

INBREEDING DEPRESSION FOR GROWTH, WOOD AND FECUNDITY TRAITS IN *EUCALYPTUS NITENS*

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ABSTRACT

Controlled cross pollinated (outcross), self and natural open-pollinated (OP) progenies from 12 parents of *Eucalyptus nitens* were used to estimate inbreeding depression for growth, wood and fecundity traits to 9 years of age. Growth traits (height, DBH, basal area and volume) exhibited significant inbreeding depression. Inbreeding depression was also present for number of flower buds. However, inbreeding depression was absent for wood density, relative bark thickness, frost damage, and proportion of reproductively mature individuals. In general, the magnitude of inbreeding depression was positively related to the level of quantitative dominance. Most growth traits exhibited high levels of dominance while wood density and bark thickness appeared to be almost entirely controlled by genes with additive effects. The extent to which heritabilities estimated from OPs were inflated relative to outcross estimates also appeared to be related to the level of inbreeding depression. These results suggest that variation in the extent of inbreeding depression amongst traits may affect the choice of mating design for genetic parameter estimation and tree improvement.

Keywords: *Eucalyptus nitens*, self-fertilization, open-pollination, dominance variation, specific-combining ability, fitness, breeding strategy

INTRODUCTION

Eucalyptus nitens (Deane & Maiden) Maiden is an important species for the establishment of short rotation plantations for pulpwood, particularly because of its frost hardiness (TIBBITS 1986, ELDRIDGE *et al.* 1993). In eucalypt breeding programs, inbreeding may arise when: (i) open-pollinated (OP) families are collected from the wild (HARDNER *et al.* 1996) or from seed orchards (MORAN *et al.* 1989, MONCUR *et al.* 1995); (ii) relatives are mated, especially when effective population sizes are reduced by intensive selection; or (iii) when specifically employed to purge deleterious alleles or increase selection response (reviewed in WILLIAMS & SAVOLAINEN 1996).

The most commonly reported consequence of inbreeding is a reduction in the vigor of inbred progeny, or inbreeding depression. However, inbreeding depression may not be expressed to the same degree across all traits. From a simple theoretical point of view, inbreeding depression may be related to level of dominance of a trait and may be absent for traits under purely additive genetic control (FALCONER 1989). On the other hand, inbreeding depression may occur for

strongly additive traits if rare deleterious alleles with major effect, that are masked and not expressed under outcrossing, are exposed in the homozygous state with inbreeding (RITLAND 1996). Several authors have also suggested that traits that are closely linked with fitness exhibit large dominance variation and inbreeding depression (FALCONER 1989, CRNOKRAK & ROFF 1995). Clearly, the extent of inbreeding depression for a trait will impact on the strategy employed for its improvement.

While there is considerable literature on inbreeding depression in conifers (reviewed in WILLIAMS & SAVOLAINEN 1996; DUREL *et al.* 1996), this is not the case for *Eucalyptus*, and *E. nitens* in particular. TIBBITS (1989) reports severe inbreeding depression in seed set for *E. nitens* (72 %). However, there is no published information of the effects of inbreeding for growth in the field, an important breeding objective in *E. nitens* (GREAVES *et al.* 1996a). In *E. globulus* (HARDNER & POTTS 1995a), *E. regnans* (GRIFFIN & COTTERILL 1988) and *E. grandis* (HODGSON 1976), significant inbreeding depression due to selfing developed within two years after planting and OP progenies were intermediate. In addition, there was significant inbreeding depression in

survival from 4 years after planting in *E. regnans* (HARDNER & POTTS 1997). There is also little information on inbreeding depression in wood density and fecundity in eucalypts. Wood density is an important breeding objective for pulp production from eucalypts (GREAVES *et al.* 1996a) and, if present, inbreeding depression in fecundity could compromise the success of tree improvement programs (WILLIAMS & SAVO-LAINEN 1996).

In this paper, we examine the level of inbreeding depression to 9 years after planting in an old *E. nitens* trial. This trial provides key information on the extent and possible genetic control of inbreeding depression for later age growth, fecundity and wood density traits for a commercially important species, information that is important for the development of breeding strategies for this species.

METHODS

Crossing, field design and measurements

The founding parents used in this study were 5–11 year old *E. nitens* progenies from open-pollinated seed lots collected in several localities (Table 1) that were edge trees to plantations. The eight trees used as both seed and pollen parents have been described in TIBBITS (1989). Four other trees were used as additional pollen parents. Fifteen full-sib families and 5 self families were produced (Table 1). Open-pollinated (OP) families were also collected from all parents. The only basis for selection of parents was accessibility and hence it is assumed that both parents and families represent a random sample with respect to the characters of inter-

est. Seeds from the three cross-types were germinated and transplanted into pots and grown in a heated greenhouse. Mature seedlings were planted in the field in 12 complete blocks with each family represented by a single tree plot at a spacing of 4 × 3m.

Survival was recorded at 1, 2, 4, 7, and 9 years after planting and the average proportion of planted seedlings alive at each date calculated for each cross type and family. Height was measured at planting to 4 years of age. Individual heights were adjusted for differences at planting, and the absolute height growth rate per year was calculated for each individual. Over bark diameter at breast height (DBH) was measured from age 4. Basal area was calculated from DBH, and conic volume from height and basal area at age 4 (HARDNER & POTTS 1995a).

Each individual was scored for percentage frost damage at 2 years. At the 4th, 6th, and 7th year after planting, the number of flower buds was assessed on a five point scale (0, 1–10, 11–100, 101–1,000 and greater than 1,000) and used to calculate the cumulative percentage of individuals flowering at each age. At 9 years, wood density was indirectly assessed by measuring pilodyn penetration at breast height. Pilodyn is an instrument that drives a steel pin into wood at a known force. Previous studies in *E. nitens* have shown a good relationship between pilodyn penetration and wood density in *E. nitens* (GREAVES *et al.* 1996b). A small window in the bark on the North-West side of the tree was made and 2 pilodyn readings taken and averaged. Bark thickness at breast height was measured at 7 and 9 years and relative bark thickness calculated as the ratio of bark thickness to DBH.

Table 1. Mating design for the production of controlled self (s) and outcross (x) progenies from 12 *E. nitens* parents (see TIBBITS 1989 for further information) sampled from several populations (Errinunda Plateau Victoria, E; Macalister Victoria, M; Northern New South Wales, N; Rubicon Victoria, R; Southern New South Wales, S; and Toorongo Plateau Victoria, T). Reciprocal crosses are marked with an asterisk (*). Open-pollinated families were also collected from all parents.

♀ \ ♂	E125	E130	M119	N121	R115	S127	S131	S132	T122	T123
E130			x	x*						x
M119	x	x*	s	x*	x	s	x	x	x*	x*
N121		x*	x*	s					x	s
S120		x	x						x	
S127			x*						x	
S128									s	
T122									x*	
T123										

Analysis

Chi-squared statistics were used to test for significant differences in survival over specific intervals among cross types and differences in percentage of individuals flowering among cross types and years.

The significance of inbreeding depression for different traits was examined using the procedure PROC MIXED in the statistical package SAS (SAS 1992) with a univariate linear model that included fixed block and cross-type (outcross, OP, self) effects, the random effect of families within cross-type and a random residual effect:

$$y = \mu + \text{block} + \text{cross type} + \text{family}(\text{cross type}) + \text{residual.}$$

Residuals were tested for normality and appropriate transformations were employed for traits where the distribution was not normal. Least square means (and their standard errors) were estimated for each cross-type, and two contrasts: (i) self against outcross; and (ii) OP against outcross, were undertaken to test the significance of the difference between cross types. While the magnitude of the residual variances may differ between self and outcross progenies under this model (LYNCH 1989, HARDNER & POTTS 1995a), the approach adopted here appears to be a reasonable approximation for hypothesis testing without the introduction of a more complicated genetic model (cf. DE BOER & HOESCHELE 1993). In addition, as the self and outcross families are assumed to represent a random sample, estimates of inbreeding depression should not be greatly biased by the incomplete mating design.

Inbreeding depression was estimated as:

$$ID \% = (X_{\text{out}} - X_{\text{inbred}}) / X_{\text{out}} * 100$$

where X_{out} was the least square mean for the controlled outcrosses and X_{inbred} was the least square mean for the inbred progenies (self or OP), with the standard error calculated after JOHNSTON and SHOEN (1994).

To investigate the relationship between inbreeding depression and mode of genetic control of a trait, phenotypic variance components and their standard errors were estimated for each trait from outcross progenies. The computer program REML VCE v3.2 (GROENEVELD 1995) was used with a univariate mixed linear model that included blocks (fixed), the additive genetic effect of each individual tree (a_i , random), the effect of each specific combination of parents (SCA, random), and residual effects (R , random):

$$y = \mu + \text{blocks} + a_i + \text{SCA} + R$$

Additive genetic variation (σ_A^2) was estimated directly in the analysis and dominance genetic variation (σ_D^2) was estimated by assuming that σ_{sca}^2 represented 1/4 of σ_D^2 (FALCONER 1989), and thus environmental variance (σ_E^2) was ($\sigma_R^2 - 3/4 * \sigma_D^2$). Epistatic effects were ignored as most of this variance would be expected to occur within the environmental variance. Narrow sense and broad sense heritability were estimated as:

$$h^2 = \sigma_A^2 / (\sigma_A^2 + \sigma_D^2 + \sigma_E^2) \text{ and;}$$

$$H^2 = (\sigma_A^2 + \sigma_D^2) / (\sigma_A^2 + \sigma_D^2 + \sigma_E^2) \text{ , respectively.}$$

The level of dominance was estimated as:

$$D = \sigma_D^2 / (\sigma_A^2 + \sigma_D^2)$$

For comparison, additive genetic variation and narrow sense heritabilities were also estimated using a purely additive model where effectively SCA variation was set to zero. The difference between the fit to the data of the full (additive+sca) and purely additive model was compared using the likelihood ratio test, where twice the negative difference of the log likelihoods approximates a χ^2 -square statistic with one degree of freedom (MCCULLAGH & NEDLER 1989). Standard errors for the above parameters were obtained directly from the analysis or calculated after KENDALL & STUART (1963).

To examine the relationship between inbreeding depression and OP heritabilities for different traits, phenotypic variance components were estimated from OP progenies using PROC MIXED with a simple model which only included fixed block effects, the random effect of parents (σ_{PAR}) and residual effects (σ_{Rop}). Narrow sense heritability was estimated from OP progenies (h_{OP}^2) by assuming a purely additive model (SQUILLACE 1974) and 30% selfing (WHITEMAN *et al.* 1992) such that:

$$H_{\text{OP}}^2 = 2.5 * \sigma_{\text{PAR}}^2 / (\sigma_{\text{PAR}}^2 + \sigma_{\text{Rop}}^2)$$

Standard errors for h_{OP}^2 were calculated after KENDALL & STUART (1963).

RESULTS

Survival of planted seedlings to 9 years was high for all

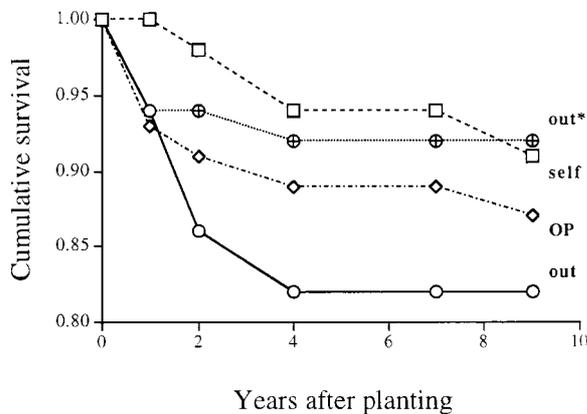


Figure 1. Proportion of planted seedlings surviving to different ages for self, open-pollinated (OP) and outcross (out, including family E130*M119; out*, excluding family E130*M119) progenies of *E. nitens*.

cross-types and there was no evidence of inbreeding depression for survival. Overall, selfs survived better than outcrosses and OPs were intermediate (Fig. 1), although the differences between cross types were not significant ($P > 0.1$). However, the poorer survival of the outcrosses was almost entirely due to the near complete failure of cross E130*M119 (including reciprocal) by 4 years of age. When this family was excluded from the analysis, the survival to 9 years of the selfs and outcrosses was very similar. The growth between planting and 2 years of family E130*M119 was also much poorer than the other outcross families and hence it was also excluded from the analyses of the other traits.

Significant inbreeding depression due to selfing had developed in height by 1 year after planting (27 %, Table 2). At two years of age inbreeding depression was 30 % (Table 2), but declined to 14 % by 4 years of age (Table 3). This was due to a reduction in inbreeding depression for height growth between 2 and 4 years of age (9 %, Table 3) compared to that between 1 and 2 years of age (31 %, Table 2).

Table 2 Least square means of outcross (Out), open-pollinated (OP) and self progenies of *E. nitens* for height (after adjusting for differences at planting, m) and height growth per year (m.yr^{-1}) at different ages. Superscripts to inbreeding depression represent the level of significance of the contrast between inbred (i.e. self or OP) and outcross progenies (ns $P > 0.05$, * $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$), and standard errors are given in parentheses.

Height	Out	OP	Self	Inbreeding depression	
				Self	OP
1 year	0.59	0.56	0.43	27** (10)	5 ^{ns} (6)
2 years	2.62	2.49	1.88	28*** (10)	5 ^{ns} (5)
growth (1-2 years)	2.04	1.92	1.45	31** (7)	9 ^{ns} (5)
4 years	9.13	8.59	7.82	14*** (3)	6 ^{ns} (3)
growth (2-4 years)	3.22	3.04	2.93	9 ^{ns} (5)	5 ^{ns} (3)

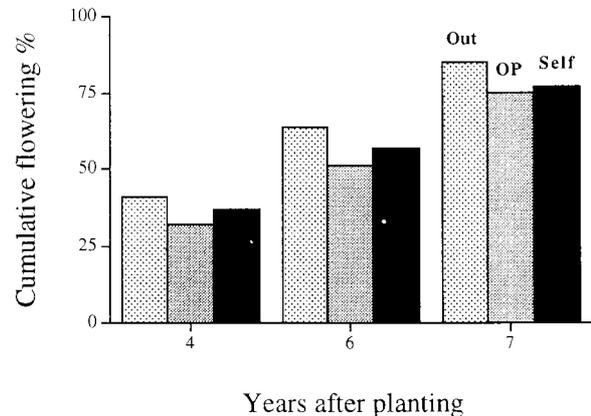


Figure 2. Percentage of plants that had flowered by different ages for outcross (Out), self and open-pollinated (OP) progenies of *E. nitens*.

At 4 years of age, inbreeding depression for DBH was 22 % and increased slightly to 28 % by 9 years after planting (Table 3). Inbreeding depression was more severe for basal area (37 %) and conic volume (40 %) at 4 years.

On the other hand, inbreeding depression was negligible for relative bark thickness, wood density at 9 years as assessed by pilodyn penetration, and percentage frost damage at 2 years (Table 4). Inbreeding depression was also not significant for time to first flowering (never flowered $\chi^2_2 = 3.38$, $P > 0.1$) (Fig. 2). Inbreeding depression for the number of buds at 7 years was high (37 %), but the contrast between self and outcross progenies was not significant and may be a consequence of the greater genetic variation within the self families leading to conservative tests under the statistical model employed in this study.

In general, there was a trend for traits with a high estimated level of dominance to also exhibit a large level of inbreeding depression, despite the poor precision involved in the estimation of these parameters (Fig. 3, Tables 3 and 4). All traits appeared to be under

Table 3. Estimated least square means, inbreeding depression, and components and ratios of variation for diameter at breast height (DBH, cm), basal area (BA, cm²) and conic volume (Vol, m³x10⁴) at different ages for outcross (Out), open-pollinated (OP) and self *E. nitens* progenies. Superscripts to inbreeding depression represent the level of significance of the contrast between the mean of the inbred progenies (ie. self or OP) and the mean of the outcross progenies (^{ns} P > 0.05; * P < 0.05; ** P < 0.01; *** P < 0.001). Additive (σ^2_A), dominance (σ^2_D) and non-genetic (σ^2_E) variation, narrow sense (h^2) and broad sense (H^2) heritabilities, and the level of dominance (D) were estimated from outcross progenies. Also shown are negative twice the log_e likelihoods ($-2\log_e L$) calculated when variance components were estimated for each trait with: (i) a full genetic model (*Full*); and (ii) a purely additive (*Add*) genetic model. Narrow sense heritability was also estimated from OP progenies (h^2_{OP}) by assuming a coefficient of relationship of 1/2.5. The standard error for each estimate is given in the second line in italics.

Trait	Means			Inbreeding depression		Variance components								
	Out	OP	Self	Self	OP	Model	Outcross						-2logL	h^2_{OP}
							σ^2_A	σ^2_D	σ^2_E	h^2	H^2	D		
DBH (4 yr)	12.3	10.7	9.6	22**	13*	Full	1.8	3.6	3.7	0.21	0.61	0.67	319.3	0.82
					6		3		2.5	3.0	2.7	0.28		0.46
						Add	6.1		3.9	0.36			320.3	
							2.9		1.6	0.18				
DBH (7 yr)	22.5	19.9	17.0	24**	11ns	Full	3.7	4.4	12.9	0.18	0.39	0.54	337.1	0.85
					5		3		4.5	5.3	4.8	0.19		0.29
						Add	8.1		14.1	0.24			337.5	
							4.7		2.9	0.17				
DBH (9 yr)	28.6	25.0	21.0	28**	13*	Full	0	14.0	18.0	0	0.44	1.00	333.3	0.78
					5		3		0.0	7.2	5.9	0.00		0.18
						Add	8.1		25.8	0.24			337.0	
							6.2		4.2	0.17				
BA (4yr)	125	98	81	35**	21ns	Full	1269	732	904	0.39	0.61	0.37	321.8	0.86
					10		6		1430	1196	1154	0.38		0.55
						Add	2330		1281	0.65			322.0	
							978		540	0.19				
Vol (4 yr)	250	190	150	40**	24*	Full	8.2	1.2	8.6	0.46	0.51	0.13	326.5	1.30
					11		7		7.0	4.0	4.6	0.28		0.35
						Add	9.9			0.53			326.5	
							4.4			0.18				

at least a moderate degree of genetic control, as broad sense heritability (H^2) estimates ranged between 0.20 (height 2 years after planting) and 0.66 (Tables 3 and 4; $H^2 = 0.23$ for height growth between 2 to 4 years). For height at 4 years, height growth between 2 and 4 years, relative bark thickness at 7 and 9 years, and wood density at 9 years, traits that exhibited low inbreeding depression, the estimated level of dominance was near zero. The estimated level of dominance was moderate for diameter at breast height at 4 and 7 years, and for

basal area at 4 years, traits for which inbreeding depression was moderate to high. For number of flower buds at 7 years and diameter at breast height at 9 years, the estimated level of dominance was near one with inbreeding depression also high. The exception to this trend was volume at 4 years, for which the estimated level of dominance was low but inbreeding depression was high. Estimates of the level of dominance for height from planting to 2 years were greater than 1 and are not presented. These results may be due to impre-

Table 4. Estimated least square means, inbreeding depression, and components and ratios of variation for percentage frost damage (Frost), number of flower buds (Buds), relative bark thickness (Bark, mm.cm⁻¹) and wood density as measured by pilodyn penetration (Dens, mm) at different ages for outcross (Out), open-pollinated (OP) and self *E. nitens* progenies. Means for percentage frost damage and number of flower buds are expressed on the back transformed scale, while all the other parameters refer to the scale at which the analysis was undertaken (Frost^{0.5} and Buds^{1.25}). Abbreviations and symbols follow those of Table 3.

Trait	Means			Inbreeding depression		Model	Variance components							
	Out	OP	Self	Self	OP		Outcross				OP			
							σ^2_A	σ^2_D	σ^2_E	h^2	H^2	D	$-2\log_e L$	h^2_{OP}
Frost (2 yr)	1.73	1.89	1.66	4 ns	-9 ns	Full	0.00	0.28	0.20	0.00	0.60	1.00	280.1	0.11
				7	5		0.00	0.12	0.09	0.00	0.24	0.00		0.15
						Add	0.22		0.31	0.42			281.5	
							0.11		0.07	0.17				
Buds (7 yr)	2.54	1.90	1.59	37 ns	25 ns	Full	0.14	1.84	1.24	0.04	0.60	0.93	343.5	0.89
				15	10		0.56	1.16	0.94	0.17	0.40	0.27		0.30
						Add	1.54		2.00	0.44			345.6	
							0.75		0.44	0.17				
Bark (7 yr)	456	468	472	4 ns	3 ns	Full	2.5	0.4	3.7	0.38	0.42	0.14	355.0	0.85
				4	3		1.0	0.8	0.9	0.13	0.18	0.24		0.31
						Add	2.5		4.1	0.38			355.1	
							1.0		0.7	0.13				
Bark (9 yr)	422	405	385	9 ns	4 ns	Full	4.2	0.0	2.2	0.66	0.66	0.00	302.0	0.78
				5	3		1.4	0.0	0.8	0.16	0.16	0.00		0.31
						Add	4.2		2.2	0.66			302.0	
							1.4		0.8	0.16				
Dens (9 yr)	11.1	11.9	12.0	8 ns	7 ns	Full	1.33	0.0	1.79	0.42	0.42	0.00	314.3	0.57
				12	8		0.51	0.0	0.31	0.13	0.13	0.00		0.27
						Add	1.33		1.79	0.42			314.3	
							0.56		0.34	0.13				

cise estimation of the genetic parameters or failure to account for other factors such as growth differences from the grouping of families in the nursery. These factors could also explain the finding of low inbreeding depression despite a high level of dominance for percentage frost damage at 2 years (Table 4).

Obviously, the standard errors on the genetic parameters estimated from the outcross progenies are large due to the small number of parents and families used in the mating design. However, for traits where the estimated level of dominance was large, the full genetic model (additive + SCA effects) provided a better fit to the data than a purely additive model as judged by the comparison of the log likelihoods (Tables

3 and 4), although the difference was not significant for any trait ($\chi^2_1 = 3.83$ for a one tailed test at $P < 0.05$). Heritabilities also differed considerably under the two different models for those traits with large dominance variance component estimates.

There also appeared to be a tendency for narrow sense heritability estimated from OP progenies to be greatly inflated, relative to heritabilities estimated from outcross progenies, for traits with large inbreeding depression (Tables 3 and 4). This was particularly the case for diameter at breast height at 4 and 9 years, basal area and volume at 4 years, and number of flower buds at 7 years. On the other hand, for traits such as: frost damage at 2 years; bark thickness at 7 and 9 years; and

Table 5 Published estimates of inbreeding depression due to selfing (%) for growth traits (DBH, diameter at breast height) in *Eucalyptus* species calculated using all survivors at each age (¹ HARDNER & POTTS 1995a; ² GRIFFIN & COTTERILL 1988; ³ HARDNER & POTTS 1995b; ⁴ HODGSON 1976; ⁵ POTTS *et al.* 1987; ⁶ this study) (ns $P > 0.05$; * $P < 0.05$; ** $P < 0.01$; *** $P < 0.001$).

Traits	<i>E. globulus</i> ¹	<i>E. regnans</i>	<i>E. grandis</i> ⁴	<i>E. gunnii</i> ⁵	<i>E. nitens</i> ⁶
Height					
planting		10 ²			
1 year	17*	22 ²	38**	13***	27 (10)**
2 years	22*	17 ²			28 (10)***
4 years	26*	11 ²			14 (3)**
DBH					
4 years	24*	18 ²			22 (6)**
Basal area					
4 years		50* ³			37 (10)**
Volume					
4 years	48*	37* ²			40 (11)**

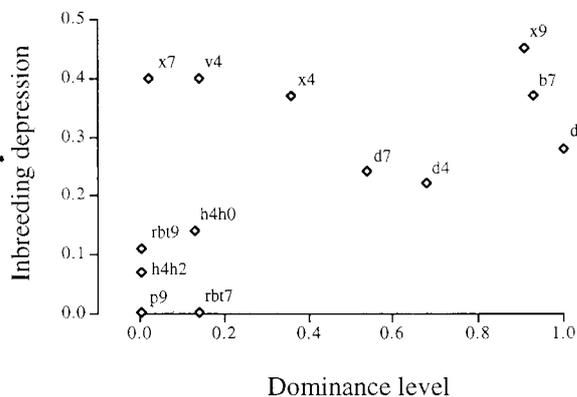


Figure 3. Inbreeding depression due to selfing against level of dominance estimated from outcross progenies for different traits in *E. nitens* (h4h0, height at 4 years adjusted for planting height; h4h2, height growth between 2 and 4 years; d4, d7 and d9, DBH at 4, 7 and 9 years; x4 and v4, basal area and conic volume at 4 years, rbt7 and rbt9, relative bark thickness at 7 and 9 years; b7 number of buds at 7 years; w9, wood density as assessed by Pilodyn penetration at 9 years.

wood density at 9 years, where inbreeding depression was low, OP estimates of narrow sense heritability were similar to outcross estimates.

DISCUSSION

Inbreeding depression

The failure to detect inbreeding depression for survival in this trial contrasts with some other studies with eucalypt species. In *E. gunnii* (POTTS *et al.* 1987) and *E. regnans* (HARDNER & POTTS 1997), significant

inbreeding depression had developed by 1 year of age. In addition, only 38 % of the planted selfs and 60 % of the planted outcrosses had survived by age 10 in the *E. regnans* trial. In *E. globulus*, survival rates to 4 years were comparable to our levels and there was no significant difference between selfs and outcrosses, although there was a trend for outcross survival to be higher (HARDNER & POTTS 1995a). The higher survival rates in this study relative to the *E. regnans* trial may be a consequence of: (i) a lower competition intensity as this study was planted at wider spacings (4 × 3m compared to 3 × 2m in the *E. regnans* trial) and maintained generally free of competing weed growth; or (ii) a higher number or greater severity of deleterious genes in *E. regnans*; or both (iii).

The near complete failure of a single outcross family E130*M119 including the reciprocal by age 4 is of interest. TIBBITS (1988) also noted the poor nursery growth of this family. Individual E130 is from the Errinunda Plateau (Table 1) and since the establishment of this trial the Errinunda population has been reclassified as a separate species, *E. denticulata* (COOK & LADIGES 1991). However, this may not fully explain the poor performance of E130*M119 as cross incompatibility among closely related species appears to be rare (POTTS *et al.* 1987, TIBBITS 1988) and other families (Table 1) produced by crossing with E130, and another Errinunda parent (E125), do not show such poor performance.

Inbreeding depression for growth traits in *E. nitens* reported in this study are generally similar to the levels reported in studies of other species of *Eucalyptus*

(Table 5). Despite the small number of parents and families examined in this study, inbreeding depression is moderate for height and diameter at breast height, but severe for basal area and conic volume. Across all species, inbreeding depression due to selfing for height is generally manifest by the end of the first growing season. For diameter at breast height at 4 years, inbreeding depression due to selfing appears to be consistently around 20 % in *E. globulus*, *E. regnans*, and *E. nitens*. Inbreeding depression for basal area and conic volume is also severe in these three species. Variation in estimates among studies may partly be due to differences in growth patterns and trial design or poor estimation due to a limited genetic sample (*E. globulus*, HARDNER & POTTS 1995a; this study).

This study is the first to report the absence of inbreeding depression for wood density as assessed by pilodyn penetration in *Eucalyptus*. Similar to our result, inbreeding depression for wood density assessed by pilodyn penetration was absent in *Pinus radiata* (WILCOX 1983). We have also found little evidence of inbreeding depression in relative bark thickness, frost damage and time to first flowering. Consistent with an absence of inbreeding depression, VOLKER *et al.* (1994) found no difference in the frost damage between OP and controlled cross nursery-raised *E. nitens* seedlings, although these authors did report a difference for *E. globulus* seedlings. The absence of inbreeding depression for time to first flowering in *E. nitens* is consistent with results for *E. globulus* (HARDNER & POTTS 1995a). On the other hand, we did find large inbreeding depression (although not significant) for the number of flower buds at 7 years which is consistent with inbreeding depression in cone number reported for conifer species (WILLIAMS & SAVOLAINEN 1996).

Relationship between inbreeding depression and genetic variation

The absence of inbreeding depression in some traits is clearly not due to an absence of genetic variation as all traits appeared to be under at least moderate genetic control ($H^2 > 0.20$, Tables 3 and 4). However, the level of inbreeding depression for a trait was generally related to its level of dominance estimated from the outcross progenies, despite the low precision of the genetic parameter estimates. WILCOX (1983) also reports low dominance genetic variation for wood density as assessed by pilodyn penetration (a trait for which inbreeding depression is absent) in *P. radiata*. At the other extreme, moderate levels of dominance have been reported for volume at 9 years in *E. grandis* (VAN WYK 1990), similar to our findings for growth traits after age 4, traits which exhibit severe inbreeding

depression.

A relationship between inbreeding depression and the level of dominance variation in outcross progeny for a given trait is not unexpected as inbreeding depression arises if quantitative dominance is directional (FALCONER 1989). However, inbreeding depression may also be due to the exposure of deleterious mutations which are carried as the heterozygote where the effect is masked such as reported in *E. gunnii* (POTTS 1990). If these mutations are rare in the population, then crossing among unrelated individuals (particularly those from different populations) may not unite recessive alleles and hence dominance will not be apparent under outcrossing. This may explain the failure to detect dominance from the factorial analysis despite the presence of inbreeding depression for some traits, although, again, this could be due to poor estimation of the level of dominance for these traits.

In addition, it is interesting to note that in this study some of the traits which exhibit high levels of dominance variation (e.g. flower bud number at 7 years, and later age growth traits) can be considered as important fitness traits, while others which exhibit little dominance (e.g. wood density, relative bark thickness) may be less important. This is consistent with the hypothesis that the importance of a trait to fitness and its level of inbreeding depression are related (FALCONER 1989, CRNOKRAK & ROFF 1995).

The response of OP progenies

The intermediate performance of the OP progenies is consistent with the presence of a mixture of outcross and inbred progeny within the same OP family. Levels in OP inbreeding depression for growth traits in *E. nitens* to 4 years of age are comparable to *E. globulus* (HARDNER & POTTS 1995a), but are higher than for *E. regnans* (GRIFFIN & COTTERILL 1988). HODGE *et al.* (1996) also found depression in *E. nitens* OP progenies for volume at 2 years of age. Levels for basal area in *E. nitens* after age 4 are comparable to those for *E. regnans* (HARDNER & POTTS 1997). However, there are problems comparing OP inbreeding depression across studies. Firstly, the genetic quality of the outcrosses in the OPs may not be comparable to that of the controlled outcrosses used to calculate inbreeding depression. For example, the OPs in this study were sampled from plantations, not from natural populations, and the controlled outcrosses were produced by inter-population crosses. Secondly, outcrossing rates may vary among studies.

Inflated heritabilities for growth traits estimated from OP families compared to estimates from controlled cross progenies is considered to be a conse-

quence of a mixture of inbred and outcross progenies in OP families, a variation in the proportion of each cross type among families and significant inbreeding depression for these traits (GRIFFIN & COTTERILL 1988, BORRALHO & POTTS 1996, HODGE *et al.* 1996). Despite the low precision of our variance component estimates, all growth traits had inflated OP heritability estimates and significant inbreeding depression. This trend was also evident for the number of flower buds at 7 years. On the other hand, there was little inflation of heritabilities estimated from OP progenies for relative bark thickness at 7 years and pilodyn penetration at 9 years, traits which exhibited no inbreeding depression. In this context, our OP estimate of 0.56 for the heritability for wood density as assessed by pilodyn penetration is well within the range estimated from *E. nitens* OP progenies (GREAVES *et al.* 1996b).

Implications for tree breeding

The results from this study suggest that the level of inbreeding depression for a trait may influence the mating system employed to estimate other genetic parameters for the trait. While OP is a commonly used mating design because of its low cost, for traits that exhibit dominance and inbreeding depression OP heritabilities can be expected to be inflated, particularly if outcrossing rates vary among families (HARDNER *et al.* 1996). In addition, OP progenies tend to underestimate the level of G × E interaction and the correlation between breeding values under OP and under outcrossing may be poor for growth traits (GRIFFIN & COTTERILL 1988, HARDNER & POTTS 1995a, HARDNER & POTTS 1995b, HODGE *et al.* 1996). On the other hand, OP may be an efficient mating design for traits such as wood density where inbreeding depression appears to be absent, if account is made for the inflation of the expression of additive variation due to inbreeding and if the outcross progeny within the OP families are from several male parents (SQUILLACE 1974, ASKEW & EL-KASSABY 1994).

The level of dominance and inbreeding depression for particular traits may also influence the choice of breeding strategy. For traits such as wood density that exhibit little inbreeding depression, OP based strategies can be expected to produce reasonable gains at low cost as progeny performance will not be biased by inbreeding depression. However, enforced inbreeding may lead to higher gains, as the expression of additive variation is greater than under outcrossing (FALCONER 1989). On the other hand, OP strategies may not be efficient for growth traits, such as DBH, where dominance and inbreeding depression appear to be large and may confound the expression of additive genetic effects

(HARDNER & POTTS 1995a). In these cases, controlled pollination strategies would be expected to produce greater gains from breeding. While reciprocal recurrent selection strategies typically involve crossing among superior unrelated parents, if inbreeding depression is due to rare deleterious alleles with major effect, enforced inbreeding may enable these alleles to be purged from the breeding population in early generations (WILLIAMS & SAVOLAINEN 1996). However, this strategy may be compromised in *E. nitens* due to inbreeding depression in the number of flowering buds. In addition, purging may not be effective if inbreeding depression is mainly due to quantitative dominance (HEDRICK 1994), as suggested for some traits in this study.

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