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Effects of Inbreeding on Growth, Stem Form and Rust Resistance in *Pinus elliottii*

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Abstract

Effects of relatively low levels of inbreeding were studied in a slash pine (*Pinus elliottii* ENGELM. var. *elliottii*) experiment established over 4 test sites in 1980 and 1981. There were 3 levels of inbreeding: outcrosses (F=0), half-sib (F=0.125) and backcrosses (F=0.25). Measurements of growth, rust incidence, stemform and forking were taken in 1982, 1983, 1986, 1988 and 1993. Grandparental contribution to the progeny of each crossing type was not the same, i.e. there were some grandparents included in outcrosses that were not included in backcrosses. For this reason, adjustment to growth measurements based on a priori prediction of volume growth breeding value was carried out through covariance analysis. Adjustment for rust breeding value to rust scores had no effect.

Inbreeding depression was found to be linearly related to the inbreeding coefficient F with no significant quadratic effects for

any trait. Inbred families were shorter and had smaller diameters and volumes than those that were outcrossed. On the other hand, analysis of the standard deviations and coefficients of variation of the traits showed significant quadratic effects, such that inbred families had higher variance than those that were outcrossed, but backcrosses did not necessarily have higher variances than half-sib crosses.

Inbreeding depression for plot volume was not the same for families derived from 2 grandparents of similar breeding value.

The implications of these results on inclusion of relatives in wind-pollinated seed orchards were investigated. It was better to include parent-offspring pairs than either full-sib or half-sib families because the proportion of related matings was less. Fewer siblings in the full-sib or half-sib families together with more families would have the same effect of reducing related matings. It was better to include half-sibs than full-sibs because the increased number of half-sib survivors was outweighed by the greater depression exhibited by full-sibs.

Key words: inbreeding depression, slash pine, growth, stem form, rust resistance.

FDC: 165.41; 181.6; 174.7 *Pinus elliottii*.

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Introduction

Among predominantly outcrossing species, inbreeding has long been known to cause reduced performance for traits related to fitness such as vigour and fertility (FALCONER, 1981). This means that when mating occurs between relatives, offspring tend on average to be less vigorous and less fertile than outcrosses. This inbreeding depression is entirely removed when inbred individuals are outcrossed, and in some circumstances performance is enhanced through hybrid vigour. Inbreeding depression does not affect all traits to the same extent. FALCONER (1981) gives examples of inbreeding depression varying from 0.6% per 0.1 units of inbreeding coefficient (F) for mouse body weight to 7.2% for mouse litter size. JONES (1939) found that ear height in maize was unaffected by 5 generations of selfing, but yield was reduced by 79% and plant height by 29% after 30 generations of selfing.

Under normal circumstances, inbreeding tends to increase homozygosity. Selfing a heterozygote at a particular locus produces both homozygotes as well as heterozygotes in the progeny. In the absence of dominance this should not affect the population mean since both homozygotes are produced with the same frequency on average. But where there is dominance, selfing the heterozygote (which looks like the dominant homozygote) produces homozygote recessives which look different from the homozygote dominant and the heterozygote. If the locus affects a quantitative trait and the recessive allele has less effect than the dominant, selfing will reduce the mean. Over many such loci, in which the dominance is always in the same direction, the situation would be called directional dominance and is usually present when inbreeding depression occurs (FALCONER, 1981; WILLIAMS and SAVOLAINEN, 1993).

According to FISHER (1930), dominance at a locus evolves through the action of modifiers in response to selection and that it is in the direction of the favoured allele. BREESE and MATHER (1960) found evidence that this is generalised over many loci into directional dominance in response to directional selection. Traits exhibiting the strongest directional selection are fitness traits such as viability, numbers of seeds, litter size and fertility, and should therefore exhibit the greatest inbreeding depression. Seed yield in maize would be expected to be closely related to fitness and therefore exhibit directional selection and high levels of inbreeding depression (see JONES, 1939). Traits with no directional dominance should be those in which selection is for an intermediate optimum, and there should be little or no inbreeding depression. For example, one would expect that intermediate ear height in maize would be an advantage because too large an ear would cause the stem to break and too small an ear would not be pollinated.

Overdominance has been invoked as a mechanism for inbreeding depression by ZIEHE and ROBERDS (1989). Here, the frequency of heterozygotes (which have a mean higher than either homozygote) is reduced under inbreeding thus reducing the population mean. There are very few examples of overdominance for pines (see BARNES *et al.*, 1987) and its presence is not necessary for there to be inbreeding depression (see above).

Epistasis can also act to produce inbreeding depression, but only in the presence of directional dominance (CROW and KIMURA, 1970). This seems reasonable if FISHER's view of the evolution of directional dominance arises through inter-locus interactions modifying dominance. The existence of epistasis is indicated when the relationship between inbreeding depression and F is not linear (FALCONER, 1981). However, even if the relationship is linear, then positive and negative epistatic effects may still be present but cancel each other out (HALLAUER and MIRANDA, 1981; WILLIAMS and SAVOLAINEN, 1993).

When deleterious alleles are recessive, selection is against them when in the homozygous state and they will therefore have low frequency in a population. Most such alleles will occur in heterozygotes, also at low frequency. Homozygote recessives will be very rare except in the progeny of relatives in which case their mean will be very much lower than the population mean, thereby exhibiting high inbreeding depression. If the deleterious alleles merely reduce growth and there is not strong selection against them, it is possible for their frequency to be high. In this case, the frequency of homozygote recessives would also be high, producing a low population mean close to the mean of inbred progeny. The similarity of these 2 means would mean that the observed effects of inbreeding depression would also be low.

Variance within inbred lines becomes small as lines become more inbred (FALCONER, 1981). As homozygosity within lines increases, allele frequencies become either 0 or 1. However, early in the inbreeding process, variance within lines for a single recessive allele with frequency 0.1 increases before falling after the first or second generation (ROBERTSON, 1952). Extension to many loci would be expected to delay the fall in within-line variance; RUDOLPH (1981) found no difference between inbreds and outcrosses of jack pine for within-line variation in height.

Most studies of inbreeding in forest trees relate to studies involving selfs (e.g. SQUILLACE and KRAUS, 1962; ERIKSSON *et al.*, 1973; SORENSON and MILES, 1974; WILCOX, 1983; LUNDKVIST *et al.*, 1987) although there are a few studies involving other levels of inbreeding (e.g. GANSEL, 1971; ANDERSSON *et al.*, 1974; LAYTON and GODDARD, 1983; SNIEZKO and ZOBEL, 1988). The most obvious effect in conifers is reduced seed yield (FRANKLIN, 1970; GRIFFIN and LINDGREN, 1985) where selfing reduces the proportion of filled seed by about half for *Pinus radiata* (GRIFFIN and LINDGREN, 1985) and about 66% in other pine species (FRANKLIN, 1970). Growth rates are also affected by inbreeding depression. SORENSON and MILES (1982) quote figures of between 1.7% and 4.7% per 0.1 F for height from other studies of selfs in several conifer species. ANDERSSON *et al.* (1974) found effects of inbreeding depend on the trait under consideration and the age at which it was measured. WILCOX (1983) found that the effects of inbreeding in radiata pine seemed to vary with age, it was 3.6% per 0.1 F for nursery height and 2.8% for height at 4 years, measured on the same trees. SORENSON and MILES (1982) found the reverse for height in Douglas-fir (4.8% at planting, 5.8% at 10 years), ponderosa pine (6.0% and 7.2%) and noble fir (5.4% and 6.0%). In WILCOX' (1983) study, the effects of inbreeding on diameter did not seem to change between 4 and 7 years of age.

Studies in trees on traits other than growth seem very sparse. WILCOX (1983) reports inbreeding depression of less than 1% per 0.1 F for stem straightness and malformation, 3.97% for branching quality and essentially zero for wood density in radiata pine.

This paper reports results of a comparison of growth, survival, rust resistance, stem straightness and forking in control-pollinated families with several mild inbreeding levels. The effects of inbreeding depression on these traits and how they change with time are presented together with evidence on the significance of epistasis and allele frequencies. Effects of inbreeding in families derived from two different grandparents is also reported. Results on germination, early growth and survival from the experiments reported here were presented by LAYTON and GODDARD (1983). Implications of the growth results are discussed for several seed orchard designs in which relatives are included.

Materials and Methods

Controlled pollinations, beginning in 1975, were carried out in a clonal seed-orchard at White Springs, Florida to establish groups of families with 3 levels of inbreeding: $F=0$ (outcrosses, OC), $F = 0.125$ (half-sib matings, HS) and $F = 0.25$ (backcross matings, BC). The orchard consisted mainly of second-generation selections from open-pollinated progeny tests, with between 2 and 5 clones from each of 11 families used for crossing (LAYTON and GODDARD, 1983). Outcrosses were formed from crosses between clones originating from different open-pollinated families. Half-sibs were formed by crossing clones derived from the same open-pollinated family and back crosses were obtained by crossing an orchard clone with its female parent. For half-sibs we assume that the probability of any 2 clones used for these crosses having the same pollen parent is small and that the actual inbreeding level was the same as the theoretical level (i.e. $F=0.125$).

No attempt was made to balance family representation among the 3 types of crosses so it is possible that observed differences between cross types merely reflect differences in genetic background (BURROWS and ASKEW, 1982). Parents involved in this study are also represented in many other tests and so their performance is reasonably well-known through their breeding values (estimated by best linear prediction, BLP – WHITE and HODGE, 1989). We attempted to take account of parental imbalance by using breeding values for the parental clones (i.e. their value judged from the performance of their progeny in other tests) as shown below.

A total of four field tests was planted near Lake Palestine, Florida (Table 1), on cleared and mounded sites. Each planting had 10 blocks arranged in a split-plot configuration in which the main plots in each block were cross type (OC, HS, or BC) and subplots in each main plot contained 4 or 5 different families of each type, each family represented by a 4- or 5-tree row plot. Two field tests (3A and 3B) were planted in 1980 on adjacent sites using 27 families representing all 3 crossing types (21 families in 3A and 14 in 3B). A further 2 field tests (4A and 4B) were planted in 1981 again on adjacent sites close to but on wetter land than tests 3A and 3B. There were 25 families in test 4A (only half-sib and outcrosses represented) and 40 in test 4B. Only 2 families were common to both 1980 and 1981 plantings.

Measurements were carried out in 1981 for tests 3A and 3B and on all 4 tests in 1982, 1983, 1986, 1988 and 1993. This meant that measurements (except at 2 years) are on trees 1 year older for tests 3A and 3B compared with tests 4A and 4B. Heights and survival were measured on all occasions, diameters and various scores in 1986 and thereafter. Scores were presence (=1), absence (=0) of rust, forks (or ramicornes) and straight stems. Diameters were measured at breast height (1.3m) using a diameter tape and heights were measured using a Suunto clinometer. Tree volumes were calculated using a standard volume equation for slash pine in Florida (GODDARD and STRICKLAND, 1968). Plot volumes were calculated by multiplying mean tree volume for each 4- or 5-tree plot by proportion survival of that plot.

Statistical Treatment

Because of differences in family representation among the 4 tests and different numbers of trees per plot and numbers of cross types per block between tests (Table 1), no attempt was made to combine information across tests or years. We attempted to take account of differences in family representation among different cross types by including breeding values of parents as a covariate in the model to adjust for parental differences in breeding value. Breeding values for all selections in the Cooperative Forest Genetics Research Program, including those used in this experiment, were calculated for both volume and resistance to fusiform rust by WHITE and HODGE (1988). First we examined the association between breeding values (for volume and rust score separately) and each measured variable. In general, although there was some positive association between measured growth traits and volume breeding values ($R^2 > 5\%$), there was none for rust breeding value and any variable. For this reason we carried out analyses for rust without any covariance adjustment but fitted models both including and excluding volume breeding value for growth measurements.

All analyses were carried out on plot means (average of the 4 or 5 trees representing each family in a given family plot) and in the case of the scores, the angular transformation was used together with a correction for continuity (SNEDECOR and COCHRAN, 1967).

Table 1. – Summarised features of each test. Tabulated data are from the latest measurement.

Test	3A	3B	4A	4B
# blocks	10	10	10	10
# cross types	3	3	2	4 ¹
# cross types/block	3	3	2	2
# families/plot	6	4	10	10
# trees/subplot	4	4	5	5
# outcrosses	9	6	12	14
# half-sib crosses	6	4	12	21
# backcrosses	6	4	0	5
# known grandparents	8	8	10	10
# trees in test	704	480	796	796
Year planted	1980	1980	1981	1981
Age of latest measurement	13	13	12	12
% survival	86.6	87.6	83.7	82.5
% trees with rust	3.2	6.5	17.6	8.7
% trees forked	7.7	10.9	14.5	12.2
% trees straight	87.2	68.8	83.0	81.4

¹) A single self was planted in a few plots of Test 4B but was omitted from the analysis.

An algebraic representation of the model (without the breeding values covariate fitted) was:

$$y = \mu + \text{Block}_i + \text{Cross type}_j + \text{Block} \times \text{Cross type}_{ij} + \text{Family-within-cross type}_{k(j)} + \text{Residual}_{ik(j)}$$

in which all terms were considered random except μ and cross type and the residual is the block \times family interaction.

Analyses were carried out using type III sums of squares (partial sums of squares – in which each model factor is adjusted for the presence of all other factors) from PROC GLM in SAS (SAS, 1988) to try to account for the imbalance in each test. In addition for each trait, the cross type mean squares were partitioned into linear and quadratic components using polynomial regression to examine the regression on level of inbreeding. Pseudo-F (i.e. pseudo variance ratio) tests constructed from mean squares for block \times cross type, families within cross type and residual (SATTERTHWAITE, 1946; NETER and WASSERMAN, 1974) were used to test significance of cross types and the linear and quadratic regressions on inbreeding coefficient (F). For growth traits, significant negative linear regressions imply the presence of inbreeding depression where growth is poorer with increasing inbreeding levels. Significant negative quadratic regressions indicate that growth is poorer at higher levels of inbreeding than expected under a linear model. Where growth under higher levels of inbreeding is not much different from growth under moderate levels, this would lead to positive quadratic regressions.

Because the breeding values were completely aliased with families, it was not possible to include families within cross type in the model with the covariate and so tests of significance for cross types were not possible for this analysis.

The linear regression coefficient (b) for each trait on increasing levels of inbreeding (i.e. F=0, 0.125, 0.25) is frequently used as a measure of the effects of inbreeding depression. It is commonly presented as a percentage reduction in growth per 0.1 increase in F (FALCONER, 1981). We calculated b in this way for all variates and, in addition, calculated b from the analyses for growth variates adjusted for breeding value as discussed above. We also calculated quadratic regressions in order to test for departures from linearity with increasing levels of F which would indicate the presence of epistasis. Similar analyses (not adjusted for breeding value) were carried out for plot standard deviation and plot coefficient of variation for growth traits to test for differences in within-family variation among cross types (RUDOLPH, 1981).

We attempted to estimate the operational impact of inbreeding depression for a tree-improvement program by examining four hypothetical orchard compositions spanning a range of different degrees of relatedness.

- 1) a 40-clone orchard in with no relatives other than ramets of the same clone (control),
- 2) a 40-clone orchard derived from 10 half-sib families each contributing 4 selections to the orchard,
- 3) a 40-clone orchard derived from 10 full-sib families each contributing 4 selections to the orchard,
- 4) a 40-clone orchard in which there were 20 unrelated pairs of parent-offspring, each pair consisting of a parent and one of its offspring (as in HODGE and WHITE, 1993).

Except for the control, the other 3 types of orchards above would have two kinds of related matings: selfing among ramets of the same clone and related matings among selections from the same family (half-sib, full-sib or parent-offspring). The total number of pairwise mating combinations is $40 \times 39/2 = 780$ of which 40 are selfings. For orchard 2 there are 60 different kinds of half-sib matings. For orchard 3 there are 60 different

kinds of full-sib matings. For Orchard 4 there are 20 kinds of related matings. The inbreeding coefficient for selfs is 0.5, for full-sibs and backcrosses is 0.25 and for half-sibs is 0.125.

There is likely to be reduced seed viability and germination in seeds derived from related matings (SQUILLACE and KRAUS, 1962; LAYTON and GODDARD, 1983). LAYTON and GODDARD (1983) found that half-sibs had 77.9% germination compared with 91.3% for outcrosses and 93.9% for backcrosses. Pre-germination mortality was not taken into account in these figures, but could well be high. For example, MATHESON (1980) found there were 75% fewer selfs than expected among progeny produced using mixtures of self and outcross pollen in *Pinus radiata*. We examined the effects of inbreeding for 3 levels of pre-germination mortality among selfs; 75%, 50% and 25% (37.5%, 25% and 12.5% for full-sibs and backcrosses, 18.75%, 12.5% and 6.25% for half-sibs). These correspond to linear regressions of 15%, 10% and 5% pre-germination mortality per 0.1F respectively in this series of experiments. At the highest level of pre-germination mortality, we expect only 25% of selfs, 62.5% of progeny of full-sibs and backcrosses and 81.25% of progeny of half-sibs to contribute to the next generation.

Of the 780 possible matings in all orchards, we expect 40 to result from selfings in all orchards, 60 from half- or full-sib matings in orchards 2 and 3, and 20 to result from parent-offspring matings (backcrosses) in orchard 4. At the highest level of pre-germination mortality the proportion (p) of resulting seed will therefore be:

$40/780 \times 0.25$ for selfs in all orchards (i.e. $p_S = 0.0128$),

$60/780 \times 0.8125$ for half-sibs (i.e. $p_H = 0.0640$ in orchard 2, = 0 in other orchards),

$60/780 \times 0.625$ for full-sibs (i.e. $p_F = 0.0480$ in orchard 3, = 0 in other orchards),

$20/780 \times 0.625$ for backcrosses (i.e. $p_B = 0.0160$ in orchard 4, = 0 in other orchards).

We compared performance (of any trait affected by inbreeding depression) for orchards 2,3 and 4 with orchard 1 and expressed the result as a percentage impact as follows:

$$\left(1 - \frac{[1-p_S \cdot 0.5b - p_H \cdot 0.125b - p_F \cdot 0.25b - p_B \cdot 0.25b]}{[1-p_S \cdot 0.5b]}\right) \cdot 100$$

where b is the regression coefficient relating plot volume growth to inbreeding level for each test used and 0.5, 0.125, 0.5 and 0.5 are the inbreeding levels for selfs, half-sibs, full-sibs and backcrosses respectively. In this formula, b is expressed as the proportion (not percentage) reduction per 1 unit F (not per 0.1 unit F). Thus $b\% = 18\%$ per 0.1F would be $b = 1.8$ above.

Results

There were highly significant differences among cross types (i.e. between outcrosses, half-sibs and backcrosses) for growth, but not generally for the other traits (except survival in Test 3A). Differences among families within cross types were significant for growth, rust and survival, but were variable for forking and straightness (families significant only in Test 4A, for straightness only in Test 3B). These results were reflected in regression lines relating cross types to theoretical inbreeding level (Table 2). Significant linear regression coefficients were found for growth traits in all tests, but only in Test 3A for survival (Table 2). Other significant linear regression coefficients were straightness at age 6 (Test 3A), straightness at ages 7 and 12 and forking at age 12 (Test 4A), forking at ages 7 and 12 and straightness at age 7 (Test 4B). Significant non-growth regressions were positive, indicating higher incidence of both straight and forked trees among inbred progeny. Although inbred trees seemed straighter than outcrossed trees in Test

3A, particularly at young ages, this effect tended to disappear as trees became older. This trend seems to be the reverse in Tests 4A and 4B and may be associated with tree size.

In general, the difference between outcrosses and half-sibs is greater than between half-sibs and backcrosses (*Fig. 1*). However, these differences in apparent slope were generally not significant in this experiment. Only for diameter at year 12 in Test 4B (Prob=0.047), and for straightness at age 5 in Test 4B (Prob=0.021) were the quadratic terms significant. Comparison of tests 3A, 3B and 4B in *figure 1* shows that inbreds grew relatively poorer in 3B and 4B than in 3A, most strongly seen for plot volume. This is likely to be a scale effect since plot volume is a linear combination of heights and squared diameters in the volume equation used (GODDARD and STRICKLAND, 1968). It is also clear from *figure 1* that most of the growth in plot volume has occurred in the last 5 years.

Adjustment of growth data to take account of differences in breeding value between different cross types made some difference to regression coefficients (*Fig. 2*). Adjustments to Tests 3A and 3B generally had the effect of increasing differences between cross types and thereby increasing the magnitude of the linear regression coefficient. However, because of different parentage and a different suite of breeding values used as covariates, adjustment in Tests 4A and 4B had the reverse effect, reducing differences between cross types. In no case did adjustment alter the conclusions drawn from the data.

Inbreeding depression tended to remain much the same over different ages from 2 to 13 years for height and diameter. If anything, depression for height seems to decrease slightly and for diameter to increase slightly (*Table 2*) when expressed as a percentage. There was a slight tendency for depression to increase slightly with age for survival. However, depression

increased consistently with age for both tree volume and plot volume (from 15.6% to 17.5% for volume in Test 3B, 9.2% to 11.9% for plot volume in Test 4B).

In general, inbred progeny had higher within-plot standard deviations for growth traits than outcrossed progeny, largest for 12- or 13-year height (*Table 3*). Coefficient of variation (CV) was mostly higher for inbred progeny, with highly significant linear effects for height and tree volumes in Test 4A. The regression of CV on F tended strongly to increase with age, particularly in Tests 3A and 3B. Negative quadratic regressions were observed for standard deviations and CVs in general with SDs and CVs of backcrosses frequently lower or similar to those of half-sib crosses.

It was possible to test for differential depression in two grandparents, namely 6 and 8, that contributed families to each cross type. Families of these two grandparents showed difference in depression for plot volume consistently across tests although the actual crosses involved in Tests 3A and 3B differed from those in Tests 4A and 4B. Plot volumes at age 13 (Test 3A) and at age 12 (Tests 4A and 4B) are shown in *table 4*. The difference was significant in 2 of the 3 tests where it was estimable. Even where not significant, 8 showed more depression than 6.

Results of projecting these observations onto hypothetical seed-orchards are given in *table 5*. In all cases the deleterious effects of including full-sibs were greater than those of including half-sibs. Even with increased pre-germination mortality with higher levels of inbreeding, greater inbreeding depression among full-sibs predominated. The effects of including parent-offspring pairs, as in orchard 4, were always less than the effects of including half- or full-sibs because the number of related matings was always smaller (20/780 rather than 60/780).

Table 2. – Summarised regression analyses. Tabulated are significance of linear (lin) and quadratic (quad) regressions together with the regression coefficients. Significance levels of linear and quadratic mean squares were tested against a synthetic mean square (see text), where 'ns' (not significant) means that the probability was greater than 0.05. Percentage inbreeding depression per 0.1 units of theoretical inbreeding coefficient, not adjusted (b%) and adjusted (b% (adj)) for volume breeding value differences between cross types. Traits are given with the age at which they were measured; heights in metres (ht), diameters in cm (dbh), volumes in m³ (vol), plot volumes in m³ (pvol) and the arcsine-transformed traits: survival % (surv), rust % (rust), forks % (fork) and straightness (strt).

Trait	Test 3A			Test 3B			Test 4A			Test 4B		
	Prob	b %	b % (adj)	Prob	b %	b % (adj)	Prob	b %	b % (adj)	Prob	b %	b % (adj)
ht2	ns	-1.75	-4.25	ns	-4.70	-8.79	ns	-2.2	-2.11	ns	-3.78	-3.11
ht6	ns	-5.87	-7.29	ns	-3.70	-7.12	ns	-2.25	-2.25	ns	-4.12	-3.51
ht8	ns	-4.67	-5.30	ns	-3.68	-6.50	ns	-2.0	-1.77	ns	-3.57	-2.94
ht13	0.049	-5.22	-5.12	0.003	-4.91	-6.17	ns	-2.4	-2.14	0.007	-5.03	-4.27
dbh6	0.03	-9.41	-10.84	0.06	-5.82	-8.52	ns	-3.7	-3.40	0.048	-5.11	-4.92
dbh8	0.03	-6.27	-6.64	0.008	-6.62	-8.19	ns	-3.7	-3.38	0.02	-4.93	-4.66
dbh13	0.02	-5.84	-6.57	0.001	-7.90	-8.53	ns	-4.1	-3.92	0.003	-5.48	-5.24
vol6	ns	-12.12	-14.82	ns	-10.95	-15.55	ns	-7.1	-6.34	0.04	-10.03	-8.91
vol8	ns	-12.08	-12.64	0.0008	-12.90	-16.11	ns	-7.4	-6.67	0.03	-10.58	-9.10
vol13	0.011	-12.91	-13.99	0.0003	-16.38	-17.47	ns	-9.1	-8.53	0.003	-13.27	-11.51
pvol6	0.07	-14.81	-16.51	0.03	-13.21	-17.36	ns	-4.8	-4.73	ns	-11.08	-9.15
pvol8	0.02	-16.05	-16.18	0.006	-14.61	-18.01	ns	-5.6	-5.49	0.02	-12.93	-9.67
pvol13	0.002	-17.84	-17.49	0.0011	-17.14	-19.32	ns	-7.0	-6.89	0.003	-15.96	-11.94
surv2	ns	-4.03		ns	0.72		ns	1.2		ns	-0.98	
surv6	ns	-5.36		ns	-1.95		ns	1.6		ns	-1.07	
surv8	0.03	-7.00		ns	-1.66		ns	1.5		ns	-1.61	
surv13	0.02	-7.27		ns	-1.06		ns	1.8		ns	-2.16	
rust6	ns	-1.02		ns	6.41		ns	-7.3		ns	1.19	
rust8	ns	-3.18		ns	9.44		ns	-3.3		ns	6.13	
rust13	ns	4.91		ns	8.95		ns	-4.4		ns	8.05	
fork6	ns	-2.12		ns	4.12		ns	-0.9		ns	2.67	
fork8	ns	-3.50		ns	9.33		ns	1.0		0.02	12.37	
fork13	ns	4.35		ns	11.64		ns	10.5		0.011	17.46	
strt6	0.005	14.26		ns	-0.53		ns	2.8		ns	0.02	1.81
strt8	ns	3.86		ns	-1.29		ns	4.1		0.03	ns	4.84
strt13	ns	2.39		ns	2.35		ns	5.7		ns	ns	4.81

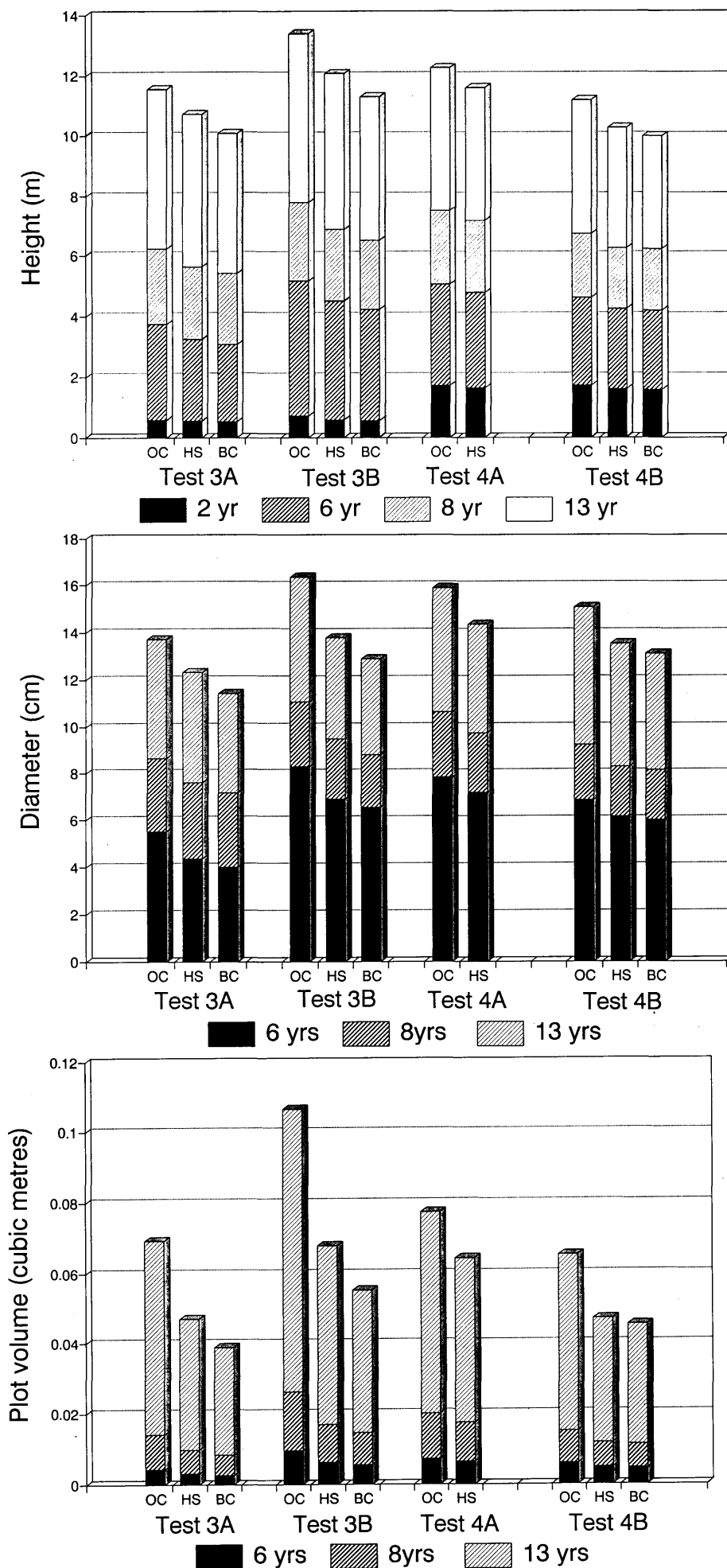


Figure 1. - Cumulative growth results for each test at each age plotted against inbreeding level. Figure 1A gives results for height in metres, figure 1B gives results for diameters and figure 1C gives results for plot volume. OC, HS and BC represent outcrosses half-sib and backcrosses respectively. Results presented here were adjusted for differences in breeding value of parents involved in each crossing type.

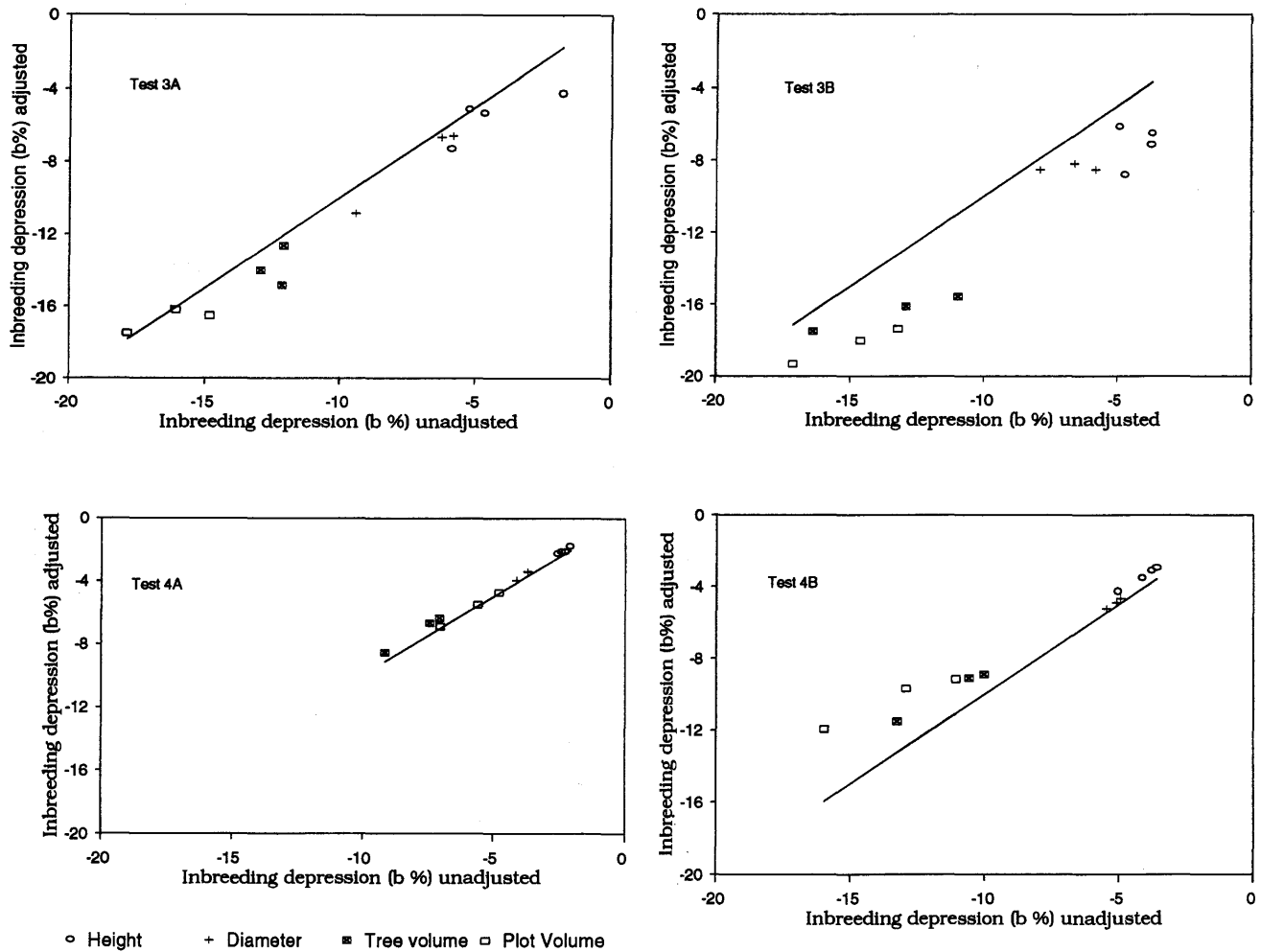


Figure 2. – Regression coefficients relating growth traits to theoretical inbreeding level (0.1 units of F). Unadjusted values (X-axis) are plotted against values adjusted for breeding value of parents (Y-axis). The straight line represents no adjustments; points below the line indicate where the estimate of inbreeding depression has increased following adjustment. Points above the line indicate where the estimate of inbreeding depression has decreased following adjustment.

Table 3. – Summarised regression analyses for standard deviations and coefficients of variation of heights and tree volumes. Columns are as in table 2.

Trait	Test 3A			Test 3B			Trait	Test 4A			Test 4B
	Prob	quad	b%	Prob	quad	b%		Prob	lin	quad	
sdh2	ns	ns	3.56	0.029	ns	-10.38	sdh2	ns	ns	ns	1.51
sdh6	ns	0.012	3.43	ns	ns	1.94	sdh5	0.042	ns	ns	-3.53
sdh8	ns	0.021	7.99	ns	ns	7.62	sdh7	0.031	ns	ns	7.50
sdh13	ns	ns	18.55	0.013	0.027	23.04	sdh12	0.004	ns	ns	2.93
sdvol6	ns	ns	-0.17	ns	ns	-8.50	sdvol5	ns	ns	ns	-4.61
sdvol8	ns	ns	-2.53	ns	ns	-9.35	sdvol7	ns	ns	ns	-5.69
sdvol13	ns	ns	0.41	ns	0.004	-6.39	sdvol12	ns	ns	ns	-10.81
cvh2	ns	ns	5.44	ns	ns	-6.54	cvh2	ns	ns	ns	6.35
cvh6	ns	0.007	10.94	ns	ns	6.31	cvh5	0.01	ns	ns	9.22
cvh8	ns	0.0225	14.97	ns	ns	15.67	cvh7	0.01	ns	0.033	11.81
cvh13	ns	ns	25.14	0.014	ns	34.09	cvh12	0.002	ns	0.042	9.77
cvvol6	ns	ns	6.41	ns	ns	-1.26	cvvol5	0.003	ns	ns	11.36
cvvol8	ns	0.052	18.45	ns	ns	6.02	cvvol7	0.003	ns	ns	10.66
cvvol13	0.051	ns	21.07	0.044	ns	15.32	cvvol12	0.002	ns	ns	8.92

Table 4. – Mean plot volumes in m³ (with % reduction of half-sibs in parentheses) for families from grandparents 6 and 8 in 3 tests. Mean plot volume was obtained by multiplying mean tree volume by survival for each plot. The grandparent x cross type interaction was significant in Tests 3A and 4B indicating more inbreeding depression in grandparent 8 than grandparent 6. The interaction was non-estimable in Test 4A and there were too few families from these grandparents in Test 3B.

	Grandparent 6		Grandparent 8	
	Half-sib	Outcross	Half-sib	Outcross
Test 3A (13 years)	0.055 (3.5%)	0.057	0.041 (40.6%)	0.069
Test 4A (12 years)	0.074 (8.6%)	0.081	0.055 (28.6%)	0.077
Test 4B (12 years)	0.061 (3.2%)	0.063	0.038 (42.4%)	0.066

Table 5. – Percentage reduction in performance resulting from the inclusion of relatives in 3 orchards of 40 clones. Performance is any trait affected by inbreeding depression. Orchard 1 consists of 40 unrelated clones, Orchard 2 consists of 10 half-sib families each contributing 4 half-sibs, Orchard 3 consists of 10 full-sib families each contributing 4 full-sibs and Orchard 4 consists of 40 clones forming 20 parent-offspring pairs. Calculations were based on 3 levels of pre-germination mortality among inbreds (75 %, 50 % and 25 % deaths among selfs, others proportionately less) and inbreeding depression (b) of 1.8, 1.0 and 0.2 per unit F¹. Reduction in Orchards 2, 3 and 4 are given relative to Orchard 1.

Rate of inbreeding depression (b)	Orchard 2 (HS) % reduction	Orchard 3 (FS) % reduction	Orchard 4 (BC) % reduction
	75% deaths among selfs		
1.8	1.42	2.19	0.73
1.0	0.79	1.21	0.40
0.2	0.16	0.24	0.08
	50% deaths among selfs		
1.8	1.55	2.66	0.89
1.0	0.85	1.46	0.49
0.2	0.17	0.29	0.10
	25% deaths among selfs		
1.8	1.68	3.14	1.05
1.0	0.92	1.72	0.57
0.2	0.18	0.34	0.11

¹⁾ The b values used here are expressed in proportion per 1 unit F and correspond to b % values of 18 %, 10 % and 2 % in tables 2 and 3.

Discussion

BURROWS and ASKEW (1982) have pointed out that many studies suffer from a lack of balance in parental contribution to each inbreeding level. They also suffer because family sizes are frequently smaller for inbred families than outcrossed ones. This means that inbreeding effects could be due either to inbreeding itself or to differential parental contribution. BURROWS and ASKEW (1982) re-analysed results of SQUILLACE and KRAUS (1962) to show that inbreeding depression was over-estimated because of these effects. The experiments reported here suffer from imbalance in the crossing pattern, but not from differences in family size. Our attempts to adjust growth traits for parental contribution based on breeding value for volume increased the estimates of inbreeding depression in Tests 3A and 3B and decreased them in Tests 4A and 4B. However, adjustment made little difference to the conclusions even though differential parental breeding values between crossing types had a significant effect on the analyses. Adjustment for breeding value for rust had no significant effect on the results

for rust scores, perhaps because the incidence of rust in these tests was fairly low (< 10 %, except Test 4A – Table 1).

The experiments reported here suggest that the effect of inbreeding on growth is mostly negative and linear, and that it increases with age only for tree and plot volume. Volume was calculated as a linear combination of height, diameter and diameter squared and so the age-related changes may be an effect of scale, as suggested by LINDGREN and GREGORIUS (1976). There was no tendency for inbreeding depression for growth to disappear with age, as suggested by SORENSEN and MILES (1974, 1982). Effects of inbreeding on survival were small and significant only in Test 3A at 8 and 13 years. Clearly, increased mortality among inbred progeny would tend to reduce the effects of inbreeding depression on vigour with increasing age if smaller inbred trees were the ones to die. But this could not explain SORENSEN and MILES' (1982) results or WILCOX' (1983) results for diameter.

We found little or no evidence of non-linearity over the range of inbreeding levels used in this study. Significant quadratic regressions were sparse among the traits themselves, suggesting that epistasis, if present, is non-directional. Causes of directional epistasis are presumably the same as those leading to directional dominance (FISHER 1930; BREESE and MATHER, 1960), but it seems difficult to detect non-directional epistasis. There were significant quadratic regressions when within-plot standard deviations and Cvs were analysed as traits. Negative quadratic regressions, as observed here, result from families with higher levels of inbreeding having lower levels of variability than expected from a linear model. ROBERTSON (1952) showed for a single locus with the recessive allele at a frequency of less than 0.41 and with no selection, that low levels of inbreeding increased the genetic variance, but that higher levels decreased it again. These results carry through to the multiple-locus situation, but are accentuated by selection. Selection will have the effect of decreasing the variance among inbreds roughly in proportion to F. We calculated theoretical within-family variances using ROBERTSON'S (1952) methods with various allele frequencies and presence and absence of selection. Our observed within-family variances were similar to the theoretical ones when allele frequencies were about 0.3 and when there was selection against recessive homozygotes.

It was surprising to find little evidence of inbreeding depression for survival. Except at ages 8 and 13 in Test 3A, none of the regressions on F, linear or quadratic, were significant. Of all the traits reported here, survival is most closely related to fitness and should therefore have been subject to the greatest directional selection leading to the greatest directional dominance. Possible explanations could be 1) that there might be no genetic variation in the population for survival under the conditions in the tests or 2) mean survival was high (69% to 87%) so the variance in this binomial trait was low. However, in all tests except Test 3A after 2 years of age, differences among families within cross types for survival were always highly significant. It seems possible that much of the differential mortality between cross types had occurred before planting out, either before germination or in the nursery. There was an excess of seedlings in the nursery for many families and some nursery culling may have taken place.

Effects of inbreeding do not appear the same for the families of the 2 grandparents compared here. Families from grandparent 8 showed much greater inbreeding depression for growth than those from grandparent 6. The 2 grandparents had similar breeding values and so our results are consistent with those of GRIFFIN et al. (unpublished data) who found

heterogeneity of regression coefficients of growth on F and that the regressions were unrelated to GCA estimates.

Inbreeding depression must be dynamic over generations because deleterious alleles are selected against more strongly under inbreeding. Each generation, under the partial dominance model, some purging of the population takes place so the frequency of deleterious alleles is reduced. Once deleterious allele frequency drops, the effects of inbreeding must become less. There is evidence in this study that this happens even at low levels of inbreeding from the backcrosses that had lower plot standard deviations and CVs than half-sibs. FALCONER (1981) observed that this meant inbreeding depression could be measured precisely only during the early stages of an inbreeding program. On the other hand, the long-term implications of inbreeding can be revealed through study over several generations (WILLIAMS and SAVOLAINEN, 1993). Populations purge themselves of deleterious alleles through inbreeding (NAMKOONG and KANG, 1990; BARRETT and CHARLESWORTH, 1991) so the effects diminish with time. This has important implications for advanced-generation breeding programs in which the conflict between genetic progress and effective population size is evident. Sublining as a tool for managing inbreeding has been adopted in a number of breeding programs (VAN BUIJTENEN and LOWE, 1979; MCKEAND and BEINEKE, 1980; BURDON and NAMKOONG, 1983; PURNELL and KELLISON, 1983; LOWE and van BUIJTENEN, 1986; CARSON *et al.*, 1990) where inbreeding within lines is allowed, but removed by crossing between lines for deployment to plantations. Evaluation and selection within a line become difficult after a few generations when inbreeding levels build up. However, systematic mild inbreeding with selection may purge each line of deleterious alleles and reduce the effects of inbreeding to acceptable levels (NAMKOONG and KANG, 1990).

SQUILLACE (1973) proposed the idea of including relatives in seed orchards. LINDGREN and MATHESON (1986) went a little further by suggesting inclusion of clones in varying proportions. Lower levels of inbreeding may have a more negative effect on seed orchard progeny than selfing because more seedlings survive to affect the plantation (SNYDER 1968; FRANKLIN, 1970). The results of our calculations involving hypothetical orchards with different levels of inbreeding do not support this idea. The small difference in pre-germination mortality between full- and half-sib matings does not outweigh the difference the resulting progeny make to the population. Orchard 4, with parents and offspring, illustrates another point. The inbreeding coefficient (F) for full-sib matings and for backcrosses is the same (0.25). Orchard 3 and Orchard 4 differ only in the way relatives are apportioned. Orchard 3 has four relatives per family and 10 families, whereas Orchard 4 has 2 relatives per family and 20 families. The proportion of related matings in Orchard 3 is 60/780 compared with 20/780 for Orchard 4. The effect of including both parents and offspring in an orchard is small (between 1% and 2%) and may be an advantage for operational reasons (HODGE and WHITE, 1993). However, including more than 1 offspring with the parent is not recommended.

Conclusion

Inbreeding depression (adjusted for differences in parental breeding value) of between 4% and 8% for each 0.1F was estimated for height growth, a little more (5% to 9%) for diameter and still more for tree and plot volume (6% to 19%). There was little evidence that inbreeding depression declined with age; on the contrary, it increased with age for tree and plot volume, perhaps an effect of scale. There was little evi-

dence for inbreeding depression affecting survival, rust incidence, stem straightness or forking. This could be due to low incidence of these binomially-scored traits.

In general, inbreeding depression was linear on F for all traits, but there was a significant quadratic coefficient for standard deviation and CVs of some growth traits. Inbreds had higher SDs and CVs than outcrosses, but the quadratic term arose because plot SD and CV for backcrosses was usually lower than or at least similar to that of half-sibs.

The effect of including relatives in seed orchards is greater for full-sibs than half-sibs although fewer seeds survive to germinate at higher inbreeding levels. It is better to include few relatives many times than many relatives few times in a seed orchard. To make the orchards comparable, we have assumed they all have similar breeding value. Orchards with fewer families, however, may have higher breeding value than orchards with more families thus mitigating some of the higher inbreeding effects (see LINDGREN and MATHESON, 1986).

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Inbreeding Depression and Changes in Variation After Selfing in *Eucalyptus globulus* ssp. *globulus*

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Summary

The effect of self pollination (selfing), open-pollination (OP) and controlled cross pollination (outcrossing) on progeny of 11 *Eucalyptus globulus* ssp. *globulus* seed parents was examined from seed set to 43 months after planting. Selfing severely depressed seed set and field growth relative to outcrossing. No effect was found on germination percent and rate, nursery and field survival, nor the proportion of plants possessing adult foliage or flower buds. Inbreeding depression for height first occurred between germination and 8 months after planting but occurred later for diameter. There was a trend for inbreeding depression to increase with age. The performance of OP families was intermediate between self and outcross treatments but was only significantly different from outcrossing for volume at 43 months after planting. Selfing also appeared to increase variation between and within families relative to outcrossing. The results are discussed in terms of the biology and genetics of the species and the relevance to tree improvement programs.

Key words: inbreeding depression, selfing, genetic variances, open-pollination, progeny testing, tree improvement, *Eucalyptus globulus*, breeding.

FDC: 165.41; 165.53; 232.11; 176.1 *Eucalyptus globulus*.

Introduction

A knowledge of the effects of inbreeding can be important in tree improvement programs. On the one hand, potential genetic gains may be compromised by inbreeding depression (MESKIMEN, 1983; GRIFFIN, 1989); on the other hand, employing inbreeding offers benefits not otherwise available (BARKER and LIBBY, 1974; GULLBERG and KANG, 1985; LINDGREN, 1975; KANG and NIENSTAEDT, 1987; GRIFFIN and COTTERILL, 1988; NAMKOONG *et al.*, 1988). Theory also predicts that inbreeding may change the distribution of variation within a population (ROBERTSON, 1952; WEIR and COCKERHAM, 1977), yet this effect has received little attention in forest trees (*e. g.* SORENSON and WHITE, 1988).

The reproductive system of eucalypts offers ample opportunity for self pollination (GRIFFIN and HAND, 1979; ELDRIDGE *et al.*, 1993). The effects of selfing in eucalypts have been reported to include reduced seed set (HODGSON, 1976a; PRYOR, 1976; GRIFFIN *et al.*, 1987; POTTS and SAVVA, 1988a; SEDGLEY and SMITH, 1989; SEDGLEY *et al.*, 1989; TIBBITS, 1989), decreased germination percent (ELDRIDGE, 1978; ELDRIDGE and GRIFFIN, 1983), increased frequency of abnormal phenotypes (HODGSON, 1976b; POTTS *et al.*, 1987), depressed field growth and vigour