GENETIC PARAMETERS AND GENOTYPE-BY-ENVIRONMENT INTERACTIONS FOR PULP YIELD PREDICTED USING NEAR INFRARED REFLECTANCE ANALYSIS AND PULP PRODUCTIVITY IN *EUCALYPTUS GLOBULUS*

C. A. Raymond[1,2], L. R. Schimleck[3], A. Muneri[2,4] & A. J. Michell[3]

[1)] CSIRO Forestry and Forest Products, GPO Box 252-12, Hobart TAS 7001 Australia
[2)] CRC for Sustainable Production Forestry, GPO Box 252-12, Hobart TAS 7001 Australia
[3)] CSIRO Forestry and Forest Products, Private Bag 10, Clayton South MDC, VIC 3169, Australia
[4)] Current Address: Queensland Forest Research Institute, P. O. Box 631, Indooroopilly, QLD 4068 Australia

Author for Correspondence : Carolyn Raymond, CSIRO Forestry and Forest Products , GPO Box 252-12 Hobart TAS 7001 Australia, Phone: 03 6226 7948, Fax: 03 6226 7901 Email: Carolyn.Raymond@ffp.csiro.au

Received September 1, 2000; accepted August 2, 2001

ABSTRACT

Near infrared reflectance analysis was used to predict kraft pulp yield of open-pollinated progenies from 70 families collected from six populations (subraces) across the natural distribution of *E. globulus*: West Otways, Strzelecki Ranges, Furneaux Islands, King Island, north east Tasmania and south east Tasmania. Three sites were sampled and all sample trees were also assessed for diameter and wood basic density. Genetic parameters for predicted pulp yield, pulpwood productivity (basic density multiplied by predicted pulp yield) and pulp production index (pulpwood productivity multiplied by tree diameter) were estimated together with the degree and practical importance of genotype by environment interactions for each trait.

Predicted pulp yield was under moderate genetic control (h^2 ranging from 0.33 to 0.58), pulpwood productivity was under strong genetic control (h^2 ranging from 0.80 to 0.84) and pulp production index generally under weak genetic control (h^2 ranging from 0.17 to 0.33). Genetic correlations between diameter and predicted pulp yield were variable (ranging from –0.16 to –0.43) as were genetic correlations between basic density and predicted pulp yield (range 0.00 to 0.74).

Genotype by environment interaction was not considered a major problem for predicted pulp yield and pulpwood productivity. Although significant interaction between the subraces and sites was found for predicted pulp yield there were no significant differences among subraces at one site and the range of subrace means was small. For predicted pulp yield the family by site interaction was not significant, genetic correlations between the sites were very high and the heritability estimated for the combined data set was similar to the average of the individual site estimates.

Combining tree diameter, basic density and predicted pulp yield data into a single variable (pulp production index) is not recommended as the heritability was generally low and it was subject to significant genotype by environment interaction.

Keywords: *E. globulus*, wood properties, pulp yield, NIR analysis, heritability, genetic correlations, genotype by environment interaction

INTRODUCTION

Eucalyptus globulus is predominantly planted as a crop for production of kraft pulp. Kraft pulping involves cooking wood chips in an alkaline solution at elevated temperature and pressure to dissolve lignin and leave fibres, which are composed of cellulose and hemicellulose, intact (SMOOK 1982). The yield of pulp per unit input of wood is a major determinant of the economics of such an operation. Traditional assessment of pulp yield by cooking wood chips to a fixed kappa number in a laboratory digester is slow and expensive, restricting the number of samples that may be processed. The recent development of near-infrared reflectance analysis (NIR analysis) for predicting pulp yield (MICHELL 1995; SCHIMLECK & MICHELL 1998) provides a rapid, cost effective alternative technique to traditional assessment of kraft pulp yield.

NIR analysis involves measuring the spectra of a large number of samples whose kraft pulp yield is known, developing a model that relates the NIR spectra of each sample to its pulp yield at the desired kappa number and then using the model to predict the pulp yield for a new sample from its NIR spectra. NIR

analysis is potentially of value in tree breeding programs as the quantity of wood required is very small (about 3 g air-dry) allowing the prediction of pulp yield from small wood samples, such as increment cores.

Little information is available regarding the heritability of kraft pulp yield or its correlation with other growth or wood traits for *E. globulus*. The two published heritability estimates differ: DEAN *et al.* (1990) reported an estimate of 0.56 whilst the estimate of BORRALHO *et al.* (1993) was lower at 0.30. Both authors found pulp yield to have a positive genetic correlation with basic density (0.67 and 0.30 for DEAN *et al.* and BORRALHO *et al.*, respectively) and a negative correlation with tree growth (–0.54 with diameter and –0.13 with height for DEAN *et al.* 1990 and –0.05 with volume for BORRALHO *et al.* 1993).

Determination of the best races or seedlots to use in a breeding program is complex when several different traits are desired but show different patterns of variation. To attempt to overcome this problem one or more traits may be combined and selection may be conducted on the complex trait. For assessment for pulp production CROMER *et al.* (1998) used the complex traits of pulpwood productivity and forest productivity. Pulpwood productivity was defined as the product of basic density and pulp yield whilst forest productivity was defined as the product of pulpwood productivity and mean annual increment of volume growth. In the current study data was available for basic density and predicted pulp yield from increment core samples and for tree diameter. Pulpwood productivity could be calculated and examined but forest productivity as defined by CROMER *et al.* (1998) could not be calculated. However, a new term, called pulp production index was calculated as the product of basic density, predicted pulp yield and breast height diameter.

As *E. globulus* is planted as a crop for kraft pulping it is vital to determine the degree of genetic control for these traits and to determine the size and practical importance of genotype by environment interactions (GEI). The presence of GEI will reduce the rate of progress possible in a breeding program. Studies of GEI in other temperate eucalypt species have largely been restricted to tree growth but have indicated a wide range of different patterns of interaction from very strong, geographically based interactions in *E. delegatensis* (GARNIER-GERE *et al.* 1995) to relatively small and inconsistent interactions in *E. regnans* (RAYMOND *et al.* 1997). As wood properties are generally under much stronger genetic control than tree growth it may be expected that the magnitude and patterns of GEI may differ from those seen for growth traits. Using the same material as in this current study, MUNERI & RAYMOND (2000) found a significant family by site interaction for basic density in *E. globulus* but no interaction between race and site for either basic density or pilodyn penetration.

GEI may be caused by two factors: different variances between the sites, or changes in the ranking of genotypes across sites. The significance and practical importance of GEI may be assessed in a number of ways, starting with significance of the interaction term in an across site analysis of variance. However, statistical significance of the interaction term does not provide information on the practical importance of the interaction to a breeding program. SHELBOURNE (1972) proposed that if the ratio of the interaction component of variance to the genetic component of variance was greater than 0.5 then the interactions were considered to be a threat to the rate of genetic progress in a breeding program. Another way of examining the importance of GEI is to calculate the genetic correlations between pairs of sites for each trait (BURDON 1977). If correlations are large, then GEI would be expected to have little impact on the rate of progress. Alternatively, the data can be examined to determine whether any geographic patterns are present or whether the interactions are due to particular sites or families. If only a few families are causing the interaction, the potential problem caused by GEI may be avoided by not including these families in the breeding population.

Each of the above approaches was used to examine genotype by environment interactions for pulp productivity traits. Three open-pollinated progeny trials of *E. globulus* were sampled and results for predicted pulp yield, pulpwood productivity and pulp production index are reported here. As only three sites were available for analysis it was not feasible to undertake more sophisticated analyses of GEI such as joint regression analysis.

MATERIAL AND METHODS

Field sampling

This study was based on removal of 12 mm diameter bark-to-bark increment cores using a motor driven coring machine, which leaves a 22 mm hole through the tree. As we did not wish to kill the sampled trees we were restricted to sampling only those sites where trees were sufficiently large. The minimum diameter over bark at breast height (DBHOB) for sampled trees was set at 15 cm. Three trials provided suitable material and were sampled: Massy Greene in Tasmania and Mt - Worth and Flynn in Victoria (Table 1). Massy Greene trial was established using 5 replicates, each containing 25 incomplete blocks of 24 open-pollinated families in two-tree line plots. The Victorian trials have 5 sublines, each with 20 randomised complete blocks of 39 to 47

open-pollinated families, planted as single tree plots. Each subline was planted as a distinct unit and separated from other sublines by a minimum of 100 m. Two families in each subline were *E. globulus* control seedlots, which were planted in all sublines. Wood samples were collected from these control families in each subline and the data used to determine differences due to subline location within site.

The study aimed to sample as much of the natural range of the species as possible. Using the race classification of JORDAN *et al.* (1994) and pilodyn information from an earlier study (MACDONALD *et al.* 1997), six races were selected to represent a wide range of pilodyn penetration – King Island for high penetration, Furneaux, South Eastern Tasmania and North Eastern Tasmania for intermediate penetration and, West Otways and Strzelecki for low penetration. Seventy families (Table 2) representing 15 collection areas (seedlot localities defined by JORDAN *et al.* (1994)) representing seven subraces (according to a new racial classification of DUTKOWSKI *et al.* (1997)) were selected across these six races. All 70 selected familes were sampled at all three sites.

To reduce potential sources of bias in the study we selected families on the basis that they occurred at each site and little emphasis was placed on average diameter of each family. Six trees were sampled per family, with sampling commencing from the first replicate and the first 6 acceptable trees (unforked and greater than 15 cm DBHOB) in each family were sampled. Each tree was measured for DBHOB and two bark-to-bark increment cores were extracted one above the other at 1.1 m following sampling recommendations of RAYMOND *et al.* (2001). Basic density was determined on one core using the water displacement method (MUNERI & RAYMOND 2000). The second core was used for determining predicted pulp yield using near infrared reflectance analysis. To minimise seasonal effects all trials were sampled in late winter and early spring, prior to active growth commencing. The trials were sampled consecutively, with all sampling done within a five-week period.

Determination of predicted pulp yield

Laboratory Processing

Each core was broken into pieces and then reduced to small fragments using an ESSA 200 mm disc pulveriser and then reduced to wood meal in a Wiley Mill. Grinding of all samples was carried out through a 1.0 mm screen for one minute.

Prediction of pulp yield

NIR spectra were measured on two subsamples of the wood meal for each core and the predicted pulp yield calculated as described by MICHELL (1995) and SCHIMLECK & MICHELL (1998). The NIR spectra were measured in diffuse reflectance in a scanning spectrophotometer (NIRSystems Inc. Model 5000). The instrument reference was a ceramic standard. The NIR spectra were collected at 2 nm intervals over the wavelength range 1100–2500 nm. Fifty scans, in total, were accumulated for each of the duplicate samples and the results averaged. The data were converted before analysis to the second derivative mode by using the instrument's NSAS software. A segment width of 10 nm and a gap width of 20 nm were used for the conversion.

NIR analysis relies on developing a calibration model that relates the NIR spectra of a large number of samples to their known kraft pulp yield. This model is then used to predict the pulp yield of further samples based on their NIR spectrum. Currently, it is not possible to reliably pulp very small wood samples such as increment cores. The calibration models used in this study are based on pulping whole trees. One potential problem is that the range of variation in pulp yield of small samples taken from specific locations within a tree may be greater than that seen for the whole tree samples. Care should be taken in interpreting the actual yield figures presented as there may be some over or

Table 1. Location, rianfall and elevation for each site.

Trial	Massy Greene	Mt Worth	Flynn
Location	Tasmania	Victoria	Victoria
Owner	North Forest Products	Australian Paper Plantations	Australian Paper Plantations
Latitude (South)	41° 05'	38° 16'	38 °13'
Longitude (East)	145° 54'	146° 02'	146° 16'
Elevation (m)	120	380	60–80
Rainfall (mm)	1130	1200	700
Age (years)	7	8	8

Table 2. Number of families sampled within subrace and seedlot locality. The bold figures indicate the total number of families from each subrace.

Subrace number	Name	Locality	Numbers of families
2	West Otways	Cannan Spur	6
		Otway State Forest	6
		Parker Spur	8
			20
5	Strzelecki	Bowden Road	4
		Jeeralang	3
		Jeeralang North	8
			15
6	Madalya Road (Gippsland)	Madalya Road	**5**
9	Furneaux	Central Flinders Island	4
		Central North Flinders Island	2
		North Flinders Island	2
		South Flinders Island	2
			10
11	NE Tasmania	Royal George	**5**
16	SE Tasmania	North Maria Island	5
22	King Island	Central King Island	5
		South King Island	5
			10

underestimation at the extremes of the range of yields.

It is implicit in this technique that the "training" sets on which the models are based contain all of the kinds of variation in the samples to be analysed. Difficulties arise in multi-site work as pulp yield depends on the tree species, its genetic makeup and the growing conditions. In addition, differences exist between laboratories in the method used for evaluating pulp yield. Prediction of pulp yield using the NIR methodology is only valid for samples that are chemically similar to samples that have been included in the calibration set for a particular pulping laboratory.

When seeking to match a new sample to an existing calibration based on a training set it is necessary to determine whether the new sample falls within the membership of that set. This can be done by analysing the bands in the NIR spectrum of the new sample and comparing them with those in the spectra of samples in the training set. Comparisons at 700 points, as in the raw spectra, are however unmanageable, so the variation in the data has to be accounted for by using fewer variables. This is done by using Principal Components Analysis which compresses the variation into fewer variables. In the case of NIR spectra of eucalypt woods some 90 % of the variation can often be accounted for in as few as three derived variables. Differences between the spectra can then be expressed in terms of distances in the space defined by these variables. We have chosen to use the distance called the Mahalanobis distance (MAHALANOBIS 1936; WEISBERG 1985; ANON 1996). If the Mahalanobis distance is greater than 3 (3 standard deviations from the mean) then there is a probability of 0.01 or less that the sample belongs to the set and can be classified as a non-member of the training set (ANON 1996).

The model used for predicting pulp yield was based on the training set for North Forest Products pulping laboratory. The degree of fit of data from each site to the model was very good with more than 70 % of the samples falling within a Mahalanobis distance of 3 (71.3 % for Flynn, 88.8 % for Mt Worth and 95.3 % for Massy Greene).

Estimation of genetic parameters

Two additional variables were calculated: Pulpwood productivity (PP in units of $kg \cdot m^{-3}$ dry pulp) and pulp production index (PPI) in which the pulpwood productivity is weighted by tree diameter (DBH).

PP = Density * pulp yield / 100

PPI = PP * DBH/10

Prior to proceeding with any analysis, the distribution of these variables was checked and found to be approximately normal.

To determine whether the restriction on tree size had resulted in a biased sample, the distribution of diameters of the cored trees was compared to the diameters of all available trees within the selected families at each site using data from the last complete diameter assessment of the trials (1993). Comparison of the histograms of tree sizes indicated a normal distribution and that no systematic bias was present.

The two Victorian trials, Mt Worth and Flynn, were sampled across 5 sublines with families being nested within sublines. Subline effects for all traits were calculated using data from the two control families and fitting the following model:

$$\mathbf{y}_c = \mathbf{X}_1\mathbf{c} + \mathbf{X}_2\mathbf{s} + \mathbf{e} \quad [1]$$

where $\mathbf{y}_c$ is the vector of individual tree observations for the control families, **c** is a vector of fixed control family effects, **s** is a vector of fixed subline effects and $\mathbf{X}_1$ and $\mathbf{X}_2$ are incidence matrices for the fixed effects. The analysis indicated a significant difference ($P < 0.05$) between sublines for predicted pulp yield at Mt Worth, suggesting the need to adjust the relevant data. As the sublines had been established by grouping seedlots by geographic region, the subrace effect was confounded with the subline and replicate within subline effects. If the data had been adjusted for differences between the control families and subsequently analysed it would not have been possible to separate the subrace and replicate within subline effects in the analysis. To overcome this problem the two control families were defined as a new subrace (subrace 1) and in the subsequent analysis the model was structured so that all other data were fitted at deviations from subrace 1.

Data for each trial were analysed using the model, in matrix notation:

$$\mathbf{y} = \mathbf{X}_1\mathbf{r} + \mathbf{X}_2\mathbf{b} + \mathbf{Z}_1\mathbf{f} + \mathbf{e} \quad [2]$$

where **y** is the vector of individual tree data for predicted pulp yield, pulpwood productivity and pulp production index, **r** is a vector of fixed subrace effects, **b** is a vector of fixed replicate effects, **f** is a vector of random family within subrace effects, **e** is the vector of residuals and $\mathbf{X}_1$, $\mathbf{X}_2$, and $\mathbf{Z}_1$, are incidence matrices relating the observations to the effects in the model.

To test the significance of genotype by environment interaction the following model was fitted:

$$\mathbf{y} = \mathbf{X}_1\mathbf{r} + \mathbf{X}_2\mathbf{b} + \mathbf{X}_3\mathbf{s} + \mathbf{X}_4\mathbf{c} + \mathbf{Z}_1\mathbf{f} + \mathbf{Z}_2\mathbf{i} + \mathbf{e} \quad [3]$$

where **b** is a vector of fixed replicate within site effects, **s** and **c** are respectively vectors of fixed site and subrace by site interaction effects, and **i** is a vector of random family by site interaction effects. $\mathbf{X}_3$, $\mathbf{X}_4$ and $\mathbf{Z}_2$ are the incidence matrices for these effects, and other symbols are the same as those in Equation 2.

For all models, the best linear unbiased estimates (BLUEs) of subrace effects and best linear unbiased predictors (BLUPs) of family means were estimated using REML in GENSTAT. All variance components and genetic parameters and appropriate standard errors were estimated using ASREML (GILMOUR *et al.* 1998).

Individual narrow-sense heritabilities were calculated, assuming a coefficient of relatedness of 0.4 (VOLKER *et al.* 1990) as:

$$h^2 = \frac{\sigma_f^2}{r\,(\sigma_f^2 + \sigma_e^2)} \quad [4]$$

for the single site analysis and,

$$h^2 = \frac{\sigma_f^2}{r\,(\sigma_f^2 + \sigma_i^2 + \sigma_e^2)} \quad [5]$$

for combined sites analysis, and where r is the coefficient of relatedness, s^2_f is the between family variance component, s^2_i family by site interaction variance and s^2_e is the error variance.

Genetic correlations were estimated between all traits within each site and between sites for each trait.

$$r_g = \frac{\mathrm{cov}(f_1, f_2)}{\sqrt{\sigma_{f1}^2 + \sigma_{f2}^2}} \quad [6]$$

where cov (f_1, f_2) is the family covariance between traits 1 and 2, or sites 1 and 2. Phenotypic correlations between traits were estimated on an individual tree basis for each site.

Genotype by environment interaction

The practical importance of the genotype by environment interaction was evaluated by examining:

1) Whether the interaction terms were significant in the across site analysis of variance.

2) Potential causes due to differing variances or changes in rankings. Subraces and families that contributed most to site interaction were identified on the basis of rank changes (MATHESON & RAYMOND 1984). The ranking of each subrace and family for all traits were calculated for each site as well as across the three sites. Mean rank deviation was estimated as the sum of the absolute rank deviation from the across site rank divided by the number of sites.

Table 3. Subrace means, standard deviations (SD) and ranking for diameter (cm), basic density (kg·m^{-3}), predicted pulp yield (PPY in percentage), pulpwood productivity (PP in kg of pulp per m^3 of wood) and pulp production index (PPI which is the product of PP and diameter/10).

Trait	Subrace	Massy Greene		Mt Worth		Flynn	
		Mean (SD)	Rank	Mean (SD)	Rank	Mean (SD)	Rank
Diameter	West Otways	23.0 (3.9)	2	25.8 (4.6)	1	19.7 (2.6)	1
	Strzelecki	22.1 (3.2)	4	22.0 (4.2)	7	17.0 (2.4)	6
	Madalya Road	23.7 (3.9)	1	24.2 (5.2)	6	16.7 (2.3)	7
	Furneaux	21.2 (3.0)	5	24.4 (4.2)	5	18.7 (2.3)	3
	NE Tasmania	18.7 (2.7)	7	24.5 (3.1)	4	17.5 (1.9)	5
	SE Tasmania	20.1 (3.7)	6	24.7 (3.6)	3	18.7 (2.1)	4
	King Island	22.6 (4.1)	3	25.1 (5.1)	2	19.6 (2.6)	2
	Site mean (SD)	21.8 (3.7)		24.0 (4.6)		18.5 (2.4)	
	Range for subrace BLUEs	18.7–23.7		22.0–25.8		16.7–19.7	
	Range for family BLUPs	19.8–23.6		22.7–25.4		17.5–19.1	
Density	West Otways	469 (33)	6	465 (31)	3	554 (31)	3
	Strzelecki	504 (37)	1	474 (30)	1	571 (37)	1
	Madalya Road	487 (34)	2	463 (33)	4	559 (34)	2
	Furneaux	481 (36)	4	468 (33)	2	533 (32)	5
	NE Tasmania	485 (41)	3	455 (32)	5	538 (28)	4
	SE Tasmania	471 (44)	5	442 (27)	6	504 (32)	6
	King Island	428 (35)	7	423 (34)	7	489 (33)	7
	Site mean (SD)	473 (42)		460 (35)		543 (41)	
	Range for subrace BLUEs	428–504		423–474		489–571	
	Range for family BLUPs	436–500		420–491		473–581	
PPY	West Otways	52.0 (1.2)	2	52.3 (1.2)	4	52.1 (1.4)	2
	Strzelecki	51.8 (1.0)	5	53.1 (1.3)	2	51.1 (1.3)	7
	Madalya Road	51.9 (1.1)	3	53.4 (1.3)	1	51.1 (1.4)	6
	Furneaux	51.8 (1.2)	6	52.5 (1.4)	3	51.6 (1.3)	5
	NE Tasmania	51.8 (1.2)	7	51.2 (0.9)	7	51.7 (1.1)	4
	SE Tasmania	52.1 (1.2)	1	52.1 (1.3)	6	52.3 (1.3)	1
	King Island	51.8 (1.2)	4	52.2 (1.2)	5	52.0 (1.4)	3
	Site mean (SD)	51.9 (1.2)		52.2 (1.4)		51.9 (1.5)	
	Range for subrace BLUEs	51.8–52.1		51.2–53.4		51.1–52.3	
	Range for family BLUPs	50.9–52.5		51.1–53.4		51.0–52.5	
PP	West Otways	244 (18)	6	243 (18)	4	288 (17)	2
	Strzelecki	261 (20)	1	252 (17)	1	291 (20)	1
	Madalya Road	253 (20)	2	247 (18)	2	286 (19)	3
	Furneaux	249 (22)	4	246 (21)	3	275 (19)	5
	NE Tasmania	251 (25)	3	233 (17)	5	278 (16)	4
	SE Tasmania	245 (27)	5	230 (19)	6	264 (19)	6
	King Island	222 (20)	7	221 (17)	7	254 (18)	7
	Site mean (SD)	245 (24)		240 (20)		281 (21)	
	Range for subrace BLUEs	222–261		221–252		254–291	
	Range for family BLUPs	218–264		214–254		251–298	

3) The ratio of the variance components for the interaction term and families to determine whether it was greater than 0.5 (SHELBOURNE 1972).

4) Genetic correlations between the sites (BURDON 1977).

To explore potential causes for the observed site by family interaction the data was subdivided in several ways:

- Geographic groups of subraces by state – Victoria versus Tasmania with the Bass Strait Islands included in the Tasmanian group.
- The most interactive subrace based on average rank deviations was deleted.
- The most interactive families were sequentially deleted.

Table 3. (continued)

Trait	Subrace	Massy Greene		Mt Worth		Flynn	
		Mean (SD)	Rank	Mean (SD)	Rank	Mean (SD)	Rank
PPI	West Otways	561 (108)	3	626 (112)	1	564 (76)	1
	Strzelecki	574 (97)	2	559 (111)	6	494 (76)	5
	Madalya Road	596 (105)	1	602 (143)	2	475 (62)	7
	Furneaux	527 (87)	4	599 (112)	3	514 (65)	2
	NE Tasmania	475 (86)	7	571 (76)	4	489 (63)	6
	SE Tasmania	493 (92)	6	571 (115)	5	496 (65)	4
	King Island	501 (103)	5	553 (111)	7	498 (70)	3
	Site mean (SD)	534 (104)		577 (118)		521 (72)	
	Range for subrace BLUEs	475–596		553–626		475–564	
	Range for family BLUPs	472–583		550–617		481–531	

RESULTS

Detailed results for basic density and diameter over bark were presented in an earlier paper (MUNERI & RAYMOND 2000). Results presented here concentrate on the traits related to pulp yield.

Site differences

Sites differed significantly for predicted pulp yield, pulpwood productivity and pulp production index (Table 6), with Mt Worth having the highest predicted pulp yield and pulp production index (Table 3). Massy Greene and Flynn had the same average predicted pulp yield but Flynn had a higher pulpwood productivity, reflecting the higher basic density at this site. The range of predicted pulp yields within site was generally low, with both the subrace BLUEs and the family BLUPs having a range of around 2 %. The range for pulpwood productivity was greater (around 50 $kg \cdot m^{-3}$) reflecting the greater variation for basic density.

Subrace effects

Subraces differed significantly for both pulpwood productivity and pulp production index at each site but differences between the subraces were not significant for predicted pulp yield at Massy Greene. At the other two sites the ranking of subraces (Table 3) for predicted pulp yield varied considerably with Strzelecki ranking second at Mt Worth and last at Flynn and SE Tasmania ranking top at Flynn and second last at Mt Worth.

In contrast the ranking of subraces for pulpwood productivity were largely stable across the sites reflecting the more stable rankings for basic density. The Strzelecki subrace ranked highest for pulpwood productivity at each site and King Island ranked lowest. Ranking of subraces for pulp production index were more variable due to the introduction of diameter into the trait.

Genetic parameter estimates

Heritabilities for predicted pulp yield (Table 4) were moderate at all sites ranging from 0.33 to 0.58 and fell within the range previously published for this species of 0.30 to 0.67 (BORRALHO *et al.* 1993 and DEAN *et al.* 1990). When predicted pulp yield is combined with basic density to form pulpwood productivity the heritability estimates increased to more than 0.80 for all sites. However, when diameter is introduced heritability falls dramatically, with heritabilities for pulp production index ranging from 0.17 to 0.33.

Genetic correlations between diameter and predicted pulp yield (Table 5) were negative at all sites, ranging from –0.43 at Massy Greene and Flynn to –0.16 at Mt Worth and slightly lower than the previously published estimate of –0.54 from DEAN *et al.* (1990). The respective phenotypic correlations were very close to zero.

Correlations between basic density and predicted pulp yield (Table 5) present a mixed picture. Both the genetic and phenotypic correlations were small and non-significant for Mt Worth and Flynn. However, at Massy Greene, the genetic correlation between basic density and predicted pulp yield was strong and positive indicating that denser trees had higher predicted pulp yield. The current estimates fall outside the range of the two previously published estimates (0.30 and 0.67) perhaps indicating large differences between sites for this correlation.

Genotype × Environment interaction

The practical importance of the genotype by environment interaction were evaluated by examining the significance of the interaction term in the analysis of variance, potential causes due to differing variances or changes in rankings, the ratio of the variance compo-

Table 4. Variance components and heritabilities (standard error) for diameter, basic density, predicted pulp yield (PPY), pulpwood productivity (PP in kg of pulp per m^3 of wood) and pulp production index (PPI which is the product of PP and diameter/10) at each site.

Trait	Genetic parameters	Massy Greene	Mt Worth	Flynn
Diameter	Family variance	1.55	1.09	0.346
	Residual variance	10.39	17.40	5.069
	Heritability (s.e)	0.33 (0.11)	0.15 (0.12)	0.16 (0.10)
Basic Density	Family variance	338.98	343.67	428.27
	Residual variance	930.32	643.31	641.05
	Heritability (s.e)	0.67 (0.13)	0.87 (0.16)	1.00 (0.15)
PPY	Family variance	0.274	0.391	0.237
	Residual variance	1.113	1.296	1.566
	Heritability (s.e)	0.49 (0.13)	0.58 (0.15)	0.33 (0.12)
PP	Family variance	146	105	109
	Residual variance	288	219	230
	Heritability (s.e)	0.84 (0.15)	0.81 (0.16)	0.80 (0.15)
PPI	Family variance	1301	929	326
	Residual variance	8559	11115	4489
	Heritability (s.e)	0.33 (0.12)	0.19 (0.13)	0.17 (0.10)

Table 5. Genetic correlations (standard error) and phenotypic cerrelations (below diagonal) between diameter, basic density and predicted pulp yield (PPY) at each site.

Site	Trait	Diameter	Basic density	PPY
Massy Greene	Diameter		0.00 (0.21)	-0.43 (0.24)
	Basic Density	0.09		0.74 (0.14)
	PPY	-0.05	0.19	
Mt Worth	Diameter		-0.22 (0.34)	-0.16 (0.35)
	Basic Density	0.03		0.08 (0.20)
	PPY	-0.05	0.15	
Flynn	Diameter		-0.44 (0.25)	-0.43 (0.35)
	Basic Density	-0.07		0.00 (0.22)
	PPY	-0.12	-0.20	

nents for the interaction term and families and genetic correlations between the sites. The data was then subdivided to determine what families or sites were causing the interaction.

The across site analyses of variance (Table 6) indicate that the subrace by site interaction term was significant for predicted pulp yield but not for the other two traits. In contrast, the family by site interaction term was significant for pulpwood productivity and pulp production index but not for predicted pulp yield.

As GEI may be caused by scale differences between sites it was essential to check that the standard deviations and residual variance for each site are relatively homogeneous. Standard deviations (Table 3) and residual variance components (Table 4) for predicted pulp yield were very similar across sites. Another possible cause of GEI is changes in ranking of genotypes across sites. Table 3 indicates that ranking of the subraces changed considerably for predicted pulp yield but that rankings were relatively constant across sites for pulpwood productivity. Families also changed rankings across sites for each trait.

Variance components obtained from the combined analysis (Table 7) indicated that the family by site interaction term was very small for predicted pulp yield and approximately 25 % of the family variance for pulpwood productivity. However, for pulp production index the interaction component was larger than the

Table 6. Across-site analysis of variance for predicted pulp yield (PPY), pulpwood productivity (PP in kg of pulp per m^3 of wood) and pulp production index (PPI which is the product of PP and diameter/10).

Source of variation	Degrees of freedom	Mean Squares		
		PPY	PP	PPI
All sites				
Site	2	17.037**	227258**	382879**
Replicate within sites	174	3.190**	709**	13899**
Subrace	7	3.695**	13097**	85890**
Subrace by site	13	2.866**	464	14639
Family within	64	5.666**	1861**	15882**
subrace	126	1.534	371**	11353**
Family by site	933	1.318	246	7924
Residual				

* $P < 0.05$, ** $P < 0.01$

Table 7. Variance components and heritabilities (standard error) for diameter, basic density, predicted pulp yield (PPY), pulpwood productivity (PP in kg of pulp per m^3 of wood) and pulp production index (PPI which is the product of PP and diameter/10) for combined data from Massy Greene, Mt Worth and Flynn.

Genetic parameters	Diameter	Basic density	PPY	PP	PPI
Family variance	0.31	280.4	0.265	95.8	326
Family by site	0.70	99.6	0.027	22.1	534
variance	11.04	740.9	1.330	247.5	7994
Residual variance	0.06 (0.05)	0.63 (0.11)	0.41 (0.09)	0.66 (0.11)	0.09 (0.05)
Heritability (s.e)					

Table 8. Genetic correlations (standard error) between sites for diameter, basic density, predicted pulp yield (PPY), pulpwood productivity (PP in kg of pulp per m^3 of wood) and pulp production index (PPI which is the product of PP and diameter/10).

Trait	Site	Mt Worth	Flynn
Diameter	Massy Greene	0.12 (0.39)	0.29 (0.37)
	Mt Worth		0.76 (0.44)
Basic density	Massy Greene	0.83 (0.11)	0.91 (0.08)
	Mt Worth		0.46 (0.14)
PPY	Massy Greene	0.80 (0.16)	0.99 (0.19)
	Mt Worth		0.89 (0.19)
PP	Massy Greene	0.82 (0.10)	1.02 (0.07)
	Mt Worth		0.56 (0.14)
PPI	Massy Greene	0.04 (0.35)	0.95 (0.35)
	Mt Worth		0.58 (0.37)

family component of variance. The heritability estimate for the combined data for predicted pulp yield (Table 7) was similar to the average of the individual site estimates, reflecting the lack of a significant family by site interaction. For pulpwood productivity and pulp production index the heritabilities estimated across all sites were lower than the individual site estimates (Table 4) due to the presence of genotype by site interaction.

Genetic correlations between sites (Table 8) were

very strong for predicted pulp yield and pulpwood productivity. However, correlations were highly variable for pulp production index, ranging from 0.04 to 0.95 with relatively large standard errors.

Subdividing the data into groups on a geographic basis yielded different results for predicted pulp yield, pulpwood productivity and pulp production index. When the Tasmanian subraces were analysed alone, the site by subrace interaction for predicted pulp yield became non-significant whilst for the Victorian subraces it remained significant. For pulp productivity, the geographic grouping did not have any effect with the site by family interaction remaining significant for both the Tasmanian and Victorian groups. For pulp production index the Tasmanian subraces alone showed no significant family by site interaction whilst this term remained significant for the Victorian subraces.

Removal of the most interactive subrace, based on average rank deviations, had no effect for any of the traits with the interactions between site and subrace remaining significant. However sequential deletion of interactive families was found to be effective in reducing the interaction for pulpwood productivity and pulp production index but not for predicted pulp yield. For pulpwood productivity, deleting the 5 most interactive families resulted in the site by family interaction becoming non-significant but the site by subrace term became significant. Deletion of an additional family led to both terms being non-significant. The families removed were scattered across a range of subraces (2 from Strzelecki and SE Tasmania and one each from West Otways and NE Tasmania). For pulp production index removing 3 families led to the site by family interaction becoming non-significant but the site by subrace term became significant. In this case 15 families were removed before both interaction terms became non-significant (6 from West Otways, 5 from Strzelecki, 2 from Madalya and one each from Furneaux and King Island).

The site by subrace interaction appeared to be more complex for the predicted pulp yield data as it could not be explained in terms of geographic groups or by removing the most interactive subrace. Sequential deletion of the most interactive families also failed, with the interaction still being significant after more than half the families had been removed. The next step was to analyse the sites in paired groups to see if one of the site was causing the site by subrace interaction. For Massy Greene and Flynn there was no significant interactions but for the other pairings with Mt Worth the interactions were significant indicating that Mt Worth is behaving differently to the other two sites.

DISCUSSION

A study of this nature would not have been possible using traditional kraft pulping technology due to the need to destroy valuable breeding stock and the huge cost involved in pulping so many individual tree samples. Previous studies on genetic control of pulp yield have generally been restricted to a relatively small number of families on a single site (for example DEAN *et al.* 1990 sampled only 18 families). The advent of near infrared reflectance analysis models for pulp yield allows for much larger studies to be undertaken in a relatively inexpensive and rapid manner and for many trees and families to be sampled by removing wood cores in a non-destructive manner. Genetic parameters for predicted pulp yield can now be estimated using larger numbers of families and across multiple sites.

However, the validity of such studies on predicted pulp yield relies on the effectiveness of the calibration models used. The technique assumes that the training set used to develop the models will contain all of the kinds of variation expected in the samples to be analysed. Whether this is a valid assumption for multi-site studies is uncertain but the degree of fit of the data from each site to the model used in this study was very good. In addition, the heritability estimates for predicted pulp yield from the current study were within the range of those previously published, indicating that the degree of expression of genetic control was similar for both traditional laboratory pulping and predicted pulp yield.

The patterns of genetic architecture found for predicted pulp yield differ to those found for diameter and basic density by MUNERI & RAYMOND (2000) for the same trees. For diameter, small differences were found amongst the subraces and the within-race heritabilities were relatively low. For predicted pulp yield there were again small differences between the subraces but the heritability estimates were in the moderate range and much higher than those for diameter. In contrast, large subrace differences were found for basic density together with very high within-race heritability estimates.

Genotype by environment interaction was not considered a major problem for predicted pulp yield. Although there was a significant interaction between subraces and sites there were no significant differences among subraces at one site and the range of subrace means was small. The family by site interaction was not significant, genetic correlations between the sites were very high (range 0.80 to 0.99) and the heritability estimated for the combined data set was similar to the average of the individual site estimates.

Similarly, in terms of practical importance, the genotype by environment interaction for pulpwood productivity would appear to be minor. The site by

family interaction term was significant in the analysis of variance but contributed little to the overall variation. Removal of the six most interactive families resulted in no significant interactions between site and subraces or families. Genetic correlations between sites were again generally strong indicating that families which performed well at one site also did well at other sites. The heritability estimate for the combined data (0.66) was slightly lower than that for the individual sites (range 0.80 to 0.84) but would still be regarded as a high heritability.

For pulp production index the picture becomes less clear with the addition of tree diameter to the combination of pulp yield and density leading to a much more complex outcome for genotype by environment interactions. The site by family interaction was significant and larger than the family variance. Removal of over 20 % of families was necessary before the interactions of site with subraces and families became non-significant. Genetic correlations between sites were highly variable, ranging from 0.04 to 0.95 and the across site estimate of heritability relatively small (0.09) and lower than any of the individual site estimates.

Diameter, density and pulp yield appear to follow different patterns of variation, with different patterns of genetic variation and differences in the top ranking subraces for each trait and often for each site. The issue of whether to use combined multiple traits in a breeding program then becomes critical. For future data analysis, when data is available for all three traits (diameter, basic density and predicted pulp yield) it would appear sensible to treat diameter as a separate trait and either analyse basic density and predicted pulp yield separately or to use pulpwood productivity to give a measure of productivity of dry pulp per unit green wood. The genetic and phenotypic correlations of diameter with both basic density and predicted pulp yield were generally low and variable indicating no strong relationships. Both predicted pulp yield and pulpwood productivity had much higher heritabilities than pulp production index and the family by site interactions were not considered to be of major importance. Combining tree diameter, basic density and predicted pulp yield data into a single variable (pulp production index) is not recommended as the heritability was generally low and it was subject to significant genotype by environment interaction.

ACKNOWLEDGEMENTS

The able assistance of Andrew MacDonald and Jason Lawson in collecting and processing the wood samples and of Ms L Nagy in obtaining the NIR spectra of the cores is gratefully acknowledged. Thanks also to those collaborators from Australian Paper Plantations and North Forest Products who kindly let us sample their trees and provided assistance with organising and undertaking this study. Finally, the authors wish to thank Greg Dutkowski for his assistance with selection of seedlots for sampling and for his ongoing interest and comments on this study.

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