5	0.2	0.9	9.0	7.1	17.2	14.0	37	39	17.2
37	9501162	B98	12	81	4.0	5.1	0.3	2.2	7.6	8.1	18.3	29.5	39	41	18.8
38	9503476	B98	13	118	3.6	5.5	0.2	0.5	7.7	7.5	15.8	13.4	36	40	17.8
39	9501698	B98	12	13	4.4	5.2	0.2	1.1	9.4	9.5	20.2	38.4	35	35	18.1
40	9503883	B98	14	33	4.1	5.3	0.2	0.6	8.5	8.6	18.0	27.4	36	34	18.0