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Report No. 9

Comparison among control-pollinated and open-pollinated seedlots of *Eucalyptus nitens* in New Zealand

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Summary

This paper reports the results at age 7 years of a *Eucalyptus nitens* genetic gain trial established in the central North Island, New Zealand, with a range of improved seedlots. The material tested included control-pollinated families among parents forward selected from native population families in Australia, and two sets of open-pollinated families from forward selected clones, again from native population families from a New Zealand seed orchard. Differences among families in diameter growth, wood density, and stem form were observed in some but not all of the family collections. Heritability estimates strongly differed among these family sets, and showed high standard errors, probably because of the low number of families and individuals per family in each set. No significant differences were observed among the different seed sources, suggesting that no extra gain should be expected from using the advanced improved Australian material in New Zealand. The lack of correspondence between the observed performance of the OP families, and that expected from the parental breeding-value estimates, suggests a poor accuracy of previous breeding-value estimation based on open-pollinated progeny testing. However, the results presented here should be managed with care due the insufficient number of families and individuals per family tested.

Keywords: Open-pollinated families, control-pollinated families, genetic gain, progeny testing, genetic parameters.

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Introduction

Eucalyptus nitens is planted in New Zealand both for the production of short-fibre pulp and, to a lesser degree, solid-wood end uses. Together with *E. fastigata*, it is one of the eucalypts that have been the focus for genetic improvement, with the first major introduction and testing of *E. nitens* open-pollinated families in 1978 (King and Wilcox 1988).

Breeding programs for this species have been developed both in Australia and New Zealand, with several clonal seed orchards established for seed production, and open- and control-pollinated progeny testing for estimating genetic parameters and for further breeding. In 1999, a trial was established in New Zealand to compare the performance of different subsets of open- and control-pollinated families from these breeding populations. The results of this trial for diameter growth, stem form and wood density at age 7 years are reported.

Material and Methods

The trial

The trial was established by Tasman Forest Industries and is located north of Lake Taupo. The site, known as McCauleys, is ex-pasture, flat topography and at 368masl. The trial design is a modified randomized complete block design with five blocks and two-tree row-plots. Two types of improved material were tested: control-pollinated (CP) families from the Southern Tree Breeding Association (STBA) breeding population in Australia, and open-pollinated (OP) families from the Proseed New Zealand clonal seed orchard at Waikuku. Blocks were subdivided in two sub-blocks, and the two-tree family plots of each group randomly distributed within each sub-block. The two seed sources were located in the same relative position with respect to each other in each replicate. A total of 25 Australian CP families and 12 New Zealand OP families were included in the trial. In addition, 12 extra New Zealand OP families were established adjacent in an independent randomized complete block design with three blocks and two-tree row-plots. The two New Zealand OP family sets will be referred to as OP-set1 and OP-set2. The crossing design of the Australian CP families is outlined in Appendix 1.

Assessments

Diameter at breast height (DBH) was measured in all live and non-suppressed trees 7 years after planting. Trees were also visually scored for stem form (FORM) using a 9-point scale (from 1 - very poor form, to 9 - straight form). Wood density (DEN) was evaluated in a total of 3-4 trees of each family. Basic

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wood density was determined on 5 mm bark to bark cores using the maximum moisture content procedure (Smith, 1954).

Statistical analysis

Plot means for each trait were analyzed independently for each set of families using the GLM procedure of SAS (Type III sum of squares) (SAS-Institute 1999) and the following model:

$Y_{ij} = \mu + B_i + F_j + \varepsilon_{ij}$

where Y_{ij} is the plot mean of one of the studied traits, μ is the overall mean, B_i is the effect of block i, F_j is the fixed effect of the jth family, and ε_{ij} is the experimental random error. Because of the scarce number of blocks and families, all factors were considered fixed. We used type III sum of squares because of the missing data for some plots, especially in the case of wood density, a trait just measured in a few trees per family.

When the family effect was significant ($p < 0.05$) variance components were estimated running again the model with the MIXED procedure of SAS (SAS-Institute 1999) and considering all factors random. Individual heritability was calculated from the resulting variance-component estimates as:

$$h_i^2 = \frac{r \cdot \sigma_f^2}{\sigma_f^2 + \sigma_e^2}$$

where σ_f^2 and σ_e^2 are the family and error variance components, respectively, and r is the coefficient of relationship which was assumed to be 3 for the OP families and 2 for the CP families. The use of a coefficient of 3 for OP families instead of the theoretical 4, has been discussed by many authors and is the most commonly used for insect-pollinated species such as *Eucalyptus* (e.g. Borralho et al. 1992; Costa-Silva et al. 2006; Gea et al. 1997). Approximate standard errors of individual heritability were estimated according to Wright (1976).

Because the two seed sources were not randomized within each replicate, a test of significance of source differences may be not appropriate. Regardless, we attempted across-sources analyses, assuming randomization, and using the overall means of Australian CP and the New Zealand OP-set1 families within each block.

Previous estimates of parental breeding values (BV) for diameter, stem straightness and pilodyn resistance were available for some genotypes of the New Zealand breeding population. Scatter plots between these BV estimates and the performance of the corresponding families (best linear unbiased predictor, BLUP) in the studied trial were used to visually explore the

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correspondence between the two types of estimate for those traits for which significance differences among families were detected.

Results and Discussion

The analyses of variance show significant differences for diameter and wood density among the Australian CP families, and for diameter and stem form among the New Zealand OP-set1 families. No significant differences among families were observed in the New Zealand OP-set2 material for any trait (Table 1).

Table 1. Summary of the analyses of variance for the control-pollinated (CP) Australian families and the two sets of New Zealand (NZ) open-pollinated (OP) families. F ratios, significance levels[1] and estimated variance components[2] (σ^2) are presented.

Variable / Effect	Australian CP			NZ OP-set1			NZ OP-set2		
	DF	F	σ^2	DF	F	σ^2	DF	F	σ^2
Diameter (mm)									
Block	4	1.35	27.8	4	0.50	0.0	2	1.76	45.5
Family	24	1.73 *	254.2	11	3.25 **	419.2	11	1.27	66.1
Error	74		1403.0	44		877.5	22		722.3
Wood density (kg m^{-3})									
Block	4	2.11	0.0	3	0.90	4.0	2	0.28	0.0
Family	24	1.90 *	142.9	11	1.74	142.4	11	1.72	150.8
Error	31		522.3	10		326.5	13		356.8
Stem form (sc ore 1-9)									
Block	4	0.55	0.00	4	3.79 *	0.25	2	2.79	0.82
Family	24	1.23	0.30	11	2.80 **	0.37	11	0.34	0.00
Error	70		3.78	43		1.00	21		3.74

[1] Significance levels*: p<0.05; **: p<0.01; ***: p<0.001

[2] Variance components were estimated with the REML method using the MIXED procedure of SAS. Because of the high imbalance in the dataset, especially in the case of wood density, these components differed from those obtained resolving the expected mean square equations. This may explain the apparent incongruence between the F-ratio and the estimated variance components for wood density.

Individual-tree heritability estimates were 0.31±0.16 and 0.65±0.38 for diameter of CP and OP-set1 families, respectively, 0.43±0.27 for wood density in the CP material, and 0.54±0.37 for stem form in the OP-set1. All these estimates show high standard errors because of the low number of families and individuals per family in the trial. This small sample size may also explain the lack of significant differences among families in the OP-set2 material, while it is probably partly responsible for the low genetic variation detected for wood density, a trait that usually shows high heritability in this species (Gea et al. 1997). Moreover, the 25 CP families were based on 22 parents (see

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Appendix 1.), meaning that the CP families were not disconnected pair-crosses, but shared common parents. Thus, the coefficient of relationship used to estimate the additive genetic variance was probably not adequate, so the precision of the estimate of heritability from this set of families is less than expected. This heritability estimate is also subject to bias according to the relative importance of the non-additive genetic variance component within these related pair-crosses. On the other hand, the heritability estimates derived from the open-pollinated family sets are also subject to some uncertainty. Despite the use of a coefficient of relationship of 3, commonly accepted for insect pollinated species such as Eucalypts (e.g. Borralho et al. 1992; Costa-Silva et al. 2006; Gea et al. 1997), this value is arbitrarily set, and the appropriate coefficient would depend on the unknown levels of selfing, full-sibbing, and neighbourhood inbreeding within these OP families. The results obtained here should be considered very imprecise and thus should be used with care.

The Australian CP and the New Zealand OP-set1 materials showed no significant differences for any trait (Table 2, Figure 1). Statistical comparisons with the OP-set2 were not possible because of the experimental design. The method and intensity of selection of the families represented in the trial within each breeding population, and the possible incidence of genotype $\times$ environment interactions, could explain this lack of differences. Nevertheless, these results indicate that advanced improved material from Australia may not perform as well as expected (on the basis of the pollen parents being selected) in New Zealand.

Table 2. Analysis of variance for comparing overall means between Australian CP families and New Zealand OP-set1 families.

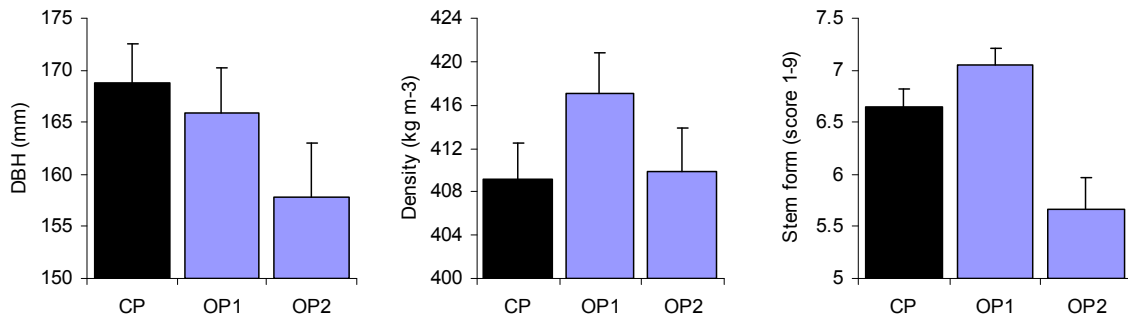| Variable / Effect	DF	DF error	F	p<F
Diameter				
Seedlot type	1	4	0.56	0.4953
Block	4	4	1.98	0.2627
Wood density				
Seedlot type	1	3	4.21	0.1324
Block	4	3	4.05	0.1401
Stem form				
Seedlot type	1	4	3.55	0.1328
Block	4	4	1.63	0.3231

Figure 1. Overall means (± s.e.) of the Australian control-pollinated (CP) families and the two sets of New Zealand open-pollinated families (OP1 and OP2).

Figure 2 shows the relationship between the previous parental breeding-value estimates for diameter and stem form, and the performance in the studied trial of the corresponding open-pollinated families. Only OP-set1 families were considered because they were the only ones that showed significant differences in these traits. No trend can be observed in any case. Even allowing for the low number of points in these figures and the low individuals per family in the studied trial, these results indicate a poor accuracy of the previous breeding-value estimation. First-generation material of insect-pollinated species such eucalypts have been reported to suffer high levels of inbreeding that may affect the estimation of breeding values in open-pollinated progeny trials, especially when the seeds are collected in natural stands (Hodge et al. 1996; Volker et al. 1994). Nevertheless, the results presented here should be interpreted with care because of the small sample size.
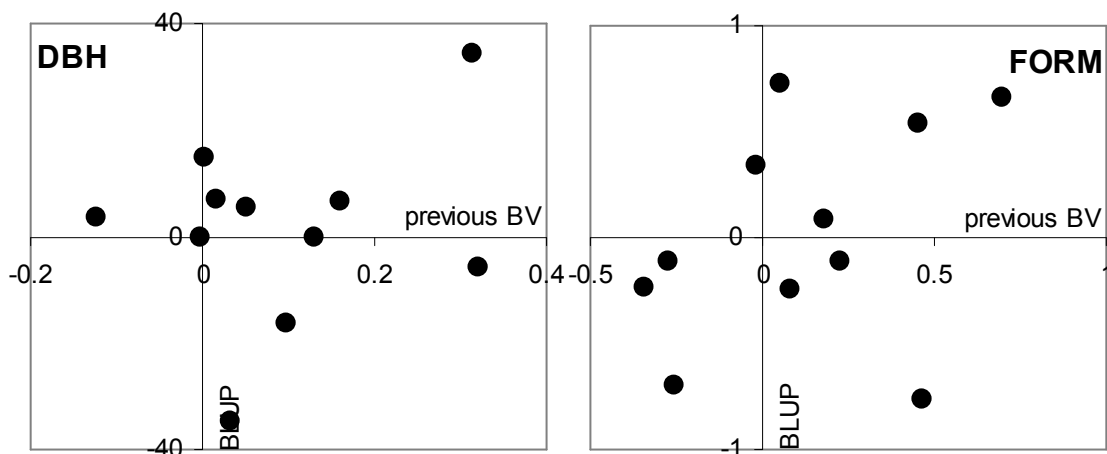

Figure 2. Relationships between the performance of 11 OP New Zealand families (from set 1) in the studied trial and the corresponding previous parental breeding-value estimates.

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Conclusions

Significant differences among families were observed in diameter, wood density and stem form in some but not all of the family collections represented in the trial. Heritability estimates showed high standard errors and were clearly unreliable. The low numbers of families and individuals per family in the trial preclude any precise analysis.

The Australian CP families were not significantly superior to the OP material from New Zealand for any trait. The results do not give any evidence of larger genetic gains of using the advanced improved Australian material in New Zealand.

A poor correspondence between OP family performance and the previous parental breeding-value estimations were observed for both diameter and stem form. Despite the low number of families available for this analysis, these results indicate a poor accuracy of the previous breeding-value estimation.

Acknowledgments

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APPENDIX 1.

Crossing scheme for the Australian control-pollinated families. Codes for parents are those established by the STBA, whereas codes for families where the temporary codes used in the present study.

Female parent	Male parent												
	13172	13832	13851	13884	13947	13962	14023	14201	14221	14241	14280	14290	14347
10068			11	13									
13556													21
13559	14												
13564										9			
13686					17	15							
13742		25	31	22	28	19						26	
13894		23					30		32			16	24
14009							20	29	27		12	18	33
14063										10			8[*]

[*] Family coded 8 was not included in the analyses because it was only represented by three individuals in the trial.