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EUCALYPT COOPERATIVE

Report No. 8

Realized gain and accuracy of breeding-value estimation for first-generation *Eucalyptus nitens* planted in New Zealand

by
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Date: April 2007

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Table of Contents

ensis

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Summary

This paper reports the results at age seven years of two *Eucalyptus nitens* genetic gain trials established in New Zealand with different seedlots of open-pollinated first-generation families grouped according to the parental breeding values (BV) for volume and wood density. Realized genetic gain in diameter growth was 6-8% for those parents with medium- and high- volume BV, but no significant differences were observed between the offspring of parents with low-volume BV and the unimproved control. The strong genotype-by-environment interaction also indicated that realized gain was strongly environment-dependent. On the other hand, no significant differences were observed in wood density among the offspring of the parents of different BV categories. The lack of correspondence between the observed and expected performance of the different seedlots indicates a high degree of uncertainty of the previous BV estimates. As observed previously for other *Eucalyptus* species, the estimation of breeding values based on open-pollinated progeny tests, may be affected by high levels of inbreeding from selfing and/or related matings. According to these results, selections based on open-pollinated testing should be used with care in *E. nitens*, as previously warned for other *Eucalyptus* species.

Key words: Genetic gain, growth, wood density, *Eucalyptus nitens*, breeding-value accuracy, open-pollinated progeny testing.

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Introduction

Demonstrating realized genetic gain is the best way to justify the investment in genetic improvement and to quantify the progress in wood production and economic returns (Zobel and Talbert 1984). Genetic gain trials are not very common for many forest tree species because of the high costs, the long time needed for results, and the fact that the genetic material for which the results apply are usually no longer in the breeding program when the results are finally obtained (Vergara et al. 2004). Nevertheless, genetic gain trials are the only reliable way to demonstrate the real genetic gain obtained with a particular improved seedlot.

Realized gains are quantified comparing different improved seedlots against controls designed to represent unimproved material. The accuracy of the estimates of breeding values (BV) of different improved genotypes can be also explored in genetic gain trials providing that identities of the parent trees are known.

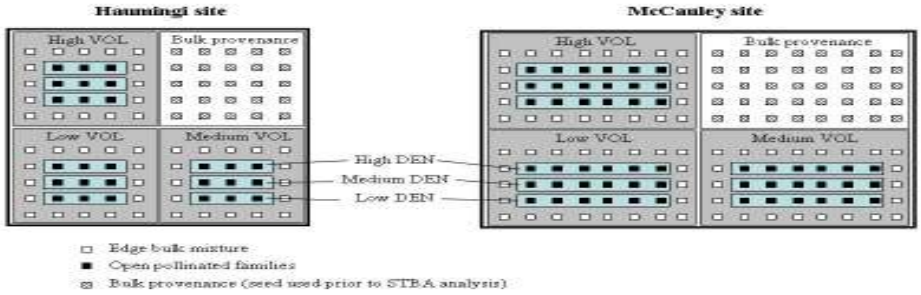
The breeding programme for *Eucalyptus nitens* in New Zealand began with a major introduction and testing of *E. nitens* open-pollinated families in 1978 (King and Wilcox 1988). This breeding population was advanced in 1990 with 310 open-pollinated families established as a single-tree-plot progeny test on two sites (Gea et al. 1997b). A similar breeding programme has been underway since the 1970's in Australia utilising many of the same genotypes. In 1999, two genetic gain trials were established in New Zealand, with the aim to estimate the realized genetic gain of different seedlots of open-pollinated families grouped according to the parental breeding values for volume and wood density. The seedlots and trial design were supplied by the Southern Tree Breeding Association (STBA). This report summarizes the results of the analysis of these trials assessed 7 years after planting. The output of this study will provide an estimate of the realized genetic gain from selections in the *E. nitens* breeding program, and will allow us to test how precise were the previous estimates of the breeding values for volume and wood density.

Material and methods

The trials

The plant material tested came from a first-generation breeding population of *E. nitens* in Australia, plus a bulk provenance seedlot (Mt Erica, Toorongo (Ex. AE O'Connor)) as an unimproved control. Parental trees were selected in native populations in Northern and Southern Central Victoria (See Appendix 1), and their parental breeding-value (BV) for volume and wood density estimated upon several progeny trials established in Australia. Parental trees were grouped into nine categories according to their breeding-value (BV) estimates for volume and wood density. Six parents of each of three categories (low, medium and high BV) for each trait were selected (see Appendix 2) and their open-pollinated offspring planted at two sites in New Zealand, Haumingi and McCauley in 1999. The Haumingi site is located on the southern side of Lake Rotoiti at 320masl. The McCauley site is north of Lake Taupo at 368masl. Both sites are ex-pasture and were prepared for planting by ripping. The McCauley site was considered to be more suited to *E.nitens* and therefore contains more families and replications. *Paropsis charybdis* has not caused any significant defoliation at either site. The experimental design at each site is a split-plot design replicated in six blocks, with the three categories of BV for volume plus the control seedlot acting as the main factor, and the three categories of BV for density as the split factor. Split-plots were composed of one tree of each of three (Haumingi site) or six (McCauley site) open-pollinated families of the corresponding BV-category group. At both sites, whole plots were surrounded by an edge line composed of a bulk mixture of families of the corresponding BV category for volume. Whole plots were 5×5 plants at Haumingi and 5×8 plants at the McCauley site. A schematic representation of the experimental layout is presented in Figure 1.

Figure 1. Scheme of the experimental layout for one of the six blocks of each site.



Assessments

Diameter at breast height (DBH) was measured in all alive and non-suppressed trees at both sites 7 years after planting. Total height (H) was measured in two randomly selected trees of each whole-plot in Haumingi, and in four trees of each whole-plot of Blocks 2, 4 and 6 in McCauley. Wood density (DEN) was evaluated from 5mm bark to bark cores in 1-5 trees of each split-plot. A volume index ($V=H \cdot DBH^2$) was also calculated for those trees for which height was measured. Trees were also scored for stem form using a 9-point scale (from 1 - very poor form, to 9 - straight form).

Statistical analysis

Whole-plot means were analyzed on a single-site basis, assuming a randomize complete block design, and using the GLM procedure of SAS (SAS-Institute 1999) and the following model:

$$Y_{ij} = \mu + B_i + BVv_j + \varepsilon_{ij} \quad (1)$$

where Y_{ij} is the whole-plot mean of one of the studied traits, μ is the overall mean, B_i is the effect of block i (i = 1, 2,…, 6) , BVv_j is the fixed effect of the j^{th} treatment (BV categories for volume plus the control seedlot, j = 1, …, 4), and ε_{ij} is the experimental random error. All factors were considered fixed. The error term would comprise the micro-site variation, and both within and among family variation. Including the family effect in the model was not considered

because it will reduce too much the analyzable data, since no family identification was available for the trees in the edge lines of the whole-plots.

A combined site analysis was also carried out using the following model:

$$Yij = \mu + Si + B(S)j(i) + BVvk + S{\cdot}BVvik + \varepsilon ij \quad (2)$$

where Si is the fixed effect of the site, B(S)i(j) is the effect of block j within site i, and S·BVvik is the interaction between site and the BV category for volume.

Statistical comparison between BV categories were conducted with the LSD test (SAS-Institute, 1999) using a significance level of α=0.01.

Split-plot means were analyzed assuming the split-plot design described above, using the MIXED procedure of SAS and the following model:

$$Y_{ij} = \mu + B_i + BVv_j + B{\cdot}BV_{ij} + BVd_k + BVv{\cdot}BVd_{jk} + \varepsilon_{ij} \quad (3)$$

where, in addition to the factors assumed before, BVdk is the BV category for density, and B·BVvij, B·BVdik, and BVv·BVdjk are the corresponding interactions. To analyze each factor with the appropriate error term all factors were considered fixed except the B·BVvij interaction which was considered random (Littell et al. 1996). There was insufficient height data to analyze with this model. The family structure was not considered in the model because it would imply a high imbalance as a result of mortality and the low number of measured individuals per family. Consequently, the error term comprised the micro-environmental, and within and among family variation.

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Results and Discussion

When analyzing all data (models 1 and 2), diameter growth and wood density of the offspring of parents of different breeding values categories for volume were significantly different at the two sites (Table 1) and when analyzing both sites together (Table 2). However, no significant differences were observed among the different seedlots in height and volume. Too few trees were measured, which did not allow an accurate analysis.

Across-sites diameter growth of the offspring of parents with medium and high breeding values for volume was significantly higher than that of the unimproved control (Figure 2a). This superiority in diameter growth corresponds with a genetic gain of 6-8%. However, diameter growth of the offspring of the low-volume BV parents did not significantly differ from that of the unimproved control.

Table 1. Summary of the analysis of variance for the whole-plot means (model 1) at each site. F ratios and significant levels are presented.

Site/Source	DF	DBH		Height	Volume	Density		Form
Haumingi								
Block	5	1.05		0.92	1.75	0.73		0.22
BV vol	3	5.09	*	0.65	2.01	14.36	***	1.14
McCauley								
Block	5[1]	4.12	*	0.53	0.21	0.26		1.45
BV vol	3	12.99	***	2.24	0.71	5.39	*	0.43

1 For H, V and DEN in McCauley, the DF for blocks are just 2

Significance levels*: p<0.05; **: p<0.01; ***: p<0.001

Combined-site analysis also revealed highly significant site-by-volume interaction for BV category, suggesting that the relative performance of the different seedlots varied among sites (Table 2). This can be easily seen in Figure 3 where important rank changes between sites are evident. At the Haumingi site, the offspring of medium- and low-volume BV parents were significantly larger than the unimproved control, whereas that of high volume BV parents did not significantly differ from the control. At McCauley, however, high and medium BV led to offspring not significantly different from the control, whereas low-BV parents led to significantly smaller trees (Figure 3). These unexpected results indicated that genetic gain estimates are not consistent across sites, and that the superiority of the improved material depends on the site where is going to be planted. Furthermore, the rank of the different seedlots were consistent with the previous parent BV estimates at McCauley (High>Medium>Low) but not at Haumingi. It can be concluded that

the accuracy of previous BV estimates was poor. The low heritability of growth traits in *E. nitens* (e.g. Gea et al. 1997a), the incidence of inbreeding in first-generation open-pollinated material (e.g. Hodge et al. 1996), and/or the incidence of genotype-by-environment interaction between New Zealand and Australia sites, may be affecting these erratic results.

The inclusion of different families in the two sites may have also contributed to the strong site-by-seedlot interaction. However, results were almost the same when the analysis was restricted to the three common families, site-by-seedlot interaction remaining statistically significant (data not shown).

Table 2. Summary of the combined-site analysis of variance for the whole-plot means (model 2). F ratios and significance levels are presented.

Source	DF	DBH		Height	Volume	Density		Form	
Site	1	7.59	**	0.70	1.82	26.53	***	14.55	***
Block(Site)	10	1.53		0.86	1.56	0.67		1.08	
BVvol	3	4.46	*	0.68	0.58	12.84	***	0.94	
S×BVvol	3	8.22	***	0.43	1.34	1.90		0.35	

Significance levels*: p<0.05; **: p<0.01; ***: p<0.001

The different-volume BV categories also led to different wood densities among their offspring (Table 1, Table 2). The improved material showed higher density than the unimproved control except in the case of the offspring of high volume BV parents (Figure 2b). Contrary to what occurred with diameter, the S × BVvol interaction was not significant for this trait.

Differences in stem form among different volume BV categories were in all cases non-significant (Table 1, Table 2)

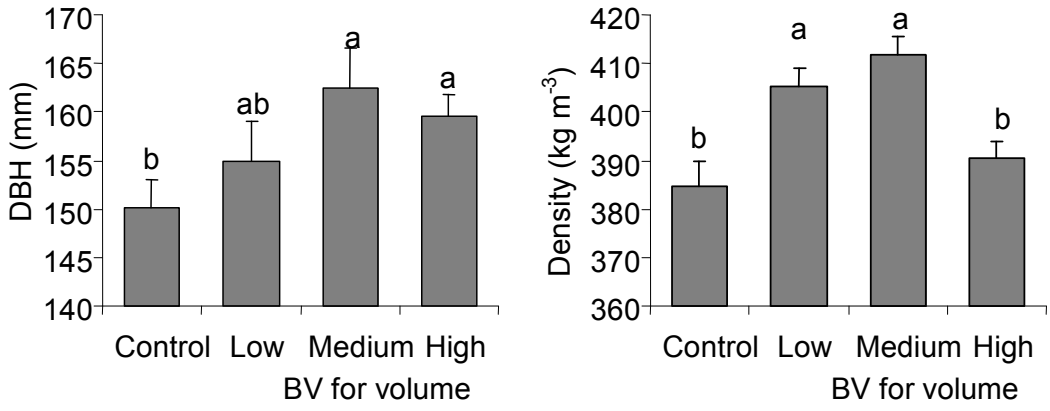

Figure 2. Across-sites diameter and wood density means (± s.e.) of the offspring of parents with high-, medium- and low estimated breeding-value for volume and of control unimproved material. Different letters denote significant (p<0.01) LSD differences among treatment means.

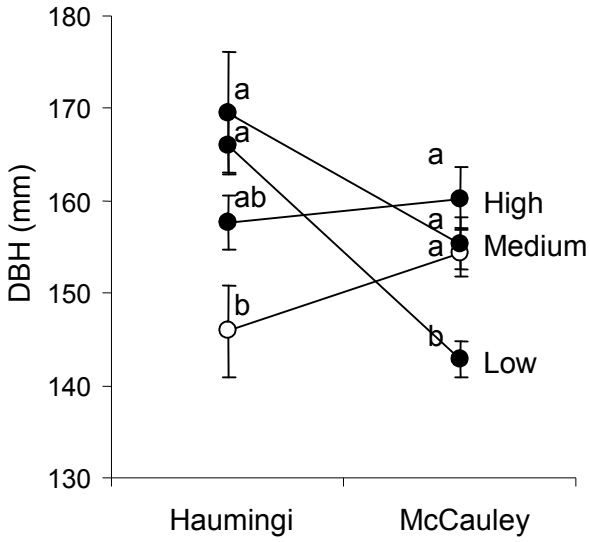

Figure 3. Diameter growth (mean ± s.e.) of the offspring of parents with high, medium and low breeding values estimates for volume (black dots) and of control unimproved material (white circles) in two NZ sites. Different lower-case letters within each site denote significant (p<0.01) LSD differences among treatment means.

Despite the higher heritability of wood density (Gea et al. 1997a), the three different groups of parents according to their BV for wood density led to offspring that did not differ in this trait (Table 3). Although the sample size for this analysis is not very high for powerful analysis, these results are again indicating poor accuracy in the estimation of the BV for this trait.

Table 3. Summary of the single-site analyses of the split-plot design (model 3). F ratios and significance levels are presented.

Source	DF	Error Term	Haumingi		McCauley	
			DBH	Density	DBH	Density
Block	5[1]	BVvol×Block	0.60	0.59	2.12	0.68
BVvol	2	BVvol×Block	0.25	1.59	6.05 *	2.54
BVden	2	error	4.04 *	1.57	5.04 *	1.05
BVvol*BVden	4	error	1.28	1.68	3.55 *	0.13

[1] DF for blocks for analyzing Density in McCauley is just 2

Significance levels*: p<0.05; **: p<0.01; ***: p<0.001

Poor accuracy of breeding-value estimates in *Eucalyptus* open-pollinated tests using seed collected in natural stands have been reported before and explained in terms of higher levels of selfing, full-sibbing and neighbourhood inbreeding in open-pollinated progeny (Hodge et al. 1996; Volker et al. 1994). These effects led to inflated additive genetic variance estimates in open-pollinated trials, and a lack of correlation between breeding values estimated from open- and control-pollinated progeny tests. Previous research indicates that these effects seem to be less important in *E. nitens* than in other *Eucalyptus* species (Hodge et al. 1996). Results presented here, however, where the performance of the different seedlots did not correspond with previous predictions, is evidence that this may be very relevant for *E. nitens*.

Conclusions

Across-site analysis revealed a realized genetic gain in diameter growth for those parents with medium- and high- volume BV of 6-8% in relation to the unimproved control. However the strong genotype-by-environment interaction indicates that results were not consistent across sites, and the realized genetic gain is strongly environment-dependent. The improved material was significantly better than the unimproved bulk provenance at one site only.

The lack of correspondence between the previous parental BV estimates for volume and wood density, and the results in these trials, indicates that previous BV estimates based on open-pollinated progeny tests were clearly unreliable, probably due to high levels of inbreeding from selfing and/or related matings, and/or the incidence of genotype-by-environment interaction. Selections based on open-pollinated testing should be used with care in *E. nitens*, as previously warned for other *Eucalyptus* species.

Acknowledgments

Comments and suggestions by Drs. Rowland Burdon, Tony Shelbourne, Luis Gea, and Satish Kumar are greatly acknowledged.

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APPENDIX 1

Origin of the parental trees tested in the two NZ sites

ID	Pederick Provenance	Subrace[1]	Locality	Population
1*	Toorongo	SCV	Thomson Valley	Thomson Valley - Mount Saint Gwinear Rd
2	Toorongo	SCV	Thomson Valley	Thomson Valley - Mount Saint Gwinear Rd
3	Toorongo	SCV	Thomson Valley	Thomson Valley - Creeks
4*	Toorongo	SCV	Thomson Valley	Thomson Valley - Mount Saint Gwinear Rd
5	Toorongo	SCV	Thomson Valley	Thomson Valley - Mount Saint Gwinear Rd
6*	Toorongo	SCV	Upper Yarra	Upper Yarra - Newlands Road
7*	Rubicon	NCV	Rubicon	Rubicon - Snobs Creek
8*	Rubicon	NCV	Rubicon	Rubicon - Snobs Creek
9	Rubicon	NCV	Rubicon	Rubicon - Little River
10	Rubicon	NCV	Rubicon	Rubicon - Little River
11*	Macalister	NCV	Mount Wellington	Mount Wellington - Miller Gap
12	Rubicon	NCV	Rubicon	Rubicon - Little River
13	Macalister	NCV	Mount Wellington	Mount Wellington - Miller Gap
14	Macalister	NCV	Mt Skene/Barkly River	Mount Skene - Lazarini Creek
15*	Rubicon	NCV	Rubicon	Rubicon - Snobs Creek
16*	Macalister	NCV	Mt Skene/Barkly River	Mount Skene - Lazarini Creek
17*	Rubicon	NCV	Rubicon	Rubicon - Little River
18	Rubicon	NCV	Rubicon	Rubicon - Barnewall Plains
19	Toorongo	SCV	Thomson Valley	Thomson Valley - Creeks
20*	Toorongo	SCV	Thomson Valley	Thomson Valley - Mount Saint Gwinear Rd
21	Macalister	CP	Connors Plain	Connors Plain - Plateau
22*	Toorongo	SCV	Thomson Valley	Thomson Valley - Marshall Spur
23	Toorongo	SCV	Upper Yarra	Upper Yarra - Newlands Road
24*	Rubicon	NCV	Rubicon	Rubicon - Royston River
25	Toorongo	SCV	Thomson Valley	Thomson Valley - Marshall Spur
26*	Toorongo	NCV	Mount Wellington	Mount Wellington - Miller Gap
27*	Toorongo	SCV	Thomson Valley	Thomson Valley - Creeks
28	Toorongo	SCV	Upper Latrobe	Upper Latrobe - Mount MacDonald
29	Toorongo	SCV	Thomson Valley	Thomson Valley - Creeks
30*	Toorongo	SCV	Thomson Valley	Thomson Valley - Marshall Spur
31	Toorongo	NCV	Mt Skene/Barkly River	Mount Skene - Lazarini Creek
32	Toorongo	SCV	Upper Latrobe	Upper Latrobe - South of Toorongo
33*	Toorongo	NCV	Upper Yarra	Upper Yarra - Mount Gregory
34	Toorongo	SCV	Upper Latrobe	Upper Latrobe - South of Toorongo
35*	Toorongo	NCV	Upper Yarra	Upper Yarra - Mount Gregory
36*	Toorongo	SCV	Upper Latrobe	Upper Latrobe - Mount MacDonald
37*	Toorongo	SCV	Thomson Valley	Thomson Valley - Mount Saint Gwinear Rd
38*	Toorongo	SCV	Thomson Valley	Thomson Valley - Mount Saint Gwinear Rd
39	Rubicon	NCV	Rubicon	Rubicon - Snobs Creek

ID	Pederick Provenance	Subrace[1]	Locality	Population
40*	Rubicon	NCV	Rubicon	Rubicon - Royston River
41	Rubicon	NCV	Rubicon	Rubicon - Quartz Creek
42*	Toorongo	SCV	Upper Latrobe	Upper Latrobe Unknown
43*	Toorongo	SCV	Thomson Valley	Thomson Valley - Marshall Spur
44	Toorongo	SCV	Upper Latrobe denticulata	Toorongo - No. 3 Road denticulata
45	Macalister	NCV	Mt Skene/Barkly River	Mount Skene - East
46*	Rubicon	NCV	Rubicon	Rubicon - Little River
47	Toorongo	SCV	Thomson Valley	Thomson Valley - Creeks
48*	Rubicon	NCV	Rubicon	Rubicon - Snobs Creek
49	Rubicon	NCV	Rubicon	Rubicon - Barnewall Plains
50	Rubicon	NCV	Rubicon	Rubicon - Little River
51*	Toorongo	SCV	SCV Unknown	Southern Central Victoria Unknown
52	Toorongo	SCV	Starling Gap	Starling Gap
53*	Rubicon	NCV	Rubicon	Rubicon - Barnewall Plains
54	Toorongo	SCV	Upper Latrobe	Upper Latrobe - Toorongo Town

[1] NCV: Northern Central Victoria, SCV: Southern Central Victorial, CP: Connors Plain.
Families included in both trials are marked with an asterisk.

APPENDIX 2

Parent trees' breeding values and associated group categories.[1]

High-volume BV				Medium-volume BV				Low-volume BV			
ID	BVv	BVd	BVden	ID	BVv	BVd	BVden	ID	BVv	BVd	BVden
37*	28.7	-2.6	M	1*	-0.1	0.0	M	19	-34.5	3.2	M
38*	26.1	-2.4	M	2	5.3	-0.5	M	20*	-15.9	1.5	M
39	26.1	-2.4	M	3	9.8	-0.9	M	21	-24.6	1.4	M
40*	37.5	-2.8	M	4*	14.3	-1.3	M	22*	-67.7	-0.4	M
41	28.9	3.8	M	5	8.6	-0.8	M	23	-38.6	0.8	M
42*	26.2	0.8	M	6*	4.0	-1.9	M	24*	-25.2	2.1	M
43*	33.3	-10.4	L	7*	22.7	-29.5	L	25	-29.9	-12.3	L
44	45.5	-8.6	L	8*	20.8	-19.4	L	26*	-24.5	-11.0	L
45	36.4	-27.6	L	9	17.7	-5.2	L	27*	-17.4	-9.9	L
46*	23.2	-18.9	L	10	12.6	-4.7	L	28	-65.0	-5.5	L
47	24.7	-16.3	L	11*	7.1	-3.9	L	29	-19.9	-17.4	L
48*	33.7	-43.2	L	12	15.6	-3.6	L	30*	-40.1	-7.3	L
49	29.3	7.8	H	13	1.0	7.7	H	31	-25.7	8.2	H
50	52.8	9.5	H	14	16.4	12.2	H	32	-74.2	8.2	H
51*	49.8	12.3	H	15*	10.8	15.5	H	33*	-87.7	14.4	H
52	23.4	13.0	H	16*	-7.1	16.0	H	34	-84.7	15.4	H
53*	39.1	15.4	H	17*	1.5	17.8	H	35*	-49.0	16.0	H
54	64.1	29.4	H	18	6.4	21.3	H	36*	-67.0	18.8	H

[1] *BVv* and *BVd* are the parental breeding values for volume and density, respectively. *BVden* is the category in which the *BVd* were grouped (H: high, M: medium, L: low). ID is the code for the open-pollinated families. Families included in both trials are marked with an asterisk.