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Quantitative and molecular genetics of juvenile wood traits in radiata and slash/Caribbean pines

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www.fwpa.com.au
FWPA Level 4, 10-16 Queen Street,
Melbourne VIC 3000, Australia
T +61 (0)3 9614 7544 F +61 (0)3 9614 6822
E info@fwpa.com.au W www.fwpa.com.au

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Prepared for

Forest & Wood Products Australia

by

H. Wu, T. McRae, S. Southerton, W. Gapare, B. Baltunis, X. Li, S. Dillon, M. Ivkovic, M. Powell, M. Dieters, K. Harding, C. Matherson and J. Ilic

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Researcher:
H. Wu, S. Southerton, W. Gapare, B. Baltunis, X. Li, S. Dillon, M. Ivkovic, M. Powell, and C. Matherson
CSIRO Materials Science and Engineering
Locked Bag 10
Clayton South VIC 3169

T. McRae
Southern Tree Breeders Association (STBA)
PO Box 1811
MT GAMBIER, SA, 5290

M. Dieters
School of Land, Crop and Food Science
University of Brisbane
BRISBANE, QLD, 4072

K. Harding
DEEDI
QLD Primary Industries and Fisheries
PO Box 1085
TOWNSVILLE, QLD, 4810

J. Ilic
CSIRO Division of Forestry and
Forest Products (forma)
Private Bag 10
CLAYTON SOUTH, VIC, 3069

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Forest & Wood Products Australia Limited
Level 4, 10-16 Queen St, Melbourne, Victoria, 3000
T +61 3 9614 7544 F +61 3 9614 6822
E info@fwpa.com.au
W www.fwpa.com.au

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EXECUTIVE SUMMARY

The Juvenile Wood Initiative (JWI) project has been running successfully since July 2003 under a Research Agreement with FWPA and Letters of Association with the consortium partners STBA (Southern Tree Breeding Association), ArborGen and FPQ (Forestry Plantations Queensland).

Over the last five and half years, JWI scientists in CSIRO, FPQ, and STBA have completed all 12 major milestones and 28 component milestones according to the project schedule. We have made benchmark progress in understanding the genetic control of wood formation and interrelationships among wood traits. The project has made 15 primary scientific findings and several results have been adopted by industry as summarized below. This progress was detailed in 10 technical reports to funding organizations and industry clients. Team scientists produced 16 scientific manuscripts (8 published, 1 in press, 2 submitted, and several others in the process of submission) and 15 conference papers or presentations.

Primary Scientific Findings

The 15 major scientific findings related to wood science, inheritance and the genetic basis of juvenile wood traits are:

1. An optimal method to predict stiffness of standing trees in slash/Caribbean pine is to combine gravimetric basic density from 12 mm increment cores with a standing tree prediction of MoE using a time of flight acoustic tool. This was the most accurate and cheapest way to rank trees for breeding selection for slash/Caribbean hybrid pine. This method was also recommended for radiata pine.
2. Wood density breeding values were predicted for the first time in the STBA breeding population using a large sample of 7,078 trees (increment cores) and it was estimated that selection of the best 250 trees for deployment will produce wood density gains of 12.4%.
3. Large genetic variation for a suite of wood quality traits including density, MFA, spiral grain, shrinkage, acoustic and non-acoustic stiffness (MoE) for clear wood and standing trees were observed. Genetic gains of between 8 and 49% were predicted for these wood quality traits with selection intensity between 1 to 10% for radiata pine.
4. Site had a major effect on juvenile-mature wood transition age and the effect of selective breeding for a shorter juvenile wood formation phase was only moderate (about 10% genetic gain with 10% selection intensity, equivalent to about 2 years reduction of juvenile wood).
5. The study found no usable site by genotype interactions for the wood quality traits of density, MFA and MoE for both radiata and slash/Caribbean pines, suggesting that assessment of wood properties on one or two sites will provide reliable estimates of the genetic worth of individuals for use in future breeding.
6. There were significant and sizable genotype by environment interactions between the mainland and Tasmanian regions and within Tasmania for DBH and branch size.
7. Strong genetic correlations between rings for density, MFA and MoE for both radiata and slash/Caribbean pines were observed. This suggests that selection for improved wood properties in the innermost rings would also result in improvement of wood properties in the subsequent rings, as well as improved average performance of the entire core.
8. Strong genetic correlations between pure species and hybrid performance for each of the wood quality traits were observed in the hybrid pines. Parental performance can be used to

identify the hybrid families which are most likely to have superior juvenile wood properties of the slash/Caribbean F1 hybrid in southeast Queensland.
9. Large unfavourable genetic correlations between growth and wood quality traits were a prominent feature in radiata pine, indicating that overcoming this unfavourable genetic correlation will be a major technical issue in progressing radiata pine breeding.
10. The project created the first radiata pine 18 k cDNA microarray and generated 5,952 radiata pine xylogenesis expressed sequence tags (ESTs) which assembled into 3,304 unigenes.
11. A total of 348 genes were identified as preferentially expressed genes in earlywood or latewood while a total of 168 genes were identified as preferentially expressed genes in either juvenile or mature wood.
12. Juvenile earlywood has a distinct transcriptome relative to other stages of wood development.
13. Discovered rapid decay of linkage disequilibrium (LD) in radiata pine with LD decaying to approximately 50% within 1,700 base pairs (within a typical gene). A total of 913 SNPS from sequencing 177,380 base pairs were identified for association genetic studies.
14. 149 SNPs from 44 genes and 255 SNPs from a further 51 genes (total 95 genes) were selected for association analysis with 62 wood traits, and 30 SNPs were shortlisted for their significant association with variation of wood quality traits (density, MFA and MoE) with individual significant SNPs accounting for between 1.9 and 9.7% of the total genetic variation in traits.
15. Index selection using breeding objectives was the most profitable selection method for radiata pine, but in the long term it may not be the most effective in dealing with negative genetic correlations between wood volume and quality traits. A combination of economic and biological approaches may be needed to deal with the strong adverse correlation.

Industry Adoption and Impact

1. The breeding values for 7,078 trees from the STBA breeding population for deployment and breeding purposes in the third generation of breeding were adopted since 2004 with an expected gain of 12.4% in wood density.
2. The best log stiffness predictor for slash/Caribbean pine and radiata pine (combining gravimetric basic density of 12 mm increment cores with time of flight acoustic wave on a standing tree) was adopted by STBA and FPQ.
3. Breeding values of standing tree stiffness, and dynamic and static MoE for more than 4,000 trees estimated from the STBA trials were integrated with the TreePlan data base and selection index.
4. The estimated net present value was more than $A400 million for selection from progeny of the second generation to third generation of breeding population for STBA members and more than $A800 million for the entire radiata pine plantation estate using genetic results from JWI in combination with results from breeding objective project.
5. CSIRO and STBA are leading wood quality genetics research, by (1) initiating a joint IUFRO Southern Pine and inaugural Australasia Forest Genetic Conference dedicated to "Breeding for Wood Quality in 2007 and (2) visits by overseas scientists including Dr John Mackay, Dr Alvin Yanchuk from Canada and upcoming visits of Dr Leopoldo Sanchez, and Dr John Russell from France and Canada for collaboration, and (3) invitations for presentations to international conferences including a Keynote speech in the 2008 IUFRO Genetics Conference for Wood Quality Breeding in Quebec, Canada.

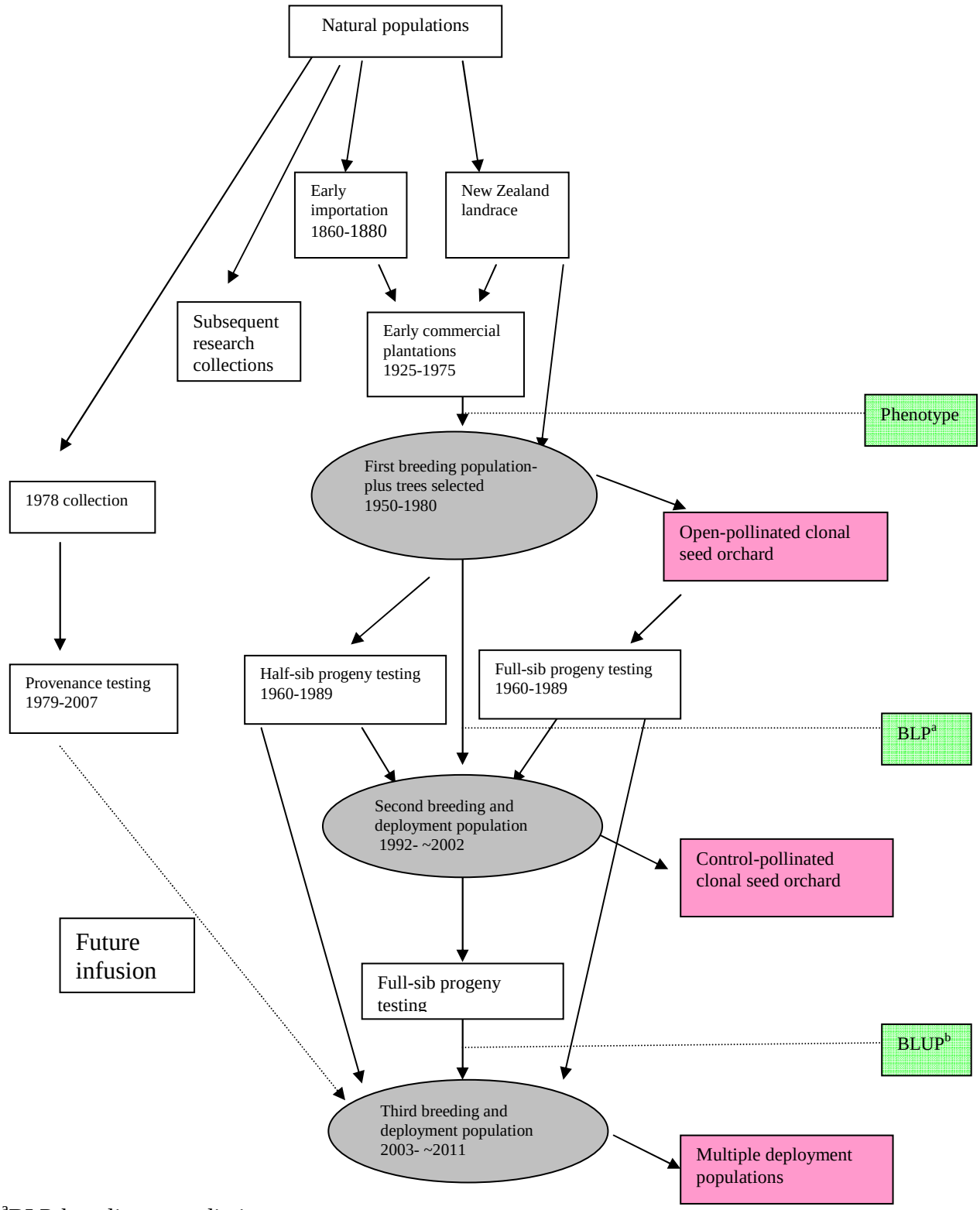
INTRODUCTION

Genetic improvement of radiata pine (*Pinus radiata* D. Don) in Australia was initiated in 1950s through selection of plus trees. The subsequent tree breeding work in radiata pine followed the conventional conifer breeding methods: selection, grafting of plus trees and establishment of clonal seed orchards with concurrent progeny testing. The first clonal seed orchard of radiata pine was established in 1957. By 1970s, more than 350 ha of seed orchards and 300 progeny trials had been established across New South Wales, the Australia Capital Territory (ACT), Victoria, South Australia, Tasmania, Queensland and Western Australia. At the same time, the South Australian and Queensland Forest Services were the first radiata pine forestry organisations to use genetically improved radiata planting stock exclusively. By 1985, seed orchard seed was used for planting most Australian radiata pine plantations (Wu *et al.* 2007).

In the 1970s and early 1980s, radiata pine breeding was mainly carried out by separate programs of the six State Forestry Services and private companies, with direct or indirect involvement of CSIRO (Eldridge 1983). Research Working Group One (RWG1 Forest Genetics) of the Australian Forestry Council played a significant role in fostering material exchange and information sharing among breeding programs. Recognising the importance of a coordinated program for advanced generation breeding, in the late 1970s CSIRO initiated an Australia-wide diallel mating and testing program. In 1982, the Southern Tree Breeding Association (STBA, an incorporated non-profit breeding organisation funded by industry organisations) was established with the assistance of CSIRO to conduct a radiata pine breeding program for two radiata pine companies and the State Government of South Australia; it eventually expanded into a national breeding organisation. A second generation breeding population was established in the 1990s for the advanced breeding program (White *et al.* 1999). The progress of the radiata pine tree improvement program is summarized in Figure 1.

Genetic improvement of radiata pine by CSIRO, STBA and other collaborators has generated significant economic benefits for Australian forestry industries. An independent economic evaluation (Sultech Report 1999) found that Australian investment in radiata pine breeding to 1999 had a net present value of A$927 million, through increased productivity and quality of the plantations produced. Realised genetic gain up to 33% was reported for volume at age 15 years from the first generation selections in one seed orchard (Matheson *et al*. 1986). Most first generation gain trials measured at 10-15 years old indicated an average of 20 to 25% volume gains (Eldridge 1982, Johnson *et al.* 1992). There is also a trend for gain to increase with the age. An internal rate of return of 20% was estimated as the economic return from first generation breeding (Wright and Eldridge 1985). Boomsma (1992) estimated that the best control-pollinated families could produce about 23% gain on top of about 24% gain from rogued seed orchards (G1.5) on an average site quality of four in Mount Gambier. Also, gains from organic matter conservation, nutrients (use of fertiliser), and moisture conservation (control of weeds) could be additive. Predicted genetic gains between 4 and 17% in diameter for the second-generation selections indicated a volume gain of more than 10% (Wu and Matheson 2005, White *et al*. 1999).

Figure 1. The generalised flow chart of radiata pine improvement in Australia.



[a]BLP-best linear prediction,
[b]BLUP- best linear unbiased prediction

Breeding for growth rate and tree form has reduced wood density slightly in radiata pine due to the negative genetic correlation between growth rate and wood density (Dean 1990,

Cotterill and Dean 1990, Wu *et al.* 2008). However, slightly reduced density may not be a large concern for mature wood which is of relatively high density. Mature wood has desirable characteristics for structural timber such as high wood density, low microfibril angle (MFA) and high stiffness (MoE- Modulus of Elasticity, Walker and Nakada 1999).

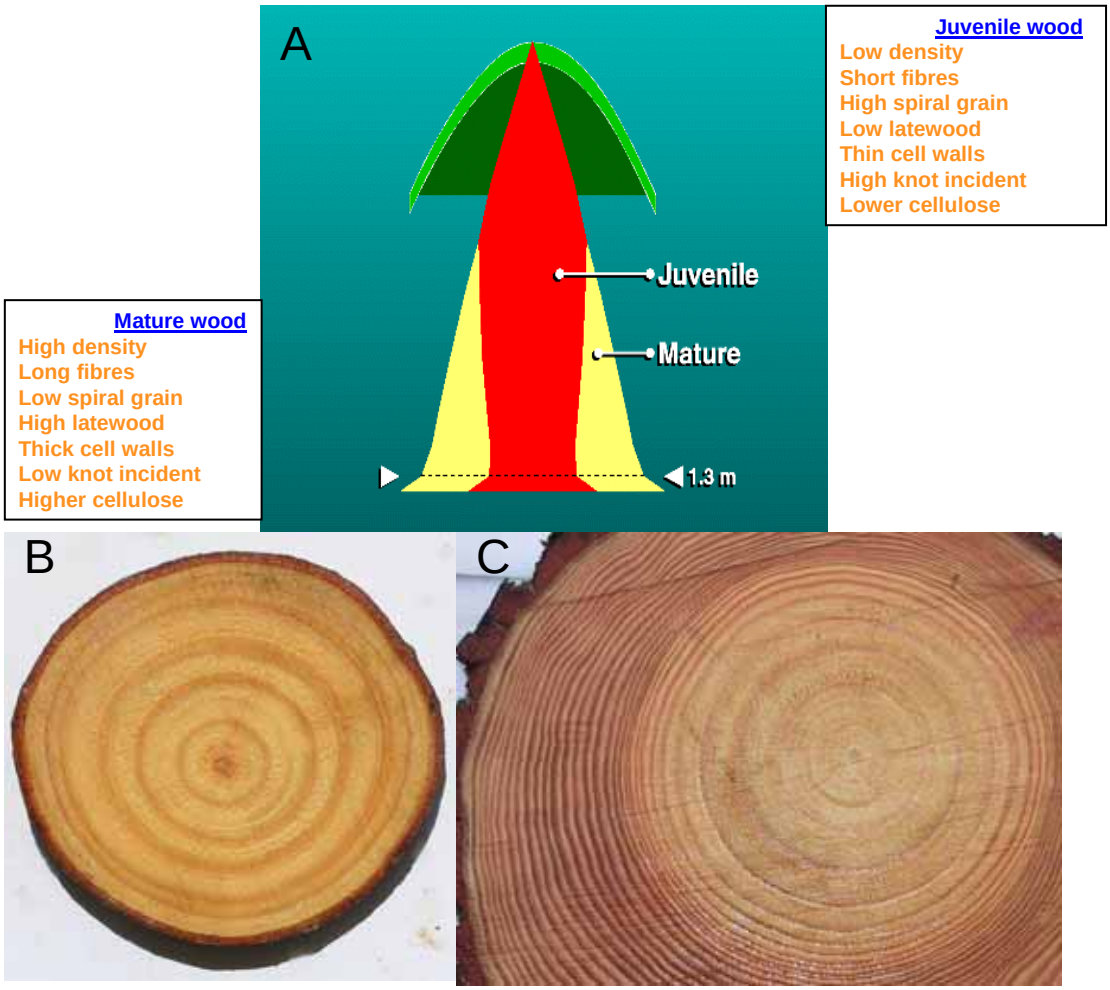


Figure 2. Schematic location and properties of *P. radiata* juvenile (corewood) and mature wood (outerwood) in a tree (A) and a 7-year-old disk of juvenile tree (B) and a 33-year-old disk with juvenile and mature wood (C).

The biggest concern for faster-growing pines is the higher proportion of juvenile wood in the harvested logs resulting from a reduced rotation age. The typical rotation age for radiata pine has been reduced from about age 40-45 to about age 30 throughout much of the radiata pine estate in Australia (e.g. the typical rotation age has been reduced from age 40 to age 27 in the Green Triangle region). Similarly, the rotation age for slash and Caribbean pines has also been reduced. This reduction of rotation age has resulted in a substantial increase in the proportion of juvenile wood (also called corewood by Zobel, Webb and Henson 1959). Juvenile wood (Figure 2) is defined as wood produced in the first five to 25 growth rings, depending on species and sites (Haygreen and Bowyer 1982, p.106). For practical purposes, juvenile wood in radiata pine from plantation timber is often referred to as the first 10 annual growth layers (Harris and Cown 1991), and a similar definition is applied to wood of slash/Caribbean hybrid pine. Recently, Burdon *et al.* (2004) categorised radiata and loblolly

pine wood into nine classes based on radial (pith to bark) and vertical (or axial) variation in the stem. By their definitions, juvenile wood can be divided into juvenile, transitional, and mature corewood based on tree height and maturation. Whether using terminology of juvenile wood or corewood, radiata pine at rotation age 27 has a significant amount of corewood, particularly juvenile corewood, about one third to one half of the harvested log. The term juvenile wood, referred to juvenile corewood in most studies, is used in this report.

Juvenile wood is recognised as likely to be of inferior quality and has the following characteristics (Bendtson 1978, Zobel and van Buijtenen 1989): high levels of spiral grain, low basic density, high moisture content before heartwood formation, a high microfibril angle in the S2 layer, above average amounts of compression wood, moderate to high longitudinal shrinkage, timber prone to warp, a low percentage of cellulose and short tracheids. These characteristics extend up the tree and affect the quality of timber from logs with a high proportion of juvenile wood, including poorer grade recovery, lower strength, more distortion and surface checks, and poorer finishing properties for structural timber (Macalister 1997, Gaunt 1998, Walford 1996, 1999). The effects of low density and high spiral grain, coupled with knots, abnormal longitudinal shrinkage, and compression wood, have already given "young" logs a poor reputation in some sawmills in New Zealand and Australia (Cown 1992, Chambers *et al.* 2000). It is important to determine how these undesirable characteristics are distributed axially as well as from pith to bark as they have a major effect on processing recoveries.

For these reasons the focus of radiata pine breeding for the third generation has shifted to wood quality traits. In 2003 CSIRO in partnership with STBA, FPQ and Forest and Wood Products Australia (FWPA) and ArborGen began work on the "Juvenile Wood Initiative" project covering radiata and the slash/Caribbean hybrid pines. This project aims to significantly increase the value of Australia's pine wood production by reducing the proportion of juvenile wood produced in each tree (decreasing the age of transition from juvenile wood to mature wood) and by improving the quality of the wood (for example, increasing the inherent stiffness of the juvenile wood) through integration of quantitative genetics, molecular genetics and biotechnology.

OVERALL AIMS

The project aims to improve juvenile wood of radiata pine and slash/Caribbean hybrid pine in the STBA and FPQ breeding and deployment populations to substantially increase timber value for the Australian softwood industry.

Specific objectives are as follows:

- Develop non-destructive method, including acoustic techniques, to predict juvenile wood properties and stiffness (MoE- Modulus of Elasticity) for breeding and deployment purposes
- Quantitative genetic analysis of juvenile wood characteristics (MoE, basic density, MFA-Microfibril Angle and other traits) in radiata and slash/Caribbean hybrid pine populations
- Quantify genetic control of the transition from juvenile to mature wood in radiata pine populations
- Estimate parental and individual breeding values of third-generation progeny in radiata pine breeding populations
- Identify candidate genes for juvenile wood traits through differential gene expression
- Characterise selected genes by sequencing and expression analysis
- Develop single nucleotide polymorphic (SNP) markers and map candidate genes selected from microarrays and sequence databases in radiata pine populations
- Conduct quantitative trait loci (QTL) or association genetics analyses for wood traits using SNP and microsatellite markers
- Develop preliminary selection strategies to integrate quantitative and molecular information into breeding programs for improved juvenile wood in radiata pine

METHODOLODY

1. Methodological Development for Measuring Stiffness of Juvenile Wood in Radiata Pine

1.1. Stiffness of standing trees

One of the constraints in improving stiffness of radiata pine is the high cost of directly measuring stiffness in young standing trees and damage to the measured trees if a destructive static measure was used. While a direct measure of the bending stiffness is the most accurate, indirect measures that are far less destructive and expensive are the most desirable for breeding purposes. Recent work has shown that stiffness can be indirectly measured by either using mechanical and chemical properties of wood or using component wood quality traits. Acoustic methods are less destructive for measuring stiffness in standing trees than other techniques such as the removal of either axial strips or radial increment cores. In this project, different acoustics tools and methods were tested to develop optimal methods (quick, low cost and accurate) to screen large numbers of trees for stiffness traits and breeding purposes.

Two young radiata pine trials (BR9611 at Flynn and BR9705 at Kromelite) from the STBA breeding population planted in 1996 and 1997, respectively, were used for determining the best method for measuring stiffness of young standing trees in radiata pine and for detailed studies for inheritance of wood density, MFA, spiral grain, shrinkage, dynamic and static MoE. Flynn site had 250 families, consisting of 88 polycross families, 157 full-sib families, and 5 controls, and Kromelite site had 110 families, consisting of 70 polycross families and 40 full-sib families). There were 41 parents and 16 full-sib families common to both sites.

Table 1. Details of the Flynn and Kromelite sites selected for the Juvenile Wood Initiative project

Details	**Flynn**	**Kromelite**
Experiment number	BR9611	BR9705
Date planted	6/1996	7/1997
Cambial age at time of sampling	7	6
Spacing	3.6 m x 2.5 m	2.74 m x 2.5 m
Latitude	38^{o} 14’S	37^{o} 50’S
Longitude	146^{o} 45’E	140^{o} 55’E
Elevation (m)	166	55
Annual rainfall (mm)	760	900
Soil type	Sandy loam	Sandy clay-loam
Site type	2nd pine rotation	2nd pine rotation
Families	250	110
Blocks	4	3
Columns within blocks	25	11
Rows within a column	25	30
Samples used for SilviScan	980 (4 per family)	660 (6 per family)
Samples used for shrinkage	466 (2)	308 (3)
Samples used for spiral grain	628 (4)	316 (3)

In order to determine the optimal instrument and method to measure tree stiffness, the following five acoustic and non-acoustic instruments were tested for the project:
Fakopp stress wave timer (www.fakopp.com). This involves inserting two probes, each with a sensor. The bottom probe is tapped with a small hammer and the time for the stress wave to travel between the probes is measured. The distance between probes is usually about 1 m, but must be known accurately. Fakopp was used only on one side of the tree.
IML Hammer stress wave timer (www.walesch.ch). This involves inserting two generic probes (or screws) and attaching a sensor to the top probe. The bottom probe is tapped with the IML hammer (Figure 3A). The IML hammer contains a strain gauge to detect the travel time of the stress wave. The distance between the probes must be known and is usually about 1 m. The IML was used on two opposing sides of the trees.
Krautkramer USD10-NS Ultrasound flaw detector (www.geinspectiontechnologies.com). This involves inserting two probes a known distance apart and applying ultrasound waves to the bottom probe and timing their arrival at the top probe. Because the wavelength is small, it is necessary to avoid branches and insert the probes in clearwood. Probes are typically about 300 mm apart.
Tree sway. This involved manually swaying the trees and filming the frequency of sway with a digital movie camera. The movie is viewed frame-by-frame to determine the frequency of sway.
Dynamic MoE of axial beams (paddle pop). This involves cutting axial specimens approximately 120 mm x 10-15 mm x 2 mm from the outerwood of a tree. In this case, they were cut from disks removed from the logs during the sampling process. These are tested directly in the laboratory for dynamic MoE (Ilic 2001, Figure 3B).

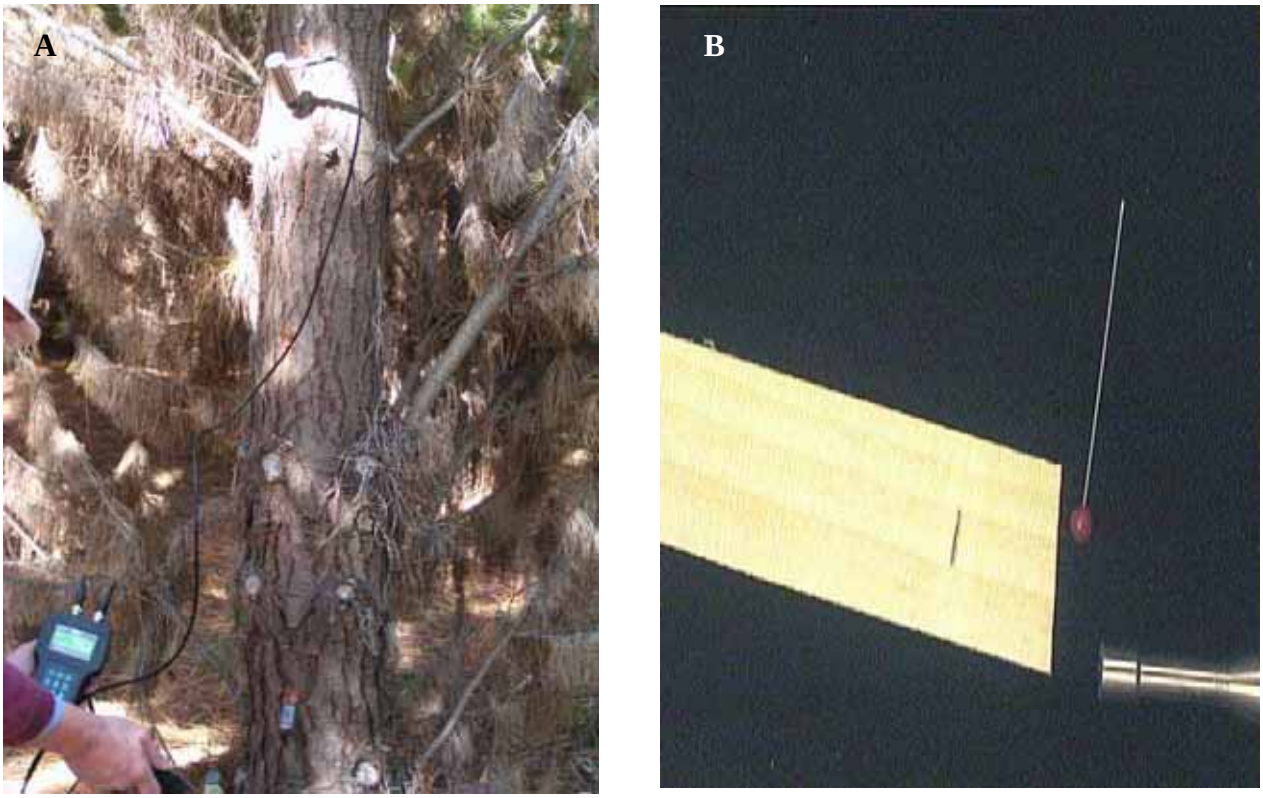


Figure 3. Acoustic measurement of stiffness in a standing tree of radiata pine using IML hammer (A) and an axial beam (paddle pop) sample (B).

Measurements on standing trees were compared with benchmark measurements based on logs cut down from the same trees and using the following two instruments:
Director HM200 (www.fibre-gen.com). This is an instrument developed by CCH Fibre-gen as a variation of Hitman to segregate logs based on their resonance. Director is placed against the lower cut surface and the log is hit with a hammer. The resonant frequency is detected with a microphone and recorded.
HP Dual Channel Dynamic Signal Analyzer 35665A. This instrument works in a manner analogous to the Director HM200. Its microphone detects resonances from the log and displays it with detected harmonics on a screen. The dominant harmonic can be determined visually and its frequency recorded.

1.2 Stiffness of clearwood board

In addition to predicting stiffness of harvested logs from standing trees , a second objective is to further examine whether stiffness, strength and stability of clearwood board sawn from logs of young radiata pine trees can be reliably predicted from field acoustic measurements and the component wood quality traits of harvested logs. Studies were conducted to relate stiffness, strength, and shrinkage of clearwood boards with the component measurements of wood quality traits using the same two young radiata pine trials (Flynn and Kromelite). A total of 850 trees were sampled for the study (Figure 4).

Figure 4. A total of 18.6 tons of logs from 850 trees were sampled from the Flynn and Kromelite sites for relating stiffness of boards with component wood quality traits and for studing the genetics of juvenile wood traits.

The harvested logs were segmented for measurement of component wood quality traits (Figure 5). The component wood quality traits used include wood density, MFA, MoE measurement from 12 mm increment cores, standing tree stiffness measurement using IML readings, spiral grain measurement on samples (A), dynamic MoE measurement on shrinkage samples (B, C, and D), dynamic and static MoE measurement on clearwood samples (E, F, and G) and dynamic MoE measurement using paddle pop sample (H). Multiple regression and path analyses were used to relate the component wood quality traits with stiffness, strength and stability of clearwood board and to develop a juvenile wood stiffness index for breeding selection purposes.

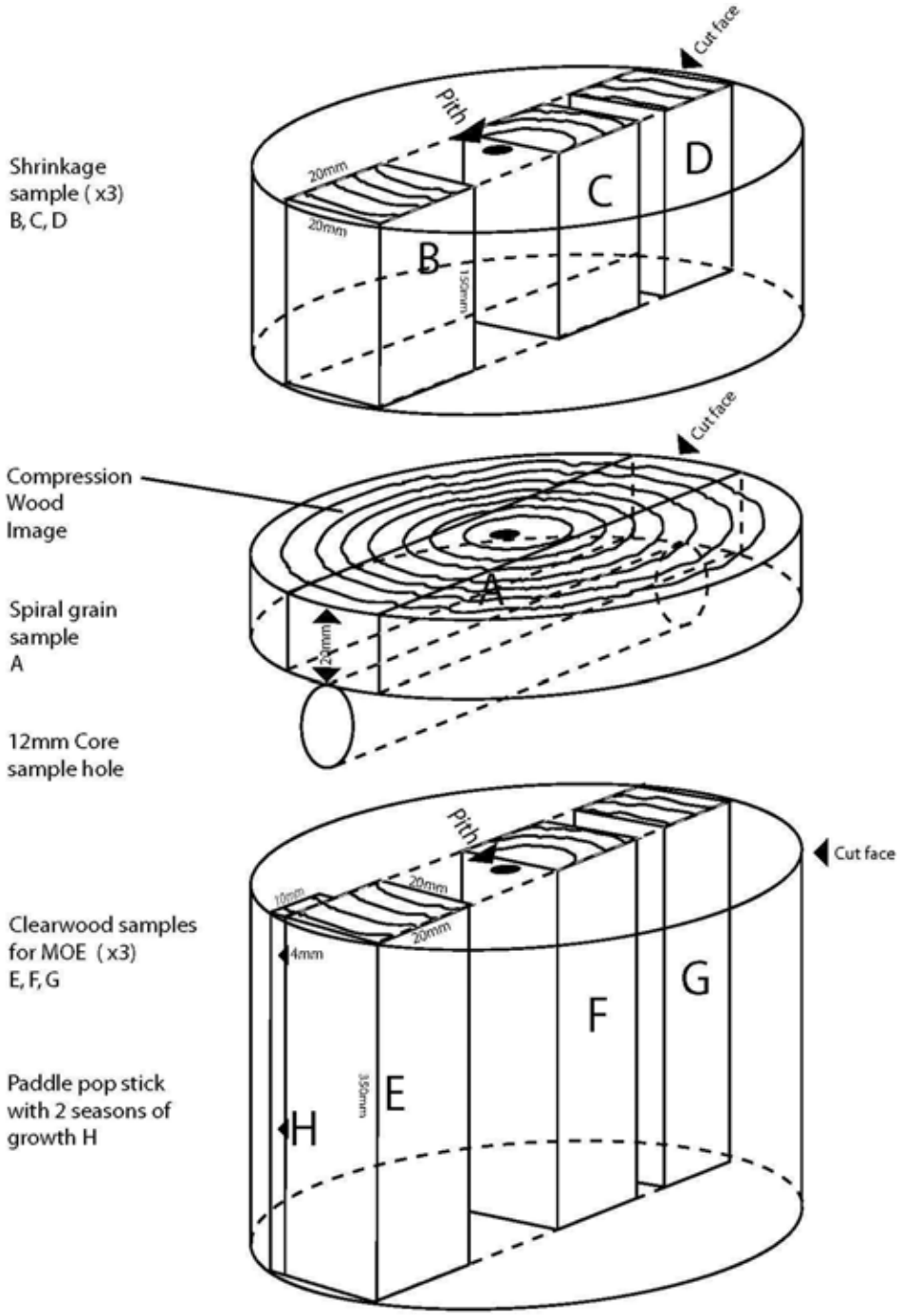


Figure 5. Sub-sampling of logs (stem sections) harvested from Flynn and Kromelite sites.

2. Methodological Development for Measuring Stiffness of Juvenile Wood in Slash and Caribbean Pines, and Their F1 Hybrid

A clonal trial planted with two clonal blocks (clone 545 and 887) was sampled for this project. A single 12 mm increment core was taken from each tree at approximately 1.3 m height at age 6 years. A stratified sample of 25 ramets from clone 545 and 19 ramets from clone 887 was selected across a range of basic density for this study. Prior to felling (April 2002, age 7.25 years), all stems were assessed using a Fakopp stress wave velocity tool. A 'matching' increment core was removed at breast height, in the vicinity of the previous core removed for density assessment in 2001, and used for SilviScan analysis. A 3 m butt log was

docked from each stem and a disk was collected at the top of this 3 m log to assess spiral grain for comparison with breast height values. The stress wave velocity of the butt logs was assessed with a WoodSpec instrument. The butt logs were sawn into structural framing with warp and stress wave velocity measured. The structure framings were cut into three test blocks for measuring stiffness (MoE), strength (MoR), and other traits (Figure 6). Data for regression analyses included:

- Fakopp (standing tree) stress wave velocity
- SilviScan data (ring density-DEN_s, MFA_s, MoE_s)
- Spiral grain, dynamic MoE and MoR for log and boards
- Air-dry density.
- Bow, Spring, and Twist measurements

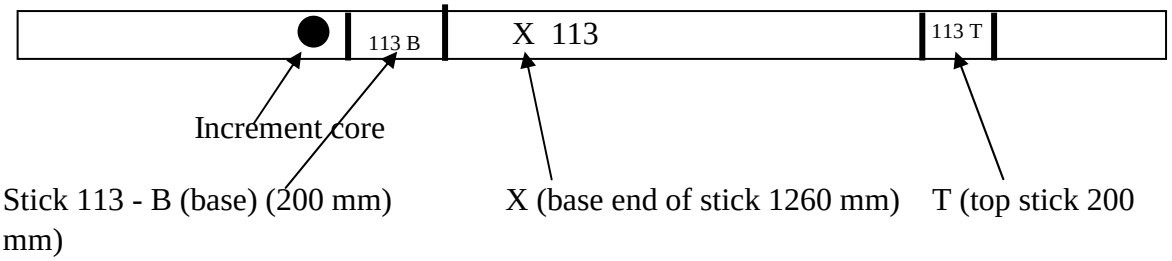


Figure 6. Schematic example of a 3 m stick docked into sub-samples.

Regression equations between component wood characteristics and board MoE were developed to determine which component trait or combination of traits is the best surrogate (phenotypic stiffness index) for determining MoE for boards and standing trees. The most economic and reliable methods of assessing MoE for slash/Caribbean pines was incorporated into FPQ breeding and clonal testing programs.

3. Inheritance and Quantitative Genetics of Juvenile Wood in Radiata Pine

Genetic parameters (heritability, additive and non-additive genetic variance and covariance) and breeding values are critical population parameters to understand genetic control of wood quality traits and to develop efficient selection and breeding strategies for radiata pine. In the Juvenile Wood Initiative project, genetic parameters for transition from juvenile to mature wood, wood density, MFA, MoE, spiral grain and shrinkage were estimated using progeny of the first and second generations of the radiata pine breeding population. These include (1) estimation of genetic control of transition from juvenile to mature wood, (2) prediction of breeding values for wood density for the progeny of the second generation breeding population and selection of the best families for STBA's third-generation deployment and breeding populations, (3) estimation of heritability and genetic variance and covariance for juvenile wood traits (wood density, MFA, MoE, spiral grain, shrinkage), (4) estimation of genotype by environment interaction for juvenile wood quality traits. These genetic parameters were also used for the development of juvenile wood stiffness index to predict board stiffness and study of selection efficiency.

3.1. Genetics of transition age from juvenile to mature wood

To study genetic variation of transition age from juvenile to mature wood, two full-sib family trials with half-diallel mating design, located in Gippsland, Victoria (VRC52 of 100 families

and VRC54 of 52 families at age 16) and two half-sib family trials (PT47 of 33 family at age 27, located in Gippsland, Victoria and PT5042 of 36 family at age 33, located in Tantanoola, South Australia) were harvested for characterizing age profile of wood density (Figure 7). After the wood disks were harvested at breast height, bark-to-bark through the pith flitches of 2 mm thick were sampled to obtain density profile from pith to bark using X-Ray densitometry and the WinDENDRO software package (Regent Instruments Inc, 2001). Three transition ages were statistically determined by using three interrelated approaches: (i) iterative solution, (ii) segmented regression/model, and (iii) constrained solution.

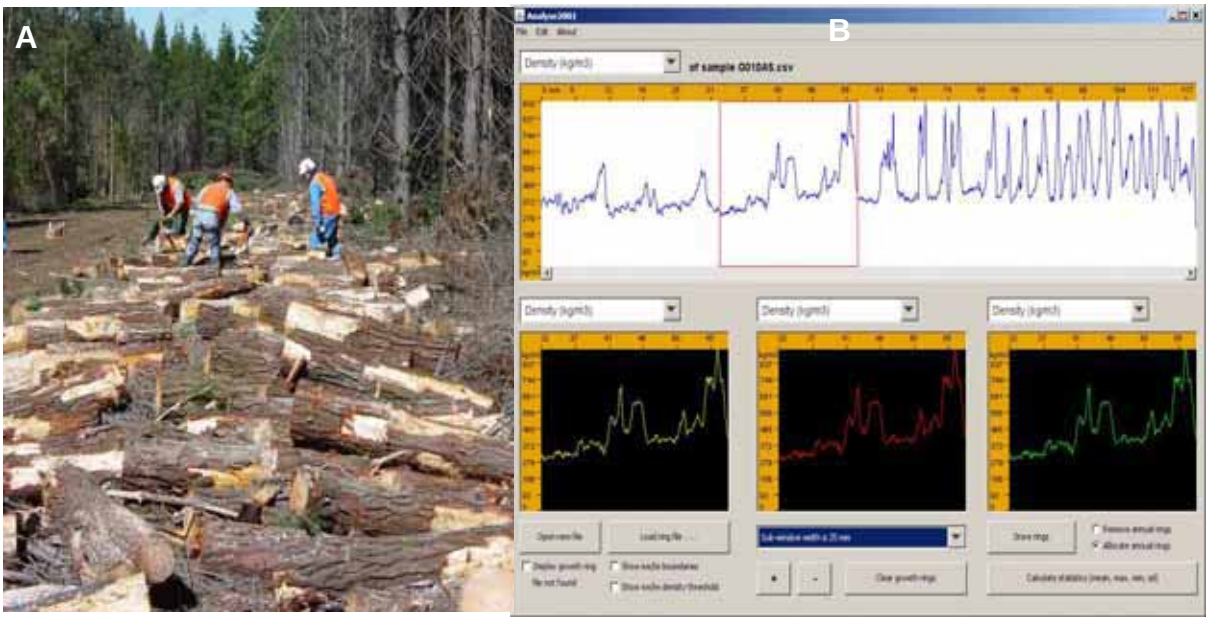


Figure 7. Sampling at VRC54 for genetics of transition from juvenile to mature wood (A) and wood density profile for site PT47 (B).

3.2. Wood density breeding values for progeny of STBA's second generation breeding population

A total of 7,078 increment cores at age 6 and 7 were sampled from six STBA genetic trials in Victoria (VIC), South Australia (SA), Tasmania (TAS) and Western Australia (WA, Table 2, Figure 8). Gravimetric density of these increment cores was measured and breeding values were estimated using ASREML (Gilmore *et al.* 2005) and TreePlan.

Table 2. Details of the six genetic trials selected for breeding value prediction for juvenile wood density

Site	**BR9601**	**BR9611**	**BR9615**	**BR9701**	**BR9705**	**BR9709**
Location	SA	VIC	TAS	WA	SA	VIC
Family	252	247	236	252	110	108
Replicate	5	5	5	5	5	5
Number of core	1274	1933	1192	1285	880	514
Mean density (kg/m3)	334	364	335	333	317	341
Range of density	254-428	295-487	271-415	269-427	264-429	285-438
Heritability	0.718	0.765	0.854	0.494	0.513	0.656

Figure 8. A subset of 7078 increment cores for estimation of breeding values for wood density in radiata pine.

3.3. Inheritance of juvenile wood traits in progeny of STBA's second generation breeding population

To study inheritance of four important juvenile wood traits (ring width, density, MFA, and MoE), a total of 1640 wood increment cores were sampled from Flynn and Kromelite (Table 1). Ring width, density, MFA, and MoE from pith to bark were assessed by SilviScan. Growth rate was greater at BR9705 for the first few growth rings than at BR9611, but wood density was higher at BR9611.

To study genetic variation of juvenile wood spiral grain angle, a total of 944 samples from disks and increment cores collected in Flynn (628) and Kromelite (316, Table 1) were measured for spiral grain using a pivoting digital protractor attached to a fixed platform (Figure 9).

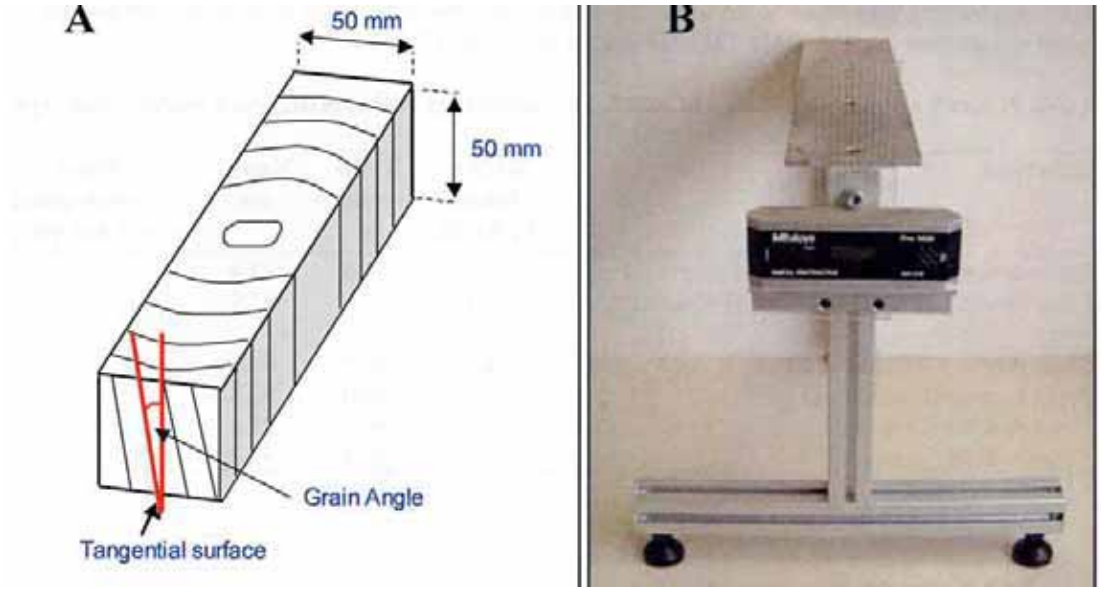


Figure 9. Diametrical strip showing grain angle (A) and the fixed platform and digital protractor used to measure the spiral grain (B).

Inheritance of shrinkage (radial, tangential and longitudinal shrinkage) in juvenile wood was examined using 774 samples collected from the Flynn and Kromelite sites. Boards of 30 mm thickness were cut from the disks with a bandsaw from bark-to-bark through the pith along the North-South axis or perpendicular to the prevailing wind direction, avoiding compression wood and other imperfection (like knots, rots and shakes). Using a thicknessing machine and a table saw, three sub-samples with dimensions 20 x 20 x 150 mm were taken from three positions along each board (B, C and D, Figure 10).

The samples were measured initially in a green state and subsequently oven dried at 103 ± 2°C to determine shrinkage (Figure 10). Moisture content based on oven-dry weight was determined before and after reconditioning. Radial, tangential and longitudinal dimensions were measured using a digital displacement gauge with readings graduated to 0.001 mm. The shrinkage value for radial, tangential and longitudinal expressed as a percentage (%) of the green measurement.

Statistical analyses were carried out using an individual tree model implemented in ASREML (Gilmour *et al.* 2005) for estimating the additive and non-additive genetic variance and covariance, heritability and genetic correlations for data from individual ring and average values.

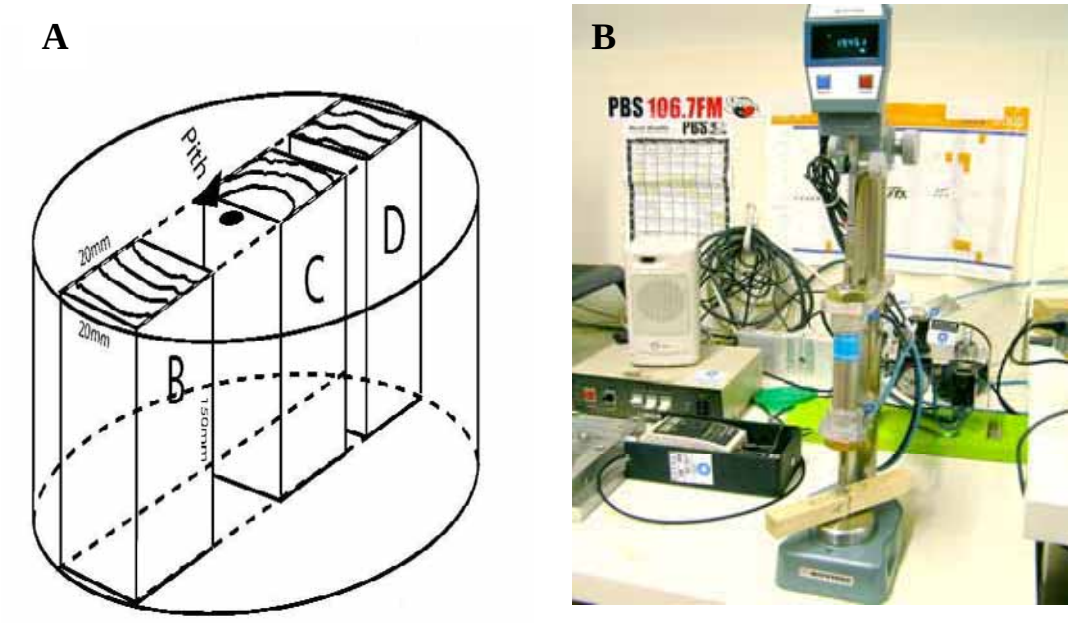


Figure 10. Schematic position of sub-samples (B, C, & D) taken from three positions on each log (A) and digital displacement gauge used to measure shrinkage with pneumatic ram and air pressure meter (B).

3.4. Genotype by environment interactions (G by E) for wood density, growth and form traits

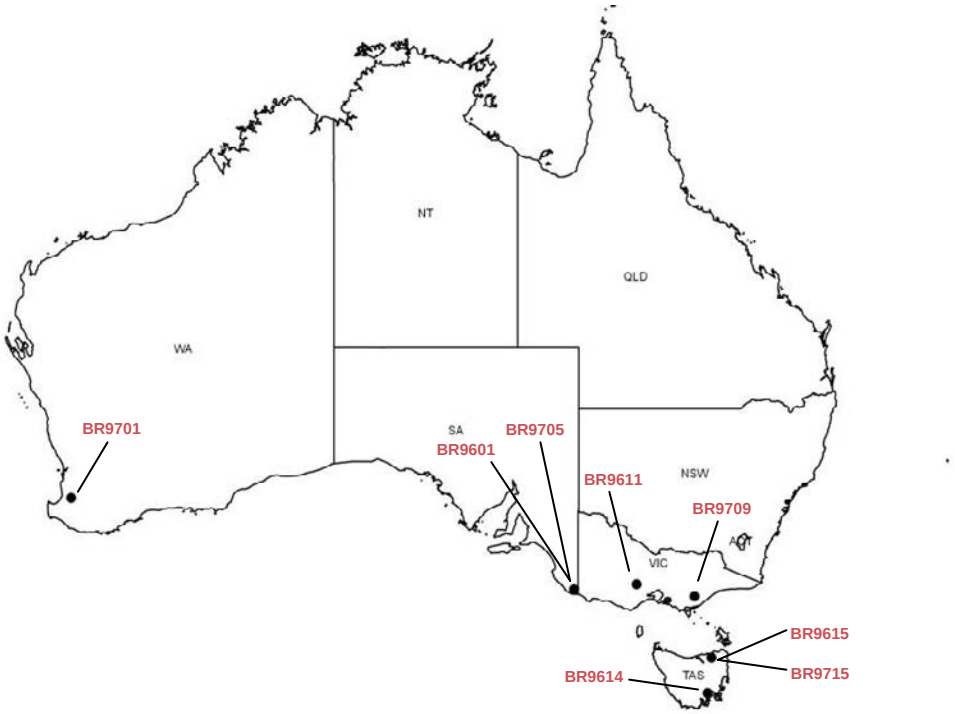


Figure 11. Distribution of eight second-generation genetic trials used for G by E analyses.

Genotype by environment interactions for wood density, growth, branching characteristics and stem straightness were investigated at eight sites located on the mainland (five sites) and Tasmania (three sites) (Figure 11). Among the eight sites used, six sites were common to the wood density breeding value prediction (Table 2), BR9614 (age 7 years) and BR9715 (age 6 years) were selected from Tasmania to increase representations from the Island State.

DBH was measured at 1.3 m above ground. Basic density was estimated from 12 mm cores. Form traits had the following classifications: Branch angle was scored on a scale of 1 to 6 with 1 = steepest branch angle, and 6 = flattest branch angle; Branch size was scored on a scale of 1 to 6 with 1 = largest branches, and 6 = smallest branches; Stem straightness was scored on a scale of 1 to 6 with 1 = most crooked stems, and 6 = straightest stems. An individual tree mixed model (Gilmore *et al.* 2005) was used for G by E analyses and type B genetic correlations were estimated for pairwise sites and for among two regions (Mainland and Tasmania).

4. Inheritance and Quantitative Genetics of Juvenile Wood in Slash and Caribbean Pines and Their F1 hybrid

A total of 1,170 wood samples collected from five replicates at both the Beerwah and Tuan sites (Table 3) were processed through SilviScan to study the patterns of genetic control of three key wood properties (density, MFA and MoE). The experiment involved 12 unrelated PEE (*Pinus elliottii* var. *elliottii*) and 12 unrelated PCH (*P. caribaea* var. *hondurensis*) parents. These 24 parents were crossed together by factorial mating design to produce 36 families of each parental species, and 144 F_1 hybrid families.

Samples were collected in 1998 when the trees were 11 years old (from planting) either by felling and removing a disk from near breast height (1.3 m) or removal of a 12 mm increment core (at a similar sampling position) from standing trees. One tree in each of the 36 pure-

species families was sampled, while only 48 of the 144 F1 families were sampled from each of 10 replicates at the Beerwah site and 7 replicates at the Tuan site. For the purposes of this study, samples were selected from five replicates at both the Beerwah and Tuan sites. Statistical analyses were conducted using ASREML (Gilmour *et al.* 2005) using an individual tree model to estimate the additive genetic variance. Preliminary across-sites analyses indicated that genotype by environment interaction was negligible for most traits; therefore the data were pooled across the sites.

Table 3. Site and experiment details of the two tests in Exp674TBS sampled for the study of juvenile wood properties.

	Beerwah Site	**Tuan Site**
Latitude (°S)	26°52’	25°38’
Longitude (°E)	152°58’	152°50’
Altitude (m asl)	30	14
Rainfall (mm/yr ave.)	1665	1340
Soil Type	Well-drained; yellow earth	Poorly-drained; lateritic – gleyed podzolic
Planting Date	May-June 1987	April-May 1987
Number replicates	12	16
Planting spacing (r × t, m)	4.0 × 2.7	4.5 × 2.4
Initial Stocking (sph)	926	926

5. Gene Discovery in Juvenile Wood Development of Radiata Pine

5.1. Construction of the 18 K cDNA microarrays and sequencing of xylogenesis ESTs

To provide molecular tools for the third generation breeding of radiata pine, a gene discovery subproject on juvenile wood formation of radiata pine was organized as a major component of the Juvenile Wood Initiative. A strategy integrating expressed sequence tags (ESTs) and cDNA microarrays was proposed for the gene discovery subproject (Figure 12).

Wood formation at six critical development stages across a rotation period of radiata pine was studied: earlywood (in spring) and latewood (in autumn) from trees at juvenile (7 yrs), transition (11 yrs) and mature (30 yrs) ages. Total RNA was extracted from sampled tissues using the method of Chang *et al.* (1993) with slight modification. The mRNA was purified for construction of six cDNA libraries. Approximately 3,000 clones from each of the six libraries were randomly isolated either by tooth pick or using a VersArray colony picking robot (Bio-Rad, USA). The isolated cDNA clones were PCR-amplified using the universal M_{13} primers. All PCR products were purified and transferred into 384-well plates using a TECAN GENESIS workstation 200. A total of 18,432 purified PCR products containing 35 reference genes were spotted on GAP II coated slides (Corning Incorporated, USA) using the SDDC2 arrayer (ESI, Toronto, Canada) and Chipmaker 3 pins (Telechem International, CA). Microarray slides were stored in a slide holder at room temperature and treated with UV cross-linking at 300 mJ/cm^3 before using for hybridization. About 10,000 cDNA clones either randomly picked from the six libraries or identified as preferentially expressed clones from various microarray experiments were sequenced for generating a large EST resource for wood formation study in radiata pine.

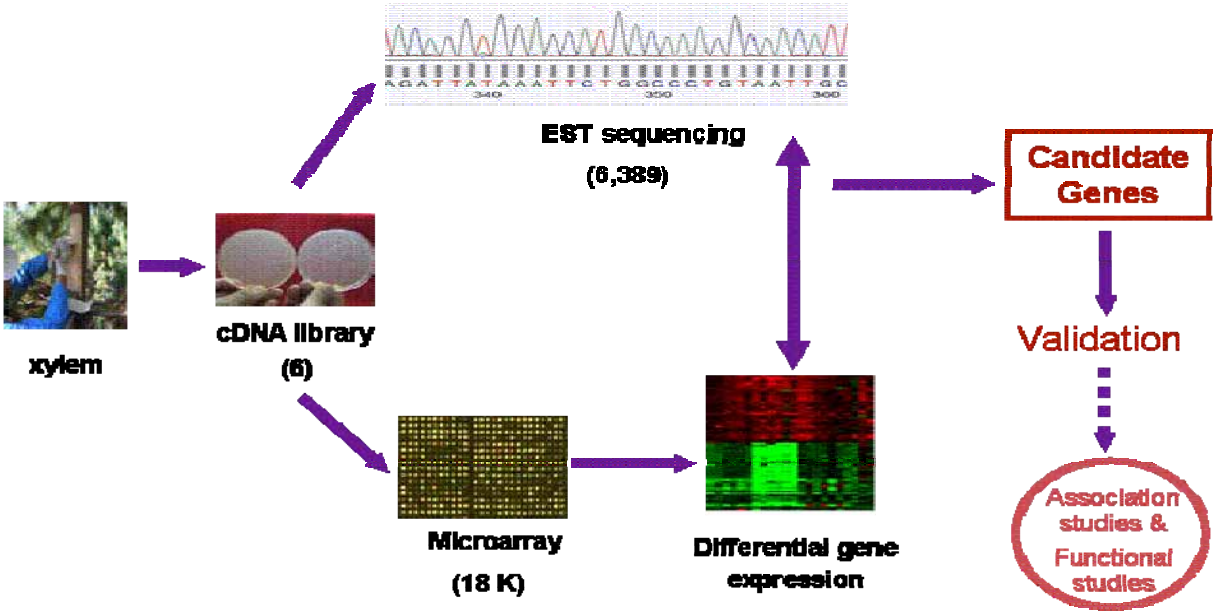


Figure 12. Strategy of gene discovery in radiata pine

5.2 Identification of differentially expressed genes using the 18 k cDNA microarrays

To identify genes that control juvenile wood property traits in radiata pine is one of the main aims of the project. We used the 18 k cDNA microarrays to investigate differential gene expression during seasonal wood development (earlywood/latewood) and wood maturation (juvenile/mature wood). Stiffness and density are the most important wood traits in the solid timber industry and were targeted as another priority in microarray experiments. Four types of microarray hybridizations were carried out in the project (Table 4). To compare gene expression of juvenile versus mature wood, high versus low stiffness and high versus low density wood, 10 trees from each category were sampled and pooled for microarray hybridizations. For earlywood/latewood comparisons, three trees were sampled for each category.

Table 4. Types of microarray experiments performed

Form of experiment	Type of experiment	Notes
Wood development	Juvenile wood vs. Mature wood	Including two comparisons: at spring (earlywood) and autumn (latewood)
	Earlywood vs. Latewood	Including juvenile (5 yrs), transition (9 yrs), mature (13 yrs) and rotation (30 yrs) ages
Wood quality traits	High stiffness vs. Low stiffness	Including two comparisons: earlywood (Flynn, Kromelite) and latewood (Flynn)
	High density vs. Low density	Latewood (Flynn)

Equal amounts of total RNA (20 or 30 µg) of the two samples for comparison were reverse transcribed using Superscript Ⅲ reverse transcriptase, and fluorescently labeled cDNA generated, following the protocol of the SuperScript^{TM} Plus Direst cDNA Labeling System

(Invitrogen, CA). Dye swaps were simultaneously performed in the microarray hybridizations as the technical replicates. The two purified probes were combined and dried, and resuspended in 60 ul hybridization buffer. Microarray hybridization was carried out at 42 ^{o}C overnight, followed by stringent washing procedures as described in the manual of GAP II Coated Slides (Corning Incorporated, USA). Hybridized slides were scanned using a GenePix Personal 4100A microarray scanner (Axon Instruments, CA). Wavelengths from both channels were adjusted and image pre-processing was performed using GenePix® Pro 4.0 and Acuity software (Axon Instruments, CA). Medians of the fluorescence intensity of the red and green colour were used to generate the fluorescence ratio representing differential gene expression in the two samples under comparison. The GenePix results of scanned slides were imputed into GEPAS v3.1 to perform print-tip loss and slide scale normalization. Normalized log2 ratios were used for statistical analysis.

Two thresholds for the percentage of clones showing similar expression patterns in a contig were established. By setting the first threshold (A %) all clones from the same contig are being judged as consistent expression in all replicates. By setting the second threshold (B %) all clones of the same contig are being judged as not randomly expression in different replicates. When a contig is positive against these two thresholds, the average expression ratio from the clones at 1.2 and 0.83 were used as the third threshold for indicating up- and down-regulated unigenes.

6. SNP Discovery and Association Genetics for Juvenile Wood Traits of Radiata Pine

6.1 Population, phenotypic traits and SNP discovery

Three populations were selected for association genetics analysis. The first population is comprised of 447 25-year-old unrelated trees derived from the mainland native populations: Monterey, Año Nuevo and Cambria, maintained as a provenance trial in Batlow, NSW. The second consisted of 458 second generation breeding selections maintained by Hancock Victorian Plantations in Flynn, VIC. The third population was a second generation F2 population including two parents and 96 progeny growing at Bondo, NSW. Needles and wood cores were collected at each site to be used for genetic and phenotypic analyses, respectively.

Phenotypic data were measured from wood strips using SilviScan. Nine traits were measured, including three wood quality traits: density, modulus of elasticity (MoE) and microfibril angle (MFA), by x-ray diffraction and point measurements; and six physical fibre dimensions: fibre wall thickness, fibre wall tangential diameter, fibre wall radial diameter, fibre coarseness, fibre specific surface area and cell population were determined from combined image analysis and x-ray diffraction/point measurement data. A set of 62 wood quality related traits (13 primary traits and 49 derived traits) were studied in association genetics.

Ninety five candidate genes were selected based on their demonstrated involvement in the determination of cell wall/ wood fibre properties in the literature or differential expression in developing xylem. Their putative functions were categorized in six major developmental pathways (Figure 13 and Table 5).

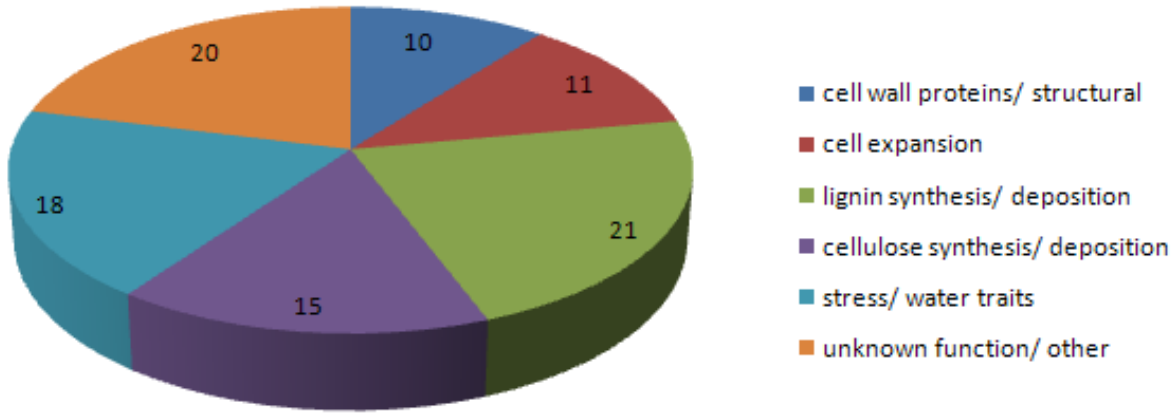


Figure 13. Summary of 95 candidate genes selected for the association study based on putative function.

One hundred and seventy four amplicons covering 177,380 bp in total of DNA sequence from 95 genes were assessed for SNP identification**.** Standard PCR was performed from a *P. radiata* DNA bulk prepared from genomic DNA of 200 individuals in the Batlow population. PCR products obtained from the DNA bulk PCR products were cloned and then DNA isolated from individual colonies sequenced by the chain termination method (Sanger *et al.* 1977). Chromatogram quality was checked either visually, or using quality scores generated from Phred Phrap. DNA sequence alignments were constructed using CLUSTLW implemented in MEGA3.1 (Kumar *et al.* 2004), or Sequencher Software version 4.7 (Gene Codes Inc. Ann Arbor, MI).

Table 5. Summary of genes and regions sequenced for SNP discovery

Candidate gene	Gene description	Basis for selection	Batch	Seq length
4CH1	cinnamate 4-hydroxylase-1	UC Davis	1	440
4CH2	cinnamate 4-hydroxylase-2	UC Davis	1	943
EXP1	alpha expansin-1	UC Davis	1	2616
AGP4	Pt. Arabino galactan protein-4	UC Davis	1	1938
AGP6	Pt.Arabino galactan protein-6	UC Davis	1	930
ARA556	putative arabinogalactan protein	UC Davis	1	383
TUB1	alpha tubulin	UC Davis	1	2813
C3H	coumaroyl-quinate/shikimate 3-hydroxylase	UC Davis	1	510
CAD	Cinamoyl alcohol dehydrogenase	database/ literature	1	2113
CCR1	hydroxycinnamoyl-CoA reductase	database/ literature	1	1857
COBL4	AtCOBRA homologue	database/ literature	1	3700
COMT2	caffeate 3-O-methyltransferase	UC Davis	1	1290
DH2	dehydrin	micro array	1	543
DH7-8	dehydrin	micro array	1	232
FRA1	Fragile fibre mutant 2 (katanin)	database/ literature	1	3000
FRA2	Fragile fibre mutant 1 (kinesin motor protein)	database/ literature	1	1000
GlyHMT	glycine hydroxymethyl transferase	UC Davis	1	1974
Korrigan	Endo-1,4-(Lecouls AC *et al.*)-Glucanase	database/ literature	1	3000
LIM	LIM trasncription factor - 1F	database/ literature	1	2167

lp5	drought-inducible gene (LP5)	UC Davis	1	720
lp8	drought-inducible gene (LP8)	UC Davis	1	430
porin	aquaporin (MIP)	micro array	1	2000
MYB4	MYB transcription factor -1F	database/ literature	1	1266
nh 3702	no hit	micro array	1	289
nh3473	no hit (possibly AGP)	micro array	1	367
NIR	nitrite oxide reductase	UC Davis	1	265
PAL1	Penylalinine amonium lyase	UC Davis	1	2284
PAL2	Penylalinine amonium lyase	UC Davis	1	826
PCBER2	phenylcoumaran benzylic ether reductase	UC Davis	1	572
peroxidase precursor	peroxidase precursor	micro array	1	812
prp1	proline rich protein 1	micro array	1	835
PtATHB-8	HD-ZIP trasncription factor	database/ literature	1	1697
PtATHB-X	HD-ZIP trasncription factor	database/ literature	1	470
PtCesA1	Pt. cellulose synthase 1	database/ literature	1	7226
PtCesA2	Pt. cellulose synthase 2	database/ literature	1	800
PtCesA3	Pt. cellulose synthase 3	database/ literature	1	4592
PtMyb1	MYB transcription factor	database/ literature	1	1256
Rac13	Gh. rac13 GTPase	database/ literature	1	4040
SAHH1	S-adenosyl-L-homocysteine hydrolase	UC Davis	1	650
SAM1	S-adenosylmethionine synthase	UC Davis	1	1816
SAM2	S-adenosylmethionine synthase	UC Davis	1	2057
SODchl	chloroplastic superoxide dismutase (oxidative stress response)	UC Davis	1	720
SuSy	Sucrose synthase	database/ literature	1	1200
XET1	xyloglucan endotransglycosylase	database/ literature	1	1752
pectate lyase	pectate lyase	micro array	2	3295
ankyrin	ankyrin like protein	micro array	2	2791
prp2	proline rich protein 2	micro array	2	2281
Kn4	homeobox Kn4	micro array	2	2907
LP3-3	drought-inducible gene (LP3-3)	micro array	2	1473
LP3-2	drought-inducible gene (LP3-2)	micro array	2	750
Aux/IAA	Aux/IAA protein (transc factor)	micro array	2	990
porin2	aquaporin-like	micro array	2	2333
transaldolase	transaldolase	micro array	2	2215
TRD2	thioredoxin 2	micro array	2	1328
phytocyanin	phytocyanin	micro array	2	4039
Actin	actin	micro array	2	3444
peroxidase	peroxidase	micro array	2	3045
actin depolymerising factor	actin depolymerising factor	micro array	2	2568
aquaporin-like protein	aquaporin-like protein	micro array	2	2526
Chitinase	chitinase	micro array	2	2977
cysteine protease	cysteine protease	micro array	2	2809
expansin	expansin	micro array	2	3992
GASA5-like protein	gibberellin-regulated protein	micro array	2	1874
laccase 3	laccase	micro array	2	3099
UDP-glucose dehydrogenase	UDP-glucose dehydrogenase	micro array	2	2472
xyloglucan endotransglycosylase	xyloglucan endotransglycosylase	micro array	2	3104
serine threonine protein kinase	serine threonine protein kinase	micro array	2	1675
Phytochrome	phytochrome	micro array	2	2018
pectinacetylesterase protein	pectinacetylesterase protein	micro array	2	3073
mykiss insulin like growth factor	mykiss insulin like growth factor	micro array	2	1853
metalothionein like protein	metalothionein like protein	micro array	2	2548

LP6	drought-inducible gene (L6)	micro array	2	2042
glycosyl transferase family protein	glycosyl transferase family protein	micro array	2	2021
Glycine rich RNA binding protein	glycine rich RNA binding protein	micro array	2	2305
gibberellin receptor	gibberellin receptor	micro array	2	1690
putative arabinogalactan	putative arabinogalactan	eSNP	2	1702
Pt. celulose synthase 1	cellulose synthase	eSNP	2	1475
Phytochrome	phytochrome	eSNP	2	431
elongation factor-1 alpha 3	elongation factor-1 alpha 3	eSNP	2	2139
Actin-depolymerizing factor 7 (ADF-7)	actin-depolymerizing factor 7	eSNP	2	2139
putative TOM20	putative TOM20	eSNP	2	2059
putative proline-rich arabinogalactan protein	proline-rich arabinogalactan protein	eSNP	2	963
putative plasma membrane intrinsic protein	plasma membrane intrinsic protein	eSNP	2	1281
delta-tonoplast intrinsic protein	delta-tonoplast intrinsic protein	eSNP	2	1101
hypothetical protein	hypothetical protein	eSNP	2	1677
PRP	putative proline-rich arabinogalactan protein	eSNP	2	1154
chitinase	chitinase	eSNP	2	1347
caffeoyl CoA O-methyltransferase	caffeoyl CoA O-methyltransferase	eSNP	2	1122
S-adenosylmethionine synthetase	S-adenosylmethionine synthetase	eSNP	2	1495
expansin 2	alpha expansin 2	eSNP	2	1375
NHL1 (NDR1/HIN1-like 1)	NHL1 (NDR1/HIN1-like 1)	eSNP	2	1416
putative ADP ATP carrier protein	ADP-ATP carrier protein	eSNP	2	1586
putative ADP ATP carrier protein	ADP-ATP carrier protein	eSNP	2	1586
glycosyltransferase	glycosyltransferase	eSNP	2	1887
CAMT_PINTA	Caffeoyl-CoA O-methyltransferase	eSNP	2	1441
zinc finger protein 774	zinc finger protein	eSNP	2	2076

6.2 SNP selection, genotyping, association genetics and genetic mapping

Two approaches were applied to select single nucleotide polymorphisms (SNPs) for association testing. Following the first approach (Figure 14A), SNP positions were initially identified and recorded from DNA alignments. Haplotype tagging analysis was performed for each alignment using HtSNPer (Ding *et al.* 2005) and HapBlock (Zhang *et al.* 2005), and was applied to sequences amplified in batch 1 only (Table 5). 518 SNPs were selected from 44 genes covering 128,000 base pairs of DNA sequence.

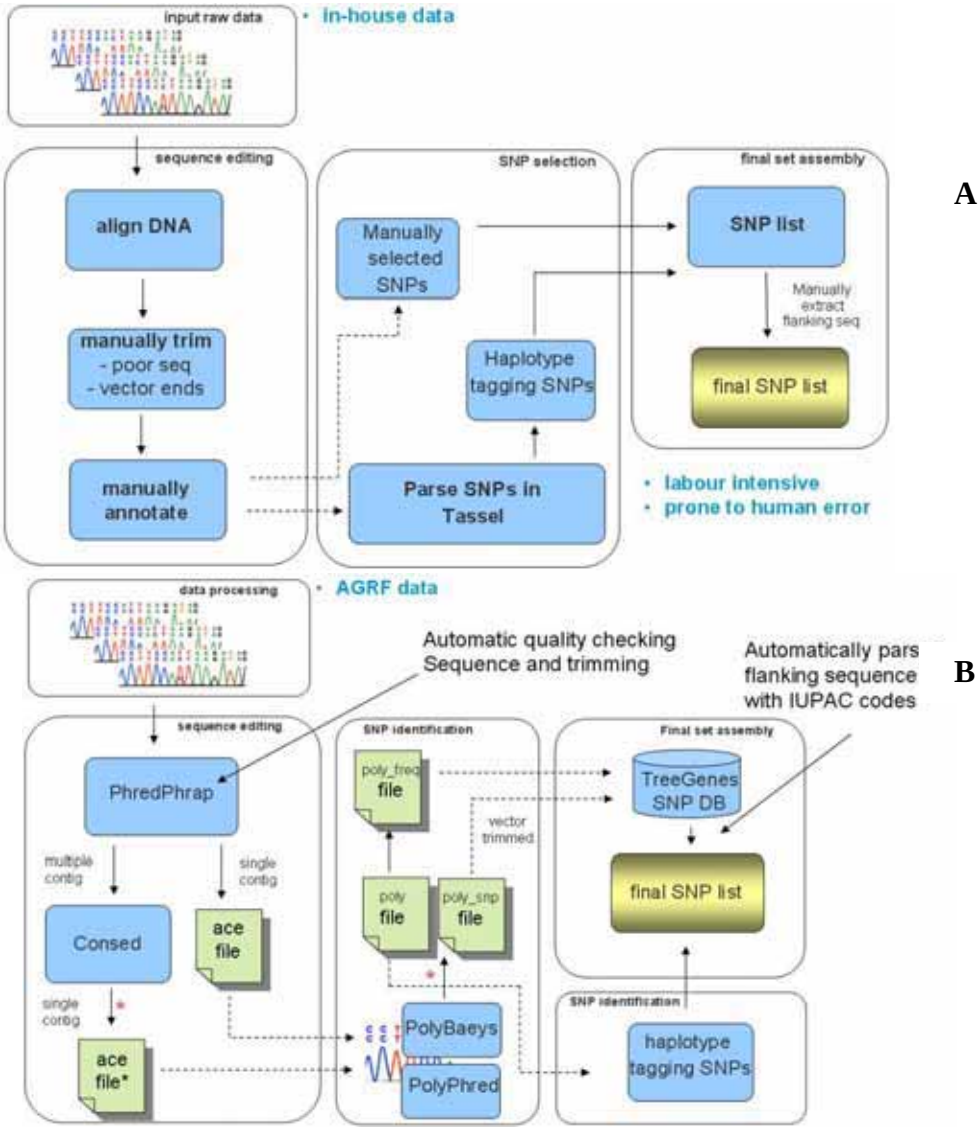


Figure 14. Haplotype tagging SNP selection pipeline with 126,000 bp assessed from 44 genes (batch 1) yielding 518 candidate SNPs (A); Phred Phrap SNP selection pipeline and 50,000 bp assessed from 53 genes (batch 2) yielding 395 candidate SNPs (B).

In the second approach (Figure 14B), DNA sequence alignments from batch 2 (Table 4) were assembled using the Phred-Phrap (Ewing and Green 1998) sequence aligner and quality scorer. SNPs were identified and selected according to user-defined criteria via a machine learning algorithm encompassing features of both PolyBaeys and PolyPhred (Ewing and Green 1998).

Genotyping was performed on genomic DNA extracted from trees from Batlow and Flynn and the mapping cross parents using the Illumina GoldenGate assay (Shen *et al.* 2005) . SNPs identified in batch 1 were analysed using the Illumina design tool which yielded 384 SNPs suitable for the GoldenGate genotyping assay. SNPs identified in batch 2 were similarly analysed using the Illumina design tool, and also yielded a minimum of 384 SNPs suitable for testing. In total 404 (53%) SNPs were of satisfactory quality for further analysis. Of these 404 SNPs, 60 were found to be polymorphic in the mapping cross parents and exhibited genotype combinations that would be useful for pedigree mapping (i.e. AAxAB, ABxAB,

ABxAA). These 60 SNPs represented 52 of the 95 genes examined in this study. Genotyping of diploid F2 DNA from the mapping pedigree was outsourced to Sequenom Pty Ltd. Of the resulting genotypes, 31 were deemed suitable for mapping. The remaining 29 SNPs were removed from analysis due to either the poor quality of the genotype result, the non-informative nature of the genotypes for mapping (i.e. AA x BB), or because the genotype did not segregate in the progeny.

A number of population genetic analyses were conducted in the Batlow population to obtain a description of nucleotide diversity, structure and past population demography in the Californian mainland populations. Only those analyses pertinent to association testing are presented here. The extent of linkage disequilibrium (LD) was assessed based on DNA sequences from 28 amplicons. The squared correlation of allele frequencies (r^2) and their significance was calculated using TASSEL software. The population recombination rate ($4N_er$) from this equation was estimated from a least squares fit of the expectation according to Press *et al.* (1992), implemented in SAS9.1. The expected decay was derived per base pair over all loci without adjustment for locus specific variation in mutation rate, selection or structure. Genetic structure was tested via a model-based Bayesian approach using Structure software (Pritchard *et al.* 2000) with population admixture assumed. Because SNPs were selected following the haplotype block partitioning approach, and LD was low among the data set, allele frequencies were treated as independent. Population and individual *Q*-matrices were computed from 5 replicates using CLUMPP (Jakobsson and Rosenberg 2007), and individual *Q*-matrices subsequently plotted using DISTRUCT (Rosenberg 2004).

Associations between 62 traits and 149 SNPs in Batlow; and 255 SNPs and 3 traits (MFA, MoE and density) in Batlow and Flynn, were tested via a least squares fixed effects general linear model (GLM) constructed in Tassel, with and without adjustment for population structure (GQ) and permutation (GPQ). The statistical model is described by: $y = X\beta + e$, where y is a vector for the observed dependant variable (trait); β is a vector containing independent fixed effects, including genetic marker and population structure; X is the known design matrix; and e is the unobserved vector of random residual (error). Population genetic structure was incorporated as a Q-matrix containing assignments of individuals to K=3 hypothetical populations. Population structure was shown to be most accurately described by the 3 population model (Figure 15). P-values were adjusted for experiment wise error following 2000 permutations of the SNP and trait data. Association testing was performed at Batlow for the whole population and within subpopulations (Año Nuevo (155), Monterey (210) and Cambria (82).

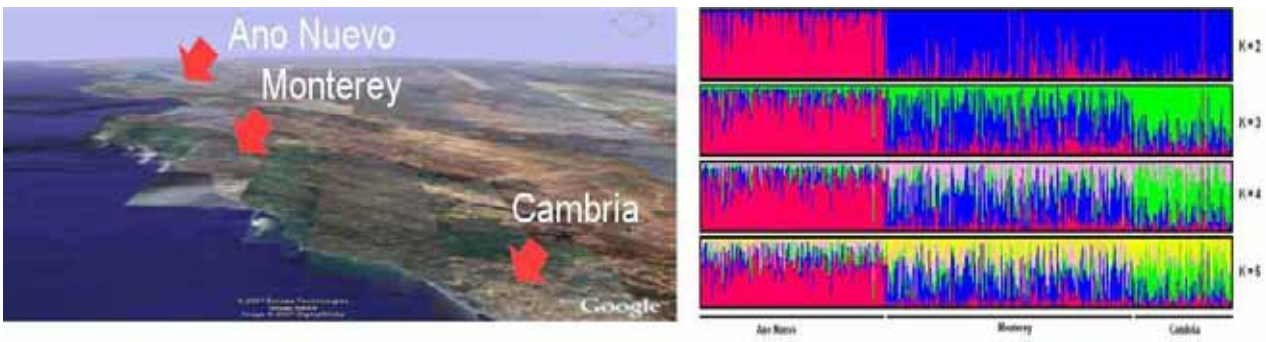


Figure 15. Population structure revealed in the mainland native provenances of *Pinus radiata* based on SNP segregation in the Batlow population.

At Flynn, testing was conducted upon the whole population. Pedigree structure, or kinship, was assumed to be low among trees due to their sampling from a widely distributed natural population. Analysis of the pairwise kinship coefficient among Batlow trees using genotype data in SPAGeDi (http://www.ulb.be/sciences/ecoevol/spagedi.html) detected a low level of pedigree structure, suggested by the large proportion of comparisons where the coefficient equaled zero. Consequently, analysis of associations as a mixed linear model with kinship, previously shown to be most effective for association testing in the presence of structure in Arabidopsis, was not attempted. Kinship was expected within the Flynn breeding selections, and the proportion of pairwise kinship coefficients >0 was consequently higher than in Batlow. Although not reported here, associations in Flynn should be re-examined using a mixed linear model incorporating this kinship matrix. The FDR and q-value were calculated for individual associations.

Multipoint linkage analysis was performed using OutMap version 1.0. Maternal and paternal co-dominant markers were mapped as a single data set. Markers were initially grouped using a LOD threshold of 3.0 and minimum recombination threshold of 0.3, except for groups 1 and 3 which were grouped using less stringent conditions, LOD 2.0 and minimum recombination 0.5. Phased data was then used to determine the order of markers along each linkage group (or hypothetical chromosome) using an 'accuracy" of 0.1 and ordering option of "two-opt" in OutMap (Voorrips 2002).

7. Incorporating Genomic and Quantitative Genetic Data into Breeding Programs

7.1 Incorporating quantitative genetic data into breeding programs

Juvenile wood density assessments in six 1996 and 1997 STBA progeny trials (BR9601 Airport, BR9611 Flynn, BR9615 Koomeela, BR9701 Bussells, BR9705 Kromelite and BR9709 Bradvale) that were completed as part of this project were incorporated into DataPlan in mid-2004 and contributed significantly to the improvement in wood density breeding values predicted by TreePlan in October 2004. This represented the first tangible benefit for the STBA radiata pine breeding program and came quite early in the project. These results have contributed positively to selection and deployment decisions since that time.

Acoustic stiffness measurements in the two main progeny trials (BR9611 Flynn and BR9705 Kromelite) were incorporated in the TreePlan analysis in December 2005. That TreePlan run also incorporated the results from the Breeding Objectives (PN01.1904) project by redefining the clearfall traits of commercial interest in the economic breeding objective and index reported in terms of Net Present Value (NPV). Results of density derived from 12 mm cores and acoustic stiffness from various/available standing tree tools are used in DataPlan and TreePlan to predict MoE as one of the breeding objective traits. Additive genetic correlations between density and stiffness measurements, summarised from a number of sources, along with additive genetic correlations between these and other traits (regional growth, branch quality, branch size, branch angle and stem straightness) all contribute to the final prediction of breeding values by TreePlan in a multivariate analysis.

Other wood property data (spiral grain, transition age, shrinkage and MFA) from the project is being incorporated and will result in improved breeding values from future TreePlan runs.

7.2 Incorporating SNPs with quantitative genetic data and SNP-aided selection

As reliable associations between economically important quantitative traits and polymorphic loci such as SNPs are identified, the potential to develop molecular breeding strategies becomes apparent. As most allelic variants account for a small amount of the total phenotypic variation for agronomic traits, molecular breeding strategies will ideally incorporate those combinations of SNP markers which best describe the trait variation with quantitative genetic data. As a first step in developing a molecular breeding strategy, we present methodology for testing for significant additive and dominance effects of SNPs by incorporating each SNP sequentially with quantitative genetic data for juvenile density, modulus of elasticity, microfibril angle, and pith-to-bark core length using an individual tree model.

The model used for estimating the additive and dominance effects associated with each SNP, and the genetic variance components is

[1] $y_{ijkl} = \mu + r_i + a_j + d_j + tree_k + e_{ijkl}$

where y_{ijkl} is the phenotypic value of a trait, μ is the overall mean, r_i is the fixed effect of the i^{th} replicate, a_j is the fixed additive effect of the j^{th} SNP, d_j is the fixed dominance effect of the j^{th} SNP, $tree_k$ is the random additive genetic effect ~ N(0, $\hat{\sigma}_a^2$), and e_{ijkl} is the residual error ~ N(0, $\hat{\sigma}_e^2$).

The additive and dominance genetic variances associated with each SNP and for each trait were defined as

[2] $V_{A\text{-}SNP} = 2pq[a + d(q - p)]^2$ and

[3] $V_{D\text{-}SNP} = (2pqd)^2$, respectively (Falconer and Mackay 1996).

Assuming independence of additive effects, the remaining additive genetic variance associated with each trait was estimated as

[4] $V_A = \hat{\sigma}_a^2$.

The residual error (V_E) and phenotypic (V_{P*}) variances were estimated as

[5] $V_E = \hat{\sigma}_e^2$ and

[6] $V_{P*} = V_A + V_{A\text{-}SNP} + V_{D\text{-}SNP} + V_E$, respectively.

In addition, the approximate percent variation controlled by the additive genetic variation of each SNP was estimated.

[7] $\frac{V_{A\text{-}SNP}}{V_{P*}} \times 100\%$.

Once reliable and significant SNPs were identified, the next step is to use SNP-aided selection to increase efficiency of selection for breeding and deployment. To incorporate SNP effects into selective breeding, one method is to use a selection index. SNP markers can be used for early selection alone as well as assisting in early phenotypic selection. This method of incorporating molecular markers in a selection index is preferable for increasing genetic gain. Incorporating SNP markers for stiffness at rotation age into an early selection index may not only increase genetic gain, but also has the advantage of shortening generation time. This can be done through an index selection approach as

[8] $I = ax + bm$,

where x is an early measurement of stiffness trait, m represents the SNP score (Wu 2002), and a and b are the estimated index coefficients for early stiffness trait x and SNP score m. The SNP score m could be derived from multiple regressions of SNPs on phenotypes. The efficiencies from such index selection are explored for stiffness trait at rotation age.

7.3. Optimal selection strategy

The objective of dissecting quantitative and molecular genetic bases of economically important traits is to develop optimal and efficient selection and breeding strategies. We had collected large data on inheritance and genetic parameters on several wood quality traits, particularly on genetic correlations between stiffness, density, microfibril angle, spiral grain, shrinkage in the juvenile core and DBH growth in radiata pine. We can now use this data to explore optimal selection and breeding strategies for radiata pine. These strategies are particularly useful for dealing with multiple breeding objective traits, and adverse genetic correlations between stiffness and growth traits in radiata pine.

The optimal selection strategy was defined by the optimal breeding objective response in terms of profitability. Responses in the breeding objective trait mean annual increment (MAI_{OBJ}) and stiffness (MoE_{OBJ}) at rotation age were evaluated through index selection based on two juvenile traits (MoE and DBH). Economic weights for the breeding objective traits for an integrated company were estimated to be $977 per one GPa increase of rotation-aged stiffness and $416 per one m^3/year/ hectare of MAI at rotation age (Ivković *et al.* 2006a). Three different selection scenarios were considered: A) Index selection using MoE and DBH as selection traits and maximising profitability; B) Restricted index selection keeping juvenile wood MoE constant; C) Restricted index selection where selection is restricted to genotypes with positive breeding values for both MoE and DBH (correlation breakers).

Genetic gain for net present value of the vertically integrated enterprises and for each individual wood quality trait is evaluated for the three selection scenarios.

RESULTS

The detailed results were reported in the ten client technical reports. Only summary results are presented in the following seven sections.

1. Optimal Method for Measuring Log and Clearwood Stiffness of Young Trees in Radiata Pine

1.1 Optimal methods for measuring log stiffness of young radiata pine trees

Acoustic and non-acoustic measurements of stiffness on standing trees and on harvested logs were compared and pairwise product-moment correlation coefficients were calculated between measurements obtained for each tree (Table 6). Correlations (above 0.94) indicate that IML was the best acoustic tool for predicting acoustic stiffness of logs based on measurements on standing trees. For a detailed comparison, see Appendix 1 (Genetic control of juvenile-mature wood transition and acoustic method to predict standing tree stiffness in radiata pine).

Table 6. Correlations between MoE measurements on standing trees and harvested logs (n=38).

Sample		**Standing trees**				**Wood**	**Logs**
	Correlation	Fakopp	IML	Ultrasound	Sway	Paddle Pop	Director
Standing trees	Fakopp	1					
	IML	0.89	1				
	Ultrasound	0.25	0.43	1			
	Sway	0.39	0.55	0.56	1		
Wood	Paddle Pop	0.70	0.70	0.41	0.64	1	
Logs	Director	0.85	0.94	0.25	0.42	0.64	
	HP	0.84	0.94	0.27	0.45	0.58	0.95

1.2 Optimal method for measuring clear-wood stiffness and strength of young radiata pine trees

For clearwood samples from harvested log, wood density (DEN) had a higher correlation with MoR than with MoE, while MFA had a higher correlation with MoE than with MoR. Spiral grain (SG) had a higher significant correlation with MoE than with MoR. Further path analyses revealed direct effect of DEN on MoR was greater than MFA while direct effect of MFA on MoE was greater than DEN. Direct effect of DEN on radial shrinkage (RS) and tangential shrinkage (TS) were greater than MFA (Figure 16).

For wood stiffness, paddle pop gave the best prediction of stem MoE at Flynn due to its vicinity to the benchmark log sample. SilviScan measurements showed good prediction for MoE for both sites. IML hammer alone and IML hammer plus core density showed a similarly good fit as SilviScan measurements. These correlations indicate that IML hammer plus core density and paddle pop measurement are the preferred methods for predicting stiffness of boards sawn from young trees in radiata pine (Table 7).

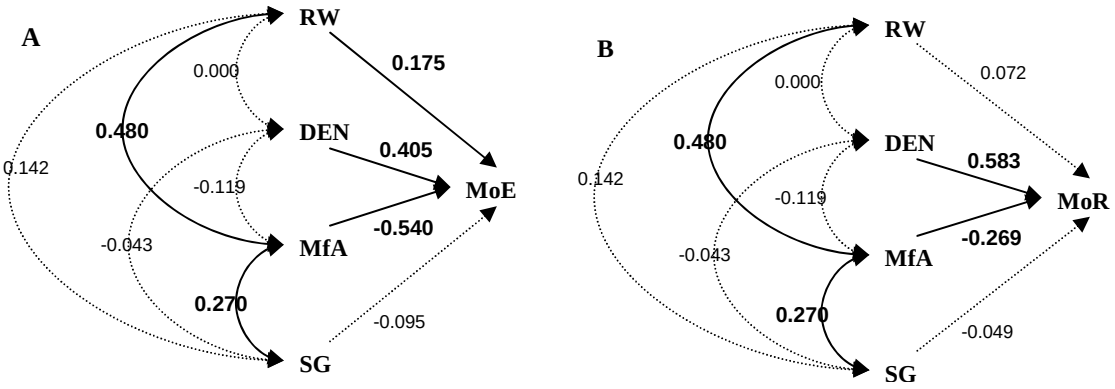


Figure 16. Path analyses for Flynn site: A. MoE_{stem} (multiple R^2=0.49) and B. MoR_{stem} (multiple R^2=0.45) with direct effects indicated in straight arrows and indirect paths indicated in curved lines with correlation coefficients.

Table 7. Prediction goodness of fit statistics R^2 for stiffness of stem, outer-ring and inner-ring samples (MoE_{stem}, MoE_{out} and MoE_{in}) and strength of stem, outer-ring and inner-ring samples (MoR_{stem}, MoR_{out} and MoR_{in}). Predictive models include: 1) SilviScan ring width and density (RW_s and DEN_s) 2) SilviScan RW_s, DEN_s and MoE_s; 3) IML® hammer MoE (MoE_{iml}); 4) MoE_{iml} and increment core density (DEN_c); 5) MoE_{iml}, DEN_c, DBH, Stem straightness(STEM), Branch size (BRS); Branch angle (BRA); 6) Paddle-pop dynamic MoE (MoE_{pp}); and 7) MoE_{pp} and DEN_c for Flynn (F) and Kromelite site (K).

		Independent variables						
	Site	**RW_s and DEN_s**	**RW_s, DEN_s and MoE_s**	**MoE_{iml}**	**MoE_{iml} and DEN_s**	**MoE_{iml} DBH, STEM, BRS, BRA, and DEN_s**	**MoE_{pp}**	**MoE_{pp} and DEN_s**
	F	0.147	0.506	0.472	0.484	0.494	0.707	0.708
MoE_{stem}	K	0.059	0.49	0.401	0.404	0.423	0.31	0.297
	F	0.154	0.507	0.519	0.529	0.537	0.713	0.715
MoE_{out}	K	0.091	0.535	0.425	0.44	0.452	0.289	0.321
	F	0.136	0.263	0.201	0.215	0.319	0.393	0.405
MoE_{in}	K	0.079	0.309	0.158	0.193	0.204	0.139	0.224
	F	0.126	0.222	0.13	0.176	0.179	0.613	0.619
MoR_{stem}	K	0.069	0.191	0.128	0.149	0.163	0.033	0.158
	F	0.132	0.243	0.184	0.234	0.237	0.651	0.654
MoR_{out}	K	0.092	0.217	0.155	0.194	0.202	0.162	0.174
	F	0.059	0.11	0.041	0.098	0.142	0.4	0.403
MoR_{in}	K	0.042	0.086	0.017	0.051	0.067	0.075	0.094

For wood strength, multiple regression predictions were weak. Only IML hammer plus core density or paddle pop measurement showed some potential to predict stem strength. For shrinkage, multiple regressions were weak. IML hammer plus core density combined could be a preferred method for prediction of shrinkage. However, the R^2 was not very high for all these predictions. Better control of sample position (similar height) and alignment of static MoE samples with samples for wood components may improve the regression. For a detailed examination of these relationships, see Appendix 2 (Prediction of wood stiffness, strength, and shrinkage in juvenile wood of radiata pine: Juvenile wood index).

2. Optimal Method for Measuring Log and Clearwood Stiffness of Young Trees in Slash and Caribbean Pines, and Their F1 Hybrid

2.1 Optimal methods for measuring log stiffness of young slash and Caribbean pine trees

Predictability of density and MFA were estimated using correlation parameters (Table 8). It was observed that wood density had higher correlations with board MoE while MFA had higher correlations with tree and log MoE.

Table 8. Correlation coefficients (with probabilities in parentheses) for whole core average density (Density) and weighted average microfibril angle (MFA) and stem or log estimates of stiffness (n = 44).

	Tree_MoE	Log_MoE	Board_MoE_F	Board_MoE_E
Density	0.518	0.630	0.652	0.750
MFA	-0.675	-0.718	-0.405	-0.395

* Tree_MoE = standing tree MoE prediction from Fakopp time of flight reading
Log_MoE = 3m log MoE estimate from Wood Spec time of flight reading
MoE_F = average MoE of the boards sawn from each log and tested on flat on a Shimadzu timber testing machine
MoE_E = average MoE of the boards sawn from each log and tested on edge on a Shimadzu timber testing machine

A regression using whole core basic density and Fakopp MoE prediction was very highly significant (Pr>F = <0.0001) in predicting 80 % of the variation in Wood Spec MoE of harvested logs (R^2 = 0.807). This indicates that we have a strong prediction of log MoE from the gravimetric assessment of basic density using a 12 mm increment core combined with a standing tree prediction of MoE using a time of flight acoustic tool. As log MoE is also linearly associated with the average MoE of sawn boards (R^2 = 0.702) we have some reliable capacity to rank trees into broad quality classes for juvenile wood stiffness which is a primary focus for juvenile wood quality improvement. Additionally, the much more expensive information obtained from SilviScan analysis of cores does not appear to add significant value to this prediction once a mean density and a standing tree acoustic velocity is obtained. This suggests a greatly improved capacity to screen larger numbers of progeny or ramets with low cost tools before identifying the most superior part of the population for more intensive evaluation with SilviScan and for grain spirality. The impact of MFA and spiral grain on warp in solid timber makes this a final screening or evaluation priority to ensure that all selections are both stable during drying and in use as well as having high stiffness properties.

2.2 Optimal method for measuring clearwood stiffness and strength of young slash and Caribbean pine trees

The best regression (stiffness index) for board stiffness (on flat) using seven significant traits from a total of 12 measured wood traits was derived as:

MoE_{board} = 6.8975-0.11045*Spring-0.03440*Bow-0.16363* MFA_s
+ 0.00962* DEN_s

where MFA_s and DEN_s are SilviScan microfibril angle and density weighted by area,. All four variables are statistically significant at the 5% probability level and the R^2 is 0.492. For further detailed methodology and results, see Appendix 3 (Juvenile wood index and the best MoE measurement method for use in assessment of slash/Caribbean hybrid pine).

3. Inheritance and Quantitative Genetics of Juvenile Wood in Radiata Pine

3.1 Genetics of transition age from juvenile to mature wood in radiata pine

Among age profiles studied for three wood density measures (earlywood, latewood, and ring wood density), it was found that age profiles for latewood was most profound with a clear age trend (Figure 17). The latewood density increased rapidly for about the first 4 years, and thereafter either remained high depending on family and sites. For the purpose of determining juvenile-mature wood transition, only the latewood density data gave reasonable results, and produced visibly identifiable breakpoints in segmented regression models applied to pith-to-bark density profiles. Latewood density is a characteristic that is closely related to stiffness (MoE) which in turn is one of the most important mechanical properties for solid wood end uses (Mamdy *et al*. 1999; Rosenberg *et al.* 1999). Therefore, latewood density was used to study the age transition from juvenile to mature wood.

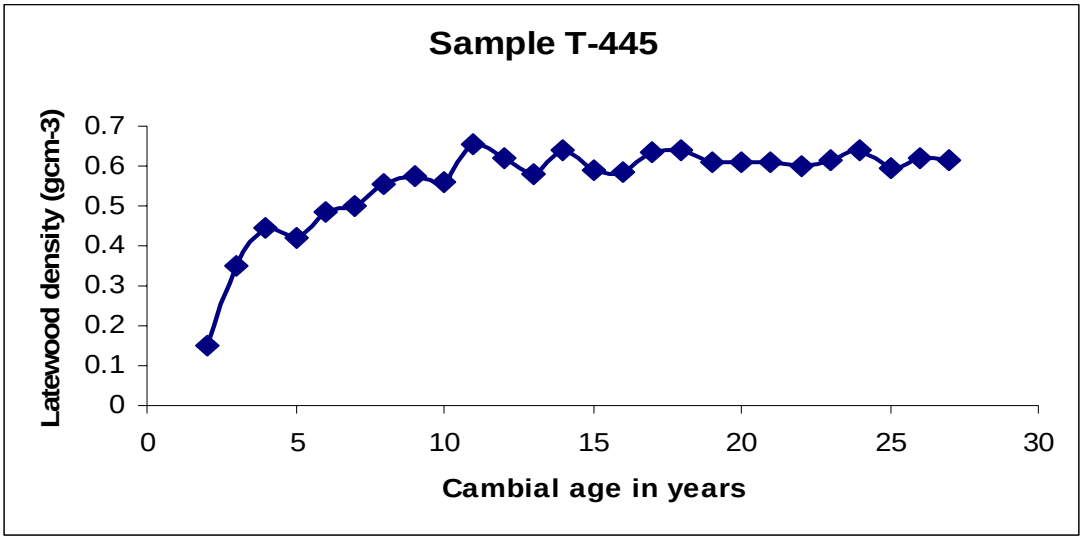


Figure 17. Development of latewood density from pith-to-bark for sample T-445.

Average latewood density values at VRC52 and VRC54 stabilised at ring 7 with a value of 0.597 g cm^{-3} with no further significant increase or decrease in latewood density (Figure 18). Latewood density at PT47 increased from 0.241 g cm^{-3} at cambial age two and stabilized at cambial age nine with a latewood density of 0.658 g cm^{-3}. In contrast, latewood density at PT5042 increased from 0.226 g cm^{-3} at cambial age two to 0.584 g cm^{-3} at cambial age 14. Average latewood density values at PT5042 stabilized at ring 12 with a value of 0.576 g cm^{-3}. Trees reaching an early plateau in latewood density would have a shorter period of juvenile wood formation (Figure 18). The profile patterns in our data are typical of a transition from juvenile to mature wood.

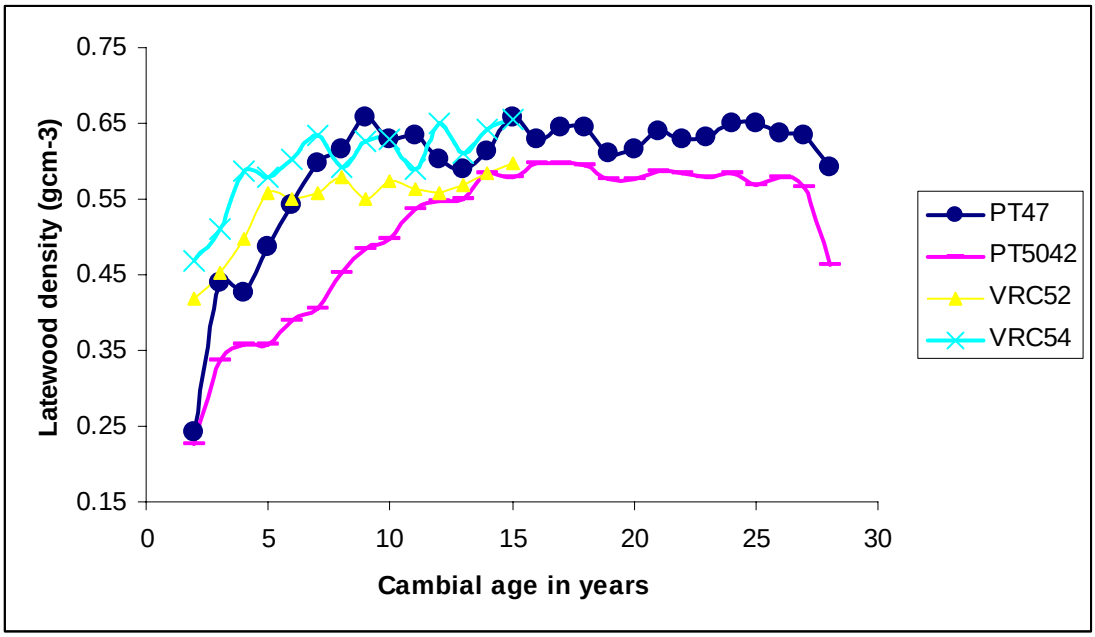


Figure 18. Trends in latewood density from pith to bark at PT5042, PT47, VRC52, and VRC54.

There were significant additive genetic variance estimates at all four sites for the transition age. Narrow-sense heritability for transition age at the two full-sib sites were 0.13 ± 0.04 (VRC52) and 0.23 ± 0.08 (VRC54) and at the two half-sib sites were 0.17 ± 0.05 (PT47) and 0.33 ± 0.04 (PT5042) (Table 9). These heritabilities were comparable with estimates in slash pine (0.22 and 0.17 for latewood density and ring density transition age, respectively), and loblolly pine (0.12 for ring density transition age, Loo *et al.* 1985). These heritabilities were also comparable to growth traits such as DBH (Wu *et al*. 2007 and 2008).

Table 9. Individual trial estimates of mean transition age (years), additive genetic (σ^2a), specific combining ability (σ^2s) and residual (σ^2e) variances, heritabilities (h^2_i) and genetic gain (Δ_G) for transition age in four trials of *Pinus radiata*. The approximate standard errors for the estimated parameters are given in parenthesis.

Trial	Min - Max	Mean	σ^2a[a]	σ^2s	σ^2e	h^2_i	Δ_G (%)
VRC52	5.6 – 14.8	7.7	0.546	0.23E-5[b]	3.689	0.13	5.2
		(1.4)	(0.08)		(0.71)	(0.04)	
VRC54	5.9 – 11.2	7.2	0.268	0.85E-6[b]	0.910	0.23	6.6
		(1.2)	(0.13)		(0.17)	(0.08)	
PT47	5.1 – 11.5	7.5	0.185	-	0.912	0.17	4.6
		(1.6)	(0.07)		(0.13)	(0.05)	
PT5042	6.3 – 21.6	12.6	1.738	-	3.576	0.33	10.1
		(2.7)	(0.73)		(0.63)	(0.04)	

[a] Additive genetic variance estimates were all significantly (P≤0.05) different from zero.

[b] SCA effects not insignificantly different from zero.

Predicted genetic gains, estimated using individual tree breeding values, for a shorter juvenile wood formation phase are reasonable (Table 9). Assuming a selection intensity of one in ten, genetic gains of up to 10% per breeding cycle are possible. These gains can be interpreted as the change in population mean that could be achieved by selection in the field trials.

Although in practice the selection method may be different, these gains provide some indication of the change possible in the population, from a selection intensity of only 10%. Predicted genetic gains of 10.1% at PT5042 would be equivalent to shortening the juvenile wood formation phase by 22 months compared to the population mean in one generation.

For further details of the study, see Appendix 1 (Genetic control of juvenile-mature wood transition and acoustic method to predict standing tree stiffness in radiata pine).

3.2 Wood density breeding values for progeny of STBA's second generation breeding population

Gravimetric densities of the 7,078 cores were measured using standard water replacement techniques. Among six sites, BR9611 had the highest average density (364 kg/m3) while BR9705 had the lowest mean density (317 kg/m3, Table 2). Estimated heritability was high among six sites, from 0.49 at BR9701 to 0.85 at BR9615, with a mean of 0.67. Breeding values were predicted for density using ASREML software for the joint site analyses. The breeding values varied from 300 to 422 kg/m3 with combined six site heritability of 0.63. Based on the estimated breeding values, initial selection of STBA population was made for 250 trees with high density for deployment and breeding purpose with expected wood density gain estimated at 12.4%.

3.3 Inheritance of juvenile wood traits in radiata pine

3.3.1 Inheritance of growth, wood density, MFA, and MoE

Detailed genetic analyses using 1640 trees from 343 families of the two sites (BR9611 Flynn and BR9705 Kromelite) for wood density, MFA, and MoE revealed that all three juvenile wood properties had substantial genetic variation. Juvenile wood core length had the lowest heritability (h^2 =0.09 - 0.23) while wood density showed the highest heritability (h^2 =0.63 - 0.77). MFA and MoE also had high heritability (h^2 =0.43 - 0.63 for MFA, h^2 =0.36 - 0.67 for MoE) (Figure 19).

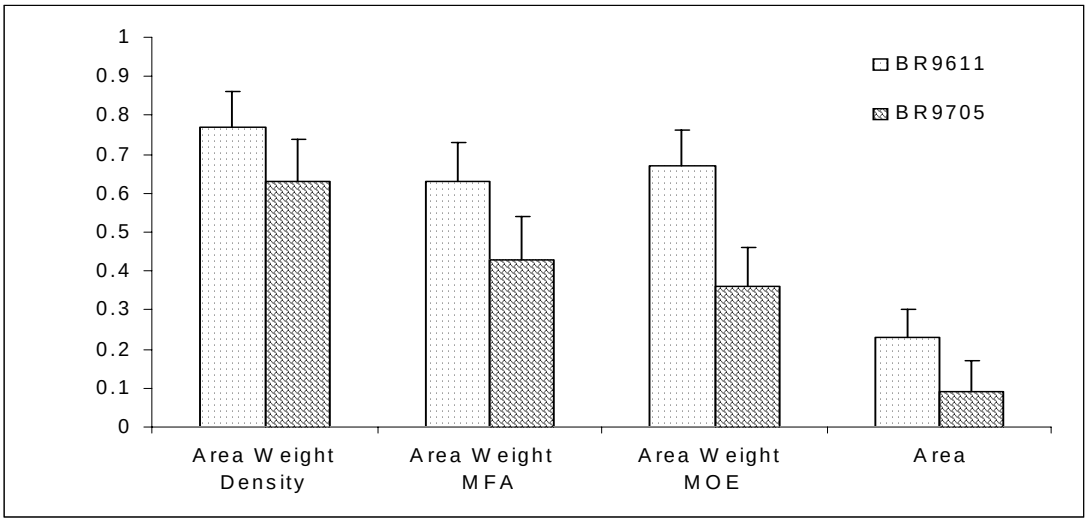


Figure 19. Narrow-sense heritability estimates ($\hat{h}^2$) for area weighted density, MFA, MoE, and area of juvenile radiata pine whole core measurements from trials BR9611 and BR9705. Standard error bars are shown.

Analyses from the 41 common parents among the two sites indicate that there was little genotype by environment interaction for the three juvenile wood properties across the two sites (Figure 20).

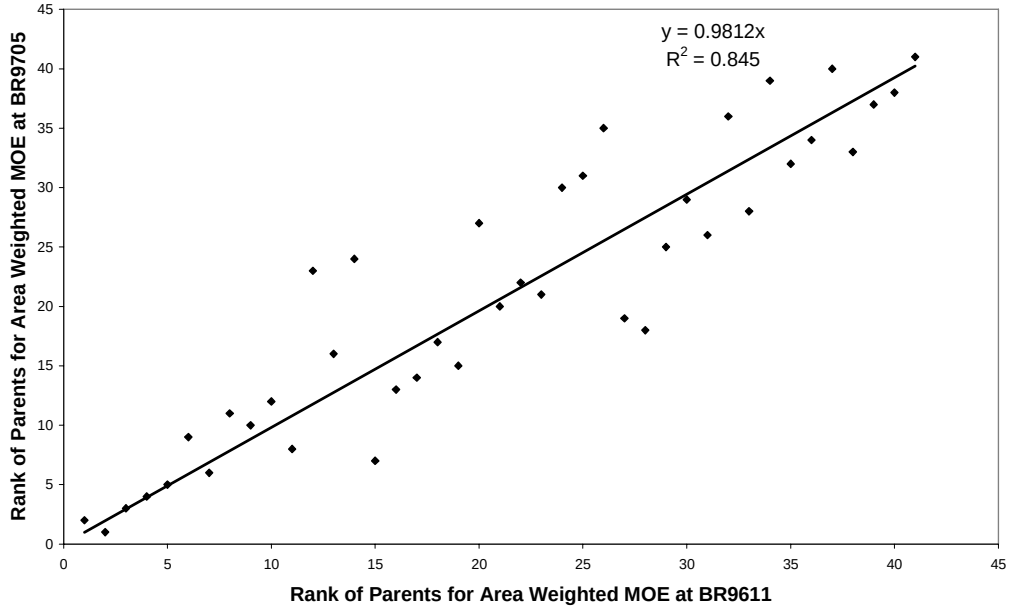


Figure 20. Rank-rank plot depicting the parental ranks for area weighted MoE for the 41 parents with progeny tested on both sites (Spearman's rank coefficient $\rho = 0.92$).

It was observed that MoE and MFA, and MoE and density had favourable genetic correlations, selection for MoE directly will produce the greatest improvement in overall stiffness of the corewood in radiata pine with concurrent increase of wood density and reduction of microfibril angle. Genetic gains between 18 and 28% are predicted for whole core MoE with selection intensity between 1 to 10% (Table 10).

Table 10. Genetic response over the entire population mean (%) for direct (bold diagonal) and indirect (off diagonal) selection. Genetic parameter and variance component estimates came from analyses of combined data across the two sites assuming homogeneous additive and residual variances.

				Target Trait (y)			
Selected Trait (x)	$\hat{h}^2$	$\hat{\sigma}_P^2$	Selection Intensity (%)	Density	MFA	MoE	Core Length
Density	0.68	749.83	10	**7.4**	-1.7	9.0	-4.7
MFA	0.51	12.01	10	0.9	**-10.4**	16.8	-1.7
MoE	0.51	2.01	10	2.7	-9.6	**18.3**	-3.3
Core Length	0.17	95.47	10	-2.2	1.6	-5.3	**3.9**
Density	0.68	749.83	1	**10.8**	-2.6	13.8	-7.1
MFA	0.51	12.01	1	-1.3	**-15.9**	25.6	-2.6
MoE	0.51	2.01	1	4.0	-14.6	**27.8**	-5.1
Core Length	0.17	95.47	1	-3.3	2.4	-8.0	**5.9**

Three juvenile wood quality traits had adverse genetic correlations with growth (-0.61, 0.28, -0.54 between growth and density, MFA, and MoE, respectively, Figure 21 and Table 11).

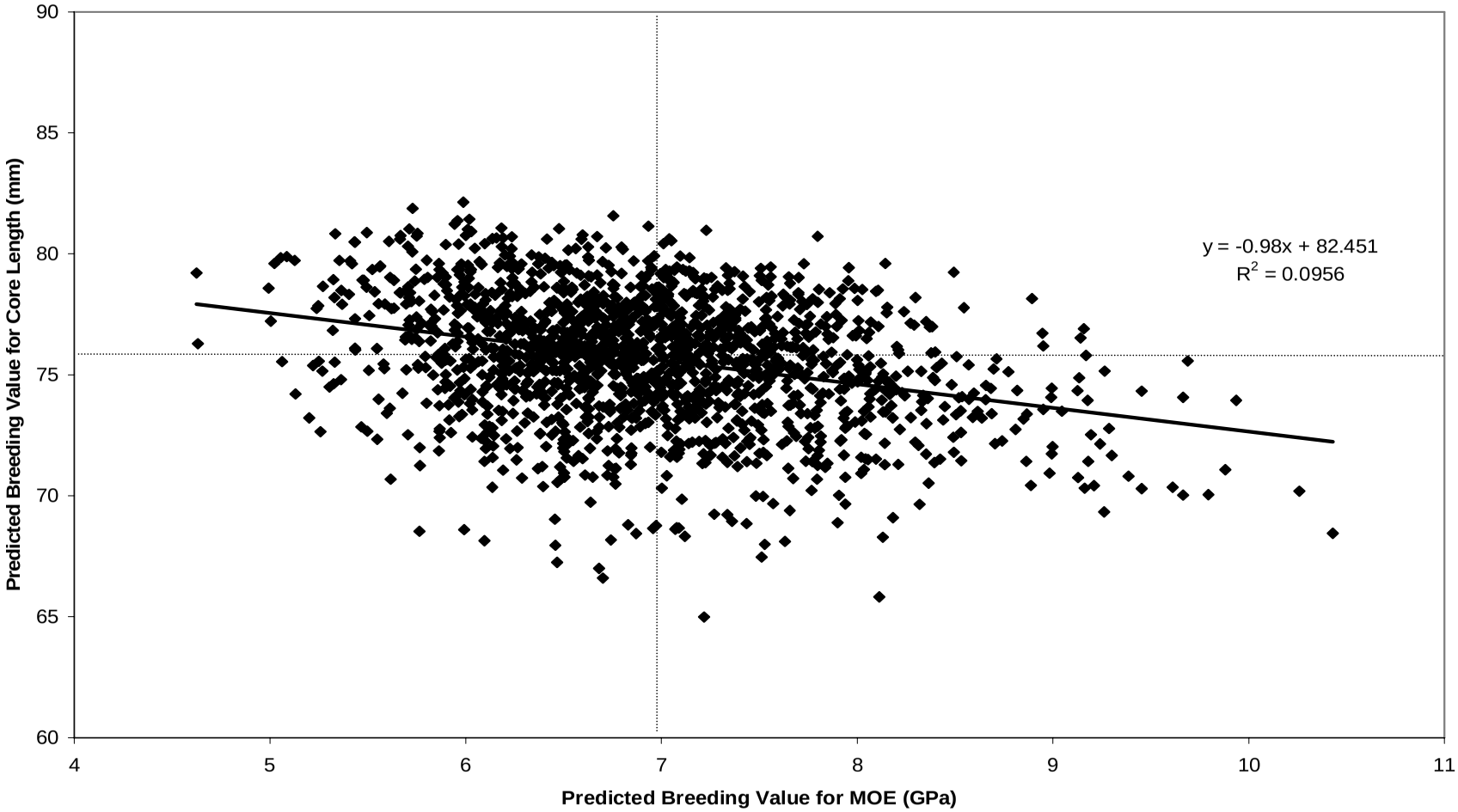


Figure 21. Predicted breeding values for MoE versus core length. Dashed lines represent population means for the respective traits. With such a negative genetic correlation, there is limited scope to select genotypes with relatively high MoE and growth.

With such adverse genetic correlation, selection for growth alone would result in decreases in density and MoE and an increase in average microfibril angle throughout the juvenile wood. For example, selection for area weighted MoE at BR9611 would result in a 4.5 to 6.8% genetic loss in core length with 10 to 1% selection intensities, respectively (Figure 22).

Table 11. Genetic (above diagonal) and phenotypic (below diagonal) correlations among radiata pine four juvenile wood properties from whole core measurements from trials BR9611 and BR9705 (joint site analyses). Standard errors are given in parentheses.

	Area	**Area-Weighted-Density**	**Area-Weighted-MFA**	**Area-Weighted-MoE**
Area		-0.61 (0.12)	0.28 (0.16)	-0.54 (0.14)
Area-Weighted-Density	-0.18 (0.03)		-0.14 (0.11)	0.43 (0.09)
Area-Weighted-MFA	0.28 (0.03)	-0.08 (0.03)		-0.92 (0.02)
Area-Weighted-MoE	-0.32 (0.02)	0.4 (0.03)	-0.89 (0.01)	

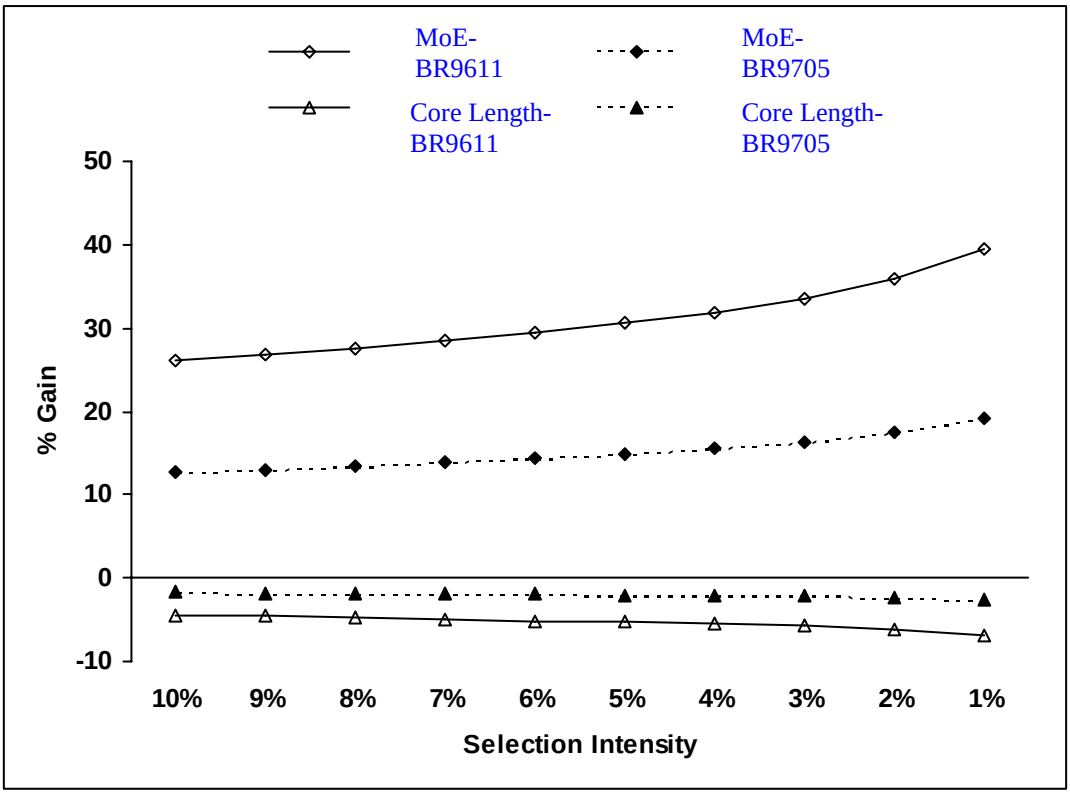


Figure 22. Genetic gain associated with direct selection for MoE and its effect on core length in juvenile radiata pine at trials BR9611 and BR9705.

Selection for optimal balance between growth and wood quality traits is important for further breeding of radiata pine, and selection and breeding strategies to overcome such high negative genetic correlations need to be developed. For further details of the study, see Appendix 4 (Genetic control of juvenile wood properties in radiata pine, as determined by SilviScan).

3.3.2 Inheritance of spiral grain

Spiral grain at breast height was assessed in two related progeny tests of radiata pine (BR9611 Fynn and BR9705 Kromelite). Radial trends for grain angle at the two sites were similar. Mean spiral grain (MSG) across the two trials was 4.3º with a standard deviation of 1.5° and a range of 0.8 to 10º. Estimates of individual tree heritabilities on a single-site basis for individual rings and mean spiral grain suggested that spiral grain is moderately or highly inherited (h^2 = 0.11 ± 0.08 to 0.66 ± 0.21 for individual rings and 0.44 ± 0.12 for mean spiral grain, Table 12 for Flynn site). Additive genotypic correlations between individual ring grain angles and mean spiral grain (MSG) were generally high, above 0.71, suggesting a favourable expected correlated response of mean grain angle in the juvenile wood to selection for grain angle of individual rings.

Table 12. Genetic parameter estimates at Flynn from single-site analysis of spiral grain traits.

Ring	N	Mean (s.d.)*	σ^2_A	σ^2_e	h^2 ± s.e.	r_A ± s.e.†	Cov_A‡
1	598	4.7 (2.01)	0.46	3.58	0.11 ± 0.08	0.71 ± 0.23	0.496
2	628	5.6 (2.30)	0.95	4.16	0.19 ± 0.09	0.85 ± 0.12	0.844
3	628	5.0 (2.13)	0.74	3.73	0.17 ± 0.09	1.05 ± 0.05	0.822
4	628	3.5 (2.06)	2.10	2.26	0.48 ± 0.13	0.96 ± 0.03	1.382
5	628	2.8 (2.00)	2.02	2.10	0.49 ± 0.12	0.91 ± 0.05	1.294
6	626	2.5 (1.90)	1.52	2.15	0.41 ± 0.12	0.99 ± 0.04	1.183
MSG	628	4.0 (1.50)	1.00	1.25	0.44 ± 0.12		

Note: * arithmetic mean and standard deviation of the absolute values of spiral grain measurements in each ring; † additive genetic correlation between individual ring number and mean spiral grain; ‡ additive genetic covariance between individual ring number and mean spiral grain.

Table 13. Predicted genetic gain from direct and indirect selection in individual rings and mean spiral grain (MSG).

	Flynn				Kromelite			
Ring	$Gain_{IND}$ (°)	$Gain_{IND}$ (%)	$Gain_{MSG}$ (°)	$Gain_{MSG}$ (%)	$Gain_{IND}$ (°)	$Gain_{IND}$ (%)	$Gain_{MSG}$ (°)	$Gain_{MSG}$ (%)
1	0.73	15.5	0.78	17.4	1.24	27.4	0.23	5
2	1.13	19.9	1.00	22.3	1.48	23.6	0.83	17.9
3	0.90	18.2	1.00	22.3	1.14	22.1	0.83	17.8
4	1.52	45.8	0.99	22.3	1.04	27.3	0.84	18.1
5	1.32	49.6	0.85	18.8	0.85	26.1	0.66	14.2
6	1.13	46.1	0.88	19.6				
MSG			1.13	25.2			1.25	27.1

Note: $Gain_{IND}$ is the predicted genetic gain in individual-ring spiral grain from direct selection (i = 1.755) in that ring, expressed both in trait units (degrees) and as a percentage relative to mean spiral grain in that ring; $Gain_{MSG}$ is the predicted correlated genetic gain in mean spiral grain resulting from selection on the spiral grain trait described on the corresponding row. $Gain_{MSG}$ is expressed both in degrees and as a percentage relative to the mean of MSG.

Selection to reduce spiral grain on any of rings 2-4 (at a selection intensity of 1.755, that is, selecting the best 10% of trees) would result in a predicted correlated genetic gain in MSG of 1.0° (Table 13). Our results suggest that selection could be performed in any of the individual rings 2, 3 or 4 (equivalent to ages 4-6) and still achieve at least 75% of the genetic gain possible from selection on the mean of all rings 1-5 (MSG). This suggests that there is

an optimum stage (rings 2-4) in which selection for this trait should take place. Our results suggest that a reduction in spiral grain angle in the juvenile core is one strategy to reduce the amount of degrade due to twist.

3.3.3. Inheritance of shrinkage

Estimates of average values for tangential, radial, longitudinal shrinkage, ratio of tangential to radial shrinkage, and the longitudinal gradient from pith-to-bark at Flynn and Kromelite are presented in Table 14. Tangential shrinkage for outer-rings (4 to 6) at Flynn averaged 6.1%, ranging from 3.8% to 7.9%. Similarly, tangential shrinkage for outer-rings (3 to 5) at Kromelite averaged 5.7%, ranging from 2.9% to 7.8% (Table 13). As expected, radial shrinkage (perpendicular to or across annual rings) for outer rings at both sites was approximately half that of tangential shrinkage (shrinkage parallel to the annual growth rings). Similarly, mean longitudinal shrinkage for the outer rings was similar at both sites (0.3%, ranging from 0.1 to 1.9 at Flynn and 0.4%, ranging from 0.02 to 1.6, at Kromelite). Mean longitudinal shrinkage for the inner rings was 4 times greater than that of the outer rings at both sites (1.3% and 1.4% at Flynn and Kromelite, respectively). The magnitude of the gradient of longitudinal shrinkage from pith-to-bark (-1.04 to 2.9%) is large enough to cause distortion problems including twist, during drying of sawn boards. This indicates that observed longitudinal shrinkage and its gradient in the juvenile core in radiata pine is large enough to cause warping and twisting in solid wood planks after drying. These values also suggest that shrinkage in the juvenile core of radiata pine is of major economic importance and therefore, should be improved either through genetics or silviculture.

Table 14. Mean estimates of radial, tangential and longitudinal shrinkage estimates at Flynn and Kromelite sites

Measured shrinkage property	**Abbrev**	**Flynn**		**Kromelite**	
		Mean (%)	**Range (%)**	**Mean (%)**	**Range (%)**
Tangential (outer rings)	TS	6.1	3.8 – 7.9	5.7	2.9 – 7.8
Radial (outer rings)	RS	3.6	1.9 – 5.2	3.3	1.6 – 5.0
Tangential/Radial Ratio	TRR	1.7	1.1 – 2.6	1.8	0.9 – 2.9
Longitudinal (outer rings)	LSO	0.3	0.1 – 1.9	0.4	0.02 – 1.6
Longitudinal (inner rings)	LCI	1.3	0.3 – 3.3	1.4	0.24 – 3.9
Longi Gradient (Pith to bark)	LGR	0.97	-1.04 – 2.9	0.87	-0.36 – 2.23

Narrow-sense individual tree heritability for tangential and radial shrinkage in the outer rings (4-6) was moderate at Flynn (0.24 ± 0.09 and 0.26 ± 0.07, respectively, Table 15). There was a lack of significant genetic variation for longitudinal shrinkage in the outer rings but significant genetic control for the inner rings (1-2) (0.26 ± 0.07). Larger size of samples is required to detect significant genetic variation for shrinkage traits due to higher noise in sampling and measuring shrinkage traits relative to other wood quality traits such as density, MFA, spiral grain and MoE.

Table 15. Estimates of individual tree heritability for shrinkage traits at Flynn and Kromelite sites

Measured shrinkage property	Abbrev	Heritability estimate	
		Flynn	Kromelite
Tangential (outer rings)	TS	0.24 ± 0.09	0.00
Radial (outer rings)	RS	0.26 ± 0.07	0.00
Tangential/Radial Ratio	TRR	0.48 ± 0.12	0.29 ± 0.33
Longitudinal (outer rings)	LSO	0.05 ± 0.08	0.00
Longitudinal (inner rings)	LCI	0.25 ± 0.06	0.00
Longitudinal Gradient (Pith to bark)	LGR	0.20 ± 0.11	0.21 ± 0.30

3.4 Genotype by environmental interaction in juvenile wood traits of radiata pine

Among the five traits (DBH, wood density, branch angle, branch size and stem straightness) studied, branch angle had the least genotype by site interaction (average type B genetic correlation $\hat{r}_B$ = 0.94), followed by wood density ($\hat{r}_B$ = 0.92), and stem straightness ($\hat{r}_B$ = 0.75). Branch size and DBH had the highest genotype by environment interactions ($\hat{r}_B$ = 0.50 and $\hat{r}_B$ = 0.67, respectively).

Among the five mainland trials, there was little evidence for genotype by environment interaction for DBH except between trials BR9611 and BR9701 where type B correlation was estimated as 0.48 (Table 15). Type B additive genetic correlations ranged from 0.48 to 1.4 between pairs of trials in the mainland. However, there was some indication that parental rankings were unstable in the Tasmanian trials indicating genotype by environment interaction for DBH with type B correlations ranging from 0.02 to 1.2. Within Tasmania, only type B genetic correlations between trial BR9715 and BR9601, BR9705, BR9709, and BR9614 were > 0.71, all other type B genetic correlations for DBH involving measurements from trials BR9615 and BR9614 were all less than 0.66 indicating that genotype by environment interaction was present (Table 16). When trials were grouped within one of two regions, the average type B genetic correlation was estimated as 0.63 for DBH.

There was no evidence for genotype by environment interaction for density across all pairwise combinations of trials (Table 17). Significant type B additive genetic correlations ranged from 0.74 to 1.0 for density with an average of 0.98. Generally, wood quality traits are believed to be genetically stable across trials. For example, type B genetic correlations were > 0.77 for density, MoE, and MFA based on SilviScan measurements between trials BR9601 and BR9705. Such high genetic correlations between sites for density indicate that parental rankings are stable, further suggesting that fewer trials may be necessary for ranking and selecting genotypes for density.

Table 16. Pairwise type B genetic correlations for DBH among eight mainland and Tasmania sites (standard error).

	Region 1 – Mainland				Region 2 - Tasmania		
	BR9611	**BR9701**	**BR9705**	**BR9709**	**BR9615**	**BR9614**	**BR9715**
BR9601	0.82 (0.12)	0.8 (0.15)	1.1 (0.06)	1.4 (1.9)	0.39 (0.18)	0.51 (0.17)	0.86 (0.23)
BR9611		0.48 (0.23)	0.91 (0.15)	0.98 (0.6)	0.48 (0.2)	0.24 (0.23)	0.56 (0.23)
BR9701			0.97 (0.17)	0.6 (0.5)	0.32 (0.25)	0.33 (0.23)	0.32 (0.29)
BR9705				1.1 (0.48)	0.63 (0.2)	0.33 (0.27)	0.85 (0.12)
BR9709					0.66 (0.62)	0.02 (0.88)	1.2 (1.1)
BR9615						0.44 (0.21)	---
BR9614							0.71 (0.23)

Table 17. Pairwise type B genetic correlations for wood density among eight mainland and Tasmania sites (standard error).

	Region 1 – Mainland				Region 2 - Tasmania		
	BR9611	**BR9701**	**BR9705**	**BR9709**	**BR9615**	**BR9614**	**BR9715**
BR9601	0.99 (0.02)	0.97 (0.06)	0.98 (0.06)	0.83 (0.14)	0.96 (0.03)	1 (0.03)	---
BR9611		0.92 (0.07)	0.79 (0.13)	0.78 (0.12)	0.99 (0.02)	1 (0.06)	---
BR9701			1 (0.08)	0.88 (0.13)	0.9 (0.07)	0.8 (0.15)	---
BR9705				0.99 (0.08)	0.88 (0.09)	1 (0.11)	---
BR9709					0.74 (0.14)	1 (0.17)	---
BR9615						1 (0.05)	---
BR9614							---

Genotype by regional interactions (mainland vs Tasmania) revealed that wood density and branch angle had the least interactions ($\hat{r}_B$ = 0.98 and $\hat{r}_B$ = 0.95, respectively). Branch size and DBH had the highest interactions among the two regions ($\hat{r}_B$ = 0.55 and $\hat{r}_B$ = 0.63, respectively). Within Tasmania, only branch size and DBH had a sizable interaction within the three sites ($\hat{r}_B$ = 0.50 and $\hat{r}_B$ = 0.58, respectively). In contrast, there was little interaction for DBH ($\hat{r}_B$ = 0.92) among the five sites on the mainland. Branch size in the mainland trials

had a similar size of interaction ($\hat{r}_B$ = 0.64) as in Tasmania. Interactions for the other three traits (wood density, branch angle, and stem straightness) were small within the two regions.

The above analysis indicates there are three patterns of genotype by environment interactions in these trials:
(1) There were little interactions for wood density within and among regions;
(2) There were sizable interactions between two regions and within Tasmania for DBH;
(3) There were large interactions between two regions and within the mainland and Tasmania for branch size.

For further details of the study, see Appendix 5 (Genotype by environmental interaction for DBH, wood density, branch angle, branch size, and stem straightness in eight young *Pinus radiata* D. Don trials in Australia).

4. Inheritance and Quantitative Genetics of Juvenile Wood in Slash and Caribbean Pines, and Their F1 Hybrid

The trends in wood density, MFA, and MoE for the pure species and hybrid pine at two sites (Beerwah and Tuan) are presented in Figures 23, 24 and 25. Pith-to-bark trends revealed that by ring 3-5 average ring density has risen above 450 kg/m3, average values of MFA have fallen well below 30^o, and predicted MoEs were close to or above 8 MPa in all three taxa. This indicates that the stiffness and distortion problems associated with low density and high MFA in the juvenile core are mostly a function of the innermost rings in PEE, PCH and their F1 hybrid and that selection and breeding should focus on the inner few rings of the juvenile core in PEE, PCH and their F1 hybrid in southeast Queensland. It also seemed that PEE had higher wood density, lower MFA, and higher MoE at early ages than PCH, and F1 hybrid was more or less in between, particularly for MoE.

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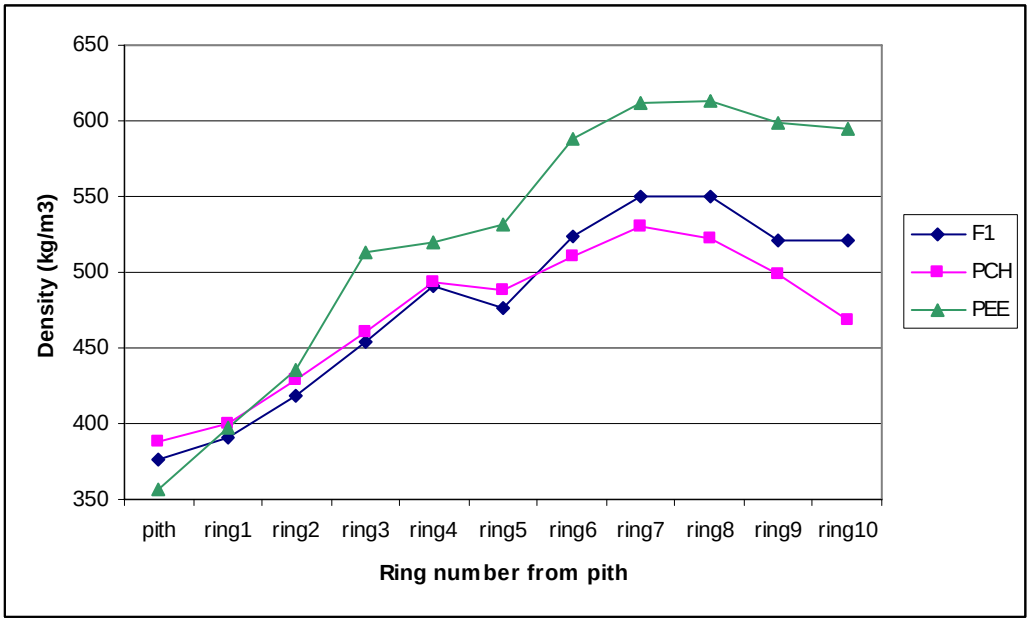

Figure 23. Mean ring density from pith to bark in 11 year-old trees of *Pinus elliottii* (PEE), *P. caribaea* var. *hondurensis* (PCH), and the F_1 hybrid between PEE and PCH (F1), averaged from two sites (Beerwah and Tuan) in south-east Queensland.

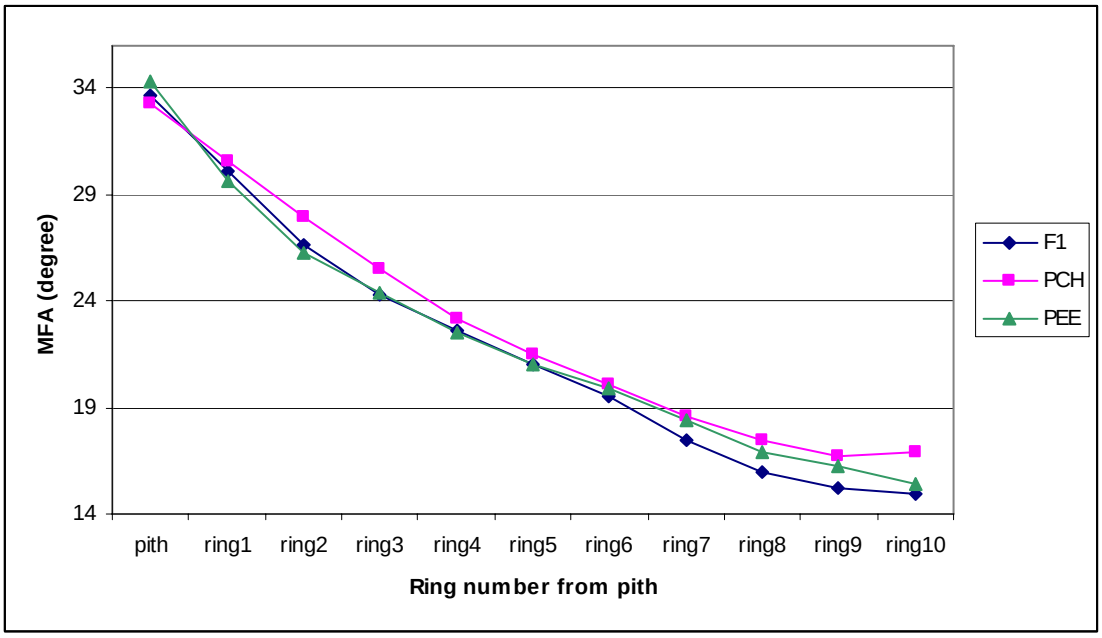

Figure 24. Mean ring microfibril angle (MFA) from pith to bark in 11 year-old trees of *Pinus elliottii* (PEE), *P. caribaea* var. *hondurensis* (PCH), and the F_1 hybrid between PEE and PCH (F1), average from two sites (Beerwah and Tuan) in southeast Queensland.

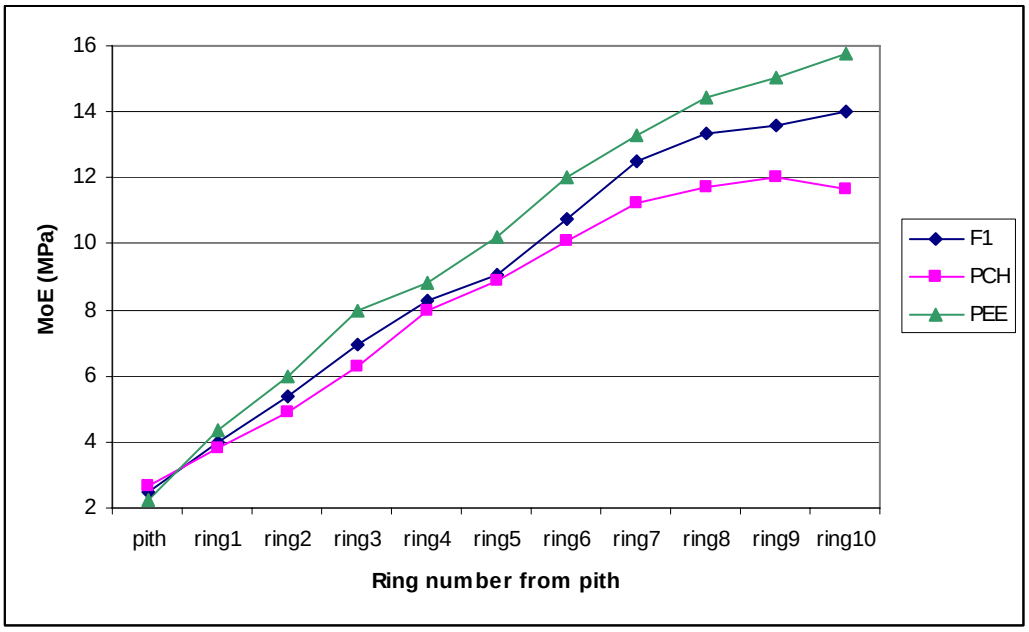

Figure 25. Mean ring modulus of elasticity (MoE) from pith to bark in 11 year-old trees of *Pinus elliottii* (PEE), *P. caribaea* var. *hondurensis* (PCH), and the F_1 hybrid between PEE and PCH (F1), averaged from two sites (Beerwah and Tuan) in southeast Queensland.

The heritability of density, MFA and MoE fluctuated greatly from ring to ring; in general the heritability appeared to maximize in rings 1 – 3, particularly for MFA (Figures 26).

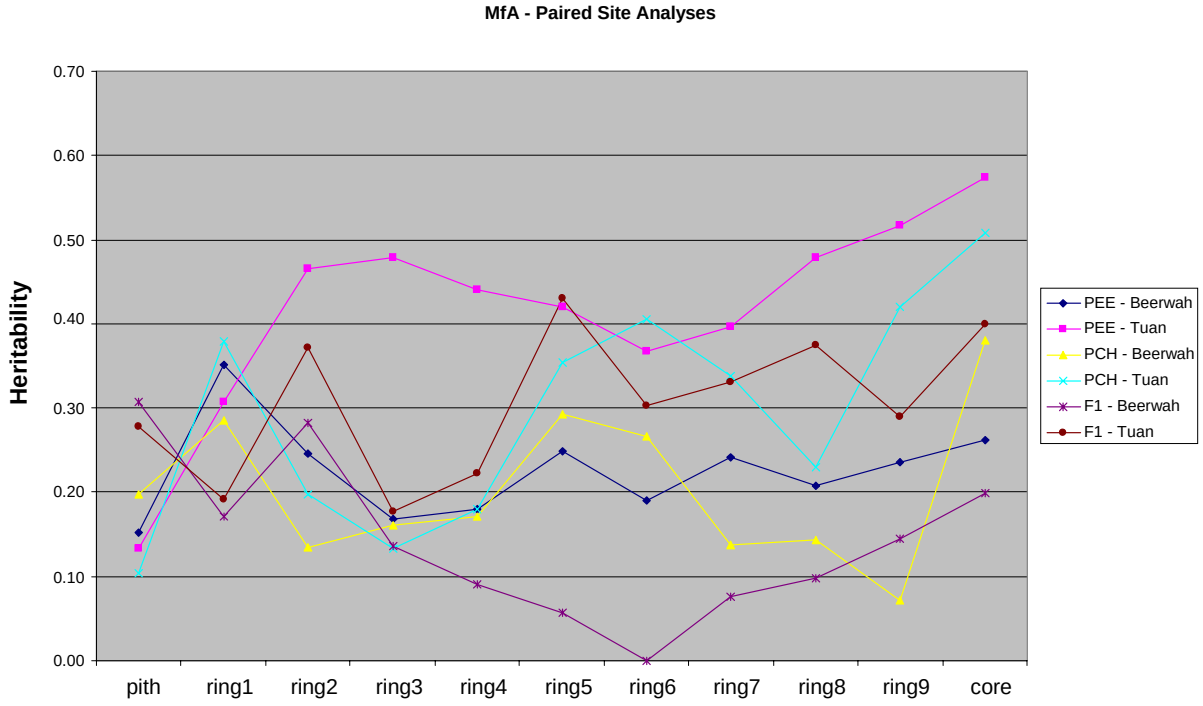

Figure 26. Heritability of microfibril angle (MFA) estimated for each ring (pith to ring9), and area weighted MFA of the whole core for *P. elliottii* (PEE), *P. caribaea* var. *hondurensis* (PCH) and their F_1 hybrid grown on two sites in southeast Queensland.

Wood density had higher heritability for all taxa/site combinations except for PCH at the more poorly drained Tuan site (Figure 27). MoE had the lowest heritability on average.

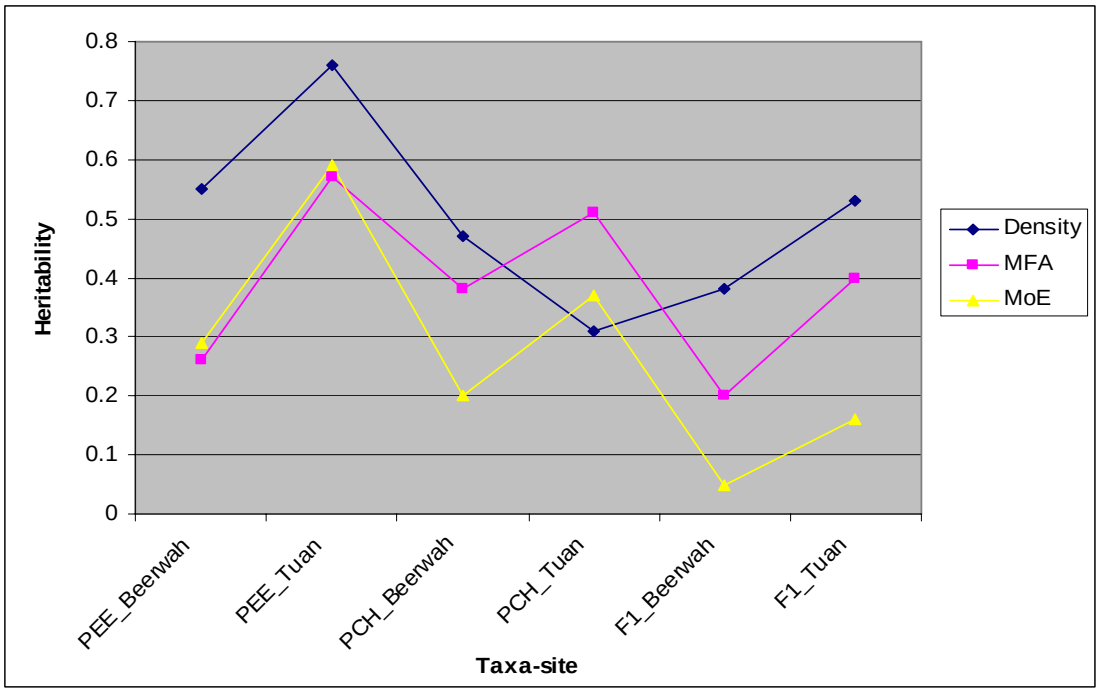

Figure 27. Heritability of wood density, microfibril angle (MFA), and modulus of elasticity (MoE) for whole core for *P. elliottii* (PEE), *P. caribaea* var. *hondurensis* (PCH) and their F_1 hybrid (F1) for Beerwah and Tuan site in southeast Queensland.

This suggests that selection for density and MFA in ring 3 (i.e. in 4 – 5 year old trees), is likely to have a greater impact on improving MoE than delaying selection until later ages. Given the lower heritability of MoE (than either density or MFA) and the much greater cost of estimating MoE (i.e. via SilviScan), selection based on whole core density (generally higher heritability than individual ring measurements) and an acoustic measure of MFA/stiffness will optimize genetic gain in MoE.

Juvenile wood properties as measured by density, MFA and MoE from SilvaScan were very stable across sites, suggesting that assessment of wood properties on one or two sites will provide reliable estimates of the genetic worth of individuals for use in future breeding. The phenotypic and genetic correlations between rings for density, MFA and MoE indicate that the values observed in the inner rings (1 – 5) were strongly and positively correlated with ring 7 and the whole core This suggests that selection for improved wood properties in the innermost rings would also result in improvement in wood properties in the subsequent rings, as well as improved average performance of the entire juvenile core (i.e. wood formed up to 10 years from planting).

Further analyses also demonstrated strong genetic correlations between pure species and hybrid performance for each of the wood quality traits (Table 18). Under this scenario, information on pure species performance can be used to reliably predict hybrid performance. This confirms the decision to collect information on the parents which are being crossed for initiation of future cycles of clonal testing – because of these strong genetic correlations, parental performance can be used to identify the hybrid families which are most likely to have superior juvenile wood properties of the slash/Caribbean F1 hybrid in southeast Queensland. The stability of genetic parameters estimated from the current study with relative small size of sample (12 parents for pure species) remains to be verified from a larger population.

Table 18. Additive genetic correlations (r_A) between pure species (*P. elliottii*, PEE, and *P. caribaea* var. *hondurensis*, PCH) and their F_1 hybrid, for key wood quality traits (wood density, MFA, and MoE) and cross-sectional area (Area), determined using standardized data pooled across the Beerwah and Tuan sites. (Standard errors in parentheses.)

Trait	**Ring(s)**	**r_A PEE-F_1**		**r_A PCH-F_1**	
Area	core	0.59	(0.32)	0.78	(0.30)
Density	1	1.13	(0.07)	1.13	(0.07)
	3	1.16	(0.24)	1.31	(0.30)
	5	1.20	(0.13)	0.87	(0.21)
	7	0.98	(0.13)	0.99	(0.15)
	core	0.99	(0.07)	0.98	(0.09)
MFA	1	0.76	(0.25)	1.24	(0.12)
	3	0.86	(0.22)	0.85	(0.24)
	5	-0.05	(0.44)	0.83	(0.20)
	7	0.77	(0.25)	0.92	(0.20)
	core	0.75	(0.22)	0.89	(0.14)
MoE	1	1.07	(0.12)	1.09	(0.12)
	3	1.59	(0.37)	0.99	(0.59)
	5	0.68	(0.41)	0.38	(0.42)
	7	1.37	(0.24)	0.60	(0.40)
	core	1.06	(0.08)	0.69	(0.25)

For further details of the study, see Appendix 6 (Genetic control of juvenile wood properties (density, microfibril angle and predicted modulus of elasticity) in slash (*Pinus elliottii* var. *elliottii)* and Caribbean (*P. caribaea* var. *hondurensis*) pines and their F1 hybrid, as determined by SilviScan.).

5. Gene Discovery in Juvenile Wood Formation

5.1 Generation and analysis of xylogenesis ESTs in radiata pine

A total of 6,389 high quality ESTs of at least 100 bp in length were collected from approximately 8,000 raw sequences. The 6,389 ESTs were sequenced from 5,952 different cDNA clones in six developing xylem cDNA libraries. Average size of all 6,389 ESTs and the 5,952 ESTs from different clones was 624 bp and 636 bp, respectively. The number of ESTs in each library ranged from 694 in earlywood at transition age to 1,636 in latewood at juvenile age. The highest proportion of ESTs were from juvenile wood as it is the focus of this study. The assembly of all ESTs from 5′ end sequences generated 3,304 xylogenesis unigenes, which included 952 contigs and 2,352 singletons (Table 19). The 3,304 unigenes have an average length of 702 bp. Of the 952 contigs, 270 have four or more transcripts and the three deepest contigs included 69-79 transcripts. Blast searches of the 5,952 ESTs indicate that a total of 139 ESTs (2.3%) showed no matches in the current public databases, thus some of them are likely to represent novel ESTs in radiata pine wood formation.

Table 19. Assembly of radiata pine xylogenesis ESTs from six cDNA libraries

Assembly	EST	Contig	Singleton	Unigene	Redundancy (%) [c]
Assembly for six libraries [a]	5,952	952	2,352	3,304	47.8
Assembly for each library [b]					
Juvenile earlywood	1,259	198	711	909	27.8
Juvenile latewood	1,636	241	935	1,176	28.1
Transition earlywood	694	92	410	502	27.7
Transition latewood	799	73	371	444	44.4
Mature earlywood	837	128	454	582	30.5
Mature latewood	727	65	559	624	14.2
Total of each library	5,952	797	3,440	4,237	28.8 [d]

[a] ESTs from the 5′ ends of 5,952 clones were used in the assembly;
[b] Only 5′ end ESTs in each library were used in the assemblies;
[c] Redundancy was estimated by: 1- (number of unigenes / number of ESTs);
[d] The average redundancy from each of six cDNA libraries.

Of the 3,304 xylogenesis unigenes, 68.1% have matches in the NCBI nr database with blastx at E-value $\leq 10^{-5}$, however, 77.3% of all matches are unknowns or uncharacterized proteins. In contrast, a total of 96.1% of the unigenes matched sequences in the UniProt (with blastx) and TIGR (with blastn) databases and only 42.9% of all matches were not assigned GO terms. The results blasted with unigenes are similar to those with ESTs. In the functional

classification with GO terms, 89.1% of the 1,813 unigenes with assigned GO terms have molecular functions, 74.6% are involved in a biological process, and 47.8% are cellular components. The three categories of GO terms fell predominantly into one or two sub-categories. In the molecular function category with 1,616 unigenes 56.4% and 57.2% have binding and catalytic activity, respectively. Of the 867 unigenes in the cellular component, 98.9% are related to cell components. As for the 1,353 unigenes involved in a biological process, 87.7% and 96.2% have functions in cellular process and physiological process, respectively.

In the 5,952 ESTs, only 6.8% have homologs (blastx, E-value $\leq 10^{-5}$) in the Cell Wall Navigator, a primary wall gene database of *Arabidopsis*. However, all 18 categories of primary and secondary wall genes in the MAIZEWALL database were represented in the radiata pine EST resource, including 1,070 ESTs classified into 91 cell wall gene families. Therefore, genes related to secondary cell walls are highly abundant in the radiata pine EST resource. The 1,070 cell wall related ESTs of radiata pine were previously assembled into 826 contigs and 19 singletons, which matched sequences in the UniProt and TIGR databases with 557 non-redundant accession numbers, suggesting possibly 557 cell wall-related genes occurred in the radiata pine EST resource. The most abundant cell wall gene is *cellulose synthase* (*CesA*), with a total of 175 ESTs (2.9%). The 30 most highly abundant cell wall related genes are listed in Table 20.

Table 20. Thirty highly abundant genes (or gene families) in the 5,952 xylogenesis ESTs of radiata pine.

Gene or gene family	**ESTs**	**%**
Cellulose synthase (*CesA*)	175	2.94
Tubulin (*TUB*)	102	1.71
Aquaporin	102	1.71
Arabinogalactan protein (*AGP*)	89	1.50
Phytochrome	75	1.26
Actin	58	0.97
S-adenosylmethionine synthetase (*SAMS*)	46	0.77
Methionine synthase (*cobalamin-independent*)(*MetE*)	41	0.69
Elongation factor	38	0.64
Photoassimilate-responsive protein (*PAR*)	38	0.64
Laccase	36	0.60
Pectate lyase	35	0.59
Auxin-induced protein	32	0.54
Caffeoyl-CoA O-methyltransferase (*CCoAMT*)	29	0.49
Unknown (*Emb|CAB86899.1*)	29	0.49
Endo-1,4-beta-D-glucanase (*cellulase*)	29	0.49
Unknown (*Os07g0462200*)	29	0.49
Phytocyanin	29	0.49
Ubiquitin	28	0.47

Cytokinin-binding protein	27	0.45
Sucrose synthase (*SuSy*)	26	0.44
Eukaryotic translation initiation factor	25	0.42
Zinc finger	25	0.42
Chitinase	24	0.40
RNA-binding protein	24	0.40
Metallothionein-like protein class II (*MT-II*)	23	0.39
Pollen-specific protein C13	23	0.39
Expansin	21	0.35
UDP-glucose dehydrogenase	21	0.35

PlantTFDB, a recently developed database of transcription factor (TF) families for 22 plant species, was used to identify putative transcription factors expressed during radiata pine wood formation. Blastx searches revealed 358 ESTs (assembled into 284 unigenes) of radiata pine with matches in PlantTFDB at E-value $\leq 10^{-5}$. These homologs fell into 41 families and represented 64.1% of the 64 TF families in the poplar genome, suggesting extensive involvement of transcription factors in the regulation of xylogenesis gene expression. The most abundant TF family in radiata pine wood formation is PHD (Cys4–His–Cys3 zinc finger) with 55 ESTs.

Comparative genomic analysis revealed highly conserved xylogenesis transcriptome in conifers, and distinctly divergent transcriptome in poplars and *Arabidopsis*. However, the functional domains of the pine xylogenesis transcriptomes are moderately conserved in poplars and *Arabidopsis*, suggesting a common transcriptome ancestor for gymnosperm and angiosperm wood formation. There were 290 putative pine xylogenesis-specific unigenes and 699 putative xylogenesis orthologs, which suggest considerable differences in gene regulation in gymnosperm and angiosperm during wood formation.

5.2 Genes preferentially expressed in different wood development stage and different wood

Microarray gene expression studies have revealed a time series of transcriptome reorganization through seasonal wood development in a rotation period. Of the xylogenesis unigenes presented in the microarrays, 11.5-29.9% are preferentially expressed in either earlywood or latewood tissues (Figure 29), suggesting transcriptome reorganization involved in earlywood and latewood formation in response to seasonal change. The transition age (i.e. 9 yrs) is likely to have the most extensive transcriptome reorganization during earlywood/latewood transition with 29.9% of the transcriptome differentially expressed. In the juvenile age (5 yrs) 20.7% of the transcriptome are reorganized in response to seasonal change, thus juvenile wood also involves extensive transcriptome reorganization. In contrast, only 11.5% and 12.7% of the transcriptome is reorganized in mature age (13 yrs) and rotation age (30 yrs) trees, respectively, which is about 30-50% of the level in transition age (9 yrs) and juvenile age (5 yrs), suggesting transcriptome reorganization in response to seasonal change is likely to decline rapidly with wood maturation processes in trees. When only considering the xylogenesis contigs and using higher thresholds, 1.5-5.7% were identified as preferentially expressed genes in either earlywood or latewood formation (Figure 28). These

preferentially expressed contigs detected at higher thresholds are putative candidate genes specifically expressed in earlywood or latewood formation. At both higher and lower thresholds, the number or percentage of preferentially expressed genes in earlywood is relatively similar to that in latewood for each of the four wood maturation stages.

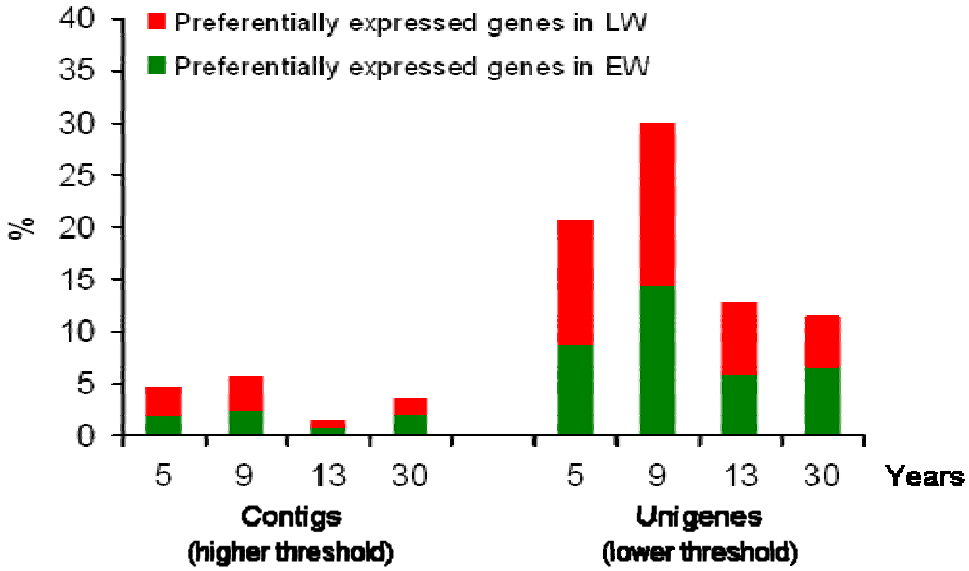

Figure 28. Percentage of genes preferentially expressed in earlywood and latewood at four distinct stages of wood maturation

The clustering analyses for unigenes (3,304 contigs + singletons) and contigs (952) both generated two clusters, which clearly showed the similarity of transcriptome reorganization during earlywood/latewood transition between juvenile age (5 yrs) and transition ages (9 yrs), as well as between mature age (13 yrs) and rotation age (30 yrs). The similar transcriptome reorganization within each cluster suggests that the types of differentially expressed genes during earlywood/latewood transition and their expression values are similar in juvenile and transition wood, as well as in mature and rotation wood. Interestingly, the four stages of wood maturation (5, 9, 13 and 30 yrs) were separated into two clusters, as the early maturation stage at 13-year-old was grouped with rotation age (30 yrs) (Figure 29). Therefore in a rotation period of radiata pine the transcriptome reorganization during earlywood/latewood transition tends to have two distinct phases, the early phase before mature age (juvenile and transition ages, <12 yrs) and the late phase from mature age (mature and rotation ages, ≥ 13 yrs).

To identify differentially expressed genes in earlywood and latewood formation with higher confidence level, we only used xylogenesis contigs using higher thresholds. A total of 348 genes were identified as preferentially expressed genes in earlywood and latewood. Of the 348 preferentially expressed genes, 163 and 128 genes are specifically expressed in earlywood and latewood, respectively, in at least one of the four stages of wood maturation, while the remaining 57 genes are preferentially expressed in either earlywood and latewood at different stages of wood maturation. Cell wall-related genes specifically expressed in earlywood include genes involved in cell division (cyclin-like F-box and profiling-1), cell differentiation (clavata-like receptor), cell expansion (three expansin genes), actin skeleton (three actins) cell wall proteins (AGP4, FLA8, FLA16, beta-1,3-glucanase and proline-rich

proteins), pectin pathway (pectate lyases, pectinesterase, UDP-apiose/xylose synthase and UDP-glucose pyrophosphorylase), cellulose (cellulose 8, glycosyl transferase NTGT5a) and lignin (peroxidase, laccase, chitinase-like 1, dirigent-like, methionine synthase 2 and uclacyanin 3-like). In contrast, most cell wall-related genes specifically expressed in latewood are involved in cellulose synthesis (CesA3, CesA7, sucrose synthase and callose synthase-like), lignin pathway (4CL, C3H, C4H, CAD, CCoAOMT, COMT, laccase, chitinase-like, endochitinase, phenylcoumaran benzylic ether reductase and plastocyanin-like), cell skeleton (four tubulins), cell wall protein (AGP5) and cell death (metacaspase type II). Some signalling genes responsive to auxin and ABA (auxin-regulated protein, cullin 1A, cullin-like, rac-like GTP binding protein, 14-3-3 and 14-3-3-like) are specifically expressed in earlywood formation, while genes responsive to ethylene (ethylene-responsive element binding factor and ethylene-forming enzyme) are specifically expressed in latewood. Among the genes related to water transport and drought stress, aquaporins and water deficit inducible protein are specifically expressed in earlywood, while dehydrins, aquaporin-like, LEA and LP6 are specifically expressed in latewood.

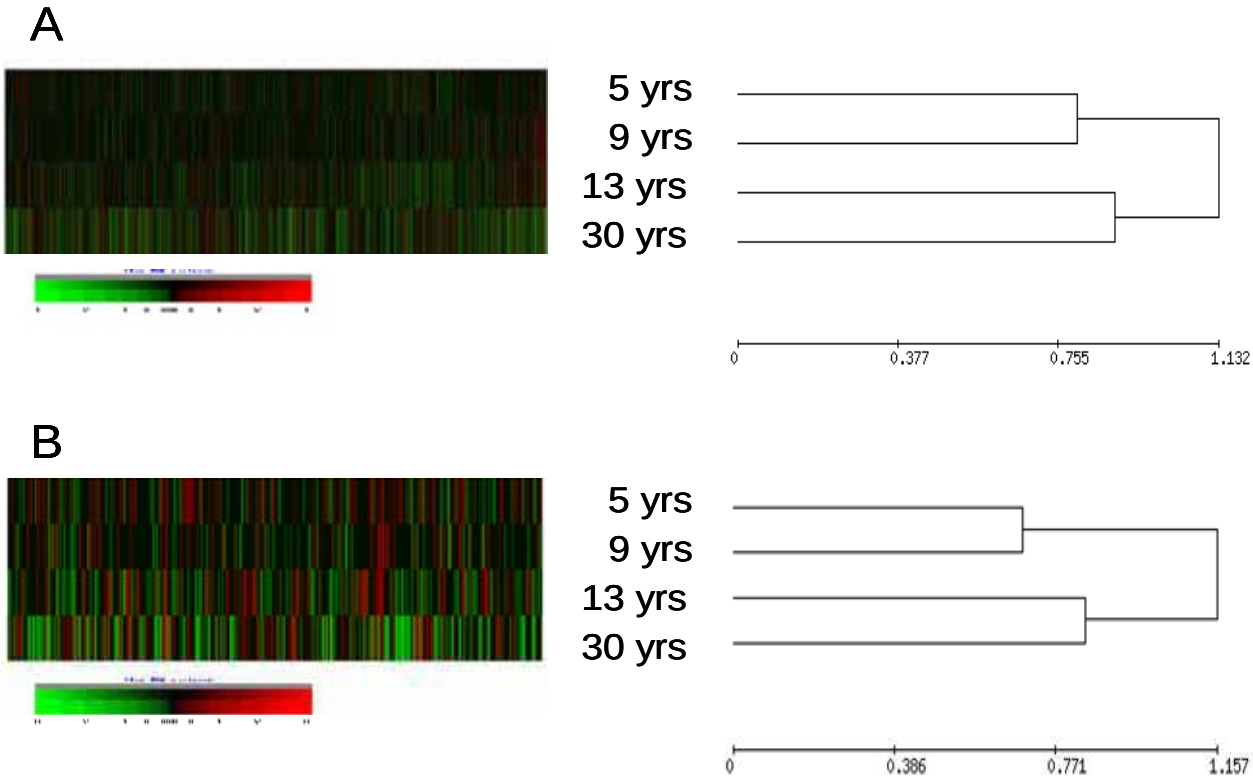

Figure 29. Complete linkage (maximum distance) clustering trees based on correlation measure of distance (un-centered**)** for unigenes (lower threshold, A) and for contigs (higher threshold, B).

Transcriptome reorganization during wood maturation across a growing season was also revealed by microarray gene expression studies. Of the 3,304 xylogenesis unigenes, 19.5% and 9.2% are preferentially expressed in either juvenile wood or mature wood in spring and autumn, respectively (Figure 30), suggesting genes differentially expressed in juvenile and mature wood in spring were about twice the number identified in autumn. When only considering the xylogenesis contigs and using higher thresholds, 4.6% and 1.3% were identified as preferentially expressed genes in either juvenile wood or mature wood (Figure 30). These preferentially expressed contigs selected at higher thresholds are putative candidate genes specifically expressed during juvenile or mature wood formation.

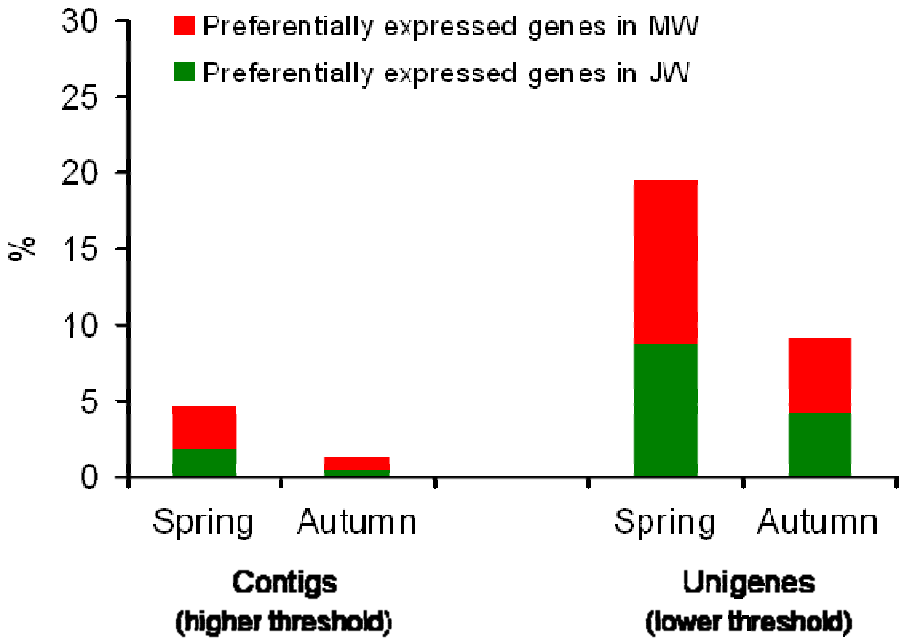

Figure 30. Percentage of genes preferentially expressed in juvenile and mature wood at spring and autumn

Of the xylogenesis contigs selected at higher thresholds, a total of 168 genes with unique accession numbers in the Uniprot and TIGR databases were identified as preferentially expressed genes in juvenile and mature wood at spring and autumn. Of the 168 preferentially expressed genes, 99 and 68 genes are specifically expressed in juvenile and mature wood, respectively, in at least one season (spring or autumn), while a single gene (cytokinin-binding protein) is preferentially expressed in juvenile wood in autumn and mature wood in spring.

Genes preferentially expressed in high/low stiffness and high/low density were also identified in microarray experiments. For further details of the study, see Appendix 7 (Gene discovery in juvenile wood formation of *Pinus radiata*.).

6. SNP Discovery and Association Genetics for Juvenile Wood Traits

6.1 Population genetics

Linkage disequilibrium (LD) was characterized in the three mainland populations of *P. radiata.* Figure 31 depicts the distribution of r^2 for 2279 SNP site pairs. From the fitted curve of r^2 to its expectation ($E(r^2)$), with an adjustment for average sample size (Hill and Weir 1988), LD decays to approximately 50% within 1700 bp (from r^2 = 0.48 to 0.27), or within the length of a typical gene. This is comparable to estimates in *P. taeda* (Brown *et al.* 2004). Applying an alternative cut off for LD decay (r^2 = 0.1) (Remington *et al.* 2001), the extent of LD predicted by E(r^2) would exceed the length of these fragments. The extent of LD across the genome will permit high resolution mapping of candidate gene SNPs with traits, but will demand a high density of SNPs tested per gene if the majority of haplotype variation is to be captured. The parameters and proportional memberships generated in STRUCTURE provided reliable evidence for the existence of three discrete populations. The log probability as a function of K began to plateau between K priori 3 and 4. The modal value for the

distribution of a second ad hoc indicator, ΔK (Evano *et al.* 2005), verified the presence of three discrete populations. Bar plots generated by STRUCTURE for K = 2-6 illustrate the proportion of each individual with ancestry in the inferred clusters (Figure 15). Beyond K=3 the ancestral integrity of individuals visibly degrades, and the distribution of additional clusters within the three populations appears uniform.

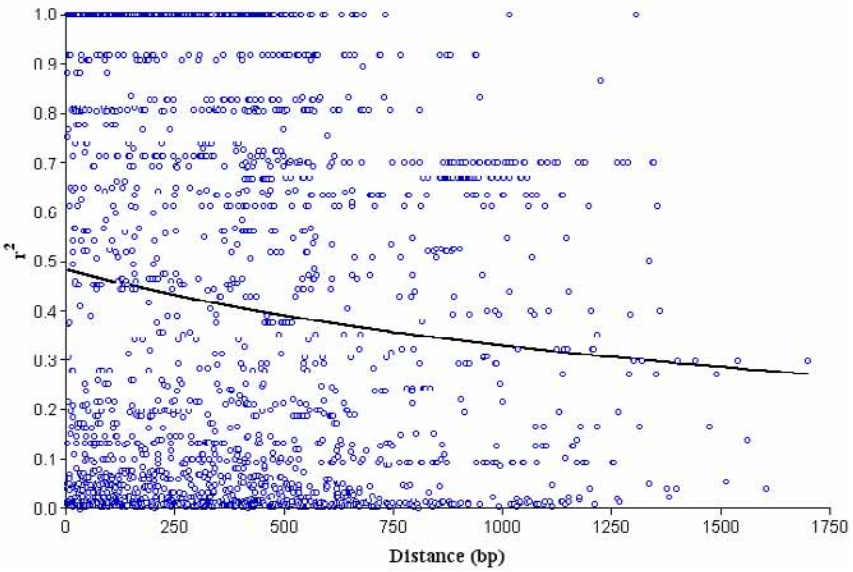

Figure 31. Distribution of the squared correlations of allele frequencies (r^2) for 28 amplicons. In total 2,279 pairwise SNP comparisons are mapped against distance (bp). The fitted curve describes the least squares fit of r^2 to its expectation $E(r^2)$.

6.2 Association genetics

6.2.1 Batlow site (149 SNPs)

The effect of regime (permutations with and without the Q-matrix) on the level of significant associations was observed from the cumulative p-value distribution between 0 and 1 is shown in Figure 32.

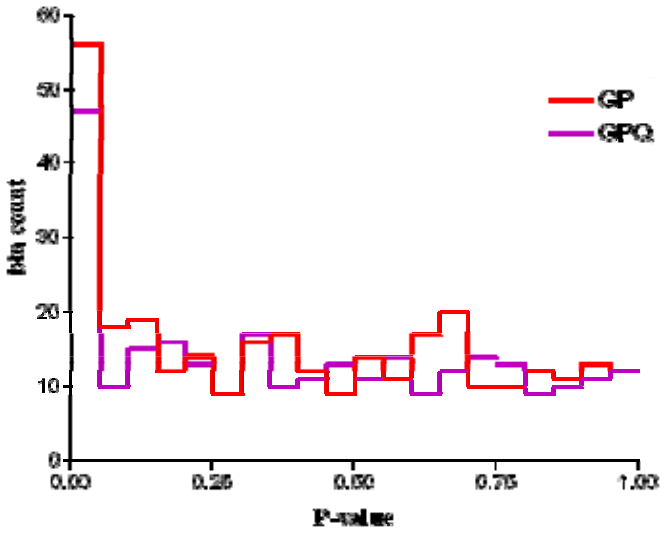

Figure 32. Proportion of truly alternative features (π^o: ~20%) estimated from the cumulative P-value distribution, assuming that null *p* values are uniformly distributed.

Naïve analysis (no structure or permutation) produced a large proportion of significant associations for P < 0.05 (GP). The number of associations with P < 0.05 dropped noticeably following perturbation of the data set (GPQ, Figure 33). An alternative statistic, q-value, was

estimated to measure the strength of each association (feature) in terms of the false discovery rate (FDR; Storey and Tibshirani 2003). Modal bins were observed between $P \leq 0$ and 0.05 for three distributions (GL, GLP and GLPQ) which were skewed towards zero, whereas the distributions for GL regimes were generally flat for values of P between 0 and 1 (data not shown). Both the GLP and GLPQ regimes afforded between 10 and 11% of q-values low enough to be considered significant (< 0.1). Based on the p-value distribution the estimated pie (π) indicated that between 21 and 17 percent of association tests for GLP and GLPQ respectively were expected to be truly significant.

Based on the results it appeared that the most reliable associations would be obtained with the GLM by incorporating both structure and permutations, with the FDR applied subsequently. This approach was used to create a shortlist of 30 SNP associations, from 15 genes, at the mixed population level (Table 21). The proportion of genetic control on a trait (R) attributed to each SNP and the corresponding percent improvement afforded is also listed. Individual SNPs described between 1.9 and 6.5% of total genetic variation (3.3% average). Undesirable, large negative correlations between shortlisted SNP associations and growth were not detected.

Table 21. 30 SNPs shortlisted after association testing

SNP #	SNP	Trait	p value	q - val	Rsq marker	population
2	PrRac132F04	mature MFA	0.0015	0.0633	0.065	Batlow
2	PrRac132F04	min MFA	0.0035	0.0779	0.063	Batlow
2	PrRac132F04	max MFA	0.0055	0.0779	0.064	Batlow
11	PrPorinMP134	PCA2	0.0025	0.0633	0.031	Batlow
11	PrPorinMP134	coarseness	0.0045	0.0779	0.031	Batlow
17	PrPaL1301	cell population	0.0105	0.0957	0.019	Batlow
29	AGP424	max density	0.0115	0.1007	0.0289	Batlow
42	CAD1R1	wall thickness	0.0105	0.0957	0.028	Batlow
45	COMT213	min MOE	0.0065	0.0779	0.0264	Batlow
45	COMT213	min density	0.012	0.1012	0.0294	Batlow
45	COMT213	PCA1	0.0125	0.1017	0.0282	Batlow
45	COMT213	density	0.0135	0.102	0.029	Batlow
50	EXPAN11R6	wall thickness	0.0025	0.0633	0.0298	Batlow
50	EXPAN11R6	wall thickness	0.0045	0.0779	0.0266	Batlow
57	Lp519	wall thickness	0.002	0.0633	0.0318	Batlow
57	Lp519	wall thickness	0.0055	0.0779	0.033	Batlow
57	Lp519	specific surface area	0.0065	0.0779	0.036	Batlow
57	Lp519	specific surface area	0.0075	0.0813	0.037	Batlow
58	MYB121	min density	0.0005	0.0379	1.42E-07	Batlow
60	PAL111	% juvenile density of mature density	0.0005	0.0379	0.020	Batlow
61	PAL131	cell population	0.0135	0.1021	0.019	Batlow
69	PrATHB8102	cell population	0.008	0.0828	0.027	Batlow
84	PrCesA12R01	juvenile rings	0.0075	0.0813	0.023	Batlow
118	PrRac131F01	cell population	0.006	0.0779	0.028	Batlow
118	PrRac131F01	cell population	0.0065	0.0779	0.029	Batlow
131	SAHH119	PCA3	0.0015	0.0633	0.0311	Batlow

133	SAM132	max density 1997 (dry year)	0.0065	0.0779	0.034	Batlow
133	SAM132	max density	0.0095	0.0941	0.042	Batlow
133	SAM132	MFA	0.0005	0.0379	5.47E-08	Batlow
146	PrSusy1Fg218	max density 1991 (dry year)	0.002	0.0633	0.0284	Batlow
13	GBP_2F_02	density	0.0005	0.0015	0.023	Batlow and Flynn
59	GIR_11133X	density	0.026	0.067	0.017	Batlow and Flynn
77	PEL_1F461**	MFA	0.0005	0.0015	0.024	Batlow and Flynn
77	PEL_1F461**	density	0.026	0.067	0.016	Batlow and Flynn
80	Rac13_4g1_44**	MFA	0.0005	0.0015	0.043	Batlow and Flynn
80	Rac13_4g1_44**	MOE	0.0005	0.0015	0.038	Batlow and Flynn
80	Rac13_4g1_44**	density	0.0005	0.0015	0.061	Batlow and Flynn
121	ALP_1F415**	MFA	0.0005	0.0015	0.02	Batlow and Flynn
133	SAM132	density	0.0005	0.0015	0.017	Batlow and Flynn
139	CYP_2670**	density	0.0005	0.0015	0.029	Batlow and Flynn
141	LAC_2F221	MFA	0.0005	0.0015	0.027	Batlow and Flynn
141	LAC_2F221	MOE	0.0005	0.0015	0.024	Batlow and Flynn
141	LAC_2F221	density	0.0005	0.0015	0.054	Batlow and Flynn
152	KN4_3F779	MFA	0.0005	0.0015	0.024	Batlow and Flynn
152	KN4_3F779	MOE	0.025	0.067	0.017	Batlow and Flynn
152	KN4_3F779	density	0.0005	0.0015	0.028	Batlow and Flynn
179	GBP_2R_09	MFA	0.025	0.067	0.018	Batlow and Flynn
179	GBP_2R_09	density	0.0005	0.0015	0.023	Batlow and Flynn
189	Rac13_4g3_42**	MFA	0.021	0.062	0.049	Batlow and Flynn
189	Rac13_4g3_42**	MOE	0.0005	0.0015	0.055	Batlow and Flynn
189	Rac13_4g3_42**	density	0.0005	0.0015	0.079	Batlow and Flynn
210	ADF_F2529	MOE	0.025	0.067	0.025	Batlow and Flynn
210	ADF_F2529	density	0.0005	0.0015	0.016	Batlow and Flynn
210	ADF_F2529	MFA	0.0005	0.0015	0.05	Batlow and Flynn
236	AQP_2F548	MFA	0.0005	0.0015	0.025	Batlow and Flynn
236	AQP_2F548	MOE	0.0005	0.0015	0.022	Batlow and Flynn
236	AQP_2F548	density	0.0005	0.0015	0.022	Batlow and Flynn
238	Lac_3218**	density	0.0005	0.0015	0.018	Batlow and Flynn
239	ALP_2F546**	MFA	0.0005	0.0015	0.019	Batlow and Flynn
239	ALP_2F546**	MOE	0.0005	0.0015	0.02	Batlow and Flynn
239	ALP_2F546**	density	0.0005	0.0015	0.024	Batlow and Flynn
240	GIR_1768	MFA	0.0005	0.0015	0.056	Batlow and Flynn
240	GIR_1768	MOE	0.0005	0.0015	0.035	Batlow and Flynn
240	GIR_1768	density	0.0005	0.0015	0.028	Batlow and Flynn
270	Thioh_2F607**	MOE	0.022	0.062	0.015	Batlow and Flynn
308	LAC_1_F656	MFA	0.027	0.068	0.018	Batlow and Flynn
323	EST_1314_1	MFA	0.0005	0.0015	0.021	Batlow and Flynn
335	LAC_2F201	density	0.0005	0.0015	0.021	Batlow and Flynn
344	IAA_1F872	MFA	0.0005	0.0015	0.019	Batlow and Flynn
344	EST_1305_1	MOE	0.0005	0.0015	0.017	Batlow and Flynn
344	EST_1305_1	density	0.0005	0.0015	0.015	Batlow and Flynn
358	EXP_1F2106	MFA	0.0005	0.0015	0.019	Batlow and Flynn
365	ADF_F2475	MFA	0.0005	0.0015	0.041	Batlow and Flynn

365	ADF_F2475	MOE	0.0005	0.0015	0.034	Batlow and Flynn
365	ADF_F2475	density	0.0005	0.0015	0.05	Batlow and Flynn
371	COBL4_3F475	MFA	0.0005	0.0015	0.02	Batlow and Flynn
374	KN4_2F792	MFA	0.0005	0.0015	0.047	Batlow and Flynn
374	KN4_2F792	MOE	0.0005	0.0015	0.037	Batlow and Flynn
374	KN4_2F792	density	0.0005	0.0015	0.065	Batlow and Flynn

Elevated levels (2.3 fold) of significance were observed for SNPs showing allelic structure. Adjustment of the model residual error following inclusion of the Q-matrix resulted in a 44% drop in the number of significant associations among structured SNPs. This was best seen from the cumulative p-value distributions for structured and non-structured SNPs, before and after adjustment for structure (Figure 33). Associations detected with SNPs demonstrating allelic structure may also have contributed to an inflated false positive rate, consistent with the reduction in associations between $P \leq 0$ and 0.05 following inclusion of the Q-matrix (Figure 33).

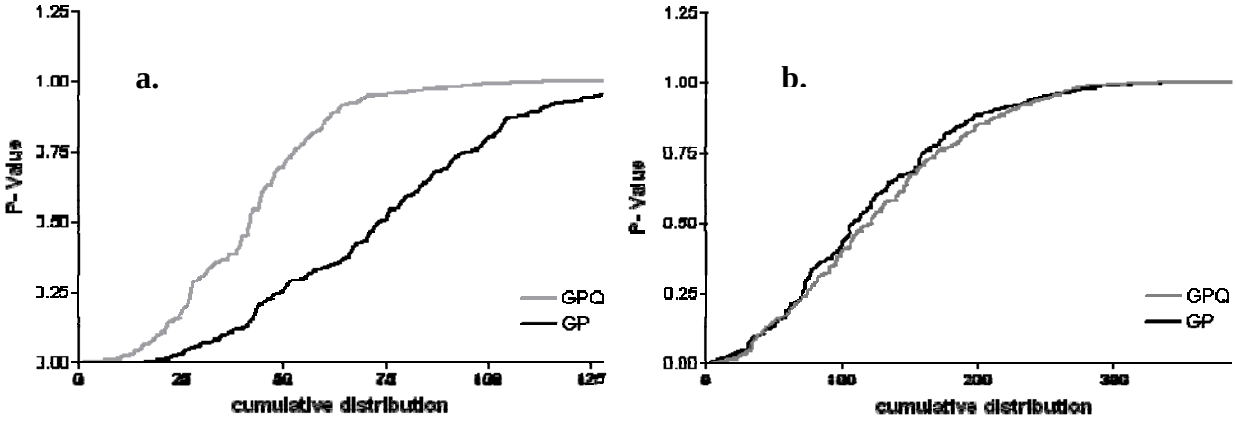

Figure 33. P-value distributions for (a) structured and (b) non-structured SNPs with and without model adjustment for the Q-matrix.

Although sample sizes for the sub-populations were small (n = 82 – 220), 86% of associations shortlisted in Table 20 were significantly associated in one or more of the sub-populations – or in the absence of structure. This suggests that structure has not driven false discoveries in these cases. To test whether SNP population structure was likely to have been driven by selection, the proportion of significant associations for structured and non-structured SNPs were compared in the sub-populations (Figure 34).

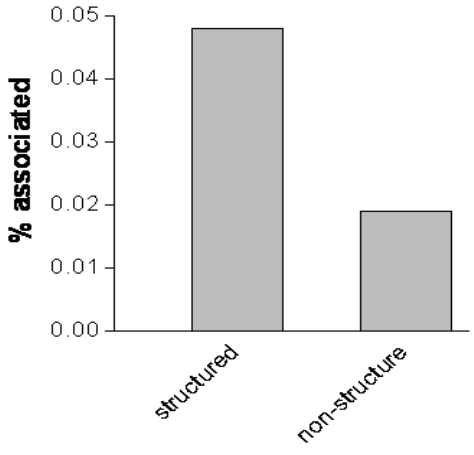

Figure 34. The proportion of significant associations (P< 0.05) when SNPs tested in the absence of structure (in sub-populations) are classed as structured or non-structured at the mixed population level.

This analysis revealed a 2.6 fold higher level of significant associations for structured SNPs, suggesting selection has been directed at wood properties for a significant proportion of structured SNPs in this data set. Consequently a proportion of the associations ruled out by the Q-matrix may have been real. This raises an important question as to how best to handle population structure in association analysis in order that false associations are not excluded at the cost of true association driven by natural selection.

Several possibly meaningful trends were observed among the significant associations ($P < 0.05$) when assessed in the GLPQ and GLP models, that may strengthen their validity. When associations were grouped according to the position of the SNP in the ORF (i.e. intron vs. exon vs. promotor vs. 3'UTR), the proportion of significant associations were greatest for SNPs in the 3'UTR and exons (Figure 35) as we might expect. SNPs from introns and 5'UTR/ promotor regions exhibited the fewest associations. In addition, the proportion of exon associations with silent SNPs yielded half the number of significant associations as compared to non-synonymous (change amino acid) SNPs (Figure 35). We expect that SNPs causing an amino acid change to have a higher probability of affecting protein function than non-coding or synonymous polymorphisms.

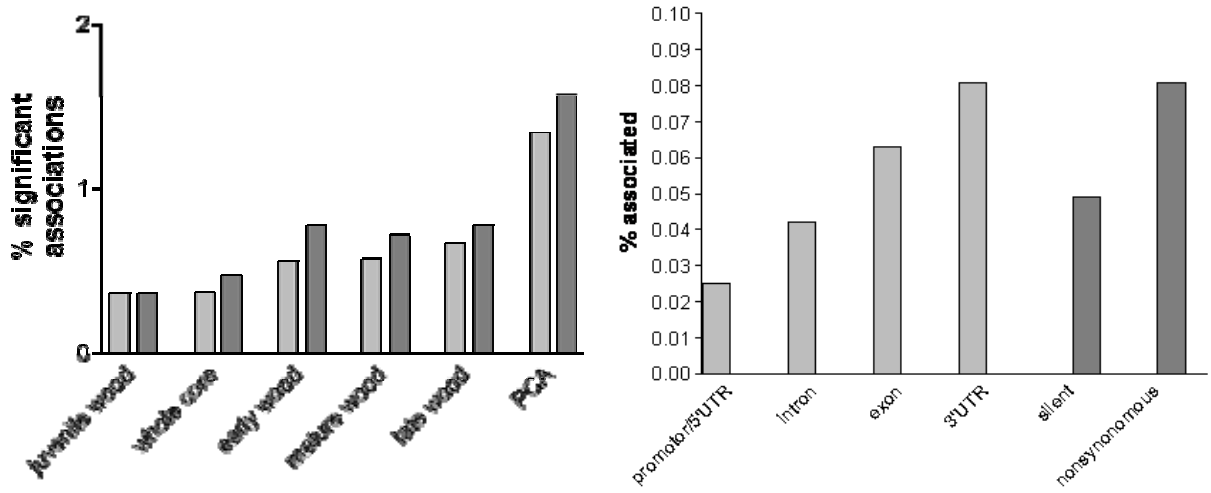

Figure 35. The proportion of significant tests related to trait and marker type (based on 149 SNP markers)

6.2.2 Joint Flynn and Batlow sites (255 SNPs)

255 SNPs from 51 putative pine cell wall candidate genes were assessed for evidence of genetic structure and kinship across the three data sets. Association testing via GLM produced 51 and 151 associations with (GPQ) and without (GP) adjustment for the detected genetic structure, respectively (Figure 36). The p-value distribution which was clearly skewed towards 0 indicated strong evidence for associations with $P < 0.05$. The false discovery rate was consequently low for many associations and q-values for significant SNPs ranged from 0.0006 to 0.045. Significant associations arising from permuted analysis, with population structure, on the combined population were shortlisted where q-values were less than 0.07 (Appendix 8). More detailed results can be found in Appendix 8 (SNP discovery and association genetics in juvenile wood of radiata pine).

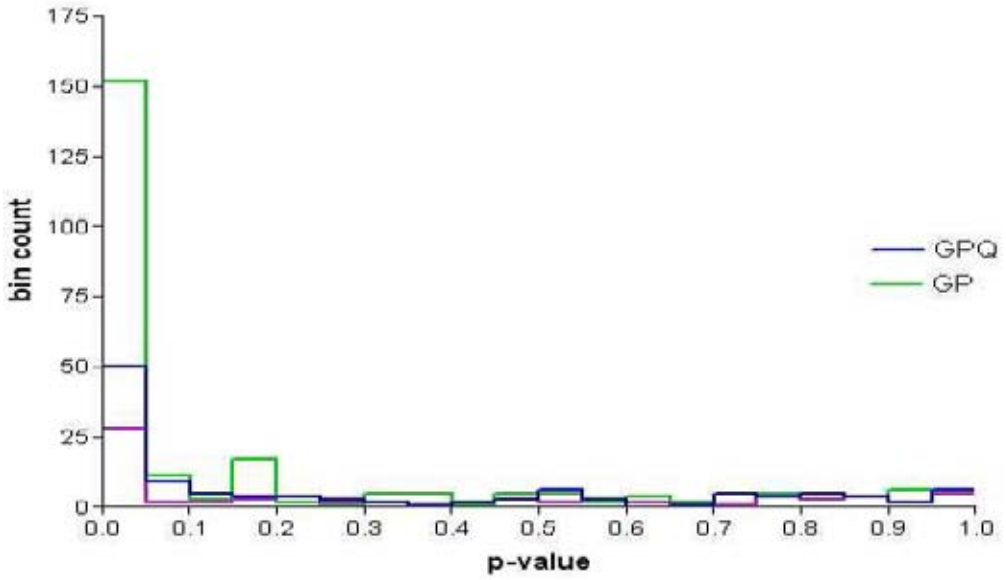

Figure 36. Proportion of truly alternative features estimated from the cumulative P-value distribution, assuming that that null *p* values are uniformly distributed.

6.3 Genetic mapping

Linkage analysis on SNPs genotyped on the Bondo mapping population revealed a total of 9 linkage groups from a final number of 60 markers which covered 543.45 centimorgans in total. Map distances were expressed in Kosambi centimorgans (Figure 37). Linkage groups were visualised using Mapchart software (Voorrips 2002). The number of linkage groups in the map corresponds closely to the actual number of chromosomes for radiata pine (12). Those markers that did not map in this experiment, due to insufficient recombination information with other loci, have a high probability of mapping to one of the existing linkage groups once more markers are added to this map (see conclusion for comments on additional mapping of 400 SNPs).

The smaller groups on the framework map presented may represent smaller fragments of one or more of the other groups identified. Similarly several of the larger groups (2, 3 and 7) which exhibit large centimorgans distances towards their centre suggest these groups may represent two groups spuriously combined. Such groups may fall apart once additional markers have been added to the map. We did not attempt to assign QTLs for traits collected across the progeny at this stage, due to the small number of total markers mapped, but will proceed with QTL analysis once additional SNP data for these individuals has been made available.

In order to increase the resolution of the current map, the existing mapping pedigree will be genotyped with up to 400 candidate gene SNP markers, identified from candidate gene re-sequencing efforts, and mapped into the existing framework of microsatelite markers. This work is part of an ongoing collaboration with the Forest Genomics Group UC Davis, California, headed by David Neale. More detailed results can be found in Appendix 9 (Genetic mapping of candidate genes for vascular development in *Pinus radiata* D. Don).

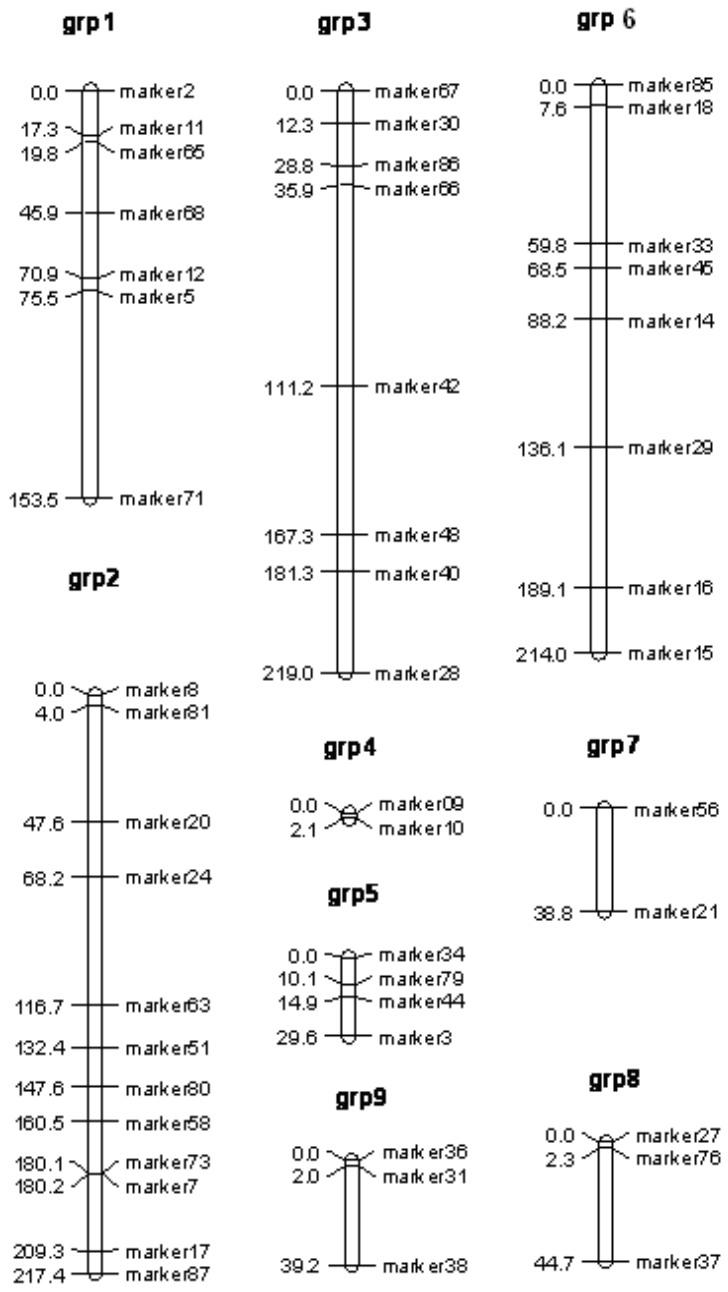

Figure 37. Linkage groups through 1 to 9 mapped with a combination of SNP and microsatellite markers

Summary of SNP discovery and association genetic studies

- SNPs associated with variation in several important wood properties were identified after adjustment for population genetic structure and multiple testing error.
- The Flynn population should be analysed separately using a mixed liner model accounting for kinship.
- When testing associations in the presence of structure, model adjustments may remove spurious results, but overlook associations resulting from population dependant selection.

- If sample sizes are suitably large, testing within subpopulations is recommended to avoid structure and reduce the risk of omitting true associations resulting from selection.
- SNPs are currently being validated in other populations, and their functions studied.
- We are currently modeling the efficiency gained by including SNP markers in breeding selection.

7. Incorporating Genomics and Quantitative Data into Breeding Program

7.1. Incorporating quantitative genetic data into radiata pine breeding program

Major genetic parameters derived in the project have been added to TreePlan for estimating breeding values and selection for breeding and deployment. Wood density data and stiffness data were incorporated in the DataPlan records for the trials physically sampled as well as in refining the generic matrix utilized by TreePlan. These genetic parameters along with the results from the Breeding Objectives (PN01.1904) project have contributed significantly to the improvement in wood density breeding values predicted by TreePlan since October 2004 and MoE stiffness breeding values since December 2005. Results of density derived from 12 mm cores and acoustic stiffness from various/available standing tree tools were used in DataPlan and TreePlan to predict MoE as one of the breeding objective traits. Net Present Values (NPV) were calculated using these results for about 250,000 trees (Figure 38). This gave an estimated NPV of more than $A400 million for selection from the average of the third generation (progeny of the second generation) to the breeding population of the third generation (selected trees from the progeny of the second generation) for STBA members and more than $A800 million for the entire radiata pine plantation estate.

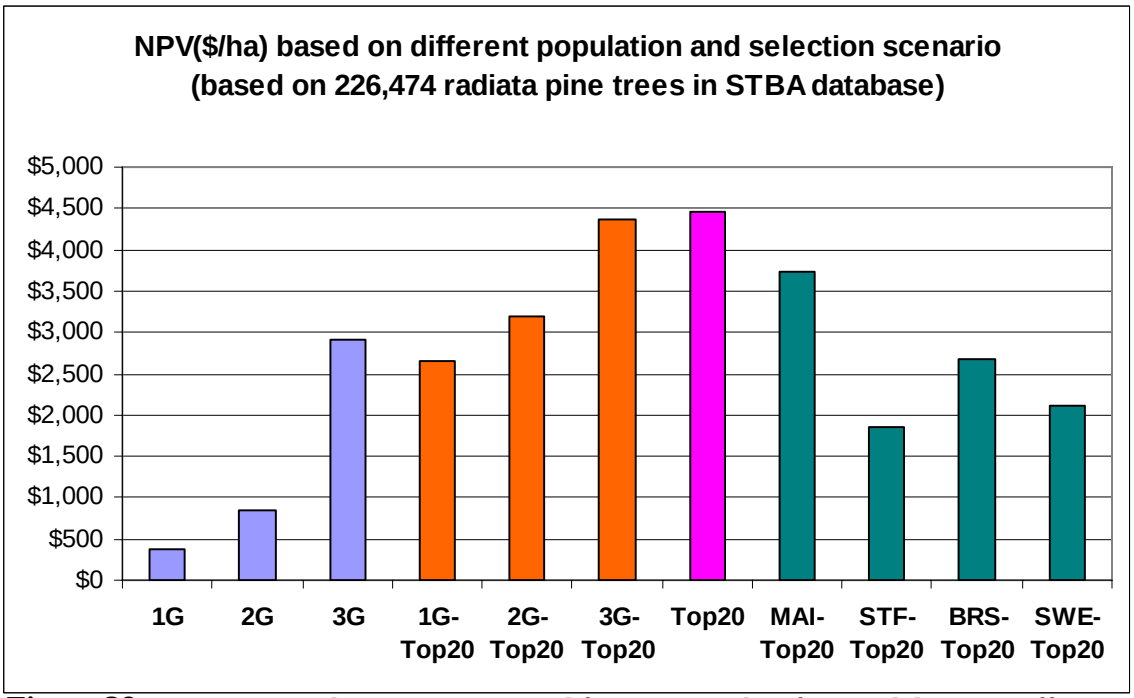

Figure 38. Net Present Value (NPV) estimated from genetic data for wood density, stiffness and data from economic breeding objectives (1G to 3G representing average of the first to third generation of radiata pine breeding. Top20 refers to selection of top 20 trees in the different generations based on four breeding objective traits (MAI-mean annual increment, STF-stiffness, BRS-branch size, and SWE-stem straightness) combined, or independently).

More genetic gains will contribute to the radiata improvement program as further integration of wood quality data from the current project is used in selection, grafting and crossing options using new tools such as the MatePlan and SeedPlan systems.

Selection decisions for STBA breeding crosses and STBA Member deployment is an annual process. MoE prediction methodology developed in the Juvenile Wood Initiative (and associated data) has contributed significantly to both these elements and has been encapsulated in STBA procedures. As more trial assessments are finalised using these procedures, and as new trials come on stream and are assessed, more benefits directly attributable to the Juvenile Wood Initiative will flow into the breeding program as well as into Members' plantations.

7.2 SNP-aided selection

Of the 255 single nucleotide polymorphisms identified in the Flynn population, 14 showed significant additive effects (p-value < 0.05) associated with average density. Four of these SNPs also had significant dominance effects associated with average density. The approximate percent phenotypic variation for density explained by each of the 14 significant SNPs was low, ranging from 0.03% to 1.61% with a mean of 0.66%. Twenty-six SNPs had significant additive effects for microfibril angle. Six of these SNPs also had significant dominance effects. For microfibril angle the favourable allele (A_1) is the allele that decreases microfibril angle with respect to the cell axis (e.g. Barnett and Bonham 2004). For example, individuals with genotype A_1A_1 at SNP 240 have a statistically lower mean microfibril angle than A_1A_2 and A_2A_2 genotypes (Figure 39).

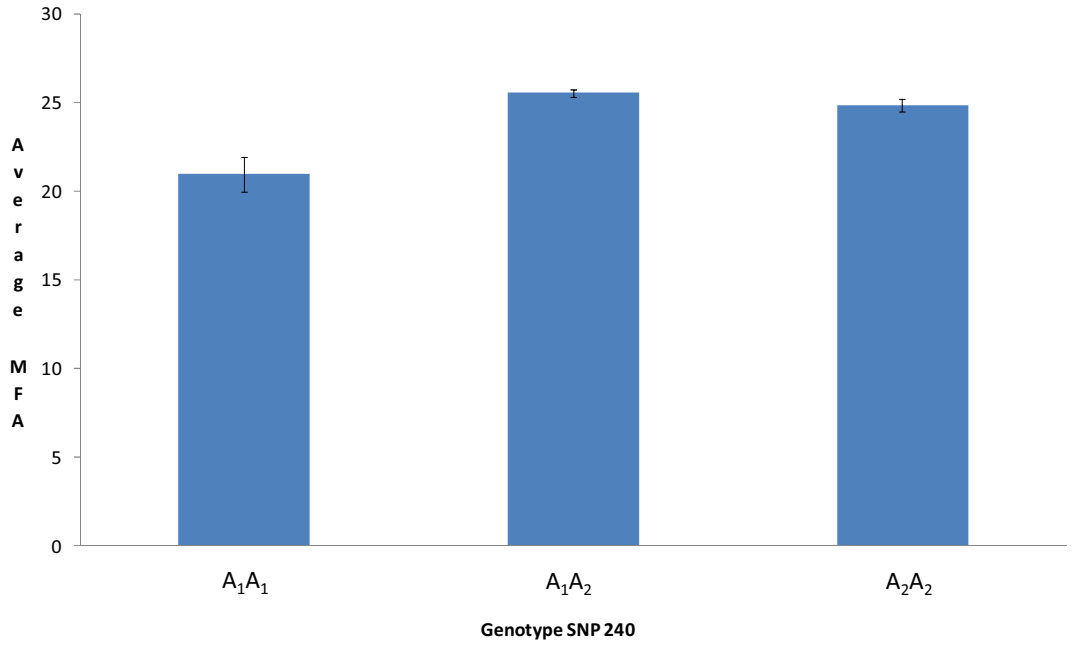

Figure 39. Average microfibril angle (MFA) for three genotypes at SNP 240.

The percent variation explained by additive effects of the 26 significant SNPs ranged from 0.04% to 6.31% with a mean of 1.65%. Eighteen significant SNPs showed correspondence between microfibril angle and modulus of elasticity suggesting possible pleiotropy of this locus. Significant SNP associations were also detected for average modulus of elasticity

(Table 22). Twenty-nine SNPs had significant additive effects, but of these, only one SNP also had a significant dominance effect. The percent variation explained by additive effects of these 29 SNPs ranged from 0.45% to 8.23% with a mean of 1.53%. Twelve SNPs showed significant additive effects associated with pith-to-bark core length, and 1/3 of these also had significant dominance effects. The percent variation in core length explained by additive effects of SNPs was low and ranged from 0.01% to 1.65% with a mean of 0.73%.

Table 22. SNPs with significant additive effects ($p \leq 0.05$) for MoE averaged over rings 4-7 from single-SNP analyses.

SNP ID	Freq of A1 (p)	a effect	d effect	Va-snp	Vd-snp	Va	Ve	Vp	Va-snp/Vp
6	0.56	0.303	-0.02	0.046	0	2.621	1.374	4.041	1.14%
13	0.467	0.282	-0.006	0.039	0	3.706	0.539	4.284	0.92%
52	0.611	0.267	0.191	0.024	0.008	2.624	1.449	4.106	0.59%
55	0.298	0.579	-0.551	0.053	0.053	1.914	2.49	4.51	1.17%
75	0.859	0.569	0.5	0.011	0.015	2.655	1.368	4.049	0.26%
85	0.657	0.411	0	0.076	0	2.554	1.555	4.185	1.82%
90	0.491	0.341	-0.298	0.056	0.022	2.547	1.636	4.261	1.32%
119	0.566	0.29	-0.135	0.047	0.004	2.542	1.382	3.975	1.17%
133	0.52	0.296	-0.262	0.047	0.017	2.507	1.495	4.065	1.15%
176	0.327	0.443	-0.024	0.083	0	2.503	1.389	3.975	2.09%
179	0.613	0.369	-0.098	0.073	0.002	2.831	1.21	4.116	1.77%
197	0.719	0.595	0.416	0.069	0.028	2.628	1.393	4.118	1.67%
212	0.372	0.305	-0.186	0.031	0.008	2.96	1.141	4.139	0.75%
238	0.48	0.325	-0.286	0.049	0.02	2.423	1.591	4.084	1.21%
240	0.375	1.276	-1.444	0.392	0.458	2.691	1.228	4.769	8.23%
247	0.371	0.362	0.162	0.076	0.006	2.648	1.496	4.226	1.80%
270	0.465	0.371	0.052	0.07	0.001	2.53	1.473	4.074	1.72%
278	0.528	0.265	-0.003	0.035	0	2.732	1.332	4.099	0.86%
280	0.705	0.295	-0.082	0.045	0.001	2.841	1.321	4.208	1.07%
307	0.637	0.324	-0.012	0.049	0	2.698	1.476	4.223	1.17%
308	0.657	0.297	-0.186	0.057	0.007	2.023	1.663	3.75	1.52%
318	0.549	0.392	-0.198	0.084	0.01	2.919	1.102	4.115	2.04%
335	0.747	0.805	0.428	0.133	0.026	2.482	1.598	4.239	3.14%
338	0.054	1.316	-1.066	0.014	0.012	2.882	1.061	3.969	0.35%
342	0.307	0.509	-0.315	0.064	0.018	2.587	1.416	4.085	1.56%
373	0.417	0.253	-0.357	0.018	0.03	2.945	1.109	4.103	0.45%
377	0.338	0.346	-0.139	0.04	0.004	2.476	1.393	3.913	1.03%
382	0.1	0.99	-0.591	0.049	0.011	2.658	1.279	3.997	1.21%
383	0.699	0.347	-0.021	0.053	0	2.689	1.253	3.993	1.33%

Efficiency of early SNP-aided selection for MoE was analysed for radiata pine using the MoE data. The required proportion of genetic variances accounted for by SNPs for the same efficiency as selection directly on MoE at early age (age 6) for a rotation-aged MoE is presented in Figure 40. The estimated heritability for MoE was 0.5 at early age (Wu *et al*. 2007) and the genetic correlation between an early age (age 6) and rotation age (age 30) was 0.8 (Wu *et al.* 2008). Therefore, only SNPs that accounted for 32% or more genetic variance for rotation-aged MoE can have the same or more efficiency as early selection based on MoE of young trees for the target trait (rotation-aged MoE). We observed that selection based on individual SNPs will not have the same efficiency as selection based on early MoE

measurement since individual effects of SNPs for MoE was equal to or below 8.23%. If the effects of all 29 SNPs for MoE in Table 22 were real without estimation error and were independent for genetic variation (no collineality), then selection based on the cumulated genetic variation of the 29 SNPs (44.5%) would be more efficient than selection based on MoE of young trees alone.

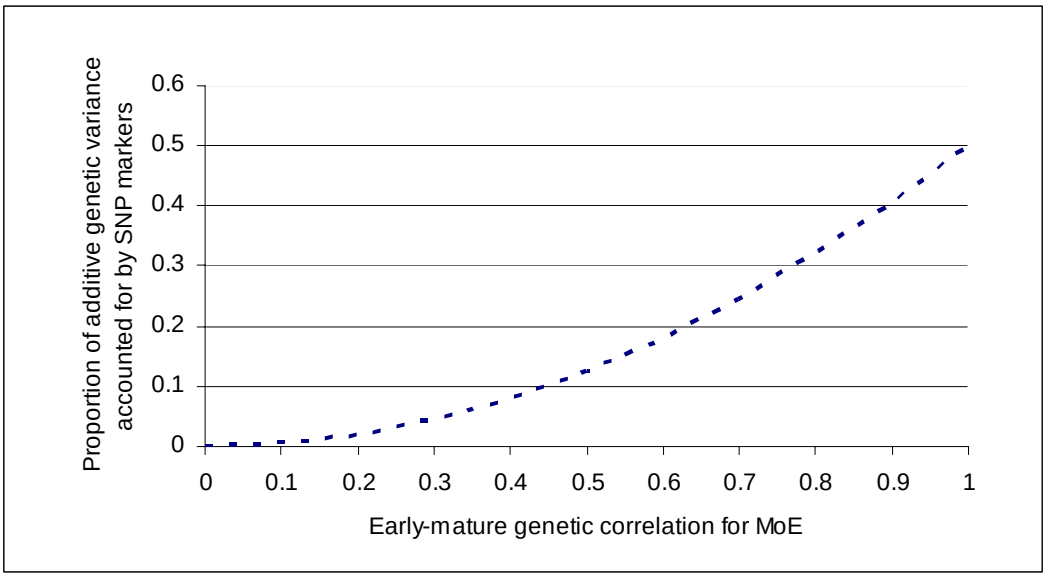

Figure 40. Threshold for the proportion of additive genetic variance accounted for by SNPs for MoE so that selection based on SNPs alone is more efficient than selection based on MoE at early age (age 6) with the estimated heritability of 0.50.

The preferred way to use SNP markers is to incorporate SNP markers for mature traits into an early selection index. The relationships between gain and genetic parameters are illustrated in Figures 41 and 42.

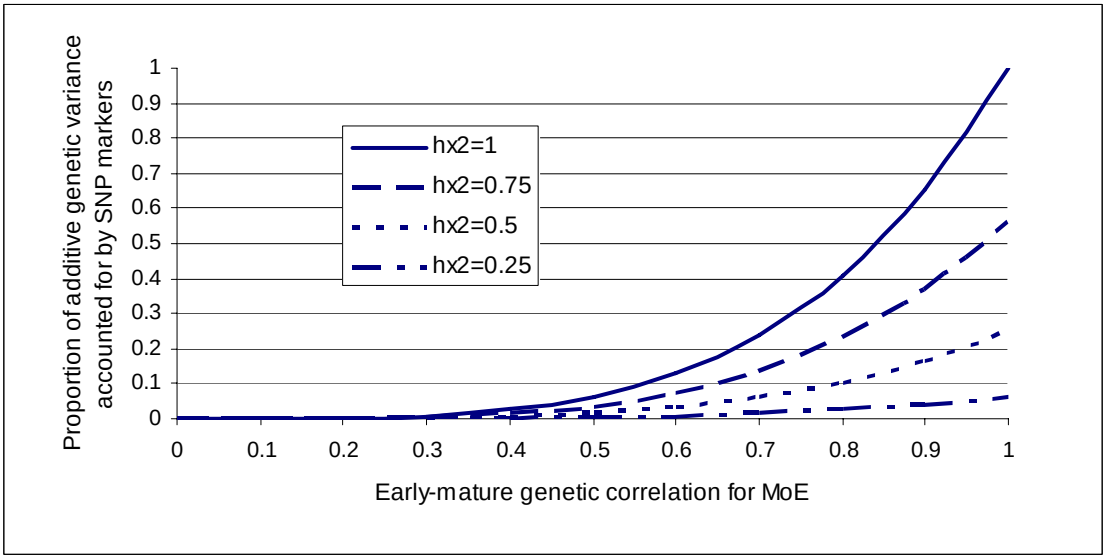

Figure 41. Threshold for the proportion of additive genetic variance accounted for by SNPs so that SNP-aided early selection is more efficient than selection based on a single MoE trait at early age alone under four heritbilities of MoE

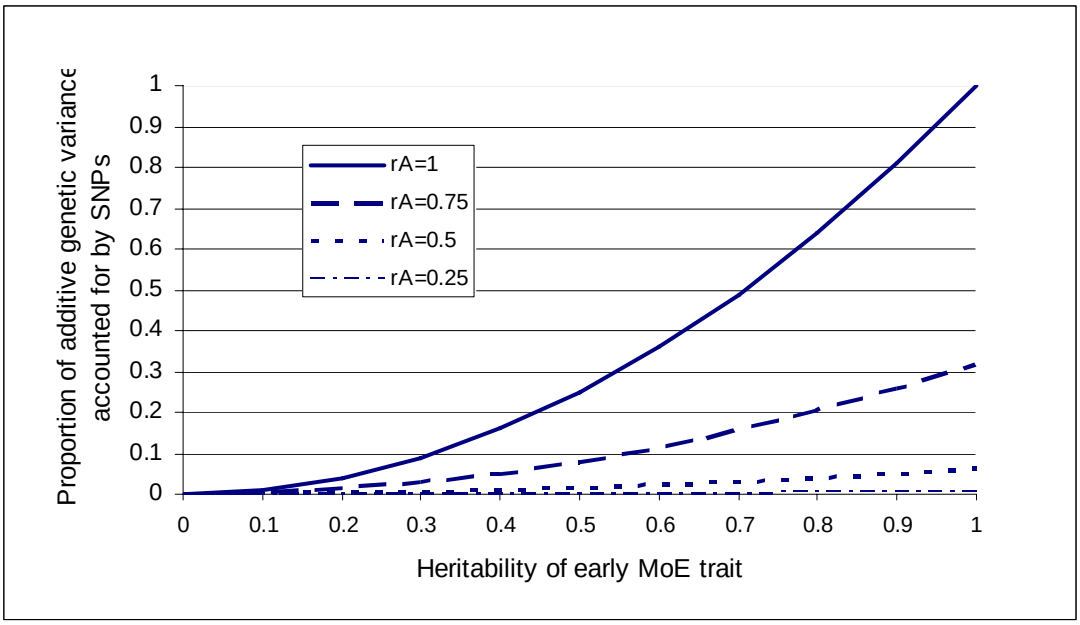

Figure 42. Threshold for the proportion of additive genetic variance accounted for by SNPs so that SNP-aided early selection is more efficient than selection based on a single MoE trait alone at early age under four early-mature genetic correlations for MoE.

The required proportion of genetic variance accounted for by SNPs in order for SNP-aided early selection to be more efficient than selection based on a single early MoE trait alone is illustrated for four heritabilities of MoE at early age (Figure 41) (h_x^2=0.25. 0.5. 0.75, 1) and for four levels of early-mature genetic correlations for MoE (Figure 42) (r_A=0.25, 0.5, 0.75, 1). For our early selection for stiffness (MoE) at rotation, if SNPs could account for 10.24% or more of genetic variation for stiffness at rotation age, then SNP-aided early selection could be more efficient than selection based on MoE at early age. Therefore, for more efficient SNP-aided early selection for stiffness, SNP that accounts for more than 10.24% of independent genetic effects for rotation aged MoE are preferred. More detailed results can be found in Appendix 10 (Incorporating single nucleotide polymorphisms with quantitative genetic data and SNP-aided selection).

7.3 Optimal selection strategy

Index selection with optimal economic breeding objectives drives profitability for multiple-trait breeding programs (Ivković *et al.* 2006). Selection indices used in this study were based on juvenile selection traits (i.e. DBH and MoE), and the measure of efficiency of index selection was profitability, rather than the genetic responses of individual traits. Therefore, genetic responses for individual traits could be favourable or unfavourable under such selection scenarios. Selection scenario A (selection index with economic weights) was the most optimal with profitability of A$2409/ha/yr (53.1% gain), based on juvenile genetic parameters from this study. However, there was a -3.7% and -5.3% decrease in juvenile wood density (DEN) and MoE, respectively (Table 23). The small reduction in MoE is counterbalanced by the larger increase in DBH growth. The responses in other juvenile wood properties were also unfavourable while for growth rate the genetic response was positive (6.8%).

When MoE was held constant by applying the restricted selection index (scenario B, restricted index), there was a slight decrease in the genetic response in DBH compared to scenario A. Although this decrease was small, there were less unfavorable responses in other wood properties. For example, MoE increased relative to scenario A, whereas, MFA decreased (favorable for stiffness). By using index selection only within the genotypes with positive breeding values for both MoE and DBH (scenario C, correlation breakers), there was an 11% increase in genetic response in MoE compared to scenario A (Table 23).

Table 23. Predicted genetic responses and percentage gain at 10% selection intensity in juvenile growth and wood quality traits and net present value profitability (breeding objective response) for index selection using genetic parameters determined in this study. Three different scenarios were considered: A) Index selection using modulus of elasticity (MoE) and diameter (DBH) as selection traits and maximising profitability; B) Restricted index selection keeping juvenile wood MoE constant; C) Restricted index selection among the genotypes with positive breeding values for both MoE and DBH.

Predicted Genetic Response								
Selection Scenario		**DEN** Kg/m3	**DBH** mm	**MoE** GPa	**MFA** deg	**SG** deg	**LSH** %	**NPV** A$/ha
Index with economic weights	**gain**	**-13**	**10.7**	**-0.25**	**0.56**	**0.37**	**0.06**	**2,409**
	%	**(-3.7%)**	**6.8%**	**(-5.3%)**	**1.9%**	**9.4%**	**4.4%**	**53.1%**
Restricted Index	**gain**	**-10.37**	**10.44**	**-0.04**	**-0.2**	**0.18**	**0.05**	**2,282**
	%	**-2.9%**	**6.6%**	**(-0.5%)**	**(-0.7%)**	**4.5%**	**3.9%**	**50.3%**
Correlation breakers	**gain**	**-3.51**	**7.02**	**0.29**	**-1.29**	**-0.16**	**0.02**	**1498**
	%	**(-1.0%)**	**4.5%**	**6.00%**	**(-4.4%)**	**(-4.1%)**	**1.8%**	**33.0%**

However, there was a reduction in the production system profitability for alternative scenarios (B and C) compared with index selection scenario A. For example, if the response in juvenile MoE was restricted to 0 (no further increase from current levels), the index value expressed as per hectare net present value (NPV) profit of an integrated radiata pine production system decreased from A$2409 to A$2282 at 10% selection intensity. Similarly, if the selections were made only from the genotypes with positive breeding values for both growth and MoE, profitability decreased from A$2409 to A$1498. From a purely biological perspective, selection scenario B and C would be preferred since scenario C improves both MoE and growth rate, while scenario B improves DBH and maintains MoE at current levels. However, from the economic perspective of enterprises, scenarios B and C were less advantageous than scenario A, even though in scenario A, juvenile wood quality traits showed unfavorable responses. For detailed description and exploration of selection strategies, see Appendix 11 (Genetic correlations among juvenile wood quality and growth traits and implications for selection strategy in *Pinus radiata* D. Don).

SUMMARY

The Juvenile Wood Initiative project has made significant progress in understanding of genetic control of wood formation and interrelationship among wood traits. These new progresses were summarized in the 15 significant scientific findings and several industry adoptions which were documented in ten client technical reports to funding organizations and industry clients and in 16 scientific manuscripts (8 published, 1 in press, 2 submitted, and others in process of submission), and 15 conference papers or presentations. Primary scientific findings, industry adoptions, and technical and scientific reports are summarized below.

Scientific Findings

The 15 primary scientific findings related to wood science, inheritance and genetic bases of juvenile wood traits are summarised below.

1. The best method to predict stiffness of standing tree in slash/Caribbean pine: use gravimetric basic density from 12 mm increment cores combined with a standing tree prediction of MoE using a time of flight acoustic tool. This was the most accurate and cheapest way to rank trees for breeding selection for slash/Caribbean hybrid pine. This method was also recommended for radiata pine.
2. Wood density breeding values were predicted in the first time for STBA breeding population using a large sample of 7,078 trees (increment cores) and it was estimated that selection of the best 250 trees for deployment will produce wood density gain of 12.4%.
3. Large genetic variation for a suite of wood quality traits including density, MFA, spiral grain, shrinkage, acoustic and non-acoustic stiffness (MoE) for clear wood and standing trees were observed. Genetic gains between 8 and 49% were predicted for these wood quality traits with selection intensity between 1 to 10% for radiata pine.
4. Site had a major effect on juvenile-mature wood transition age and effect of selective breeding for a shorter juvenile wood formation phase was only moderate (about 10% genetic gain with 10% selection intensity, equivalent to about 2 years reduction of juvenile wood).
5. The study found no usable site by genotype interactions for wood quality traits of density, MFA and MoE for both radiata and slash/Caribbean pines, suggesting that assessment of wood properties on one or two sites will provide reliable estimates of the genetic worth of individuals for use in future breeding.
6. There was significant and sizable genotype by environment interactions between the Mainland and Tasmania regions and within Tasmania for DBH and branch size.
7. Strong genetic correlations between rings for density, MFA and MoE for both radiata and slash/Caribbean pines were observed. This suggests that selection for improved wood properties in the innermost rings would also result in improvement in wood properties in the subsequent rings, as well as improved average performance of the entire core.
8. Strong genetic correlations between pure species and hybrid performance for each of the wood quality traits were observed in the hybrid pines. Parental performance can be used to identify the hybrid families which are most likely to have superior juvenile wood properties of the slash/Caribbean F1 hybrid in southeast Queensland.
9. Large unfavourable genetic correlations between growth and wood quality traits were a prominent feature in radiata pine, indicating overcoming this unfavourable genetic correlation will be a major technical issue in progressing radiata pine breeding.

10. The project created the first radiata pine 18 k cDNA microarray and assembled xylogenesis 5,952 ESTs and found 3,304 unigenes for radiata pine.
11. A total of 348 genes were identified as preferentially expressed genes in earlywood or latewood while a total of 168 genes were identified as preferentially expressed genes in juvenile or mature wood.
12. Juvenile earlywood has a distinct transcriptome relative to other wood development stages.
13. Discovered rapid decay of linkage disequilibrium (LD) in radiata pine and LD decayed to approximately 50% within 1700 bp (within a typical gene). A total of 913 SNPS from sequencing 177,380 base pairs were selected for association genetic studies.
14. 149 SNPs from 44 genes and 255 SNPs from remaining 51 genes (total 95 genes) were studied for association with 62 wood traits, and 30 SNPs were shortlisted for their significant association with variation of wood quality traits (density, MFA and MoE) with individual significant SNPs accounting for between 1.9 and 9.7 percent of the total genetic variation in traits.
15. Index selection using breeding objective was the most profitable selection method for radiata pine in dealing with negative genetic correlation between wood volume and quality traits.

Industry Adoption and Impact

1. The breeding values for the 7,078 trees for STBA breeding population for deployment and breeding purpose in the third generation breeding were adopted since 2004 with expected gain of 12.4% in wood density.
2. The best log stiffness predictor for salsh/Caribean pine and radiata pine (combining gravimetric basic density of 12 mm increment cores with time of flight acoustic wave on a standing tree) was adopted by STBA and FPQ.
3. Breeding values of standing tree stiffness and dynamic and static MoE for more than 4,000 trees estimated from the STBA trials and were integrated with TreePlan data base and selection index.
4. The estimated net present value was more than $A400 million for selection from progeny of the second generation to third generation of breeding population for STBA members and more than $A800 million for the entire radiata pine plantation estate using genetic results from JWI in combination with results from breeding objective project.
5. CSIRO and STBA are leading wood quality genetics research, through (1) initiating a joint IUFRO Southern Pine and inaugural Australasia Forest Genetic Conference dedicated to "Breeding for Wood Quality) in 2007 and (2) visits by overseas scientists including Dr John Mackay, Dr Alvin Yanchuk from Canada and upcoming visits from Dr Leopoldo Sanchez, and Dr John Russell from France and Canada for collaboration, and (3) invitations for presentation to international conference including a Keynote speech in the 2008 IUFRO Genetics Conference for Wood Quality Breeding in Quebec, Canada.

Client Technical Reports:

1. Juvenile Wood Initiative: Genetic Control of Juvenile-Mature Wood Transition and Acoustic Methods to Predict Standing Tree Stiffness in Radiata Pine. (FWPA and STBA).
2. Juvenile Wood Initiative: Genetic Control of Juvenile Wood Properties in Slash and Caribbean Pines and their F1 Hybrid, as Determined by SilviScan. (FWPA and FPQ).

3. Juvenile Wood Initiative: Juvenile Wood Index and the Best MoE Measurement Method for Use in Assessment of Slash x Caribbean Hybrid Pine. (FWPA and FPQ).
4. Juvenile Wood Initiative: Genetic Control of Juvenile Wood Properties in Radiata Pine, as Determined by SilviScan. (FWPA and STBA).
5. Juvenile Wood Initiative: Gene Discovery of Juvenile Wood Formation of *Pinus radiata* Carr. (FWPA, STBA and ArborGen).
6. Juvenile Wood Initiative: Prediction of Wood Stiffness, Strength, and Shrinkage in Juvenile Wood of Radiata Pine: Juvenile Wood Index. (FWPA and STBA).
7. Juvenile Wood Initiative: SNP discovery and Association Genetics in Juvenile Wood of Radiata Pine (FWPA and STBA).
8. Juvenile Wood Initiative: Genotype by Environmental Interaction for DBH, Wood Density, Branch Angle, Branch Size, and Stem Straightness in Eight Young *Pinus radiata* D. Don Trials in Australia (FWPA and STBA).
9. Juvenile Wood Initiative: Incorporating Single Nucleotide Polymorphisms with Quantitative Genetic Data and SNP-aided Selection (FWPA and STBA).
10. Juvenile Wood Initiative: Genetic Mapping of Candidate Genes for Vascular Development in *Pinus radiata* D. Don (FWPA and STBA).

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Juvenile Wood Initiative:

Genetic Control of Juvenile-Mature Wood Transition and Acoustic Methods to Predict Standing Tree Stiffness in Radiata Pine

Washington, J. Gapare, A. Colin Matheson, Aljoy

Abarquez, and Harry X. Wu

June *2006*

COMMERCIAL IN CONFIDENCE

CSIRO Forestry and Forest Products
PO Box E4008
Kingston ACT 2604 AUSTRALIA
Telephone: 02 62818211 Fax: 02 62818312

Executive Summary

Juvenile wood has undesirable properties such low density, low stiffness. Juvenile wood quality may be improved by breeding for an early transition age from juvenile to mature wood and/or for increased stiffness of juvenile wood. The number of years a tree produces juvenile wood at a fixed height can be defined by the age of the wood at which properties change from juvenile to mature wood. Wood stiffness is an important characteristic of radiata pine for structural products. To select high stiffness radiata pine for breeding purpose, rapid, inexpensive methods for measuring wood stiffness are desirable. This report is in two parts – the first part reports on the genetic control of transition from juvenile to mature wood in radiata pine. The second part reports on the inheritance and genetic gain in wood stiffness measured acoustically in young standing trees.

The genetic control of transition from juvenile to mature wood in radiata pine was investigated using wood samples from two 16-year old Australia-Wide Diallel (AWD) radiata pine tests and two-28 year-old open-pollinated (OP) progeny tests for wood density. Iterative solution and segmented regression approach were used to estimate the age of transition from juvenile to mature wood for pith-to-bark profiles of ring density, earlywood density, latewood density and percent latewood density. Precisely determining the transition age between juvenile and mature wood was difficult because transition is gradual. Latewood density was the only variable that could be readily divided into juvenile and mature wood.

Transition age varied between sites in Gippsland, Victoria (mean = 7.5 years) and at Tantanoola in Green Triangle, South Australia (mean = 12.6 years). This finding suggests that site has a major effect on juvenile-mature transition in radiata pine. We detected moderate levels of genetic control in latewood density transition age that would allow for selective breeding for a shorter juvenile wood formation phase. Assuming a selection intensity of one in ten, genetic gains of up to 10% per breeding cycle are possible and would be equivalent to shortening the juvenile wood formation phase by almost 2 years.

To investigate the inheritance and genetic gain in wood stiffness in young standing trees, a preliminary study evaluated 5 various acoustic and non-acoustic methods for determining optimal method for measuring stiffness of standing young trees. The preliminary study suggested that the IML hammer was the most cost-effective instrument in acoustic testing. Time of flight was recorded in standing trees in two progeny trials, one in eastern Victoria (Flynn) aged 7 years and the other in South Australia (Kromelite) aged 6 years. Average time-of-flight at Kromelite was higher than at Flynn, (519 µs compared to 463µs) which corresponds to 3.71 GPa and 4.67 GPa for MoE, respectively. We observed positive genetic correlation between stem diameter at breast height (DBH) and time-of-flight, suggesting that tree size and wood stiffness are negatively genetically correlated. Heritability for time-of-flight was higher at Flynn ($h^2 = 0.67$) than at Kromelite ($h^2 = 0.44$). Selection of the best 10% for time-of-flight based on pooled data would result in 21% genetic gain. Selection for correlation breakers between stiffness and growth would be effective for the Australian radiata pine breeding population used.

PART I:

Genetic Control of Juvenile-Mature Wood Transition in Radiata Pine

Introduction

Selective breeding in *Pinus radiata* D. Don for the first two generations has produced more than 30% improvement in growth rate with substantial benefits from the increased volume of harvested wood in pine plantations in Australia (Wright *et al*. 1985; Matheson *et al*. 1986; Wu and Matheson, 2002), with substantial benefits from the increased volume of harvested wood. A major concern for faster-grown radiata pines is the occurrence of higher proportion of juvenile wood in the logs partly due to reduced rotation age. The typical rotation age for radiata pine has been shortened from 40-45 years to 25-30 years throughout much of the radiata plantation in Australia. Because of this, juvenile wood now accounts for a much higher proportion of harvested wood than it has in the past. Juvenile wood in fast growing conifers has lower density and strength than mature wood (Cown, 1992, Burdon *et al.* 1992). Thus, there is a need for a genetic study to determine the time of transition from juvenile to mature wood and quantify genetic control in time of transition from juvenile to mature wood.

Juvenile wood formed near the pith throughout the trunk of a tree can have different properties from wood produced in the outer rings, termed mature wood. However, definition of juvenile wood can be difficult and is to some degree subjective; it is often described in terms of the number of rings from the pith (Cown, 1992). The demarcation boundary in the stem between juvenile and mature wood is diffuse and the region where one type of wood starts and the other stops, frequently referred to as transition wood (Zobel and Sprague, 1998). Generally, transition from juvenile to mature wood occurs gradually over two to five growth rings depending on the wood property Zobel and Jett, 1995). Harris and Cown (1991) describe juvenile wood in radiata pine as the first 10 growth rings from the pith, mainly on the basis of the known variation in wood density and outer wood properties. As such, varying criteria have been used to delineate juvenile and mature wood.

Juvenile wood quality can be improved through breeding or through silvicultural management. Improvement by breeding has become a priority along with growth rate for the third-generation selections of radiata pine in Australia. Log quality may be improved through reduction of juvenile wood and increasing the stiffness of juvenile wood (Nicholls *et al*. 1980; Vargas-Hernandez and Adams, 1991). It is well understood that the stiffness of juvenile wood in radiata pine can be improved through breeding, either through improvement of MoE or other component traits such as wood density and microfibril angle (Matheson and Dungey, 2004). Numerous studies on inheritance of wood quality in radiata pine have shown high heritabilities for wood density, and other stiffness related traits such as microfibril angle (Wu and Matheson, 2002; Wu *et al*. 2004a; Wu *et al.* 2004b). With the invention of new wood technologies for measuring wood properties such as SilviScan® (Evans *et al*. 1996), and acoustic tools, e.g., IML® hammer, TreeTap®, Director ST300® (Tsehaye *et al*. 2000; Carter and Lausberg, 2001; Andrews, 2004), estimation of inheritance on MoE

and stiffness became possible. There is considerable genetic variation for MoE in radiata pine and selection would be effective (Wu *et al*. 2004a; Matheson and Dungey, 2004).

In addition to breeding trees with improved juvenile wood properties, it may be possible to breed for an early transition age from juvenile wood to mature wood. We define transition age as the age at which the transition from juvenile wood to mature wood is completed, leading to a stable wood density in growth rings. Reducing the volume of juvenile wood would increase the overall wood density and the quality of certain wood products (Szymanski and Tauer, 1991; Zamudio *et al*. 2005). Thus, reducing the proportion of low density wood by selecting for a shorter juvenile wood formation phase is an attractive option for improving wood quality.

To use transition age as a selection criterion for improving wood quality in radiata pine, an understanding of the genetic variability of transition age is critical. Few studies have examined the genetic mechanisms influencing transition age in fast growing pines. For example, Loo *et al*. (1985) reported a family heritability estimate for wood specific gravity transition-age of 0.36 in *Pinus taeda* and suggested moderate gains (earlier transition ages) could be obtained by selecting on a family mean basis. For *Pinus elliottii*, Hodge and Purnell (1993) reported an average transition age for ring density, latewood density, and latewood percentage of 9.4, 7.5 and 10.3 years, respectively; and the heritability of these traits ranged from 0.16 to 0.22. Genetic control of earlywood density, latewood density and latewood percentage in radiata pine is moderately heritable (Zobel and Jett, 1995; Kumar, 2002). However, if we can ascertain the genetic control in time of transition from juvenile to mature wood in radiata pine, there may be an opportunity to select for a reduction in transition age and therefore decrease the proportion of the log containing juvenile wood.

A prelude to an accurate estimation of the proportion of juvenile wood in a tree is to be able to detect the boundary between juvenile and mature wood. Several traits (e.g., fiber length, microfibril angle, ring density, latewood density) have been used to determine the point of demarcation between juvenile and mature wood (Loo et al. 1985; Hodge and Purnell, 1993; Abdel-Gadir and Krahmer, 1993). However, the issue is complicated by the fact that the transition point from juvenile to mature wood varies with the trait under investigation (Bendtsen and Senft, 1986). Several methods have been proposed to estimate transition age, including mathematical approaches such as the Gompertz function, iterative and constrained solutions and segmented regression techniques (Abdel-Gadir and Krahmer, 1993, Loo *et al*. 1985; Szymanski and Tauer, 1991).

The objectives of this study on radiata pine were to: 1) estimate age of transition from juvenile to mature wood; 2) quantify genetic control in time of transition from juvenile to mature wood; and 3) explore the possibility of selection for a shorter juvenile wood formation phase.

Materials and methods

Sample origin and sampling procedures

APPENDIX 1

Table 1 provides general information about the field trials examined in this study. Two half-diallel trials comprised families from seven sets of 16-years old 6 x 6 half-diallels in the Gippsland region, Victoria. The half-diallel tests were part of Australia-Wide-Diallel (AWD) tests originally designed to provide reliable estimates of GCA (General Combining Ability) and SCA (Specific Combining Ability) for radiata pine in Australia. Details of the Australia-Wide-Diallel program are given in Wu and Matheson (2002). Summary results on growth and form traits are summarised in Wu and Matheson (2002). VRC52 was planted in 1986 at Flynn with 100 families of which 13 were controls and four tree plot in four replications. VRC54 was planted at Silver Creek in 1986 with 52 families of which 4 were controls and single tree plot in 20 replications. The average number of crosses per full-sub family was five. However, there were less than 15 crosses common across the two trials. There were differences in previous land-use between the two sites: VRC54 is a second-rotation site and VRC52 is an ex-pasture first-rotation site. Ex-pasture sites are usually more fertile than second-rotation sites (Wu and Matheson, 2002).

Table 1. Site characteristics, details of families, number of blocks sampled, sample size of wood disks and trial age for juvenile-mature wood transition study for four *Pinus radiata* progeny trials in Australia.

Trial	VRC52	VRC54	PT47	PT5042
Site	Flynn Creek-Diallel	Silver Creek-Diallel	Flynn Creek-OP	Tantanoola-OP
Year of planting	1986	1986	1969	1971
Age at harvest (years)	16	16	31	27
Latitude	38^0 14’ S	38^0 15’ S	38^0 12’ S	37^0 29’ S
Longitude	146^0 41’ E	146^0 39’ E	146^0 40’ E	140^0 40’ E
Elevation	93	184	100	62
Mean annual rainfall (mm)	760	785	760	900
Soil type	Sandy loam	Sandy loam	Sandy loam	Sandy c.l.
Site type[a]	Ex-pasture	2nd PR	Ex-pasture	2nd PR
Family structure	Full-sib	Full-sib	OP	OP
No. families	100	52	36	33
No. blocks sampled	3	6	3	6
No. trees/ family sampled	6	6	18	22
No. trees sampled	600	312	648	780

[a] 2nd PR – second rotation of radiata pine crop

The second two trials comprised 30 open-pollinated families excluding controls. The field designs of the open-pollinated trials were randomized complete blocks with 10-tree row-plots in 6 replications planted in 1971 at Tantanoola, South Australia (PT5042) and harvested at age 27 years and a 2x3 tree plots in 9 blocks planted in 1969 at Flynn, Gippsland, Victoria (PT47) and harvested at age 31 years. Soils at PT47 are characterised as sandy loam whereas at PT5042, they are characterized as sandy clay loam.

Variable sampling strategies were applied depending on trial design, number of blocks, families and trees per family. At VRC52, wood disks at breast height (1.3

meters) were collected from two trees per plot from the first three blocks, giving a sample size of 600, using a systematic approach, i.e., sampling trees 2 and 4 in every plot. In VRC54, the single tree per plot was sampled from the first 6 blocks and giving the overall sample size of 312 (Table 1). At PT47 trial, two trees were selected from each of 6-tree plots and harvested at age 31 years from planting, giving a sample size of 648. At PT5042, three out of 10 trees per plot were sampled at age 27 years, in three blocks and all trees were sampled in the remaining three blocks, giving a sample size of 780. A total of 2340 cross-sectional disks were collected at the four sites, but only 1904 samples were used after exclusion of control lots and several dozen disks that had incomplete outside rings.

Sample preparation
From the wood discs, bark-to-bark-through-pith flitches of 2 mm thick were prepared using a specially designed electric twin-blade saw. In order to obtain density values that are not an overestimate in the juvenile wood section (initial growth rings from the pith), it was necessary to extract resins from the samples in which heartwood was well developed and highly resinous, particularly in the first three growth rings. Absolute value of optimally determined density may be an overestimate if resin is not extracted (Nyakuengama, 1997). Resin was extracted by boiling the samples in acetone for 24 hours and the samples were air-dried to 10% moisture content.

Wood density measurement
Wood density of 2 mm flitch was measured using X-Ray densitometry and WinDENDRO software package (Regent Instruments Inc, 2001). Wood density was measured from pith to bark of the two radii. The average density of the two radii was taken to represent a sample tree. Densitometry readings were calibrated with samples of known wood density. Comparisons of gravimetric density of X-ray samples with density determined by direct scanning densitometry by SilviScan® gave R^2 =0.94. In this study, wood density is expressed in g cm^{-3}. Using the indirect reading X-Ray densitometer (Polge, 1963), the samples were scanned to determine the basic wood density (oven-dry weight / green volume) for each ring from pith to bark. The first and last annual rings of each sample were rejected because they were too narrow for densitometry analysis.

For each annual ring, earlywood and latewood boundary was delineated. The boundary between earlywood and latewood was defined as the point within a growth ring at which positive slope of the densitometric trace exceeds 50%. In most cases this boundary coincided almost exactly with the midpoint between average earlywood density and average latewood density of the ring. Ring and latewood boundaries assigned automatically by WinDENDRO were edited to remove false rings and other obvious aberrations. The data obtained and processed, consist of average values for each growth ring; ring width-RW, ring density-RD, minimum density-MINDEN, maximum density-MAXDEN earlywood density-EWD, latewood density-LWD and latewood percentage-LWP.

Determination of transition age
Three transition ages were statistically determined by using three interrelated approaches: (i) iterative solution, (ii) segmented regression/model, and (iii) constrained solution.

A. Iterative solution

An iterative solution approach has been used to determine the age of transition from juvenile to mature wood (Loo *et al.* 1985; Tasissa and Burkhart, 1998). It is assumed that the radial development of latewood density from pith-to-bark can be described by two functions/models, one for the steep slope over the first years beginning at the pith (juvenile wood) and the second for the later part of the curve (mature wood). These models characterize change of slope in the radial density trend. In this approach, the data are divided into two parts using one of the ages as the transition age, a linear regression is fitted, and the mean-squared error is estimated. From sequential linear regression, the transition age with minimum mean-squared error was considered to be the approximate transition age. The fitted regression model takes form:

$$Y_i = \beta_0 + \beta_1 X_i + \beta_2 (X_i - \alpha) I + \epsilon_i \qquad (1)$$

where

Y_i is the independent variable for wood property,

X_i is ring number,

α is the ring number at which wood changes from juvenile to mature wood,

I is an indicator variable where $I = 1$ if $X_i - \alpha \geq 0$, or $I = 0$ otherwise,

β_0 is the intercept of the line of the juvenile wood

B_i are regression coefficients, and

ϵ_i 's are errors.

If α was known, Eq. (1) is a linear regression, and its parameters (B_i 's) is estimated using piece-wise linear regression (Tasissa and Burkhart, 1998). Thus for $X_i \leq \alpha$ (juvenile wood),

$$E(Y_i) = \beta_0 + \beta_1 X_i \qquad (2)$$

And for $X_i \geq \alpha$ (mature wood),

$$\begin{aligned} E(Y_i) &= \beta_0 + \beta_1 X_i + \beta_2 (X_i - \alpha) \\ &= (\beta_0 - \beta_2 \alpha) + (\beta_1 + \beta_2) X_i \\ &= \gamma_0 + \gamma_1 X_i \end{aligned} \qquad (3)$$

In this formulation, the two regression lines are continuous at the join point (α). Consequently, at the boundary Eq. (2) yields $Y_i = \beta_0 + \beta_1 \alpha$, and Eq. (3) becomes $(\beta_0 - \beta_2 \alpha) + (\beta_1 + \beta_2)\, \alpha = \beta_0 + \beta_1 \alpha$.

B. Segmented regression analysis

When the joint point (or transition point - α) in Eq. (1) is unknown, the problem becomes nonlinear and has to be estimated along with the regression parameters. With segmented regression, a statistical model (Eq 1), can simultaneously estimate parameters of the two curves and a breakpoint between juvenile and mature wood (Abdel-Gadir and Krahmer, 1993). A solution can be directly obtained by using nonlinear least squares procedures (PROC NLIN) in SAS (SAS Institute Inc, 1990) with the transition age being the ring number which minimizes the mean squared error. Since the transition age is unknown, the least squares procedure in SAS (SAS Institute Inc, 1990) was used to obtain estimates of the regression parameters and the transition age (join point).

The fitted regression model takes the form:

$$Y_i = \beta_0 + \beta_1 X_i + \beta_2 (X_i - \alpha) I + \epsilon_i \qquad (4)$$

where

Y_i is the independent variable for wood property,

X_i is ring number,

α is the ring number at which wood changes from juvenile to mature wood,
I is an indicator variable where I =1 if $X_i - \alpha \geq 0$, or I =0 otherwise,
β_0 is the intercept of the line of the juvenile wood
β_1 are regression coefficients, and
ϵ_i 's are errors.

In order to use iterative and segmented regression approaches to determine transition age, the pith-to-bark profiles of the six density related variables (ring width-RW, ring density-RD, minimum density-MINDEN, maximum density-MAXDEN, earlywood density-EWD, latewood density-LWD, and latewood percentage-LWP were plotted using the GPLOT procedure (SAS Institute Inc, 1990). Preliminary analyses indicated that ring width-RW, ring density-RD, minimum density-MINDEN, maximum density-MAXDEN earlywood density-EWD, and latewood percentage-LWP were not suitable for a clear differentiation between juvenile and mature wood. For latewood density, it can be readily divided into juvenile and mature wood and two separate regressions can be reasonably fitted for the whole profile from pith-to-bark of latewood. Therefore, transition age was estimated for latewood density only.

Genetic analyses
Single-site analyses of variance components for diallel data were carried out using an individual tree model. The fitted model was:

$$y = Xb + Z_1a + Z_2s + W_1m + e \qquad (5)$$

where y is the vector of individual tree observations; b is the vector of block fixed effects; a is the vector of random general combining ability (GCA) effects of individual trees; s is the vector of random specific combining ability (SCA) effects due to specific combinations of males and females; m is the vector of random plot effects; e is the vector of random residual deviations of individual trees; X, Z_1, Z_2 and W_1 are incidence matrices relating to the model effects. It is assumed that the random terms are jointly normal with moments:

$$E(a) = E(s) = E(m) = E(e) = 0$$

$$\text{VAR}\begin{bmatrix} a \\ s \\ m \\ e \end{bmatrix} = A\,\sigma^2 a \oplus I\,\sigma^2 s \oplus I\,\sigma^2 m \oplus I\,\sigma^2 e$$

where $\oplus$ is the direct sum of matrices related to the random terms in the model; A is the additive genetic relationship matrix between trees and I is an identity matrix; σ^2a is the additive genetic variance; σ^2s is the variance due to specific combinations of males and females; σ^2m is the plot variance; σ^2e is the residual variance. In the case of open-pollinated data, the s term was dropped from model (5).

Restricted maximum likelihood (REML) estimates of variance components and their standard errors were obtained by using the average information REML algorithm (Gilmour *et al*. 2005). Narrow-sense heritabilities and residual were estimated according to standard formulae (Falconer and Mackay, 1996). The standard errors of the estimated parameters were calculated from variance of ratios, using an approximation based on a Taylor series expansion. The significance of the variance component was determined by the ratio of the component relative to its standard error. If the variance component was more than two standard errors from zero, then the

variance component was considered significant. If the variance component was less than one standard error from zero, then the variance component was considered insignificant. For variance components between 1 or 2 standard error from zero, Likelihood ratio test (LRT) was used to test for any significant differences among the effects (e.g., Gilmour *et al*. 2005). The Log-likelihoods were compared as LRT = $2(\text{Log } L_{p+g} - \text{Log } L_p)$ where Log L_{p+g} is the Log-likelihood with the variance component and $p+g$ degrees of freedom and Log L_p is the Log-likelihood without the variance component with p degrees of freedom. The LRT was distributed as a χ_q^2 with q degrees of freedom.

BLUPs of the additive genetic values (i.e., predicted breeding values, PBV) for individual trees for transition age were predicted from model (5). Individual trees were ranked on PBVs based on early transition age for each trial. 10% of the individuals in each trial were selected to give an indication of the genetic gain expected from selecting individuals having the shortest juvenile wood formation phase.

Results and Discussion

Comparison of statistical methods

The segmented regression analysis yielded decimal transition ages with minimum mean-squared error, whereas in the iterative solution method, integer transition age values were obtained. When the transition age estimates from segmented regression analysis were rounded off to the nearest integer, the methods provided similar results. Although the segmented regression may give a negative slope due to variability of latewood density, its estimated transition ages were similar to the iterative method. We have presented results from segmented regression approach.

The applicability of segmented regression to patterns of intra-ring characteristics is obvious, since all patterns follow two straight line relationships on opposite sides of an undetermined switch point. However, use of this technique requires caution; if the intra-ring density of a particular trait does not show an increase from pith to some maximum value with increasing ring number, then it is not possible to objectively measure transition age. For example, earlywood density profiles showed low variation and curves without increasing or decreasing trends.

Search for a suitable ringwood variable as indicator of juvenile-mature wood transition zone

Previous studies examining transition age from juvenile to mature wood have considered several traits as indicators of juvenile-mature wood transition zone. These include density, microfibril angle, fiber length, lignin/cellulose ratio and latewood density (Abdel-Gadir and Krahmer, 1993; Hodge and Purnell, 1993; Vargas-Hernandez and Adams, 1991). These characteristics are closely related to tracheid differentiation, which changes with cambial age. While the transition zone for each of these characteristics is of scientific interest, for practical purposes, we are more concerned with those properties that are closely related to end-product quality and that can be repeatedly and economically measured.

The radial development of wood density components (ring width-RW, ring density-RD, minimum density-MINDEN, maximum density-MAXDEN, earlywood density-

EWD, and latewood percentage-LWP showed considerable fluctuations with age, making it unclear as to where the demarcation between juvenile and mature wood could be drawn. For example, earlywood density (EWD) showed low variation from pith to bark with no obvious change (e.g., sample # T-445, Figure 1). This type of curve characterised all sample trees, with very few exceptions. These findings are similar to those for Douglas-fir (Abdel-Gadir and Krahmer, 1993). The same pattern of changes in EWD was also mentioned for radiata pine grown in Chile (Zamudio *et al.* 2005). EWD trends from pith to bark showed that early wood density was not suitable for a clear differentiation between juvenile and mature wood.

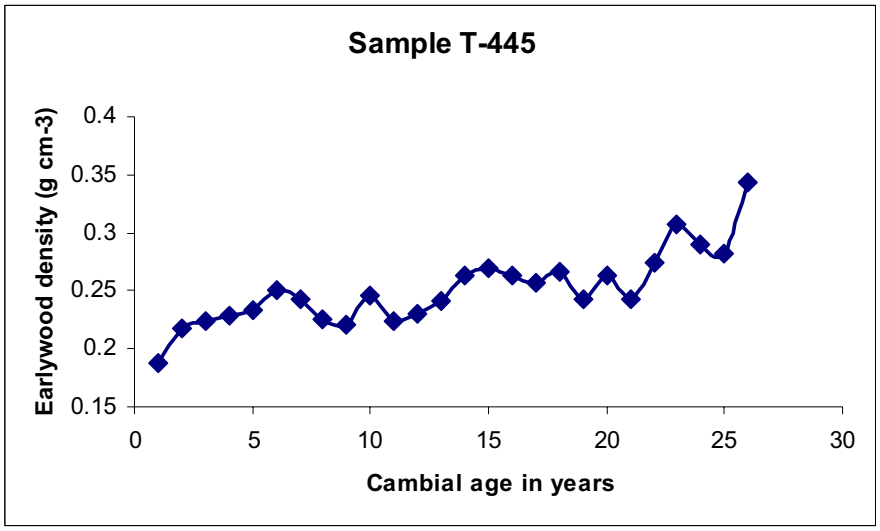

Figure 1. Development of earlywood density from pith-to-bark for sample T-445.

Ring density increased linearly from pith-to-bark showing little variation (e.g., sample # T-445, Figure 2) and we considered it unsuitable for differentiating between juvenile and mature wood. When the segmented regression models were applied, it was deduced that the use of ring density was not appropriate, because of low coefficients of determination and large range of ages for transition from juvenile to mature wood. Such a trend was unexpected as other studies on fast growing conifers were able to use ring density to estimate transition age (Hodge and Purnell, 1993; Loo *et al.* 1985). Cown and Parker (1979) reported that radiata pine, like most coniferous species show a tendency to increase value in ring density, outwards from the pith.

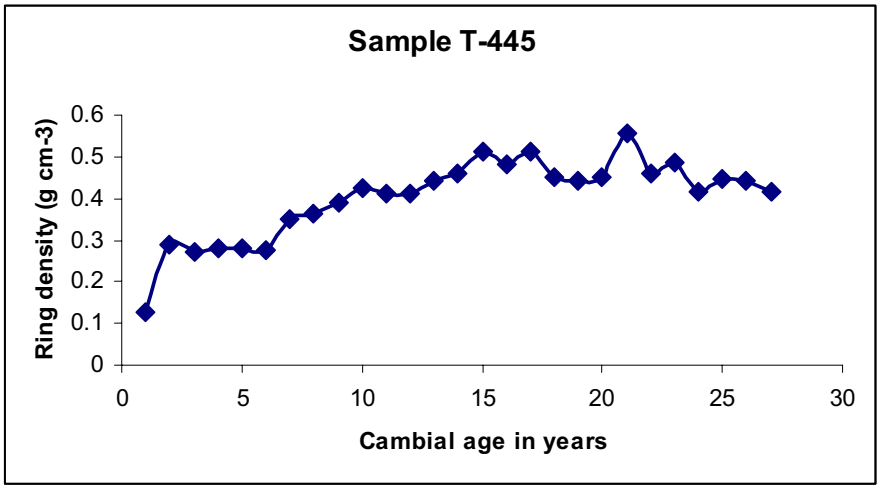

Figure 2. Development of ring density from pith-to-bark for sample T-445.

Latewood density increased rapidly for about the first 4 years, and thereafter either remained high (e.g., sample # T-445, Figure 3). For the purpose of determining juvenile-mature wood transition, only the latewood density data gave reasonable results, and produced visibly identifiable breakpoints in segmented regression models applied to pith-to-bark density profiles. Latewood density is a characteristic that is closely related to stiffness (MoE) which in turn is one of the most important mechanical properties for most end uses of wood-based biomaterials (Mamdy *et al.* 1999; Rosenberg *et al.* 1999).

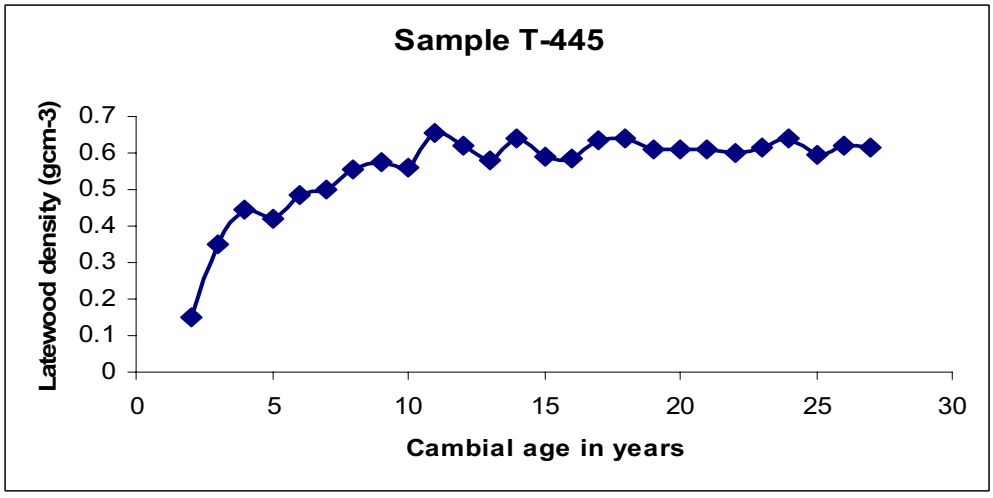

Figure 3. Development of latewood density from pith-to-bark for sample T-445.

Trends in latewood density

Average latewood density values at VRC52 and VRC54 stabilised at ring 7 with a value of 0.597 g cm^{-3} with no further significant increase or decrease in latewood density (Figure 4). Latewood density at PT47 increased from 0.241 g cm^{-3} at cambial age two and stabilized at cambial age nine with a latewood density of 0.658 g cm^{-3}. In contrast, latewood density at PT5042 increased from 0.226 g cm^{-3} at cambial age two

to 0.584 g cm^{-3} at cambial age 14 (Figure 4). Average latewood density values at PT5042 stabilized at ring 12 with a value of 0.576 g cm^{-3}. Trees reaching an early plateau in latewood density would have a shorter period of juvenile wood formation (Figure 4). The profile patterns in our data can be described as typical of a transition from juvenile to mature wood (e.g., Hodge and Purnell, 1993; Zamudio *et al*. 2002).

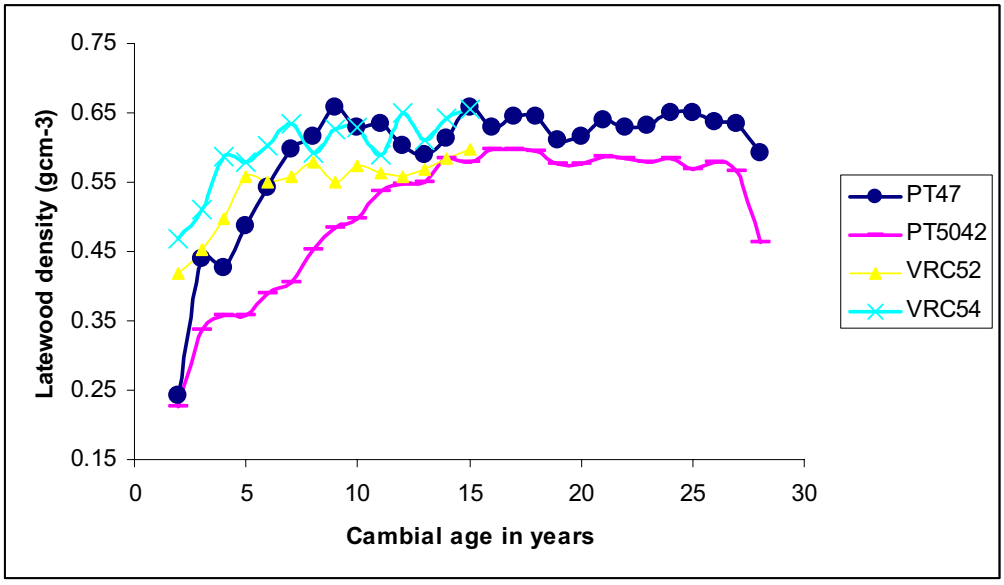

Figure 4. Trends in latewood density from pith to bark at PT5042, PT47, VRC52, and VRC54.

Several studies have reported that some coniferous species show a tendency to increase values of ring density components outward from the pith (Cown and Parker, 1979; Zobel and Jett, 1995; Zobel and Sprague, 1998; Zamudio *et al*. 2005; Wang *et al*. 2000). Zamudio et al. 2005 observed that latewood density in radiata pine increased with cambial age in 31 open-pollinated families. A similar pattern was observed by Wang et al. (2000) in families of lodgepole pine that showed that latewood density increased during the first years, reached its maximum at age six. Latewood density for loblolly pine grown in the south-east USA was found to increase rapidly with ring number from the pith, stabilizing at ring five (Loo *et al*. 1985). Similar trends in latewood density changes from pith to bark have been reported by Zobel and Sprague (1998) for other conifers.

Transition age at VRC52 varied from 4.3 to 9.3 years, with a mean around 7.7 years, and at VRC54 it varied from 5.9 to 11.2 years, with a mean around 7.2 years. Similarly, transition-age for the OP material at PT47 varied from of 5.1 to 11.5 years with a mean around 7.5 years. However, transition age for OP material at PT5042 varied from 6.3 to 21.6 years with a mean around 12.6 years (Table 2). The first three trials were located in Gippsland, Victoria whereas PT5042 was planted in Green Triangle in South Australia (Table 1). Differences in transition age between PT47 and PT5042 would seem to suggest that transition age may be site-specific.
Our results are in general agreement with those of other fast growing conifers. For example, Hodge and Purnell (1983) reported a transition age for latewood density to

be 7.5 rings from the pith. Loo *et al*. (1985) used similar approach to investigate transition age for specific gravity and tracheid length in loblolly pine. They reported mean ages of transition of 11.5 and 10.4 years for specific gravity and tracheid length, respectively. Szymanski and Tauer (1991) reported a higher transition age of 12.7 years for east Texas sources than the average transition age (11. 5 years) for east Texas families of loblolly pine reported by Loo *et al.* (1985). This suggests that the transition from juvenile to mature wood varies not only among species, but among families, traits and sites. Cown and Ball (2001) also reported the average age of onset of mature wood formation (in this study referred to as transition age) as varying among sites, ranging from five years at one site to 20 years at other sites.

The rate of change in wood density from pith to bark determines the size of the juvenile wood zone and has a major effect on the uniformity of the wood within the bole. Jayawickrama *et al* (1997) reported that *Pinus taeda* L. families that grow in height later into the growing season start forming latewood later, often leading to lower wood specific gravity at the genotype level. Dodd and Power (1994) attributed the variation pattern in specific gravity from pith to bark to earlywood width, which was more important than latewood width. They hypothesized that time of shoot growth initiation controlled the transition from earlywood to latewood production and thus the slope of the juvenile wood curve. Together, these studies provide evidence for an association between height growth cessation and latewood transition at family and individual tree level. In addition, time of latewood transition at family and individual tree level does help to explain differences in percent latewood and density in fast growing radiata pine trees (Nyakuengama, 1997).

Genetic control of transition age

Additive genetic variance (GCA) estimates at VRC52 and VRC54 were significantly different from zero whereas the SCA effects were not significantly different from zero (Table 2). Additive genetic variance estimates for the open-pollinated material (PT47 & PT5042) were also significantly different from zero. Narrow-sense heritability for transition age at the two full-sib sites were 0.13 ± 0.04 (VRC52) and 0.23 ± 0.08) (VRC54) and at the two OP sites were 0.17 ± 0.05 (PT47) and 0.33 ± 0.04 (PT5042) (Table 2). In comparison, individual tree narrow-sense heritability estimates in slash pine were 0.22 and 0.17 for latewood density and ring density transition age, respectively, [15]. Similarly, Loo *et al* (1985) reported individual tree narrow-sense heritability of 0.12 for ring density transition age in loblolly pine.

Table 2. Individual trial estimates of mean transition age (years), additive genetic (σ^2a), specific combining ability (σ^2s) and residual (σ^2e) variances, heritabilities (h^2_i) and genetic gain (Δ_G) for transition age in four trials of *Pinus radiata*. The approximate standard errors for the estimated parameters are given in parenthesis.

Trial	Min - Max	Mean	σ^2a[a]	σ^2s	σ^2e	h^2_i	Δ_G (%)
VRC52	5.6 – 14.8	7.7	0.546	0.23E-5[b]	3.689	0.13	5.2
		(1.4)	(0.08)		(0.71)	(0.04)	
VRC54	5.9 – 11.2	7.2	0.268	0.85E-6[b]	0.910	0.23	6.6
		(1.2)	(0.13)		(0.17)	(0.08)	
PT47	5.1 – 11.5	7.5	0.185	-	0.912	0.17	4.6
		(1.6)	(0.07)		(0.13)	(0.05)	
PT5042	6.3 – 21.6	12.6	1.738	-	3.576	0.33	10.1
		(2.7)	(0.73)		(0.63)	(0.04)	

[a] Additive genetic variance estimates were all significantly ($P \leq 0.05$) different from zero.

[b] SCA effects not insignificantly different from zero.

Prediction of breeding values

Predicted genetic gains, estimated using individual tree breeding values, for a shorter juvenile wood formation phase are reasonable (Table 2). Assuming a selection intensity of one in ten, genetic gains of up to 10% per breeding cycle are possible. These gains can be interpreted as the change in population mean that could be achieved by selection in the field trials. Although in practice the selection method may be different, these gains provide some indication of the change possible in the population, from a selection intensity of only 10%. Predicted genetic gains of 10.1% at PT5042 would be equivalent to shortening the juvenile wood formation phase by 22 months compared to population mean in one generation.

Implication of Results

Results from this study provide some useful information that may be incorporated into breeding strategies for radiata pine. Breeding for growth or wood quality is a controversial matter, and many approaches have been suggested (Zobel and van Buijtenen, 1989). One plausible approach would be to select for high wood density within families with high growth rate. If valid, this approach should maximize wood production with acceptable, or even superior, wood density. However, this approach may result in the production of non-uniform wood, or wood with a high proportion of juvenile wood. Thus, integrating other criteria into this approach would be beneficial for wood quality. Using radial profiles of latewood density, it would be possible to identify radiata pine individuals and families with high growth rate, high latewood density, low juvenile wood proportion, and uniform wood. A selection index integrating all these traits would certainly help develop radiata pine varieties possessing all desired traits. This approach would be useful to improve wood properties of fast-grown plantation trees known to have a high proportion of juvenile wood and low density.

Conclusions

Segmented regression analysis proved to be a practical and objective method to estimate cambial age of transition from juvenile to mature wood in a study of radiata

pine. The age of transition from juvenile to mature wood in radiata pine can be estimated only with reference to a particular wood property such as latewood density. Transition age for radiata pine is under moderate genetic control and may be site specific. A comprehensive study to examine transition age across the Australian radiata pine plantation environment would identify the best genotypes for early transition age in the different environments.

Acknowledgments

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PART II

Inheritance and genetic gain in wood stiffness measured acoustically in young standing trees

Introduction

Australia has an advanced breeding program for *Pinus radiata* (Powell *et al*. 2005), which has over the last 50 years significantly improved many characteristics of this widely planted fast growing conifer (Matheson *et al*. 1986; Cotterill and Dean 1990, Wu *et al.* 2004). Traditionally trees have been selected based on visual qualities including diameter, height, branching, straightness, observable defects, age and site characteristics (Wu and Matheson 2002). However, neither site nor visual characteristics are good predictors of the mechanical properties of the wood products. In addition, as the main uses of the products are structural applications either as solid wood or as engineered wood products, there is a demand to plant trees with high stiffness. In the framework of a genetic improvement program, visually unobservable characteristics such as wood stiffness could be considered as a selection criteria in the same way as growth or form, if it can be measured inexpensively, to maintain or improve the mechanical properties of wood produced.

Wood stiffness, a measure in terms of its modulus of elasticity (MoE) is one of the most important mechanical properties for most end uses of wood-based biomaterials. Based on an industry survey, Ivkovich *et al.* (2005) recommended MoE as one of the major breeding objective traits for radiata pine in Australia. MoE has a significant impact on structural timber grade outturn. This is particularly driven by the aim to increase the recovery of structural- and appearance grade products, which gives a higher economic return (Dungey *et al*. 2006). Unfortunately, in Australia radiata pine, the juvenile wood (also called corewood), i.e., ranging from the first seven to the first 13 rings from the pith (Gapare *et al.* 2006), often presents problems for utilisation due to the poorer mechanical properties and the high distortion. The low-stiffness wood zone becomes a strategic research topic for improving radiata pine wood quality in order to achieve shorter rotations with high stiffness wood.

In forest tree breeding, mature performance is customarily predicted using attributes measured in juvenile field trials. Selection at early ages can be expected to yield higher genetic gain per unit time than direct selection for harvest age performance if there are sizable age-age genetic correlations (e.g., Matheson *et al*. 1994). The advantages of pre-rotation selection comprise easier measurement and lower costs per tree, and there is also a quicker incorporation of genetically improved materials into forestry. The optimum age for selection for growth and wood density in radiata pine is about age 6 (Cotterill and Dean 1988, Matheson *et al.* 1994, Li and Wu 2005) at which age destructive sampling for wood properties is undesirable and expensive. Non-destructive and cheaper surrogates for wood stiffness in standing trees would be an advantage for tree selection both for deployment and further breeding. To help capture the opportunities the potential stiffness of products needs to be identified at an early age of the trees.

Genetic variation for stiffness based on direct bending test or using SilviScan prediction has been observed (Matheson *et al.* 1997, Shelbourne *et al.* 1997, Matheson and Dungey 2003, Wu *et al*. 2004, Dungey *et al*. 2006). But direct measurement of the bending MoE (known as the static MoE, usually performed on normalized specimens) requires destructive sampling and is expensive and time-consuming. While a direct measure of the bending stiffness is the most accurate, indirect measures that are far less destructive and expensive are the most desirable for breeding purposes. Recent work has shown that stiffness can be indirectly measured by either using mechanical and chemical properties of wood or using component wood quality traits. Schimleck *et al.* (2002) estimated wood stiffness of increment cores using near-infrared spectroscopy. Instruments based on acoustic waves showed great promise for measuring stiffness of standing trees, logs (Carter 2005), and small axial specimens from outer wood (Ilic 2001a, 2003), and for logs segregation in radiata pine (Walker and Nakada 1999, Matheson *et al.* 2002). Stiffness of wood is known to be related to dry-wood density, microfibril angle (MfA) and slope of grain arising from spiral grain (Cave 1969). Indirect prediction of stiffness using these component traits is therefore also possible (Evan and Ilic 2001).

Acoustic methods are the least destructive for measuring stiffness in standing trees as the tree receives only two small holes driven approximately 25-30mm into the xylem tissue. Other techniques require the removal of either axial strips or radial increment cores. Normally wood stiffness measured this way provides an indication of stiffness only in the outerwood; the overall stiffness of the tree or of the logs from the tree is represented by a "geometric average" of velocities because stiffness varies (increases) from pith to bark, furthermore there is variation (reduction up the stem) along the length of the stem as well. Usually the acoustic velocity from logs is measured by tapping the log-ends which provides such a geometric average. However, while both techniques provide a measure of an acoustic velocity, the latter approach gives a better indication of the log stiffness. This stiffness differs from the one used for assessing standing trees but fortunately, the two are related, usually quite well (Kumar 2004), ~ r^2=0.9) when the log or felled stem (full length) incorporates the measured standing tree position.

The standing tree time of flight technique provides an acoustic wave velocity for the stem. The acoustic velocity is related to MOE of the wood according to equation (1).

$V^2 = MoE/D$ ……..(1)

Where:
V = Velocity ms^{-1} (V = 1/T; T = time of flight (s^{-1}))
MoE = Modulus of elasticity (Pa)
D = Bulk density (kgm^{-3}), usually assumed to be 1000 kg/m^3 for radiata pine.

When MOE is measured in this way it is known as dynamic MOE in contrast to static MOE which is measured in bending. The dynamic and static measured MOE values are highly related in green and dry wood (Booker and Sorensson 1999; Ilic 2001b).

Variation in the time of flight for repeated measurements is expected depending on the nature and sharpness of the induced stress waves, to some extent that can be operator dependent, i.e. how hard or how rapid the impact is on the first transducer. A

second source of error comes can come from the threshold setting of the electronic timer. This is particularly important if different brands of instruments are used for assessments.

The Southern Tree Breeding Association (STBA), which works cooperatively to breed and improve radiata pine for its 20 members, has now begun selection for the third-generation breeding population among trees in second-generation progeny tests planted in 1996 and 1997. Currently, there are no estimates of genetic parameters for wood stiffness based in acoustics measurements in Australia. Wood density and microfibril angle are the key drivers of MoE in radiata pine, thus initial work to improve stiffness is through improvement of these component traits (Dungey *et al*. 2006). However, direct improvement of MoE would be more efficient if MoE can be measured accurately. Previous work on small numbers of samples have shown high heritabilities of wood stiffness for New Zealand radiata pine based on acoustics measurements (Kumar *et al.* 2002 and Kumar 2004). Selection for MoE, using indirect methods such as acoustic measurements would remove the need to assess MfA through SilviScan in order to improve the stiffness of corewood in radiata pine. The main objective of this study was to estimate the amount of genetic variability in wood stiffness from standing trees based on measuring the velocity of acoustic waves. To measure stiffness of standing trees accurately, a preliminary study was necessary to identify which particular time of flight instrument combined relative low cost with ease of use and accuracy.

Materials and Methods

Two progeny trials (BR9611, located at Flynn, Victoria and managed by Hancock Victorian Plantations and BR9705, located at Kromelite, South Australia and managed by Green Triangle Forest Products) were selected for this study. These radiata pine tests are part of the progeny test series following controlled pollinations based on the Nucleus Breeding Strategy of White *et al.* (1999). The broad objectives of the progeny test series were to (i) provide genetic parameters needed for selection index development focusing on growth and wood quality traits; (ii) provide groups of genetically connected progeny for use in advanced generation selections and breeding in Australia; and (iii) provide rankings of families and individuals for use in forward selections for the following generation progeny trials. The trials consisted of all families generated from crosses of the second generation selections. A mixed mating structure was used to generate full-sib, and half-sib families (White *et al.* 1999).

Flynn was planted in June, 1996 with 250 families, using a 10x25 row-column design with 5 replicates and four-tree row plots. Kromelite was planted in July, 1997 with 110 families, with a 10x11 row-column design with 5 replicates and four-tree row plots. Every third row at each site was an outrow. Both sites were second radiata pine rotation sites. Only 16 families were common to both sites, but there is more genetic connection through common parents and grandparents for some other families. Site was prepared by ploughing and mounding with good soil drainage (Table 3).

Table 3. Details of the Flynn (BR9611) and Kromelite (BR9705) sites used for this study.

Site	BR9611 (Flynn)	BR9705 (Kromelite)
Date planted	6/1996	7/1997
Cambial age at time of sampling	8	7
Spacing	3.6m x 2.5m	2.74m x 2.5m
Latitude	38^{o} 14'S	37^{o} 50'S
Longitude	146^{o} 45'E	140^{o} 55'E
Elevation (m)	166	55
Annual rainfall (mm)	760	900
Soil type	Sandy loam	Sandy clay-loam
Site type	2nd rotation	2nd rotation
Total number of families	250	110
Number of replicates	4	3
Number of columns within blocks	25	11
Number of rows within a column	25	30

Preliminary study to identify optimal instrument for breeding purposes

In order to determine the optimal instrument to measure tree stiffness, the following five acoustic and non-acoustic instruments were tested for the project:
Fakopp stress wave timer[1] (www.fakopp.com). This involves inserting two probes, each with a sensor. The bottom probe is tapped with a small hammer and the time for the stress wave to travel between the probes is measured. The distance between probes is usually about 1m, but must be known accurately. Fakopp was used only on one side of the tree.
IML Hammer stress wave timer (www.walesch.ch). This involves inserting two generic probes (initially screws) and attaching a sensor to the top probe. The bottom probe is tapped with the IML hammer. The IML hammer contains a strain gauge to detect the travel time of the stress wave. The distance between the probes must be known and is usually about 1m. The IML was used on two opposite sides of the trees.
Krautkramer USD10-NS Ultrasound flaw detector (www.geinspectiontechnologies.com). This involves inserting two probes a known distance apart and applying ultrasound waves to the bottom probe and timing their arrival at the top probe. Because the wavelength is small, it is necessary to avoid branches and insert the probes in clear wood. Probes are typically about 300mm apart.
Tree sway. This involved manually swaying the trees and filming the frequency of sway with a digital movie camera. The movie is viewed frame-by-frame to determine the frequency of sway.
Dynamic MoE of axial beams. This involves cutting axial specimens approximately 120mm x 10-15mm x 2mm from the outerwood of a tree. In this case, they were cut from discs removed from the logs during the sampling process. These are tested directly in the laboratory for dynamic MoE (Ilic 2003).

[1] Mention of a commercial product in no way implies endorsement of the product.

These measurements on standing trees were compared with benchmark measurements based on logs cut down from the same trees and using following two instrument:.
Director HM200 (www.fibre-gen.com). This is an instrument developed by CCH Fibre-gen as a variation of Hitman to segregate logs based on their resonance. Director is placed against the lower cut surface and the log is hit with a hammer. The resonant frequency is detected with a microphone and recorded.
HP Dual Channel Dynamic Signal Analyzer 35665A. This is an off-the-shelf product and works in a manner analogous to the Director HM200. Its microphone detects resonances from the log and displays it with detected harmonics on a screen. The dominant harmonic can be determined visually and its frequency recorded.

Thirty-eight trees in outrows between treatment trees at the Flynn site were used to compare measurements made by each technique in December 2003. Lower branches were first cut off with an axe and a Fakopp measurement made on one side of the tree with probes 1m apart, avoiding placing probes into branch whorls. An IML measurement was then made using the same holes as the Fakopp on one side of the tree and a further measurement was made on the opposite side of the tree with probes again 1m apart. An Ultrasound measurement was then made on a portion of the stem 300mm long between the two Fakopp probe holes and not encompassing branch stubs. Trees were then manually swayed and the decay of the swaying recorded on a digital camcorder. The tree was then cut down, the log length measured and a disc about 150mm thick taken at 2/3 of the tree height. A Director measurement was then taken on the log representing 2/3 of the tree height, followed by an HP signal analyser measurement. Axial specimens were cut from the discs in the laboratory and tested for dynamic MoE as described in Ilic (2003). A fault in the HP signal analyser meant that the two measurements on logs were carried out a few weeks later than the others.

Pairwise product-moment correlation coefficients were calculated between measurements obtained for each tree. Correlations from this preliminary study are given in Table 4.

Table 4. Correlations between MoE measurements by different instruments on 38 trees derived from the outrows in the Flynn trial.

	Fakopp	IML	Ultrasound	Sway	Director	HP
IML	0.891	1.000				
Ultrasound	0.248	0.434	1.000			
Sway	0.386	0.552	0.557	1.000		
Director	0.849	0.941	0.249	0.419	1.000	
HP	0.838	0.940	0.270	0.445	0.945	1.000
Axial beam	0.700	0.701	0.412	0.643	0.636	0.577

For two logs, the HP Signal Analyzer corrected the Director result which had selected the incorrect harmonic. Results included the corrected values rather than the originals. The IML results are for the average of measurements taken on the two sides of the tree.

The Director was regarded as the main benchmark, partly because it is used in industry. Table 4 indicated that the IML was the most accurate (r = 0.941 with the Director). Correlations between the two IML measurements taken separately and the Director were 0.903 and 0.916. IML also showed a high correlation with HP (0.94). Consequently, it was resolved to measure stiffness in the progeny tests using the IML hammer on one side of the tree to optimise cost and accuracy.

Methodology for acoustic data collection

For the purpose of this study, two trees in each plot of all five replicates were tested at Flynn. At Kromelite, all four trees in three of the five replicates were used for acoustic measurement.

The IML hammer was used measuring the timing and stiffness. The two probes in IML were modified from the original screw probes so they could be hammered into trees rather then being screwed. The bottom probe was hammered into a tree to be tested, angled upwards at about 45º. The top probe was hammered into the tree 1m above the lower probe and angled downwards at 45º. A special sleeve was used when hammering in the upper probe to avoid damaging the threaded hole in the probe. The detector was screwed into the top probe and connected to the electronic timer box. The special hammer was also connected to the electronic timer box. The bottom probe was tapped with the special hammer a number of times and the time of flight displayed for each tap by the electronic box in microseconds (μs). When a consistent reading was obtained, that reading was recorded.

Methodology for data analysis.

Traits

Assuming the density of green wood is 1000kg/m^3 the Modulus of Elasticity can be calculated as $v^2/10^6$ in GPa where v is the velocity of the stress wave in the tree (in m/s). Examination of residuals for MoE suggested that transformation of the raw data would be required. For this reason and the one-to-one relationship between time of flight and MoE, only time of flight was analysed. Time of flight could be regarded itself as a transformation for MoE. Longer flight times are associated with lower stiffness values. Unless explicitly stated, analyses of DBH (provided by STBA) were conducted only for trees which had a time of flight measurement.

Individual sites

Data were analysed using an individual tree model (Borralho 1995) with genetic founder groups (Quaas 1988, Westell *et al.* 1988) in the restricted maximum likelihood (reml) statistical program ASReml (Gilmour *et al* 2005), adjusting for incomplete row and column effects. Replicate effects and genetic founder groups were regarded as fixed and other effects considered random. The genetic founder groups describe whether the ancestors of the parent trees in the trials were pre- or post-1970 selections, in Australia or NZ, elite selections or not as well as the line (A or B) and the part of the line from which they were drawn (Nucleus or Main) as described above.

Individual tree models in ASReml (Gilmour *et al*. 2005) involve the development of a relationship matrix from a supplied pedigree file in which the ancestry of every individual in the trial(s) is described. This is extremely flexible and enables estimates of genetic parameters from very diverse trials such as these in which some genetic entries were the result of polycrosses and some were full sibs. Most genetic analysis computer programs would require families to be divided into groups with common genetic structure (i.e., separate polycrosses from full-sibs) so appropriate coefficients of relationship can be applied to variance components when estimating genetic parameters. The linear format of the full model used for analyses within each site was as follows:

$$y = \mu + R + row + col + P + A + SCA + e \qquad (1)$$

where y represents each individual observation, R represents the fixed replicate effects, *row*, *col* and *P* represent the random effects of the rows, columns and plots within replicates (each row or column forming an incomplete block), *A* represents the random additive genetic effects, *SCA* represents the random specific combining ability effects (non-additive genetic effects containing dominance and some epistatic effects) and *e* represents the residual.

A number of decisions had to be made during the sequential analysis process. The fixed effects were tested using Wald tests and non-significant terms omitted from the model. For random terms, the first indication of their significance was given by the ratio of the variance components to their corresponding standard error. Terms for which this ratio was >2 were regarded as significant. Terms for which the ratio was <1 were regarded as not significant. For ratios between 1 and 2, the likelihood ratio test was applied (-2*(difference between log likelihoods including and excluding the term) ~ χ^2) (Gilmour *et al*. 2005). Non-significant terms were dropped one-by-one. Occasionally, extremely small terms were observed to have been estimated at a boundary and were dropped as well. The model initially fitted contained fixed terms for replicates and random terms for rows and columns within replicates (incomplete block effects), plots, families and individual trees.

Models with significant terms were then used to predict the individual tree breeding values and to calculate heritabilities as follows:

$$h^2 = \frac{V_A}{V_A + V_{SCA} + V_P + V_e} \qquad (2)$$

in which V_A, V_{SCA}, V_P and V_e represent the additive genetic, specific combining ability, plot and residual variances, respectively. Neglecting higher order epistatic interactions, the SCA variance is 0.25 * non-additive variance (Becker 1984) and the sum of the plot and residual variances estimates 0.75 * non-additive variance + environmental variance. Standard errors were calculated as in Gilmour *et al* (2005). Replicate and incomplete block effects are not included in the phenotypic variance (the denominator) because it is assumed selection will be made on data adjusted for replicate and incomplete block effects (Williams *et al*. 2002).

Genetic correlation with stem diameter

A file containing diameters from an earlier measurement of diameter at breast height (DBH), measured on 6 June 2004 was obtained from STBA. Bivariate individual tree models were fitted using model 1, and non-significant blocking terms omitted from the model as described above. Genetic correlations were obtained as follows:

$$r_{A_{xy}} = \frac{C_{A_{xy}}}{\sqrt{V_{A_x} V_{A_y}}} \quad (3)$$

in which $C_{A_{xy}}$ represents the additive covariance component between traits *x* and *y*, V_{A_x} and V_{A_y} represent the additive variance components for traits *x* and *y* respectively. Standard errors were calculated as in Gilmour *et al* (2005).

Correlated change in stem diameter following selection for lower time-of-flight was calculated from the bivariate analysis of both traits as:

$$CR = ih_d h_t r_{A_{dt}} \sigma_{P_d} \quad (4)$$

where i is the intensity of selection, h_d and h_t are the square roots of heritabilities for diameter and time-of-flight respectively, $r_{A_{dt}}$ is the additive genetic correlation between stem diameter and time-of-flight and σ_{P_d} is the phenotypic standard deviation for stem diameter.

Combined analyses.

Although the single-site analyses indicated that the residual variances were different at each site, the additive variances were similar. To test whether this was significant, individual-tree models were fitted with a single error term and a separate error term for each site. An Akaike Information Criterion (AIC) was calculated for each model as –2ln(maximum likelihood) + 2*(number of fitted parameters) in which models yielding a lower AIC are regarded as more parsimonious (Lynch and Walsh 1998).

Two models were fitted; one to estimate the additive and non-additive genetic correlations between sites and the other to provide estimates of breeding values of individual trees for selection purposes that were comparable across sites. The first was a bivariate model in which the times of flight at each site were treated as different traits. Effects for site and replicate within site were treated as fixed, other effects (rows and columns within replicates at each site separately, plots at each site separately, SCA and additive effects for each site separately and with a covariance term) were treated as random. This initial model can be summarised as:

$$y=\mu+S+R(S)+row(R)_F+row(R)_K+col(R)_F+col(R)_K+P(R)_K+P(R)_F+SCA_F+SCA_K+SCA_C+A_F+A_K+A_C+e_F+e_K \quad (5)$$

where the subscripts *F, K* and *C* refer to the Flynn and Kromelite sites and the covariance respectively. *S* and *R(S)* refer to the fixed site and replicate within site

effects respectively. *row(R)* and *col(R)* refer to the random effects of rows and columns within replicates. *P(R)* refers to the random effect of plots within replicates, *SCA* and *A* refer to the random non-additive and additive effects respectively and *e* refers to the residual term. Non-significant random terms were omitted as before (where warranted) and the additive genetic correlation was calculated as in (2), and standard errors as before (Gilmour *et al.* 2005).

For the second combined analysis model, a univariate model was fitted such that combined estimates of additive and non-additive genetic effects were obtained. This meant that the BLUP estimates of breeding values for each tree at each site were comparable. Effects were treated as fixed or random and criteria for retaining in the model were the same as above. The initial model can be summarised as:

$$y=\mu+S+S(R)+row(SR)+col(SR)+P(SR)+SCA+SCA*S+A+A*S+e \quad (6)$$

where *S* and *S(R)* represent the fixed effects of site and replicates within sites. *row(SR)* and *col(SR)* represent the random effects of rows and columns within sites and replicates respectively. *SCA* and *SCA*S* represent the random non-additive and non-additive*site effects respectively. *A* and *A*S* represent the random additive and additive*site effects respectively. The term *e* represents the residual term.

Results

Individual site analyses

Flynn

The mean time of flight was 463 µs over a 1m distance, corresponding to an velocity of 2160 ms^{-1} and an MoE of 4.67 GPa. Mean diameter was 154.1 mm. Results from the analyses are presented in Table 3. All the variance components were significant except for the plot effect in DBH. A number of possible outliers were identified. Five observations had residuals above 4.0 sd from the fitted values and were removed, reducing the residual variance of the fitted model from 543 to 442 (22%). It is clear that the row-column design has been successful at improving the precision of the experiment for both traits.

In this analysis, the non-additive variance is estimated by four times the SCA variance component. The non-additive variance (194) at Flynn is a little over 15% of the additive variance (1280). However, for DBH, the non-additive variance (17) is over one third (37%) of the additive variance (45). The heritability estimates for time of flight was high, but for DBH was low.

Table 5 Sources of variation, estimated variance components and their standard errors for univariate analyses at Flynn following removal of outliers. Rep.row and Rep.col refer to the incomplete blocking structures rows and columns within replicates respectively.

A. Time of flight (μs) [IML]				
Source	Component	se	h^2	se (h^2)
Rep.row	51.38	18.55		
Rep.col	120.75	27.69		
SCA	193.68	41.21		
Plot	172.43	45.02		
Additive	1280.12	252.49		
Residual	442.57	153.67	0.6680	0.1020
B. Diameter at Breast Height (cm) [DBH]				
Rep.row	17.60	5.40		
Rep.col	13.39	4.36		
SCA	16.86	6.15		
Plot	12.68	11.02		
Additive	44.96	17.70		
Residual	298.97	17.06	0.1246	0.0474

Gains from selection and genetic correlation with DBH

Genetic correlations between time of flight and DBH were estimated using the multivariate reml facilities in ASReml. This fits a model using a multivariate (in this case bivariate) normal distribution. The genetic correlation was 0.3302 ± 0.2311. This suggests that families with long flight times (low MoE) also have large diameters and hence that wood stiffness and diameter are significantly negatively genetically correlated at Flynn. Selection of the best 10% for time-of-flight would result in a decrease of 11.6% in time-of-flight, corresponding to an increase in stiffness of 1.3GPa, accompanied by a loss of stem diameter of 1.7% (2.7mm out of 154mm).

Kromelite

The mean time of flight was 519 μs, corresponding to a velocity of 0.00193 ms^{-1} and an MoE of 3.71 GPa. The mean diameter was 170.3mm, larger than at Flynn even though trees were a year younger. Variance components for row within replicate, SCA and plot within replicate were not found to be significant for time-of-flight and so were dropped from the final model for analysis. One value with a large residual was dropped from the analyses whose results are given in Table 5. Neither of the incomplete blocking structures were significant for DBH, but were column within replicate (rep.col) was left in the model for analysis of covariance with time-of-flight.

Table 6. Sources of variation, estimated variance components, heritabilities (h^2) and their standard errors for univariate analyses at Kromelite. Rep.col refers to the blocking structure row within replicate.

A. Time of Flight (μs)

Source	Component	SE	h^2	se(h^2)
Rep.col	278.36	89.506		
Additive	1043.85	237.239		
Residual	1329.91	180.694	0.4397	0.0871

B. DBH (mm)

Source	Component	SE	h^2	se(h^2)
Rep.col	2.52	4.00		
Additive	63.44	26.77		
Residual	398.83	27.21	0.1372	0.0563

The heritability estimate for time of flight (44%) was clearly less than at Flynn (67%) even though the estimate of additive variance was much the same. The heritability for DBH is almost the same at Kromelite as for Flynn (13.7% cf 12.5%).

Gains from selection and genetic correlations with DBH

As for the Flynn site, genetic correlations between time of flight and stem diameter growth (DBH) was carried out using the multivariate reml procedures in ASReml. The estimate was –0.115 ± 0.214, opposite in sign from that at Flynn, but with a large standard error and hence not significantly different from 0. Selection of the best 10% for time-of-flight would result in a decrease of 7.2% in time-of-flight, corresponding to an increase in stiffness of 0.6GPa.

Combined analysis

There was a significant difference between the sites, Flynn having a predicted mean time of flight 56.3 μs lower than Kromelite (462.8 μs vs 519.1 μs), corresponding to a difference of 0.96 GPa in MoE. In addition, there were significant differences between replicates within sites. Replicate 2 at Kromelite was 15 μs faster than replicate 1 and 9 μs faster than replicate 3. Replicates 1 and 3 were not significantly different. At Flynn, replicate 5 was 20 μs faster than replicate 1 and significantly faster than all other replicates. Some of the differences between replicates are probably due to operator effects. The analysis to estimate genetic correlation (from equation (5)) between sites is presented in Table 7. The combined individual tree analysis (from equation (6)) is presented in Table 6.

In Table 7, rows, columns and plots within replicates were unique to each site and so had no covariance term. The results for these terms were essentially similar to those for the analyses of individual sites. The variance component for non-additive effects was not significant at Kromelite, but was left in the model to permit its estimation at Flynn which was similar to the single site estimate. The estimate of the non-additive genetic correlation was greater than unity (>1.6), probably because the estimate for non-additive effects at Kromelite (in the denominator of equation (2)) was smaller than its standard error and so was not significantly different from zero.

The phenotypic correlation between predicted family mean times of flight at each site for the common 16 families was 0.804 (Fig 1). The additive genetic correlation (r_G) between the sites, estimated from covariance components in Table 7, was 0.946 ± 0.086. The additive genetic covariance term contains information not only from the common families at each site, but the common parents and grandparents. The extremely high values for both the additive genetic and the phenotypic correlation indicate the lack of additive x site interaction in this case. We do not regard this as definitive because the genetic links across sites were not extensive.

Residual variance estimates (error) were similar to the estimates from the analyses of individual sites.

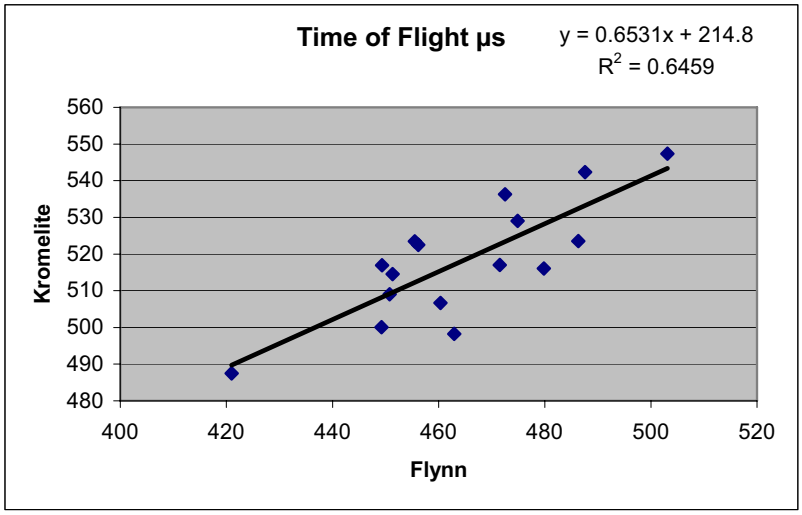

Fig 5. Comparison between predicted family mean times of flight at each site. Each point represents the predicted means of one of the 16 families common to both sites.

The Akaike Information Criterion (AIC – see Lynch and Walsh 1998) for a model with a separate residual variance for each site was 11774.94 and for the model with a single residual variance the AIC was 11733.98. For this reason, the single residual variance model was chosen as it was the most parsimonious.

Table 7. Bivariate analysis across sites. In this analysis, sites and times of flight at each site were treated as different traits in order to estimate genetic correlations. Components for rows and plots within replicate at Kromelite were essentially zero and so standard errors were meaningless. Rep.row and Rep.col refer to the incomplete blocking structures rows and columns within replicates respectively. Plot refers to plots within replicate.

Source	Component	Standard Error
Rep.row (Kromelite)	0.0011	
Rep.row (Flynn)	52.63	18.73
Rep.col (Kromelite)	269.22	86.56
Rep.col (Flynn)	123.42	27.92
Plot (Kromelite)	0.0011	
Plot (Flynn)	171.78	44.85
Sca (Kromelite)	62.54	67.25
Sca (Covariance)	173.88	73.69
Sca (Flynn)	186.29	39.72
Additive (Kromelite)	958.33	283.53
Additive (Covariance)	1053.71	209.90
Additive (Flynn)	1294.94	239.36
Residual (Kromelite)	1373.16	200.46
Residual (Flynn)	433.35	146.40

Table 8 presents the combined analysis obtained from fitting equation (6), leading to common breeding value estimates for each individual in both sites expressed on a single scale. These breeding value estimates allow direct estimates of gain as they combine individual and family information across both sites. Overall, including the row and column effects improved the estimates of genetic parameters, indicating the importance of good experimental design. The estimate of additive variance was similar to the two estimates from individual sites. The two estimates of residual variance were different, in particular the combined estimate was almost 50% bigger than Flynn's single site estimate, and was less than half of the estimate at Kromelite.

Table 8. Combined individual tree analysis across sites. In this analysis, time of flight was treated as a trait with pooled estimates of variance components across sites.

Source	Component	Standard Error	h^2	se(h^2)
Site.Rep.Col	165.57	28.45		
Site.Rep.Row	36.19	12.61		
Sca	164.46	32.18		
Additive	1168.64	202.53	0.5357	0.0754
Residual	848.26	128.14		

Predicted gain was calculated from selecting the 10% of trees with the highest predicted breeding values obtained by fitting equation (5). This was estimated to decrease the time-of-flight by 44μs, corresponding to an increase in stiffness of 0.90 GPa, a gain of 21.0%. The additive genetic correlation between time-of-flight and stem diameter across both sites was 0.56 ± 0.08. Using equation (4), it was estimated

there would be a concomitant reduction of 4.2% in stem diameter following selection as above for time-of-flight.

Discussion

In the analyses presented here, we have used time-of-flight as the analytical variate rather than transforming these times to stiffness (MoE). The MoE trait (in gigapascals – GPa) is calculated from the time of flight as $10^6/t^2$ where t is the time of flight in microseconds. This calculation assumes the density of green wood is 1000 kg/m^3. As this is not a linear transformation of the time of flight, the scales are not exactly equivalent. Preliminary analyses indicated that the residuals for MoE were not normally distributed and would have required transformation before analysis, introducing difficulties of interpretation. For this reason, analyses were carried out on times-of-flight rather than on MoE although means were converted for comparison.

Choice of acoustic tool

Acoustic means of measuring wood stiffness in logs and cut wood pieces have existed for some time (e.g., Booker and Sorensson 1999, Walker and Nakada, 1999). Newer tools for measuring time-of-flight in standing trees have been adapted for measurements of wood stiffness and the latest tools have been designed for the purpose (Carter 2005, Wang *et al*. 2004).

The preliminary investigations reported here indicate that the acoustic tools all give very similar estimates of time-of-flight and hence MoE in standing trees as was found by Kumar (2004). Decisions about which tool to use are then based on convenience and consistency. Most tools involve an operator tapping a probe with a hammer. Our experience showed that this is a learned skill and that differences in tapping technique lead to differences in times-of-flight even for the same operator. Changes in design of the tools to allow for a standard tapping force applied in exactly the same way would appear to be one way of reducing variability between techniques and between operators.

Phenotypic correlations between the flight times and dynamic MoE measured in small axial beams were high for all acoustic tools, but highest for the tapping tools (Fakopp and IML). Both IML and Fakopp measured flight times over 1000mm and sometimes encompassed branch whorls. The axial beams measured MoE over only a very short distance of clearwood (about 150mm) up the stem at 1.3m above ground and so perfect correlation with the stress wave timers would not be expected.

Differences between sites

Despite being a year younger, trees measured acoustically at Kromelite (average dbh = 170mm) were larger than those at Flynn (average dbh = 154mm). The average time-of-flight at Kromelite was higher than at Flynn, (average times 519µs cf 463µs) corresponding to 3.71GPa and 4.67GPa for MoE, respectively. This was consistent with the positive genetic correlation, between tree diameter and time-of-flight at Flynn (i.e. larger trees had longer flight times), suggesting that tree size and wood stiffness are negatively genetically correlated. However, the lack of a significant

genetic correlation at Kromelite indicates that this relationship, like other genetic parameters, depends on site and size of sample.

In the analyses of separate sites, the heritability estimate for acoustic time-of-flight was higher at Flynn ($h^2 = 0.67$) than at Kromelite ($h^2 = 0.44$), primarily because the residual variance was lower (443 cf 1330). Although these residual variances appeared different, the Akaike Information Criterion indicated that a combined model with pooled residual variance was more parsimonious than one with separate residuals for each site. The within-site estimate of heritability for time-of-flight at Kromelite is similar to that obtained by Kumar (2004) for a single site in New Zealand ($h^2 = 0.37$), but that obtained at Flynn is considerably higher. It is possible that some environmental differences within the Kromelite site were not taken into account by the row-column blocking structures. These structures appear to have been successful at accounting for trends at Flynn where there was a slope both between and within replicates so that columns would account for variation within replicates and replicates would account for the rest of the variation due to slope. There was apparently some variation within replicates across the slope because the variance component for rows was also significant. This was not so at Kromelite where none of the within-replicate blocking structures were significant. The site was level and apparently very uniform, but there could have been a patchy environment on a scale less than either rows or columns (Gezan 2005). This effect might also have been caused by only two trees per plot in all five replicates being measured at Flynn, but all four trees per plot measured in only three of the five replicates at Kromelite.

Estimates of the specific combining ability variance component was significant (the ratio of the component to its standard error was 4.7) at Flynn for time-of-flight but not at Kromelite where it was so small that it was dropped from the model. The specific combining ability variance contains mostly dominance variance with a smaller amount of epistatic variance (Costa e Silva *et al*. 2004).

The separate-site analyses of stem diameter were quite similar to each other, yielding very similar estimates of heritability. The residual variances for this trait were not very different either (298 at Flynn and 398 at Kromelite). The estimate of SCA variance was significant at Flynn (ratio of component to its standard error was 2.47) but was effectively zero at Kromelite, similar to the result for time-of-flight. Genetic correlations between time-of-flight and dbh were positive (i.e., smaller trees had shorter times and therefore had stiffer wood) at Flynn but effectively zero at Kromelite.

Because of its higher heritability for time-of-flight, the Flynn site would clearly be the preferred site to test and select for wood stiffness even though the trees were growing slower than at Kromelite. More sites would have to be used to make broader inferences about slower-growing sites provided a better testing environment.

Combined results

The estimated heritability for the combined model (6) was 0.54, lying between the estimates for the two sites taken separately. The gain (21%) estimated from selection of only the best 10% of trees is considerable. The trees measured here were only 6 (at Kromelite) and 7 (Flynn) years old and so the wood being tested almost certainly

would be defined as 'corewood' (Burdon *et al*. 2004). Dungey *et al*. (2006) found there was considerable variation and high heritability estimates in wood stiffness in corewood, but less variation and lower heritability estimates in outerwood. Their estimates, one at a site in New Zealand and the other in NSW, Australia were very similar at age 8. Our estimated specific combining ability variance was about 1/7 of the additive genetic variance, indicating that dominance effects were unimportant for stiffness. The additive genetic correlation between DBH and stiffness was -0.56, indicating a strong negative relationship between wood stiffness and stem diameter. This value, from the data combined over both sites is greater than either of the values obtained from each site alone, but should be more reliable (it has a lower standard error than either site alone) since it is based on more trees.

There was very little evidence for genotype x site interaction. This term in the analysis was often dropped from fitted models because it was not significant. In addition, the additive genetic correlation between the sites was extremely high (r_A =0.96). However, there were very few common families (see Fig 5) and this result should not be taken as definitive.

The negative genetic correlation between growth and wood stiffness presents some problems for selection in a breeding program. Simply selecting for greatest MoE (or smallest time-of-flight), some account must be taken of stem diameter. A suitable means to achieve this would be to construct a selection index aimed at maximising benefits from these two traits including not only their genetic parameters but also an estimate of the effects of each on the economic outcome for an appropriate breeding objective (see Ivkovic *et al.* 2005).

Truncation selection based on IML<426µs and DBH>160mm at Flynn yielded the best 14 correlation-breaking trees in the trial. It is noteworthy that 7 of these 14 trees belonged to a single full-sib family. At Kromelite, with truncation selection at IML<490µs and DBH>175mm, three of the 18 selections belonged to another single full-sib family and another selection had the same male parent, five belonged to a single polycross family and four had the same parent either as a male or female parent.

BLUP predicted breeding values combined over sites were also used to investigate the effects of selection over both trials. Of the top 40 selection for time-of-flight BLUP BVs, 20 had the same female parent of which 7 were selfs and a further 9 had the same (different from the selfs) male parent.

These two results reinforce the need to have strong pedigree control when crossing parents selected for stiffness to produce the next breeding population or for deployment. Selection for correlation breakers is also a possibility.

Results from the preliminary study on choice of tool indicated that there was a good phenotypic relationship between acoustic time-of-flight and a measure of dynamic MoE from small axial beams ($r \approx 0.7$). The axial beams were approximately 150mm long and were sampled from approximately 1.3m up the stem and about 15mm radially towards the pith from the cambium. Variation in stiffness up or down the stem is one factor that could reduce the correlation, as could radial variation. Although high type B genetic correlations were obtained between dynamic MoE

measured acoustically and static MoE by Kumar (2004), they were not measured on the same trees and so direct comparison with the phenotypic results obtained here is not possible. Comparison between static MoE (in boards or in special samples) and acoustic measures in standing trees should be explored to verify how good acoustic measurement in standing trees are at predicting stiffness of harvested boards.

Conclusion

- The IML hammer has proved to be a workable field instrument for measuring time of flight in real situations. Using a team of 3 people per instrument it was possible to measure a tree approximately each 40 seconds and a large trial in two days.
- Heritabilities for the time of flight were very high and there was no appreciable genotype x environment interaction between the two sites measured. This indicates that large gains in wood stiffness are possible in trees of this age, although the lack of interactions could be because of low connectivity across sites.
- There appears to be a negative genetic correlation between growth and time of flight (at least at one site as well as across both sites). This suggests that a form of index selection to find correlation breakers would be necessary for breeding.
- Correlation breakers combining stiff wood and fast growth came predominantly from a few families.
- The relationship between the acoustic measures of stiffness from the standing tree and more direct static or dynamic MoE measurements are required as an essential step.

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APPENDIX 2

CSIRO Forestry and Forest Products
CLIENT REPORT: No. 1733

Juvenile Wood Initiative:

Prediction of Wood Stiffness, Strength, and Shrinkage in Juvenile Wood of Radiata Pine: Juvenile Wood Index

M. Ivković, W.J. Gapare, A.C. Matheson, A. Abarquez

M.B. Powell, T.A. McRae, and H.X. Wu

***December* 2006**

COMMERCIAL IN CONFIDENCE

CSIRO Forestry and Forest Products
PO Box E4008
Kingston ACT 2604 AUSTRALIA
Telephone: 02 62818211 Fax: 02 62818312

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Executive Summary

Wood stiffness, strength and stability are important for all wood products, and particularly for structural timber products. In radiata pine, the juvenile wood often presents issues for utilisation due to its poor mechanical properties and high distortion. Development of optimal ways to predict juvenile wood stiffness, strength and stability using wood quality traits that can be measured with relative ease and low cost is a priority for tree breeding. The objectives of this study were to examine: 1) within-disk variability of wood stiffness and strength, and shrinkage; 2) examine hypothesis of causal relationship of stiffness, strength and shrinkage with basic wood properties using path analyses; and 3) compare different methods for prediction of stiffness, strength and shrinkage, namely SilviScan, IML hammer, and paddle-pop sample measurements.

Wood property data for this study were sourced from two progeny tests aged at 6 (Kromelite) and 7 (Flynn). Wood traits including Modulus of Elasticity (MoE), Modulus of Rupture (MoR), time of flight or sound wave velocity, spiral grain (SG), radial, tangential and longitudinal shrinkage (RS, TS, LS), wood density (DEN), and micro-fibril angle (MfA) were measured from a series of samples. Key results include:

- MoE and MoR were lower (50%) in inner-rings (rings 1-2 from pith) than for outer-rings (rings 3-6 from pith).
- RS and TS were higher (30-50%) for outer-rings than inner-rings, but LS decreased rapidly (>200%) from inner-rings to outer-rings. The ratio of TS:RS:LS was about 20:10:1 in outer-rings.
- Variation between inner- and outer-rings was larger than that among trees for stiffness, strength and shrinkage.
- DEN had a higher correlation with MoR than with MoE, while MfA had a higher correlation with MoE than with MoR. SG had higher significant correlation with MoE than with MoR.
- RS and TS had a weak, significant linear relationship with DEN and MoE while LS had a strong negative non-linear relationship with MoE.
- Path analyses revealed direct effect of DEN on MoR was greater than MfA while direct effect of MfA on MoE was greater than DEN. Direct effect of DEN on RS and TS were greater than MfA.
- For wood stiffness, paddle-pop had the best prediction to stem MoE at Flynn due to its vicinity to the benchmark log sample. SilviScan measurements showed good prediction for MoE for both sites. IML hammer alone and IML hammer plus core density showed similar good fit as SilviScan measurements. IML hammer plus core density or paddle-pop measurement is preferred methods for predicting stem stiffness.
- For wood strength, multiple regression predictions were weak, only IML hammer plus core density or paddle-pop measurement showed some potential to predict stem strength.

- For shrinkage, multiple regressions were weak. IML hammer plus core density combined was preferred method for prediction.

Introduction

In radiata pine, the juvenile wood, which is also called corewood (Burdon *et al.* 2004), comprises anywhere from the first seven to the first 13 rings from the pith (Gapare *et al.* 2006), and often presents issues for utilisation due to its poor mechanical properties and high distortion. As radiata pine crop rotation becomes shorter due to genetic improvement and better silvicultural regime, the proportion of juvenile wood will be higher. Besides lower wood stiffness and strength, dimensional instability is also a problem in juvenile wood, in which microfibril angle and spiral grain are larger and coupled with a significant amount of compression wood. Defects such twists, bow and crook often occur in products made from trees with a high proportion of juvenile wood harvested in fast grown plantations (e.g. Zobel and Sprague 1998). Differential wood shrinkage within a piece of timber can cause such timber deformations. Twist is the main form of instability of radiata pine timber (Cown *et al.* 1996b), and it can be explained by variation in longitudinal and tangential shrinkage (Johansson and Bäkström 2002). Bow and crook in timber can be explained mainly by variation in longitudinal shrinkage (Johansson 2003). Consequently, one of the main impediments for greater market acceptance of fast grown radiata pine wood is the dimensional instability of its juvenile core (Cown and vanWyk 2004). Therefore, wood stiffness, strength, and stability are regarded as three important traits for structural timber products. In Australia, structural timber is machine-stress-graded using Australian and New Zealand standard (AS/NZS 4063:1992). Wood stiffness, measured as Modulus of Elasticity (MoE), is one of the most important mechanical properties for structural end-uses and has a direct impact on structural timber grade outturn. Wood strength measured as Modulus of Rupture (MoR) for mechanically stress-graded timber are given in AS/NZS 1748:1997. In this report, wood stability is measured using three measures of shrinkage (Tangential, Radial, and Longitudinal shrinkage, denoted as TS, RS, and LS, respectively).

One of objectives in Juvenile Wood Initiative is to develop optimal ways to predict juvenile wood stiffness, strength and stability using wood quality traits that can be measured with relative ease and low cost. These optimal methods then can be used for breeding purpose to rank selections. To predict juvenile wood stiffness, strength and stability of radiata pine tree, consideration must be given to within-tree variation in stiffness, strength and shrinkage.

Wood stiffness (MoE) in radiata pine increases radially from pith to bark, but the greatest change occurs in the wood near the pith (Downes *et al.* 2002, Xu and Walker 2004, Wu *et al.,* 2004). In corewood zone, a rapid increase of wood stiffness occurs also in vertical direction (Xu *et al.* 2004). In general, wood stiffness follows distributions of basic wood traits such as cell wall thickness, wood density and micro-fibril angle in both radial and axial directions (Megraw *et al.* 1999). Maps of wood properties including MoE have been created using SilviScan® data in radiata pine (McKinley *et al.* 2003).

Wood shrinkage varies in tangential, radial and longitudinal directions at approximate ratios of TS:RS:LS = 20:10:1. Variation in shrinkage has been shown a predictable

pattern within a tree (Cown *et al.* 1991). Transverse (radial and tangential) shrinkage values are lower in the juvenile wood zone (near the pith), but longitudinal shrinkage values are higher. There is also variation along the stem vertical axis. Variation between two aspects within a stem may in fact reflect variation between compression and opposite wood (Harris 1997).

To predict juvenile wood stiffness, strength, and stability, understanding of relationship between mechanical performance of clear wood and physical and anatomical properties of wood such as wood density (DEN), cell wall thickness, microfibril angle (MfA), and spiral grain (SG) (Cave, 1969) would assist the selection of predictive traits. Wood density is a measure of the relative amount of solid cell wall material, and therefore an important trait for predicting strength properties (Panshin and de Zeeuw 1980). However, microfibril orientation in S2 layer of cell wall is considered to be an even more important factor contributing to wood stiffness especially in corewood zone (Xu and Walker 2004). The negative influence of spiral grain on mechanical properties of clear wood samples is also significant (Cown *et al.* 1996a, Tsehaye and Walker 1996, Cown *et al.* 1999).

Indirect prediction of stiffness using component traits has been shown to be possible (Evans and Ilic, 2001). The relationship between wood density and stiffness (MoE) or strength (MoR) was shown to be strong in radiata pine clear samples. The relationship was influenced by the position (radial and longitudinal) of the specimen within a stem (Cown *et al.* 1999). Wood density and microfibril angle have a major influence on mechanical performance of clear samples, and the two traits combined can predict MoE and MoR reliably (Donaldson 1996, Cown *et al.* 1999). One issue with using MfA is that it is relatively expensive to measure for a large population such as breeding population with progenies. Lower wood density and higher MfA are likely to be the cause of low strength in juvenile wood of radiata pine. Higher spiral grain angle also reduces the strength because wood is much stronger along the grain than across the grain. This holds in general for "cross grain" in structural lumber and boards, but, corewood stiffness seems to be less sensitive to spiral grain than outerwood stiffness (Tsehaye and Walker 1996).

Besides density and MfA, edge knots in lumber are also considered as primary determinants of its strength (Rajeshwar *et al*. 1997). Accuracy of prediction of MOR using MoE was shown to be independent of lumber density, but was improved by including knot area and knot position in the regression (Grant *et al.* 1984). While the average bending strength and stiffness of the timber were dependent on the basic density of the log, the lower fifth-percentile strength may be more dependent on knot characteristics (Bier 1986). Such influence of knots is included in the standards for visual stress-grading AS 2858-2001(2001).

The causes of shrinkage can be theoretically examined at molecular, ultrastructural, microscopic and macroscopic levels (Astley *et al.* 1997). At the molecular level, cellulose and hemicellulose are responsible for adsorption and desorption of water molecules, while lignin and extractives are retarding water penetration into cellulose. At the higher levels of wood structure, shrinkage of wood cells is considered to be dominated by tracheid structure, cell wall thickness, lumen shape, effects of rays and bordered pits, and by the microfibril angle in the S2 layer. In multi layers of wood with variable shrinkage properties (e.g. earlywood and latewood, or juvenile and

mature wood), stresses are generated due to constraints between layers (Barber and Meylan 1964, Cave 1972, Peng 2002). A hypothesis has been proposed for explaining the anisotropic transverse shrinkage, based on the result that radial cell walls in pine latewood are about 25% thicker than tangential walls. Furthermore, there is a tendency of preferential orientation of the fibrils in both cell walls in the general tangential direction (Gu *et al.* 2001). Based on wood structure, slope of grain does not cause longitudinal shrinkage directly, but a portion of tangential shrinkage is transferred in a longitudinal direction (Haslett *et al.* 1991). Similar conjecture could be made at individual cell level using microfibril angle, because individual fibers would also shrink more transversally than longitudinally (Panshin and De Zeeuw 1980). In juvenile corewood of fast grown trees with wide rings, ring curvature has a similar effect as spiral grain.

Although statistical methods cannot explain the idiosyncratic behaviour of any particular wood cut, they can predict the average trends. Partial regressions have been used to untangle the relationships between traits causing wood shrinkage. Several authors reported a non-linear relationship between longitudinal shrinkage (LS) and MfA in pines, with no relationship between LS and MfA until angles reach about 35 degrees after which the shrinkage exponentially increases with increasing MfA (Harris and Meylan 1965, Meylan 1967, Megraw 1998). Other authors found that both SG and MfA would be the best predictors of LS (Ying *et al.* 1994). Cown *et al.* (1991) found that segregation of stems into high, medium and low density classes accounted for a significant proportion of variation in volumetric shrinkage (which is in small samples dominated by transverse shrinkage). They also showed significant regression trends of increase in transverse shrinkage with increase in basic density. Significant genetic correlations between tangential shrinkage, density and MfA have also been found (Matheson *et al.* 1997). More recently, Ilic (2004) has concluded that acoustic velocity is probably the most practical measure of longitudinal shrinkage, with potential utility for identifying not only low stiffness but also distortion-prone wood.

Direct assessment of stiffness, strength, and shrinkage is time consuming expensive and destructive. Development of optimal ways to predict clear wood stiffness, strength, and shrinkage within a tree using non-destructive measurements would be of great importance to wood processors and tree breeders. The specific objectives of this study were:

- examine pith-to-bark variability of wood stiffness and strength, and tangential, radial and longitudinal shrinkage;
- examine causal relationship between stiffness, strength, shrinkage and basic wood properties using path analyses;
- develop optimal ways for prediction of clear wood stiffness, strength and shrinkage using component wood properties measurable in non-destructive ways (i.e. juvenile wood index).

Materials and Methods

The study was based on two genetic trials located in Victoria (Flynn- BR9611) and South Australia (Kromelite-BR9705) and the details of site characteristics are provided in Table 1.

Table 1. Characteristcs of the Flynn (BR9611) and Kromelite (BR9705) sites used for this study.

Site	BR9611 (Flynn)	BR9705 (Kromelite)
Date planted	6/1996	7/1997
Cambial age at time of sampling	8	7
Spacing	3.6m x 2.5m	2.74m x 2.5m
Latitude	38^{o} 14'S	37^{o} 50'S
Longitude	146^{o} 45'E	140^{o} 55'E
Elevation (m)	166	55
Annual rainfall (mm)	760	900
Soil type	Sandy loam	Sandy clay-loam
Site type	2nd rotation	2nd rotation

There were 250 treatments in 5 replications in BR9611. One tree each from replication 1 and 4 was felled to sample a stem section about 700 cm at breast height (between 0.5m and 2.0m from ground). The stem section was cut into three sub-samples for shrinkage, spiral grain and stiffness measrements. BR9705 only had 110 treatments with 5 replications. One tree each from the first three replications was sampled. Trees to be sampled in each trial have been previously increment cored with a 12 mm core and have had acoustic stress-wave measurements of the whole stem. Most plots have had two trees cored. The first trees in each plot with a 12 mm core hole and acoustically measured were sampled. The stem sections were shipped to Yarralumla for sampling stratification according to Figure A1. The measurements on standing trees, increment cores, and subsequent samples were done as follows

Wood density, MfA, MoE mesurement from increment cores: Twelve millimetre bark-to-bark increment cores were collected at breast height (1.3 m) from 980 trees at Flynn and 660 trees at Kromelite in 2003, before the stem sections were sampled in 2004. Full core density (DENc) was assessed on those samples using gravimetric method. The cores from 830 trees felled in 2004 were also assessed by SilviScan. Density (DEN_s) was obtained at 50 μm intervals, while MfA_s was averaged over 5 mm intervals, and these estimates were used to predict dynamic MoE_S (Evans 2003).

Standing tree stiffness measurement using IML readings: An IML hammer (stress wave timer (www.walesch.ch) was used to measure the standing tree time-of-flight in all trees that were assessed for density and MfA. The standing tree time-of-flight technique provides an acoustic wave velocity for the stem. This involves inserting two generic probes and attaching a sensor to the top probe. The bottom probe is tapped with the IML hammer. The IML hammer contains a strain gauge to detect the travel time of the stress wave. The distance between the probes must be known and is usually about 1m. .

The acoustic velocity is related to MoE of the wood according to the following equation:

$SWV^2 = MoE/D$

where SWV = Velocity ms^{-1} (SWV = 1/T; T = time of flight (s^{-1})); MoE = Modulus of elasticity (Pa) and D = Bulk density (kgm^{-3}), usually assumed to be 1000 kg/m^3 for radiata pine. When MoE is measured in this way it is known as dynamic MoE_{iml} in contrast to static MoE which is measured by bending. The dynamic and static measured MoE values are highly related in green and dry wood (Booker and Sorensson, 1999; Ilic, 2001).

Spiral grain measurement: A total of 466 and 308 trees were available at Flynn and Kromelite, respectively, for spiral grain sampling and measurement. Stem discs were air dried at 25±5°C, for 6 months. Each disc sample was sawn into a 3.5×5 cm diametrical flitch following a north-south axis through the stem, including at least part of the pith. Along the length of each flitch, a flat surface was created using a belt sander, to provide a plane of reference for spiral grain measurement (Figure 1 (A)). Growth rings were numbered according to their calendar year of formation, counting inwards from the cambium. A chisel and mallet were used to split the samples on the outermost boundary of each annual latewood band. Spiral grain angle was measured using a pivoting digital protractor attached to a fixed platform (Figure 1 (B)). Mean grain angle for each ring was obtained by adding the measurements on two opposing radii and dividing by two. The mean grain angle in each ring can be considered a measure of average grain angle deviation from the vertical axis of the cambial cylinder in each year of growth (e.g., Hansen and Roulund 1998).

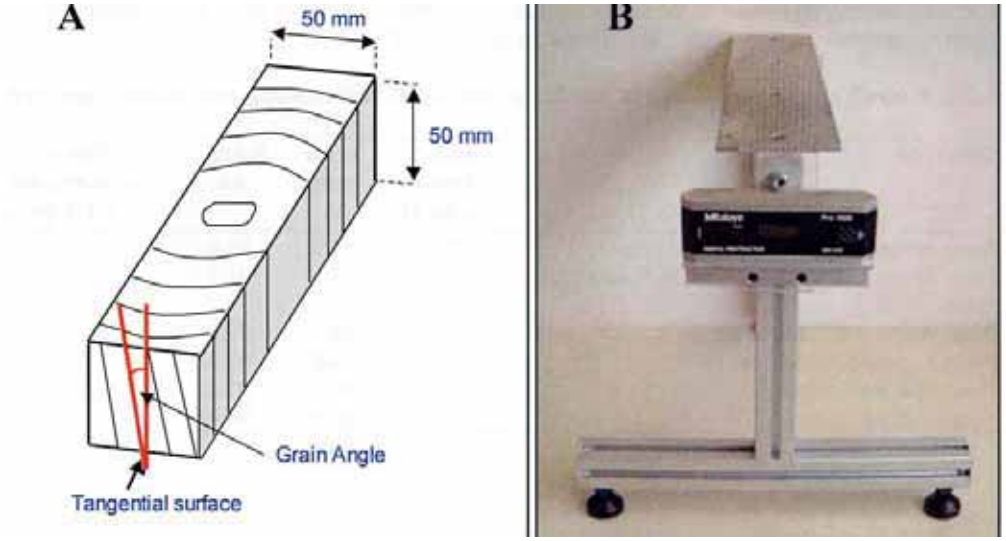

Figure 1 (A) Diametrical strip showing grain angle and (B) the fixed platform and digital protractor used to measure the spiral grain.

Dynamic MoE and shrinkage measurement on shrinkage samples:
The procedures for determining shrinkage were similar to those used by Kingston and Risdon (1961). The three samples (B, C, and D) cut from the shrinkage billet (see Figure A1) were measured initially in a green state and subsequently oven dried at 103 ± 2°C to determine shrinkage. Dynamic MoE was also measured on samples B, C, and D. Moisture content based on oven-dry weight was determined before and after reconditioning. For each of the three samples (Figure 2A), radial, tangential and

longitudinal dimensions were measured using a digital displacement gauge with readings graduated to 0.001 mm. A pneumatic ram using 200 KPa of air pressure was applied to the upper contact point, a 10 mm flat disc, while the specimens rested on a 10 mm flat disc as shown in Figure 2 (B). The shrinkage value for radial (RS), tangential (TS) and longitudinal (LS) expressed as a percentage (%) of the green measurement. Anisotropic shrinkage ratio was calculated as TS/RS. Average values of boards B and D represented the outer rings (rings 4 to 6 at Flynn; and rings 3 to 5 at Kromelite) whereas C represented the inner rings (rings 1 and 2) close to the pith. Radial gradient (pith to bark) was defined as the difference in shrinkage between outer and inner rings. However, due to ring angle caused by the wandering pith in sample C and to some extent, ring curvature close to the pith; it is generally not possible to get valid tangential and radial shrinkage values for the inner-rings close to the pith. This is partly because shrinkage close to the pith will be a function of both radial and tangential shrinkage. We made adjustments for ring curvature following Dumail and Castera (1997), but this had small overall effects.

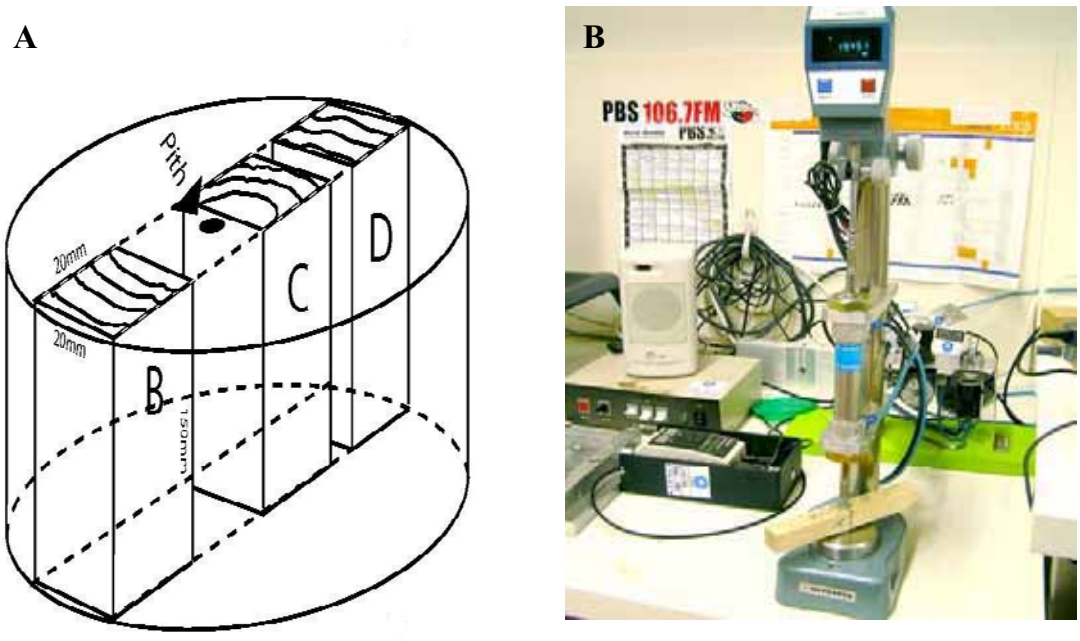

Figure 2. (A). Schematic position of sub-samples (B, C, & D) taken from three positions on each billet. (B) Digital displacement gauge used to measure shrinkage with pneumatic ram and air pressure meter.

Because samples were generated from juvenile wood, the oven dried material was in many cases distorted. This presented a problem in accurately measuring the length of samples as the curvature present exaggerated the extent of measured shrinkage. This necessitated the measurement of the maximum point of curvature and the incorporation of a correction factor to the final length measurement. A distorted sample is illustrated on Figure 3(A). The maximum curvature is measured by the jig which is shown in Figure 3 (B). The gauge is set to zero by a standard bar before a measurement is taken.

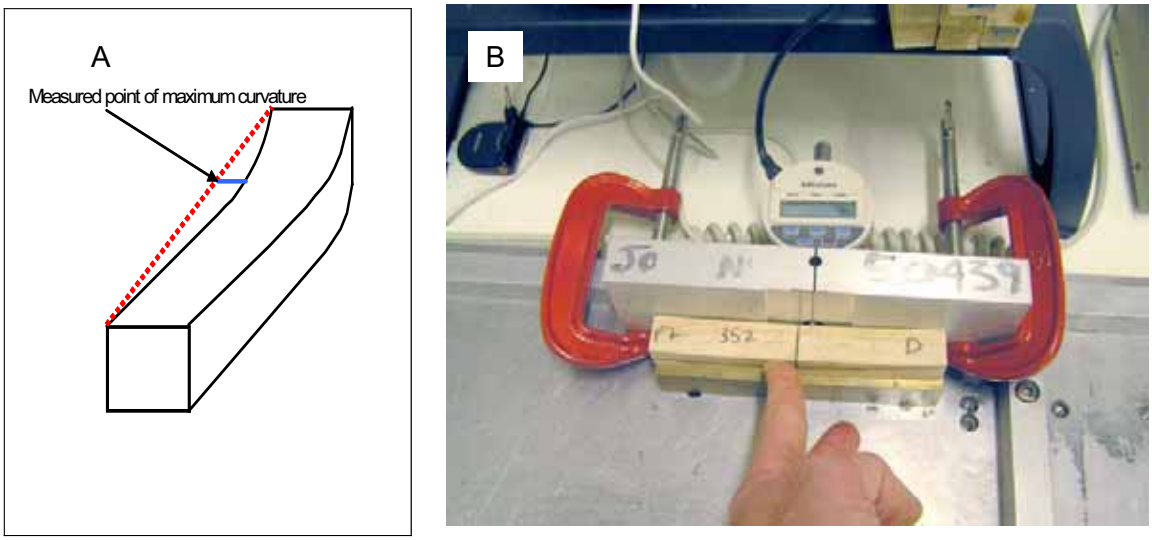

Figure 3. Distortion measurement procedure; (A) distorted sample, (B) maximum curvature measured by the jig. The gauge was set to zero by a standard bar before a measurement is taken.

Dynamic and static MoE measurement on clear wood samples: Three samples (E, F, and G) with 20 mm x 20 mm in cross section and 350 mm in length were cut from the stiffness billet (see Figure A1, bottom). Dynamic and static Modulus of Elasticity (MoE) was measured on each of the three samples. Static bending tests were carried out according to the procedure outlined by Mack (1979).

Dynamic MoE measurement using paddle-pop sample: A small clear specimen (sample H, 350 mm x 10 mm x 4 mm) was cut radially along bottom billet near the bark (Figure A1). The purpose is to assess predictivity of surface sample for the log billet. Ilic (2001b) had successfully performed such testing.

STATISTICAL ANALYSES

Calculation of benchmark stiffness, strength, and shrinkage: Before embarking on analyses, the benchmark stiffness, strength and shrinkage that we would like to predict needed to be determined. We selected area-weighted average for the sampled stem (log) as the benchmark values. Their computations are listed as below.

Average values of stiffness for stem were calculated from measurements on samples B, C, and D (acoustic) and E, F, and G (static and acoustic). Three average values of stiffness were computed for inner-rings, outer-rings and whole stem. For inner-rings (ring 1-2 from pith), the average (MoE_{in}) was obtained by averaging values for samples C and F. The F sample had two measurements (static and acoustic) averaged and the average value was given double weight due to its double size relative to sample C. The outer rings (ring 3-6 from the pith) stiffness (MoE_{out}) was obtained by averaging values for samples B, D, E, and G. The E and G samples had two measurements (static and acoustic) averaged and the average was given double weight

due to its double size relative to volume of sample B and D. The whole stem value (MoE_{stem}) was obtained by area-weighting MoE_{in} and MoE_{out} using respective core length.

Average values of strength for stem were calculated from samples E, F, and G. For inner rings, MoR_{in} was the values taken from sample F. For outer rings, MoR_{out} was obtained by averaging values for samples E and G. Whole stem average (MoR_{stem}) was obtained by area-weighting MoR_{in} and MoR_{out} using respective core length.

Average values of shrinkage (RS, TS, and LS) for stem were calculated from samples B, C, and D. For inner rings, RS_{in}, TS_{in}, and LS_{in} were the values obtained from sample C. For outer rings, values RS_{out}, TS_{out}, and LS_{out} were obtained by averaging measurements for samples B and D. The whole stem averages (RS_{stem}, TS_{stem}, and LS_{stem}) were obtained by weighting values for inner and outer rings using respective core length.

Variable transformations: Distributions of the measured traits were checked for normality using SAS interactive data analyses package (SAS Institute Inc. 2005). Most of the traits conformed to normality tests (KS statistic at $p>0.01$, significance level) except for longitudinal shrinkage and ratio of tangential shrinkage to radial shrinkage (TS/RS). Log transformation was applied to LS in order to obtain an approximately normally distributed variable, and square-root arc-sine transformation was applied to TS/RS to normalize the data and stabilise the variance.

Pith-to-bark variation analysis: Pith-to-bark variation was analysed using inner-rings and outer-rings. Procedures ANOVA and VARCOMP in SAS 9.1 (SAS Institute Inc. 2005) were used to analyse variation from inner-rings to outer-rings and from two aspects (North and South).

Path analysis: Path analyses were used to examine casual models involving direct and indirect effects of independent variables and dependent response variables (e.g., Downes *et al.* 2002). Correlation analysis quantifies linear relationship between two variables, but does not specify any cause/effect relationship. Path analysis partitions a correlation coefficient into direct effect of the casual variable and indirect effects through alternate pathways to the response. We used standardized partial regression coefficients to indicate strength and direction of direct effects (Li 1981). The standardized regression coefficient equals the value of correlation coefficient between the variable of interest and the residuals from the regression, if the variable were omitted. The objective of our path analyses was to examine how much component wood quality traits such as ring width (RW_s), DEN_s, MfA_s, SG can explain wood stiffness, strength and shrinkage.

Multiple regression analyses: To search for optimal combination of component wood variables to predict wood stiffness, strength, and shrinkage, multiple regression analyses were used. The RW_s, DEN_s, MfA_s, from increment cores, standing tree stiffness measurement from IML hammer (MoE_{iml}), dynamic MoE_{pp} from paddle-pop measurements and spiral grain angle (SG) were used as independent variables. We used the procedure PROC REG in SAS 9.1 (SAS Institute Inc. 2005) to fit multiple linear regression models. For each model, as an overall test of significance, we used multiple R^2 (r^2 for the measured y's vs. the predicted y's) and root mean square error

(RMSE). For each independent variable, we obtained a regression coefficient (*b*), with associated *t* and *p* values for conditional significance given all the other variables are in the model. Exploratory data analyses showed that dependent and independent variables were approximately normally distributed (KS statistic $p>0.05$). Independent variables were also tested for co-linearity, and inter-correlations between independent variables would make estimation difficult (SAS Institute Inc. 2005). Residual plots were examined for patterns and outliers.

RESULTS AND DISCUSSION

Wood Stiffness and Strength

Pith-to-bark variation and phenotypic correlation among wood traits: Trait means for RW_s, DEN, SG, MfA_s, MoE and MoR based on inner-rings and outer-rings and weighted averages for whole stem are given in Table 2. Variation between inner-rings and outer-rings of same tree was higher than variation between trees (for MoE VAR_{ring} =5.6 vs. VAR_{tree}=0.77). Correlations between inner and outer samples were all significant at $p<0.0001$ and were 0.56 for DEN, 0.61 for MoE, and 0.68 for MoR.

Table 2. Sample means for inner-rings (rings 1-2) and outer-rings (rings 3-6) and weighted averages for whole stem (stem) at Flynn (F) and Kromelite (K) sites.

Trait	Site	RW_s[1] (mm)	DEN[2] (kg/m^3)	SG[3] (deg)	MfA_s[1] (deg)	MoE[2] (GPa)	MoR[4] (MPa)
Stem	F	12.5	388	4.0	28.5	5.8	50.7
	K	15.1	350	3.8	31.7	4.5	40.9
Inner-rings	F	17.6	346	4.9	37.0	3.2	33.6
	K	23.7	310	4.6	37.2	2.6	30.9
Outer-rings	F	9.6	403	2.7	22.1	6.7	56.5
	K	8.8	367	3.4	27.3	5.1	49.2

[1]RW_s and MfA_s are based only on SilviScan increment cores; [2]DEN and MoE based on 7 clear-wood samples (BCDEFGH). [3]SG based on shrinkage disk samples. [4] MoR based on 3 clear-wood samples (EFG).

Generally, between-traits correlations were similar at Flynn and Kromelite for inner-rings, outer-rings or whole stem (Table 3). For whole stem, RW had non-significant correlations with DEN and MoR, and low but significant negative correlation with MoE, at both sites ($r>-0.15$). For inner-rings, RW had significant correlations with MoE (r=-0.31 at Flynn and r=-0.21 at Kromelite). Also for whole stem, DEN had a higher correlation with MoR (r=0.62 at Flynn and r=0.70 at Kromelite) than with MoE (r=0.41 at Flynn and r=0.50 at Kromelite). In contrast, MfA had a higher correlation with MoE (r=-0.60 at Flynn and r=-0.50 at Kromelite) than with MoR (r= -0.32 at Flynn and r= -0.27 at Kromelite). Similarly, SG had higher significant correlation with MoE (r=-0.24 at Flynn and r= -0.15 at Kromelite, than with MoR (r=-0.16 at Flynn and r= -0.18 at Kromelite).

Table 3. Correlations between six wood traits for inner-rings, outer-rings and whole stem (above-diagonal for Flynn and below-diagonal for Kromelite, values for stem, outer-rings, and inner-rings listed from top to bottom, correlations in bold are significant at $p<0.001$).

MoE	**0.707** **0.731** **0.663**	-0.150 -0.164 **-0.308**	**0.406** **0.390** **0.499**	**-0.604** **-0.626** **-0.418**	**-0.242** **-0.206** -0.093
0.713 **0.716** **0.661**	**MoR**	-0.075 -0.074 **-0.167**	**0.621** **0.613** **0.583**	**-0.319** **-0.351** **-0.240**	-0.160 -0.117 -0.055
-0.119 0.168 **-0.212**	0.045 0.124 -0.120	**RW**	-0.000 0.030 **-0.203**	**0.480** **0.414** **0.452**	0.142 0.035 -0.035
0.498 **0.360** **0.366**	**0.701** **0.693** **0.638**	0.008 0.003 -0.036	**DEN**	-0.119 -0.094 -0.094	-0.043 -0.022 -0.026
-0.499 **-0.565** **-0.365**	**-0.266** **-0.265** -0.123	**0.190** **0.252** **0.244**	0.046 0.033 0.12021	**MfA**	**0.270** **0.210** -0.066
-0.150 **-0.223** -0.061	-0.181 **-0.199** -0.104	0.030 0.042 **0.216**	0.042 -0.092 -0.006	0.068 0.132 -0.051	**SG**

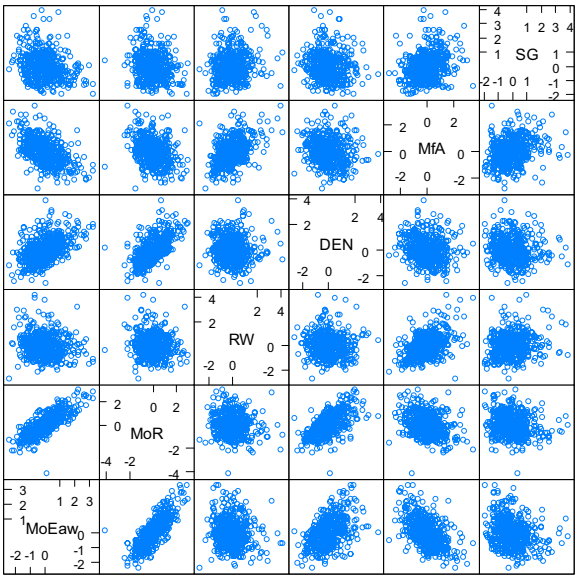

Figure 4. Average relationships between six wood variables used in path analyses.

Causation from path analyses: Six wood variables listed in Table 2 (RW_s, DEN, SG, MfA_s, MoE_{stem} and MoR_{stem}) for whole stem were standardised for path analyses (Figure 4). Path diagrams are presented in Figure 5. RW had a positive direct effect (path coefficient) on MoE $pc_{RW,MoE}$=0.175, but a negative indirect effect trough path

connecting RW, MfA and MoE (Figure 6a). The indirect effect of RW on MoE was a result of the significant positive correlation between the two traits (Table 3). Direct effect of DEN on MoE was positive and significant but less than the effect of MfA (eg. Flynn stem $pc_{DEN,MoE}$=0.405 vs. $pc_{MfA,MoE}$=-0.540). That relationship held overall at average and outer sampling strata, but for inner samples the DEN effect was equal or even higher then MfA (eg. Flynn_in $pc_{DEN,MoE}$=0.458 vs. $pc_{MfA,MoE}$=-0.353); On the other hand, direct effect of DEN on MoR was consistently higher then the effect of MfA at all sample strata (eg. Flynn stems $pc_{DEN,MoR}$=0.583 vs. $pc_{MfA,MoR}$=-0.269).

MfA had a negative and significant effect on MoE($pc_{RW,MfA}$ =-0.540, and it was higher than the effect of DEN. Direct effect of MfA on MoR was consistently negative and significant, but less so than the effect of DEN (Figure 6b). The stronger relationship of MoR with DEN than with MfA was also indirectly confirmed by comparing the relationship between MoR and sound wave velocity (SVW), which was also consistently less related to MoR than DEN (*eg.* pc_{VEL_MoE} =0.21 pc_{DEN_MoE} =0.40, not shown).

When average stem values were used to estimate MoE, MfA had a smaller direct effect on MoE than predicted MoE_s from SilviScan (pc_{MfA_MoEs}=-0.87 vs. pc_{MfA_MoE} =-0.64). This may imply that SilviScan® estimates overestimated the influence of MfA on MoE relative to other traits. Downes *et al.* (2002) also found similar relationships in their path analyses.

Direct effect of SG on either MoE or MoR was insignificant. However, SG and MfA may act jointly because of significant positive correlation between them (Table 3 and Figure 5).

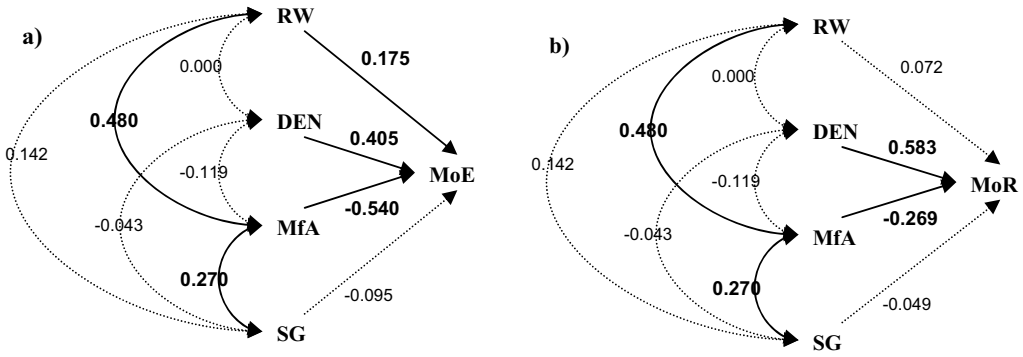

Figure 5. Path analyses for Flynn site. (A) MoE_{stem} (multiple R^2=0.49 and (B) MoR_{stem} (multiple R^2=0.45) with direct effects indicated in straight arrows and indirect paths indicated in curved lines with correlation coefficients.

Prediction of stem stiffness and strength using component wood quality traits: Paddle-pop dynamic MoE_{pp} had the best prediction to stem MoE_{stem} at Flynn (R^2=0.707, Table 4). However, MoE_{pp} had lower prediction level at Kromelite (R^2=0.310). Adding core DEN_c to MoE_{pp} did not increase R^2 significantly. Combination of RW_s, DEN_s and MoE_s showed good prediction for MoE_{stem} at Flynn (R^2=0.506) and Kromelite (R^2=0.490). Adding spiral grain to RW_s, DEN_s and MoE_s measurements did not significantly improve the regression (result not presented).

Both IML® hammer estimate (MoE_{iml}) and MoE_{iml} plus full increment core density DEN_c showed almost as good fit (R^2=0.472 and R^2=0.484 for Flynn, respectively and R^2=0.401 and R^2=0.404 for Kromelite, respectively) as the combined data from SilviScan increment core measurements. Inclusion of other growth and quality traits such as DBH and stem straightness, branch size and branch angle scores only slightly increased the R^2 values. Table 4 also indicates that, whether using increment core measurements, IML or Paddle-pop readings, prediction R^2 is always higher for outer-rings than for inner-ring samples.

Since using SilviScan measurement MoE_s would incur a higher cost relative to measurement of MoE_{pp}, MoE_{iml} and density (gravimetric method), use of MoE_{pp} or MoE_{iml} and gravimetric density for prediction of stem MoE_{stem} are preferred methods. However, one concern is that paddle-pop samples were more adjacent to samples used for calculating stem MoE_{stem}. This proximity may render the paddle-pop measurements an advantage relative to SilviScan and IML measurements.

For wood strength (MoR) multiple regression prediction was less precise (Table 4). Paddle-pop dynamic MoE_{pp} had the best prediction to stem MoR_{stem} at Flynn (R^2=0.613). The increment core measurements using SilviScan or IML hammer measurement had regression of R^2=0.222 and R^2=0.179, respectively for Flynn site. Based these weak regression relationships for strength, only Paddle-pop MoE_{pp} or IML measurement MoE_{iml} plus DEN_c (gravimetric method) might be useful to predict stem strength.

Table 4**.** Prediction goodness of fit statistics R^2 for stiffness of stem, outer-rings and inner-rings samples (MoE_{stem}, MoE_{out} and MoE_{in}) and strength of stem, outer-rings and inner-rings samples (MoR_{stem}, MoR_{out} and MoR_{in}). Predictive models include: 1) SilviScan RW_s and DEN_s; 2) SilviScan RW_s, DEN_s and MoE_s; 3) IML® hammer MoE_{iml}; 4) MoE_{iml} and increment core DEN_c; 5) MoE_{iml}, DEN_c, DBH, STEM (Stem straightness), BRS (Branch size); BRA (Branch angle) and ; 6) Paddle-pop dynamic MoE_{pp}; and 7) MoE_{pp} and DEN_c for Flynn (F) and Kromelite (K).

		RW_s and DEN_s	**RW_s, DEN_s and MoE_s**	**MoE_{iml}**	**MoE_{iml} and DEN_s**	**MoE_{iml} DBH, STEM, BRS, BRA, and DEN_s**	**MoE_{pp}**	**MoE_{pp} and DEN_s**
MoE_{stem}	F	0.147	0.506	0.472	0.484	0.494	0.707	0.708
	K	0.059	0.490	0.401	0.404	0.423	0.310	0.297
MoE_{out}	F	0.154	0.507	0.519	0.529	0.537	0.713	0.715
	K	0.091	0.535	0.425	0.440	0.452	0.289	0.321
MoE_{in}	F	0.136	0.263	0.201	0.215	0.319	0.393	0.405
	K	0.079	0.309	0.158	0.193	0.204	0.139	0.224
MoR_{stem}	F	0.126	0.222	0.130	0.176	0.179	0.613	0.619
	K	0.069	0.191	0.128	0.149	0.163	0.033	0.158
MoR_{out}	F	0.132	0.243	0.184	0.234	0.237	0.651	0.654
	K	0.092	0.217	0.155	0.194	0.202	0.162	0.174
MoR_{in}	F	0.059	0.110	0.041	0.098	0.142	0.400	0.403
	K	0.042	0.086	0.017	0.051	0.067	0.075	0.094

Wood Shrinkage

Pith-to-bark variation and correlation between shrinkage and other wood traits: Average for RS, TS, and the ratio TS/RS, LS, basic density (DEN) of the samples, dynamic MoE, RW_s and MfA_s from increment cores, and spiral grain (SG) are listed for the whole stem, inner-rings, and outer-rings in Table 5. Correlations between inner-rings and outer-rings (0.183 for RS, 0.167 for TS and 0.321 for LS) were low but significant at p=0.01 level.

Table 5 Sample means for inner-rings (rings 1-2) and outer-rings (rings 3-6) and weighted averages for whole stem (stem) at Flynn (F) and Kromelite (K) site

Trait		RS (%)	TS (%)	TS/RS	LS (%)	RW_s (mm)	DEN (kg/m^3)	SG (deg)	MfA_s (deg)	MoE (GPa)
Stem	F	3.44	5.64	1.67	0.59	12.5	375	4.0	28.5	5.33
	K	3.10	5.25	1.75	0.71	15.1	333	3.8	31.7	4.90
Inner-rings	F	2.86	4.35	1.58	1.31	17.6	329	4.9	37.0	3.33
	K	2.58	3.98	1.61	1.37	23.7	299	4.6	37.2	2.86
Outer-rings	F	3.64	6.10	1.71	0.33	9.6	392	2.7	22.1	6.17
	K	3.29	5.76	1.99	0.47	8.8	346	3.4	27.3	5.64

Due to relative large errors in measuring RS and TS of the rings next to the pith, the relationships between shrinkage and other wood traits were examined only for outer rings (3-6). RS and TS had a weak linear relationship with DEN (r^2=0.15 and r^2=0.13, respectively) and MoE (r^2=0.16, r^2=0.13, respectively, Figure 6). However, LS had a strong negative non-linear relationship with MoE (r^2>0.58) and acoustic velocity (r^2>0.77).

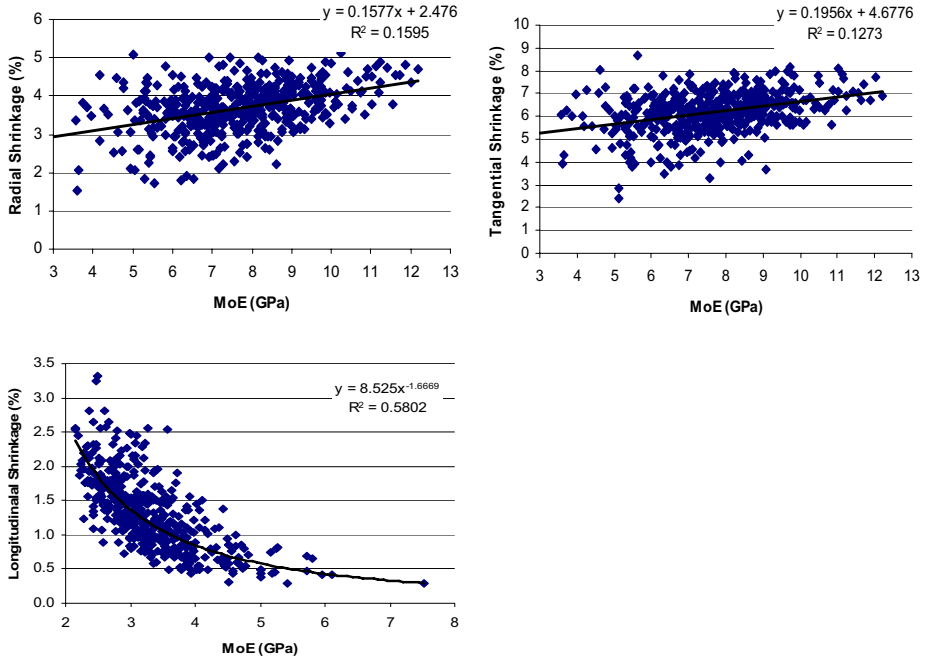

Figure 6. Relationship between RS, TS, and LS and MoE. The upper panels are for TS and RS of outer wood samples (rings 3-6) at Flynn, and the lower panel represents data for the inner (rings 1-2) samples at Flynn.

Correlations among shrinkage and wood quality traits of the outer rings (rings 3-6) are listed in Table 6. RS and TS were positively correlated, but they were both negatively correlated with LS. Correlation between LS and SG was insignificant. RS and TS were positively correlated with wood density, while longitudinal shrinkage was strongly negatively correlated with MoE and positively correlated with MfA. Correlations generally similar in magnitude were obtained for LS with the other traits using the inner samples (rings 1-2).

Table 6. Correlations for outer-rings (upper-diagonal for Flynn and lower-diagonal for Kromelite. Correlations in bold are p<0.001.

RS	**0.539**	**-0.640**	**-0.422**	-0.063	**0.460**	**0.360**	**-0.171**	-0.034
0.378	**TS**	**0.270**	**-0.527**	**-0.174**	**0.249**	**0.367**	**-0.174**	-0.010
-0.691	**0.318**	**T/R[1]**	0.005	-0.087	**-0.279**	-0.076	0.033	0.036
-0.436	**-0.409**	0.145	**LS[2]**	0.141	-0.081	**-0.656**	**0.423**	0.150
0.082	-0.086	-0.144	**-0.224**	**RW**	0.030	**-0.164**	**0.414**	0.035
0.352	0.123	**-0.216**	-0.020	-0.032	**DEN**	**0.390**	-0.094	-0.022
0.371	**0.325**	-0.119	**-0.768**	0.123	**0.274**	**MoE**	**-0.626 <.0001**	**-0.206 <.0001**
-0.053	-0.146	-0.064	**0.415**	**0.252**	0.031	**-0.618**	**MfA**	**0.210**
-0.081	-0.093	0.005	**0.190**	0.042	-0.060	**-0.244**	0.132	**SG**

[1] Square-root arc-sine transformed data. [2] Log transformed data

Path analyses: Path diagrams for RS, TS, and LS for outer rings at Flynn are presented in the Figure 7 (A, B and C). Path analyses revealed that RS was influenced mainly by DEN ($pc_{DEN,RS}$ = 0.441, p<0.001) and to a lesser extent by MfA ($pc_{MfA,RS}$=-0.119, p=0.012), with multiple R^2= 0.224. RW had a small indirect effect through MfA (Figure 7A).

TS was influenced mainly by DEN ($pc_{DEN,TS}$ = 0.225, p<0.001) and to a lesser extent by RW ($pc_{RW,TS}$=-0.114, p=0.002) and MfA ($pc_{RW,TS}$ =-0.103, p=0.043) with multiple of only R^2= 0.099. RW had small indirect effect through MfA (Figure 7B).

LS at Flynn (outer rings), was significantly influenced only by MfA ($pc_{MfA,LS}$ = 0.424, p<0.001). However, at Kromelite there was evidence of influence by RW (p<0.001), MfA (p<0.001) and SG (p<0.005). Multiple of R^2= 0.31. Again RW might also have some small indirect effect through MfA (Figure 7C). For inner rings at Flynn LS was significantly influenced by MfA ($pc_{MfA,LS}$ = 0.302, p<0.001) and DEN ($pc_{DEN,LS}$ = 0.212, p<0.001) (Figure 7D). At Kromelite, besides MfA and DEN, there was also evidence of influence by RW (p<0.005, R^2= 0.30).

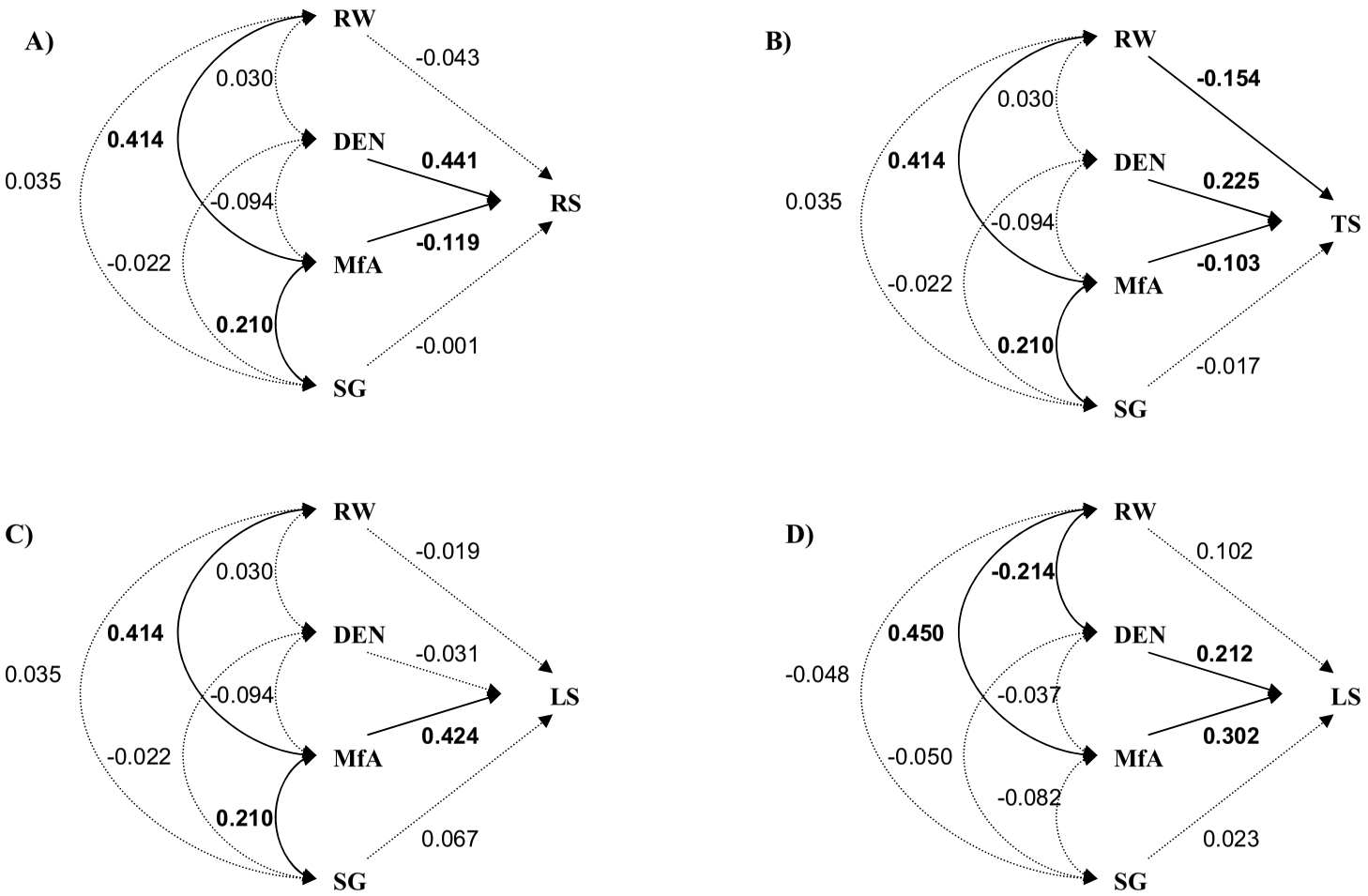

Figure 7. Path analyses diagrams for Flynn samples. (A) RS, (B) TS, (C) LS for outer-rings, and (D) LS for inner-rings.

Although R^2 values were generally low with about 20% of variation explained by RS and TS and around 30% explained by LS, the F values and model significance generally high (p<0.001).

Prediction of RS, TS, and LS using component wood traits: The IML hammer measurement MoE_{iml} plus core gravimetric denensity (DEN_c) were significant predictiors for shrinkage traits at Flynn site (Table 7). Increment core measurement RWs, DENs and MfAs using SilviScan were significant predictors for Kromelite site. All predictions only accounted for less 30% of variation except for LS_{out} at Kromelite.

Table 7**.** Prediction goodness of fit statistics R^2 for RS_{out}, TS_{out}, and LS_{out} of outer-rings and LS of inner-rings. Predictive models include: 1) increment core RW_s and DEN_s, 2) increment core RW_s, DEN_s and MfA_s; 3) IML® hammer MoE_{iml}, and 4) MoE_{iml} plus DEN_s for Flynn (F) and Kromelite (K). Models with R^2 significant are bolded.

	Site	RW_s and DEN_s	RW_s, DEN_s and MfA_s	MoE_{iml}	MoE_{iml} and DEN_s	MoE_{pp}
RS_{out}	F	0.161	0.178	0.056	**0.178**	0.096
	K	0.156	**0.170**	0.012	0.124	(0.031)
TS_{out}	F	0.077	0.090	0.063	**0.094**	0.081
	K	0.052	**0.075**	0.040	0.068	(0.033)
LS_{out}[1]	F	0.023	0.203	0.214	**0.222**	**0.262**
	K	0.064	**0.318**	0.182	0.211	(0.159)
LS_{in}[1]	F	0.043	0.128	0.066	**0.129**	0.080
	K	0.041	**0.234**	0.056	0.072	(0.107)

[1] Log-transformed data

Conclusions

Pith-to-bark variability of wood stiffness, strength, and tangential, radial and longitudinal shrinkage was very high. The variation in those wood properties followed the typical patterns described for radiata pine (Cown *et al.* 1991). There was an increase in wood density, stiffness, strength and transverse shrinkage, and a rapid decrease in longitudinal shrinkage from pith-to-bark. There was also significant variation of samples between North and South aspects. Such high within-disk variation renders sampling, measurement and prediction of whole stem averages using component wood traits highly challenging (Downes *et al.* 1977).

Stiffness and strength of wood were correlated with micro-fibril angle and wood density, as previously found in pines (Megraw *et al.* 1998, Megraw *et al.* 1999, Cown 1999). Both micro-fibril angle and wood density were important to stiffness, but micro-fibril angle had a stronger direct effect on stiffness (Cave and Walker 1994, Downes *et al.* 2002). At the same time, wood density in our study showed more direct effect on wood strength than micro-fibril angle. Density also influenced transverse shrinkage more, but micro-fibril angle had stronger effect on longitudinal shrinkage.

The velocity of sound wave propagation has been shown to be a good predictor of radiata pine wood stiffness (Ilic 2004, Wu *et al.* 2004). Acoustic tools for measuring

wood stiffness in logs and boards have been developed in the last few years (Walker and Nakada, 1999). Tools for measuring sound wave velocity in standing trees such as IML Hammer, Director ST300, and Fakoop are commercialy available (Carter 2005, FibreGen 2006). The different acoustic tools give similar estimates of velocity and MoE in standing trees (Kumar et al. 2002, 2004, Matheson *et al.* 2005). Phenotypic correlations between velocity and static MoE measured in small axial beams were typically very high for all acoustic tools. Using axial beams, modulus of elasticity is measured over only a very short distance of clearwood (about 150-300 mm), while standing tree MoE sometimes encompasses branch whorls up the stem and so perfect correlation with the stress wave velocity would not be expected.

For breeding purpose, indirect (acoustic) measurements of stiffness may be more effective than measurements of component traits such density or microfibril angle (Kumar 2004, Dungey *et al*. 2006, Kumar *et al.* 2006, Matheson *et al.* 2006). Breeding to improve wood properties requires large numbers standing trees (progenies) to be evaluated non-destructively so that superior individuals can be selected as parents (Dungey *et al*. 2005). Based on our results, we recommend the use of acoustic tools such as IML hammer together with increment core density or paddle-pop measurements for prediction of wood stiffness in evaluations of genetic trials.

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APPENDIX A

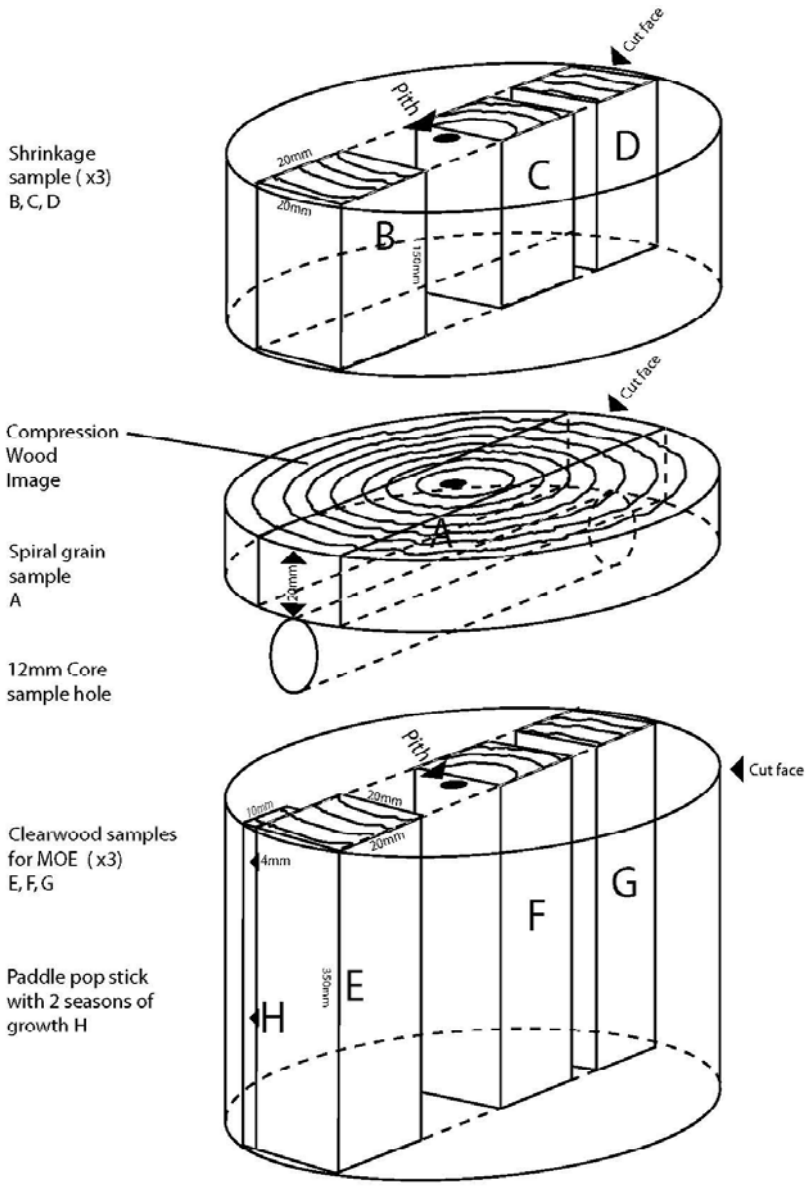

Figure A1. Sub-sampling of billets (stem sections) harvested from Flynn and Keomelite sites.

CSIRO Forestry and Forest Products
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Juvenile Wood Initiative:

Juvenile Wood Index and the Best MOE Measurement Method for Use in Assessment of Slash x Caribbean Hybrid Pine

Kevin J. Harding, Terry R. Copley, Paul J. Toon and Harry X. Wu

September *2004*

COMMERCIAL IN CONFIDENCE

CSIRO Forestry and Forest Products
PO Box E4008
Kingston ACT 2604 AUSTRALIA
Telephone: 02 62818211 Fax: 02 62818312

Executive Summary

The predictive power of regression models for wood stiffness based on acoustic velocity in logs found in the Queensland hybrid pine study are of a similar order of magnitude to those found by the project team working with radiata pine. This suggests that despite the different acoustic screening tools used and the different pine species assessed we would appear to be observing similar and perhaps somewhat generic variation in stiffness patterns and our capacity to predict them. The Fakopp standing tree tool used in this study is somewhat less sophisticated than the IML hammer used in the radiata pine part of this project or the recently developed FibreGen ST300 tool. One would hope that these newer more sophisticated tools might improve the power of prediction and add further value to the work undertaken here.

We can predict log MOE using gravimetric basic density results from 12 mm increment cores combined with a standing tree prediction of MOE using a time of flight acoustic tool. As log MOE is also linearly associated with the average MOE of sawn boards ($r = 0.702$) we have identified some capacity to rank trees into broad quality classes for juvenile wood stiffness, which is a primary focus for juvenile wood quality improvement. Additionally, the much more expensive information obtained from SilviScan analysis of cores does not appear to add significant value to this prediction once a mean density and a standing tree acoustic velocity is obtained. This provides a greatly improved capacity to screen larger numbers of progeny or ramets with low cost tools before identifying the most superior part of the population for more intensive evaluation with SilviScan and for grain spirality. The impact of MfA and spiral grain on warp in solid timber makes these more expensive to assess properties a final screening or evaluation priority to ensure that all selections are both stable during drying and in use, as well as having the high stiffness properties needed for structural applications.

Models to predict bow, spring and twist were statistically significant but their power of prediction was poor (r^2 from 0.09 for bow to 0.22 for spring) reflecting the small sample size of 120 boards recovered. This sample size would appear to have been too small to provide enough variation to obtain large differences between trait results for in-grade and out-of grade populations. It has not been suitable for modeling to develop an index of critical trait values or limits to apply when screening clones for juvenile wood quality. It seems that a different approach is needed to investigate this further and provide more definitively useful data. Two approaches that might be considered are: (a) to use trial material with a broader genetic base with more variability in wood properties, or (b) undertaking a detailed examination of heart-in boards from a production sawmill to define the characteristics of boards that achieve acceptable grade or fail to reach grade standards. The first approach requires detailed characterization of the wood properties a large sample of clones or families; this would be very expensive if this information is not already available. The latter approach should be more cost-effective whilst ensuring that a wide range of performance and quality is sampled in the study material and would therefore lend itself to modeling the interaction between the key traits that determine structural grade.

Juvenile Wood Index and the Best MOE Measurement Method for Use in Assessment of Slash x Caribbean Hybrid Pine

(This is a report for Milestone 3 of Schedule 4 for FWPRDC Juvenile Wood Initiative Project)

K.J. Harding[1], T.R.Copley[1], P.J Toon[2] and H.X. Wu[3]

[1] Horticulture and Forestry Sciences, Department of Primary Industries and Fisheries, Indooroopilly
[2] Department of Primary Industries Forestry, Department of Primary Industries and Fisheries, Gympie
[3] CSIRO, Forestry and Forest Products, Canberra

BACKGROUND

An intensive study of two slash pine X Caribbean pine F_1 hybrid (*Pinus elliottii var. elliottii* × *P. caribaea var. hondurensis*) clones was undertaken at age 7 years to investigate a range of juvenile wood properties and how they might be assessed to select for superior juvenile wood quality. Both non-destructive and destructive sampling methods were used. The primary goal for juvenile wood improvement is superior sawn wood stiffness and stability during drying to produce structural dimension lumber with the stiffness and strength properties required for MGP10 grade and with warp within the grade acceptability limits required.

This study was undertaken as part of a portfolio of studies initiated for the Forestry and Wood Products Research and Development Corporation (FWPRDC) Juvenile Wood Initiative Project, managed by CSIRO Forestry and Forest Products in partnership with the Southern Tree Breeding Association (STBA) and Queensland's Department of Primary Industries Forestry (DPIF) business group. This report draws heavily on a conference paper prepared in 2002 (Harding *et al.*, 2002).

MATERIAL AND METHODS

The two clones sampled, clones 545 and 887, were two of the best performing clones for growth and form from the first series of clonal tests established by the then Queensland Forestry Research Institute (QFRI) as part of the DPIF clonal forestry program and they share the same pedigree. This January 1995 planting was the first operational deployment of these two clones, established as two adjacent monoclonal blocks of 2.5 ha (clone 545) and 0.5 ha (clone 887). The 400m long block changes gradually from a moderately drained "grey podzolic" soil to a poorly drained (problematic) "podzol" soil type (Taylor et al. 1997). The 2.6-year good performance for growth across this site reported by Taylor et al. in 1997 was no longer evident in February 2001 (age 6 years) when the clones were increment core sampled to investigate wood density (P.G. Toon, DPIF, pers. comm.). At age 6, the trees planted on the problematic podzol soil displayed signs of yellowing foliage and reduced growth.

The clonal blocks of 545 and 887 were divided into three broad sections based on the development of the trees: best, average and worst. The decline in tree development was associated with the gradual decline in soil quality from the grey podzolic to the podzol. Within each of these three sections, six tree plots were established to sample the environmental gradient on the site. A single 12 mm diametral core was taken

from each tree at approximately 1.3 m height. Considerable variation in basic density was observed amongst the 108 (c545) and 72 (c887) ramets sampled with individual mean basic densities ranging from 323 to 406 kg/m^3 in c545 (coefficient of variation = 5.46%) and 351 to 426 kg/m^3 in c887 (coefficient of variation = 3.46%) (Toon, 2004). A stratified sample of 25 ramets from c545 and 19 ramets from c887 was selected across these basic density ranges so that the 44 ramets sampled for this study ranged from 323 to 426 kg/m^3 (that is 103 kg/m^3).

Prior to felling (April 2002, age 7.25 years), all stems were assessed using a Fakopp® stress wave velocity tool. A 'matching' increment core was removed at breast height, in the vicinity of the previous core removed for density assessment in 2001, and used for x-ray densitometric analysis on the CSIRO Division of Forestry and Forest Products SilviScan densitometer (Evans and Ilic, 2001). SilviScan results for density and microfibril angle were obtained from the best radius (least compression wood and/or other imperfections). A 3m butt log was docked from each stem and a disc was collected at the top of this 3m log to assess spiral grain for comparison with breast height values. The stress wave velocity of the butt logs was assessed with a WoodSpec® tool provided by Industrial Research Limited (Lower Hutt, New Zealand).

The butt logs were sawn into 80 x 40 mm (green dimension) structural framing at the Department of Primary Industries and Fisheries' (DPI&F) Salisbury Research Centre. The recovery varied with stem size from one to three sticks from the centre cant, with the bigger stems also yielding one or two side boards. All recovered sticks were dried and dressed to 70 x 35mm. Warp was measured on each stick with twist assessed at both the bottom and top of the stick. The stress wave velocity of each stick was assessed in the 3m length with the WoodSpec tool. A test length of 1260 mm was then docked from above the 1.3m height to avoid increment core holes, with 200mm block samples removed above and below each test length. The 1260mm test pieces were also assessed with the WoodSpec.

The block samples were assessed for air-dry density, slope of grain on inner and outer stick faces, average ring width and the ring numbers from the pith were identified so corresponding ring data values from the SilviScan and spiral grain assessments could be related to the sticks.

The test lengths were tested for stiffness (MOE) on flat and on edge, and for strength (MOR) on a Shimadzu Universal Testing Machine under four point loading in accordance with Australian/New Zealand Standard AS4063-1992 (1992).
Therefore, data available for analysis included:

- Fakopp (standing tree) stress wave velocity
- SilviScan area weighted cross-sectional and mean growth ring density
- SilviScan area weighted cross-sectional and mean growth ring microfibril angle
- SilviScan predicted MOE (from density and MfA results)
- Spiral grain from increment cores at breast height (1.3m) and 3.0m disc samples
- 3m butt log WoodSpec stress wave velocity and predicted MOE
- 3m sawn board WoodSpec stress wave velocity and predicted MOE
- 1260mm test sample WoodSpec stress wave velocity and predicted MOE

- air-dry density of 200mm long samples taken above and below each 1260mm test sample docked from each 70x35mm board (refer schematic example in Figure 1 and image in Figure 2).
- slope of grain on the inner and outer faces of the test samples assessed on the 200mm samples removed above and below the test samples
- MOE on flat and on edge for each 1260mm sample length
- MOR for each 1260mm sample length
- Bow and Spring measurements to 0.5mm on each 3m board
- Twist measurements to 0.5mm on each 3m board at both top and bottom ends.

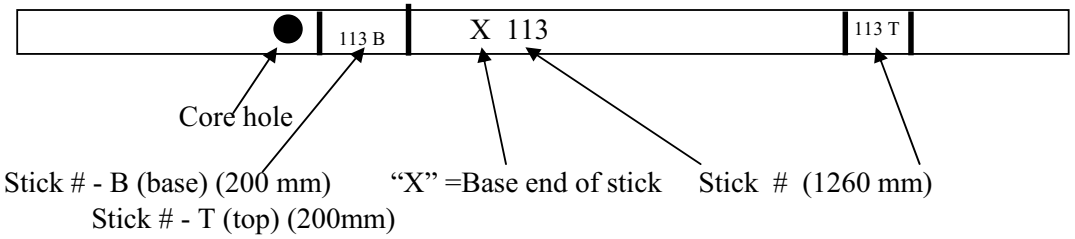

Figure 1: Schematic example of a 3m stick docked into sub-samples.

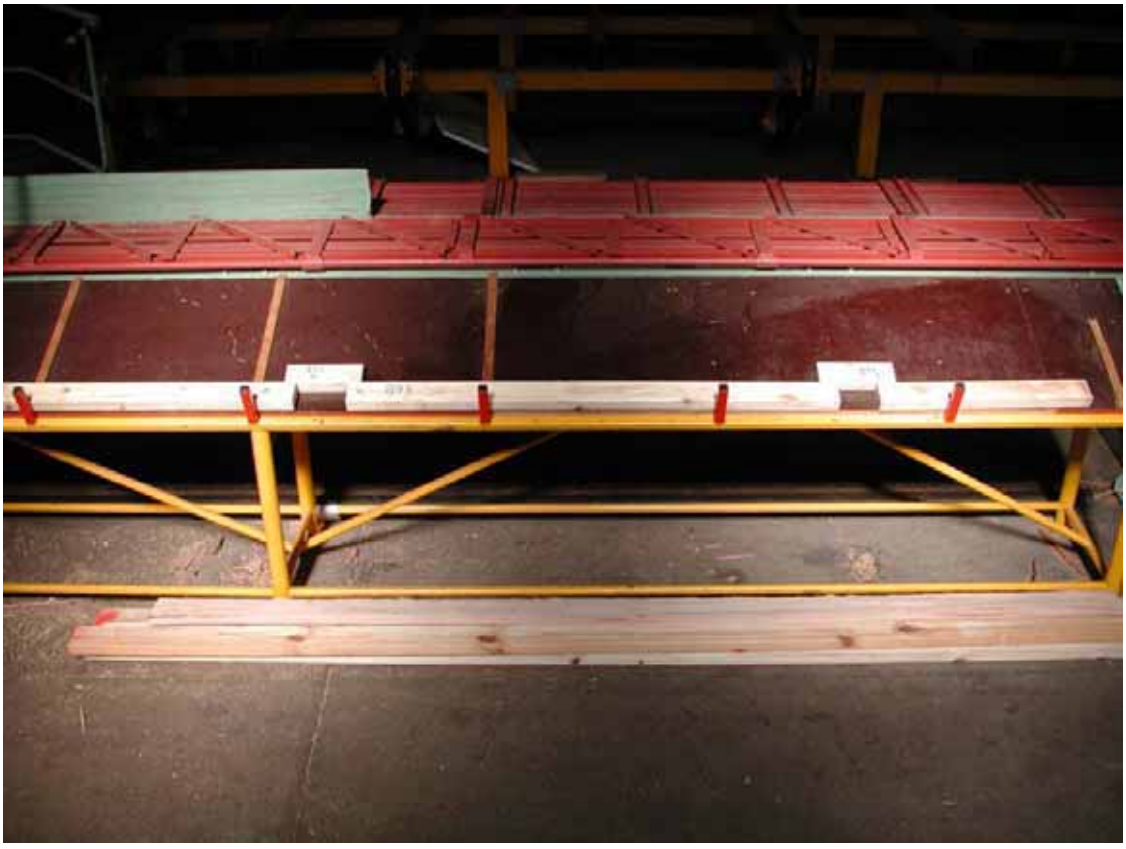

Figure 2: An example of a stick cut into the sample lengths indicated in the Figure 1 schematic.

RESULTS and DISCUSSION

In-grade recovery

The total recovery of 70 x 35 mm boards from the 44 ramets sampled was 120 boards. Twenty-eight of these boards were passed in-grade under machine-graded pine (MGP10) criteria (AS 1720.1 – 1997) for stiffness and strength but 5 of these failed the in-grade limits (AS/NZS 1748: 1997) for warp (four for twist and one for spring). Therefore, 23 boards (19.2% of total dried dressed recovery) were in-grade for MGP10, the base grade for load-bearing structural pine timber in Australia. The average air-dry density of these in-grade boards was 487 kg/m^3 (range 405 to 574 kg/m^3) and their average microfibril angle (MfA) was 18.6° (range 14.1° to 25.0°). By comparison the out-of grade boards averaged 473 kg/m^3 (range 435 to 526 kg/m^3) and their average microfibril angle (MfA) was 21.9° (range 19.5° to 24.8°).

Five ramets produced all MGP10 recovery. However, three of these were very small trees from which only a single board was recovered and the other two in-grade ramets produced two boards each, so were also smaller stems. Their average core basic density was 387 kg/m^3 (ramet values 359, 373, 391, 405 and 406 kg/m^3), average area-weighted air dry density was 519 kg/m^3 (ramet values 494, 497, 517, 517 and 572 kg/m^3) and average area-weighted MfA was 16.1° (range 11.8° to 20.3°). The complexities facing tree breeders are exemplified in these results with the ramet with the lowest density (494 kg/m^3) predicted to have the highest mean MOE (14.56 Mpa) of these ramets because it had the lowest mean MfA (11.8°). In contrast, the ramet with the highest density (572 kg/m^3) was predicted to have considerably lower stiffness (10.77 MPA) because it also had the highest mean MfA (20.3°) of these ramets. Clearly it would be unwise to apply individual trait acceptability limits independently of their interactions with other important traits when the objective trait of interest results from an interaction of their effects, as in the case of timber stiffness. An index is required to assess the combined impact of traits effecting stiffness.

SilviScan results are summarized in Table 1 for these two clones. The additional variability observed in clone 545 is not surprising given the larger sample size and planting area for this clone. However, the mean MOE values predicted using SilviScan density and microfibril angle results are high when the actual in-grade recovery results are considered. However, the SilviScan predictions use basal area-weighted results for all 6-7 growth rings in the samples assessed. During sawing it is rare for the outer 1 or 2 growth rings to be recovered in any of the sawn boards whereas these growth rings results will tend to increase the radial core means quite significantly due to the weighting. Additionally, SilviScan samples are small clear radial increment cores or cross-sections so are free from the influence of knots, sloping grain and other factors that may influence MOE results from full length sawn products. Nevertheless, these Sliviscan predictions are of value in assessing the relative merit of clones.

The definitive method of determining stiffness is to saw a tree and test the boards on a timber testing machine such as the Shimadzu timber tester used in this study. However, this is very expensive and a key goal of this study was to assess the efficacy of less costly methods of predicting stiffness by assessing individual traits or using indirect non-destructive assessment techniques.

Table 1: Comparison of means, ranges and coefficients of variation for traits measured or predicted from results obtained using the SilviScan x-ray densitometer.

		Basal area (mm^2)	Basal Area-weighted Air-dry Density (kg/m^3)	Basal Area-weighted MfA (degrees)	Predicted MOE (MPa)
CLONE 545	Mean	14875	495	18.8	10
	Min	7074	410	13.5	7.1
	Max	25762	599	23.8	12.7
	C.V.	39.39%	9.19%	15.31%	15.18%
CLONE 887	Mean	16987	519	15.4	12.4
	Min	10137	446	11.8	9.5
	Max	28208	602	20.4	15.1
	C.V.	25.22%	7.12%	12.75%	11.56%

Predicting timber stiffness

Stiffness is a function of the interaction of several wood properties and the physical structure of the tree. Clear wood properties such as density , microfibril angle (MfA) and grain spirality can interact to influence timber stiffness, which is also impacted by branch size, position and frequency as well as stem straightness and slope of grain resulting from sawing logs with sweep.

The correlation between whole core density and MfA and estimates or predictions of log stiffness in this trial are summarized in Table 2.

Table 2: Correlation coefficients (with probabilities in parentheses) for whole core average density and weighted average microfibril angle and stem or log estimates of stiffness (n = 44).

	Fak_MOE	**WS_MOE**	**A_MOE_F**	**MOE_E**	**A_S_MOE**	**S_W_MOE**
WC_EBD*	0.518 (0.0003)	0.630 (<0.0001)	0.652 (<0.0001)	0.750 (<0.0001)	0.672 (<0.0001)	0.674 (<0.0001)
S_W_MFA	- 0.675 (<0.0001)	- 0.718 (<0.0001)	- 0.405 (0.0064)	- 0.395 (0.008)	- 0.773 (<0.0001)	- 0.822 (<0.0001)

* WC_EBD = whole core extracted basic density at breast height
S_W_MFA = SilviScan basal area weighted microfibril angle
Fak_MOE = standing tree MOE prediction from Fakopp time of flight reading
WS_MOE = 3m log MOE estimate from Wood Spec time of flight reading
A_MOE_F = average MOE of the sticks sawn from each log and tested on a Shimadzu timber testing machine – average of on flat tests in each direction

MOE_E = average MOE of the sticks sawn from each log and tested on edge on a Shimadzu timber testing machine
A_S_MOE = average of the SilviScan MOE predictions for each growth ring
S_W_MOE = SilviScan basal area weighted average MOE prediction

It is clear that whole core basic density is a better predictor of MOE on edge rather than on flat. It also displays a similar level of correlation with log (WoodSpec) and SilviScan predictions of MOE and a lower correlation with standing tree (Fakopp) predictions. The correlations between SilviScan MfA and acoustic tool predictions of MOE are understandably stronger than density given that acoustic velocity is a function of the square of MfA and density. For this Queensland pine hybrid material the relative strength of correlation between density and Shimadzu MOE averages versus the MfA correlations indicates that density is a stronger prediction of early juvenile wood stiffness. The latter reflects a higher average density than radiata pine and the generally low microfibril angle observed in this material.

To develop a stiffness index (regression equation) to predict stiffness of the boards (sticks) sawn from each log, correlation and stepwise regression analyses were conducted using wood characteristics measured in this project. Seven significant traits were selected from a correlation study of 12 wood traits measured on each board and used in a stepwise regression. The best regression (stiffness index) for board stiffness (on flat) was derived as:

MoE_board = 6.8975-0.11045*Spring-0.03440*Bow-0.16363* S_W_MFA
+ 0.00962* S_W_DEN

where S_W_MFA and , S_W_DEN are SilviScan microfibril angle and density weighted by area,. All four variables are statistically significantly at 5% probability level and the R^2 is 0.492.

Wood Spec predicted MOE of log was very highly significantly and moderately to strongly correlated with Shimadzu MOE on flat (0.702), and standing tree Fakopp MOE (0.874). Given these relationships we decided to evaluate predictive regression models using the REG procedure of SAS STAT to predict Wood Spec log MOE. This mirrored work in radiata pine being undertaken for this project (C. Matheson, pers. comm.), which assessed log acoustic velocities with a Director tool and assessed the predictive capacity of several standing tree tools. A regression using whole core basic density and Fakopp MOE prediction was very highly significant (Pr>F = <0.0001) in predicting 80 % of the variation in Wood Spec MOE (r^2 = 0.8073). Adding SilviScan variables (weighted density, MfA and predicted MOE) improved this coefficient of prediction (r^2 = 0.8386) but the model parameters were not significant except for Fakopp.

These r^2 values are of a similar order of magnitude to those found by the project team working with radiata pine (Matheson, pers. comm.). This gives us some confidence that despite the different tools used and the different pine species assessed we would appear to be observing similar and perhaps somewhat generic variation.

It therefore appears that we have a strong prediction of log MOE from the gravimetric assessment of basic density using a 12 mm increment core combined with a standing tree prediction of MOE using a time of flight acoustic tool. As log MOE is also linearly associated with the average MOE of sawn boards (r = 0.702) we have some reliable capacity to rank trees into broad quality classes for juvenile wood stiffness which is a primary focus for juvenile wood quality improvement. Additionally, the much more expensive information obtained from SilviScan analysis of cores does not appear to add significant value to this prediction once a mean density and a standing tree acoustic velocity is obtained. This suggests a greatly improved capacity to screen larger numbers of progeny or ramets with low cost tools before identifying the most superior part of the population for more intensive evaluation with SilviScan and for grain spirality. The impact of MfA and spiral grain on warp in solid timber makes this a final screening or evaluation priority to ensure that all selections are both stable during drying and in use as well as having high stiffness properties.

Warp

Nearly a third of the recovered boards (39 out of 120) exceeded maximum permissible warp allowances (AS/NZS 1748: 1997), mostly for twist and spring. This result was expected given the age of this material when sampled (7 years), meaning that all boards sawn from these ramets consisted entirely of juvenile wood. It was hoped that, given the large variation observed in basic density, significant levels of variation might be found in other wood traits resulting in a broad range of sawn board properties and grades. However, the correlations between wood traits and warp results have not been consistent (refer to Tables 3 and 4) or have displayed only small differences between in-grade and out-of-grade boards. The permissible allowances under the standard are 7mm and 9mm respectively for twist and spring. The mean differences between the in-grade and out-of-grade boards are large but their magnitude is not reflected in the spiral grain or microfibril angle results that would be expected to influence expression of twist and spring respectively.

The sample size would appear to have been too small and narrowly based to provide enough variation to obtain large differences between trait results for in-grade and out-of grade populations. It has not been suitable for modeling to develop an index of critical trait values or limits to apply when screening clones for juvenile wood quality. It seems that a different approach is needed. Two approaches considered are: (a) to use trial material with a broader genetic base with more variability in wood properties, or (b) undertaking a detailed examination of heart-in boards from a production sawmill to define the characteristics of boards that achieve acceptable grade or fail to reach grade standards. The first approach requires detailed characterization of the wood properties a large sample of clones or families; this would be very expensive if this information is not already available. The latter approach should be more cost-effective whilst ensuring that a wide range of performance and quality is sampled in the study material and would therefore lend itself to modeling the interaction between the key traits that determine structural grade.

Table 3: Trait means and ranges for wood properties relating to 107 in-grade versus 13 out-of-grade boards assessed for permissible twist allowance in 3.0m long structural size (70x35mm) boards sawn from forty-four 7-year-old ramets of two slash × Caribbean pine clones.

GRADE		TWIST (mm)	Mean air dry density (kg/m^3)	Maximum Slope of Grain (degrees)	Mean MfA (degrees)	Standard Deviation of Growth Ring MfA means	Average 1.3m and 3.0m core/disc spiral grain (degrees)	Standard deviation of 1.3m and 3.0m core/disc spiral grain	Average of Increment core Spiral grain at 1.3m (degrees)	Standard Deviation of Increment core Spiral grain at 1.3m	Average of Spiral grain at 3.0m (degrees)	Standard Deviation of Spiral grain at 3.0m
IN	Mean	3.7	407	2.9	21.11	4.73	2.79	1.64	2.74	1.39	2.85	1.16
	Maximum	7.0	572	6.5	31.74	10.41	5.04	2.97	5.58	3.45	5.80	4.09
	Minimum	0.0	346	0.5	13.65	0.72	0.81	0.28	0.28	0.04	0.76	0.03
OUT	Mean	11.8	416	3.2	22.10	6.77	3.14	1.05	3.00	1.19	3.28	0.73
	Maximum	19.0	469	5.0	25.14	11.05	4.96	1.91	5.01	2.72	5.27	1.38
	Minimum	8.0	375	2.0	17.80	4.30	1.49	0.56	0.92	0.29	1.88	0.21

Table 4: Trait means and ranges for microfibril angle mean and standard deviation of growth ring values relating to 94 in-grade versus 26 out-of-grade boards assessed for permissible spring allowance in 3.0m long structural size (70x35mm) boards sawn from forty-four 7-year-old ramets of two slash × Caribbean pine clones.

GRADE		SPRING (mm)	Mean MfA (degrees)	Standard Deviation of Growth Ring MfA means
IN	Mean	4.3	20.80	4.94
	Max	9.0	29.93	11.05
	Min	1.0	14.08	0.72
OUT	Mean	12.4	22.72	5.00
	Max	19.5	31.74	9.92
	Min	9.5	13.65	0.98

The REG procedure of SAS STAT was used to predict bow, spring and twist using combinations of variables that displayed significant correlations with these measures of warp, such as: MfA, average spiral grain, Wood Spec MOE predictions in 3m boards and 1.2 m long sample tests boards. Although the regressions models produced were significant their power of prediction was poor (r^2 from 0.09 for bow to 0.22 for spring) reflecting the small sample size and other issues discussed above.

CONCLUSION

The predictive power of regression models for wood stiffness based on acoustic velocity in logs found in the Queensland hybrid pine study are of a similar order of magnitude to those found by the project team working with radiata pine. This suggests that despite the different acoustic screening tools used and the different pine species assessed we would appear to be observing similar and perhaps somewhat generic variation in stiffness patterns and our capacity to predict them. The Fakopp standing tree tool used in this study is somewhat less sophisticated than the IML hammer used in the radiata pine part of this project or the recently developed FibreGen ST300 tool. One would hope that these newer more sophisticated tools might improve the power of prediction and add further value to the work undertaken here.

We can predict log MOE using gravimetric basic density results from 12 mm increment cores combined with a standing tree prediction of MOE using a time of flight acoustic tool. As log MOE is also linearly associated with the average MOE of sawn boards ($r = 0.702$) we have identified some capacity to rank trees into broad quality classes for juvenile wood stiffness, which is a primary focus for juvenile wood quality improvement. Additionally, the much more expensive information obtained from SilviScan analysis of cores does not appear to add significant value to this prediction once a mean density and a standing tree acoustic velocity is obtained. This provides a greatly improved capacity to screen larger numbers of progeny or ramets with low cost tools before identifying the most superior part of the population for

more intensive evaluation with SilviScan and for grain spirality. The impact of MfA and spiral grain on warp in solid timber makes these more expensive to assess properties a final screening or evaluation priority to ensure that all selections are both stable during drying and in use, as well as having the high stiffness properties needed for structural applications.

Models to predict bow, spring and twist were statistically significant but their power of prediction was poor (r^2 from 0.09 for bow to 0.22 for spring) reflecting the small sample size of 120 boards recovered. This sample size would appear to have been too small to provide enough variation to obtain large differences between trait results for in-grade and out-of grade populations. It has not been suitable for modeling to develop an index of critical trait values or limits to apply when screening clones for juvenile wood quality. It seems that a different approach is needed to investigate this further and provide more definitively useful data. Two approaches that might be considered are: (a) to use trial material with a broader genetic base with more variability in wood properties, or (b) undertaking a detailed examination of heart-in boards from a production sawmill to define the characteristics of boards that achieve acceptable grade or fail to reach grade standards. The first approach requires detailed characterization of the wood properties a large sample of clones or families; this would be very expensive if this information is not already available. The latter approach should be more cost-effective whilst ensuring that a wide range of performance and quality is sampled in the study material and would therefore lend itself to modeling the interaction between the key traits that determine structural grade.

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Juvenile Wood Initiative:

Genetic Control of Juvenile Wood Properties in Radiata Pine, as Determined by SilviScan

Brian S. Baltunis, Mike. B. Powell and Harry X. Wu

***June* 2006**

COMMERCIAL IN CONFIDENCE

CSIRO Forestry and Forest Products
PO Box E4008
Kingston ACT 2604 AUSTRALIA
Telephone: 02 62818211 Fax: 02 62818312

Executive Summary

A total of 1640 wood increment cores were sampled from 343 families in two STBA genetic trials at Flynn, Victoria (9 years old) and Kromelite, South Australia (8 years old BR9705). Ring width, density, MFA, and MOE from pith to bark were assessed by SilviScan. Growth rate was greater at BR9705 for the first few growth rings than at BR9611, but wood density was higher at BR9611. All three juvenile wood properties showed substantial genetic variation. Juvenile wood core length had the lowest heritability (h^2 =0.09 - 0.23) while wood density showed the highest heritability (h^2 =0.63 - 0.77). MFA and MOE also had high heritability (h^2 =0.43 - 0.63 for MFA, h^2 =0.36 - 0.67 for MOE). There was little genotype x environment interaction for the three juvenile wood properties across two sites. Among density, MFA, and MOE, the highest genetic gains could be achieved by selecting for whole core area weighted MOE. Since MOE and MFA, and MOE and density had favourable genetic correlations, selection for MOE directly will produce the greatest improvement in overall stiffness of the corewood in radiata pine while still increasing density and reducing microfibril angle. These results indicate selection for increased stiffness and density and reduced microfibril angle in juvenile radiata pine are achievable. Genetic gains between 15 and 40% are predicted for whole core MOE with selection intensity between 1 to 10%.

All of the juvenile wood quality traits had unfavourable genetic correlations with growth (-0.6, 0.27, -0.59 between growth and density, MFA, and MOE, respectively), indicating that selection for increased density and MOE, and reduced MFA will result in a genetic loss or reduced growth. Also direct selection for core length would result in decreases in density and MOE and an increase in average microfibril angle throughout the corewood For example, selection for area weighted MOE at BR9611 would result in a 4.5 to 6.8% genetic loss in core length with 10 to 1% selection intensities, respectively. Selection for optimal balance between growth and wood quality traits is important for further radiata pine breeding program, and selection and breeding strategies to overcome such high negative genetic correlation need be developed. There may be potential for selection of correlation breakers, to improve juvenile wood properties with minimum adverse effect on growth in radiata pine.

Genetic Control of Juvenile Wood Properties in Radiata Pine, as Determined by SilviScan

Brian S. Baltunis, Mike B. Powell and Harry X. Wu

Introduction

Tree improvement programs have historically placed their primary emphasis on improving stem volume production. Secondary traits, such as disease resistance, form, and wood quality, have received less attention. The first generation of improvement of radiata pine (*Pinus radiata* D. Don) in Australia began in the 1950s. Estimated gains in volume after the first generation of breeding of radiata pine were as much as 30% over unimproved seedlots (Wright and Eldridge 1985; Matheson *et al.* 1986), and as of 1990, 100% of the annual planting of radiata pine was from improved selections (Sultech Report 1999). As a result of the intensive selection for growth rate, plantations are producing merchantable trees at a much faster rate resulting in harvesting trees at a much younger age.

A major concern with shortening the rotation is that there is a much greater proportion of juvenile wood (corewood) with much less desirable properties than mature wood (Zobel 1981). Specifically, juvenile wood has lower density, thinner cell walls, shorter tracheids, and higher microfibril angle (MFA) than mature wood (Zobel 1981; Megraw 1985; Cown 1992) leading to both a lesser quantity and lower quality of product. For example, Kibblewhite and Lloyd (1983) indicated that the lower densities and fibre dimension of juvenile radiata pine wood are expected to produce a poorer quality product. In addition, as the percent of juvenile wood increases, stiffness or modulus of elasticity decreases (Kretschmann and Bendtsen 1992). For these reasons, wood property traits have begun to receive more attention from forest industry and tree improvement programs (Jayawickrama and Carson 2000; Jayawickrama 2001; Atwood *et al.* 2002; Powell *et al.* 2004; Byram *et al.* 2005).

The one wood property that has received the most attention has been wood density or specific gravity (Zobel and van Buijtenen 1989). Density has often been considered the most important trait in describing wood quality (Zobel 1981; Megraw 1985; Burdon and Low 1992). Density can be measured relatively easily and inexpensively compared to other wood quality traits. Although density has been reported to be under strong genetic control compared to growth traits (Zobel and van Buijtenen 1989; Harding 1990; Zobel and Jett 1995), other wood quality traits such as microfibril angle and wood stiffness, may be equally or more important in improving overall product quality. Wood stiffness or modulus of elasticity (MOE) may be the most important wood quality trait for structural lumber, and is derived from both density and microfibril angle. In fact, Evans and Ilic (2001) reported that MFA together with density accounted for 96% of the variation associated with MOE in *Eucalyptus delegatensis* R.T. Baker. Similarly, nearly 93% of the variation in MOE in loblolly pine (*P. taeda* L.) was accounted for by MFA and density (Megraw *et al.* 1999). Previous reports of MFA and MOE indicate that these traits are also under strong genetic control (Lindstrom *et al.* 2004; Dungey *et al.* 2006). Furthermore, recent advances in technology such as SilviScan® (Evans 1994) have allowed these other wood quality traits to be measured more efficiently.

Juvenile wood can account for as much as 85% of the merchantable volume of 15 year old loblolly pine, and in 30 year old trees, 30% of the merchantable volume can be juvenile wood (Zobel and van Buijtenen 1989). Similarly, Cown (1992) reported that approximately 35% of volume in a 25 year old butt log of radiata pine was juvenile wood, and this proportion increased up to 90% in the uppermost logs. Consequently, any reduction of the juvenile core or genetic improvement in the quality of juvenile wood could have broad economic implications for forest industry. However, with multiple-trait breeding objectives (*e.g.*, growth, density, etc.) consideration of correlated traits will be essential. For example, in several previous studies, wood density and growth were negatively correlated, and therefore, selection for growth rate has slightly reduced wood density in radiata pine (Dean *et al.* 1983; Zobel and van Buijtenen 1989; Dean 1990; Cotterill and Dean 1990; Burdon and Low 1992; Jayawickrama 2001; Kumar 2004; Li and Wu 2005). Other unfavourable correlations may exist among juvenile wood properties and growth. In order to address unfavourably correlated traits in breeding programs, one option may be to assign selections to elite populations (Byram *et al.* 2005) or breeds (Jayawickrama and Carson 2000) based on their specific traits, *e.g.*, high wood density, structural timber, growth and form, while maintaining a broader genetic diversity in the main population.

The overall focus of this research was to explore the potential for improving juvenile wood properties in radiata pine by identifying individuals with desirable traits, *e.g.*, high juvenile density, low microfibril angle, and high stiffness (MOE). Specifically, the objectives of this study were to (*i*) determine heritability estimates of three key wood property traits in juvenile radiata pine (density, MFA , and MOE), (*ii*) determine the genetic stability of these traits by estimating the genetic correlations across sites, (*iii*) determine the genetic correlations throughout the profile of the core from pith to bark for each trait, (*iv*) determine the genetic correlations among density, MFA, MOE, and growth (as measured by ring width and core length), and (*v*) discuss the implications associated with selection of correlated traits for improvement of juvenile wood properties and growth.

Materials and Methods

Field trials and genetic material

Since the early 1980s, breeding and selection of radiata pine in Australia has been conducted by the Southern Tree Breeding Association (STBA). The STBA established a series of progeny trials in 1996 and 1997 from 2nd generation selections. A total of about 460 families were planted in 30 progeny tests in order to form the population for 3rd generation selections (Powell *et al.* 2004). Two of these 2nd generation radiata pine progeny trials were utilized in this study (Table 1). These trials contained a total of 343 families derived from both full-sib crosses from single-pair matings and polymix crosses. Sixteen families and 41 parents were common among the crosses tested across these two sites.

Table 1. Description of two radiata pine 2nd generation full-sib family progeny trials and sampling details for juvenile wood properties.

Trial	BR9611	BR970
Location	Flynn, Victoria Australia	Kromelite, South Australia Australia
Latitude	38 ^{O} 14'S	37 ^{O} 50'S

Longitude	146 ^{O} 45'E	140 ^{O} 55'E
Elevation (m)	166	55
Annual rainfall (mm)	760	900
Soil type	Sandy loam	Sandy clay-loam
Date planted	June 1996	July 1997
Cambial age	7	6
Total number of families	249	110
Replications sampled	2	3
Trees sampled per family	4	6
Total trees sampled	980	660

Sampling

In total 980 trees were sampled from BR9611, while 660 trees were sampled at BR9705 (Table 1). Two trees per family in each of two reps were sampled at BR9611, and two trees per family in each of three reps were sampled at BR9705. Twelve millimetre bark-to-bark increment cores were collected at breast height (1.4 m) from these 1640 trees and assessed by SilviScan®. Density was obtained at 50 μm intervals, while MFA was averaged over 5 mm intervals. Dynamic MOE was then predicted from these estimates (Evans 2003). Growth rings were assigned from pith to bark and ring widths measured. Basal area was then calculated for each growth ring, and measurements of density, MFA, and MOE were weighted by their individual ring basal areas. In addition whole core estimates were determined for all of the growth and wood quality traits.

Statistical analyses

All of the juvenile wood properties (*e.g.,* density, MFA, MOE) and growth (*e.g.*, core length, area) traits were analysed in ASREML (Gilmour *et al.* 2002) using an individual tree linear mixed-effects model for both sites individually and jointly. Full-sib family effects were negligible in early runs and were subsequently not included in analyses. Results from single site analyses were used to obtain starting values for the pooled site analyses. Both heterogeneous additive and error effects were assumed. In addition, bivariate analyses were conducted in order to estimate the genetic correlation between traits. The following general model was used to estimate variance components, genetic parameters, and to predict breeding values:

[1] $\mathbf{y}_i = \mathbf{X}_i\mathbf{b}_i + \mathbf{Z}_i\mathbf{a}_i + \mathbf{e}_i$,

where $\mathbf{y}_i$ is the vector of observations indexed (*i*) by trial in the case of single trait analyses across sites, or by trait in the case of bivariate analyses, $\mathbf{b}_i$ is the vector of fixed effects (*i.e.*, mean, trials and replications within trials) and $\mathbf{X}_i$ is the known incidence matrix relating the observations in $\mathbf{y}_i$ to the fixed effects in $\mathbf{b}_i$ where

$$\mathbf{X}_i\mathbf{b}_i = \begin{bmatrix} \mathbf{X}_1 & \mathbf{0} \\ \mathbf{0} & \mathbf{X}_2 \end{bmatrix} \begin{bmatrix} \mathbf{b}_1 \\ \mathbf{b}_2 \end{bmatrix},$$

$\mathbf{a}_i$ is the vector of random genetic effects of individual genotypes ~MVN $(\mathbf{0}, \mathbf{G} \otimes \mathbf{A})$ where $\mathbf{G} = \begin{bmatrix} \hat{\sigma}^2_{a_1} & \hat{\sigma}_{a_1 a_2} \\ \hat{\sigma}_{a_1 a_2} & \hat{\sigma}^2_{a_2} \end{bmatrix}$ and $\mathbf{A}$ = additive genetic numerator relationship matrix, $\mathbf{Z}_i$ is the known incidence matrix relating observations in $\mathbf{y}_i$ to the genetic effects in $\mathbf{a}_i$, $\hat{\sigma}^2_{a_i}$ is the additive genetic variance, $\hat{\sigma}_{a_1 a_2}$ is the genetic covariance between additive effects across sites, $\mathbf{e}_i$ is the random vector of residual terms ~MVN$\left(\mathbf{0}, \begin{bmatrix} \mathbf{I}_1 \hat{\sigma}^2_{e_1} & \mathbf{0} \\ \mathbf{0} & \mathbf{I}_2 \hat{\sigma}^2_{e_2} \end{bmatrix}\right)$, $\hat{\sigma}^2_{e_i}$ is the residual variance for each trait, $\mathbf{I}_i$ is the identity matrix of dimension equal to the number of observations in each trial in the case of analysis of a single trait, and $\mathbf{0}$ is the null matrix. When multiple measurements are made on the same individual then a covariance exists among the measurements. Therefore, in the case of the bivariate analyses, correlated residuals were taken into account.

Estimates of heritability were obtained for each trait at each site using the variance components from the single trait analyses across sites. Standard errors were calculated using the Taylor series expansion method (Kendall and Stuart 1963; Namkoong 1979; Huber *et al.* 1992; Dieters 1994).

[2] $\hat{h}^2_i = \dfrac{\hat{\sigma}^2_{a_i}}{\hat{\sigma}^2_{p_i}} = \dfrac{\hat{\sigma}^2_{a_i}}{\hat{\sigma}^2_{a_i} + \hat{\sigma}^2_{e_i}}$ is the individual tree narrow-sense heritability for each trait at each trial, and $\hat{\sigma}^2_{p_i}$ is the phenotypic variance.

In order to measure the extent of genotype x environment interaction for each of the traits, type B genetic correlations were calculated. Standard errors were calculated using the Taylor series expansion method (Kendall and Stuart 1963; Namkoong 1979; Huber *et al.* 1992; Dieters 1994).

[3] $\hat{r}_{B_{ADDITIVE}} = \dfrac{\hat{\sigma}_{a_1 a_2}}{\sqrt{\hat{\sigma}^2_{a_1} \hat{\sigma}^2_{a_2}}}$ is the type B genetic correlation of additive effects across sites. A value of $\hat{r}_{B_{ADDITIVE}}$ near one indicates little genotype x environment interaction, while a low $\hat{r}_{B_{ADDITIVE}}$ indicates extensive genotype x environment interaction and additive effects were not stable across sites.

Similarly, genetic correlations between multiple traits measured on the same individual (type A) were calculated from variance component estimates from the bivariate analyses in order to measure the genetic relationship between traits. In addition phenotypic correlations are also reported. Correlations between traits can range between -1 to 1, where negative values indicate a negative relationship and values close to zero indicate the two traits are independent.

[4] $\hat{r}_{A_{ADDITIVE}} = \dfrac{\hat{\sigma}_{a_x a_y}}{\sqrt{\hat{\sigma}^2_{a_x} \hat{\sigma}^2_{a_y}}}$ is the genetic correlation between traits where $\hat{\sigma}_{a_x a_y}$ is the covariance between additive effects of the two traits.

Selection for each juvenile wood property on a whole core area weighted basis was performed and the genetic response was predicted using a theoretical gain formula (Falconer and Mackay 1996) under varying selection intensities (Becker 1984).

[5] $\Delta g = i\hat{h}^2\hat{\sigma}_P$ is the genetic response for selection of each trait where i is the selection intensity associated with selection of 10%, 9%, …, 2%, or 1% of the population (see Becker 1984), $\hat{h}^2$ was defined above, and $\hat{\sigma}_P$ is the square root of the phenotypic variation as defined above.

Density is relatively easy and inexpensive to measure, and consequently, it is the most likely wood quality trait to be incorporated into a breeding program. Therefore, the correlated genetic response in MFA, MOE, and growth associated with indirect selection of area weighted density was predicted (Searle 1965). In addition the genetic response for indirect selection for other traits was determined. Indirect selection was also used to predict the genetic response in MOE throughout the profile of the core.

[6] $\Delta g = i\hat{h}_x\hat{h}_y\hat{r}_{A_{ADDITIVE}}\hat{\sigma}_{P_y}$ is the genetic response in the target trait y when trait x is selected, $\hat{h}_x$ and $\hat{h}_y$ are the square roots of heritability estimates for the selected and target traits, respectively, i and $\hat{r}_{A_{ADDITIVE}}$ as defined above, and $\hat{\sigma}_{P_y}$ is the square root of the target trait's phenotypic variance. In all cases, the genetic response was presented as the % gain over the population mean at each trial. Additionally, the genetic gain over the entire population mean was also calculated based on estimates of variance components and genetic parameters from combined-site analyses assuming homogeneous additive and residual variances. As a result, breeding value predictions across sites were then adjusted and placed on a common scale for selection and gain calculations.

Results and Discussion

General trends in growth, density, MFA, and MOE

Direct comparisons between measurements at the two trials may be somewhat misleading since the Flynn trial (BR9611) was established one year earlier than the trial at Kromelite (BR9705). Nevertheless, trait means for both sites are presented together since the trials originated from the same 2nd generation radiata pine breeding population. As a result of being one year older, overall growth was greater at BR9611 than at BR9705. The average core length at BR9611 was 75.5 mm, whereas at BR9705, the mean core length was 72.6 mm (Table 2; Table 3). Although overall growth was greater at BR9611, generally growth rate was greater at BR9705 for the first few growth rings as evidenced by individual ring widths (Figure 1). In addition, only 75% of the sampled trees at BR9611 reached breast height (1.4 m) after two years of growth, while 85% of the sampled trees reached breast height at BR9705 after two years of growth indicating that growth rate may have been less variable at BR9705. Generally, the Kromelite site was more uniform, was planted at a lower elevation, had greater rainfall, and a better soil. All of these factors may have contributed to faster and more uniform growth at BR9705 than at BR9611.

Overall core density was greater at BR9611 than at BR9705 (Table 2; Table 3) which is not surprising since faster growth rates are typically associated with lower densities in radiata pine. For example, whole core area weighted density at BR9611 ranged from 376.5 to 551.6 kg/m^3 with a mean of 459.7 kg/m^3 (Table 2). While at BR9705, mean

area weighted density of the whole core was 409 kg/m^3 and ranged from 327.6 to 502 kg/m^3 (Table 3). Similar trends are apparent in individual rings with trees at BR9611 showing higher density, on average, than trees at BR9705 for all rings (Figure 2). Density also appears to be slightly increasing with cambial age which is in agreement with published reports (Figure2). For example, Cown *et al.* (1992) plotted density trends for several half-sib families from a radiata pine trial in New Zealand demonstrating this increasing trend in density with ring number from the pith. Also, average density trends for radiata pine in New Zealand and Australia were reported to range from approximately 400 kg/m^3 in rings 1-4 to near 600 kg/m^3 in outer rings (Dungey *et al.* 2006). However, Li and Wu (2005) recently reported slightly lower age trends of density in radiata pine increasing from around 240 to 440 kg/m^3 from the pith to cambial age 14, respectively.

Table 2. Minimum, mean, maximum, and range of radiata pine juvenile wood properties from 980 whole core measurements from trial BR9611.

	Minimum	Mean	Maximum	Range
Average Density (kg/m^3)	358.0	439.2	542.5	184.5
Average MFA (^{o})	21.6	32.2	43.4	21.8
Average MOE (GPa)	2.2	6.1	10.9	8.7
Core Length (mm)	40.3	75.5	107.6	67.3
Area Wt. Density (kg/m^3)	376.5	459.7	551.6	175.1
Area Wt. MFA (^{o})	17.7	28.6	43.1	25.4
Area Wt. MOE (GPa)	2.2	7.4	13.4	11.2
Area (mm^2)	5090	18208	36343	31253

Table 3. Minimum, mean, maximum, and range of radiata pine juvenile wood properties from 660 whole core measurements from trial BR9705.

	Minimum	Mean	Maximum	Range
Average Density (kg/m^3)	325.8	392.3	485.5	159.7
Average MFA (^{o})	23.1	33.8	45.0	21.9
Average MOE (GPa)	2.4	4.5	8.2	5.8
Core Length (mm)	33.5	72.6	97.9	64.4
Area Wt. Density (kg/m^3)	327.6	409.0	502.0	174.4
Area Wt. MFA (^{o})	20.4	31.1	43.2	22.8
Area Wt. MOE (GPa)	2.4	5.5	9.5	7.1
Area (mm^2)	3516	16857	30114	26599

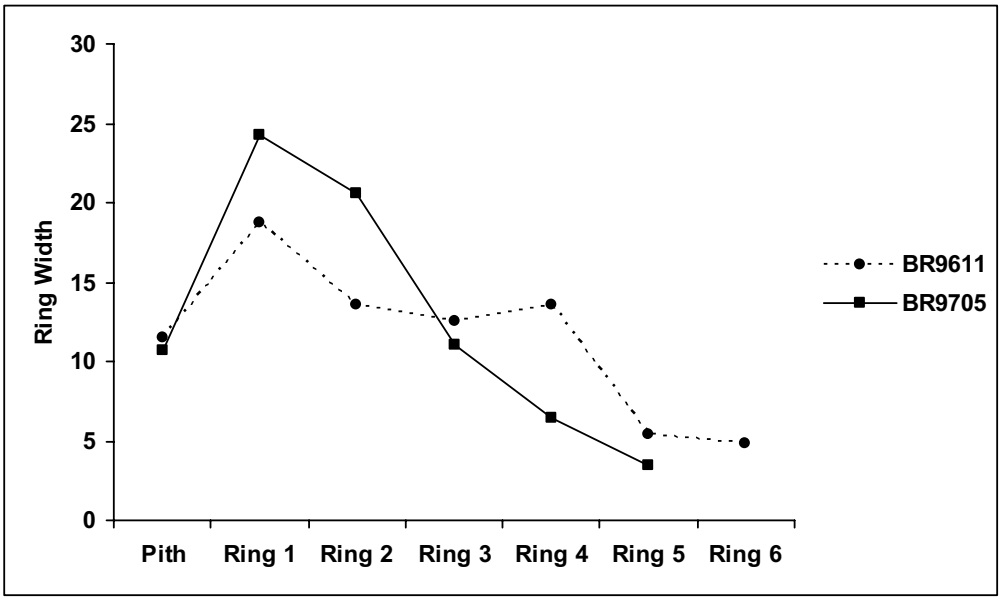

Figure 1. Width (mm) of individual rings of radiata pine from trials BR9611 and BR9705.

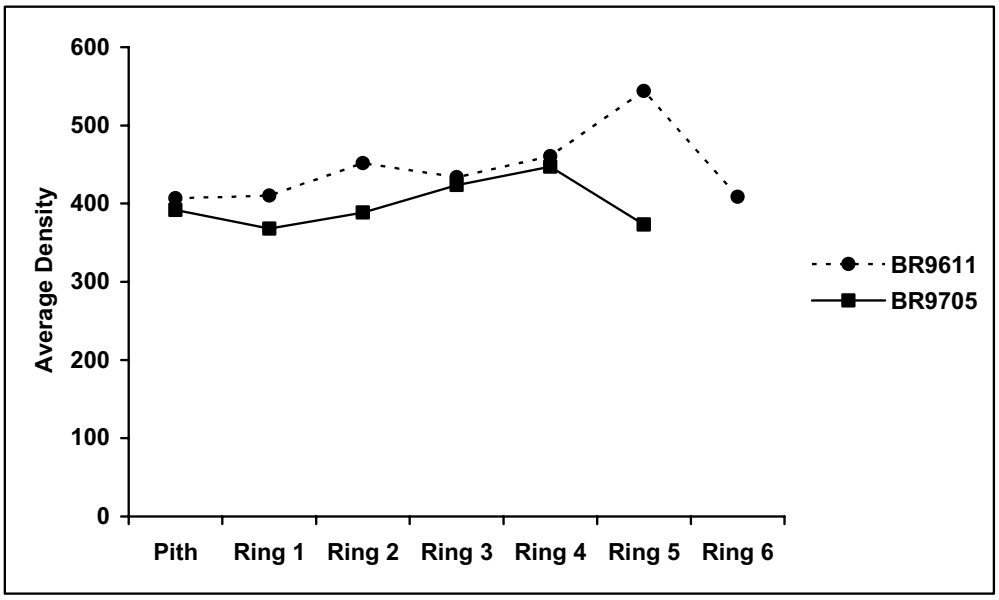

Figure 2. Average density (kg/m^3) of individual rings of radiata pine from trials BR9611 and BR9705

Microfibril angle, on the other hand, was comparatively similar at the two sites. Overall core area weighted MFA at BR9611 averaged 28.6^{O} and ranged from 17.7 to 43.1^{O} (Table 2). Mean area weighted MFA of the whole core at BR9705 was only slightly higher with a similar range in observed values (Table 3). The profiles of MFA across the whole core were nearly identical at both sites (Figure 3). Microfibril angle decreased from approximately 40^{O} in the pith to 20^{O} in the outermost ring (Figure 3). The trend for MFA from pith to bark was consistent with other studies in that microfibril angle has been shown consistently to decrease from the innermost rings

to the outermost rings (Megraw 1985). For example, Donaldson (1997) reported that MFA ranged from 30 to 50 ^{O} in radiata pine corewood, while only 15 to 25 ^{O} in the outerwood. In a radiata pine clonal study, microfibril angle was reported to decrease more or less linearly until about age 10 (Donaldson and Burdon 1995) with similar values as reported in the current study through cambial age 6. More recently, Dungey *et al.* (2006) reported mean MFA near the pith ranging from 35 to 40 ^{O} and dropping to about 30 ^{O} by about ring 6 and then continuing to decline to 11 to 13 ^{O} by ring 17 in radiata pine. Similar pith to bark trends in individual ring values for MFA have also been reported in many other species, such as loblolly pine (Megraw *et al.* 1998; Myszewski *et al.* 2004) and Norway spruce (*Picea abies* (L.) Karst.) (Herman *et al.* 1999; Lundgren 2000).

Whole core MOE was also greater at BR9611 than at BR9705 (Table 2; Table 3). Mean area weighted MOE at BR9611 was 7.4 GPa and ranged from 2.2 to 13.4 GPa, while at BR9705, mean MOE was 5.5 GPa and ranged from 2.4 to 9.5 GPa (Table 2; Table 3). Modulus of elasticity values reported in the present study showed an increasing trend from pith to bark at both sites (Figure 4). Stiffness of these progeny of 2nd generation selections appears to be greater than previously reported for New Zealand radiata pine. By cambial age 4, MOE was in excess of 8 GPa (Figure 4), whereas the average MOE in rings 6-8 of open-pollinated progeny from 72 1st generation selections of radiata pine in New Zealand was 6.3 (Kumar *et al.* 2002) to 6.6 GPa (Kumar 2004). Dungey *et al.* (2006) also reported mean MOE of radiata pine greater for radiata pine in Australia than for a different population in New Zealand.

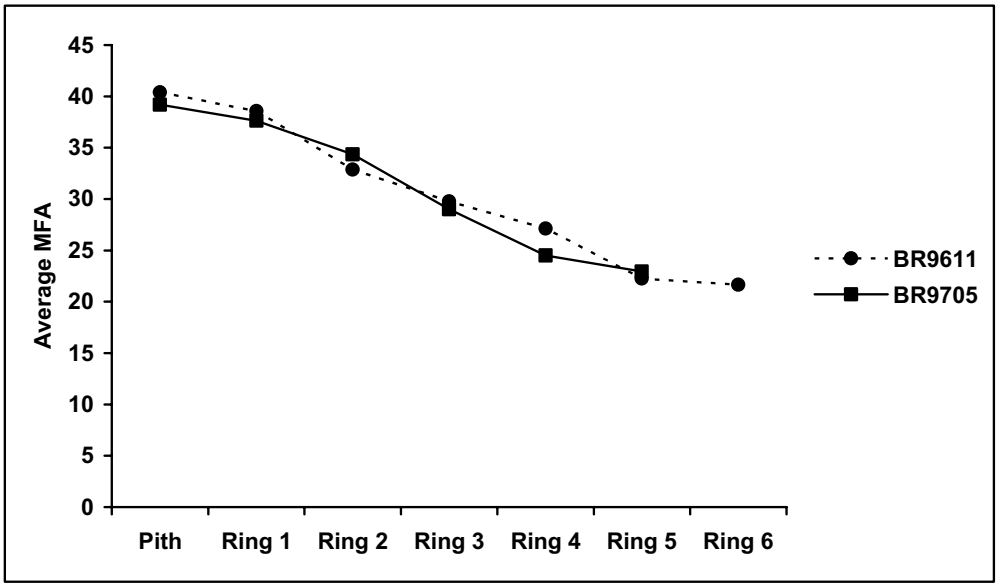

Figure 3. Average MFA (^{o}) of individual rings of radiata pine from trials BR9611 and BR9705.

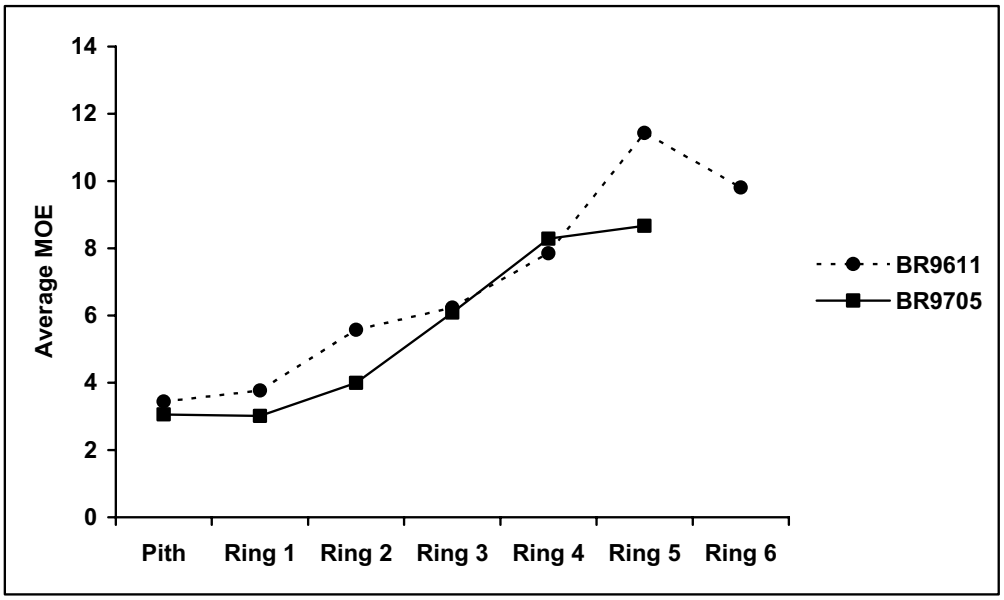

Figure 4. Average MOE (GPa) of individual rings of radiata pine from trials BR9611 and BR9705.

Heritability and genotype x environment interactions

Individual tree narrow-sense heritability was estimated for all measured traits at each site in order to indicate the amount each trait is under additive genetic control. Both average data and area weighted variables were analysed. Heritability for area weighted traits closely followed heritability estimates based on the average of a trait. For example, h^2_{9611} of average density was 0.81, and for area weighted density, h^2_{9611} was 0.77 (Table 4; Figure 5; Figure 6). The majority of the traits had higher heritability estimates at BR9611 than at BR9705 (Table 4; Figures 5-8); although, these differences may not be statistically different as indicated by standard error estimates (*e.g.*, overlapping confidence intervals).

As expected wood quality traits were more heritable than growth traits. Heritability for growth rate at BR9611 was 0.23 for core length and area, while at BR9705, $\hat{h}^2_{9705}$ was 0.06 and 0.09 for core length and area, respectively (Table 4; Figure 5; Figure 6). On the other hand, whole core estimates of density, either average density or area weighted density, were more heritable than microfibril angle and modulus of elasticity. Density was strongly controlled by additive effects with heritability estimated as 0.77 and 0.63 at sites BR9611 and BR9705, respectively (Table 4; Figure 6). These heritability estimates for density are in agreement with Burdon and Low (1992), who reported $\hat{h}^2$ for density ranging from 0.53 to 0.96 for several populations of radiata pine growing in New Zealand. Similarly, Bannister and Vine (1981) reported heritability estimates near 0.6 for density in 15 year old open-pollinated radiata pine. More recently, Kumar (2004) reported heritability for density of 72 open-pollinated families of radiata pine at two sites in New Zealand as 0.71 and 0.55. However, Li and Wu (2005) reported for radiata pine a lower heritability of whole core area weighted density of approximately 0.3, and this value was stable from cambial age 3 to 26. Additionally, Dungey *et al.* (2006) reported a lower heritability for cumulative area weighted density in radiata pine in Australia with a maximum heritability of just below 0.4 occurring at ring 22.

However, they did report $\hat{h}^2$ of area weighted density near 0.8 at rings 6 and 7 for open-pollinated radiata pine in New Zealand (Dungey *et al.* 2006).

Both microfibril angle and modulus of elasticity based on whole core measurements showed moderate to high heritability estimates, indicating that MFA and MOE of juvenile radiata pine are both under genetic control. Heritability of area weighted MFA ranged from 0.37 at BR9705 to 0.63 at BR9611, while $\hat{h}^2$ for MOE was 0.37 and 0.67 at BR9705 and BR9611, respectively (Table 4; Figure 6). MFA

Table 4. Individual tree narrow-sense heritability estimates ($\hat{h}^2$), and the across site genetic correlation between additive effects ($\hat{r}_{B_{ADDITIVE}}$) for radiata pine juvenile wood properties from trials BR9611 and BR9705. Standard errors are given in parentheses.

Variable	$\hat{h}^2_{9611}$	$\hat{r}_{B_{ADDITIVE}}$	$\hat{h}^2_{9705}$
Core Length	0.23 (0.07)	1.1 (0.57)	0.06 (0.07)
Average Density	0.81 (0.09)	0.79 (0.12)	0.64 (0.11)
Average MFA	0.62 (0.1)	0.87 (0.13)	0.37 (0.1)
Average MOE	0.69 (0.09)	0.91 (0.12)	0.37 (0.1)
Area	0.23 (0.07)	0.94 (0.45)	0.09 (0.08)
Area Wt. Density	0.77 (0.09)	0.77 (0.13)	0.63 (0.11)
Area Wt. MFA	0.63 (0.1)	0.82 (0.13)	0.43 (0.11)
Area Wt. MOE	0.67 (0.09)	0.9 (0.12)	0.36 (0.1)
Pith Density	0.37 (0.08)	0.92 (0.2)	0.26 (0.1)
Pith MFA	0.32 (0.08)	1.02 (0.19)	0.22 (0.09)
Pith MOE	0.39 (0.09)	0.45 (0.26)	0.52 (0.11)
Pith Width	0.02 (0.03)	---	0.1 (0.07
Ring 1 Density	0.53 (0.11)	0.66 (0.21)	0.55 (0.13)
Ring 1 MFA	0.69 (0.1)	0.9 (0.19)	0.25 (0.11)
Ring 1 MOE	0.6 (0.1)	0.89 (0.23)	0.23 (0.1)
Ring 1 Width	0.16 (0.08)	---	0.01 (0.07)
Ring 2 Density	0.55 (0.09)	0.74 (0.16)	0.54 (0.11)
Ring 2 MFA	0.52 (0.09)	0.81 (0.17)	0.35 (0.1)
Ring 2 MOE	0.6 (0.09)	0.95 (0.12)	0.34 (0.1)
Ring 2 Width	0.45 (0.11)	0.57 (0.35)	0.19 (0.1)
Ring 3 Density	0.6 (0.09)	0.75 (0.16)	0.53 (0.11)
Ring 3 MFA	0.61 (0.1)	0.76 (0.15)	0.5 (0.11)
Ring 3 MOE	0.59 (0.09)	0.87 (0.11)	0.5 (0.11)
Ring 3 Width	0.2 (0.07)	0.75 (0.26)	0.45 (0.12)
Ring 4 Density	0.71 (0.09)	0.76 (0.16)	0.37 (0.11)
Ring 4 MFA	0.57 (0.09)	0.87 (0.11)	0.57 (0.11)
Ring 4 MOE	0.58 (0.09)	0.93 (0.11)	0.41 (0.1)
Ring 4 Width	0.32 (0.09)	0.37 (0.33)	0.43 (0.12)
Ring 5 Density	0.47 (0.09)	0.85 (0.24)	0.18 (0.09)
Ring 5 MFA	0.53 (0.09)	0.84 (0.11)	0.6 (0.11)

Ring 5 MOE	0.51 (0.09)	0.85 (0.12)	0.48 (0.11)
Ring 5 Width	0.09 (0.05)	0.48 (0.88)	0.05 (0.07)
Ring 6 Density*	0.45 (0.09)	---	---
Ring 6 MFA*	0.57 (0.09)	---	---
Ring 6 MOE*	0.59 (0.09)	---	---
Ring 6 Width*	0.12 (0.05)	---	---

*Heritability estimates based on univariate analyses.

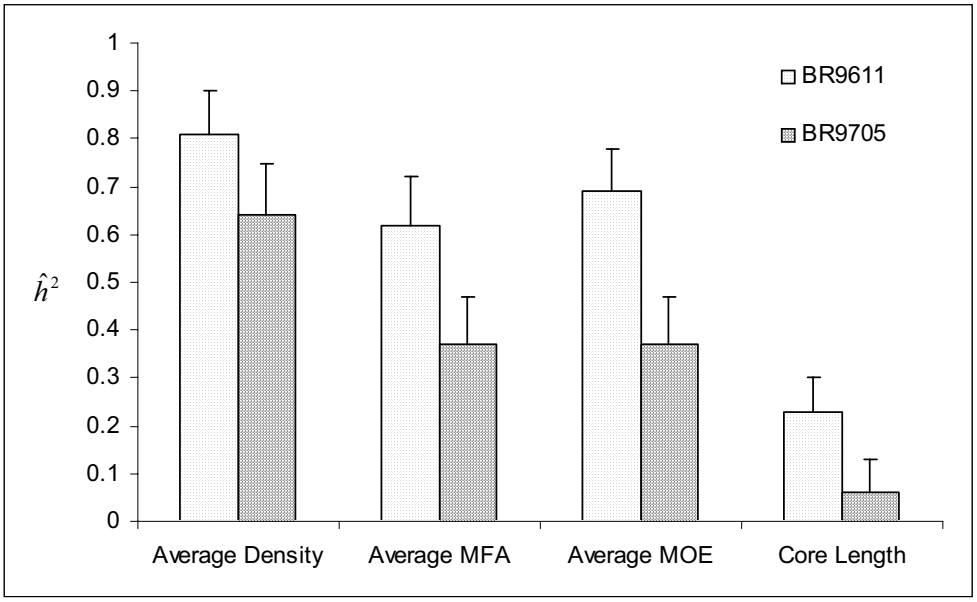

Figure 5. Narrow-sense heritability estimates ($\hat{h}^2$) for average density, MFA, MOE, and core length of juvenile radiata pine whole core measurements from trials BR9611 and BR9705. Standard error bars are shown.

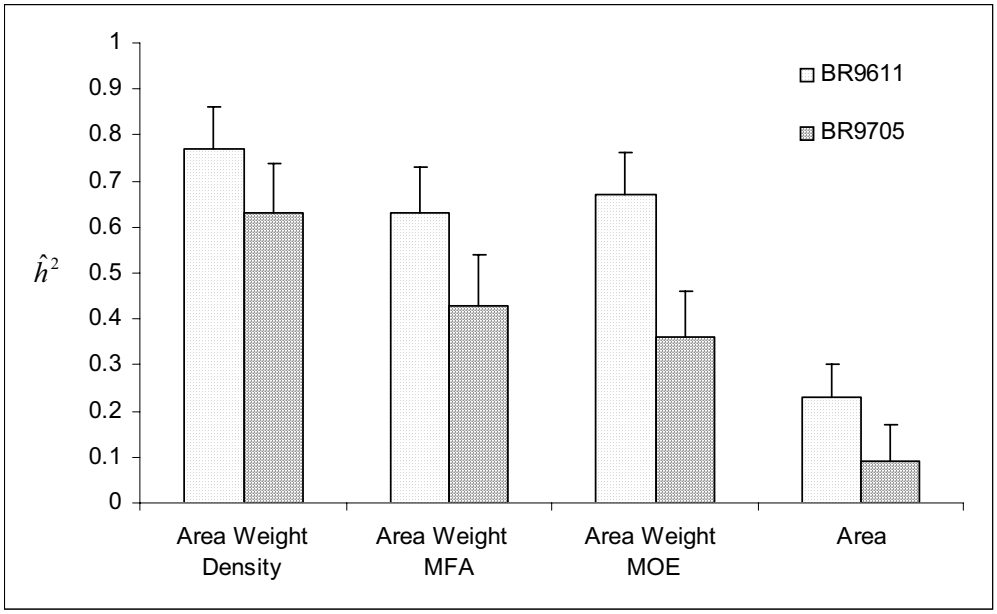

Figure 6. Narrow-sense heritability estimates ($\hat{h}^2$) for area weighted density, MFA, MOE, and area of juvenile radiata pine whole core measurements from trials BR9611 and BR9705. Standard error bars are shown.

and MOE have previously been reported to be under genetic control both in radiata pine and other conifers. For example, in a clonal trial of radiata pine, broad-sense heritability ($\hat{H}^2$) for MFA was 0.7 (Donaldson and Burdon 1995). In a separate study of three year old clones of radiata pine, $\hat{H}^2$ for MFA was 0.81, while for MOE, $\hat{H}^2$ ranged from 0.34 to 0.89 for different measurements of MOE (Lindstrom *et al.* 2004). Similarly, area weighted MFA in the corewood of radiata pine was reported to be under moderate to high genetic control (Dungey *et al.* 2006). Dungey *et al.* (2006) reported a moderate to high heritability for area weighted MOE in the corewood, but lower heritability in the outerwood for open-pollinated radiata pine in New Zealand. Individual-tree narrow-sense heritability was also reported near 0.6 for cumulative area weighted MOE at ring 6 for a small radiata pine full-sib family trial in Australia (Dungey *et al.* 2006). However, lower values of heritability for MOE have been reported in radiata pine (Kumar 2004), and for MFA in loblolly pine (Myszewski *et al.* 2004) and Norway spruce (Hannrup *et al.* 2004).

Patterns of heritability from pith to bark were also investigated for each trait. Heritability estimates of individual ring width were highly variable at both sites (Table 4; Figure 7). Lowest values were observed closest to the pith and heritability peaked between rings 2 to 4. Heritability estimates for individual ring density were lower than whole core estimates of $\hat{h}^2$ (Table 4). Generally, individual ring density was more heritable at BR9611 than at BR9705 as was the case with whole core estimates of density (Table 4; Figure 7). At BR9611, $\hat{h}^2_{9611}$ of individual ring density ranged from a low of 0.37 at the pith to a high of 0.71 by ring 4, although these estimates were more stable across the core profile than for ring width (Figure 7). Heritability of individual ring density at BR9705 also increased initially from the pith to moderately high values (> 0.5) through ring 3 before steadily declining in the outermost rings (Figure 7).

Trends in heritability for radiata pine ring density have been previously investigated. Age trends in heritability for fifty top open-pollinated selections of radiata pine in New Zealand were higher than that reported here (Kumar and Lee 2002). Kumar and Lee (2002) reported steadily increasing estimates of $\hat{h}^2$ for ring density from ~0.7 at cambial age 2 to near 1 at harvest age. Similarly, Dungey *et al.* (2006) reported increasing heritability of ring density through ring 6 before dropping in later rings in a radiata pine trial in New Zealand. In a small radiata pine full-sib family trial in Australia, a generally increasing trend in heritability of ring density was observed with a maximum heritability of 0.38 occurring in ring 16 (Dungey *et al.* 2006). Conversely, Li and Wu (2005) reported slightly lower estimates of heritability of ring density for radiata pine in Australia. Nevertheless, the fluctuations in heritability

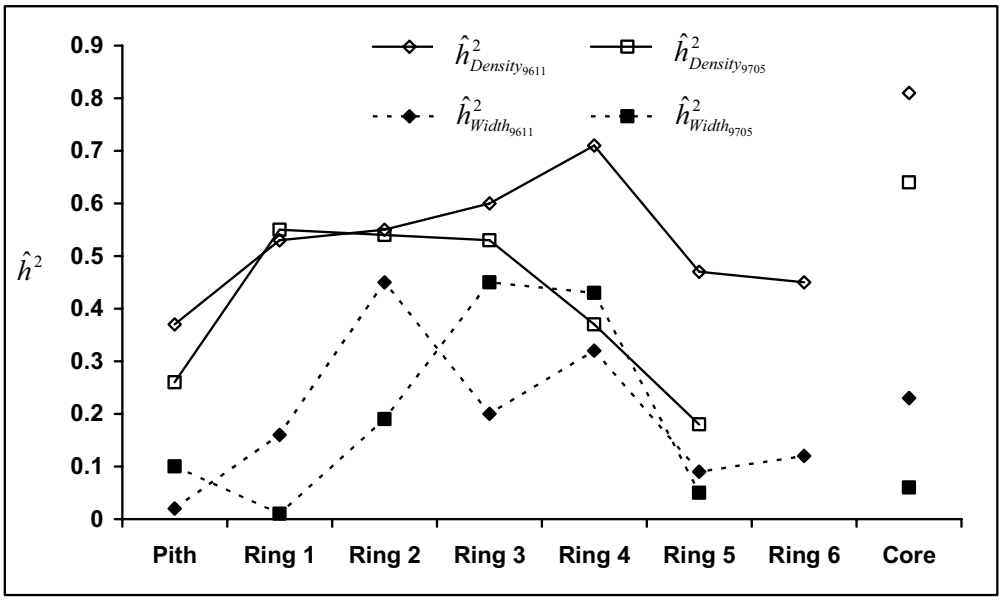

Figure 7. Individual tree narrow-sense heritability estimates ($\hat{h}^2$) for density and ring width for individual rings of radiata pine from the pooled analyses of trials BR9611 and BR9705. Whole core estimates are also shown.

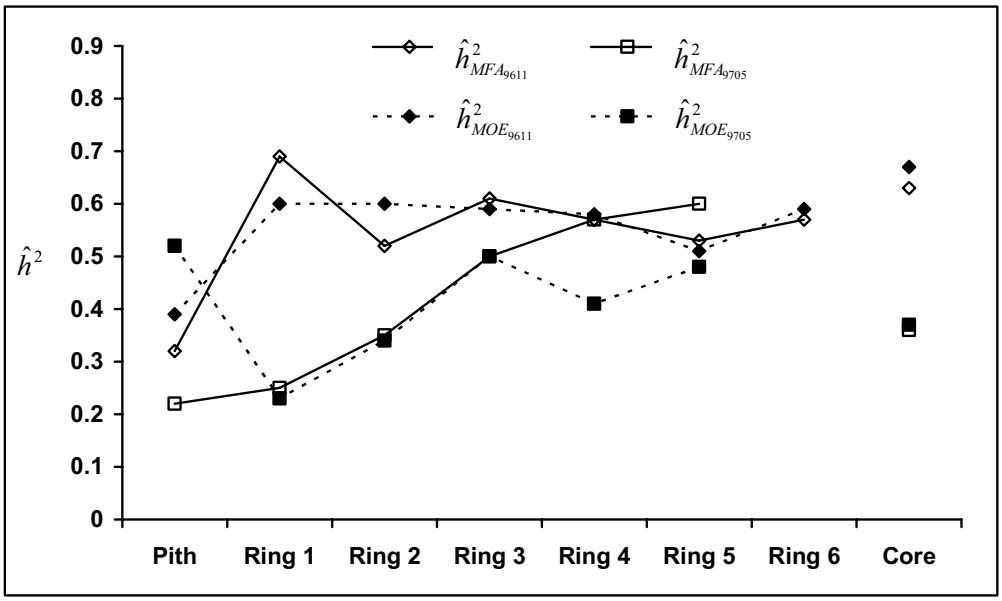

Figure 8. Individual tree narrow-sense heritability estimates ($\hat{h}^2$) for MFA and MOE for individual rings of radiata pine from the pooled analyses of trials BR9611 and BR9705. Whole core estimates are also shown.

estimates from ring to ring (Li and Wu 2005) were more similar to the trends observed in the current study as opposed to the steadily increasing trend reported by Kumar and Lee (2002).

Within each site, heritability estimates of MFA and MOE on an individual ring basis from the pith to the outermost rings were very similar (Figure 8). At BR9611, for example, heritability was lowest at the pith and then increased to relatively stable levels ($\hat{h}^2 \approx 0.6$) across the rest of the core profile for both MFA and MOE (Figure 8). For the most part, individual ring heritabilities for MFA and MOE were lower than whole core estimates of heritabilities at BR9611 (Table 4; Figure 8). The pattern of heritability for MFA and MOE from pith to bark at BR9705 was somewhat different. Generally, at BR9705 individual ring heritabilities for MFA and MOE have steadily increased from the innermost to outermost rings (Figure 8). Heritability estimates of whole core MFA and MOE were generally greater than estimates from the innermost rings but less than estimates from the outer rings at BR9705.

The extent of genotype x environment interaction was investigated for all traits in order to measure the stability of additive effects across sites. For the most part, there was little genotype x environment interaction for all of the wood quality traits both on whole core measurements and individual ring measurements (Table 4). For instance, for whole core area weighted MOE, the type B genetic correlation was 0.9 (Table 4). The closer $\hat{r}_{B_{ADDITIVE}}$ is to one the better the indication that additive effects, or estimated breeding values, should rank similarly across sites. For example, there were 41 parents with progeny tested on both sites. The ranking of these 41 parents for area weighted MOE across sites was relatively stable (Figure 9) with a Spearman's rank coefficient (ρ) = 0.92 indicating that forest managers can have a relatively high level of confidence in making selections or deployment decisions for area weighted modulus of elasticity in juvenile radiata pine.

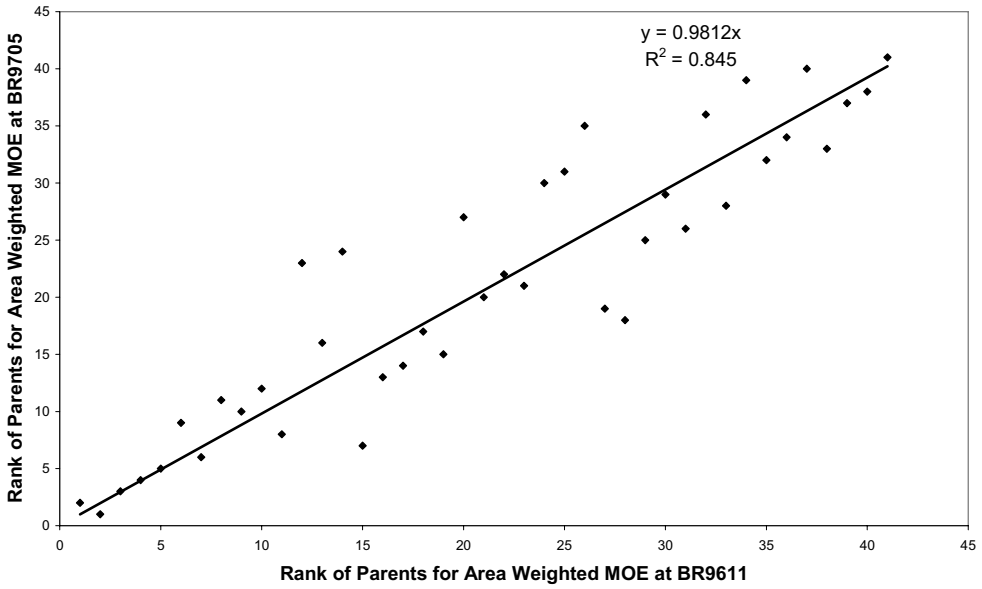

Figure 9. Rank-rank plot depicting the parental ranks for area weighted MOE for the 41 parents with progeny tested on both sites (Spearman's rank coefficient $\rho = 0.92$).

Alternatively, estimates for type B genetic correlations for growth rate were either poorly estimated with large standard errors and/or were much lower, indicating more extensive genotype x environment interaction than was observed for the wood quality traits (Table 4). Type B genetic correlations of whole core growth rate were large;

however, standard errors were also large, and in the case of core length, $\hat{r}_{B_{ADDITIVE}}$ was not different than zero based on a 95% confidence interval (Table 4). Wu and Matheson (2005) recently reported genotype x environment interaction for radiata pine which they attributed to differences in elevation (*e.g.,* primarily from snow damage at high elevation sites). These two sites in the current study were planted at a similar elevation, and therefore, elevation was probably not the contributing factor causing genotype x environment interaction for growth. Other possibilities such as unequal annual rainfall, drainage, vegetative competition control, population sample size, unequal age of samples, or low genetic control could give rise to high or imprecisely quantified genotype x environment interaction for growth rate. From a practical standpoint, the type B genetic correlations indicate that for growth traits more samples and sites should be included to more precisely estimate the stability of selections across the deployment zone, whereas, for juvenile wood quality traits, two sites may be sufficient in order to precisely quantify the additive genetic effects in order to make selections.

Genetic correlations among growth, density, MFA, and MOE

Genetic correlations between pairs of traits were calculated based on the series of bivariate analyses of the data pooled across sites. Whole core average and area weighted measurements of density, MFA, MOE, or growth were all near one indicating that both average and area weighted measurements are the same trait (Table 5). For instance, the genetic correlation between average density (AveDen) and area weighted density (ArWtDen) was 0.99 (Table 5). This tells us that the ranking of genotypes will be nearly identical for these two variables, and furthermore, that the same individuals would be selected regardless of weighting density measurements based on area. Similar results were observed for average MFA and area weighted MFA, average MOE and area weighted MOE, and core length and area (Table 5).

The genetic correlations between growth and the wood quality traits were all unfavourable (Table5; Table 6). A number of studies have reported an unfavourable genetic correlation between wood density and growth in radiata pine (Dean *et al.* 1983; Zobel and van Buijtenen 1989; Dean 1990; Cotterill and Dean 1990; Burdon and Low 1992; Jayawickrama 2001; Kumar 2004; Li and Wu 2005). Wu *et al.* (2004) recently reported an average genetic correlation (based on 17 published reports) between growth and density in radiata pine of -0.44. The results in the current study compare well with earlier studies of radiata pine in that density and growth rate were negatively correlated (Table 5; Table 6). The genetic correlation between whole core area weighted density and core length was -0.6 (Table 5), while the genetic correlation between density and ring width ranged from -0.13 to -0.78 depending on ring number from pith (Table 6; Figure 10). Similar negative genetic correlations were observed between MOE and core length (-0.5) and area (-0.54), whereas genetic correlations throughout the core ranged from -0.28 to -0.86 (Table 5; Table 6; Figure 10). Kumar *et al.* (2002) and Kumar (2004) also reported a moderate negative genetic correlation between MOE and diameter in radiata pine at three sites, while a genetic correlation near zero at a fourth site. Although not significant, the genetic correlation between whole core area weighted MFA and growth in the current study was positive (Table 5). In addition, moderately high positive and significant genetic correlations between MFA and ring width were present in rings 2-4 from the pith (Table 6; Figure 10). Positive but insignificant genetic correlations between MFA and growth have also been reported in loblolly pine (Myszewski *et al.* 2004). Unfavourable correlations between growth and density, MFA,

and MOE imply that selection for increased juvenile wood density, reduced microfibril angle, and increased stiffness will all result in decreased growth.

A negative genetic correlation does not always indicate an unfavourable relationship. For example, high microfibril angles are undesirable from a product standpoint, and consequently, any reduction in MFA would be an improvement. However, increases in growth rate, density, and MOE are sought-after. Therefore, a negative genetic correlation between MFA and these other traits would be favourable in that improvement in multiple traits is achievable. In the current study, overall core density and MFA had a slight negative genetic correlation (Table 5). However, this negative correlation was not different than zero as indicated by the standard error, and therefore, density and MFA could be considered independent traits with different underlying genes controlling them. Similarly, within an individual ring the genetic correlation between density and MFA was typically slightly negative and insignificant across the core profile (Table 6; Figure 10). Lindstrom *et al.* (2004) also reported a

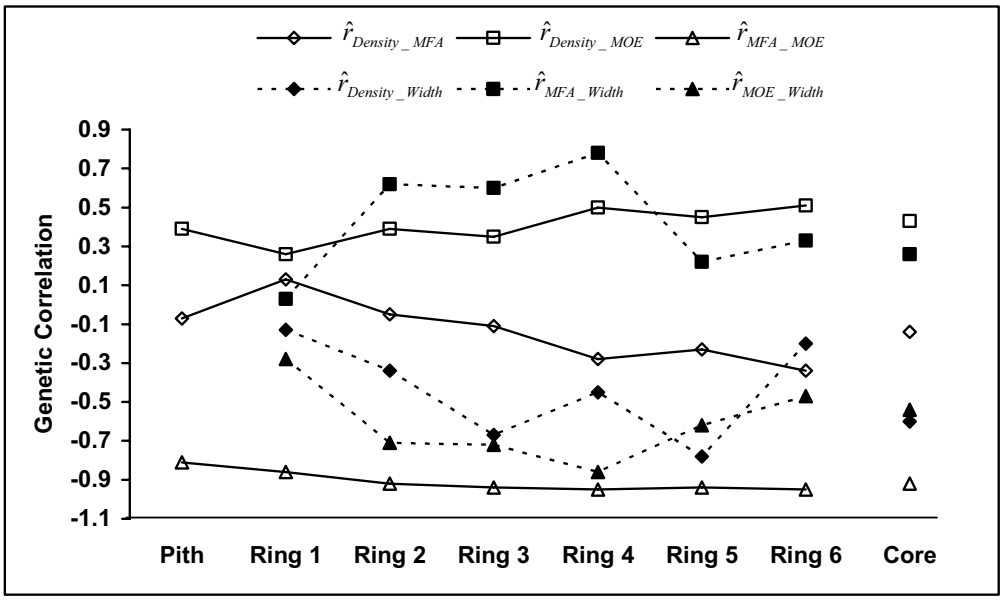

Figure 10. Genetic correlations among density, MFA, MOE, and width within individual rings of radiata pine from the pooled analyses of trials BR9611 and BR9705. Whole core genetic correlations are also shown. Ring 6 estimates are based on univariate analyses from trial BR9611.

Table 5. Genetic (above diagonal) and phenotypic (below diagonal) correlations among radiata pine juvenile wood properties from whole core measurements from trials BR9611 and BR9705 (joint site analyses). Standard errors are given in parentheses.

	AveDen	AveMFA	AveMOE	CoreLen	Area	ArWtDen	ArWtMFA	ArWtMOE
AveDen		-0.08 (0.11)	0.43 (0.09)	-0.58 (0.13)	-0.6 (0.12)	0.99 (0.003)	-0.08 (0.11)	0.4 (0.09)
AveMFA	-0.02 (0.03)		-0.9 (0.03)	0.25 (0.17)	0.27 (0.16)	-0.14 (0.11)	0.98 (0.01)	-0.9 (0.03)
AveMOE	0.37 (0.03)	-0.84 (0.01)		-0.55 (0.14)	-0.59 (0.14)	0.47 (0.09)	-0.9 (0.03)	0.99 (0.003)
CoreLen	-0.18 (0.03)	0.27 (0.03)	-0.33 (0.02)		1 (0.001)	-0.6 (0.13)	0.26 (0.16)	-0.5 (0.15)
Area	-0.19 (0.03)	0.27 (0.03)	-0.33 (0.02)	0.99 (0.001)		-0.61 (0.12)	0.28 (0.16)	-0.54 (0.14)
ArWtDen	0.97 (0.002)	-0.07 (0.03)	0.42 (0.03)	-0.17 (0.03)	-0.18 (0.03)		-0.14 (0.11)	0.43 (0.09)
ArWtMFA	-0.03 (0.03)	0.97 (0.002)	-0.87 (0.01)	0.27 (0.03)	0.28 (0.03)	-0.08 (0.03)		-0.92 (0.02)
ArWtMOE	0.34 (0.03)	-0.83 (0.01)	0.98 (0.001)	-0.32 (0.02)	-0.32 (0.02)	0.4 (0.03)	-0.89 (0.01)	

Table 6. Genetic correlations among density, MFA, MOE and width of individual rings of radiata pine from combined analyses of BR9611 and BR9705. Standard errors are given in parentheses.

	Density and MFA	Density and MOE	MFA and MOE	Density and Width	MFA and Width	MOE and Width
Pith	-0.07 (0.16)	0.39 (0.13)	-0.81 (0.07)	---	---	---
Ring 1	0.13 (0.13)	0.26 (0.13)	-0.86 (0.05)	-0.13 (0.22)	0.03 (0.22)	-0.28 (0.21)
Ring 2	-0.05 (0.13)	0.39 (0.11)	-0.92 (0.03)	-0.34 (0.14)	0.62 (0.11)	-0.71 (0.1)
Ring 3	-0.11 (0.12)	0.35 (0.11)	-0.94 (0.02)	-0.67 (0.12)	0.6 (0.11)	-0.72 (0.1)
Ring 4	-0.28 (0.11)	0.5 (0.09)	-0.95 (0.02)	-0.45 (0.14)	0.78 (0.09)	-0.86 (0.08)
Ring 5	-0.23 (0.13)	0.45 (0.11)	-0.94 (0.02)	-0.78 (0.24)	0.22 (0.24)	-0.62 (0.22)
Ring 6*	-0.34 (0.14)	0.51 (0.11)	-0.95 (0.02)	-0.2 (0.22)	0.33 (0.19)	-0.47 (0.18)

*Estimates based on analyses involving BR9611 only.

genetic correlation between basic density and MFA near zero in a 3-year old radiata pine clonal study. While Dungey *et al.* (2006) reported significant and moderately negative genetic correlations (-0.6) between density and MFA beginning around ring 7 and continuing throughout the core profile through ring 25 in radiata pine. However, insignificant genetic correlations between density (specific gravity) and microfibril angle were also reported in loblolly pine (Myszewski *et al.* 2004) and Norway spruce (Hannrup *et al.* 2004).

Highly negative and significant genetic correlations, on the other hand, exist between MFA and MOE (Table 5; Table 6; Figure 10). The genetic correlation between whole core area weighted MFA and area weighted MOE was -0.92 (Table 5), and this trend was observed throughout the profile of the core with genetic correlations between MFA and MOE within individual rings ranging from -0.81 to -0.95 (Table 6; Figure 10). Similar estimates of the relationship between MFA and MOE were reported by Lindstrom *et al.* (2004) in a clonal radiata pine study. Highly negative genetic correlations between MFA and MOE close to the values presented here were also reported at two radiata pine trials in New Zealand and Australia through ring 25 (Dungey *et al.* 2006). A negative genetic correlation implies that the same genes are controlling MFA and MOE, but that these genes are causing negative pleiotropy. This strong negative relationship is highly advantageous for making improvements in both traits. Selection for reduced microfibril angle should also lead to gains in MOE throughout the juvenile core, or *vice versa*.

The genetic correlation between MOE and density was also favourable indicating that selection for density will also lead to improvement in juvenile wood overall stiffness, or *vice versa* (Table 5; Table 6; Figure 10). The genetic correlation was moderately positive between whole core density and modulus of elasticity (0.43) (Table 5). Similarly, the genetic correlation between density and MOE across the profile of the core from pith to bark was also positive and ranged from 0.26 to 0.51 (Table 6; Figure 10). Density and MOE have been reported to be genetically correlated in previous studies with similar estimates to what was presented here. For example, Kumar (2004) reported the genetic correlation between density and MOE in radiata pine ranging from 0.44 to 0.64. However, Lindstrom *et al.* (2004) reported positive but insignificant correlations between various measures of density and MOE in clones of radiata pine. Since MOE and density (and MOE and MFA) are favourably correlated and MOE is under genetic control in the current study, then selection for MOE directly as opposed to selecting for its component traits (density and MFA) should yield improvements in stiffness of juvenile radiata pine, as well as density and MFA, as previously indicated by Dungey *et al.* 2006.

The genetic and phenotypic correlations between the same wood quality trait measured in different rings or the whole core measurement were also determined (Table 7; Table 8; Table 9). Genetic correlations were always greater than phenotypic correlations for all traits throughout the core profile. Generally, the highest correlations for the same trait measured in different rings were observed between measurements in adjacent rings and was lowest between rings furthest apart. However, all of the genetic correlations were positive and significant (low standard errors). Furthermore, whole core measurements of density, MFA, and MOE were highly correlated with individual ring measurements. For example, the genetic correlation between whole core area weighted density and the average density in the pith was 0.84 and was greater than 0.9 throughout

the rest of the core profile (Table 7). The genetic correlation between whole core MFA and individual ring measurements ranged from 0.62 (pith) to 0.97 (ring 3) (Table 8). Similarly, the genetic correlation between whole core area weighted MOE and MOE in the rings reached 0.95 by ring 2 (Table 9). This is highly relevant for selection and breeding in that improvement in juvenile wood properties across the entire profile of the corewood including the innermost rings can be achieved by selecting for an average whole core measurement.

Table 7. Genetic (above diagonal) and phenotypic (below diagonal) correlations among radiata pine individual ring density measurements and the area weighted density of the whole core from trials BR9611 and BR9705 (joint site analyses). Standard errors are given in parentheses (* indicate that estimates based on analyses involving BR9611 only).

	Pith	Ring 1	Ring 2	Ring 3	Ring 4	Ring 5	Ring 6*	Core
Pith		0.91 (0.06)	0.81 (0.07)	0.8 (0.08)	0.77 (0.08)	0.73 (0.09)	0.91 (0.08)	0.84 (0.06)
Ring 1	0.41 (0.03)		0.94 (0.03)	0.96 (0.03)	0.8 (0.07)	0.79 (0.07)	0.88 (0.07)	0.96 (0.02)
Ring 2	0.36 (0.02)	0.69 (0.02)		0.94 (0.03)	0.87 (0.05)	0.86 (0.05)	0.93 (0.04)	0.97 (0.02)
Ring 3	0.33 (0.03)	0.55 (0.02)	0.62 (0.02)		0.93 (0.03)	0.89 (0.05)	0.84 (0.07)	0.99 (0.01)
Ring 4	0.29 (0.03)	0.51 (0.02)	0.52 (0.02)	0.66 (0.02)		0.82 (0.06)	0.8 (0.07)	0.94 (0.02)
Ring 5	0.21 (0.03)	0.42 (0.03)	0.46 (0.02)	0.47 (0.02)	0.58 (0.02)		0.82 (0.04)	0.9 (0.04)
Ring 6*	0.27 (0.03)	0.5 (0.03)	0.51 (0.03)	0.52 (0.03)	0.59 (0.03)	0.69 (0.02)		0.91 (0.04)
Core	0.42 (0.02)							

Table 8. Genetic (above diagonal) and phenotypic (below diagonal) correlations among radiata pine individual ring MFA measurements and the area weighted MFA of the whole core from trials BR9611 and BR9705 (joint site analyses). Standard errors are given in parentheses (* indicate that estimates based on analyses involving BR9611 only).

	Pith	Ring 1	Ring 2	Ring3	Ring 4	Ring 5	Ring 6*	Core
Pith		0.8 (0.07)	0.64 (0.1)	0.52 (0.11)	0.46 (0.12)	0.46 (0.12)	0.65 (0.11)	0.62 (0.1)
Ring 1	0.58 (0.02)		0.91 (0.03)	0.76 (0.06)	0.7 (0.07)	0.62 (0.09)	0.67 (0.09)	0.83 (0.05)
Ring 2	0.41 (0.02)	0.75 (0.03)		0.94 (0.02)	0.9 (0.03)	0.85 (0.05)	0.9 (0.04)	0.96 (0.02)
Ring 3	0.35 (0.03)	0.62 (0.02)	0.83 (0.01)		0.98 (0.01)	0.96 (0.02)	0.97 (0.02)	0.97 (0.01)
Ring 4	0.34 (0.03)	0.54 (0.02)	0.7 (0.01)	0.89 (0.01)		0.99 (0.01)	0.98 (0.01)	0.96 (0.01)
Ring 5	0.33 (0.03)	0.49 (0.02)	0.65 (0.02)	0.81 (0.01)	0.91 (0.01)		0.97 (0.01)	0.93 (0.02)
Ring 6	0.34 (0.03)	0.5 (0.03)	0.65 (0.02)	0.77 (0.02)	0.83 (0.01)	0.92 (0.01)		0.98 (0.01)
Core	0.47 (0.02)	0.75 (0.01)	0.87 (0.01)	0.91 (0.01)	0.88 (0.01)	0.85 (0.01)	0.86 (0.01)	

Table 9. Genetic (above diagonal) and phenotypic (below diagonal) correlations among radiata pine individual ring MOE measurements and the area weighted MOE of the whole core from trials BR9611 and BR9705 (joint site analyses). Standard errors are given in parentheses(* indicate that estimates based on analyses involving BR9611 only).

	Pith	Ring 1	Ring 2	Ring 3	Ring 4	Ring 5	Ring 6*	Core
Pith		0.83 (0.06)	0.65 (0.09)	0.54 (0.1)	0.52 (0.1)	0.52 (0.1)	0.63 (0.11)	0.65 (0.08)
Ring 1	0.64 (0.02)		0.87 (0.04)	0.75 (0.06)	0.67 (0.08)	0.62 (0.09)	0.74 (0.08)	0.82 (0.05)
Ring 2	0.43 (0.02)	0.76 (0.01)		0.94 (0.02)	0.89 (0.03)	0.83 (0.05)	0.91 (0.04)	0.95 (0.02)
Ring 3	0.35 (0.03)	0.62 (0.02)	0.82 (0.01)		0.96 (0.02)	0.92 (0.03)	0.98 (0.02)	0.97 (0.01)
Ring 4	0.31 (0.03)	0.53 (0.02)	0.7 (0.01)	0.83 (0.01)		0.97 (0.01)	0.99 (0.01)	0.95 (0.02)
Ring 5	0.29 (0.03)	0.49 (0.02)	0.64 (0.02)	0.74 (0.01)	0.85 (0.01)		0.97 (0.02)	0.92 (0.02)
Ring 6*	0.3 (0.03)	0.49 (0.03)	0.62 (0.02)	0.7 (0.02)	0.78 (0.01)	0.8 (0.01)		0.99 (0.01)
Core	0.44 (0.02)	0.71 (0.02)	0.83 (0.01)	0.89 (0.01)	0.9 (0.01)	0.85 (0.01)	0.83 (0.01)	

Selection

Early tree improvement programs for radiata pine in Australia have concentrated most of their effort on improving growth and form. Even with previous selection for growth and form, considerable genetic variation and high heritabilities observed in the current study indicate the potential for improvement of juvenile wood quality in radiata pine. Similar results were observed by Ridoutt *et al.* (1998) in New Zealand where high heritabilities and substantial genetic variation were observed for wood quality traits in 20 radiata pine plus tree selections that were selected for growth and form. Also, Cown *et al.* (1992) demonstrated that intensive selection for growth and form did not have an effect on wood density in 30 families of radiata pine. In addition little genotype x environment interaction was observed in the current study for the three juvenile wood properties assessed indicating that selection of parents for each trait will be stable across multiple environments.

Density, stiffness, and microfibril angle were all under moderate to high genetic control indicating that improvement in wood properties in juvenile radiata pine can be achieved. The genetic gain associated with selection of each whole core trait was estimated for a range of selection intensities. In all cases genetic gains were greater at BR9611 than at BR9705 which should be expected since the traits were under stronger genetic control at BR9611. For example, selection for whole core area weighted density at BR9611 resulted in genetic gains of 8.3% when 10% of the population was selected (98 selections) to 12.6% gain when selection intensity was increased to 1% (~10 selections) (Figure 11). While at BR9705, genetic gains in whole core area weighted density ranged from 7 to 10.6% (Figure 11). Genetic gains over the entire population ranged from 7.1% to 10.8% in area weighted density when 10 to 1% of the population was selected, respectively (Table 10). Even higher genetic gains in selection for reduced microfibril angle could be achieved. Gains in reduced MFA at BR9611 ranged from slightly greater than 14% to nearly 22% depending on selection intensity (Figure 11). However, gains in reduced MFA at BR9705 ranged from approximately 8 to 12% (Figure 11). Genetic gains when the entire population was considered ranged from a reduction in MFA of 10.4 to 15.9% (Table 10). The greatest gains can be attained by selecting for increased stiffness. For example, genetic gains in whole core area weighted MOE ranged from 26 to 40% and from 13 to 19% at BR9611 and BR9705, respectively (Figure 12), while gains over the entire population mean were 18.3 to 27.8% in increased MOE (Table 10).

Table 10. Genetic response over the entire population mean (%) for direct (bold diagonal) and indirect (off diagonal) selection. Genetic parameter and variance component estimates came from analyses of combined data across sites assuming homogeneous additive and residual variances.

Selected Trait (x)	$\hat{h}^2$	$\hat{\sigma}_P^2$	Selection Intensity (%)	Target Trait (y) Density	MFA	MOE	Core Length
Density	0.68	749.83	10	**7.4**	-1.7	9.0	-4.7
MFA	0.51	12.01	10	0.9	**-10.4**	16.8	-1.7

MOE	0.51	2.01	10	2.7	-9.6	**18.3**	-3.3
Core Length	0.17	95.47	10	-2.2	1.6	-5.3	**3.9**
Density	0.68	749.83	5	**8.4**	-2.0	10.6	-5.5
MFA	0.51	12.01	5	1.0	**-12.2**	19.8	-2.0
MOE	0.51	2.01	5	3.1	-11.3	**21.5**	-3.9
Core Length	0.17	95.47	5	-2.6	1.8	-6.2	**4.5**
Density	0.68	749.83	1	**10.8**	-2.6	13.8	-7.1
MFA	0.51	12.01	1	-1.3	**-15.9**	25.6	-2.6
MOE	0.51	2.01	1	4.0	-14.6	**27.8**	-5.1
Core Length	0.17	95.47	1	-3.3	2.4	-8.0	**5.9**

Because here these traits are genetically correlated, maximum gains in each trait will not be possible when improvement in multiple traits is desired. Density is relatively easy and inexpensive to measure, and consequently, it has been the most likely wood quality trait to be incorporated into breeding programs. Therefore, the correlated genetic response in MFA, MOE, and growth associated with indirect selection of area weighted density was predicted using whole population estimates of genetic parameters and variance components (Table 10). When 10% of the population was selected for increased density, then this corresponded to a reduction in MFA of 1.7%, an increase in MOE of 9%, and a reduction in core length of 4.7%. If MFA was used as a surrogate trait for MOE, then gains in MOE would be nearly twice as much as those produced by selecting for density (Table 10).

On the other hand, since the ultimate goal in improving juvenile wood properties is to improve the volume recovery and properties of sawn timber products from juvenile wood, any increases in the stiffness of juvenile wood will be essential to meet this goal. Furthermore, maximization of genetic gains in MOE will still lead to gains in both density and MFA since these traits are favourably correlated (Table 10). When MOE was selected, genetic gains in density ranged from 2.7 to 4% over the entire population mean, while indirect selection of MFA resulted in reduced levels near those produced through direct selection (Table 10).

Unfortunately, all of the juvenile wood quality traits had unfavourable genetic correlations with growth indicating that selection for increased density and MOE, and reduced MFA may result in a genetic loss or reduced growth. Direct selection for core length would result in decreases in density and MOE and an increase in average microfibril angle throughout the corewood (Table 10) as reported previously for radiata pine (Dean *et al.* 1983; Zobel and van Buijtenen 1989; Dean 1990; Cotterill and Dean 1990; Burdon and Low 1992; Jayawickrama 2001; Kumar 2004; Li and Wu 2005). For example, the genetic correlation between whole core area weighted MOE and core length was -0.5. Selection for area weighted MOE at BR9611 would result in a -4.5 to -6.8% genetic loss in core length with 10 to 1% selection intensities, respectively (Figure 12). At BR9705 the genetic loss in core length was not as great when selection was for

whole core MOE (-1.7 to 2.6%) (Figure 12). A reduction in overall core length of 3.3 to 5.1% under the entire population mean would result when MOE was selected. Although, even with this genetic loss in core length, this equates to only a 1 cm reduction in diameter growth for 9-year old radiata pine at BR9611, and less than 0.5 cm reduction in diameter at BR9705. Increases in the overall stiffness of radiata pine corewood may outweigh the slight loss in growth. Similarly, selecting for reduced MFA or increased density would also result in a reduction in growth (Table 10). Another option to account for unfavourable genetic correlations between growth and juvenile wood properties would be to select for correlation breakers. For example, breeding values predictions for MOE and core length from the combined site analyses were plotted against each other (Figure 13). Even though these traits were negatively correlated, individual genotypes can be identified that have relatively high stiffness and growth (upper right quadrant of Figure 13).

There were highly positive genetic correlations between whole core juvenile wood quality traits and individual ring traits. Therefore, selection for juvenile wood properties based on whole core measurements will not only maximize gains for the whole core trait, but it will also lead to improvement of juvenile wood properties in each ring throughout the core. Selection for increased whole core area weighted MOE, for example, will also result in increases in individual ring MOE values throughout the core including the innermost rings (Figure 14; Figure 15). At BR9611, maximum gains in MOE throughout the core occurred in rings 2 to 4 from the pith, and depending on selection intensity, 27 to 44% gains in MOE of these rings can be achieved by selecting for whole core MOE (Figure 14). However, gains in stiffness of the pith and ring 1 are still possible (Figure 15). Similarly, at BR9705 the greatest gains in MOE across the profile occurred in the outermost rings (Figure 15). For example, selection of whole core area weighted MOE at BR9705 would result in improvements of more than 15 to 25% in MOE of rings 2 to 5 (Figure 15).

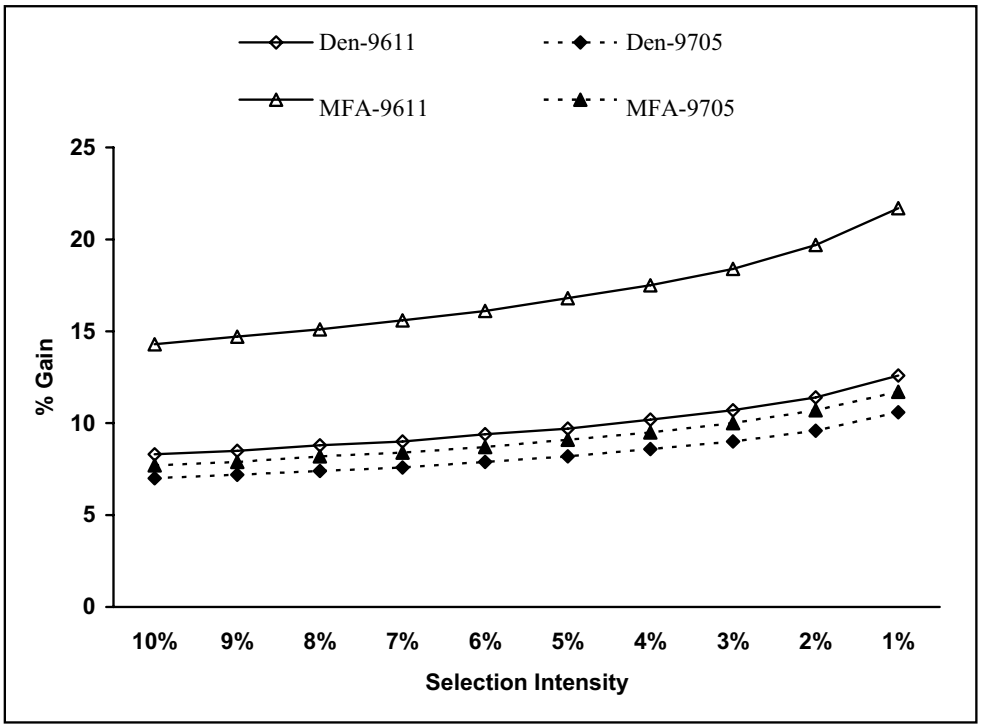

Figure 11. Genetic gains associated with direct selection for increased whole core area weighted density or reduced MFA in juvenile radiata pine at trials BR9611 and BR9705.

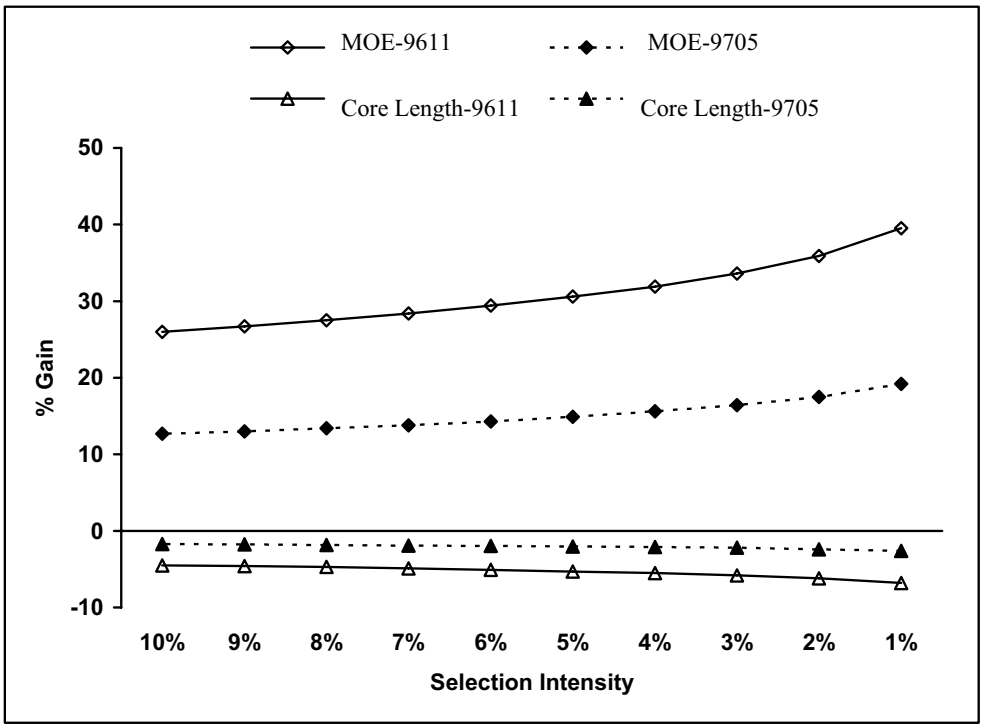

Figure 12. Genetic gain associated with direct selection for MOE and its effect on core length in juvenile radiata pine at trials BR9611 and BR9705.

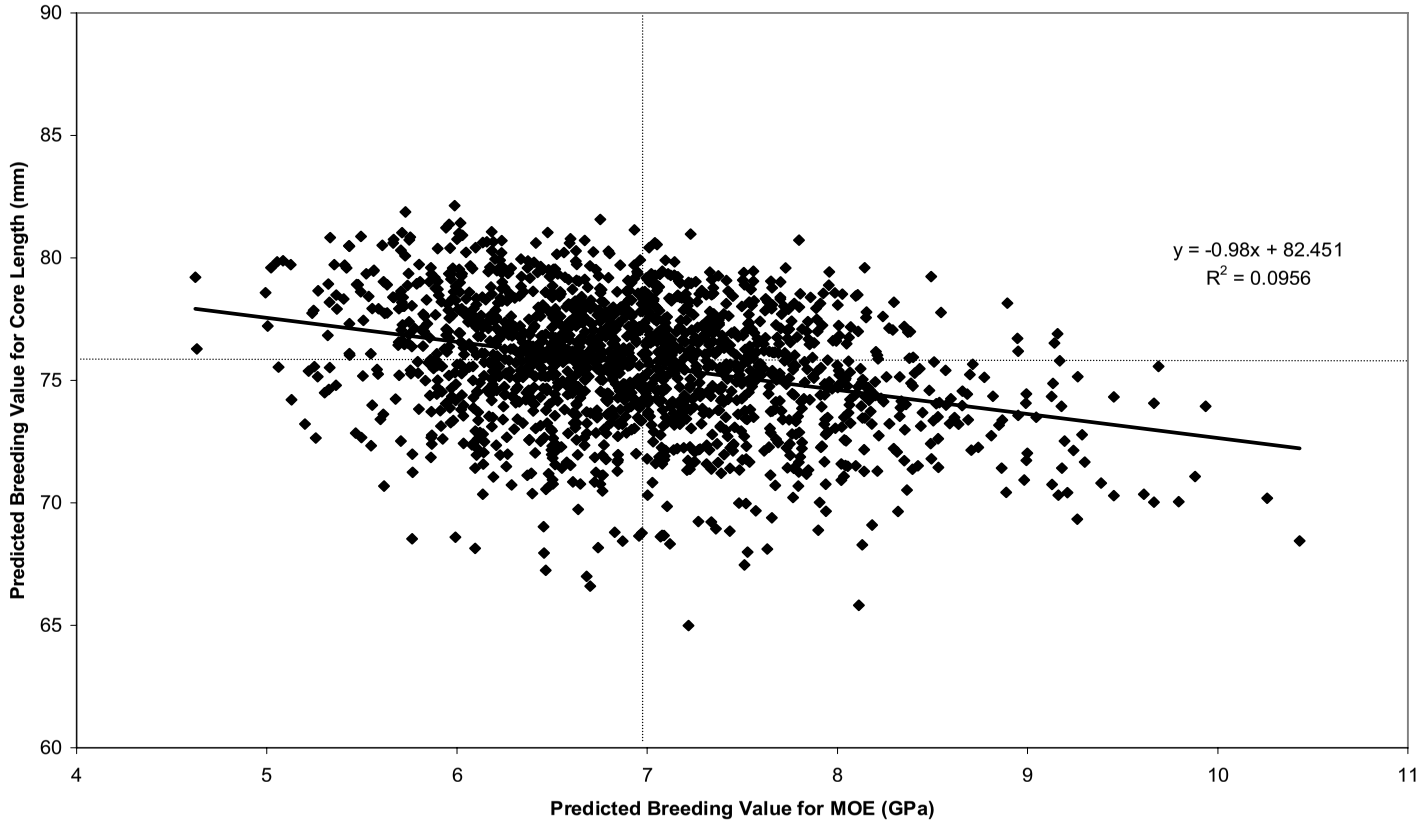

Figure 13. Predicted breeding values for MOE versus core length. Dashed lines represent population means for the respective traits. Even with a negative genetic correlation, genotypes can be selected with relatively high MOE and growth, *e.g.*, the upper right quadrant represents correlation breakers.

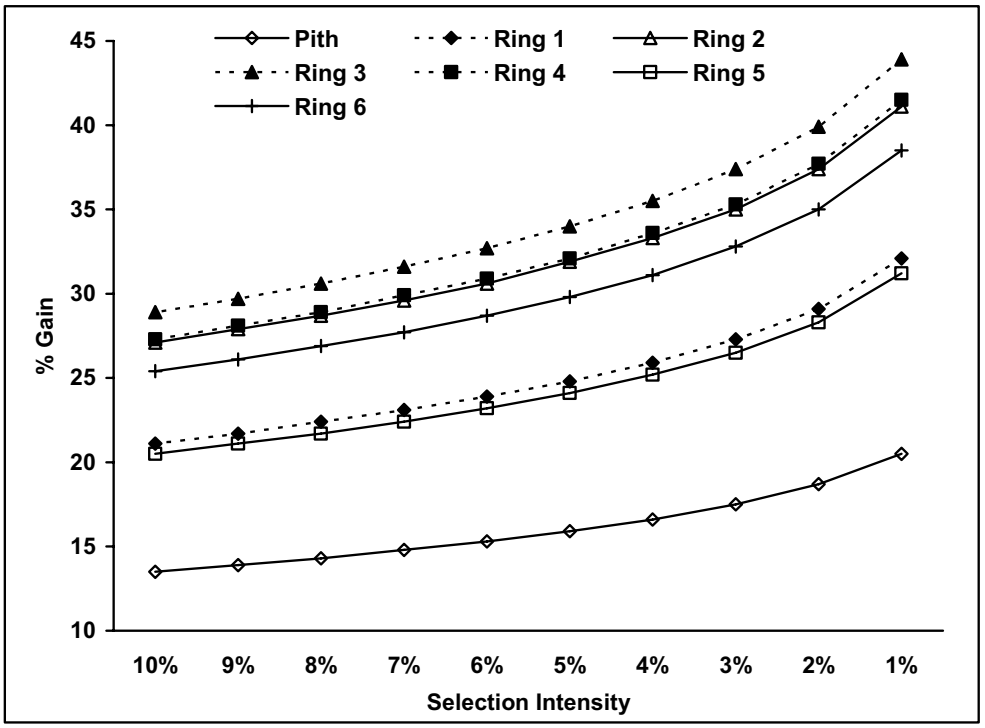

Figure 14. The genetic gain in MOE across the profile of the core when selecting for whole core area weighted MOE in juvenile radiata pine at trial BR9611.

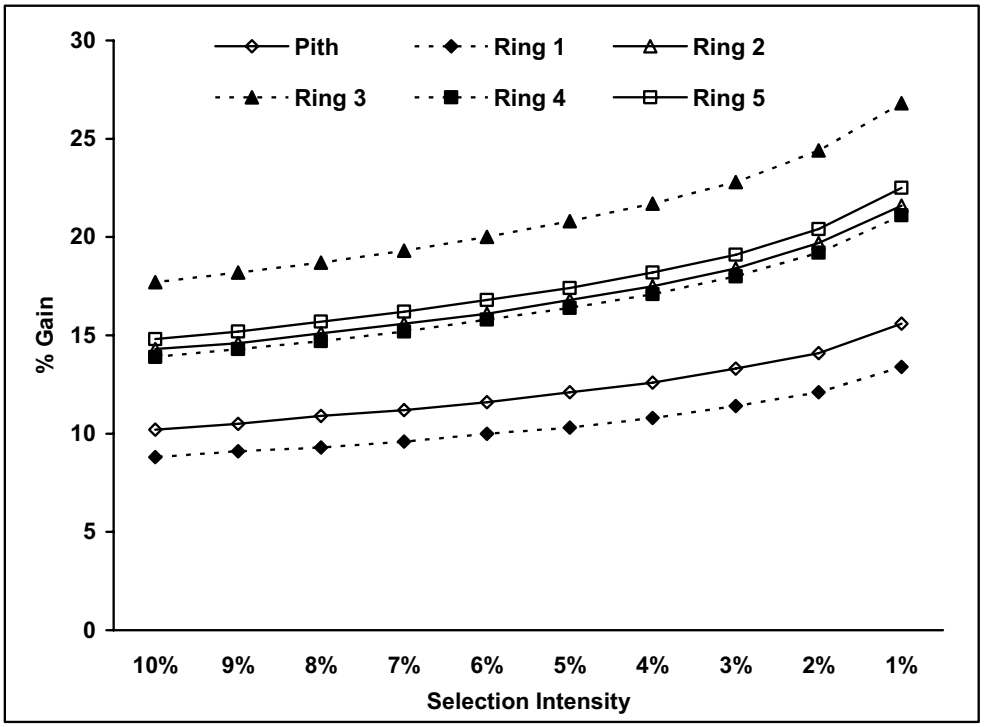

Figure 15. The genetic gain in MOE across the profile of the core when selecting for whole core area weighted MOE in juvenile radiata pine at trial BR9705.

Conclusion

There are two main methods for improving juvenile wood quality. First, any reduction in the proportion of juvenile wood in the tree would improve overall wood quality since mature wood, or outerwood, has more desirable properties. Second, since juvenile wood properties are undesirable from a product stand point, improvements in wood quality of the juvenile core, such as increased density and stiffness and reduced MFA, will lead to improvements in the overall quality of the wood. The trees used in the current study were 8 to 9 years old, and consequently, the whole core samples had wood properties associated with corewood. Therefore, this study was more suited to address improvement in the quality of juvenile wood properties.

All of the juvenile wood properties, density, MFA, and MOE, are heritable in radiata pine, and all three traits showed substantial genetic variation. These results, in conjunction with the fact that little genotype x environment interaction exists for juvenile wood properties, make these traits quite amenable to selection for increased stiffness and density and reduced microfibril angle in juvenile radiata pine. Of the three traits, the highest genetic gains could be achieved by selecting for whole core area weighted MOE. Since MOE and MFA, and MOE and density had favourable genetic correlations, selection for MOE directly will produce the greatest improvement in overall stiffness of the corewood in radiata pine while still increasing density and reducing microfibril angle. Furthermore, selection for whole core MOE will also result in improvements of MOE in individual rings throughout the core including the innermost rings. Although growth and wood quality traits were unfavourably correlated, overall reductions in growth may be of little consequence when considering the genetic gains in juvenile wood properties. Moreover, there is potential for selection of correlation breakers, thus improving juvenile wood properties without adversely affecting growth in radiata pine.

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APPENDIX 5

Juvenile Wood Initiative:

Genotype by Environmental Interaction for DBH, Wood Density, Branch Angle, Branch Size, and Stem Straightness in Eight Young *Pinus radiata* D. Don Trials in Australia

B.S. Baltunis, M.B. Powell and H.X. Wu

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COMMERCIAL IN CONFIDENCE

CSIRO Forest Bioscience
PO Box E4008
Kingston ACT 2604 AUSTRALIA
Telephone: 02 62818211 Fax: 02 62818312

Genotype by Environmental Interaction for DBH, Wood Density, Branch Angle, Branch Size, and Stem Straightness in Eight Young Pinus radiata D. Don Trials in Australia.

B.S. Baltunis, M.B. Powell and H.X. Wu

Executive Summary

Genotype by environment interactions for wood density, growth, branching characteristics and stem straightness were investigated in eight sites located in Mainland (five sites) and island state Tasmania (three sites) of Australia. Overall, branch angle had the least genotype by site interaction (average Type B genetic correlation $\hat{r}_B$ = 0.94), followed by wood density ($\hat{r}_B$ = 0.92), and stem straightness ($\hat{r}_B$ = 0.75). Branch size and DBH had the highest genotype by environment interactions ($\hat{r}_B$ = 0.50 and $\hat{r}_B$ = 0.67, respectively).

Genotype by regional interactions (Mainland vs Tasmania) revealed that wood density and branch angle had the least interactions ($\hat{r}_B$ = 0.98 and $\hat{r}_B$ = 0.95, respectively). Branch size and DBH had the highest interactions among the two regions ($\hat{r}_B$ = 0.55 and $\hat{r}_B$ = 0.63, respectively). Within Tasmania, only branch size and DBH had a sizable interaction within the three sites ($\hat{r}_B$ = 0.50 and $\hat{r}_B$ = 0.58, respectively). In contrast, there was little interaction for DBH ($\hat{r}_B$ = 0.92) among the five sites in Mainland. Branch size in the Mainland trials had a similar size of interaction ($\hat{r}_B$ = 0.64) as in Tasmania. Interactions for the other three traits (wood density, branch angle, and stem straightness) were small within the two regions.

This analysis indicates there are three patterns of genotype by environment interactions in these trials:
(1) There were little interactions for wood density within and among regions;
(2) There were sizable interactions between two regions and within Tasmania for DBH;
(3) There were large interactions between two regions and within Mainland and Tasmania for branch size.

INTRODUCTION

The genetic variation associated with DBH, density, branch angle, branch size, and stem straightness was investigated for young radiata pine growing in eight STBA 2nd generation progeny trials in Australia. Five trials were located in mainland Australia, while three were in Tasmania. To our knowledge, this is the first published reports of genetic parameters for these traits from radiata pine trials in Tasmania; and as a consequence, the extent of genotype by environment interaction for these traits is unknown between radiata pine trials in Tasmania and mainland Australia. The objectives of the study were to i) estimate heritability for DBH, density, branch angle, branch size, and stem straightness; ii) estimate genetic correlations among DBH, density, and form traits; and iii) investigate genotype by environment interaction for DBH, density, and form traits across a diverse range of sites.

METHODS AND MATERIALS

Genetic material and measurements

DBH, density, branch angle, branch size, and stem straightness were measured at eight radiata pine 2nd generation STBA progeny trials in Australia. Two trials were located in South Australia, two in Victoria, one in Western Australia, and three in Tasmania (Table 1). The trials were planted in 1996 and 1997. Trials contained five replications, incomplete blocking, and 4- or 5-tree row plots. In total progeny from 275 radiata pine selections from the STBA breeding population were tested across the eight trials. Connectedness among pairs of trials (e.g. number of parental selections in common) ranged from 13% to 100%.

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Table 1. Eight 2nd generation radiata pine progeny trials used in this study.

Trial	Alias	Location	Region
BR9601	Airport Trial25	Mt. Gambier, SA	Mainland
BR9611	Flynn Trial64	Gippsland, VIC	Mainland
BR9615	Koomeela Trial56	Scottsdale, TAS	Tasmania
BR9701	Bussels Trial258	Collie, WA	Mainland
BR9705	Kromelite Trial62	Mt. Gambier, SA	Mainland
BR9709	Bradvale Trial278	Ballarat, VIC	Mainland
BR9614	Moogara Trial734	Hobart, TAS	Tasmania
BR9715	Kamona Trial2097	Scottsdale, TAS	Tasmania

Measurements were collected in 2004. DBH was measured at 1.3 m above ground. Basic density was estimated from 12-mm cores. Form traits had the following classifications:

Branch angle was scored on a scale of 1 to 6 with 1 = steepest branch angle, …, 6 = flattest branch angle;

Branch size was scored on a scale of 1 to 6 with 1 = biggest branches, …, 6 = smallest branches;

Stem straightness was scored on a scale of 1 to 6 with 1 = most crooked stems, …, 6 = straightest stems.

Trait means at each trial are reported in the APPENDIX 1.

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Statistical analyses and genetic parameters

A series of four genetic analyses were conducted on the data in ASReml (Gilmour et al. 2005). First, each trait from all of the trials (univariate, single-site) was analysed in order to estimate the genetic variance components, individual-tree narrow-sense heritability and standard errors associated with each trait. An individual-tree linear mixed-effects model was used:

[1] $y_{ijklmno} = \mu + R_i + row_{j(i)} + col_{k(i)} + tree_l + fam_m + plot_n + e_{ijklmno}$,

where $y_{ijklmno}$ is the individual tree measurement, μ is the overall mean, R_i is the fixed effect of replication, $row_{j(i)}$ is the random effect of row $\sim N(0, \hat{\sigma}^2_{row})$, $col_{k(i)}$ is the random effect of column $\sim N(0, \hat{\sigma}^2_{col})$, $tree_l$ is the random additive genetic effect of individual tree $\sim N(0, \hat{\sigma}^2_A)$, fam_m is the random effect of family $\sim N(0, \hat{\sigma}^2_{fam})$, $plot_n$ is the random effect of plot $\sim N(0, \hat{\sigma}^2_{plot})$, and $e_{ijklmno}$ is the random residual effect $\sim N(0, \hat{\sigma}^2_E)$.

Observed variance components were used to estimate the causal variance components and individual-tree narrow-sense heritability for each trait:

$\hat{\sigma}^2_A$ = estimate of additive genetic variance,

$\hat{\sigma}^2_D = 4\hat{\sigma}^2_{fam}$ = estimate of dominance genetic variance,

$\hat{\sigma}^2_G = \hat{\sigma}^2_A + \hat{\sigma}^2_D$ = estimate of total genetic variance,

$\hat{\sigma}^2_P = \hat{\sigma}^2_A + \hat{\sigma}^2_{fam} + \hat{\sigma}^2_{plot} + \hat{\sigma}^2_E$ = estimate of phenotypic variance, and

$\hat{h}^2 = \dfrac{\hat{\sigma}^2_A}{\hat{\sigma}^2_P}$ = estimate of individual-tree narrow-sense heritability.

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Bivariate, single-site individual-tree linear mixed-effects models were used to estimate the genetic correlations between traits within a site:

[2] $$\mathbf{y}_i = \mathbf{X}_i\mathbf{b}_i + \mathbf{Z}_{m_i}\mathbf{m}_i + \mathbf{Z}_{n_i}\mathbf{n}_i + \mathbf{Z}_{a_i}\mathbf{a}_i + \mathbf{Z}_{f_i}\mathbf{f}_i + \mathbf{Z}_{p_i}\mathbf{p}_i + \mathbf{e}_i,$$

where $\mathbf{y}_i$ is the vector of observations indexed (i) by trait,

$\mathbf{b}_i$ is the vector of fixed effects (mean and replications) and $\mathbf{X}_i$ is the known incidence matrix relating the observations in $\mathbf{y}_i$ to the fixed effects in $\mathbf{b}_i$ where

$$\mathbf{X}_i\mathbf{b}_i = \begin{bmatrix} \mathbf{X}_1 & \mathbf{0} \\ \mathbf{0} & \mathbf{X}_2 \end{bmatrix}\begin{bmatrix} \mathbf{b}_1 \\ \mathbf{b}_2 \end{bmatrix},$$

$\mathbf{m}_i$ is the vector of random row within replication effects

$$\sim MVN\left(\mathbf{0}, \begin{bmatrix} \mathbf{I}_r\hat{\sigma}^2_{row_1} & \mathbf{0} \\ \mathbf{0} & \mathbf{I}_r\hat{\sigma}^2_{row_2} \end{bmatrix}\right),$$

$\mathbf{n}_i$ is the vector of random column within replication effects

$$\sim MVN\left(\mathbf{0}, \begin{bmatrix} \mathbf{I}_c\hat{\sigma}^2_{col_1} & \mathbf{0} \\ \mathbf{0} & \mathbf{I}_c\hat{\sigma}^2_{col_2} \end{bmatrix}\right),$$

$\mathbf{a}_i$ is the vector of random additive effects of individual trees $\sim MVN(\mathbf{0}, \mathbf{G} \otimes \mathbf{A})$ where $\mathbf{G} = \begin{bmatrix} \hat{\sigma}^2_{A_1} & \hat{\sigma}_{A_1A_2} \\ \hat{\sigma}_{A_1A_2} & \hat{\sigma}^2_{A_2} \end{bmatrix}$ and $\mathbf{A}$ = numerator relationship matrix generated from the pedigree, $\mathbf{f}_i$ is the vector of random effects of full-sib family (specific combining ability) $\sim MVN(\mathbf{0}, \mathbf{F} \otimes \mathbf{I}_f)$ where $\mathbf{F} = \begin{bmatrix} \hat{\sigma}^2_{fam_1} & \hat{\sigma}_{fam_1fam_2} \\ \hat{\sigma}_{fam_1fam_2} & \hat{\sigma}^2_{fam_2} \end{bmatrix}$ and $\mathbf{I}_f$ is an identity matrix equal to the number of families,

$\mathbf{p}_i$ is the vector of random plot effects $\sim MVN\left(\mathbf{0}, \begin{bmatrix} \mathbf{I}_p\hat{\sigma}^2_{plot_1} & \mathbf{0} \\ \mathbf{0} & \mathbf{I}_p\hat{\sigma}^2_{plot_2} \end{bmatrix}\right)$,

$\mathbf{e}_i$ is the random vector of residual terms $\sim MVN(\mathbf{0}, \mathbf{R} \otimes \mathbf{I})$ where $\mathbf{R} = \begin{bmatrix} \hat{\sigma}^2_{E_1} & \hat{\sigma}_{E_1E_2} \\ \hat{\sigma}_{E_1E_2} & \hat{\sigma}^2_{E_2} \end{bmatrix}$,

0 is the null matrix, **I** is the identity matrix equal to the number of observations; $\mathbf{I}_r$, $\mathbf{I}_c$, and $\mathbf{I}_p$ are identity matrices equal to the number of rows, columns, and plots, respectively; $\mathbf{Z}_{m_i}, \mathbf{Z}_{n_i}, \mathbf{Z}_{a_i}, \mathbf{Z}_{f_i}$, and $\mathbf{Z}_{p_i}$ are the known incidence matrices relating observations in $\mathbf{y}_i$ to effects in $\mathbf{m}_i$, $\mathbf{n}_i$, $\mathbf{a}_i$, $\mathbf{f}_i$, and $\mathbf{p}_i$, respectively.

The additive genetic correlation between traits was estimated as:

$$\hat{r}_A = \frac{\hat{\sigma}_{A_1A_2}}{\sqrt{\hat{\sigma}^2_{A_1}\hat{\sigma}^2_{A_2}}}.$$

A multivariate, multi-site (all traits and all trials) analysis was also conducted in order to estimate additive genetic correlations between traits across all trials using a model similar to equation [2], except that here trial is a fixed effect.

In order to determine the extent of genotype by environment interaction for each of the traits; univariate, paired-site analyses were conducted and Type B genetic correlations estimated using a model similar to equation [2], except trial is a fixed effect, and $\mathbf{y}_i$ is now defined as the vector of observations for a single trait indexed (*i*) by trial. Type B additive genetic correlations were then estimated as:

$\hat{r}_B = \frac{\hat{\sigma}_{A_1A_2}}{\sqrt{\hat{\sigma}^2_{A_1}\hat{\sigma}^2_{A_2}}}$ where higher values (> ~ 0.68) indicate little genotype by environment interaction, and lower values indicate that genotype by environment interactions exist. Additionally, the three trials in Tasmania were assumed to be a single region while the five mainland trials a second region, and Type B additive genetic correlations were estimated to look for region by genotype interactions.

RESULTS AND DISCUSSION

Genetic variation for DBH, density, and form traits

Additive and non-additive (dominance) genetic variances were estimated for each trait at each trial (Table 2). There was significant additive genetic variance ($\hat{\sigma}_A^2$) estimated for DBH at each of the trials except at trial BR9709. $\hat{\sigma}_A^2$ ranged from 10.65 to 114.5 at the eight trials (Table 2). Dominance genetic variance ($\hat{\sigma}_D^2$) was only significant at four of the trials, and ranged from 0.24 to137.0 at the eight trials (Table 2). The ratio of $\hat{\sigma}_A^2/\hat{\sigma}_D^2$ varied across the eight trials. For example, at trials BR9615 and BR9701, this ratio was less than one, indicating a majority of $\hat{\sigma}_D^2$ associated with DBH. Trials BR9601 and BR9611 had a $\hat{\sigma}_A^2/\hat{\sigma}_D^2$ for DBH equal to one, while the rest of the trials had $\hat{\sigma}_A^2/\hat{\sigma}_D^2$ greater than one for DBH.

There was substantial genetic variation for density at all of the trials (Table 2). Additive genetic variance made up the majority of the genetic variation for density at six trials and was significant at all but trial BR9715 in Tasmania (Table 2). At this trial, $\hat{\sigma}_D^2$ comprised 96% of the genetic variance for density which was rather unexpected. At the seven trials where $\hat{\sigma}_A^2$ was significant, $\hat{\sigma}_A^2$ ranged from 153.4 to 420.7 (Table 2). Dominance genetic variance was negligible for density at trials BR9601, BR9611, BR9615, BR9705, and BR9709. The ratio of $\hat{\sigma}_A^2/\hat{\sigma}_D^2$ for density was much greater than one at these trials. $\hat{\sigma}_D^2$ was significant at trials BR9701, BR9614, and BR9715. The ratio of $\hat{\sigma}_A^2/\hat{\sigma}_D^2$ for density was 1.25, 0.83, and 0.04 (essentially 0) at trials BR9614, BR9701, and BR9715, respectively.

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Table 2. Variance component and heritability estimates for DBH, DEN, BA, BS, and SS ± standard error from the single-trait-single-site analyses.

a. BR9601

	DBH	DEN	BA	BS	SS
$\hat{\sigma}_A^2$	53.32 ± 13.3	328.5 ± 58.6	0.2907 ± 0.055	0.1033 ± 0.031	0.2343 ± 0.05
$\hat{\sigma}_D^2$	50.62 ± 12.3	15.1 ± 33.7	0.0912 ± 0.034	0.115 ± 0.035	0.0343 ± 0.029
$\hat{\sigma}_G^2$	103.9 ± 15.5	343.5 ± 61.8	0.3819 ± 0.058	0.2183 ± 0.038	0.2686 ± 0.049
$\hat{\sigma}_E^2$	170.5 ± 9.3	115.1 ± 36.9	0.6999 ± 0.038	0.7883 ± 0.026	0.7898 ± 0.036
$\hat{\sigma}_P^2$	245.2 ± 6.9	447.3 ± 27.1	1.013 ± 0.028	0.9203 ± 0.022	1.033 ± 0.027
$\hat{h}^2$	0.22 ± 0.05	0.73 ± 0.10	0.29 ± 0.05	0.11 ± 0.03	0.23 ± 0.04

b. BR9611

	DBH	DEN	BA	BS	SS
$\hat{\sigma}_A^2$	55.04 ± 16.9	303.5 ± 45.9	0.4321 ± 0.08	0.2181 ± 0.057	0.2814 ± 0.065
$\hat{\sigma}_D^2$	45.3 ± 18.8	18.14 ± 21.9	0.0522 ± 0.048	0.0897 ± 0.054	0.1321 ± 0.055
$\hat{\sigma}_G^2$	100.3 ± 20.7	321.6 ± 46.8	0.4843 ± 0.083	0.3077 ± 0.066	0.4135 ± 0.07
$\hat{\sigma}_E^2$	291.2 ± 13.2	107.1 ± 27.6	0.6607 ± 0.052	0.8803 ± 0.043	0.909 ± 0.047
$\hat{\sigma}_P^2$	379.7 ± 10.3	415.1 ± 21.5	1.194 ± 0.04	1.220 ± 0.034	1.253 ± 0.035
$\hat{h}^2$	0.14 ± 0.04	0.73 ± 0.08	0.36 ± 0.06	0.18 ± 0.04	0.22 ± 0.05

c. BR9615

	DBH	DEN	BA	BS	SS
$\hat{\sigma}_A^2$	93.46 ± 31.5	420.7 ± 70.3	0.2435 ± 0.051	0.0913 ± 0.031	0.1392 ± 0.034
$\hat{\sigma}_D^2$	137.0 ± 36.3	21.67 ± 37.3	0.0243 ± 0.029	0.0342 ± 0.033	0
$\hat{\sigma}_G^2$	230.5 ± 38.0	442.4 ± 74.1	0.2678 ± 0.052	0.1255 ± 0.035	0.1392 ± 0.034
$\hat{\sigma}_E^2$	556.1 ± 24.6	81.78 ± 43.6	0.6144 ± 0.036	0.8103 ± 0.029	0.6833 ± 0.028
$\hat{\sigma}_P^2$	691.2 ± 17.9	507.9 ± 31.7	0.888 ± 0.025	0.9169 ± 0.022	0.9002 ± 0.023
$\hat{h}^2$	0.14 ± 0.04	0.83 ± 0.10	0.27 ± 0.05	0.10 ± 0.03	0.15 ± 0.04

d. BR9701

	DBH	DEN	BA	BS	SS
$\hat{\sigma}_A^2$	61.59 ± 22.0	153.4 ± 43.9	0.2751 ± 0.063	0.073 ± 0.032	0.2407 ± 0.062
$\hat{\sigma}_D^2$	105.6 ± 30.6	185.8 ± 51.7	0.1349 ± 0.055	0.1102 ± 0.047	0.07 ± 0.052
$\hat{\sigma}_G^2$	167.2 ± 29.4	339.2 ± 53.8	0.41 ± 0.063	0.1831 ± 0.043	0.3106 ± 0.059
$\hat{\sigma}_E^2$	574.6 ± 20.2	268.1 ± 31.2	0.904 ± 0.047	1.069 ± 0.033	1.174 ± 0.051
$\hat{\sigma}_P^2$	687.6 ± 15.1	467.9 ± 20.3	1.252 ± 0.03	1.215 ± 0.026	1.453 ± 0.033
$\hat{h}^2$	0.09 ± 0.03	0.33 ± 0.09	0.22 ± 0.05	0.06 ± 0.03	0.17 ± 0.04

e. BR9705

	DBH	DEN	BA	BS	SS
$\hat{\sigma}_A^2$	107.8 ± 38.1	193.2 ± 67.1	0.1163 ± 0.027	0.1011 ± 0.031	0.1118 ± 0.053
$\hat{\sigma}_D^2$	20.68 ± 33.9	17.45 ± 71.8	0	0	0.0223 ± 0.053
$\hat{\sigma}_G^2$	128.5 ± 29.7	210.7 ± 50.0	0.1163 ± 0.027	0.1011 ± 0.031	0.1341 ± 0.035
$\hat{\sigma}_E^2$	310.3 ± 28.0	203.4 ± 45.2	0.3682 ± 0.023	0.5454 ± 0.03	0.4792 ± 0.039
$\hat{\sigma}_P^2$	423.3 ± 15.2	400.9 ± 22.2	0.4845 ± 0.017	0.6465 ± 0.021	0.5966 ± 0.02
$\hat{h}^2$	0.25 ± 0.09	0.48 ± 0.15	0.24 ± 0.05	0.16 ± 0.05	0.19 ± 0.09

f. BR9709

	DBH	DEN	BA	BS	SS
$\hat{\sigma}_A^2$	10.65 ± 27.2	296.9 ± 73.3	0.2269 ± 0.115	0.0688 ± 0.038	0.0983 ± 0.071
$\hat{\sigma}_D^2$	0.24 ± 38.6	0.002 ± 0.001	0.1542 ± 0.123	0	0.0456 ± 0.077
$\hat{\sigma}_G^2$	10.89 ± 24.0	296.9 ± 73.3	0.3811 ± 0.095	0.0688 ± 0.038	0.1439 ± 0.049
$\hat{\sigma}_E^2$	716.2 ± 29.5	155.6 ± 56.1	0.8391 ± 0.084	1.18 ± 0.051	0.6408 ± 0.054
$\hat{\sigma}_P^2$	726.9 ± 22.9	452.4 ± 32.6	1.20 ± 0.043	1.257 ± 0.04	0.8056 ± 0.027
$\hat{h}^2$	0.01 ± 0.04	0.66 ± 0.13	0.19 ± 0.09	0.05 ± 0.03	0.12 ± 0.09

g. BR9614

	DBH	DEN	BA	BS	SS
$\hat{\sigma}_A^2$	83.88 ± 27.10	254.4 ± 93.3	0.1697 ± 0.051	0.1365 ± 0.054	0.1460 ± 0.047
$\hat{\sigma}_D^2$	41.93 ± 31.0	202.6 ± 135.4	0	0.0879 ± 0.069	0
$\hat{\sigma}_G^2$	125.81 ±37.1	457.1 ± 147.8	0.1697 ± 0.051	0.2244 ± 0.076	0.1460 ± 0.047
$\hat{\sigma}_E^2$	651.1 ± 27.4	160.6 ± 63.2	0.8707 ± 0.042	1.395 ± 0.056	1.169 ± 0.048
$\hat{\sigma}_P^2$	747.2 ± 23.1	465.7 ± 48.4	1.106 ± 0.036	1.579 ± 0.048	1.365 ± 0.042
$\hat{h}^2$	0.11 ± 0.03	0.55 ± 0.16	0.15 ± 0.04	0.09 ± 0.03	0.11 ± 0.03

h. BR9715

	DBH	DEN	BA	BS	SS
$\hat{\sigma}_A^2$	114.5 ± 54.6	20.73 ± 98.7	---	0.0928 ± 0.054	0.0912 ± 0.048
$\hat{\sigma}_D^2$	45.68 ± 62.8	484.9 ± 202.3	---	0.0037 ± 0.066	0.0966 ± 0.067
$\hat{\sigma}_G^2$	160.2 ± 50.0	505.7 ± 167.5	---	0.0965 ± 0.049	0.1878 ± 0.056
$\hat{\sigma}_E^2$	765.5 ± 44.4	371.6 ± 79.3	---	1.252 ± 0.053	1.011 ± 0.045
$\hat{\sigma}_P^2$	953.0 ± 26.5	513.5 ± 42.8	---	1.366 ± 0.036	1.189 ± 0.032
$\hat{h}^2$	0.12 ± 0.06	0.04 ± 0.19	---	0.07 ± 0.04	0.08 ± 0.04

Estimates of additive genetic variance for branch angle were significant at all seven trials where branch angle was measured (Table 2). The range of $\hat{\sigma}_A^2$ was between 0.1163 to 0.4321. Dominance genetic variance for branch angle was significant only at trials BR9601 and BR9701. However, the ratio of $\hat{\sigma}_A^2 / \hat{\sigma}_D^2$ for branch angle was 3.2 and 2.0 at BR9601 and BR9701, respectively. For branch size, $\hat{\sigma}_A^2$ was significant at all of the trials except BR9709, and $\hat{\sigma}_A^2$ ranged from 0.073 to 0.2181 (Table 2). Dominance genetic variance, on the other hand, ranged from 0 to 0.115,

but was only significant at trials BR9601 and BR9701 which was the same as reported above for branch angle. However, the ratio of $\hat{\sigma}_A^2/\hat{\sigma}_D^2$ for branch size at these two trials was less than one. For stem straightness, $\hat{\sigma}_A^2$ was significant at six trials, while $\hat{\sigma}_D^2$ was only significant at trial BR9611 (Table 2). Estimates of additive genetic variance for stem straightness ranged from 0.0912 to 0.2814 across all eight trials. At BR9611, the ratio of $\hat{\sigma}_A^2/\hat{\sigma}_D^2$ was in excess of 2.1.

Individual-tree narrow-sense heritability ($\hat{h}^2$)

Generally, $\hat{h}^2$ for density > branch angle > stem straightness > DBH > branch size; and significant $\hat{h}^2$ was observed for all traits and at all trials with a couple exceptions (Table 2). For example, at trial BR9709 only density and branch angle had significant heritability estimates, and basically DBH was not heritable at all (Table 2f). Additionally, at trial BR9715 only DBH and stem straightness were heritable (Table 2h). Significant values of $\hat{h}^2$ for DBH ranged from 0.09 to 0.25 across the trials (Table 2) with a mean $\hat{h}^2$ = 0.15 (or = 0.14 if include non-significant 0.01 estimate). Similar $\hat{h}^2$ for DBH was reported by Matheson and Raymond (1984) in a population of 30 open-pollinated families of radiata pine tested across multiple sites ($\hat{h}^2$ = 0.18). Moderately high and significant $\hat{h}^2$ was observed for density across all trials (Table 2) except at trial BR9715, where all of the genetic variance associated with density was non-additive (Table 2h). Significant values of $\hat{h}^2$ for density ranged from 0.33 to 0.83 (Table 2) with a mean $\hat{h}^2$ = 0.62 (or = 0.54 if include non-significant 0.04 estimate).

For branch angle, significant $\hat{h}^2$ was estimated at all of the trials where it was measured and ranged from 0.15 to 0.36 (Table 2), and mean $\hat{h}^2$ was 0.25. Branch size was not as heritable as branch angle, and $\hat{h}^2$ ranged from a non-significant 0.05 to 0.18 (Table 2). Considering only significant values of $\hat{h}^2$ for branch size, the mean $\hat{h}^2$ was 0.12 (or = 0.10 if include non-significant estimates). Significant values of $\hat{h}^2$ for stem straightness ranged from 0.08 to 0.23 (Table 2) with a mean $\hat{h}^2$ = 0.16 (if non-significant 0.12 included, then $\hat{h}^2$ was still equal to 0.16).

Genetic correlations ($\hat{r}_A$)

Statistically significant genetic correlations were estimated between DBH and density, and $\hat{r}_A$ ranged between -0.23 to -0.57 across all trials (Table 3). However, at BR9709, $\hat{r}_A$ was not significant ($\hat{r}_A$ = -0.23 ± 0.40). The genetic correlations between pairs of traits were also estimated from a multivariate, combined-site analysis (Table 4). From this analysis, DBH had a moderate, negative genetic correlation with density ($\hat{r}_A$ = -0.48 ± 0.08). The adverse genetic correlation between DBH and density observed here is well within published estimates for radiata pine (Dean et al. 1983; Cotterill and Dean 1990; Burdon and Low 1992; Jaywickrama 2001; Kumar 2004; Li and Wu 2005; Baltunis et al. 2007). Additionally, Wu et al. (2008) recently reviewed estimates of genetic parameters in radiata pine and reported an average estimate of genetic correlation of -0.48 between growth and density which is the same as our multivariate, combined-site estimate. Similar negative genetic correlations (in the range of -0.4) have been observed in other conifers (e.g., Lee 1997; Costa E Silva et al. 1998; Rozenberg and Cahalan 1998; Hannrup et al. 2000).

Table 3. Additive genetic correlation ± standard error between pairs of traits for two-trait-single site analyses.

		Trial							
Trait 1	**Trait 2**	**BR9601**	**BR9611**	**BR9615**	**BR9701**	**BR9705**	**BR9709**	**BR9614**	**BR9715**
DBH	DEN	-0.52 ± 0.13	-0.57 ± 0.13	-0.51 ± 0.16	-0.39 ± 0.19	-0.46 ± 0.14	-0.23 ± 0.40	-0.51 ± 0.21	---
DBH	BA	0.63 ± 0.11	0.24 ± 0.15	0.06 ± 0.18	0.60 ± 0.15	0.59 ± 0.18	---	0.25 ± 0.22	---
DBH	BS	-0.74 ± 0.09	-0.17 ± 0.18	-0.50 ± 0.14	-0.22 ± 0.21	-0.36 ± 0.21	---	-0.23 ± 0.23	-0.50 ± 0.17
DBH	SS	0.13 ± 0.16	-0.30 ± 0.17	0.41 ± 0.20	-0.01 ± 0.20	-0.04 ± 0.20	-0.15 ± 0.65	0.38 ± 0.20	0.12 ± 0.34
DEN	BA	-0.10 ± 0.14	-0.01 ± 0.12	0.01 ± 0.14	0.04 ± 0.17	-0.04 ± 0.17	-0.13 ± 0.23	-0.40 ± 0.22	---
DEN	BS	0.15 ± 0.17	0.09 ± 0.14	0.27 ± 0.17	0.12 ± 0.24	0.12 ± 0.19	-0.09 ± 0.28	0.38 ± 0.25	---
DEN	SS	-0.63 ± 0.10	-0.18 ± 0.13	-0.33 ± 0.14	-0.34 ± 0.17	-0.31 ± 0.21	-0.19 ± 0.23	-0.27 ± 0.24	---
BA	BS	-0.33 ± 0.15	0.55 ± 0.11	0.27 ± 0.17	0.58 ± 0.17	-0.11 ± 0.19	0.77 ± 0.16	0.46 ± 0.19	---
BA	SS	-0.04 ± 0.14	0.15 ± 0.14	0.04 ± 0.16	0.30 ± 0.14	-0.28 ± 0.17	0.09 ± 0.27	0.27 ± 0.21	---
BS	SS	0.05 ± 0.17	0.49 ± 0.13	0.02 ± 0.19	0.45 ± 0.20	0.24 ± 0.19	0.37 ± 0.29	0.43 ± 0.21	-0.20 ± 0.30

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Table 4. Additive genetic correlation ± standard error between pairs of traits from multivariate, combined site analysis.

	Density	**Branch angle**	**Branch size**	**Stem straightness**
DBH	-0.48 ± 0.08	0.34 ± 0.09	-0.26 ± 0.10	-0.03 ± 0.10
Density		-0.04 ± 0.08	0.10 ± 0.10	-0.28 ± 0.08
Branch angle			0.18 ± 0.10	-0.07 ± 0.09
Branch size				0.27 ± 0.10

DBH also had statistically significant genetic correlations with branch angle, and branch size. For example, $\hat{r}_A$ = 0.34 ± 0.09 between DBH and branch angle from the combined-site analysis (Table 4). However, $\hat{r}_A$ between DBH and branch angle ranged from a non-significant 0.06 to 0.63 across individual trials (Table 3). A positive genetic correlation between DBH and branch angle indicate a favourable relationship between these two traits, i.e. larger diameter trees tended to have flatter branch angles. Not surprisingly, larger diameter trees also tended to have bigger branches. This is indicated by the significant negative genetic correlation between DBH and branch size, $\hat{r}_A$ = -0.26 ± 0.10 (Table 4). Estimates of the genetic correlation between DBH and branch size ranged from a non-significant -0.17 to moderately high and negative -0.74 across individual trials (Table 3). The genetic correlations between DBH and stem straightness were only significant at one of the trials in Tasmania (BR9615), while non-significant at all other trials (Table 3). Additionally, based on results from the combined-site analysis, DBH and stem straightness may be considered independent traits ($\hat{r}_A$ = -0.03 ± 0.10).

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There were no significant genetic correlations between density and branch angle, and density and branch size at any of the trials (Table 3). Generally, genetic correlation estimates between these traits were low with large standard errors in all single-site analyses and in the combined-site analysis (Table 4). However, there was a significant genetic correlation between density and stem straightness based on the combined-site analysis, $\hat{r}_A$ = -0.28 ± 0.08 (Table 4). Genetic correlations between density and stem straightness ranged from a non-significant -0.13 to -0.63 across the individual trials (Table 3). This adverse genetic correlation implies that trees with higher density tended to have more crooked stems, perhaps a result of compression wood.

No clear trend was observed from the genetic correlation between branch angle and branch size based on single-site analyses. For example, although statistically significant estimates of the genetic correlation were observed, both negative and positive values were estimated, and $\hat{r}_A$ ranged from -0.33 to 0.77 across all trials (Table 3). Furthermore, the estimated genetic correlation between branch angle and branch size based on the combined-site analysis was non-significant ($\hat{r}_A$ = 0.18 ± 0.10) (Table 4). Similarly, the genetic correlation between branch angle and stem straightness was not different than zero ($\hat{r}_A$ = -0.07 ± 0.10) (Table 4), and only statistically significant at one trial (Table 3). Generally, the genetic correlations between branch size and stem straightness were positive with a range from a non-significant -0.20 to 0.49, but only significant at three trials (Table 3). However, the combined-site estimate of the genetic correlation between branch size and stem straightness was significant, $\hat{r}_A$ = 0.27 ± 0.10 (Table 4), indicating that straighter stemmed trees tended to have smaller branches.

Genotype by environment interactions and Type B genetic correlations ($\hat{r}_B$)

Among the five mainland trials, there was little evidence for genotype by environment interaction for DBH except between trials BR9611 and BR9701 where $\hat{r}_B = 0.48$ (Table 5). Type B additive genetic correlations ranged from 0.48 to 1.4 between pairs of trials in the mainland (Table 5). However, there was some indication that parental rankings were unstable in the Tasmanian trials indicating genotype by environment interaction for DBH. For example, $\hat{r}_B$ ranged from 0.02 to 1.2, however, only Type B genetic correlations between trial 2097 and BR9601, BR9705, BR9709, and 734 were > 0.71. Type B genetic correlations for DBH involving measurements from trials BR9615 and BR9614 were all less than 0.66 indicating that genotype by environment interaction was present (Table 5). When trials were grouped within one of two regions, then $\hat{r}_B = 0.63 \pm 0.11$ for DBH.

Wu and Matheson (2005) reported on genotype by environment interaction for DBH among eight Australian-Wide-Diallel trials. They observed significant interaction and $\hat{r}_B$ ranged from -0.37 to 1.00 with an average $\hat{r}_B = 0.39$ (Wu and Matheson 2005). Furthermore, significant regional effects were observed, and two trials located in New South Wales had an average Type B genetic correlation of 0.12 with non-New South Wales' trials, which may have been a result of snow damage at trials in New South Wales (Wu and Matheson 2005). Similarly, Matheson and Raymond (1984) reported significant genotype by environment interaction for DBH for 30 open-pollinated families of radiata pine tested across eleven trials in Australia with an overall $\hat{r}_B =$ 0.33. However, they recommended culling more interactive families from the breeding population rather than having distinct regionalized breeding programs (Matheson and Raymond 1984). In New Zealand, significant family by environment

interactions was observed in radiata pine between pumice and clay sites (Johnson and Burdon 1990).

Table 5. Type B genetic correlations for DBH between two sites (standard error).

	Region 1 – Mainland				**Region 2 - Tasmania**		
	BR9611	**BR9701**	**BR9705**	**BR9709**	**BR9615**	**BR9614**	**BR9715**
BR9601	0.82 (0.12)	0.80 (0.15)	1.1 (0.06)	1.4 (1.9)	0.39 (0.18)	0.51 (0.17)	0.86 (0.23)
BR9611		0.48 (0.23)	0.91 (0.15)	0.98 (0.60)	0.48 (0.20)	0.24 (0.23)	0.56 (0.23)
BR9701			0.97 (0.17)	0.60 (0.50)	0.32 (0.25)	0.33 (0.23)	0.32 (0.29)
BR9705				1.1 (0.48)	0.63 (0.20)	0.33 (0.27)	0.85 (0.12)
BR9709					0.66 (0.62)	0.02 (0.88)	1.2 (1.1)
BR9615						0.44 (0.21)	---
BR9614							0.71 (0.23)

Region 1 vs. Region 2 (all sites) $\hat{r}_B$ = 0.63 (0.11)

There was no evidence for genotype by environment interaction for density across all pairwise combination of trials (Table 6). Significant Type B additive genetic correlations ranged from 0.74 to 1.0 for density (Table 6). There was also no region by genotype interaction with $\hat{r}_B$ = 0.98 ± 0.02. Generally, wood quality traits are believed to be genetically stable across trials. For example, Baltunis et al. (2007) previously reported Type B genetic correlations > 0.77 for density, MoE, and Mfa based on SilviScan measurements between trials BR9601 and BR9705. Such high genetic correlations between sites for density indicate that parental rankings are

stable, further suggesting that fewer trials may be necessary for ranking and selecting genotypes for density.

Table 6. Type B genetic correlations for density between two sites (standard error).

	Region 1 – Mainland				**Region 2 - Tasmania**		
	BR9611	**BR9701**	**BR9705**	**BR9709**	**BR9615**	**BR9614**	**BR9715**
BR9601	0.99 (0.02)	0.97 (0.06)	0.98 (0.06)	0.83 (0.14)	0.96 (0.03)	1.0 (0.03)	---
BR9611		0.92 (0.07)	0.79 (0.11)	0.78 (0.12)	0.99 (0.02)	1.0 (0.06)	---
BR9701			1.0 (0.08)	0.88 (0.13)	0.90 (0.07)	0.80 (0.15)	---
BR9705				0.99 (0.08)	0.88 (0.09)	1.0 (0.11)	---
BR9709					0.74 (0.14)	1.0 (0.17)	---
BR9615						1.0 (0.05)	---
BR9614							---

Region 1 vs. Region 2 (all sites) $\hat{r}_B$ = 0.98 (0.02)

Similar trends were also seen for branch angle (Table 7). For branch angle, $\hat{r}_B$ ranged from 0.71 to 1.1 across all pairwise combination of trials, and no region by environment interaction was present ($\hat{r}_B$ = 0.95 ± 0.04). Wu and Matheson (2005) reported an average $\hat{r}_B$ = 0.80 for branch angle across the Australian-Wide-Diallel trials. However, there was some evidence of genotype by environment interaction for branch angle between certain pairs of trials ($\hat{r}_B$ ranged from 0.31 to 1.1), but no clear regional trend (Wu and Matheson 2005).

Table 7. Type B genetic correlations for branch angle between two sites (standard error).

	Region 1 – Mainland				Region 2 - Tasmania		
	BR9611	**BR9701**	**BR9705**	**BR9709**	**BR9615**	**BR9614**	**BR9715**
BR9601	0.93 (0.05	0.91 (0.07)	0.99 (0.07)	1.4 (0.21)	0.91 (0.05)	0.87 (0.09)	---
BR9611		0.90 (0.07)	0.87 (0.10)	1.1 (0.21)	0.88 (0.06)	0.80 (0.12)	---
BR9701			1.0 (0.07)	1.0 (0.15)	0.93 (0.07)	0.83 (0.13)	---
BR9705				0.89 (0.15)	0.96 (0.07)	0.71 (0.18)	---
BR9709					1.1 (0.18)	0.94 (0.21)	---
BR9615						0.83 (0.10)	---
BR9614							---

Region 1 vs. Region 2 (all sites $\hat{r}_B$ = 0.95 (0.04)

There was considerable genotype by environment interaction for branch size (Table 8). Type B additive genetic correlations for branch size ranged from -0.13 to 1.1. However, at some trials there was little additive genetic variance associated with branch size. Therefore, $\hat{r}_B$ had large standard errors and was non-significant in most cases (Table 8). Within the five mainland trials, when branch size measurements involved trial BR9705, there was generally low genotype by environment interaction. When trials were grouped into regions, the $\hat{r}_B = 0.55 \pm 0.15$. Similarly, an average $\hat{r}_B$ = 0.59 was reported by Wu and Matheson (2005) for branch size in the Australian-Wide-Diallel trials.

Table 8. Type B genetic correlations for branch size between two sites (standard error).

	Region 1 – Mainland				Region 2 - Tasmania		
	BR9611	**BR9701**	**BR9705**	**BR9709**	**BR9615**	**BR9614**	**BR9715**
BR9601	0.39 (0.20)	0.43 (0.26	0.96 (0.13)	-0.13 (0.41)	-0.01 (0.22)	0.56 (0.19)	0.80 (0.21)
BR9611		1.1 (0.10)	0.85 (0.20)	0.46 (0.30)	0.59 (0.16)	0.50 (0.21)	0.23 (0.28)
BR9701			1.1 (0.27)	0.70 (0.30)	0.45 (0.27)	0.56 (0.28)	0.19 (0.28)
BR9705				0.57 (0.30)	0.53 (0.24)	0.49 (0.29)	0.28 (0.27)
BR9709					0.51 (0.34)	0.21 (0.42)	0.21 (0.38)
BR9615						0.43 (0.24)	0.45 (0.31)
BR9614							0.61 (0.30)

Region 1 vs. Region 2 (all sites) $\hat{r}_B$ = 0.55 (0.15)

There was some indication of instability of parental rankings across trials for stem straightness, but generally, moderately high $\hat{r}_B$ was observed in both regions (Table 9). For example, $\hat{r}_B$ ranged from 0.41 to 1.2. Most of the lower values of $\hat{r}_B$ (ranging from 0.41 to 0.57) occurred with stem straightness measurements from Tasmanian trials (Table 9). However, when trials were grouped within regions, the $\hat{r}_B$ = 0.76 ± 0.07 indicating that overall parental rankings were stable across Tasmanian and mainland trials.

Table 9. Type B genetic correlations for stem straightness between two sites (standard error).

	Region 1 – Mainland				Region 2 - Tasmania		
	BR9611	**BR9701**	**BR9705**	**BR9709**	**BR9615**	**BR9614**	**BR9715**
BR9601	0.93 (0.05)	0.88 (0.07)	0.98 (0.07)	0.82 (0.14)	0.77 (0.09)	0.62 (0.15)	0.57 (0.23)
BR9611		0.89 (0.10)	0.84 (0.16)	0.73 (0.18)	0.75 (0.10)	0.53 (0.17)	0.68 (0.22)
BR9701			0.86 (0.10)	0.59 (0.19)	0.83 (0.10)	0.67 (0.16)	0.57 (0.22)
BR9705				0.81 (0.13)	0.55 (0.21)	0.56 (0.29)	0.72 (0.27)
BR9709					0.91 (0.16)	0.71 (0.22)	0.41 (0.33)
BR9615						0.87 (0.15)	0.87 (0.23)
BR9614							1.2 (0.20)

Region 1 vs. Region 2 (all sites) $\hat{r}_B$ = 0.76 (0.07)

CONCLUSION

DBH, density, branch angle, branch size, and stem straightness were under genetic control. Significant additive genetic variance was estimated indicating that improvement in these traits is possible through traditional breeding. Generally, $\hat{h}^2$ for density > branch angle > stem straightness > DBH > branch size; and significant $\hat{h}^2$ was observed for all traits and at all trials with only two exceptions. Significant additive genetic correlations were estimated between some of the traits. DBH had a moderate, negative genetic correlation with density ($\hat{r}_A$ = -0.48) and branch size ($\hat{r}_A$ = -0.26) (bigger trees produced bigger branches), while a positive genetic correlation with branch angle ($\hat{r}_A$ = 0.34). Density was negatively correlated with stem

straightness ($\hat{r}_A$ = -0.28), while branch size had a positive genetic correlation with stem straightness ($\hat{r}_A$ = 0.27). All other genetic correlations among traits were not significant. The presence of genotype by environment interaction can be an indicator of whether regional breeding is warranted. No to little genotype by environment interaction was present for density, branch angle, and stem straightness and when trials were grouped into one of two regions, $\hat{r}_B$ was 0.98, 0.95, and 0.76, respectively. However, there was some evidence of genotype by environment interaction for DBH in the Tasmanian trials and for branch size across all trials. When trials were grouped into one of two regions, $\hat{r}_B$ was 0.63 and 0.55 for DBH and branch size, respectively. Further research is recommended in determining the cause of this genotype by environment interaction for DBH and branch size in radiata pine.

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APPENDIX 1: Tabulation of trait means ± standard deviation (S.D.) and count for each trial.

BR9601	Trait	N	Mean ± S.D.
	DBH (mm)	4706	149.61 ± 17.3
	Density (kg/m^3)	1254	333.73 ± 23.2
	Branch angle	4706	3.4613 ± 1.03
	Branch size	4706	3.4437 ± 0.95
	Stem straightness	4705	3.4803 ± 1.02

BR9611	Trait	N	Mean ± S.D.
	DBH (mm)	3992	153.1 ± 21.1
	Density (kg/m^3)	1891	364.0 ± 24.6
	Branch angle	3992	3.4339 ± 1.12
	Branch size	3992	3.5386 ± 1.12
	Stem straightness	3992	3.553 ± 1.12

BR9701	Trait	N	Mean ± S.D.
	DBH (mm)	4936	154.3 ± 26.6
	Density (kg/m^3)	1534	334.8 ± 22.6
	Branch angle	4936	3.4808 ± 1.14
	Branch size	4936	3.3171 ± 1.12
	Stem straightness	4936	3.1564 ± 1.21

BR9705	Trait	N	Mean ± S.D.
	DBH (mm)	2091	170.5 ± 20.7
	Density (kg/m^3)	880	317.4 ±21.2
	Branch angle	2091	3.4754 ± 0.71
	Branch size	2091	3.4237 ± 0.81
	Stem straightness	2090	3.4048 ± 0.77

BR9709	Trait	N	Mean ± S.D.
	DBH (mm)	2051	171.9 ± 27.5
	Density (kg/m^3)	510	341.1 ± 21.6
	Branch angle	2051	3.3574 ± 1.10
	Branch size	2051	3.5544 ± 1.14
	Stem straightness	2047	3.6815 ± 0.90

BR9615	Trait	N	Mean ± S.D.
	DBH (mm)	4337	166.4 ± 28.4
	Density (kg/m^3)	1173	335.5 ± 24.2
	Branch angle	4340	3.4753 ± 0.95
	Branch size	4341	3.5856 ± 1.00
	Stem straightness	4340	3.5435 ± 0.96

APPENDIX 5

BR9614	Trait	N	Mean ± S.D.
	DBH (mm)	2614	174.2 ± 28.0
	Density (kg/m^3)	313	334.2 ± 23.6
	Branch angle	2616	3.4958 ± 1.08
	Branch size	2616	3.5730 ± 1.27
	Stem straightness	2616	3.3291 ± 1.21

BR9715	Trait	N	Mean ± S.D.
	DBH (mm)	3081	182.0 ±34.4
	Density (kg/m^3)	350	361.7 ± 24.6
	Branch angle	---	---
	Branch size	3072	3.4606 ± 1.18
	Stem straightness	3072	3.8844 ± 1.12

CSIRO Forestry and Forest Products
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Juvenile Wood Initiative:

Genetic Control of Juvenile Wood Properties in Slash and Caribbean Pines and their F1 Hybrid, as Determined by SilviScan

Mark J. Dieters , Keving J. Harding and Harry X. Wu

***February* 2006**

COMMERCIAL IN CONFIDENCE

CSIRO Forestry and Forest Products
PO Box E4008
Kingston ACT 2604 AUSTRALIA
Telephone: 02 62818211 Fax: 02 62818312

Executive Summary

A total 1170 wood samples collected from five replicates at both the Beerwah and Tuan sites (from three populations of PEE, PCH and the F_1 hybrid) were processed through SilviScan to study the patterns of genetic control of three key wood properties (density, microfibril angle (MfA), and predicted modulus of elasticity (MOE)). The experiment involved 12 unrelated PEE and 12 unrelated PCH parents. These 24 parents were crossed together to produce 36 families of each parental species, and 144 F_1 hybrid families.

Pith to bark trends revealed that by rings 3 – 5 average values of MfA have fallen well below 30° and ring density has risen above 400 kg/m^3, and predicted MOEs were close to or above 10 MPa in all taxa/site combinations except PCH at the more poorly drained Tuan site. This indicates the stiffness and distortion problems associated with low density and high MfA in the juvenile core are mostly a function of the innermost rings in PEE, PCH and their F_1 hybrid and that selection and breeding should focus on the inner few rings of the juvenile core in PEE, PCH and their F_1 hybrid in south-east Queensland.

The heritability of density, MfA and MOE fluctuated greatly from ring to ring; in general the heritability appeared to maximize in rings 1 – 3, particularly for MfA. This suggests that selection for density and MfA in say ring 3 (i.e. in 4 – 5 year old trees), is likely to have a greater impact on improving MOE than delaying selection until later ages. Given the lower heritability of MOE (than either density or MfA) and the much greater cost of estimating MOE (i.e. via SilviScan), selection based on whole core density (generally higher heritability than individual ring measurements) and an acoustic measure of MfA/stiffness will optimize genetic gain in MOE.

Juvenile wood properties as measured by density, MfA and MOE from SilvaScan were very stable across sites, suggesting that assessment of wood properties on one or two sites will provide reliable estimates of the genetic worth of individuals for use in future breeding.

The phenotypic and genetic correlations between rings for density, MfA and MOE indicate that the values observed in the inner rings (1 – 5) were strongly, positively correlated with ring 7 and the whole core. This suggests that selection for improved wood properties in the innermost rings would also result in improvement in wood properties in the subsequent rings, as well as improved average performance of the entire juvenile core (i.e. wood formed up to 10 years from planting).

Finally, the analyses also demonstrated strong genetic correlations between pure-species and hybrid performance for each of the wood quality traits. Under this scenario, information on pure species performance can be used to reliably predict hybrid performance. This confirms the decision to collect information on the parents which are being crossed for initiation of future cycles of clonal testing – because of these strong genetic correlations, parental performance can be used to identify the hybrid families which are most likely to have superior juvenile wood properties of the slash/Caribbean F1 hybrid in south-east Queensland.

Genetic Control of Juvenile Wood Properties (density, microfibril angle and predicted modulus of elasticity) in Slash (*Pinus elliottii* var. *elliottii*) and Caribbean (*P. caribaea* var. *hondurensis*) Pines and their F_1 Hybrid, as Determined by SilviScan.

Final report – Milestones 4.2.2 and 4.2.3, Schedule 4, Juvenile Wood Initiative – SilviScan Analysis of 1200 samples (Expt. 674 TBS – Tuan and Beerwah sites – comprising PEE, PCH and PEE × PCH F_1 hybrid) for genetic studies of juvenile wood; Genetic analysis of juvenile wood data from SilviScan; and final report.

Mark J. Dieters[1], Keving J. Harding[2] and Harry X. Wu[3]

Introduction

The first hybrids between slash (*Pinus elliottii* var. *elliottii*) and Caribbean (*P. caribaea* var. *hondurensis*) pines were produced and field tested in Queensland during the 1950s.[4] The F_1 and F_2 hybrids between these two species have demonstrated considerable advantages over either of the two pure species in terms of the hybrid's overall adaptability to a range of site types where exotic pines are commonly planted in south-east Queensland, growth rates, stem form, resistance to wind-damage and also wood properties (Rockwood et al. 1991; Nikles 1996; Powell and Nikles 1996). Selective breeding and mass propagation of superior hybrid clones has produced significant gains in growth and form traits, however gains in juvenile wood properties have been more modest. Selection for wood properties (due to costs associated with the assessment of wood quality traits) has essentially been restricted to the final stages in the selection of superior clones for commercial deployment in plantations.

This study follows on from work undertaken as part of Dominic Kain's PhD research (Kain 2003) that was supported via a post-graduate research scholarship from the Forest and Wood Products Research and Development Corporation (FWPRDC). The current study is supported by FWPRDC, Ensis/CSIRO and Queensland's Department of Primary Industries and Fisheries – Forestry (DPIF-Forestry) via the Juvenile Wood Initiative (JWI). This project utilized part (approx. 60%) of the wood samples collected for Kain's (2003) PhD research project, taken from two representative sites in south-east Queensland. Kain (2003) examined density (determined by x-ray densitometry and pilodyn penetration) and spiral grain angle (determined from 12mm cores and from bark-window assessments at breast height). Resources and facilities provided as part of the JWI allowed the samples to be assessed using the SilviScan densitometer (Evans and Ilic 2001) by Ensis Wood Quality/CSIRO.

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[1] The University of Queensland, School of Land and Food Sciences, Hartley Teakle Bldg., St Lucia, QLD 4072.
[2] Department of Primary Industries and Fisheries, Horticulture and Forestry Science, 80 Meiers Road, Indooroopilly, QLD 4068
[3] ENSIS-Genetics, CSIRO Forestry and Forest Products, PO Box E4008, Kingston, ACT 2604.
[4] For simplicity, the parental species will be referred to throughout this report simply as 'PEE' (for *P. elliottii* var. *elliottii*) and 'PCH' (for *P. caribaea* var. *hondurensis*), and the hybrid between these two species will be referred to as 'F_1' or 'hybrid pine'.

The purpose of this research project was to examine the patterns of genetic control of three key wood properties (density, microfibril angle (MfA), and predicted modulus of elasticity (MOE)) as determined on wood samples collected from 11-year-old trees of PEE, PCH and F_1 using SilviScan. Data provided by SilviScan enabled patterns of genetic control to be examined for each ring from pith to bark, as well as using average (area weighted) parameters for each tree sampled. Specifically this project sought to examine: 1) the inheritance of juvenile wood traits; 2) the optimum age for selection of juvenile wood properties in the parental species and the hybrid; 3) the stability of wood properties across sites; and 4) the relationship between wood properties determined in the parental species in comparison to the performance of their hybrid offspring. Answers to these questions will have a significant impact on design of optimal breeding and selection strategies for the improvement of juvenile wood properties in the hybrid pine.

Materials and Methods

The sampling techniques, sample preparation, and site details are described in detail by Kain (2003), and so will only be described here briefly.

Experimental design and sampling strategy
Wood samples were collected from a trial (Exp 674 TBS) established in 1987 by Queensland Department of Forestry's research branch, on three sites in south-east Queensland and a fourth site in central Queensland located within the Byfield state forest. The two sites most representative of the plantation estate were selected for sampling – a well drained site near Beerwah, and a poorly drained site near Tuan (Table 1). The experiment involved 12 unrelated PEE[5] and 12 unrelated PCH[6] parents. These 24 parents were crossed together in three separate factorial mating arrays to produce 36 families of each parental species, and 144 F_1 hybrid families. These families were planted in 2-tree non-contiguous plots with 12 and 16 replicates at Beerwah and Tuan respectively.

Table 1: Site and experiments details of the two tests in Exp674TBS sampled for the study of juvenile wood properties.

	Beerwah Site	Tuan Site
Latitude (°S)	26°52'	25°38'
Longitude (°E)	152°58'	152°50'
Altitude (m asl)	30	14
Rainfall (mm/yr ave.)	1665	1340
Soil Type	Well-drained; yellow earth	Poorly-drained; lateritic – gleyed podzolic
Planting Date	May-June 1987	April-May 1987
Number replicates	12	16
Planting spacing (r × t, m)	4.0 × 2.7	4.5 × 2.4
Initial Stocking (sph)	926	926

Samples were collected in 1998 when the trees were 11 years old (from planting) either by felling and removing a disk from near breast height (1.3m) or removal of a 12mm increment core (at a similar sampling position) from standing trees. One tree in

[5] Provenance oof *Pinus elliottii* is most likely either north Florida or southern Georgia in the USA.
[6] *Pinus caribaea* var. *hondurensis* is from the Mountain Pine Ridge provenance in Belize.

each of the 36 pure-species families was sampled, while only 48 of the 144 F_1 families were sampled[7] from each of 10 replicates at the Beerwah site and 7 replicates at the Tuan site. Disks were collected from 8 out of 10 replicates at the Beerwah site, and cores were collected from a further 2 replicates at Beerwah and 7 replicates at Tuan, giving a nominal 612, 612 and 816 samples in PEE, PCH and F_1 respectively across the two sites. However, Kain (2003, p. 104) was not able to collect samples from all nominated trees due to mortality, or the presence of malformed, leaning or highly suppressed trees within the nominated families/replicates. Resin was extracted from the samples, and each sample was planed to a 2mm thickness prior to assessment (for density) of the best radius using x-ray densitometry (Kain 2003, p.104-105). Ring boundaries were determined based on the year of formation of each ring, and confirmed by comparison of each sample against the density patterns obtained (Kain 2003, p. 106-108).

For the purposes of this study, samples were selected from five replicates at both the Beerwah and Tuan sites, giving a nominal 360, 360 and 480 samples in PEE, PCH and the F_1 hybrid, split equally between the two sites. However, in reality slightly fewer samples (1170 rather than 1200) were processed through SilviScan for this study (Table 2). Determination of ring boundaries proved time consuming and difficult with these tropical pines, and so ring boundaries previously determined by Dominic Kain were used to produce ring-by-ring values from the SilviScan output. This study utilized ring density, MfA and MOE, and area-weighted averages (i.e. average of the ring values weighted by the cross-sectional area of each ring) of these same three traits for each core, as well as the area of each core.

Statistical Analysis

Statistical analyses were conducted using ASREML (Gilmour et al. 1999) using an individual (or 'animal') model to estimate the additive genetic variance. This software was used primarily because it allows a correlated (rather than the commonly assumed diagonal) residual variance/covariance structure to be fitted. This is particularly important when examining repeated measures of the same trait on the same individual, e.g. the same trait measured in different rings of the same tree. In longitudinal data of this type a covariance exists between repeated measures on the same individuals (see for example Apiolaza et al. 2000), and it is not reasonable to assume the residuals for such traits are uncorrelated, and unless this correlation is taken into account this will lead to inflation of the estimated genetic correlations between measures (i.e. rings in this case).

A series of complementary analyses were undertaken of the data. Firstly, each trait (i.e. ring value for density, MfA and MOE, and area weighted whole-core values for density, MfA and MOE plus estimated tree area) were analyzed separately for each of the 3 taxa at each site. This provided preliminary estimates of the additive genetic variance (estimated from the variance amongst individuals after fitting the additive relationship matrix generated by ASREML for the pedigree information supplied) and dominance genetic variance (estimated as 4 × variance amongst full-sib families), plus the residual variance, for each trait, in each taxa at each site. Replicates were fitted as a fixed effect, while all other effects were fitted as random effects.

[7] Due to resource limitations, it was only possible to sample 48 of the 144 F_1 hybrid families. The subset of 48 hybrid families was selected to include 4 families from each of the 12 PEE and 12 PCH parents, forming a circular or partial factorial mating design.

Next, data were analyzed across the two sites for each trait, separately in each of the three taxa. This analysis fitted site and replicate within site as fixed effects, and fitted random effects for individual (i.e. additive genetic variance), and full-sib family (or female × male interaction). These analyses fitted separate residual and additive genetic variances for each test. As there is no reason to assume a residual correlation between trees measured in different tests, the residual correlation was taken to be zero, but a genetic covariance was fitted between the two sites. Because the single-site analyses indicated that variance between families was often very small (i.e. zero or near zero), it was decided to only fit a pooled family effect across the two sites. Post-processing options in ASREML were then used to estimate the heritability of each trait, in each taxon at each of the two sites (i.e. additive genetic variance for the trait at that site, divided by the sum of all variance components for that site – additive, family and residual variances). Similarly, the genetic correlation between sites was estimated by dividing the estimated (additive) genetic covariance between sites, by the square root of the product of the additive variances estimated for the trait at each of the two sites. ASREML provides estimates of the standard errors associated with these genetic parameters (heritability and genetic correlation) estimated using a Taylor's series approximation (Kendall et al. 1987; Dieters et al. 1995). The genetic correlation of the same trait measured in two different environments provides a measure of the importance of genotype × environment interaction (Burdon, 1977), i.e. where the correlation is near 1.0 the performance of genotypes is consistent across sites, and *vice versa* where the correlation is low the performance of genotypes is dependent on site. Heritability determines the degree of resemblance between relatives (e.g. parents and offspring) and so can be used to predict genetic progress from future cycles of selection and mating.

The across-sites analyses indicated that genotype × environment interaction was negligible for most traits (refer Results and Discussion); therefore for all subsequent analyses the data were pooled across the sites. Pooling data approximately doubled the sample size. However, to account for differences in the residual variance of each taxa at each site, prior to analysis all data were transformed by dividing each observation by the square root of the residual variance estimated for that trait, in that taxa at that site. Estimates of the residual variances obtained from the initial single-site analyses were used to standardize the data. Standardized data had a residual variance equal to 1.0 for each trait, consequently removing any scale effects.

The standardized data were used in two analyses to estimate: i) correlations between pure species and the F_1 hybrid, and ii) genetic correlations between rings and between traits within each of the three taxa. Due to difficulties accurately specifying the initial (unknown) variance/covariance matrices it was necessary to run these analyses as a series of separate bivariate analyses – e.g. PEE-F1 and PCH-F1, and ring1-ring3, ring1-ring5, ring1-ring7, ring1-core etc. for density, MfA and MOE. Nevertheless, it was still not possible to achieve convergence of log-likelihood and variance component estimates in all cases. Further, due to the large number of individual traits (pith to ring9 (plus whole core values) × 3 traits × 3 taxa) it was impractical to run analyses on data from every ring. Therefore, only data from rings 1, 3, 5 and 7 plus the whole-core area-weighted values were used in these analyses. Again, in each case test and replicate within test were fitted as fixed effects, and random effects were fitted for additive genetic effects (i.e. individual) and full-sib family. For the analyses between

taxa, a diagonal residual variance/covariance structure was fitted; however for estimating correlations between traits the residual covariances were allowed to be non-zero. Post processing options in ASREML were used to estimate the genetic correlations between taxa, and the genetic and phenotypic correlations between rings and traits within taxa. These correlations were estimated in a similar way to that described above.

Results and Discussion

Average performance of taxa across sites

In general, growth (as determined by the cross-sectional area of the samples) was slightly greater on the Beerwah site (Table 2). However, the Tuan site generally had larger minimum and maximum values for Area, with a larger range in values. Although growth rates tended to be higher on the Beerwah site, density was consistently lower at Tuan, with the greatest difference being evident in PCH (nearly 50kg/m^3, Table 2). On average the trees also had worse (i.e. higher) microfibril angle at Tuan, leading to a reduced predicted MOE. It is interesting to note that at Beerwah, these juvenile wood samples had average MOEs in all three taxa close to or exceeding 10 MPa, suggesting that this material would meet minimum standards for structural timber in all three taxa. However, on the poorly drained Tuan site where average density was lower and MfA higher, only slash pine had an average MOE exceeding 10. Nevertheless, even on this poor site, all taxa produced individuals with MOE exceeding 10 (Table 2) indicating opportunity for selection and genetic improvement within all three taxa, provided these traits are under a reasonable level of genetic control.

Table 2: Minimum, mean, maximum, range in area-weighted core density, microfibril angle (MfA), and predicted modulus of elasticity (MOE) and cross-sectional area of each sample, plus the number of observations contributing to each mean.

Taxa	Site	Trait	Minimum	Mean	Maximum	Range	No. Samples
PEE	Beerwah	Area (mm^2)	5715	16110	33370	27655	180
		Density (kg/m^3)	458.8	552.4	695.5	236.7	180
		MfA (°)	12.8	20.97	31	18.2	180
		MOE (MPa)	6.8	10.68	16.2	9.4	180
PEE	Tuan	Area (mm^2)	4466	15410	35670	31204	177
		Density (kg/m^3)	433.7	528.8	656.7	223	177
		MfA (°)	15.3	23.02	31.5	16.2	177
		MOE (MPa)	5	9.431	14.2	9.2	177
PCH	Beerwah	Area (mm^2)	6576	20000	36380	29804	177
		Density (kg/m^3)	399.5	514.7	664.3	264.8	177
		MfA (°)	13.4	20.92	31.6	18.2	177
		MOE (MPa)	5.2	9.876	15.3	10.1	177
PCH	Tuan	Area (mm^2)	6985	20880	40480	33495	170
		Density (kg/m^3)	362.1	458.7	569.1	207	170
		MfA (°)	15.5	23.24	32.4	16.9	170
		MOE (MPa)	3.8	7.551	11.8	8	170
F_1	Beerwah	Area (mm^2)	4466	22780	42060	37594	233
		Density (kg/m^3)	380.6	514.3	686.9	306.3	233
		MfA (°)	13.1	20.59	29.2	16.1	233

		MOE (MPa)	6	10.21	16.6	10.6	233
F_1	Tuan	Area (mm^2)	5411	21380	50560	45149	233
		Density (kg/m^3)	390.9	474.7	601.4	210.5	233
		MfA (°)	15.4	22.23	28.7	13.3	233
		MOE (MPa)	5.2	8.532	13.3	8.1	233

The trend of lower wood density on the poorly-drained Tuan site, compared to the well drained Beerwah site, is somewhat unusual. Previous experience suggests that wood density is lower higher, for these tropical pines, on well drained sites in south-east Queensland. There was however, a much higher proportion of wind-affected trees (i.e. leaning trees) on the Tuan site, making sampling much more difficult than at the Beerwah site. Sampling attempted to avoid wind-affect trees, and if this was not possible samples were collected to minimize the inclusion of compression wood (i.e. sample collected at a right-angle to the direction of any tree lean), and the best radius of each sample was then selected for SilviScan analysis. Nevertheless, it is possible that the higher densities observed at the Tuan site, could be due to the inclusion of compression wood in the samples – especially for PCH which is must less wind-firm than the other two taxa. The inclusion of compression wood will also account for the consistently higher MfA at the Tuan site.

Average values for each ring from the pith to the outermost ring (ring9) display similar trends to those observed from the mean values for each core (compare Table 2 to Figures 1 – 3). There is clear differentiation of taxa and sites on the basis of density in each ring (Figure 1) with the average density of each taxon at Beerwah exceeding that observed in Tuan – the only exception being 'pith' for the F_1 hybrid. Harding and Copley (2000) report similar trends in comparisons across these three taxa – typically density is highest in PEE, with PCH and the F_1 having very similar densities. Differences in ring density between sites and taxa tend to increase with ring number from the pith, with these differences maximizing by around ring5. Interestingly, while PEE consistently displayed the average highest density of the three taxa, in the early rings (pith to ring5) PCH tended to have a slightly average higher density than the F_1 hybrid grown at the same site (especially at Tuan, Figure 1), but from around ring5 the density of the F_1 hybrid tends to be intermediate between the two parental taxa. Finally, average extracted wood density tended to maximize in all taxa, at both sites, by ring7 (Figure 1). These maximum values are all well above what is considered to be the benchmark threshold value for structural timber in exotic pine grown in south-east Queensland, i.e. 400 kg/m^3 (unpublished data). This suggests that density problems associated with the production of structural timber are most likely to be related to the inner 3 – 5 rings, and that improvement of the average density of these inner rings above the 400kg/m^3 threshold (by selection and breeding) is likely to provide significant benefits.

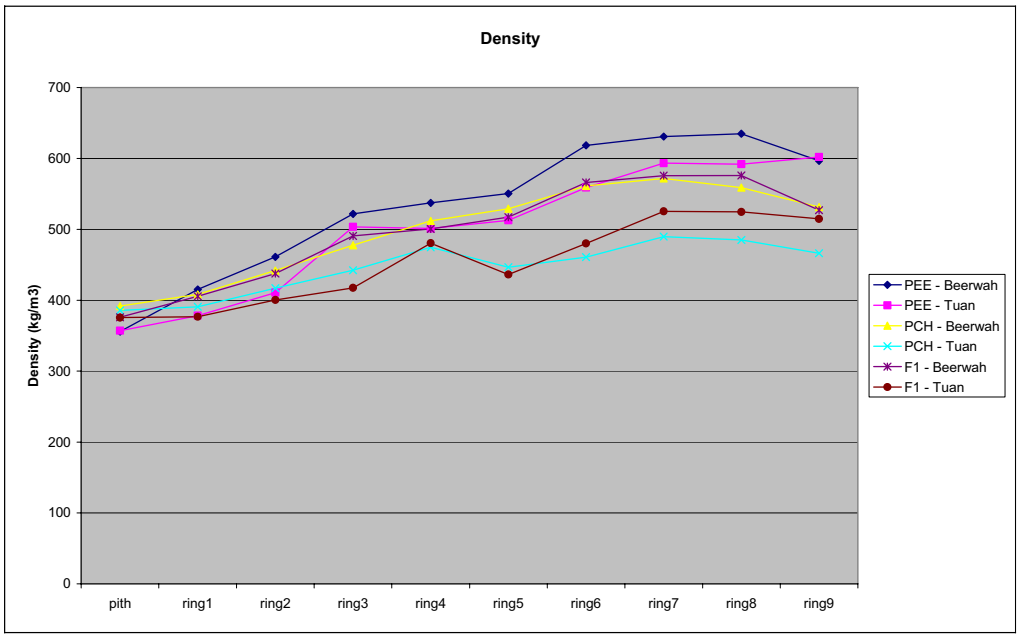

Figure 1: Average ring density estimated for each ring, from pith to bark in 11 year-old trees of *Pinus elliottii* (PEE), *P. caribaea* var. *hondurensis* (PCH), and the F_1 hybrid between PEE and PCH, grown on two sites (Beerwah and Tuan) in south-east Queensland.

The two sites also show a clear segregation in terms of microfibril angle, with the poorly drained site (Tuan), consistently producing higher (i.e. worse) microfibril angles in all three taxa from as early as ring1 (Figure 2). At both sites PCH had the highest microfibril angles, while F_1 tended be very similar to, but slightly lower than PEE. A similar trend in MfA amongst these taxa has been reported in samples from 13-year-old trees by Harding and Copley (2000), where the MfA of PCH was slightly higher on average (19.4°) than either PEE (18.9°) or the F_1 (18.6°). However, at both sites and in all taxa, mean values quickly fell below the 30° threshold (Figure 2), below which distortion problems in solid wood products, associated with excessive MfA, tend to be less important. These results again highlight the importance of focusing improvements in wood properties on the first few rings, where MfA tends to be excessive.

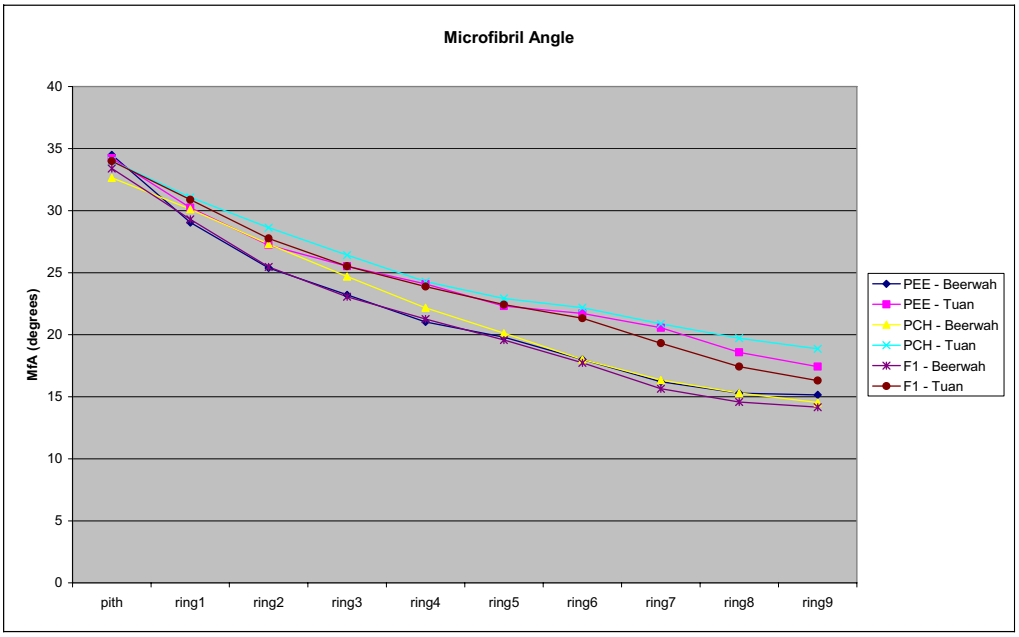

Figure 2: Average ring microfibril angle (MfA) estimated for each ring by SilviScan, from pith to bark in 11 year-old trees of *Pinus elliottii* (PEE), *P. caribaea* var. *hondurensis* (PCH), and the F_1 hybrid between PEE and PCH, grown on two sites (Beerwah and Tuan) in south-east Queensland.

Pith to bark patterns in predicted MOE (Figure 3), were as would be expected from the trends observed in density and MfA: increasing MOE being positively associated with wood density and negatively associated with MfA. At Beerwah all taxa except PCH at Tuan exceed 8 MPa by ring5: indicating potential for timber sawn from these trees to be included in a population of MGP10 structural graded pine. However, at the Tuan site, PCH takes longer reach this threshold (ring6) and only approaches 10 MPa by ring 9, suggesting that much of the juvenile wood produced by PCH at Tuan would not be sufficiently stiff to be graded as MGP10. The interrelationship of density and MfA on MOE is also reflected in the fact that predicted MOE appears to have maximised at the Beerwah site, while predicted MOE is continuing to increase in all taxa at the Tuan site (Figure 3).

These results again demonstrate that improvements in wood density and MfA are most critical in rings 1 – 5. After ring5 wood properties of exotic pines grown in south-east Queensland generally appear to be adequate to meet existing structural grades. Further, economic benefits associated with moving out-of-grade material to MGP10 (i.e. the minimum standard for machine graded pine) are likely to be considerably greater than moving MGP10 material to higher structural grades. Reiterating the importance of focusing on the genetic improvement of juvenile wood properties, particularly the inner 5 rings.

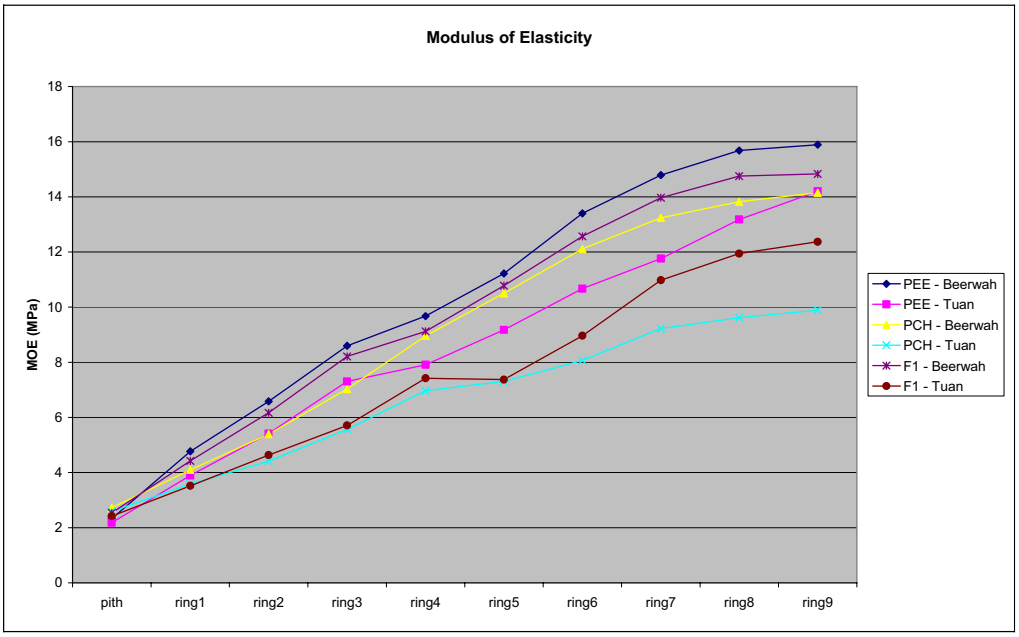

Figure 3: Average ring modulus of elasticity (MOE) estimated for each ring by SilviScan, from pith to bark in 11 year-old trees of *Pinus elliottii* (PEE), *P. caribaea* var. *hondurensis* (PCH), and the F_1 hybrid between PEE and PCH, grown on two sites (Beerwah and Tuan) in south-east Queensland.

Heritability and genetic correlations between sites

Heritability provides a measure of the relative importance of genetic variation available for utilization by selection and breeding (i.e. additive genetic variance) compared to the total amount of variation observed in a population. Consequently, it provides an indication of the relative ability of breeding to improve different traits – genetic gain being a function of heritability, the proportion selected and the observed variation in a trait (see for example Falconer and Mackay 1996, p. 189).

There were no clear trends in the heritability estimates for density, MfA, or MOE, between taxa or sites (Tables 3 – 5, Figures 4 – 6). Neither site consistently produced higher/lower heritability estimates for these wood quality traits on a ring-by-ring basis or for the whole cores, across the three taxa. Further there were no evident trends in the size of the heritability of density, MfA or MOE from pith to bark, except that perhaps heritability tended to be higher in rings 1 – 3 than in the later rings (Figures 4 – 6); however, this was not a general trend across sites and taxa. The heritability of each trait was generally higher when calculated from the area-weighted core values rather than the individual ring values. This is mostly likely because the overall values for each core will tend to average year-to-year fluctuations caused by environmental effects. However, at least part of the apparent fluctuation in heritability from ring-to-ring will be caused by random error[8]– many of the heritability estimates were less than twice the size their respective standard errors (Tables 3 – 5), suggesting that

[8] A deficiency of this study is that it involves only 12 parents from each of the parental species, and 24 parents in the case of the F_1 hybrid. Inclusion of more parents would however been impossible with the mating design used for this study. Inclusion of additional samples (i.e. trees) will improve the precision of heritability estimates, but does not address the issue of the representativeness of the parental population.

many of these estimates may not be significantly different from zero. Nevertheless, heritability values for density from the whole cores, are similar to those obtained by Kain (2003, p. 169) with twice the number of samples. Similarly, the heritability values reported here for density are similar to those reported in previous studies for these same species (0.62 pooled across two sites in PCH – Harding et al. 1991), *Pinus radiata* (0.53 – 0.89, Burdon and Low 1992; 0.65 – 0.82, Jayawickrama 2001; 0.88 (broad-sense heritability), Cown et al. 2002), *Pinus taeda* (0.18 – 0.78, Gwaze et al. 2001) and across a range of other tree species (0.5 – Cornelius 1994). This suggests that the heritability estimates obtained are indicative of the level of genetic control that can be expected in these traits, despite the relatively low precision of the estimates reported here.

The heritability of density in the first few rings was generally moderately strong (Tables 3 & 4), mostly varying between around 0.2 and 0.4. While the heritability of MOE in these same rings tended to be lower, generally ranging from around 0.15 to 0.3 (Table 5). By contrast heritability estimates for the whole cores are usually higher (as previously mentioned), being generally around 0.4 – 0.5 for density and MfA; however, heritability of MOE was still relatively low at around 0.2 – 0.3 (Table 5). This suggests that it may be better to select for the component traits of MOE (i.e. density and MfA) rather than selecting for MOE directly. By comparison, the heritability of cross-sectional area was generally similar to that for MOE from the cores (Table 5).

Dominance (i.e. non-additive genetic) variance was also estimated in these across sites analyses, but was generally very small compared to additive variance. Therefore, estimates of dominance have not been reported here. Kain (2003, p. 169) also reported very low (or zero) estimates of dominance variance in both parental species for a range of growth, form and wood property traits.

The genetic correlation between sites (r_g) provides an estimate of the importance of genotype-environment interaction (Burdon 1977) as discussed in the materials and methods. The genetic correlations between these two contrasting sites (Beerwah and Tuan) were usually very high (i.e. close to or sometimes exceeding 1.0, Tables 3 – 5) for all traits measured. Where the correlation was low (Tables 3 – 5) this usually reflected a low (near zero) heritability at one or both sites, and mostly occur in the F_1 hybrid where standard quantitative genetic assumptions are not strictly valid. The high correlations between sites, for most traits suggests that genotype-environment interaction is not important for wood quality traits in these populations of exotic pine, even when grown on very contrasting site types in south-east Queensland. This reflects the results of previous studies for wood, growth and form traits in this same experiment (Kain 2003, p. 173), growth form traits in PEE (Dieters 1996) and PCH (Woolaston et al. 1991) which found relatively little genotype-environment interaction. However, when examining genotype-environment interactions in radiata pine on sites distributed throughout Australia, Wu and Matheson (2005) regional patterns of were found, related to a difference in performance between high and low elevation sites.

Table 3: Estimated heritability (h^2) at the Beerwah and Tuan sites, and the genetic correlation (r_g) between sites for wood density, in *Pinus elliottii*, *P. caribaea* var. *hondurensis*, and their F_1 hybrid. (Standard errors indicated in parentheses.)

	Pinus elliottii var. *elliottii* (PEE)						*Pinus caribaea* var. *hondurensis* (PCH)						F_1 Hybrid					
Ring(s)	$h^2_{Beerwah}$		h^2_{Tuan}		$r_{g\ (Beerwah\text{-}Tuan)}$		$h^2_{Beerwah}$		h^2_{Tuan}		$r_{g\ (Beerwah\text{-}Tuan)}$		$h^2_{Beerwah}$		h^2_{Tuan}		$r_{g\ (Beerwah\text{-}Tuan)}$	
pith	0.05	(0.08)	0.44	(0.20)	1.36	(0.85)	0.08	(0.09)	0.01	(0.07)	4.93	(13.98)	0.44	(0.15)	0.41	(0.15)	1.08	(0.10)
ring1	0.27	(0.15)	0.57	(0.22)	0.88	(0.18)	0.18	(0.13)	0.30	(0.17)	0.99	(0.25)	0.45	(0.15)	0.14	(0.09)	1.12	(0.22)
ring2	0.30	(0.16)	0.56	(0.22)	0.96	(0.14)	0.26	(0.15)	0.32	(0.17)	0.99	(0.19)	0.23	(0.11)	0.38	(0.14)	0.85	(0.21)
ring3	0.34	(0.18)	0.45	(0.20)	0.68	(0.27)	0.18	(0.13)	0.35	(0.18)	0.01	(0.50)	0.15	(0.11)	0.00	(0.00)	0.00	(0.00)
ring4	0.34	(0.17)	0.35	(0.17)	1.18	(0.13)	0.35	(0.18)	0.15	(0.12)	0.69	(0.37)	0.18	(0.10)	0.31	(0.14)	0.90	(0.26)
ring5	0.18	(0.13)	0.43	(0.19)	0.71	(0.32)	0.59	(0.22)	0.23	(0.15)	0.79	(0.25)	0.10	(0.08)	0.16	(0.10)	1.52	(0.47)
ring6	0.41	(0.19)	0.36	(0.18)	0.83	(0.21)	0.26	(0.16)	0.31	(0.17)	0.95	(0.21)	0.22	(0.11)	0.19	(0.11)	0.95	(0.25)
ring7	0.25	(0.15)	0.60	(0.23)	0.96	(0.16)	0.34	(0.17)	0.24	(0.15)	1.03	(0.18)	0.13	(0.09)	0.44	(0.16)	0.83	(0.31)
ring8	0.40	(0.19)	0.40	(0.19)	0.69	(0.26)	0.16	(0.13)	0.30	(0.18)	0.95	(0.29)	0.12	(0.10)	0.27	(0.14)	0.87	(0.36)
ring9	0.00	(0.00)	0.17	(0.15)	0.00	(0.00)	0.11	(0.13)	0.10	(0.12)	1.24	(0.62)	0.14	(0.11)	0.32	(0.15)	0.60	(0.42)
core	0.55	(0.22)	0.76	(0.24)	0.97	(0.08)	0.47	(0.21)	0.31	(0.17)	0.86	(0.20)	0.38	(0.14)	0.53	(0.17)	0.94	(0.12)

Table 5: Estimated heritability (h^2) at the Beerwah and Tuan sites, and the genetic correlation (r_g) between sites for microfibril angle (MfA), in *Pinus elliottii*, *P. caribaea* var. *hondurensis*, and their F_1 hybrid. (Standard errors indicated in parentheses.)

	Pinus elliottii var. *elliottii* (PEE)						*Pinus caribaea* var. *hondurensis* (PCH)						F_1 Hybrid					
Ring(s)	$h^2_{Beerwah}$		h^2_{Tuan}		$r_{g\ (Beerwah\text{-}Tuan)}$		$h^2_{Beerwah}$		h^2_{Tuan}		$r_{g\ (Beerwah\text{-}Tuan)}$		$h^2_{Beerwah}$		h^2_{Tuan}		$r_{g\ (Beerwah\text{-}Tuan)}$	
pith	0.15	(0.12)	0.13	(0.11)	1.15	(0.37)	0.20	(0.13)	0.10	(0.10)	1.64	(0.57)	0.31	(0.14)	0.28	(0.14)	1.05	(0.16)
ring1	0.35	(0.18)	0.31	(0.16)	1.01	(0.15)	0.29	(0.16)	0.38	(0.18)	0.76	(0.26)	0.17	(0.11)	0.19	(0.11)	1.11	(0.30)
ring2	0.25	(0.15)	0.47	(0.20)	0.99	(0.16)	0.13	(0.11)	0.20	(0.14)	0.80	(0.42)	0.28	(0.13)	0.37	(0.15)	1.15	(0.13)
ring3	0.17	(0.12)	0.48	(0.20)	0.88	(0.25)	0.16	(0.12)	0.13	(0.12)	1.43	(0.43)	0.14	(0.10)	0.18	(0.12)	1.47	(0.36)
ring4	0.18	(0.13)	0.44	(0.20)	1.04	(0.20)	0.17	(0.12)	0.18	(0.13)	0.98	(0.34)	0.09	(0.10)	0.22	(0.13)	1.37	(0.50)
ring5	0.25	(0.15)	0.42	(0.19)	1.03	(0.16)	0.29	(0.16)	0.36	(0.18)	0.92	(0.20)	0.06	(0.08)	0.43	(0.16)	1.57	(0.76)
ring6	0.19	(0.13)	0.37	(0.18)	1.17	(0.19)	0.27	(0.15)	0.41	(0.19)	1.00	(0.17)	0.00	(0.00)	0.30	(0.16)	0.01	(0.00)
ring7	0.24	(0.15)	0.40	(0.19)	1.04	(0.17)	0.14	(0.12)	0.34	(0.18)	1.22	(0.28)	0.08	(0.09)	0.33	(0.14)	1.48	(0.57)
ring8	0.21	(0.14)	0.48	(0.21)	1.00	(0.19)	0.14	(0.12)	0.23	(0.15)	1.15	(0.31)	0.10	(0.10)	0.38	(0.15)	1.15	(0.38)
ring9	0.24	(0.17)	0.52	(0.22)	0.49	(0.39)	0.07	(0.11)	0.42	(0.20)	1.14	(0.59)	0.14	(0.13)	0.29	(0.15)	1.03	(0.32)
core	0.26	(0.15)	0.57	(0.22)	1.05	(0.13)	0.38	(0.18)	0.51	(0.21)	0.94	(0.14)	0.20	(0.12)	0.40	(0.16)	1.26	(0.17)

Table 5: Estimated heritability (h^2) at the Beerwah and Tuan sites, and the genetic correlation (r_g) between sites for predicted modulus of elasticity (MOE) and cross-sectional area (Area), in *Pinus elliottii*, *P. caribaea* var. *hondurensis*, and their F_1 hybrid. (Standard errors indicated in parentheses.)

	Pinus elliottii var. *elliottii* (PEE)						*Pinus caribaea* var. *hondurensis* (PCH)						F_1 Hybrid					
Ring(s)	$h^2_{Beerwah}$		h^2_{Tuan}		$r_{g\ (Beerwah\text{-}Tuan)}$		$h^2_{Beerwah}$		h^2_{Tuan}		$r_{g\ (Beerwah\text{-}Tuan)}$		$h^2_{Beerwah}$		h^2_{Tuan}		$r_{g\ (Beerwah\text{-}Tuan)}$	
pith	0.03	(0.07)	0.20	(0.13)	1.95	(1.77)	0.05	(0.08)	0.15	(0.12)	1.45	(0.99)	0.15	(0.12)	0.11	(0.11)	1.23	(0.43)
ring1	0.22	(0.14)	0.39	(0.19)	0.91	(0.22)	0.23	(0.14)	0.32	(0.17)	0.91	(0.24)	0.24	(0.13)	0.08	(0.10)	1.44	(0.53)
ring2	0.16	(0.13)	0.54	(0.22)	1.02	(0.20)	0.13	(0.11)	0.38	(0.19)	1.00	(0.30)	0.16	(0.11)	0.20	(0.13)	1.17	(0.27)
ring3	0.23	(0.14)	0.54	(0.21)	0.83	(0.23)	0.12	(0.11)	0.03	(0.09)	0.01	(1.23)	0.00	(0.07)	0.00	(0.08)	0.00	(0.00)
ring4	0.26	(0.15)	0.37	(0.18)	1.12	(0.14)	0.15	(0.12)	0.15	(0.12)	0.54	(0.53)	0.06	(0.08)	0.05	(0.08)	2.27	(1.93)
ring5	0.11	(0.10)	0.35	(0.17)	0.95	(0.35)	0.40	(0.19)	0.23	(0.15)	0.76	(0.28)	0.02	(0.07)	0.14	(0.12)	2.01	(2.92)
ring6	0.23	(0.15)	0.39	(0.19)	1.18	(0.15)	0.23	(0.14)	0.16	(0.12)	1.19	(0.27)	0.01	(0.07)	0.03	(0.09)	3.27	(9.39)
ring7	0.22	(0.14)	0.51	(0.21)	1.17	(0.15)	0.18	(0.13)	0.14	(0.12)	1.27	(0.35)	0.04	(0.07)	0.22	(0.12)	1.01	(0.86)
ring8	0.27	(0.16)	0.44	(0.20)	1.16	(0.13)	0.16	(0.13)	0.14	(0.13)	1.16	(0.38)	0.02	(0.07)	0.18	(0.12)	2.09	(3.92)
ring9	0.26	(0.18)	0.47	(0.22)	0.91	(0.22)	0.04	(0.10)	0.26	(0.18)	1.10	(1.08)	0.08	(0.11)	0.20	(0.14)	1.42	(0.61)
core	0.35	(0.18)	0.71	(0.24)	1.09	(0.08)	0.36	(0.18)	0.20	(0.14)	0.92	(0.24)	0.24	(0.13)	0.22	(0.14)	1.12	(0.18)
core	0.29	(0.18)	0.59	(0.23)	1.03	(0.11)	0.20	(0.14)	0.37	(0.19)	0.43	(0.41)	0.05	(0.09)	0.16	(0.11)	1.62	(1.02)

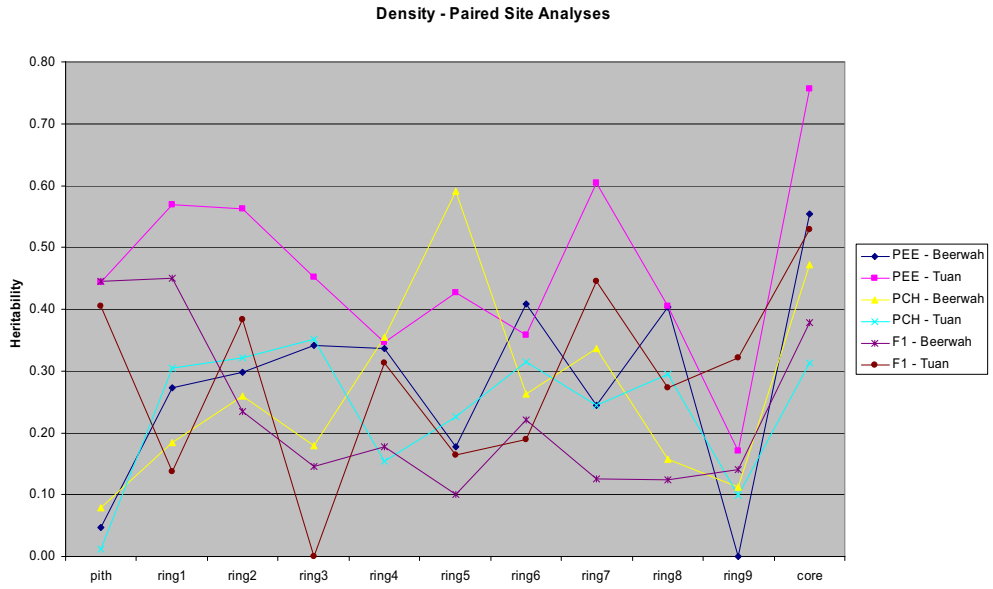

Figure 4: Heritability of wood density estimated for each ring (pith to ring9), and area weighted density of the whole core for *P. elliottii* (PEE), *P. caribaea* var. *hondurensis* (PCH) and their F_1 hybrid grown on two sites in south-east Queensland.

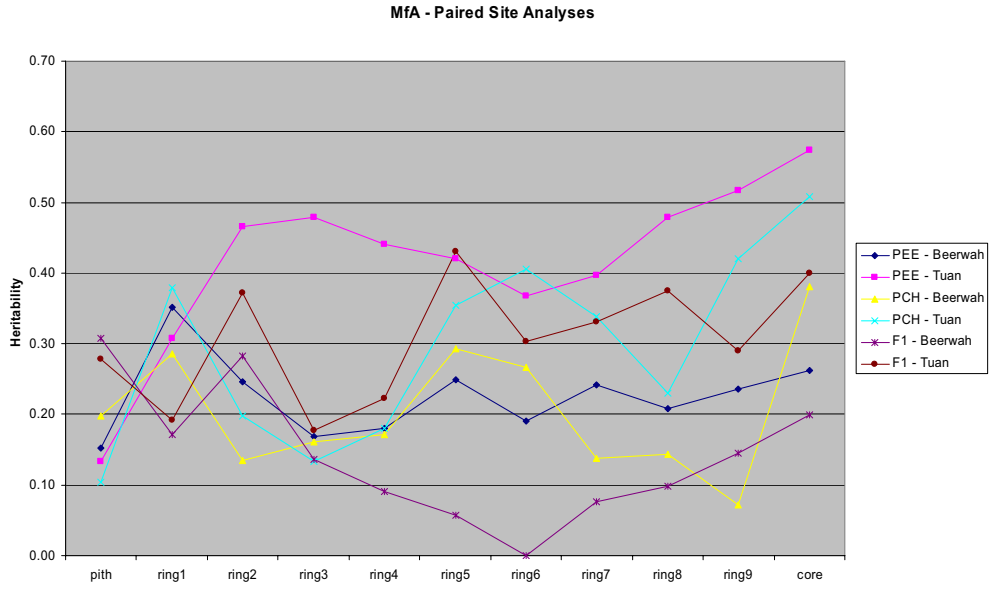

Figure 5: Heritability of microfibril angle (MfA) estimated for each ring (pith to ring9), and area weighted density of the whole core for *P. elliottii* (PEE), *P. caribaea* var. *hondurensis* (PCH) and their F_1 hybrid grown on two sites in south-east Queensland.

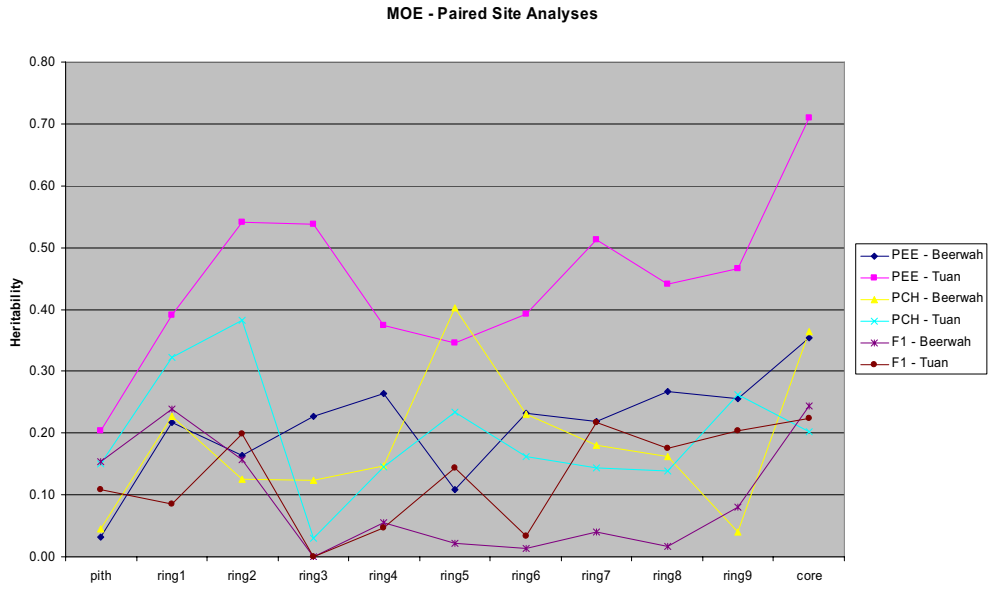

Figure 6: Heritability of predicted modulus of elasticity (MOE) estimated for each ring (pith to ring9), and area weighted density of the whole core for *P. elliottii* (PEE), *P. caribaea* var. *hondurensis* (PCH) and their F_1 hybrid grown on two sites in south-east Queensland.

Genetic correlations between taxa

An important consideration in the design of breeding and selection schemes for the development of genetically improved hybrid populations is the correlation between pure species and hybrid populations (r_{ph}). Kerr et al. (2004a & b) demonstrated by computer simulation that where the correlation between performance in pure species and hybrids was positive, then genetic gains could be maximized by a selection strategy that sort to produce a stabilized synthetic hybrid between the two species, in comparison breeding strategies which focused on recurrent selection within the pure species.

Estimates of the correlation between pure and hybrid performance for the key wood properties investigated in this study (Table 6) indicate that there is a strong relationship between the performance of related hybrid and pure-species progeny. For wood density, the correlation was very strong, both for individual rings and for the area-weighted density of the whole core, for both parental species. Similar results were reported by Kain (2003, p. 173), where estimates of the genetic correlation between pure and hybrid exceeded 0.87 for a range of density-related traits. The pure-hybrid correlations for MfA were generally lower than those for density (Table 6), with the relationship being generally stronger in PCH-F_1 (approx. 0.8 – 0.9) than in PEE-F_1 (approx. 0.75). This may reflect the slightly higher microfibril angles noted previously in PCH compared to PEE (Figure 2); suggesting that high microfibril angles in the PCH parent may have a stronger impact on MfA in the hybrid progeny than the MfA of the PEE parent. The one very low estimate of r_{ph} for ring5 between PEE-F_1 is not easily explained – if it were due to the low heritability of MfA in this ring in the F_1 (Table 5, Beerwah), this would also be expected to produce an aberrant result for

PCH-F_1 in ring5. Pure-hybrid correlations for MOE (Table 6) are quite variable from ring-to-ring, with PCH possibly exhibiting a declining association with the F_1 performance in the outer rings. The area-weighted values for the whole core also suggest that MOE in the hybrid may be more strongly influenced by PEE (1.0) than by PCH (0.7). For growth (i.e. cross-sectional area), the PCH appears to have the stronger influence on growth. However, given the standard errors associated with the r_{ph} estimates, these differences are unlikely to be significant.

Table 6: Additive genetic correlations (r_{ph}) between pure species (*P. elliottii*, PEE, and *P. caribaea* var. *hondurensis*, PCH) and their F_1 hybrid, for key wood quality traits (wood density, microfibril angle (MfA), and predicted modulus of elasticity (MOE)) and cross-sectional area (Area), determined using standardized data pooled across the Beerwah and Tuan sites. (Standard errors in parentheses.)

Trait	Ring(s)	r_{ph} PEE-F_1		r_{ph} PCH-F_1	
Area	core	0.59	(0.32)	0.78	(0.30)
Density	1	1.13	(0.07)	1.13	(0.07)
	3	1.16	(0.24)	1.31	(0.30)
	5	1.20	(0.13)	0.87	(0.21)
	7	0.98	(0.13)	0.99	(0.15)
	core	0.99	(0.07)	0.98	(0.09)
MfA	1	0.76	(0.25)	1.24	(0.12)
	3	0.86	(0.22)	0.85	(0.24)
	5	-0.05	(0.44)	0.83	(0.20)
	7	0.77	(0.25)	0.92	(0.20)
	core	0.75	(0.22)	0.89	(0.14)
MOE	1	1.07	(0.12)	1.09	(0.12)
	3	1.59	(0.37)	0.99	(0.59)
	5	0.68	(0.41)	0.38	(0.42)
	7	1.37	(0.24)	0.60	(0.40)
	core	1.06	(0.08)	0.69	(0.25)

Correlations amongst rings and with whole core

Genetic and phenotypic correlations between the same trait measured in different rings or the average (area-weighted) for the whole core, demonstrate similar patterns for each of the wood traits (density, MfA and MOE) measured in this study. In each case the phenotypic and genetic correlations were of the same sign, with the phenotypic correlation (below diagonal, Tables 7, 8 and 9) being about 0.15 to 0.25 lower than the genetic correlation (above diagonal, Tables 7, 8 and 9). The phenotypic correlations between rings declined progressively, as the rings become increasingly separated, and ring1 was always more poorly correlated (phenotypically) with the core averages than ring7. The correlations between ring values and core average increased progressively from ring1 to ring7, as would be expected due to the way the core averages were calculated. These are area-weighted averages, therefore the outer rings will contribute more to the sample average than will the inner rings, hence it would be expected that the values for the outer rings will be more strongly correlated with the core averages than the inner rings, as has been observed here (Tables 7, 8 and 9). Finally the phenotypic correlations, for each trait, were very similar in each of the three taxa.

Genetic correlations showed similar trends to those observed for the phenotypic correlations, with a general decline in the genetic correlation as rings become increasingly separated. However, as the genetic correlations were always higher than the matching phenotypic correlation, this decline was less than for the phenotypic correlations. Interestingly, the inner-ring values (i.e. rings 1 and 3) had strong genetic correlations (exceeding 0.77) with the whole core values (Tables 7, 8 and 9), indicating that selection for density, MfA and/or MOE will also improve the average-values of these traits across the whole juvenile core (i.e. up to 11 years of age when these trees were sampled). A consequence of these very strong genetic correlations is that selection at 3-5 years of age will be almost as effective as selection at 11 years of age, in terms of improving density, MfA and MOE in all three taxa.

Correlations between traits (Table 10) indicates that the cross-sectional area of the cores had only a weak phenotypic association with any of the three wood traits measured – a weak negative association between cross-sectional area and both density and MOE, and weak positive association with MfA in all three taxa. This suggests that as growth rates increase, density and MOE will decline slightly, but MfA will increase. Consequently, silvicultural regimes aimed at maximizing early growth rates, will tend to have an adverse impact on the recovery of structural grade timber from the juvenile core of these exotic pine taxa in south-east Queensland.

The genetic correlations between cross-sectional area and wood properties, appear to differ between taxa (Table 10): F_1 showed no genetic association between growth and density, a negative (i.e. favorable) association with MfA, and a positive association with MOE; while; PCH showed a strong negative association between growth and density, and weak negative associations with both MfA and MOE; and PEE showed no genetic association between growth and either density, MfA or MOE. Kain (2003, p. 176 and p. 178) reported similar genetic correlations between diameter underbark at 11 years (12 years from seed as specified by Kain, 2003): -0.33 in the F_1, -0.91 in PCH, and 0.00 in PEE, with only the genetic correlation in PCH being significantly different from zero. In comparison, radiata pine has a much clearer adverse (negative correlation) between growth (diameter) and area-weighted wood density (Li Li and Wu 2005). Consequently, if the genetic correlations observed in this experiment are assumed to be correct, then selection for increased growth rates in each of these taxa will have different consequences. In the F_1 improving growth rates by genetic selection would tend to reduce MfA and increase MOE, but in PCH this would reduce density and MOE, but have no/little impact on the wood properties of PEE.

Genetic correlations of density and MfA with MOE were consistent across the three taxa, with moderate to strong positive association between density and MOE, and strong negative correlation between MfA and MOE (Table 10). This indicates that selection for increased density and/or reduced MfA will lead to improvements in the MOE of the juvenile core. The relative merits of selection on density vs. MfA needs to be investigated in detail, since the cost of assessing density is much lower than assessing either MfA or MOE. Consequently, much greater selection pressure can be exercised if selection in based only on density (as compared to MfA alone, or density plus MfA). However, a further complication is the weak positive correlation between density and MfA in both PCH and F_1 (Table 10) indicating that selection for density (in the absence of information on MfA) in either PCH or F_1 populations will also tend

to increase MfA. By contrast in PEE the genetic correlation between density and MfA is weak and negative (i.e. favorable). Overall, this indicates that selection for growth and wood properties is likely to be relatively simple in PEE where all genetic correlations are either zero or favorable. However, in both PCH and F_1 more complex genetic relationships amongst density, MfA and MOE will make improvement of structural properties more difficult than in PEE, perhaps requiring measurement of both density and MfA. An alternative approach would be to measure density plus a surrogate measure of MfA/MOE such time-of-flight from an acoustic tool (as suggested by Harding et al. 2004).

Table 7: Genetic (above diagonal) and Phenotypic (below diagonal) correlations for wood density in rings 1, 3, 5 and 7 and the area-weighted value for each core. (Standard errors in parentheses.)

F_1	Ring1		Ring3		Ring5		Ring7		Core	
Ring1			0.82	(0.17)	0.74	(0.16)	0.77	(0.15)	0.85	(0.09)
Ring3	0.64	(0.03)			CF		CF		CF	
Ring5	0.49	(0.04)	0.60	(0.03)			0.93	(0.09)	0.94	(0.07)
Ring7	0.36	(0.05)	0.31	(0.04)	0.55	(0.04)			1.00	(0.02)
Core	0.61	(0.04)	0.56	(0.03)	0.71	(0.03)	0.79	(0.02)		

PCH	Ring1		Ring3		Ring5		Ring7		Core	
Ring1			0.92	(0.09)	0.65	(0.23)	0.72	(0.21)	0.78	(0.16)
Ring3	0.71	(0.03)			0.87	(0.14)	CF		0.97	(0.07)
Ring5	0.46	(0.06)	0.54	(0.04)			CF		0.99	(0.02)
Ring7	0.39	(0.06)	0.44	(0.04)	0.65	(0.03)			CF	
Core	0.62	(0.04)	0.71	(0.03)	0.81	(0.02)	0.83	(0.02)		

PEE	Ring1		Ring3		Ring5		Ring7		Core	
Ring1			0.95	(0.05)	0.91	(0.09)	0.63	(0.22)	0.87	(0.10)
Ring3	0.75	(0.03)			0.96	(0.06)	0.70	(0.20)	0.85	(0.11)
Ring5	0.60	(0.04)	0.63	(0.04)			0.91	(0.09)	0.99	(0.03)
Ring7	0.44	(0.07)	0.49	(0.06)	0.59	(0.05)			0.94	(0.05)
Core	0.59	(0.06)	0.62	(0.05)	0.69	(0.04)	0.79	(0.03)		

Note: CF = convergence failed, and no estimate was obtained.

Table 8: Genetic (above diagonal) and Phenotypic (below diagonal) correlations for microfibril angle (MfA) in rings 1, 3, 5 and 7 and the area-weighted value for each core. (Standard errors in parentheses.)

F_1	Ring1		Ring3		Ring5		Ring7		Core	
Ring1			CF		0.85	(0.13)	0.80	(0.15)	0.96	(0.06)
Ring3	0.56	(0.03)			0.89	(0.08)	0.90	(0.09)	0.98	(0.03)
Ring5	0.48	(0.04)	0.73	(0.02)			0.98	(0.04)	0.95	(0.04)
Ring7	0.42	(0.04)	0.61	(0.03)	0.74	(0.02)			0.97	(0.03)
Core	0.61	(0.03)	0.80	(0.02)	0.84	(0.02)	0.82	(0.02)		

PCH	Ring1		Ring3		Ring5		Ring7		Core	
Ring1			0.92	(0.10)	0.93	(0.08)	0.97	(0.07)	0.96	(0.05)

Ring3	0.62	(0.04)			0.99	(0.00)	0.93	(0.09)	0.97	(0.05)
Ring5	0.57	(0.05)	0.75	(0.02)			0.98	(0.03)	0.97	(0.03)
Ring7	0.52	(0.05)	0.65	(0.04)	0.78	(0.03)			0.97	(0.03)
Core	0.66	(0.04)	0.79	(0.02)	0.88	(0.02)	0.85	(0.02)		

PEE	Ring1		Ring3		Ring5		Ring7		Core	
Ring1			0.92	(0.08)	0.89	(0.10)	0.68	(0.21)	0.77	(0.16)
Ring3	0.60	(0.04)			0.94	(0.05)	0.78	(0.16)	0.90	(0.08)
Ring5	0.54	(0.05)	0.76	(0.03)			0.94	(0.06)	CF	
Ring7	0.40	(0.06)	0.59	(0.05)	0.75	(0.03)			0.95	(0.04)
Core	0.56	(0.05)	0.79	(0.03)	0.86	(0.01)	0.82	(0.02)		

Note: CF = convergence failed, and no estimate was obtained.

Table 9: Genetic (above diagonal) and Phenotypic (below diagonal) correlations for predicted modulus of elasticity (MOE) estimated by SilviScan in rings 1, 3, 5 and 7 and the area-weighted value for each core. (Standard errors in parentheses.)

F_1	Ring1		Ring3		Ring5		Ring7		Core	
Ring1			0.99	(0.00)	0.75	(0.20)	0.62	(0.26)	0.90	(0.09)
Ring3	0.60	(0.03)			0.66	(0.36)	0.61	(0.39)	CF	
Ring5	0.49	(0.04)	0.62	(0.03)			0.83	(0.16)	0.93	(0.10)
Ring7	0.31	(0.05)	0.43	(0.04)	0.64	(0.03)			0.96	(0.06)
Core	0.53	(0.04)	0.60	(0.03)	0.70	(0.03)	0.76	(0.02)		

PCH	Ring1		Ring3		Ring5		Ring7		Core	
Ring1			0.99	(0.00)	0.78	(0.16)	0.87	(0.14)	0.92	(0.09)
Ring3	0.69	(0.03)			CF		0.99	(0.00)	CF	
Ring5	0.58	(0.04)	0.68	(0.03)			CF		0.96	(0.04)
Ring7	0.49	(0.05)	0.59	(0.04)	0.76	(0.02)			0.99	(0.03)
Core	0.63	(0.04)	0.72	(0.02)	0.84	(0.02)	0.86	(0.02)		

PEE	Ring1		Ring3		Ring5		Ring7		Core	
Ring1			0.95	(0.05)	0.99	(0.06)	0.83	(0.14)	0.89	(0.10)
Ring3	0.70	(0.03)			1.00	(0.04)	0.83	(0.13)	0.89	(0.09)
Ring5	0.54	(0.05)	0.69	(0.03)			0.97	(0.05)	0.98	(0.05)
Ring7	0.38	(0.06)	0.50	(0.06)	0.68	(0.04)			0.97	(0.03)
Core	0.46	(0.06)	0.64	(0.05)	0.69	(0.03)	0.81	(0.03)		

Note: CF = convergence failed, and no estimate was obtained.

Table 10: Genetic (above diagonal) and phenotypic (below diagonal) correlations for cross-sectional area (Area), wood density (Density), microfibril angle (MfA) and predicted modulus of elasticity (MOE) estimated by SilviScan for each core, in each taxa. (Standard errors in parentheses.)

F_1	Area		Density		MfA		MOE	
Area			-0.04	(0.31)	-0.44	(0.30)	0.43	(0.31)
Density	-0.13	(0.05)			0.32	(0.25)	0.46	(0.21)
MfA	0.16	(0.06)	-0.03	(0.07)			-0.65	(0.16)
MOE	-0.18	(0.05)	0.59	(0.04)	-0.77	(0.03)		

PCH	Area		Density		MfA		MOE	
Area			-0.82	(0.17)	-0.14	(0.38)	-0.29	(0.37)

Density	-0.31	(0.06)			0.24	(0.34)	0.39	(0.31)
MfA	0.15	(0.07)	-0.06	(0.09)			-0.77	(0.15)
MOE	-0.27	(0.06)	0.58	(0.05)	-0.80	(0.03)		

PEE	Area		Density		MfA		MOE	
Area			-0.04	(0.34)	-0.03	(0.35)	-0.07	(0.35)
Density	-0.10	(0.10)			-0.27	(0.32)	0.77	(0.14)
MfA	0.10	(0.09)	-0.18	(0.09)			-0.82	(0.12)
MOE	-0.15	(0.10)	0.65	(0.06)	-0.83	(0.03)		

Implications for Selection and Breeding

Pith to bark trends in density, MfA and predicted MOE suggest that selection and breeding should focus on the inner few rings of the juvenile core in PEE, PCH and their F_1 hybrid in south-east Queensland. By rings 3 – 5 average values of MfA have fallen well below 30° and ring density has risen above 400 kg/m^3, and predicted MOEs were close to or above 10 MPa in all taxa/site combinations except PCH at the more poorly drained Tuan site. This indicates the stiffness and distortion problems associated with low density and high MfA in the juvenile core are mostly a function of the innermost rings in PEE, PCH and their F_1 hybrid.

Further examination of the phenotypic and genetic correlations between rings for density, MfA and MOE indicates that the values observed in the inner rings (1 – 5) were strongly, positively correlated with ring 7 and the whole core. This suggests that selection for improved wood properties in the innermost rings would also result in improvement in wood properties in the subsequent rings, as well as improved average performance of the entire juvenile core (i.e. wood formed up to 10 years from planting).

The heritability of density, MfA and MOE fluctuated greatly from ring to ring; however, in general the heritability appeared to maximize in rings 1 – 3, particularly for MfA. This suggests that selection for density and MfA in say ring 3 (i.e. in 4 – 5 year old trees), is likely to have a greater impact on improving MOE than delaying selection until later ages. Detailed investigation is however required to optimize selection efficiency – however, given the lower heritability of MOE (than either density or MfA) and the much greater cost of estimating MOE (i.e. via SilviScan), it is likely that selection based on whole core density (generally higher heritability than individual ring measurements) and an acoustic measure of MfA/stiffness will optimize genetic gain in MOE. Other work is currently evaluating the reliability of acoustic velocity measures to estimate stiffness in standing trees of 4-5 year old hybrid pine clones (Kevin Harding and Marks Nester, pers. comm.)

MfA had a much stronger genetic correlation with predicted MOE than density in both PCH and the F_1 (approx. -0.7 vs. +0.4), but MfA and density had a weak-moderate positive association in these two taxa; suggesting that improvement of juvenile wood stiffness in the F_1 hybrid can not be achieved efficiently without measurement of both density and MfA. By contrast in PEE all genetic associations were favorable or zero, indicating no impediments to improvement of growth and stiffness in this species.

The three wood properties examined (density, MfA and predicted MOE) demonstrated very little genotype-environment interaction. This allowed pooling of data across sites, to increase sample size and to improve the precision of genetic parameters. Further, this suggests that it may not be necessary to sample any more than two sites to reliably predict the genetic merit of parents for juvenile wood properties. This will have significant impacts on future assessment costs – under the current breeding plan for hybrid pine is south-east Queensland progeny tests will be established on four sites for the main population and three sites for the elite population; however only a maximum of two tests will need to be assessed for wood properties.

Finally, the analyses also demonstrated strong genetic correlations between pure-species and hybrid performance for each of the wood quality traits. Under this scenario, information on pure species performance can be used to reliably predict hybrid performance. This confirms the decision to collect information on the parents which are being crossed for initiation of future cycles of clonal testing – because of these strong genetic correlations, parental performance can be used to identify the hybrid families which are most likely to have superior juvenile wood.

Therefore, in conclusion, it appears that:

i) a selection age between 4 and 6 years after planting is likely to maximize genetic gains in juvenile wood properties;
ii) selection for both density and MfA is required in the F_1 hybrid, because of possible unfavorable associations between density and MfA – methods of assessing MfA/stiffness using a combination of whole-core density and acoustic tools, may prove to be the effective means of selection (cf. Harding et al. 2004);
iii) juvenile wood properties as measured by density, MfA and MOE from SilviScan were very stable across sites, suggesting that assessment of wood properties on two sites will provide reliable estimates of the genetic worth of individuals for use in future breeding; and,
iv) pure-species wood properties are strongly related to the performance of their hybrid progeny, indicating that any information available on the parents either in pure-species or hybrid progeny tests can be applied advantageously to improving the juvenile wood properties of the PEE × PCH F_1 hybrid in south-east Queensland.

However, further work is required to more thoroughly investigate optimization of selection strategies.

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APPENDIX 9

Juvenile wood initiative:

Genetic mapping of candidate genes for vascular development in *Pinus radiata* D. Don *II*

Dillon, S., Nolan, M., Bell, C., Wu, H., and Southerton, S.

CSIRO Forest Biosciences

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Contents

List of Acronyms:

SNP/s	Single Nucleotide Polymorphism/s
group	Linkage group
LOD	Log of odds
CM	Kosambi centimorgans

Executive Summary

This brief report is an addendum to the CSIRO Forest Biosciences client report no. 1847. Genetic mapping of candidate genes, utilised for association mapping, into a *P.radiata* framework microsatelite map is presented. The linkage map presented , which identifies the position of 22 genes, will under go further development in collaboration with the Forest Genomics Group UC Davis, to locate up to 400 additional pine candidate genes. With the location of specific genes now identified, and QTL's for several wood quality traits detected, this map will serve as a valuable tool for validation of genetic association results.

Methods and materials

Plant material

Segregating data from a two generation F2 pedigree (Figure1) including 2 parents and 96 progeny was used to construct the framework genetic linkage map. DNA was extracted from needle samples, using the method of Devey et al. (1996) and purified using Qiagen 96 well purification kit, from parents and 96 progeny from this cross which were grown at Bondo NSW (35°31'17''S; 48°08'40''E).

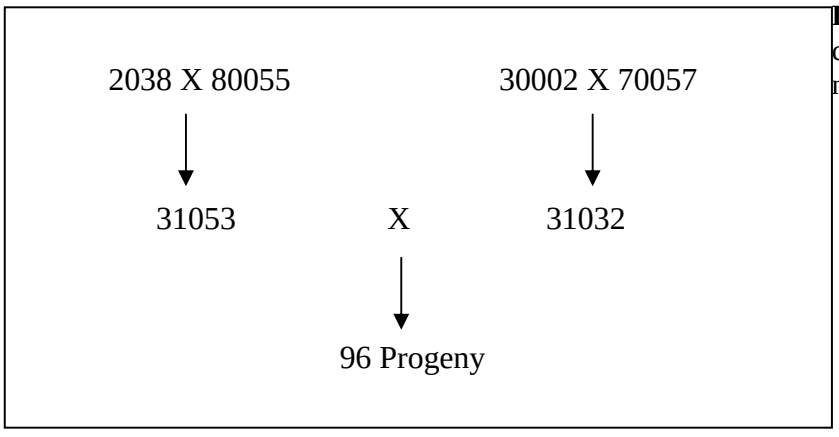


Figure 1. pedigree for mapping cross applied for linkage mapping

Phenotypic data

All selected individuals from the mapping pedigree had also been measured using for several quantitative traits, which included density (Silviscan), MOE (Silviscan), MFA (Silviscan) and fibre length. Trait measurements will be combined with linkage data to identify QTL's for wood quality.

Genotyping microsatelite markers

Microsatellites were amplified from purified DNA samples for parents and progeny, and fragments resolved and scored, according to the method of Smith and Devey (1994). In total 49 microsatelite markers were analysed in this cross (Table 1).

Genotyping SNP markers

The mapping cross parents had been screened with all SNPs presented in the earlier report using the Illumina method, concurrently to the association poualtion. Of the 404 total SNPs successfully genotyped in the *Pinus radiata* association population, 60 of these were found to be polymorphic in the mapping cross parents and showed genotype combinations that would be useful for pedigree mapping (i.e. AAxAB, ABxAB, ABxAA). These 60 SNPs represented 52 unique genes, of the 95 genes in total examined for the association study (Table 1). Genotyping was outsourced to Sequenom Pty Ltd. Of the resulting genotypes, 31 were deemed suitable for mapping. The remaining 29 SNPs were removed for analysis based on either poor quality of the genotype result, the genotype being polymorphic in the progeny but

un-useful for mapping (i.e. AA x BB), or the genotype did not segregate in the progeny. The final array of SNP (and microsatelite) markers used for linkage mapping is presented in Table 2

Table 1. Marker genotyped in 96 mapping progeny

	microsatelites	**SNPs**	**SNPs**
count	**marker**	**marker**	**Gene**
1	NZ490	4CH1_1_1	4CH1
2	gen007	AGP4_2_4	AGP4
3	gen025	AGP6_1_1	AGP6
4	gen033	ATHB8_23_1	ATHB8
5	gen036	ATHBx_1_1	ATHBx
6	gen062	CAD_3F_03	CAD
7	gen065	CCR1_3_01	CCR1
8	gen081	CCR1_promotor_06	CCR1
9	gen119a	CesA1_4g1_10	CesA1
10	gen119b	CesA1_4g2_09	CesA1
11	Pr062	CesA3_3R_26	CesA3
12	Pr203	COBL4_2_11	COBL4
13	Pr245	COMT2_2_1	COMT2
14	TX3043	DH2_38	DH2
15	Pr161	DH7_8_03	DH7
16	Pr254	EXPAN1_1R_2	EXPAN1
17	TX3032	FRA2_2F_02	FRA2
18	gen063	GlyHMT_2F_5	GlyHMT
19	gen101	KOR_2R_09	KOR
20	Pr168	LIM_1F_01	LIM
21	Pr284	Lp5_1_9	Lp5
22	gen038b	MYB4_1F_01	MYB4
23	gen094	PaL1_3_02	PaL1
24	gen108	PAL111	PAL1
25	NZ325a	PCBER2_1_2	PCBER2
26	Pr025	PorinMP1_32	PorinMP1
27	gen110	PrCAD2F01	CAD
28	gen011	PRP1(F)_04	PRP1
29	gen058	Rac13_2F_04	Rac13
30	gen095	Rac13_3Fg2_16	Rac13
31	NZ410b	DH7_8_02	DH7
32	Pr033-2	Rac131F01	Rac13
33	Pr233	SAHH1_1_10	SAHH1
34	Pr276	SAM1_4_1	SAM1
35	TX3025	SODchl_1_4	SODchl
36	Pr265	Susy1Fg2_18	Susy1
37	Pr026	TUB1_1_6	TUB1
38	gen009	XET1_1_08	XET1
	microsatelites	**SNPs**	**SNPs**

count	marker	marker	Gene
39	NZ325b	AGP_1F936	AGP
40	NZ6	ALP_1F566	ALP
41	TX3045	CHI_1F2299	CHI
42	TX2018	CYP_2327	CYP
43	Pr9.3	DH_1_2_3F727	DH1
44	Pr117	FRA1_0F871	FRA1
45	Pr294	GAS_2F355	GAS
46	Pr024	GBP_2R_08	GBP
47	Pr042	GIR_1537	GIR
48	Pr043-2	IAA_1F872	IAA
49	gen038a	KN4_3F615	KN4
50	-	Lac_3805	Lac
51	-	LP3_2_1F682	LP3
52	-	LP6_1_01	LP6
53	-	PCBER212	PCBER2
54	-	PE_F_02	PE
55	-	PEL_2F233	PEL
56	-	PeP_1453	PeP
57	-	Pero_2_01	Pero
58	-	Thioh_2F731	Thioh
59	-	UDP_2F1_05	UDP
60	-	XYL_2_02	XYL

Table 2. Marker genotyped in 96 mapping progeny

marker	marker name	gene	type	mapped
marker01	NZ490	ukn	microsatelite	-
marker02	gen007	ukn	microsatelite	yes
marker03	gen025	ukn	microsatelite	yes
marker04	gen033	ukn	microsatelite	yes
marker05	gen036	ukn	microsatelite	yes
marker06	gen062	ukn	microsatelite	yes
marker07	gen065	ukn	microsatelite	yes
marker08	gen081	ukn	microsatelite	yes
marker09	gen119a	ukn	microsatelite	yes
marker10	gen119b	ukn	microsatelite	yes
marker11	Pr062	ukn	microsatelite	yes
marker12	Pr203	ukn	microsatelite	yes
marker13	Pr245	ukn	microsatelite	-
marker14	TX3043	ukn	microsatelite	yes
marker15	Pr161	ukn	microsatelite	yes
marker16	Pr254	ukn	microsatelite	yes
marker17	TX3032	ukn	microsatelite	yes
marker18	gen063	ukn	microsatelite	yes
marker19	gen101	ukn	microsatelite	yes
marker20	Pr168	ukn	microsatelite	yes
marker21	Pr284	ukn	microsatelite	yes

marker22	gen038b	ukn	microsatelite	yes
marker	**marker name**	**gene**	**type**	**mapped**
marker23	gen094	ukn	microsatelite	-
marker24	gen108	ukn	microsatelite	yes
marker25	NZ325a	ukn	microsatelite	-
marker26	Pr025	ukn	microsatelite	yes
marker27	gen110	ukn	microsatelite	yes
marker28	gen011	ukn	microsatelite	yes
marker29	gen058	ukn	microsatelite	yes
marker30	gen095	ukn	microsatelite	yes
marker31	NZ410b	ukn	microsatelite	yes
marker32	Pr033-2	ukn	microsatelite	-
marker33	Pr233	ukn	microsatelite	yes
marker34	Pr276	ukn	microsatelite	yes
marker35	TX3025	ukn	microsatelite	-
marker36	Pr265	ukn	microsatelite	yes
marker37	Pr026	ukn	microsatelite	yes
marker38	gen009	ukn	microsatelite	yes
marker39	NZ325b	ukn	microsatelite	-
marker40	NZ6	ukn	microsatelite	yes
marker41	TX3045	ukn	microsatelite	-
marker42	TX2018	ukn	microsatelite	yes
marker43	Pr9.3	ukn	microsatelite	-
marker44	Pr117	ukn	microsatelite	yes
marker45	Pr294	ukn	microsatelite	yes
marker46	Pr024	ukn	microsatelite	-
marker47	Pr042	ukn	microsatelite	-
marker48	Pr043-2	ukn	microsatelite	yes
marker49	gen038a	ukn	microsatelite	-
marker50	CHI_1F2299	CHI	SNP	yes
marker51	DH_1_2_3F727	DH1	SNP	yes
marker53	LP6_1_01	LP6	SNP	-
marker54	FRA1_0F871	FRA1	SNP	-
marker55	PeP_1453	PeP	SNP	-
marker56	XYL_2_02	XYL	SNP	yes
marker57	GAS_2F355	GAS	SNP	-
marker58	KN4_3F615	KN4	SNP	yes
marker61	CYP_2327	CYP	SNP	-
marker62	GIR_1537	GIR	SNP	-
marker63	PCBER212	PCBER2	SNP	yes
marker64	4CH1_1	4CH1	SNP	yes
marker65	AGP4_2	AGP4	SNP	yes
marker66	AGP6_1	AGP6	SNP	yes
marker67	ATHBx_1	ATHBx	SNP	yes
marker68	CCR1_3_01	CCR1	SNP	yes
marker71	COBL4_2_11	COBL4	SNP	yes
marker72	COMT2_2	COMT2	SNP	-
marker73	DH7_8_02	DH7	SNP	yes
marker75	GlyHMT_2F	GlyHMT	SNP	yes
marker76	KOR_2R_09	KOR	SNP	yes

marker77	LIM_1F_01	LIM	SNP	-
marker78	MYB4_1F_01	MYB4	SNP	-
marker79	PAL111	PaL1	SNP	yes
marker	**marker name**	**gene**	**type**	**mapped**
marker80	PCBER2_1	PCBER2	SNP	yes
marker81	PorinMP1_32	PorinMP1	SNP	yes
marker83	Rac131F01	Rac13	SNP	yes
marker84	SAM1_4	SAM1	SNP	yes
marker85	SODchl_1	SODchl	SNP	yes
marker86	TUB1_1	TUB1	SNP	yes
marker87	XET1_1_08	XET1	SNP	yes

Linkage mapping

Multipoint linkage analysis was performed using OutMap version 1.0 (© Whitaker, D., Ling, S., Williams, E. and T. Speed 2001). Maternal and Paternal co-dominant markers were mapped as a single data set. Markers were initially grouped using a LOD threshold of 3.0 and minimum recombination threshold of 0.3, except for groups 1 and 3 which were grouped using less stringent conditions, LOD 2.0 and minimum recombination 0.5. Phased data was then used to determine the order of markers along each linkage group (or hypothetical chromosome) using an ‘accuracy” of 0.1 and ordering option of “two-opt” in OutMap.

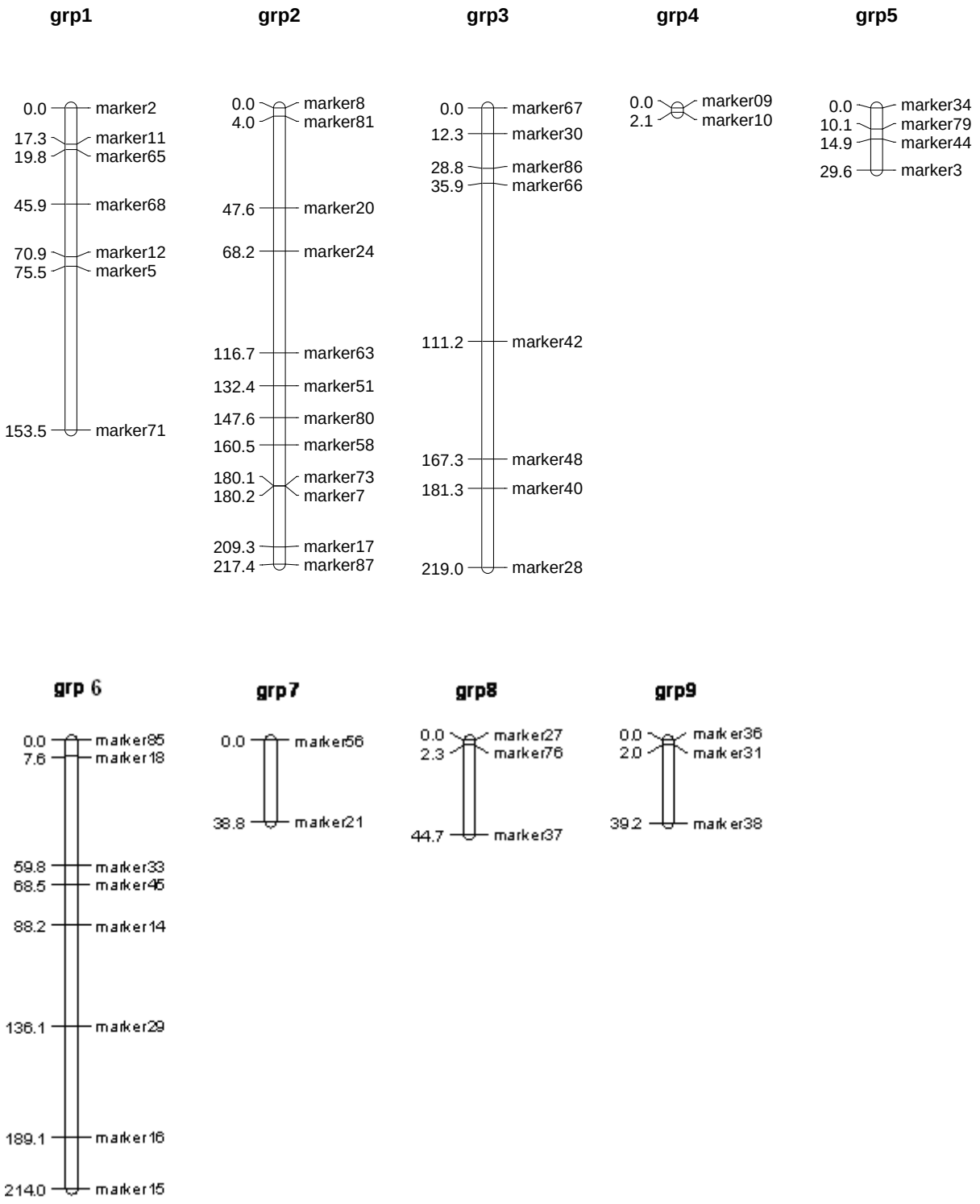
Results

In total 9 linkage groups were obtained, from a final number of 60 markers, 543.45 centimorgans in total. Map distances were expressed in Kosambi centimorgans (Figure 2.0). Linkage groups were visualised using Mapchart software (Voorrips 2002). The number of linkage groups in the map correspond closely to the actual number of chromosomes for *P. radiata* (12). Those markers that did not map in this experiment, due to insufficient recombination information with other loci, have a high probability of mapping to one of the existing linkage groups once more markers are added to this map (see conclusion for comments on additional mapping of 400 SNPs). Additional microsatelite markers from a map generated using the same pedigree, although which used different progeny, may be added into the linkage map generated here by scaling groups according to common markers present between both maps. However, many of the common markers did not map in this experiment, thus this step has been suspended until additional markers become available. Of the common markers that did map: namely, marker16 and marker15, marker17 and marker7, marker20 and marker24, and markers 36, 31 and 38, all showed comparable collocation and genetic distances to the earlier map. Certainly the smaller groups on the framework map presented may represent smaller fragments of one or more of the other groups identified. Similarly several of the larger groups (2, 3 and 7) which exhibit large centimorgans distances towards their centre suggests these groups may represent two groups spuriously combined. Such groups may fall apart once additional markers have been added to the map. We did not attempt to assign QTL' s for traits collected across the progeny at this time, due to the small number of total markers mapped, but will proceed with QTL analysis once additional SNP data for these individuals has been made available.

Conclusion

In order to update and increase the resolution of the current map, the existing mapping pedigree will be genotyped with up to 400 candidate gene SNP markers, identified from candidate gene re-sequencing efforts, and mapped into the existing framework of microsatelite markers. This work is part of an ongoing collaboration with the Forest Genomics Group UC Davis, California, headed by David Neale. The CFB group has been dependant on the delivery of this additional data from our collaborators, which had not been fulfilled at the time of this report due to technical problems. The absence of this data has impacted our ability to deliver a high resolution map at this stage. The improved map will provide an important tool for comparative genomics of *Pinus radiata*, positioning candidate genes in the genome and permitting first stage validation of SNPs associations against major QTLs.

Figure 2.0 Linkage maps generated



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APPENDIX 10

Juvenile Wood Initiative:

INCORPORATING SINGLE NUCLEOTIDE POLYMORPHISMS WITH QUANTITATIVE GENETIC DATA AND SNP-AIDED SELECTION

Brian S. Baltunis, Shannon Dillon, and Harry X. Wu.

CSIRO Plant Industry Client Report No:

Client: FWPA/STBA
Project No: PN03.1916

30th November 2008

Commercial in Confidence

Juvenile Wood Initiative:

INCORPORATING SINGLE NUCLEOTIDE POLYMORPHISMS WITH QUANTITATIVE GENETIC DATA AND SNP-AIDED SELECTION

Brian S. Baltunis, Shannon Dillon, and Harry X. Wu

Table of Content

EXECUTIVE SUMMARY

Objectives

The objectives of this study were to:

1. Document methodology for estimating additive and dominance effects of single nucleotide polymorphisms (SNPs).
2. Identify SNPs with significant additive effects for average density, modulus of elasticity, microfibril angle, and pith-to-bark core length.
3. Explore method and efficiency to incorporate SNP effect in SNP-aided Selection.

Key Results

Methodology for estimating additive and dominance effects

- The model allowed for direct estimates of additive and dominance effects of a SNP.
- ASReml syntax of the model is presented in the APPENDIX.
- An index based approach to incorporate SNP effects was explored.

Significant SNPs

- 14 SNPs had significant additive effects (p-value < 0.05) associated with average density and accounted for 0.03% to 1.61% of the total phenotypic variation.
- 29 SNPs had significant additive effects associated with average modulus of elasticity and accounted for 0.35% to 8.23% of the total phenotypic variation.
- 26 SNPs had significant additive effects associated with average microfibril angle and accounted for 0.04% to 6.31% of the total phenotypic variation. 18 of these SNPs were common between microfibril angle and modulus of elasticity suggesting pleiotropic effects.
- 12 SNPs had significant additive effects associated with pith-bark core length and accounted for 0.01% to 1.65% of the total phenotypic variation.
- However, none of the significant associations from single-SNP analyses held up when corrected for multiple testing.

SNP aided selection

- Selections based on single SNPs were not as effective as direct selection for stiffness (MoE). SNPs accounting for 32% of genetic variance for mature MoE are required to have same efficiency as early selection for mature wood MoE (stiffness).
- For an efficient SNP aided early selection for stiffness of rotation age, individual SNPs or SNP clusters that accounts for more than 10.24% of independent genetic effects for rotation age MoE should be explored using more candidate genes.

Application of Results

The methodology documented in this report can be used for estimating additive and dominance effects of single nucleotide polymorphisms. The index approach documented in this report can be used for SNP aided selection. However, results are

preliminary and significant associations need to be validated in different populations for an effective application of SNP aided selection.

Further Work

The genetic effects of individual SNPs were assumed to be independent and more analyses need to be conducted to test for multi-colinearity. In addition, significant associations need to be validated in a larger, unrelated population; and therefore, results should be viewed as preliminary. More candidate genes should be used for association genetics and it would be ideal to have enough uncorrelated SNPS that account for more than 25% of genetic variation for wood traits. We plan on doing several simulation studies, both parametric and locus-based, in order to develop and document advanced methodology and strategy for incorporating significant SNPs in a breeding strategy.

INTRODUCTION

Single Nucleotide Polymorphisms (SNPs) are a major contributing factor of trait variation (Falconer and Mackay 1996). For quantitative traits we assume that a large number of loci contribute to the genetic variation underlying the trait of interest (Infinitesimal Model; Fisher 1918). SNPs have also been shown to have major phenotypic effects in animals and plants (Spielmeyer et al. 2002; Eiberg et al. 2008). For example, the Slender 1 mutation which induces a dwarf phenotype has revolutionised the domestication of rice and barley (Spielmeyer et al. 2002). Selection and breeding aim to manipulate frequencies of desirable alleles for the genes controlling traits of interest in a population. Traditionally, this is done with no prior knowledge of genes, their frequencies or the genetic effects of individual loci. However, with identification of reliable candidate genes, their SNPs and rapid genotyping technologies the estimation of genetic effects for individual SNPs or groups of SNPs has become possible.

As further reliable associations between economically important quantitative traits and polymorphic loci are identified, the potential to develop molecular breeding strategies becomes apparent. As most allelic variants account for a small amount of the total phenotypic variation for agronomic traits, molecular breeding strategies will ideally incorporate those combinations of SNP markers which best describe the trait variation with quantitative genetic data. As a first step in developing a molecular breeding strategy, we present methodology for testing for significant additive and dominance effects of SNPs by incorporating each SNP sequentially with quantitative genetic data for

juvenile density, modulus of elasticity, microfibril angle, and pith-to-bark core length using an individual tree model.

One of objectives in detecting significant SNP is for application in breeding selection: SNP-aided selection for breeding and deployment populations. Before SNP can be used for SNP-aided selection, three assumptions must be met:

1. In large breeding population, the candidate gene with its significant SNP must be one of the QTLs that determine the genetic value of the quantitative traits. If the significant SNP was from other genes, it will be impossible to predict whether a SNP allele is in coupling or repulsion phase with the desired QTL alleles if the breeding population is at or near linkage equilibrium. Radiata pine breeding population is consisted of about 400 selections in the second generation of population (Powell et al. 2004). Thus, correlation among QTL alleles and SNP alleles must be determined separately for each pedigree of interest if SNP detected was not in QTL for the trait. If SNPs reside within QTL themselves, this becomes a less problem.

2. There are usually multiple alleles for each QTL within a breeding population. It would be useful to align SNP allele with QTL alleles for efficient gene-aided selection. This also indicates that advantageous allele of a single SNP may differ among genetic backgrounds, families and population. Therefore, the advantageous SNP allele in one pedigree or population may not be advantageous in another pedigree or population. This creates a practical problem in using QTL SNPs for selection in a breeding population.

To circumvent the problem of multiple alleles in a breeding program, it would be useful to rank all alleles in the breeding population.

3. Effect of same SNP varies with genetic background and population since SNP effect is associated with its allele frequency which may change in different populations. Significant SNPs that were detected in one population may disappear in another population simply because the change of its allele frequency, rather than its effect.

If all above three assumptions are satisfied (Candidate genes with significant SNPs are QTL themselves for the trait of interest, SNP allele are same as QTL alleles with their rank (or breeding value) being same and known between discovery and breeding population, and effect of same SNP do not change, it is possible that SNP-aided selection may result in higher genetic gain than phenotypic selection in breeding populations. The efficiency of SNP aided selection was studied using an index selection approach for tree breeding program (Wu 2002).

MATERIALS AND METHODS

Populations

Needles (for DNA extraction) and phenotypes were collected for 458 individuals from a 2nd generation STBA progeny trial in Flynn, Victoria (BR9611; see Baltunis et al. 2007 for trial details).

Phenotypic data

Phenotypic data based on pith-to-bark core length and SilviScan measurements of density, modulus of elasticity (MOE) and microfibril angle (MFA) were estimated by averaging individual ring values from the outer four of seven rings.

Genes and SNPs

Wood quality traits were the focus of this study. Therefore, genes involved in the determination of cell wall and wood fibre properties were selected. This included 51 genes, which are a subset of the 95 candidate genes reported in FWPA client report No. 1847 (Dillon et al. 2008). The selected candidate genes have putative functions in five major developmental pathways: lignin biosynthesis, cellulose biosynthesis, cell wall structure, cell expansion, and stress response.

To identify SNPs each gene was sequenced in a panel of 13-24 trees. DNA sequence alignments were assembled and SNP quality determined using Phred-Phrap (Ewing and Green 1998). SNPs were identified and selected according to user-defined criteria using a machine learning algorithm encompassing features of both PolyBayes and PolyPhred (Ewing and Green 1998) SNP selection programs. This algorithm was developed at the Conifer Research Group, Department of Plant Sciences, University of California-Davis.

Genotypic data

Up to two trees (one from each of replicates 1 and 4) from 154 full-sib families and 86 polymix families (representing 132 STBA parent selections) were selected for genotyping. In total, 458 individuals from the Flynn trial were genotyped, and 253 SNPs were identified, this included two trees from each of five check lots. 384 SNPs were genotyped in parallel across all individuals using universal bead arrays according to the manufacturers instructions (Illumina Inc) (Shen et al. 2005). Genotypes were visualised and genotype clusters manually assessed using BeadStudio© 3 software.

Estimation of additive and dominance effects

In this report, we follow the syntax of Falconer and Mackay (1996). Assume a single locus with two alleles, A_1 and A_2. Let A_1 be defined as the 'good' allele, while A_2 be defined as the 'bad' allele. The frequencies of A_1 and A_2 alleles are defined by 'p' and 'q' where $p+q=1$. Homozygous individuals with two copies of the good allele (A_1A_1) are assigned genotypic value 'a', while A_2A_2 genotypes are assigned genotypic value '-a'; and the midpoint between homozygous groups is 0 (Figure 1). Two times the additive effect (a) is the difference between means of the two homozygous groups, while the dominance effect (d) is the genotypic value assigned to heterozygous genotypes (A_1A_2).

Figure 1. Genotypic values assigned to three genotypes.

Density, modulus of elasticity, microfibril angle and core length were combined with SNP data and analysed with an individual tree mixed model in ASReml (Gilmour et al. 2005), in order to estimate the additive and dominance effects associated with each SNP, and the genetic variance components.

[1] $y_{ijkl} = \mu + r_i + a_j + d_j + tree_k + e_{ijkl}$

where y_{ijkl} is the phenotypic value of a trait, μ is the overall mean, r_i is the fixed effect of the i^{th} replicate, a_j is the fixed additive effect of the j^{th} SNP, d_j is the fixed dominance effect of the j^{th} SNP, $tree_k$ is the random additive genetic effect ~ N(0, $\hat{\sigma}_a^2$), and e_{ijkl} is the residual error ~ N(0, $\hat{\sigma}_e^2$).

The additive and dominance genetic variances associated with each SNP and for each trait were defined as

[2] $V_{\text{A-SNP}} = 2pq[a + d(q - p)]^2$ and

[3] $V_{D\text{-}SNP} = (2pqd)^2$, respectively (Falconer and Mackay 1996).

Assuming independence of additive effects, the remaining additive genetic variance associated with each trait was estimated as

[4] $V_A = \hat{\sigma}_a^2$.

The residual error (V_E) and phenotypic (V_{P*}) variances were estimated as

[5] $V_E = \hat{\sigma}_e^2$ and

[6] $V_{P*} = V_A + V_{A\text{-}SNP} + V_{D\text{-}SNP} + V_E$, respectively.

In addition, the approximate percent variation controlled by the additive genetic variation of each SNP was estimated.

[7] $\frac{V_{A\text{-}SNP}}{V_{P*}} \times 100\%$

Estimation of the false discovery rate

To account for false positives arising from multiple testing, an alternative cut-off (q-value) for the strength of each association was calculated. The q-value is a measure of significance associated with each p-value and is derived from the false discovery rate (FDR) (Storey and Tibshirani 2003). The q-value

statistic was calculated from the p-value distribution using QVALUE software (Storey and Tibshirani 2003), with the lambda parameter set between 0 to 0.9.

Efficiency of SNP-aided selection

To incorporate SNP effects into breeding selection, one method is to use selection index. SNP markers can be used for early selection alone as well as assisting in early phenotypic selection. This method of incorporating molecular markers in a selection index is preferable for increasing genetic gain. Incorporating SNP markers for stiffness at rotation age into an early selection index may not only increase genetic gain, but also has the advantage of shortening generation time. This can be done through an index selection approach as

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where x is an early measurement of stiffness trait, m represents the SNP score, and a and b are the estimated index coefficients for early stiffness trait x and SNP score m. The SNP score *m* could be derived from multiple regressions of SNPs on phenotypes. Selection will be based on the index I. The genetic gain for such index was worked out (Wu 2002). Genetic gain from this index selection relative to selection based on early stiffness trait (x) alone was derived as

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Our preliminary analyses indicated the multiple regressions of multiple SNPs on phenotypes were not able to generate a significant SNP score m due to correlation among single SNPs. Therefore, only single SNP effect was used for computation of SNP aided selection. However, this early selection index

can be extended to selection index incorporating multiple early wood traits and multiple SNP scores for multiple trait selection if more SNPs that count for higher and independent genetic variation for wood traits are discovered. Genetic parameters used for the calculation were from Wu et al. (2007, 2008).

RESULTS AND DISCUSSION

Trait means

Trait means were calculated based on measurements from all 458 genotypes. The mean density was 462.7 kg/m^3 and ranged from 381.9 to 556.1 kg/m^3. Modulus of elasticity estimates ranged from 2.41 to 16.41 GPA with a mean of 8.81 GPA. Microfibril angle ranged from 14.48 to 40.05^o with a mean of 25.31^o. Mean core length for the 457 individuals was 75.35 mm and ranged from 40.3 to 102.8 mm. These values are consistent with those reported for a larger population from the Flynn trial (Baltunis et al. 2007).

Significant SNP effects

Of the 255 single nucleotide polymorphisms identified in the Flynn population, 14 showed significant additive effects (p-value < 0.05) associated with average density (Table 1). Four of these SNPs also had significant dominance effects associated with average density (Table 1). None of the 14 SNPs were considered rare (freq of A_1 allele (p) > 0.10), while most of the 14 SNPs were at higher frequencies (p > 0.20). The approximate percent phenotypic variation for density explained by each of the 14 significant SNPs was low, ranging from 0.03% to 1.61% with a mean of 0.66%.

Significant SNP associations were also detected for average modulus of elasticity (Table 2). Most of these SNPs occurred at higher frequencies, while two SNPs were considered rare ($p \leq 0.10$). Twenty-nine SNPs had significant additive effects, but of these, only one SNP also had a significant dominance effect (Table 2). The percent variation explained by additive effects of these 29 SNPs ranged from 0.45% to 8.23% with a mean of 1.53%.

Twenty-six SNPs had significant additive effects for microfibril angle (Table 3). Six of these SNPs also had significant dominance effects. For microfibril angle the favourable allele (A_1) is the allele that decreases microfibril angle with respect to the cell axis (eg., Barnett and Bonham 2004). For example, individuals with genotype A_1A_1 at SNP 240 have a statistically lower mean microfibril angle then A_1A_2 and A_2A_2 genotypes (Figure 2). The percent variation explained by additive effects of the 26 significant SNPs ranged from 0.04% to 6.31% with a mean of 1.65%. Eighteen significant SNPs showed correspondence between microfibril angle and modulus of elasticity suggesting possible pleiotropy of this locus. The simultaneous influence of some SNPs on MFA and MOE is not surprising, since microfibril angle and modulus of elasticity are highly genetically correlated in this population (Baltunis et al. 2007).

Twelve SNPs showed significant additive effects associated with pith-to-bark core length, and 1/3 of these also had significant dominance effects (Table 4). The percent variation in core length explained by additive effects of SNPs was low and ranged from 0.01% to 1.65% with a mean of 0.73%. Two of the

significant SNPs occurred at rare frequencies (SNP 101 and SNP 207), while the remaining SNPs occurred at higher frequencies (Table 4).

Association studies are an emerging area in forest genetics. However, few studies in trees have reported significant SNP associations with traits. Thumma et al. (2005) identified significant polymorphisms in *cinnamoyl CoA reductase* affecting microfibril angle in *Eucalyptus nitens* and *E. globulus* that explained 4.6% of the variation. González-Martínez et al. (2007) identified SNPs associated with several wood property traits in *Pinus taeda* including both juvenile and mature wood density, % latewood, microfibril angle, and lignin and cellulose content. All significant SNP associations explained < 5% of the variation for wood properties which is similar to the current study (González-Martínez et al. 2007). In a recent study dealing with carbon isotope discrimination in *Pinus taeda*, significant genetic association was identified for several polymorphisms (González-Martínez et al. 2008). However, none of the associations were significant in their study after correcting for multiple testing; and the percent variation explained by SNPs was generally < 1%. The significant associations in the current report should be viewed with caution as they are preliminary and warrant further validation in additional populations. At the q = 0.05 cut-off none of the associated effects remained significant (Figure 3). This is attributed to the p-value distribution, which was not significantly different to that predicted under null expectations (dashed line). The divergence of p-values away from the null distribution for additive effects as P approaches 0 indicates that a small proportion of significant associations (π_0 ~ 20%) are likely to be real.

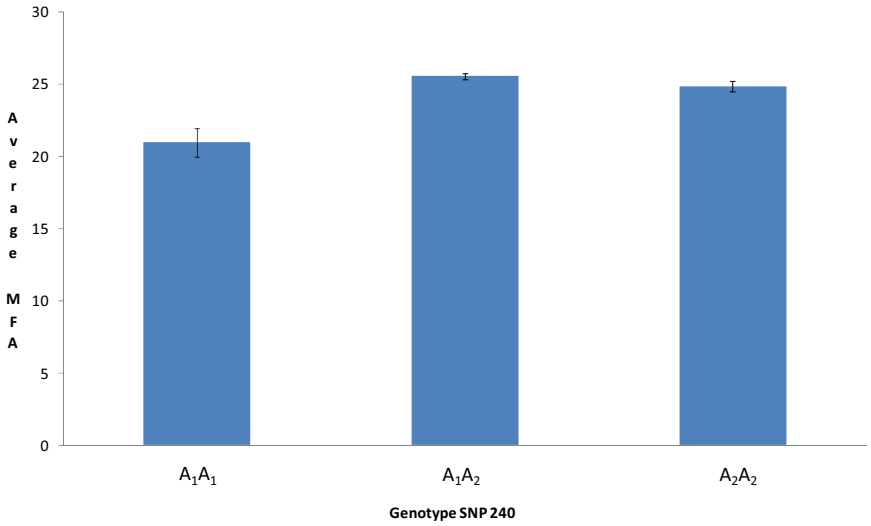


Figure 2. Average microfibril angle (MFA) for three genotypes at SNP 240.

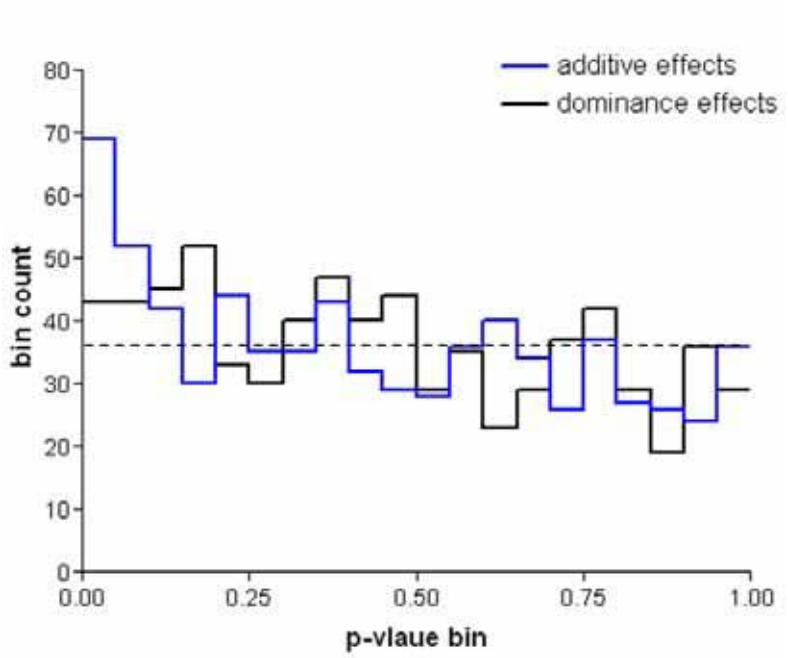


Figure 3**.** P-value distributions for all associations estimated in Q-value

Effect of allele frequencies on additive variation of SNPs

Single nucleotide polymorphisms have been identified for several traits in this study. However, the relative importance of significant SNPs in explaining the variation was generally low with a few exceptions. The percent variation

explained by additive genetic variation of a SNP is dependent on the estimates of a, d, and allele frequencies (equation 2). For example, for a single SNP (locus) when d = 0, then there are only additive effects contributing to the genetic variation of the trait. This is the case for SNP 307 and microfibril angle (Table 3). When there is no dominance then the percent variation explained by the additive genetic variation of the SNP will be maximized when the frequency of A_1 = frequency of A_2 (p = q; Figure 4a). When there is dominance present and in the case where d = -a (complete dominance of A_2 allele over A_1 allele), then the additive variation of the SNP will be maximized at p = 0.75 (Figure 4b); or when d = a, then the additive variation of the SNP will be maximized at p = 0.25.

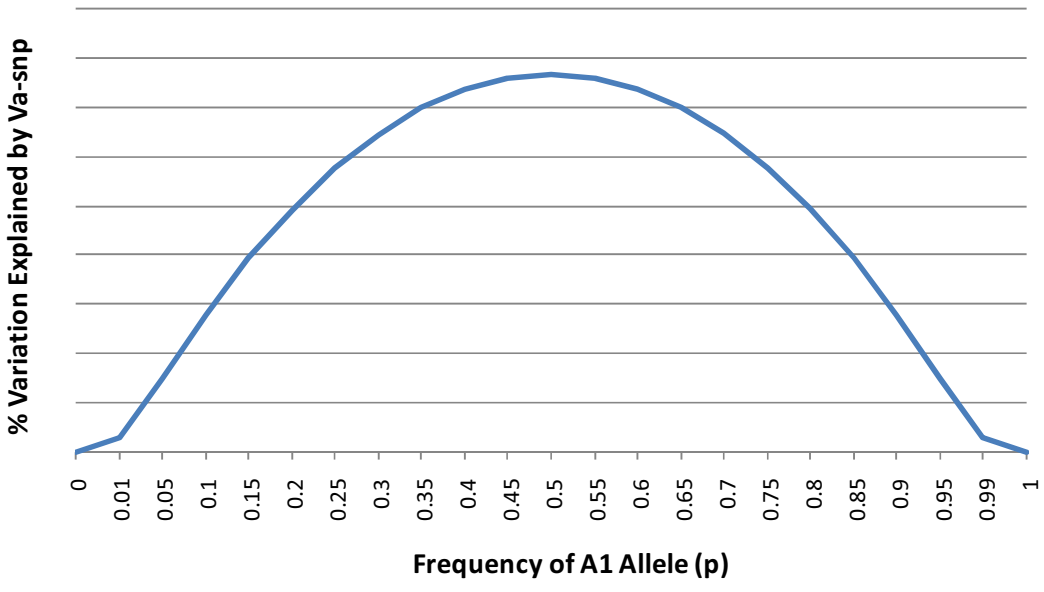


Figure 4a. The % variation explained by additive variation of a SNP is maximized when frequency of the A_1 allele (p) = 0.5 when there is no dominance (d = 0).

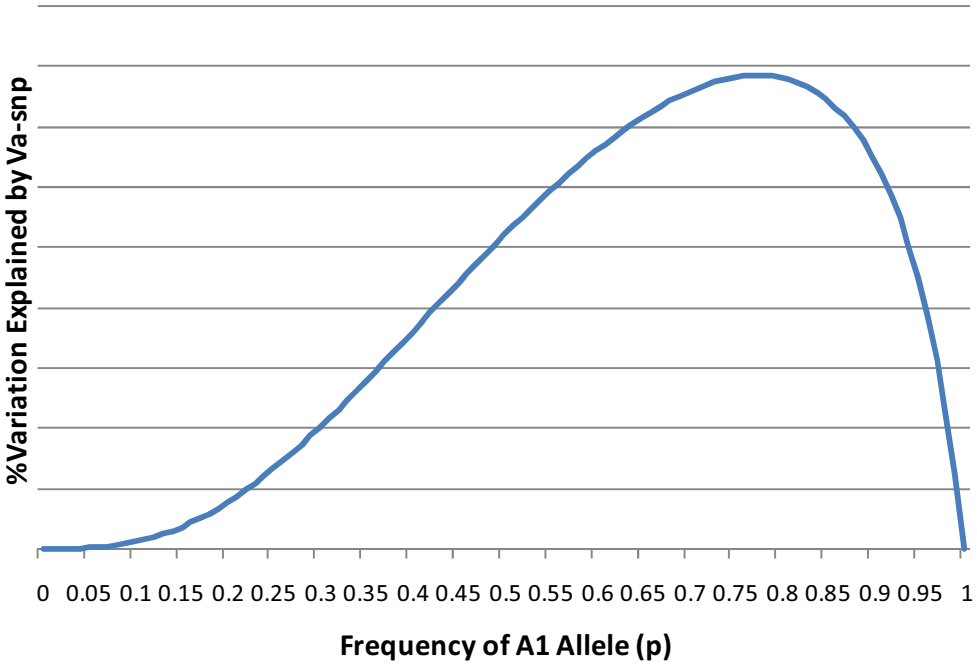


Figure 4b. The % variation explained by additive variation of a SNP is maximized when frequency of the A_1 allele (p) = 0.75 when there is complete dominance of A_2 allele over A_1 allele (d = -a).

Table 1. SNPs with significant additive effects ($p \leq 0.05$) for density averaged over rings 4-7 from single-SNP analyses.

SNP ID	Freq of A_1 (p)	Freq of A_2 (q)	a (p-value)	d (p-value)	$V_{A\text{-}SNP}$	$V_{D\text{-}SNP}$	V_A	V_E	V_{P^*}	$\frac{V_{A\text{-}SNP}}{V_{P^*}} \times 100\%$
6	0.560	0.440	4.593 (0.032)	-0.656 (0.801)	10.753	0.104	676.011	150.336	837.205	1.28%
37	0.855	0.145	7.259 (0.027)	8.797 (0.039)	0.259	4.785	671.788	188.731	865.563	0.03%
45	0.120	0.880	13.346 (0.005)	-13.866 (0.015)	1.660	8.561	701.486	107.652	819.358	0.20%
52	0.611	0.389	3.207 (0.050)	6.314 (0.017)	1.543	9.004	567.776	250.707	829.030	0.19%
63	0.807	0.193	5.591 (0.021)	6.659 (0.084)	0.708	4.313	759.977	75.669	840.667	0.08%
97	0.513	0.487	5.117 (0.003)	-3.815 (0.133)	13.589	3.634	591.921	235.131	844.275	1.61%
148	0.230	0.770	8.411 (0.024)	-8.776 (0.052)	4.783	9.670	698.759	171.949	885.161	0.54%
155	0.587	0.413	3.599 (0.045)	-2.729 (0.338)	8.051	1.751	621.071	198.397	829.270	0.97%
192	0.564	0.436	3.603 (0.045)	2.553 (0.157)	5.275	1.576	635.609	206.345	848.805	0.62%
206	0.773	0.227	10.480 (0.005)	8.274 (0.054)	12.443	8.414	536.947	259.225	817.029	1.52%
273	0.230	0.770	6.767 (0.014)	-8.661 (0.018)	1.547	9.412	763.266	79.554	853.779	0.18%
280	0.705	0.295	4.238 (0.031)	3.959 (0.235)	2.851	2.715	546.054	240.635	792.256	0.36%
306	0.409	0.591	5.065 (0.050)	-4.677 (0.127)	8.578	5.111	817.333	59.475	890.496	0.96%
340	0.546	0.454	3.544 (0.048)	-0.013 (0.996)	6.231	0.000	639.345	199.880	845.456	0.74%

Table 2. SNPs with significant additive effects ($p \leq 0.05$) for modulus of elasticity averaged over rings 4-7 from single-SNP analyses.

SNP ID	Freq of A_1 (p)	Freq of A_2 (q)	a (p-value)	d (p-value)	$V_{A\text{-}SNP}$	$V_{D\text{-}SNP}$	V_A	V_E	V_{P^*}	$\frac{V_{A\text{-}SNP}}{V_{P^*}} \times 100\%$
6	0.560	0.440	0.303 (0.050)	-0.020 (0.917)	0.046	0.000	2.621	1.374	4.041	1.14%
13	0.467	0.533	0.282 (0.028)	-0.006 (0.973)	0.039	0.000	3.706	0.539	4.284	0.92%
52	0.611	0.389	0.267 (0.022)	0.191 (0.307)	0.024	0.008	2.624	1.449	4.106	0.59%
55	0.298	0.702	0.579 (0.024)	-0.551 (0.191)	0.053	0.053	1.914	2.490	4.510	1.17%
75	0.859	0.141	0.569 (0.049)	0.500 (0.146)	0.011	0.015	2.655	1.368	4.049	0.26%
85	0.657	0.343	0.411 (0.012)	0.000 (0.999)	0.076	0.000	2.554	1.555	4.185	1.82%
90	0.491	0.509	0.341 (0.009)	-0.298 (0.182)	0.056	0.022	2.547	1.636	4.261	1.32%
119	0.566	0.434	0.290 (0.017)	-0.135 (0.474)	0.047	0.004	2.542	1.382	3.975	1.17%
133	0.520	0.480	0.296 (0.037)	-0.262 (0.175)	0.047	0.017	2.507	1.495	4.065	1.15%
176	0.327	0.673	0.443 (0.002)	-0.024 (0.903)	0.083	0.000	2.503	1.389	3.975	2.09%
179	0.613	0.387	0.369 (0.012)	-0.098 (0.620)	0.073	0.002	2.831	1.210	4.116	1.77%
197	0.719	0.281	0.595 (0.002)	0.416 (0.069)	0.069	0.028	2.628	1.393	4.118	1.67%
212	0.372	0.628	0.305 (0.032)	-0.186 (0.335)	0.031	0.008	2.960	1.141	4.139	0.75%
238	0.480	0.520	0.325 (0.024)	-0.286 (0.131)	0.049	0.020	2.423	1.591	4.084	1.21%
240	0.375	0.625	1.276 (0.011)	-1.444 (0.005)	0.392	0.458	2.691	1.228	4.769	8.23%

Table 2. continued

SNP ID	Freq of A_1 (p)	Freq of A_2 (q)	a (p-value)	d (p-value)	$V_{A\text{-}SNP}$	$V_{D\text{-}SNP}$	V_A	V_E	V_{P^*}	$\frac{V_{A\text{-}SNP}}{V_{P^*}} \times 100\%$
247	0.371	0.629	0.362 (0.012)	0.162 (0.419)	0.076	0.006	2.648	1.496	4.226	1.80%
270	0.465	0.535	0.371 (0.035)	0.052 (0.797)	0.070	0.001	2.530	1.473	4.074	1.72%
278	0.528	0.472	0.265 (0.026)	-0.003 (0.986)	0.035	0.000	2.732	1.332	4.099	0.86%
280	0.705	0.295	0.295 (0.039)	-0.082 (0.736)	0.045	0.001	2.841	1.321	4.208	1.07%
307	0.637	0.363	0.324 (0.015)	-0.012 (0.955)	0.049	0.000	2.698	1.476	4.223	1.17%
308	0.657	0.343	0.297 (0.028)	-0.186 (0.351)	0.057	0.007	2.023	1.663	3.750	1.52%
318	0.549	0.451	0.392 (0.004)	-0.198 (0.281)	0.084	0.010	2.919	1.102	4.115	2.04%
335	0.747	0.253	0.805 (0.010)	0.428 (0.192)	0.133	0.026	2.482	1.598	4.239	3.14%
338	0.054	0.946	1.316 (0.031)	-1.066 (0.128)	0.014	0.012	2.882	1.061	3.969	0.35%
342	0.307	0.693	0.509 (0.002)	-0.315 (0.122)	0.064	0.018	2.587	1.416	4.085	1.56%
373	0.417	0.583	0.253 (0.038)	-0.357 (0.119)	0.018	0.030	2.945	1.109	4.103	0.45%
377	0.338	0.662	0.346 (0.008)	-0.139 (0.471)	0.040	0.004	2.476	1.393	3.913	1.03%
382	0.100	0.900	0.990 (0.038)	-0.591 (0.257)	0.049	0.011	2.658	1.279	3.997	1.21%
383	0.699	0.301	0.347 (0.031)	-0.021 (0.917)	0.053	0.000	2.689	1.253	3.993	1.33%

Table 3. SNPs with significant additive effects ($p \leq 0.05$) for microfibril angle averaged over rings 4-7 from single-SNP analyses.

SNP ID	Freq of A_1 (p)	Freq of A_2 (q)	a (p-value)	d (p-value)	$V_{A\text{-}SNP}$	$V_{D\text{-}SNP}$	V_A	V_E	V_{P^*}	$\frac{V_{A\text{-}SNP}}{V_{P^*}} \times 100\%$
1	0.893	0.107	-1.961 (0.049)	-2.716 (0.012)	0.006	0.271	5.187	7.807	13.271	0.04%
75	0.859	0.141	-1.315 (0.019)	-1.133 (0.090)	0.061	0.075	9.659	5.388	15.183	0.40%
90	0.491	0.509	-0.720 (0.004)	0.429 (0.311)	0.253	0.046	8.304	6.491	15.094	1.68%
119	0.566	0.434	-0.505 (0.031)	0.361 (0.322)	0.150	0.031	8.651	5.620	14.452	1.04%
176	0.327	0.673	-0.915 (0.001)	-0.032 (0.934)	0.378	0.000	8.373	5.817	14.567	2.59%
197	0.719	0.281	-1.213 (0.001)	-1.056 (0.017)	0.227	0.182	9.331	5.506	15.246	1.49%
207	0.927	0.073	-2.245 (0.030)	-2.921 (0.010)	0.008	0.158	8.931	5.935	15.033	0.06%
212	0.372	0.628	-0.774 (0.005)	0.384 (0.304)	0.213	0.032	10.438	4.822	15.506	1.37%
226	0.634	0.366	-0.565 (0.022)	-0.108 (0.789)	0.133	0.003	9.612	5.323	15.070	0.88%
229	0.785	0.215	-3.910 (0.044)	-3.682 (0.060)	1.111	1.548	9.308	5.636	17.603	6.31%
238	0.480	0.520	-0.576 (0.038)	0.471 (0.196)	0.155	0.055	8.360	6.333	14.903	1.04%
240	0.375	0.625	-2.142 (0.028)	2.661 (0.008)	1.022	1.555	8.942	5.448	16.967	6.03%
247	0.371	0.629	-0.707 (0.010)	-0.206 (0.590)	0.270	0.009	9.088	5.835	15.203	1.78%
278	0.528	0.472	-0.569 (0.014)	0.196 (0.603)	0.167	0.010	8.403	6.171	14.752	1.13%
299	0.951	0.049	-3.859 (0.044)	-5.312 (0.008)	0.081	0.243	8.499	6.041	14.864	0.55%

Table 3. continued

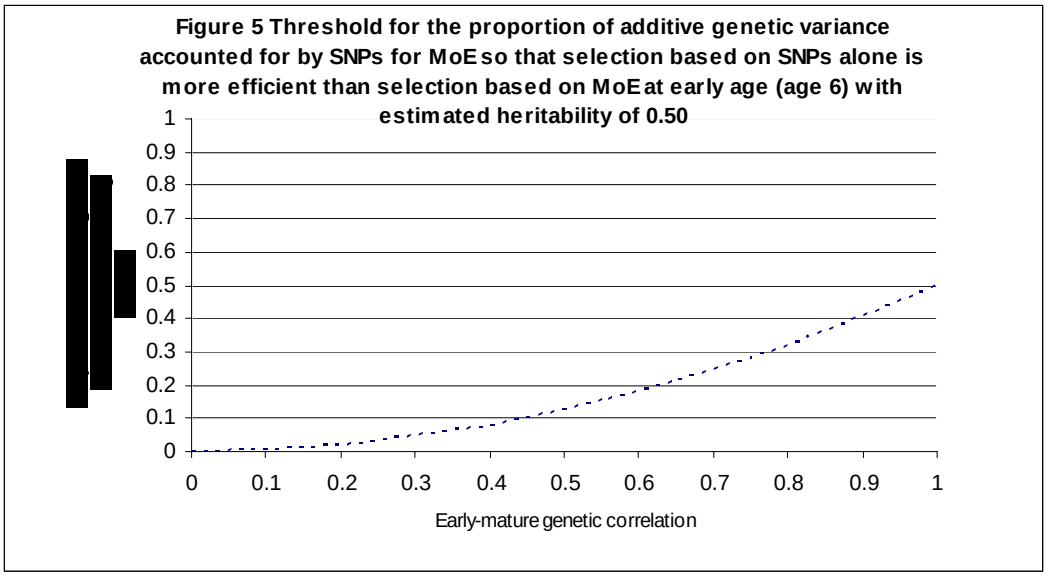
SNP ID	Freq of A_1 (p)	Freq of A_2 (q)	a (p-value)	d (p-value)	$V_{A\text{-}SNP}$	$V_{D\text{-}SNP}$	V_A	V_E	V_{P^*}	$\frac{V_{A\text{-}SNP}}{V_{P^*}} \times 100\%$
307	0.637	0.363	-0.695 (0.007)	0.001 (0.998)	0.224	0.000	9.850	5.643	15.717	1.42%
308	0.657	0.343	-0.566 (0.030)	0.259 (0.500)	0.189	0.014	7.011	6.563	13.777	1.37%
313	0.458	0.542	-0.632 (0.008)	0.380 (0.250)	0.179	0.036	9.152	5.463	14.830	1.21%
318	0.549	0.451	-0.690 (0.007)	0.463 (0.181)	0.268	0.053	10.107	4.086	14.514	1.85%
331	0.564	0.436	-0.578 (0.031)	-0.224 (0.563)	0.149	0.012	9.422	6.083	15.665	0.95%
335	0.747	0.253	-1.855 (0.002)	-1.094 (0.077)	0.652	0.171	7.333	6.513	14.668	4.44%
342	0.307	0.693	-1.017 (0.001)	0.618 (0.117)	0.258	0.069	8.705	5.942	14.974	1.72%
358	0.876	0.124	-3.753 (0.047)	-5.423 (0.005)	0.022	1.397	7.649	6.532	15.600	0.14%
377	0.338	0.662	-0.743 (0.004)	0.024 (0.949)	0.242	0.000	8.575	5.851	14.668	1.65%
382	0.100	0.900	-1.944 (0.036)	1.343 (0.186)	0.137	0.059	9.510	5.238	14.943	0.92%
383	0.699	0.301	-0.614 (0.050)	-0.190 (0.634)	0.122	0.006	9.383	5.386	14.896	0.82%

Table 4. SNPs with significant additive effects ($p \leq 0.05$) for pith-to-bark core length from single-SNP analyses

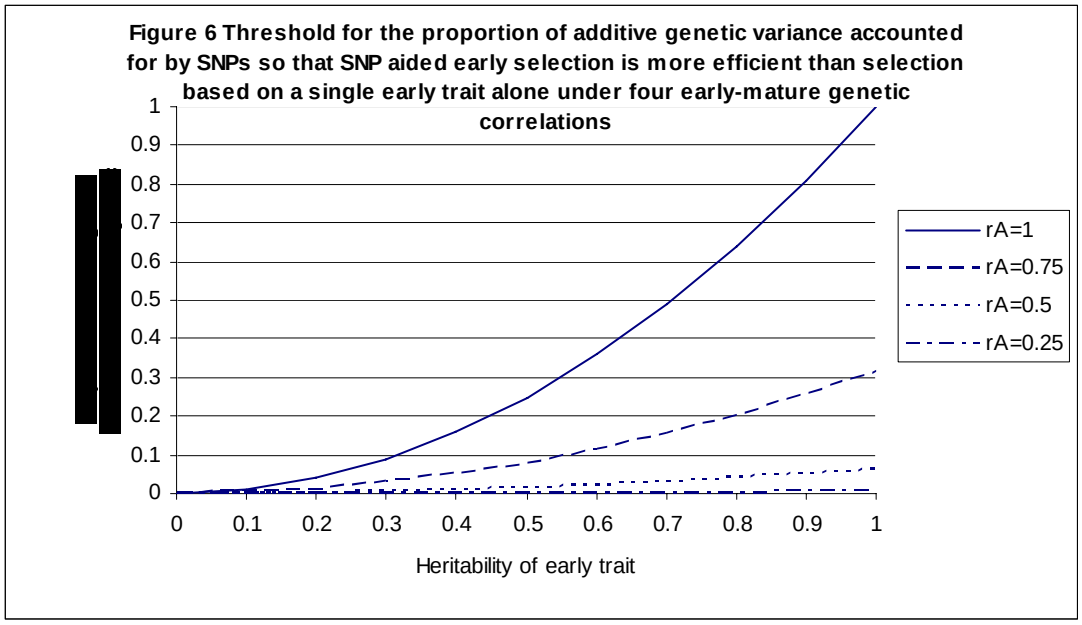
SNP ID	Freq of A_1 (p)	Freq of A_2 (q)	a (p-value)	d (p-value)	$V_{A\text{-}SNP}$	$V_{D\text{-}SNP}$	V_A	V_E	V_{P^*}	$\frac{V_{A\text{-}SNP}}{V_{P^*}} \times 100\%$
52	0.389	0.611	1.219 (0.047)	-0.147 (0.882)	0.669	0.005	36.318	64.677	101.669	0.66%
77	0.595	0.405	1.979 (0.013)	0.465 (0.674)	1.721	0.050	48.798	53.998	104.568	1.65%
101	0.064	0.936	9.209 (0.011)	-9.205 (0.017)	0.166	1.212	35.156	64.693	101.227	0.16%
126	0.500	0.500	1.282 (0.049)	0.388 (0.674)	0.821	0.038	36.326	64.549	101.734	0.81%
207	0.073	0.927	5.936 (0.035)	-4.626 (0.130)	0.539	0.397	39.404	61.467	101.806	0.53%
224	0.656	0.344	1.573 (0.023)	1.686 (0.123)	0.494	0.578	42.764	57.406	101.242	0.49%
265	0.164	0.836	3.088 (0.024)	-4.375 (0.008)	0.006	1.445	32.094	66.494	100.039	0.01%
280	0.295	0.705	1.680 (0.024)	0.023 (0.986)	1.187	0.000	30.701	67.418	99.306	1.20%
283	0.401	0.599	1.655 (0.033)	-0.885 (0.381)	1.052	0.181	28.960	68.467	98.659	1.07%
307	0.363	0.637	1.959 (0.004)	-2.777 (0.009)	0.663	1.649	38.951	59.928	101.191	0.66%
308	0.343	0.657	1.913 (0.008)	-1.668 (0.114)	0.868	0.565	36.084	60.953	98.469	0.88%
328	0.407	0.593	1.402 (0.041)	-1.130 (0.236)	0.685	0.297	37.381	62.747	101.110	0.68%

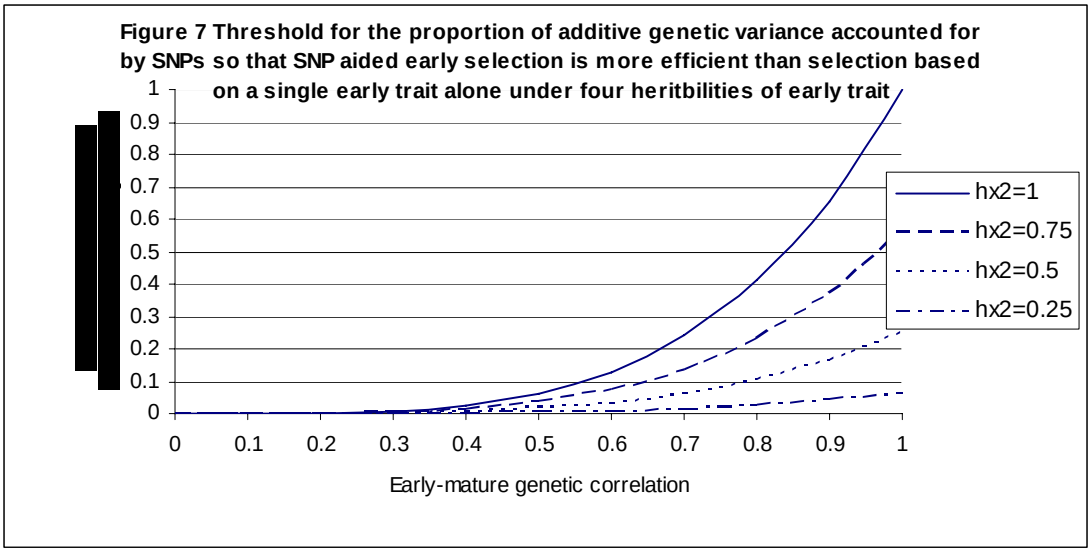
Efficiency of a single SNP aided selection for stiffness

The required proportion of genetic variances accounted for by SNPs for a same efficiency as selection directly on early age (age 6) for rotation-aged MoE was presented in Figure 5. The estimated heritability for MoE was 0.5 at early age (Wu et al. 2007) and genetic correlation between an early age (age 6) and rotation age (age 30) was 0.8 (Wu et al 2008), only SNPs that accounted for 32% of genetic variance for rotation-aged MoE can have same efficiency as early selection using MoE of young trees for rotation-aged MoE (stiffness). We observed that selection based on individual SNPs will not have same efficiency as selection based on early MoE measurement since individual effect of SNP for MoE was equal or below 8.23% (Table 2). If effects of all 29 SNPs for MoE in Table 2 were real without estimation error and were independent for genetic variation (no collineality), then selection based on the cumulated genetic variation of the 29 SNPs (44.5%) would be more efficient than selection based on MoE of young trees alone.



The more preferred way to use SNP markers is to incorporate SNP markers for mature traits into an early selection index. The relationships between gain and genetic parameters were illustrated in Figures 6 and 7 for required proportion of genetic variance accounted for by SNPs in order for SNP aided early selection to be more efficient than selection based on a single early trait alone under four early-mature genetic correlations (r_A=0.25, 0.5, 0.75, 1) and four early trait heritabilities (h_x^2=0.25. 0.5. 0.75, 1) (Wu 2002). For our early selection for stiffness (MoE) at rotation, if SNPs could account for 10.24% or more of genetic variation for stiffness at rotation age, then SNP aided early selection could be more efficient than selection based on MoE at early age. Therefore, for more efficient SNP aided early selection for stiffness, SNPs that accounts for more than 10.24% of independent genetics effects for rotation aged MoE are preferred.





CONCLUSIONS

Several studies reporting association of SNPs with quantitative traits have been published, but caution and careful consideration of statistical methods and validation controls is warranted. In this report, methodology was presented for estimating the additive and dominance effects of single nucleotide polymorphisms when included in an individual tree (animal) model. Single-SNP analyses resulted in identifying several SNPs that had significant additive effects for juvenile density, modulus of elasticity, microfibril angle, and pith-to-bark core length. Significant SNPs only accounted for little of the total phenotypic variation (generally, < 2% with a few SNPs accounting for more of the variation).

The significant SNPs that were identified in the current study need to be validated in a larger independent population, preferable with different families.

The genetic effects of individual SNPs were assumed to be independent and more analyses need to be conducted to test for multi-collinearity. In addition, none of the significant associations identified from single-SNP analyses held up when corrected for multiple testing, and therefore results should be viewed as preliminary. We plan on doing several simulation studies, both parametric and locus-based, in order to develop and document the methodology and strategy for incorporating significant SNPs in a breeding strategy.

Selection based on SNP alone was less efficient than direct or indirect selection for stiffness of radiata pine using existing SNPs observed in this project. SNPs accounting for 32% of genetic variance for rotation-aged MoE are required to have the same efficiency as early selection of MoE for rotation-aged MoE (stiffness). For an efficient SNP aided early selection for stiffness of rotation age, individual SNPs or SNP clusters that accounts for more than 10.24% of genetic effects for rotation aged MoE should be explored using more candidate genes.

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APPENDIX 11

Genetic correlations among juvenile wood quality and growth traits and implications for selection strategy in *Pinus radiata* D. Don

Washington J. GAPARE[*1], Brian S. BALTUNIS[1], Miloš IVKOVIĆ[1]& Harry X. WU

CSIRO Plant Industry, GPO Box 1600, Canberra, ACT 2601, Australia

Telephone: +61 2 6281 8327

Fax: +61 2 6281 8312

*Corresponding author: Washington.Gapare@csiro.au

[1]Authors contributed equally

Abstract

Juvenile wood quality in *Pinus radiata* is affected by factors such as low density, stiffness, and high microfibril angle, spiral grain, and shrinkage. Adverse genetic correlations between growth and wood quality traits remain as one of the main constraints in radiata pine advanced generation selection breeding program. Juvenile wood property data for this study were available from two progeny tests aged 7 and 6 years. Negative genetic correlations were found for modulus of elasticity (MoE) and density with microfibril angle, spiral grain, shrinkage, and DBH. We observed low to moderate unfavourable genetic correlations between all wood quality traits and DBH growth. These low to moderate genetic correlations suggest that there may be some genotypes which have high DBH growth performance while also having high wood stiffness and density, and that the adverse correlation between DBH and MoE may not entirely prohibit the improvement of both traits. Results indicate that, in the short term, the optimal strategy is index selection using economic weights for breeding objective traits (MAI and stiffness) in radiata pine. In the long-term, simultaneously purging of the adverse genetic correlation and optimizing index selection may be the best selection strategy in multiple-trait selection breeding programs with adverse genetic correlations.

Keywords: radiata pine, wood quality, genetic correlation, index selection, selection strategy

1. INTRODUCTION

Australia has an advanced breeding program for *Pinus radiata* D. Don which has over the last 50 years significantly improved many characteristics of this widely planted fast growing conifer (Matheson et al., 1986; Cotterill and Dean, 1990; Wu et al., 2008). Early tree improvement programs for *P. radiata* in Australia have concentrated most of their effort on improving growth and form traits, and realized gains in volume after the first generation of breeding were about 30% over unimproved seedlots (Matheson et al., 1986; Wright and Eldridge, 1985; Wu and Matheson, 2002). Considerable genetic variation and high heritabilities in both juvenile wood (also called corewood), (Burdon et al., 2004) and mature wood quality traits of *P. radiata* have been reported in Australia. For example, density, stiffness, microfibril angle, spiral grain, shrinkage and juvenile-mature wood transition have been reported to be under moderate to high genetic control (Li and Wu, 2005; Dungey et al., 2006; Baltunis et al., 2007; Gapare et al., 2006; 2007; 2008). Similarly, high heritabilities for wood quality traits were observed in New Zealand *P. radiata* populations (Jayawickrama, 2001; Kumar, 2004; Kumar et al., 2002).

As radiata pine breeding advances to third generation selections in Australia, there is an increasing need to include wood quality traits in the breeding program. This is due to the increased proportion of juvenile wood in harvested logs which is causing a variety of problems for wood utilization owing to lower stiffness (modulus of elasticity (MoE)), or strength (modulus of rupture (MOR)) (Cown, 1992; Cown and van Wyk, 2004). Besides lower wood stiffness and strength, dimensional instability is also a problem in juvenile wood, in which microfibril angle (MfA) is greater and spiral grain more pronounced, and a significant amount of compression wood is present. The low-stiffness wood zone becomes a strategic research topic for improving radiata pine wood quality in order to achieve shorter rotations with high stiffness wood.

Selection for a single trait (such as growth) not only changes the genetic variance of the trait directly selected, but also changes the genetic variances

of correlated traits and covariances between correlated traits (Bulmer, 1971). It is therefore important to study the genetic correlations of wood quality traits with traits included in the radiata pine breeding program such as growth and stem form. As an initial step in incorporating these wood quality traits into the breeding program, we have studied the genetic control in the juvenile core of radiata pine for stiffness, density, microfibril angle, (Baltunis et al., 2007), spiral grain (Gapare et al., 2007), shrinkage (Gapare et al., 2008), and dynamic MoE derived from acoustic time-of-flight measurements (Matheson et al., 2008). As might be expected for most wood quality traits (Zobel and van Buijtenen, 1989), there was evidence of significant genetic control in these traits. For example, heritability for density, MfA and MoE measured using SilviScan® (Evans and Ilic, 2001) and MoE measured acoustically were moderately high (0.70, 0.50, 0.54, and 0.53, respectively) (Baltunis et al., 2007; Matheson et al., 2008). Spiral grain and longitudinal shrinkage were moderately heritable (0.45 and 0.20, respectively) (Gapare et al., 2007; 2008).

Since most of these wood quality traits are related, a change in any of these traits is likely to have either a favourable or unfavourable effect on other traits. For example, MfA is one of the major factors that controls MoE (a major breeding objective for radiata pine breeding in Australia, see Ivković et al., 2006a) and is also a predictor of tendency to warp (Myszewski et al., 2004). A reduction in MfA and increase in MoE in the first growth rings should improve the structural and shrinkage properties of wood because lower MfA and spiral grain in the first growth rings will limit volumetric shrinkage and therefore, the drying distortion of sawn timber (e.g., Lindström et al., 2005). Ivković et al. (2008) used path analysis to examine how much component wood quality traits such as density, MfA, spiral grain and ring width could account for wood stiffness, strength and shrinkage. Their major finding was that the preferred method for predicting juvenile tree MoE was using standing tree acoustic MoE and whole core density. For the purpose of selection and breeding, it is generally desirable to include only a few traits in a selection index (e.g., Cotterill and Dean, 1990).

Genetic correlations estimate the degree of relationship between two traits owing to genetic causes. There are two biological explanations for such correlations. One is pleiotropy, where the two traits are affected by the same set of genes. Another mechanism for genetic correlation, although transient, is gametic phase linkage disequilibrium (LD), which may occur when individuals from two populations with different gene frequencies intermate, as a side effect of recent directional selection or by biased or limited sampling (e.g., Hannrup et al., 2000; Sánchez et al., 2008). In breeding programs, genetic correlations are used for predicting how selection for one or several traits will affect correlated traits in the next generation. The genetic correlations between growth rate and wood quality traits have major implications for developing selection and breeding strategies.

The specific objectives of the present study were two-fold: (i) to estimate the genetic correlations between stiffness, density, microfibril angle, spiral grain, shrinkage in the juvenile core and DBH growth in radiata pine, and (ii) to evaluate various selection scenarios to deal with multiple objective traits, particularly where adverse genetic correlations between stiffness and growth traits in radiata pine exist.

2. MATERIALS AND METHODS

2.1. Data source

The study was based on two progeny trials: BR9611, located at Flynn (latitude 38^{o} 14'S; longitude 146^{o} 45'E), Victoria and managed by Hancock Victorian Plantations, and BR9705, located at Kromelite (latitude 37^{o} 50'S; longitude 140^{o} 55'E), South Australia and managed by Green Triangle Forest Products. Both sites were initially prepared by ploughing followed by mounding, and soil drainage was considered good. Site details are presented in Table 1. There was a fertilizer application (NPK) at Flynn at a rate of 347 kg/ha in 2000, followed by another aerial fertiliser application in 2003 at a rate of 329 kg/ha. Trial maintenance at Kromelite included herbicide application in the first two

years of growth aimed at complete weed control. Unlike at Flynn, there was no fertiliser application.

Flynn was planted in June, 1996 with 250 families, consisting of 88 polycross families, 157 full-sib families, and 5 controls, planted in a 10x25 row-column experimental design (see Williams et al., 2002) with 5 blocks and four-tree row plots. Kromelite was planted in July, 1997 with 110 families, consisting of 70 polycross families, 40 full-sib families with no controls, planted in a 10x11 row-column design with 5 blocks and four-tree row plots. These trials contained a total of 344 different families from both full-sib and half-sib families from polymix crosses derived from Southern Tree Breeding Association (STBA) breeding population. There were 41 parents and only 16 full-sib families common to both sites. Stem discs and increment cores were collected from the two trials and juvenile wood traits at ages 6 and 7 years were measured including DBH. The total numbers of trees sampled per family were different for each site and so were the number of trees per trait. Generally, wood quality traits (e.g., stiffness, density, MfA) with higher levels of additive genetic control do not require large sample sizes to detect significant genetic variation. In addition, sample preparation and measurements for such traits are time consuming and expensive.

SilviScan predicted modulus of elasticity (MoE_{SS}), density (DEN) and microfibril angle (MfA)

For the assessment of SilviScan predicted modulus of elasticity (MoE_{SS}), density (DEN) and microfibril angle (MFA), twelve millimetre bark-to-bark increment cores were collected at breast height (1.3 m). 980 trees were sampled at Flynn and 660 trees were sampled at Kromelite and assessed by SilviScan® (Evans and Ilic, 2001).

Dynamic modulus of elasticity (MoE_{IML})

Acoustic measurements were recorded from standing trees at both sites. In total, measurements were available from 2454 trees at Flynn and 1284 trees

at Kromelite. The IML Electronic Hammer (Instrumenta Mechanik Labor GmbH) was used to measure the time of flight. The standing tree time-of-flight technique provides an acoustic wave velocity for the stem. Dynamic modulus of elasticity (MoE_{IML}) was estimated using velocity and green density values derived from DEN (Rolf Booker, unpublished data). When MoE is measured in this way it is known as dynamic MoE in contrast to static MoE which is measured by bending. The dynamic and static measured MoE values are highly related in green and dry wood (Booker and Sorensson, 1999; Ilic, 2001).

Spiral grain

For the assessment of spiral grain, samples were collected from 628 trees at Flynn and 316 trees at Kromelite. Spiral grain angle was measured using a pivoting digital protractor. Spiral grain angle, in degrees ±0.1°, was measured on the tangential surface of the latewood of each ring segment, as the deviation of grain angle from perpendicular to the plane of reference. The mean grain angle in each ring can be considered a measure of average grain angle deviation from the vertical axis of the cambial cylinder in each year of growth (e.g., Hansen and Roulund 1998).

Shrinkage

For shrinkage measurements, data were available from 466 trees at Flynn and 308 trees at Kromelite. The procedures for determining shrinkages for the samples were similar to those used by Kingston and Risdon (1961). Radial, tangential and longitudinal dimensions were measured using a digital displacement gauge with readings graduated to 0.001 mm. One measurement was taken in the middle of the sample and subsequently adjusted for distortion and or bow.

2.2. Data analysis

A multivariate pooled-site analysis was used in this study to estimate variance components in order to estimate genetic correlations among the wood quality traits and growth. A multivariate mixed model REML analysis using the program ASREML (Gilmour et al., 2005) was used for the multivariate pooled-site analysis:

$$\mathbf{y}_j = \mathbf{X}_j\mathbf{b}_j + \mathbf{Z}_{a_j}\mathbf{a}_j + \mathbf{Z}_{f_j}\mathbf{f}_j + \mathbf{Z}_{p_j}\mathbf{p}_j + \varepsilon_j \qquad [1]$$

where $\mathbf{y}_j$ is the vector of individual tree observations denoted (*j*) by trait, $\mathbf{b}_j$ is the vector of fixed effects (trait mean, tests and blocks within tests) and $\mathbf{X}_j$ is the known incidence matrix relating the individual tree observations in $\mathbf{y}_j$ to the fixed effects in $\mathbf{b}_j$ where

$$\mathbf{X}_j\mathbf{b}_j = \begin{bmatrix} \mathbf{X}_{MOE} & \mathbf{0} & \mathbf{0} & \mathbf{0} & \mathbf{0} & \mathbf{0} & \mathbf{0} \\ \mathbf{0} & \mathbf{X}_{IML} & \mathbf{0} & \mathbf{0} & \mathbf{0} & \mathbf{0} & \mathbf{0} \\ \mathbf{0} & \mathbf{0} & \mathbf{X}_{DEN} & \mathbf{0} & \mathbf{0} & \mathbf{0} & \mathbf{0} \\ \mathbf{0} & \mathbf{0} & \mathbf{0} & \mathbf{X}_{MFA} & \mathbf{0} & \mathbf{0} & \mathbf{0} \\ \mathbf{0} & \mathbf{0} & \mathbf{0} & \mathbf{0} & \mathbf{X}_{SG} & \mathbf{0} & \mathbf{0} \\ \mathbf{0} & \mathbf{0} & \mathbf{0} & \mathbf{0} & \mathbf{0} & \mathbf{X}_{LSH} & \mathbf{0} \\ \mathbf{0} & \mathbf{0} & \mathbf{0} & \mathbf{0} & \mathbf{0} & \mathbf{0} & \mathbf{X}_{DBH} \end{bmatrix} \begin{bmatrix} \mathbf{b}_{MOE} \\ \mathbf{b}_{IML} \\ \mathbf{b}_{DEN} \\ \mathbf{b}_{MFA} \\ \mathbf{b}_{SG} \\ \mathbf{b}_{LSH} \\ \mathbf{b}_{DBH} \end{bmatrix},$$

$\mathbf{a}_j$ is a vector of random genetic effects of individual genotypes ~MVN$(\mathbf{0}, \mathbf{G} \otimes \mathbf{A})$ where

$$\mathbf{G} = \begin{bmatrix} \sigma^2_{A_{MOE}} & \sigma_{A_{MOE}A_{IML}} & \sigma_{A_{MOE}A_{DEN}} & \sigma_{A_{MOE}A_{MFA}} & \sigma_{A_{MOE}A_{SG}} & \sigma_{A_{MOE}A_{LSH}} & \sigma_{A_{MOE}A_{DBH}} \\ \sigma_{A_{MOE}A_{IML}} & \sigma^2_{A_{IML}} & \sigma_{A_{IML}A_{DEN}} & \sigma_{A_{IML}A_{MFA}} & \sigma_{A_{IML}A_{SG}} & \sigma_{A_{IML}A_{LSH}} & \sigma_{A_{IML}A_{DBH}} \\ \sigma_{A_{MOE}A_{DEN}} & \sigma_{A_{IML}A_{DEN}} & \sigma^2_{A_{DEN}} & \sigma_{A_{DEN}A_{MFA}} & \sigma_{A_{DEN}A_{SG}} & \sigma_{A_{DEN}A_{LSH}} & \sigma_{A_{DEN}A_{DBH}} \\ \sigma_{A_{MOE}A_{IMFA}} & \sigma_{A_{IML}A_{MFA}} & \sigma_{A_{IML}A_{MFA}} & \sigma^2_{A_{MFA}} & \sigma_{A_{MFA}A_{SG}} & \sigma_{A_{MFA}A_{LSH}} & \sigma_{A_{MFA}A_{DBH}} \\ \sigma_{A_{MOE}A_{SG}} & \sigma_{A_{SG}A_{MFA}} & \sigma_{A_{SG}A_{DEN}} & \sigma_{A_{SG}A_{MFA}} & \sigma^2_{A_{SG}} & \sigma_{A_{SG}A_{LSH}} & \sigma_{A_{SG}A_{DBH}} \\ \sigma_{A_{MOE}A_{LSH}} & \sigma_{A_{MOE}A_{IML}} & \sigma_{A_{MOE}A_{DEN}} & \sigma_{A_{MOE}A_{MFA}} & \sigma_{A_{SG}A_{LSH}} & \sigma^2_{A_{LSH}} & \sigma_{A_{LSH}A_{DBH}} \\ \sigma_{A_{MOE}A_{DBH}} & \sigma_{A_{IML}A_{DBH}} & \sigma_{A_{DEN}A_{DBH}} & \sigma_{A_{IMFA}A_{DBH}} & \sigma_{A_{SG}A_{DBH}} & \sigma_{A_{LSH}A_{DBH}} & \sigma^2_{A_{DBH}} \end{bmatrix}$$

and **A** = the additive relationship matrix, $\mathbf{Z}_{a_j}$ is the known incidence matrix relating observations in $\mathbf{y}_j$ to the genetic effects in $\mathbf{a}_j$, $\sigma^2_{A_j}$ is the estimated additive genetic variance, $\sigma_{A_x A_y}$ is the estimated genetic covariance between additive effects of the two traits,

$\mathbf{f}_j$ is a vector of random effects of full-sib families ~MVN$(\mathbf{0}, \mathbf{S} \otimes \mathbf{I}_s)$ where

S =
$$\begin{bmatrix}
\sigma^2_{f_{MOE}} & \sigma_{f_{MOE}f_{IML}} & \sigma_{f_{MOE}f_{DEN}} & \sigma_{f_{MOE}f_{MFA}} & \sigma_{f_{MOE}f_{SG}} & \sigma_{f_{MOE}f_{LSH}} & \sigma_{f_{MOE}f_{DBH}} \\
\sigma_{f_{MOE}f_{IML}} & \sigma^2_{f_{IML}} & \sigma_{f_{IML}f_{DEN}} & \sigma_{f_{IML}f_{MFA}} & \sigma_{f_{IML}f_{SG}} & \sigma_{f_{IML}f_{LSH}} & \sigma_{f_{IML}f_{DBH}} \\
\sigma_{f_{MOE}f_{DEN}} & \sigma_{f_{IML}f_{DEN}} & \sigma^2_{f_{DEN}} & \sigma_{f_{DEN}f_{MFA}} & \sigma_{f_{DEN}f_{SG}} & \sigma_{f_{DEN}f_{LSH}} & \sigma_{f_{DEN}f_{DBH}} \\
\sigma_{f_{MOE}f_{MFA}} & \sigma_{f_{IML}f_{MFA}} & \sigma_{f_{IML}f_{MFA}} & \sigma^2_{f_{MFA}} & \sigma_{f_{MFA}f_{SG}} & \sigma_{f_{MFA}f_{LSH}} & \sigma_{f_{MFA}f_{DBH}} \\
\sigma_{f_{MOE}f_{SG}} & \sigma_{f_{SG}f_{MFA}} & \sigma_{E_{SG}E_{DEN}} & \sigma_{f_{SG}f_{MFA}} & \sigma^2_{f_{SG}} & \sigma_{f_{SG}f_{LSH}} & \sigma_{f_{SG}f_{DBH}} \\
\sigma_{f_{MOE}f_{LSH}} & \sigma_{f_{MOE}f_{IML}} & \sigma_{f_{MOE}f_{DEN}} & \sigma_{f_{MOE}f_{MFA}} & \sigma_{f_{SG}f_{LSH}} & \sigma^2_{f_{LSH}} & \sigma_{f_{LSH}f_{DBH}} \\
\sigma_{f_{MOE}f_{DBH}} & \sigma_{f_{IML}f_{DBH}} & \sigma_{f_{DEN}f_{DBH}} & \sigma_{f_{IMFA}f_{DBH}} & \sigma_{f_{SG}f_{DBH}} & \sigma_{f_{LSH}f_{DBH}} & \sigma^2_{f_{DBH}}
\end{bmatrix}$$

and $\mathbf{I}_s$ is an identity matrix equal to the number of full-sib families, $\mathbf{Z}_{f_j}$ is the incidence matrix relating the observations in $\mathbf{y}_j$ to the effects in $\mathbf{f}_j$, $\sigma^2_{f_j}$ is the estimated variance attributed to full-sib families (specific combining ability), and $\sigma_{f_x f_y}$ is the estimated covariance between full-sib family effects of two traits,

$\mathbf{p}_j$ is a vector of random effects of plot within block and test ~MVN$(\mathbf{0}, \mathbf{I}_{p_j}\sigma^2_{p_j})$ where $\mathbf{I}_{p_j}$ is an identity matrix equal to the number of plots, $\sigma^2_{p_j}$ is the estimated variance associated with plots within block and test,

ε_j is a random vector of residual terms ~MVN (0, R $\otimes$ I) where

$$\mathbf{R} = \begin{bmatrix} \sigma^2_{E_{MOE}} & \sigma_{E_{MOE}E_{IML}} & \sigma_{E_{MOE}E_{DEN}} & \sigma_{E_{MOE}E_{MFA}} & \sigma_{E_{MOE}E_{SG}} & \sigma_{E_{MOE}E_{LSH}} & \sigma_{E_{MOE}E_{DBH}} \\ \sigma_{E_{MOE}E_{IML}} & \sigma^2_{E_{IML}} & \sigma_{E_{IML}E_{DEN}} & \sigma_{E_{IML}E_{MFA}} & \sigma_{E_{IML}E_{SG}} & \sigma_{E_{IML}E_{LSH}} & \sigma_{E_{IML}E_{DBH}} \\ \sigma_{E_{MOE}E_{DEN}} & \sigma_{E_{IML}E_{DEN}} & \sigma^2_{E_{DEN}} & \sigma_{E_{DEN}E_{MFA}} & \sigma_{E_{DEN}E_{SG}} & \sigma_{E_{DEN}E_{LSH}} & \sigma_{E_{DEN}E_{DBH}} \\ \sigma_{E_{MOE}E_{MFA}} & \sigma_{E_{IML}E_{MFA}} & \sigma_{E_{IML}E_{MFA}} & \sigma^2_{E_{MFA}} & \sigma_{E_{MFA}E_{SG}} & \sigma_{E_{MFA}E_{LSH}} & \sigma_{E_{MFA}E_{DBH}} \\ \sigma_{E_{MOE}E_{SG}} & \sigma_{E_{SG}E_{MFA}} & \sigma_{E_{SG}E_{DEN}} & \sigma_{E_{SG}E_{MFA}} & \sigma^2_{E_{SG}} & \sigma_{E_{SG}E_{LSH}} & \sigma_{E_{SG}E_{DBH}} \\ \sigma_{E_{MOE}E_{LSH}} & \sigma_{E_{MOE}E_{IML}} & \sigma_{E_{MOE}E_{DEN}} & \sigma_{E_{MOE}E_{MFA}} & \sigma_{E_{SG}E_{LSH}} & \sigma^2_{E_{LSH}} & \sigma_{E_{LSH}E_{DBH}} \\ \sigma_{E_{MOE}E_{DBH}} & \sigma_{E_{IML}E_{DBH}} & \sigma_{E_{DEN}E_{DBH}} & \sigma_{E_{IMFA}E_{DBH}} & \sigma_{E_{SG}E_{DBH}} & \sigma_{E_{LSH}E_{DBH}} & \sigma^2_{E_{DBH}} \end{bmatrix},$$

I is the identity matrix with order equal to the number of observations, 0 is the null matrix, and $\sigma^2_{E_j}$ is the estimated residual variance for each trait and similarly, $\sigma_{E_X E_Y}$ is the estimated residual covariance between two traits. Both residual and genetic variances were assumed homogenous across sites (i.e. a single estimate of additive variance and residual variance for each trait).

Variances are not independent of the scale and the mean of the respective traits (Sokal and Rohlf, 1995). Therefore, to compare the genetic variances of the different traits, a parameter measuring the genetic coefficient of variation was calculated as:

$$\mathrm{CV}_{A_j} = \frac{100\% \times \sigma_{A_j}}{\bar{x}} \qquad [2]$$

CV_{A_j} = coefficient of additive genetic variation

σ_{A_j} = square root of the additive genetic variance for the trait

$\bar{x}$ = population mean for the trait

The CV_{A_j} expresses the genetic variance relative to the mean of the trait of interest and gives a standardized measure of the genetic variance relative to the mean of the trait. The higher the coefficient of additive genetic variation for a trait, the higher is its relative variation.

The genetic correlation r_G between two traits was estimated within the ASREML software as:

$$r_G = \frac{\sigma_{A_x A_y}}{\sqrt{(\sigma^2_{A_x} \sigma^2_{A_y})}} \qquad [3]$$

where:

$\sigma_{A_x A_y}$ = additive genetic covariance component between traits *x* and *y*;

$\sigma^2_{A_x}$ = additive genetic variance component for trait *x*;

$\sigma^2_{A_y}$ = additive genetic variance component for trait *y*

Standard errors for each of the correlations were calculated using a truncated Taylor series in ASREML (Gilmour et al., 2005).

The optimal selection strategy was defined by the optimal breeding objective response in terms of profitability. Responses in breeding objective traits mean annual increment (MAI_{OBJ}) and stiffness (MoE_{OBJ}) at rotation age were evaluated through index selection based on two juvenile traits (MoE_{SS} and DBH). Economic weights for the breeding objective traits for an integrated company were estimated to be $977 per one GPa increase of rotation-aged stiffness and $416 per one m^3/year/ hectare of MAI at rotation age (Ivković et al, 2006a). Three different selection scenarios were considered:

A) Index selection using MoE_{SS} and DBH as selection traits and maximising profitability;

B) Restricted index selection keeping juvenile wood MoE_{SS} constant;

C) Restricted index selection where selection is restricted to genotypes with positive breeding values for both MoE_{SS} and DBH.

We also created another more general index using genetic parameters for radiata pine obtained from the literature review by Wu et al., (2008), and the following scenarios based in part on Kumar (2004) and Kumar et al. (2006):

D) Index selection using average variance-covariance parameters from literature (Wu et al. 2008) for selection and objective traits;

E) Economic weight on MoE_{OBJ} (MoE as the objective trait) was increased by 50%;

F) Heritability of selection traits DEN and MoE_{SS} was reduced 50%;

G) Genetic and phenotypic correlations between objective traits MAI_{OBJ} and MOE_{OBJ} and the selection traits DEN and MoE_{SS} were reduced by 50%;

H) Heritability of selection traits DEN and MoE_{SS} was reduced 50% and correlations of the objective traits (MAI_{OBJ} and MOE_{OBJ}) with the selection traits (DEN and MoE_{SS}) were reduced by 50%.

The index coefficients for all scenario - s were calculated according to Schneeberger et al., (1992) for selection traits:

$$\mathbf{b} = \mathbf{G}_{SS}^{-1}\mathbf{G}_{SO}\mathbf{w} \qquad [4]$$

where:

$\mathbf{b}$ is a vector of index weights for the predicted breeding values for the selection criteria in the index, $\mathbf{G}_{SS}^{-1}$ is the inverse of the genetic variance-covariance matrix of the selection criteria in the index (DBH and MoE_{SS}) from current study), assumed to be known without error, $\mathbf{G}_{SO}$ is the genetic covariance matrix between the selection criteria in the index and the breeding objective traits (using genetic parameters reported by Wu et al., 2008), and $\mathbf{w}$ is the vector of economic weights for the breeding objective traits (Ivković et al., 2006a). Restriction of response in MoE_{SS} while maximising index response was achieved using the “Generalized Reduced Gradient” nonlinear optimization (Fylstra et al., 1998) implemented in the Microsoft Excel Solver®.

3. RESULTS AND DISCUSSION

3.1. Trait means and genetic variation

The overall mean values and percent coefficient of additive genetic variation for wood quality and growth traits are presented in Table II. Mean values for MoE_{SS} and MoE_{IML} in the juvenile core of radiata pine were 6.56 GPa and 4.72 GPa, respectively. The difference between the two estimates may be due to the nature of the methods used for the measurements. MoE_{SS} is an estimate of clearwood MoE and is area-weighted, while MoE_{IML} is not necessarily measured over clearwood. Similar values were reported for area-weighted MoE_{ST} (rings 3-5) in radiata pine in New Zealand (Kumar et al., 2006). Mean density was 349 kg/m^3 and was within the average density reported for radiata pine in other studies (Dungey et al., 2006; Wu et al., 2006). Similarly, MfA values were similar to those reported by Dungey et al., (2006) and Wu et al., (2006) in other radiata pine studies and other species at same age, such as loblolly pine (Megraw et al., 1998; Myszewski et al., 2004). Mean values for spiral grain (SG), longitudinal shrinkage (LSH) and DBH were in the range expected for juvenile wood in radiata pine and other conifers. For example, Cown et al., (1991) reported mean spiral grain angle of 4.7^0 in the first 10 rings from the pith in radiata pine trees grown in New Zealand.

Moderate to high levels of heritability were reported for component wood quality traits in the juvenile core of radiata pine (Table III) (see Baltunis et al., 2007; Gapare et al., 2007; 2008; Matheson et al., 2008). This suggested that there is an opportunity to improve juvenile wood quality traits as an integral part of the radiata pine breeding program in Australia. The level of genetic control of a trait and its interrelationships with other economically important traits determine the feasibility of incorporating traits in the breeding program (e.g. Kumar, 2004; Wu et al., 2008).

Both MoE_{SS} and MoE_{IML} had more genetic variation than density; that is, they had almost 3 times the coefficient of additive genetic variation (CV_A) for

density (Table II). Kumar et al., (2002) also found higher CV_A for MoE than for density in radiata pine. Similar estimates of CV_A have been reported for MoE in Douglas-fir (Johnson et al., 2006). The greater genetic variation in MoE relative to density may be a consequence of MoE being a composite trait related not only to wood density, but also to other variables such as MfA, and perhaps knots in the case of MoE_{IML}. Higher genetic variation of MoE (14.4% and 17.3%) relative to density (4.4%) with a similar heritability may indicate (1) density only contributes partially to MoE as indicated in other studies (Cave and Walker, 1994; Walker and Butterfield, 1996), and (2) direct selection based on MoE would be more effective than selection based on wood density. As might be expected, CV_A for other traits matched expectation (Wu et al., 2008), i.e., more genetic variation in DBH growth compared to wood quality traits such as longitudinal shrinkage or spiral grain (Table II).

3.2. Genetic correlations and correlated response

Table III shows genetic correlations among the wood quality traits and DBH growth. The genetic correlations between MoE_{SS} and density, and MoE_{IML} and density were 0.47 ± 0.08 and 0.41 ± 0.08, respectively. Other work on radiata pine reported the genetic correlation between density and MoE ranging from 0.44 to 0.64 (Kumar, 2004; Baltunis et al., 2007; Wu et al. 2008). As expected, the genetic correlation between MoE_{SS} and MoE_{IML} was close to unity (0.96 ± 0.02), suggesting that the measurements could be interchangeable as selection traits. Dynamic MoE, measured using ultrasound devices has been proven to strongly correlate with static bending MoE of the same clearwood samples (e.g., Booker and Sorensson, 1999). Tools such as IML hammer are therefore useful to assess acoustic stiffness on standing trees. For breeding purposes, acoustic measurements of stiffness (MoE_{IML}) may be more effective than measurements of component traits such as density and MfA as shown in this and other studies (Kumar, 2004; Dungey et al., 2006; Kumar et al., 2006; Matheson et al., 2008). In addition, MoE was recommended as one of the major breeding objective traits

for radiata pine in Australia (Ivković et al., 2006a). It may be more economical to measure standing trees using acoustic tools and density derived from increment cores (e.g., Matheson et al., 2008) than assessment of MoE_{SS}.

The genetic correlation between MoE_{SS} and MfA was highly negative (-0.93 ± 0.02). A similar negative genetic correlation was observed between MoE_{IML} and MfA (-0.94 ± 0.02). Previous work on radiata pine by Lindstrom et al., (2005) and Dungey et al., (2006) reported such negative genetic correlations between MoE and MfA. A highly positive or negative genetic correlation implies that the same genes may be responsible for the two traits (pleiotropy) (e.g., Baltunis et al., 2007) and that selection for increased MoE would lead to reduced MfA in the juvenile core of radiata pine (e.g., Kumar et al., 2004; Dungey et al., 2006). This result is encouraging as it is relatively expensive to measure MfA because of the tedious nature of the methods available including time in measurement or sample preparation, and the indirect X-ray diffraction method, which requires a more expensive technology. Spiral grain and longitudinal shrinkage were all negatively correlated to $MoE_{SS,}$ $MoE_{IML,}$ and density (Table III). Again, this suggests that selection for increased MoE would lead to reduced spiral grain and longitudinal shrinkage. Consequently, a reduction in the pith-to-bark gradient for MfA and MoE would reduce shrinkage and drying distortion of timber (e.g., Lindström et al., 2005).

We observed adverse genetic correlations between all wood quality traits and DBH growth (Table III). The genetic correlations between $MoE_{SS,}$ MoE_{IML} DEN and DBH growth were -0.34 ± 0.12, -0.26, ± 0.13, and -0.55 ± 0.10, respectively. Notably, most of the correlations between wood quality traits and DBH were estimated with large standard errors, even though large sample sizes were used in this study (Table II). Genetic correlations are functions of the magnitude of the correlation, the heritabilities and sample size. In this case, where no strong genetic correlations were found and heritabilities were low (e.g., heritability for LSH was 0.13 ± 0.08), a much larger sample size would have been required to give more precise estimates

of genetic correlations for LSH and SG (e.g., Klein et al., 1973; Gapare et al., 2008).

Several other studies have reported adverse genetic correlations between wood density or stiffness and growth in radiata pine (Dean et al., 1983; Zobel and van Buijtenen 1989; Cotterill and Dean 1990; Burdon and Low 1992; Jayawickrama 2001; Kumar 2004; Li and Wu 2005; Baltunis et al., 2007). Wu et al., (2008) reviewed estimates of genetic parameters including genetic correlations between density and growth in radiata pine and reported an average estimate of genetic correlation of -0.48. Work on other conifers such as *Pinus teada*, *P. sylvetris* and *Picea abies* has consistently found negative genetic correlations (r_A ~ -0.4) between density and DBH (Lee, 1997; Costa E Silva et al., 1998; Rozenberg and Cahalan, 1998; Hannrup et al., 2000). These low to moderate genetic correlations reflect that there may be some genotypes with high DBH growth performance, high wood stiffness and density, and that the adverse correlation between DBH and MoE_{IML} may not entirely prohibit the improvement of both traits.

3.3. Selection strategy for coping with adverse genetic correlations

Index selection with optimal economic breeding objectives drives profitability for multiple-trait breeding programs (Ivković et al., 2006a). Selection index used in this study was based on juvenile selection traits (i.e., DBH and MoE_{SS}), and the measure of efficiency of index selection was profitability and not the genetic responses of individual traits. Therefore, genetic responses for individual traits could be favourable or unfavourable under such selection scenarios. Selection scenario A was the most optimal with profitability of Aus$2409/ha/yr (53.1% gain), based on juvenile genetic parameters from this study. However, there was a -3.7% and -5.3% decrease in juvenile wood DEN and MoE_{SS}, respectively (Table IV). The small reduction in MoE_{SS} is counterbalanced by the larger increase in DBH growth. The responses in other juvenile wood properties were also unfavourable while for growth rate the genetic response was positive (6.8%) (Table IV).

When MoE_{SS} was held constant by applying the restricted selection index (scenario B), there was a slight decrease in the genetic response in DBH compared to scenario A. Although this decrease was small, there were favourable responses in other wood properties. For example, MoE_{SS} increased relative to scenario A, whereas, MfA decreased (Table IV). By using index selection only within the genotypes with positive breeding values for both MoE_{SS} and DBH (scenario C), there was an 11% increase in genetic response in MoE_{SS} compared to scenario A (Table IV).

However, there was a reduction in the production system profitability for alternative scenarios (B and C) compared with index selection scenario A. For example, if the response in juvenile MoE_{SS} was restricted to 0 (no further increase from current levels), the index value expressed as per hectare net present value (NPV) profit of an integrated radiata pine production system decreased from Aus$2409 to Aus$2282 at 10% selection intensity (Table IV). Similarly, if the selections were made only from the genotypes with positive breeding values for both growth and MoE_{SS}, profitability decreased from Aus$2409 to Aus$1498. From purely biological responses, selection scenario B and C would be preferred since scenario C improves both MoE_{SS} and growth rate, while scenario B improves DBH and maintains MoE_{SS} at current levels. However, from the economic responses for enterprises, scenarios B and C were less advantageous than scenario A, even though in scenario A, juvenile wood quality traits showed unfavourable responses.

Under more general genetic parameters (scenarios D to H, Table V) estimated from literature review by Wu et al., (2008) similar results were obtained. For example, even if genetic and phenotypic correlations between objective traits (MAI_{OBJ} and MOE_{OBJ}) and the selection traits (DEN and MoE_{SS}) where reduced by 50% (scenario G, Table V) there was a negative response in both DEN and MoE_{SS} (-1.7% and -2.3%, respectively). Under scenarios E, F and H MoE_{SS} had a positive response while DEN decreased. Generally, the genetic response in MoE_{SS} was less negative than in DEN, which was likely the consequence of the higher genetic correlation between MoE_{SS} and MoE_{OBJ} than DEN and MoE_{OBJ} (r_g =0.7 vs. 0.5). When the

economic weight on MoE_{OBJ} was increased 50% (scenario E, Table V) the response in MoE_{SS} was positive (2.9%), but the response in DEN was still slightly negative (-1.6%).

Generally, the index selection was more responsive to growth traits (DBH) as opposed to wood quality traits (MoE_{SS} and DEN) because DBH had more genetic variation. For example, DBH had almost 3-times the coefficient of additive genetic variation (CV_A) than MoE_{SS} (Table II). Moreover, as growth rate increases and rotation length decreases, there will be a higher proportion of juvenile wood with lower DEN and MoE than in the previous generations. The economic impact of reducing the quality and increasing the proportion of juvenile wood was not considered in current estimates of economic weights for radiata pine production system in Australia (Ivković et al., 2006b). We envisage that the economic weight on MoE may have to be increased relative to volume production in the next generation of selection, especially in the juvenile wood.

In general, as the mean of wood quality traits decreases, the economic value of wood quality increases relative to the economic value of volume production (Cown and van Wyk, 2004). Therefore, re-evaluation of economic weights may be necessary as the mean growth rate and the proportion of juvenile wood increases, and the overall mean of wood density and stiffness decreases (Ivković et al., 2006b). A more detailed study is needed to quantify the relationship between the proportion of juvenile wood and its impact on the production system. In this way, it may be possible to avoid reaching a critical value at which a large proportion of boards are of unacceptable quality. This should be a concern for advanced generations of radiata pine (and other conifer) tree improvement programs.

In this paper, we only considered breeding strategies dealing with adverse genetic correlations for one generation. However, development of long-term breeding strategies require an understanding of both (1) how index selection affects genetic correlations, particularly for adverse ones such as between growth rate and juvenile wood quality traits, and (2) how improvement of

biological traits affects the economic weights from one generation to the next. Selection of genotypes with positive breeding values for both traits (MoE and DBH) is not optimal using economic breeding objectives. However, more research is needed into the genetic basis of the negative genetic correlation, such as identifying possible major pleiotropic genes or close linkage. However, while linkage can be rapidly broken up by recombination, pleiotropic gene action will remain a constraint to selection for much longer (e.g., Conner, 2002). Insights from molecular genetics and association studies may enable breeders to purge the negative genetic correlation through repeated selections (King and Hansen, 1997; Sánchez et al., 2008). Simultaneously purging of the adverse genetic correlation and optimizing index selection would be the best selection strategy in multiple-trait selection breeding programs with adverse genetic correlations.

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548 Table I.

549 Site details of *Pinus radiata* progeny tests sampled for wood quality traits
550 study.

551

Details	Flynn	Kromelite
Test number	BR9611	BR9705
Date planted	6/1996	7/1997
Cambial age at time of sampling	7	6
Spacing	3.6m x 2.5m	2.74m x 2.5m
Latitude	38^{o} 14'S	37^{o} 50'S
Longitude	146^{o} 45'E	140^{o} 55'E
Elevation (m)	166	55
Annual rainfall (mm)	760	900
Soil type	Sandy loam	Sandy clay-loam
Site type	2^{nd} pine rotation	2^{nd} pine rotation
Number of families	250	110
Number of blocks	4	3

552

553 Table II.

554 Mean and percent coefficient of additive genetic variation (CV_A) of various
555 wood quality traits of *Pinus radiata*.

556

Trait	N	Mean	CV_A (%)
MoE_{SS} (GPa)	1602	6.56	17.3
MoE_{IML} (GPa)	2548	4.72	14.4
DEN (kg/m^3)	2771	349.2	4.8
MfA (degrees)	1602	29.6	8.8
SG (degrees)	932	4.16	21.9
LSH (%)	757	1.33	21.0
DBH (cm)	6083	15.9	53.2

557

Table III.

Estimates of genetic correlations among various wood quality traits in *Pinus radiata* at two test sites in Australia (heritability estimates along diagonal in italics)

	MoE_{SS}	MoE_{IML}	DEN	MfA	SG	LSH	DBH
MoE_{SS}	*0.55*	0.96	0.47	-0.92	-0.56	-0.36	-0.34
	(0.07)	(0.02)	(0.08)	(0.02)	(0.12)	(0.16)	(0.12)
MoE_{IML}		*0.48*	0.41	-0.94	-0.55	-0.36	-0.26
		(0.08)	(0.08)	(0.02)	(0.12)	(0.16)	(0.13)
DEN			*0.69*	-0.14	-0.33	-0.02	-0.55
			(0.06)	(0.09)	(0.13)	(0.16)	(0.10)
MfA				*0.53*	0.40	0.45	0.11
				(0.08)	(0.14)	(0.15)	(0.14)
SG					*0.42*	-0.01	0.37
					(0.09)	(0.22)	(0.16)
LSH						*0.24*	0.19
						(0.08)	(0.20)
DBH							*0.14*
							(0.05)

Note: Values in parentheses are approximate standard errors

565 Table IV.

566 Predicted genetic responses at 10% selection intensity (percentage in
567 parethenses) in juvenile growth and wood quality traits and net present value
568 profitability (breeding objective response) for index selection using genetic
569 parameters determined in this study. Three different scenarios were
570 considered: A) Index selection using modulus of elasticity (MoE_{SS}) and
571 diameter (DBH) as selection traits and maximising profitability; B) Restricted
572 index selection keeping juvenile wood MoE_{SS} constant; C) Restricted index
573 selection among the genotypes with positive breeding values for both MoE_{SS}
574 and DBH.

575

	Predicted Genetic Response						
Scenario	DEN (Kg/m^3)	DBH (mm)	MoE_{SS} (GPa)	MfA (deg)	SG (deg)	LSH (%)	Profitability (Aus$/ha/yr)
A)	-13.0 (-3.7%)	10.7 (6.8%)	-0.25 (-5.3%)	0.56 (1.9%)	0.37 (9.4%)	0.06 (4.4%)	2409 (53.1%)
B)	-10.37 (-2.9%)	10.44 (6.6%)	-0.04 (-0.5%)	-0.20 (-0.7%)	0.18 (4.5%)	0.05 (3.9%)	2282 (50.3%)
C)	-3.51 (-1.0%)	7.02 (4.5%)	0.29 (6.0%)	-1.29 (-4.4%)	-0.16 (-4.1%)	0.02 (1.8%)	1498 (33.0%)

576

Table V.

Predicted genetic responses at 10% selection intensity (percentage in parethenses) in juvenile growth and wood quality traits and net present value profitability (breeding objective response) for index selection based on the genetic parameters from the literature. Five different scenarios were considered: D) base scenario using variance-covariance parameters from (Wu et al. 2008) for selection and objective traits; E) Economic weight on MoE_{OBJ} (MoE as the objective trait) was increased by 50%; F) Heritability of selection traits DEN and MoE_{SS} was reduced 50%; G) Genetic and phenotypic correlations between objective traits MAI_{OBJ} and MOE_{OBJ} and the selection traits DEN and MoE_{SS} were reduced by 50%; H) Heritability of selection traits DEN and MoE_{SS} was reduced 50% and correlations of the objective traits (MAI_{OBJ} and MOE_{OBJ}) with the selection traits (DEN and MoE_{SS}) were reduced by 50%.

	Predicted Genetic Response						
Scenario	DEN (Kg/m^3)	DBH (mm)	MoE_{SS} (GPa)	MfA (deg)	SG (deg)	LSH (%)	Profitability (Aus$/ha/yr)
D)	-13.63 (-3.8%)	3.53 (2.2%)	-0.35 (-5.0%)	-0.45 (-1.5%)	0.04 (1.0%)	0.02 (1.2%)	1500 (33.0%)
E)	-5.58 (-1.6%)	2.18 (1.4%)	0.20 (2.9%)	-1.49 (-5.1%)	0.01 (2.6%)	-0.05 (-4.1%)	1387 (30.6%)
F)	-5.09 (-1.4%)	2.71 (1.7%)	0.10 (1.4%)	-1.16 (-4.0%)	0.00 (0.0%)	0.03 (2.2%)	1293 (28.5%)
G)	-6.07 (-1.7%)	3.28 (2.1%)	-0.07 (-1.0%)	-0.68 (-2.3%)	0.05 (1.2%)	-0.01 (-0.7%)	1197 (26.4%)
H)	-2.57 (-0.7%)	2.83 (1.8%)	0.13 (1.9%)	-1.02 (-3.5%)	0.04 (1.0%)	-0.01 (-1.0%)	1160 (25.6%)

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