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Inbreeding in *Pinus radiata*. I. The effect of inbreeding on growth, survival and variance

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Abstract The effects of inbreeding on growth, survival and variance in a 12-year-old radiata pine trial were studied in five populations each inbred to one of five different levels: outcross (F = 0), half-sib (F = 0.125), full-sib (F = 0.25), selfing (S_1, F = 0.5), and two-generations of selfing (S_2, F = 0.75). These five populations were derived from a founder population of eight clones. Inbreeding reduced diameter, growth, and survival but increased the variance for diameter. Inbreeding depression at F = 0.125, 0.25, 0.5, and 0.75 was 5%, 6%, 15%, and 19% respectively for DBH; − 3%, 1%, 7%, and 11% respectively, for survival. The standard deviation for diameter increased by 10%, 10%, 30%, and 25% respectively for F = 0.125, 0.25, 0.5, and 0.75 and, similarly, the coefficient of variation increased by 17%, 16%, 53%, and 55% respectively. There were significant differences among the eight founder clones in their response to inbreeding. The best clone in the trial showed no inbreeding depression. Overall, inbreeding depression was found to be linearly related to the inbreeding coefficient F with no significant quadratic effects for any trait at any population level. However, two individual clones had a quadratic relationship with F for DBH and one clone had a similar relationship for survival. A significant correlation ($r = 0.96$) between S_2 and the breeding values of founder clones was observed while the correlation ($r = 0.58$) between S_1 and breeding values was insignificant. The low inbreeding depression in radiata pine relative to other conifers may indicate that historical purging of detrimental alleles through small geographic populations, a higher degree of population subdivision, and the relative high fecundity of inbred progenies has rendered radiata pine an ideal species to use inbreeding as a breeding tool.

Key words *Pinus radiata* · Inbreeding depression · Growth · Variance · Survival · Purging

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Introduction

Inbreeding is defined as mating between relatives (Crow and Kimura 1970) and is rarely a problem in the first generation of conifer tree improvement since plus trees selected from the wild are usually unrelated. As most tree-breeding programs in conifers progress into the second or more advanced generations, the breeding population becomes smaller due to intensive selection and the trees selected might be from related families. Thus, inbreeding can become a serious issue in the management of the breeding population and in the deployment of the production population. The direct genetic consequence of inbreeding is a change in genotype frequency, i.e. an increase of homozygote frequency and a reduction of heterozygote frequency, although inbreeding alone does not change the gene frequency (Falconer 1981). The results of experimental inbreeding in trees, particularly in conifers, are usually reduced seed production and viability (Sorensen 1969; Franklin 1970), depressed growth (Sorensen and Miles 1982; Matheson et al. 1995), and decreased adult fecundity (Rudolph 1981; Durel et al. 1996). The exposure of recessive lethal or lethal-equivalent genes is believed to be the major cause of the reduction in seed production from inbreeding (Bramlett and Popham 1971; Koski 1971). Williams and Savolainen (1996) summarised estimates of the average numbers of lethal-equivalent genes per diploid conifer zygote and these

ranged from a low of 0.1 in *Pinus resinosa* (Fowler 1965) to a high of 10.8 in *Larix laricina* (Park and Fowler 1982).

Recently, Durel et al. (1996) summarised inbreeding depression values for vigour traits in tree species. For selfed progenies, the reduction in height ranged from 9% in *Pinus radiata* at age 4 (Wilcox 1983) to 61% in *Picea abies* at age 19 (Eriksson et al. 1973) compared with outcrosses. Similarly, a reduction in volume of up to 66% was observed at age 11 in *Pinus sylvestris* (Lundkvist et al. 1987) among selfed progenies. Inbreeding delayed the onset of reproduction in *Pinus banksiana* (Rudolph 1981), as well as *Pinus monticola* (Bingham 1973) and reduced the seed potential (Sniezko and Zobel 1988; Durel et al. 1996). For example, the fitness of selfed progenies, measured by the number of potential sites for seeds in female cones, was reduced by 30% in *Pinus taeda* (Sniezko 1984), and the inbreeding depression for the number of cones was 53% and 89% for the first- and second-generation selfing in *Pinus pinaster* (Durel et al. 1996).

In *P. radiata*, Pawsey (1964) noted that selfed trees were comparable in respect of the production of male and female flowers and did not show any adverse effect on cone development. However, seed production, as well as the weight, survival and size of seedlings were reduced in selfed progenies (Pawsey 1964; Griffin and Lindgren 1985). Wilcox (1983) observed that inbreeding depression from selfing appeared in both nursery and plantation stages, and the depression was 12% for diameter at age 7. He also noted that, compared with outcrossed progeny, selfed progeny had reduced stem straightness, more malformations, poorer branching quality and lower needle retention (related to *Cyclaneusma* infection), but slightly increased wood density.

To manage the adverse effects of inbreeding, a subline breeding strategy has been proposed for tree species (van Buijtenen and Lowe 1979; Burdon and Namkoong 1983; Matheson and Brown 1983; McKeand and Bridgewater 1993; White et al. 1992a; White 1993). This strategy involves subdividing the breeding population into several (unrelated) lines within which inbreeding is permitted, but crosses between lines are not permitted except for deployment. However, because each subline is a small subset of the breeding population, inbreeding would accumulate more quickly within sublines than in an undivided population. Thus, reliable estimates of inbreeding depression are essential, not only for an understanding of the genetic mechanism of inbreeding depression and the mating history of the species, but also for optimising the breeding strategy.

Most studies on inbreeding depression in forest trees deal either with selfing alone (Squillace and Kraus 1962; Fowler 1965; Snyder 1972; Eriksson et al. 1973; Libby et al. 1981; Rudolph 1981; Sorenson and Miles 1982; Wilcox 1983; Lundkvist et al. 1987) or involve an unbalanced parental and grand parental contribution at different inbreeding levels (Gansel 1971; Andersson et al. 1974; Layton and Goddard 1983; Sniezko and Zobel 1988; Matheson et al. 1995; Durel et al. 1996). With different genetic backgrounds, it is possible that observed differences between inbreeding levels could be partly due to genetic differences among parental material (Burrows and Askew 1982). The extrapolation of inbreeding depression estimates from selfing to milder inbreeding, such as half-sib and full-sib mating, may not be adequate. However, it is those milder inbreeding schemes that are more relevant to current breeding population management and seed orchard designs. Thus, examination of inbreeding effects at less severe levels of inbreeding (particularly half-sib and full-sib) with a well-balanced parental contribution is much needed.

The utility of inbreeding as a breeding tool is likely to depend largely on the mode of gene action and the evolution of the species mating system. Complete, partial and over-dominance have been proposed as genetic mechanisms for inbreeding depression or hybrid vigour (Falconer 1981). If overdominant loci are the cause of inbreeding depression, average fitness will decrease continuously over generations of inbreeding. If depression is caused by some other form of dominance, in which deleterious recessive alleles cause most of the inbreeding depression, it should be possible to eliminate or purge these recessives and fix the normal, dominant alleles. In this case, inbreeding depression will decline across generations as purging effectively removes deleterious alleles. Under the partial dominance model, it is also possible to produce very favourable genotypes with most of the dominant alleles combined together from recombination in the early generations of inbreeding. Thus, there is a pressing need for both applied and basic research on inbreeding depression in trees (Williams and Savolainen 1996).

The present study reports inbreeding effects on growth, survival and variance in a radiata pine trial. The experiment is one of most comprehensive such trials in conifers since the progenies were inbred to five levels from $F = 0$ to 0.75, mild inbreeding (half-sib and full-sib) was included, a relative balance of the grandparental contribution was achieved, and the effect of individual clones was identifiable. The following four questions will be addressed from the detailed ANOVA and regression analysis of the experiment: (1) whether inbreeding depresses growth and survival but increases variance significantly; (2) whether the inbreeding effect on growth, survival and variance is linear or nonlinear (this could determine whether epistatic effects are involved in inbreeding depression); (3) whether there are clone differences due to inbreeding, and (4) whether there is a relationship between the breeding values of founder clones and the level of inbreeding depression. The effects of inbreeding on seed production in this material has been reported previously (Griffin and Lindgren 1985).

Materials and methods

Mating design

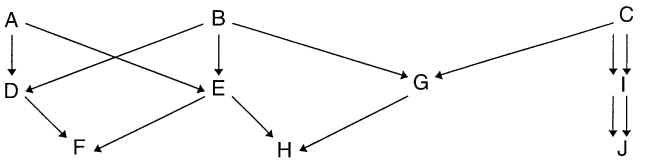
Five sets of progenies inbred to levels of F = 0, 0.125, 0.25, 0.5 and 0.75 (designated as populations 1–5, respectively) were derived from eight unrelated clones from the APM Forests Pty. Ltd. (now Australian Paper Plantations Pty. Ltd.) breeding population in Victoria, Australia, as shown in Fig. 1 (see also Griffin and Lindgren 1985).

A four by four factorial mating of the founder clones constitutes the outcrossed families (population 1, Fig. 2); these 16 full-sib families had previously been established in progeny tests and provided parents for the half-sib and full-sib matings.

Trees from four families with clone 1 as a parent (15,16,17 and 18 in Fig. 3) were crossed in a half-diallel design leading to six families with clone 1 as a common parent. This was repeated for clones 2, 3 and 4 to form population 2 (F = 0.125). Each individual used as a parent was employed for only one cross, i.e. the three families involving family 16 were each derived from a different parent within family 16, one each for crossing with 15, 17 and 18 (Fig. 3).

For full-sib matings (population 3, Fig. 4), four trees were chosen within each of 16 F_1 families and mated as two single-pair crosses, generating a total of 32 families.

The eight founder clones were self-pollinated to give eight S_1 families (population 4, Fig. 5) and between two and six trees in earlier outplantings of seven of these S_1 families were selfed to generate the S_2 families (population 5, Fig. 6).



Inbreeding level (F)	Population constitution
0	Full-sib families of unrelated parents (e.g. D)
0.125	Families from crosses of half sibs (e.g. H)
0.25	Families from crosses of full-sibs from unrelated parents (e.g. F)
0.50	Families from selfing non-inbred individuals-S_1 (e.g. I)
0.75	Families from selfing S_1 individuals-S_2 (e.g. J)

Fig. 1 Generalised diagram of crossing structure to obtain different inbreeding levels

Fig. 2 Numbers of families forming population 1 (F = 0). Female parental clones are listed at the head of the columns, male parental clones at the left of the rows. If reciprocal differences are ignored, this forms a 4 × 4 factorial set among clones 1, 2, 3 and 4 as male parents, and 5, 6, 7 and 8 as female parents

A relatively balanced grandparental contribution was achieved among the five populations except that four clones were not inbred to F = 0.125 and one clone was not available for the S_2 as described above. In choosing parent trees, no selection criteria were applied, other than that they were flowering. This might have resulted in some bias towards more vigorous individuals, especially in the S_1 progeny test where it was necessary for a candidate to produce both male and female strobili for producing S_2 progenies.

Field experiment

Seeds were extracted by hand and those from all cones of a particular family were combined. After de-winging, all normal-sized seeds were X-rayed to determine full seed yield (Griffin and Lindgren 1985).

Up to 60 seeds from each family were stratified (this number was not available for more highly inbred seedlots), with seed from each family divided into three samples of 20. Seeds were sown in open nursery beds with the seedlot location randomised within each of three replicates. All seedlings of a single seedlot were combined prior to sorting for field establishment.

The field trial of 3.2 hectares was established in June 1981 at Symmonds, an ex-pasture site owned by Softwood Holdings Ltd. (now CSR Timber Products Ltd.) near Mount Gambier, South Australia. Rows were scalped and ripped prior to planting at 2 × 3 m spacing. Weed growth was controlled with herbicide.

Since the primary interest was to compare the performance of the five inbred populations, and because these were expected to show marked variation in growth rate, seedlots were blocked according to population to alleviate competition between inbreeding levels. Thus, populations constitute main plots within each replication. Single-row six-tree sub-plots for each family were randomised within the main plots and all main plots replicated four times. Eighty five percent of 136 seedlots (families) were fully represented by 24 seedlings. For the remainder, seedlings were distributed equally among the four replicates, and empty planting spots within plots were filled with surplus stock from other seedlots of the same population.

Two families in population 1 and four families in population 4 were not planted at the Symmonds site. These were sown in the same manner 12 months later, together with six families from population 1 and four families from population 4 which had already been planted in the main Symmonds trial. These families were planted at a nearby site (Kilsbys, also owned by CSR Timber Products Ltd.) in June 1982 and the design was similar to the main trial except that there were six replicates.

The diameter at breast height (1.3 m above ground, DBH) was measured to the nearest mm in June, 1993, 12 years after planting for Symmonds and 11 years for Kilsbys.

Statistical analysis

Since two families in population 1 and four families in population 4 (half of population 4) were planted only at Kilsbys, the first step

male parental clone	female parental clone 1	2	3	4	5	6	7	8
1					1	1		
2					1	1	1	
3					1			
4						1		
5				1				
6	1							
7	1			1				
8		1	1	1				

was to incorporate these families into the analysis of the main Symmonds trial. Six families in population 1 and four families in population 4 were planted across two sites. Thus, these common families served as connections between the two plantings. For the common families of population 1 (F = 0) and population 4 (F = 0.5), a linear model was fitted to investigate family by site and family by replication interactions in the two populations separately:

$$y_{ijkl} = \mu + S_i + R_{j(i)} + F_k + SF_{ik} + RF_{jk(i)} + E_{ijkl}, \quad (1)$$

where μ is the population mean, S_i is the site effect (i = Kilsbys or i = Symmonds), $R_{j(i)}$ is the j^{th} replication effect within the i^{th} site, F_k is the k^{th} family effect, SF_{ik} is the interaction effect between the i^{th} site and the k^{th} family, $RF_{jk(i)}$ is the interaction effect between the j^{th} replication and k^{th} family, and E_{ijkl} is the residual. Non-significant interactions mean that it is possible to combine data across sites.

The interaction of family with site and family with replication were not significant in either population (populations 1 and 4) for DBH and in population 4 for survival. However, the interaction for survival of population 1 was significant. Thus, data from Kilsbys were combined with data for Symmonds for DBH for both populations and for survival in population 4 only. To adjust for the 1-year difference in age, the following equation for populations 1 and 4 was employed:

$$DBH_s = \overline{DBH_s} + \frac{\sigma_s}{\sigma_k}(DBH_k - \overline{DBH_k}), \quad (2)$$

where DBH_s is the predicted equivalent 12-year height at Symmonds, $\overline{DBH_s}$ and $\overline{DBH_k}$ are the average DBH at Symmonds and Kilsby, respectively, DBH_k is the DBH at Kilsbys and σ_s and σ_k are the standard deviations for Symmonds and Kilsbys respectively. To minimise any imbalance of replications, data from trees only in the first four replications in Kilsbys were combined with data from Symmonds.

Data were arranged into a two-way factorial format according to founder clones and populations. For populations 2, 4 and 5, families could be clearly assigned among founder clones, but for populations 1 and 3, classifying families into eight founder clones necessarily use the same families for two founder clones according to their male and female parent or grandparent. This would create some undesirable correlation between founder clones for these two populations.

Fig. 3 Numbers of families forming population 2 (F = 0.125). Family designations represent their clonal parents, e.g. family 16 represents the family formed by crossing clone 1 with clone 6. Different individuals were used in each family for each cross involving that family, e.g. three different trees were used as females from family 16 to cross with males from 15, 17 and 18

Family of male parent	Family designation of female parent											
	16	17	18	26	27	28	35	37	38	45	46	48
15	1	1	1									
17	1		1									
18	1											
25				1	2	1						
27				1		1						
28				1								
35									1			
36							1	1	1			
37									1			
45											1	1
47										1	1	1
48											1	

Fig. 4 Numbers of families forming population 3 (F = 0.25). Family designations represent their clonal parents, e.g. family 15 represents the family formed by crossing clone 1 with clone 5. Within full-sib parental families, four trees were chosen, two for use as females and two for use as males, to yield two families for most combinations

Male	Family designation of female parent															
	15	16	17	18	25	26	27	28	35	36	37	38	45	46	47	48
15	2															
16		2														
17			2													
18				2												
25					2											
26						2										
27							1									
28								3								
35									2							
36										2						
37											1					
38												2				
45													2			
46														2		
47															1	
48																2

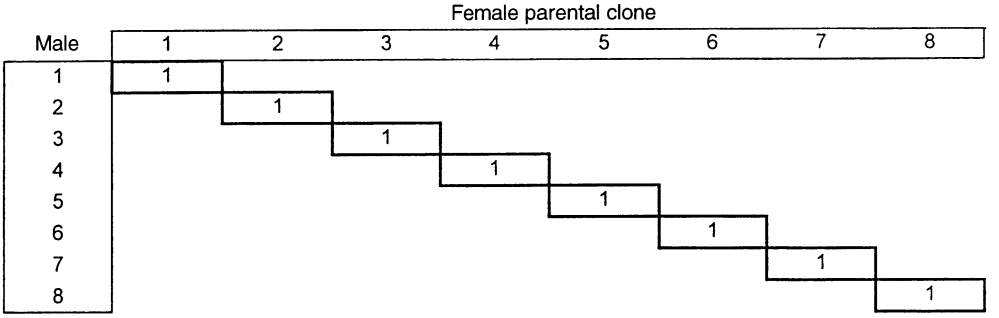


Fig. 5 Numbers of families representing population 4 (F = 0.5). Each clone was self-pollinated to produce a single family

A preliminary analysis of the combined DBH data indicated that interactions between replication and clone, and between replication and population (inbreeding level) were not significant. Thus the following linear model was used to study the effect of founder clones, population (inbreeding levels) and their interaction:

$$Y_{ijkl} = \mu + R_i + C_j + P_k + CP_{jk} + E_{ijkl}, \quad (3)$$

where μ is the grand mean, R_i is the effect of replication, C_j is the founder clone effect, P_k is the population (inbreeding level) effect, CP_{jk} is the interaction effect between clone and population, and E_{ijkl} is the residual. All effects were assumed to be random except for the grand mean and population effects. We partitioned the population effect in the following model to study the relationship between inbreeding levels and population performance:

$$P_k = \alpha F_k + \beta F_k^2 + I_k, \quad (4)$$

where F_k is the coefficient of inbreeding for the five populations, α and β are the coefficients for linear and quadratic effects for the variate F_k, I_k is the residual from the inbreeding-level effect after allowing a linear and quadratic contribution from the variate F_k The population effect and the clone by population interaction effect can also be further partitioned using the following model to study whether each clone has a different reaction to the inbreeding level:

$$P_k + CP_{jk} = \alpha_j F_k + \beta_j F_k^2 + I_{jk}, \quad (5)$$

where α_j and β_j are the coefficients for linear and quadratic effects of the inbreeding-level F_k on an individual clone and I_{jk} is the residual after accounting for differences in relation to F_k between clones.

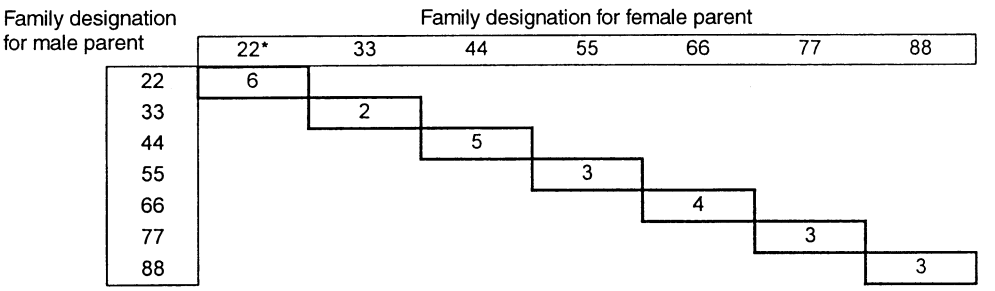


Fig. 6 Numbers of families representing population 5 (F = 0.75). Each parental family was formed from self-pollination and parent designation represents the grandparental clone (e.g. 44 refers to the family formed from selfing clone 44). * note that for all of these crosses, different individuals in parental families were selfed to produce each offspring family

Thus, the full model may be written as:

$$Y_{ijkl} = \mu + R_i + C_j + \alpha F_k + \beta F_k^2 + (\alpha_j - \alpha)\, F_k + (\beta_j - \beta)\, F_k^2 + I_{jk} + E_{ijkl}. \quad (6)$$

If the model is successful, most of the population variance will be explained by fitting the quadratic equation parameters α and β, and most of the interaction variance will be accounted for by fitting the heterogeneity of the regression parameters α_j and β_j The partitioning presented here is an extension to the frequently used genotype stability model for the study of genotype by environment interaction (Freeman and Perkins 1971).

The full model is non-linear and its statistical analysis is more complicated. The following approximate procedure was adopted to estimate parameters and in significance testing. First, we fitted model 3 to estimate the mean squares, the expected mean squares for clone, population, interaction between clone and population, and to test for significance. Second, we used the following model

$$Y_{ijkl} = \mu + R_i + C_j + \alpha F_k + \beta F_k^2 + I_k + CP_{jk} + E_{ijkl} \quad (7)$$

to estimate the mean squares for α, β and I_k, and test for their significance. Thus, the models were fitted sequentially. Regression parameters were tested for significance using synthesised mean squares from equation 3 (Satterthwaite 1946).

For the quadratic regression of individual clones, the model can be written as

$$Y_{ijkl} = \alpha + R_i + C_j + \alpha_j F_k + \beta_j F_k^2 + I_{jk} + E_{ijkl} \quad (8)$$

in which the individual parameters α_j, β_j and I_{jk} can be estimated approximately and tested based on individual clones (Bulmer 1980). The difference between models 8 and 7 can be expressed as $CP_{jk} + I_k - I_{jk} = (\alpha_j - \alpha)\, F_k + (\beta_j - \beta)\, F_k^2$, i.e. the heterogeneity of regressions. Thus, the total sum of squares for regression based on all individual clones minus the combined regression is the sum of squares for the heterogeneity of regression with a degree of freedom $C - 1$. We used this mean square to test the heterogeneity of quadratic equations among clones. There might be two other perceived biases in analysing such inbreeding data: accounting for possible unequal error variances among populations and an unequal contribution of the breeding value to different inbreeding levels. The

Table 1 Partitioning of variance, degrees of freedom and expected mean squares (EMS) in the analysis of variance for a 12-year old radiata pine inbreeding trial. n_{ijk} is number of trees within the ijk^{th} plot and C_j is number of populations (inbreeding levels) within the j^{th} clone. $k_1 = 141.16$, $k_2 = 9.56$, $k_3 = 41.31$, $k_4 = 14.49$, and $k_5 = 14.40$ for DBH (diameter at breast height). $k_1 = 138.13$, $k_2 = 9.40$, $k_3 = 40.53$, $k_4 = 14.10$, and $k_5 = 14.07$ for STD and CV (standard deviation and coefficient of variation). $k_1 = 133.09$, $k_2 = 13.68$, $k_3 = 49.46$, $k_4 = 14.15$, and $k_5 = 15.61$ for survival. σ_E^2, σ_I^2, σ_R^2, and σ_C^2 are the variances for residual, interaction, replication and clone, respectively; and Q(P) is a quadratic function of fixed population effects

Sources of variation	*df* [a]	EMS
Replication	(R–1)	$\sigma_E^2 + k_1\sigma_R^2$
Clone	(C–1)	$\sigma_E^2 + k_2\sigma_I^2 + k_3\sigma_C^2$
Population	(P–1)	$\sigma_E^2 + k_4\sigma_I^2 + Q(P)$
Linear	1	
Quadratic	1	
Deviation	(P–3)	
Clone * population	$\sum C_j - C - P + 1$	$\sigma_E^2 + k_5\sigma_I^2$
Heterogeneity of regression	$(C - 1)$	
Deviation	$\sum C_j - C - (P - 1) - (C - 1)$	
Residual	$\sum n_{ijk} - \sum C_j - R + 1$	σ_E^2
Total	$\sum n_{ijk} - 1$	

[a] *df* degree of freedom

treatment of these analytical complexities is beyond the purpose of this paper.

The overall partitioning of variances and the expected mean square is presented in Table 1. Plot means for DBH, the standard deviation (STD) and the coefficient of variation (CV) of DBH were computed for each plot and all analyses were based on plot means (there was an average of 4–6 trees for most families within a plot). STD and CV were used to represent the variation for each population. For survival data, the arcsine square-root transformation with a correction for 0 and 100% survival (Snedecor and Cochran 1967) was used before the analysis of variance. Variances for between-clone, between-family and within-family were also estimated for each population by the maximum-likelihood method (Searle 1971; SAS Institute Inc. 1989). Correlations were determined between the estimated DBH breeding values of the eight founder clones and the performances of full-sib, S_1 and S_2 families, and the linear regression coefficients for DBH and CV. The breeding values for the eight founder clones were estimated using open-pollinated progeny test data from 106 test locations Australia-wide (White et al. 1992 b).

Results

Analysis of variance

At age 12 from planting, the average DBH of radiata pine using combined data from Symmonds and Kilsbys declined from 20.2 cm for F = 0 to 19.2, 19.0, 17.1 and 16.4 cm for F = 0.125, 0.25, 0.5 and 0.75 respectively (Fig. 7). In contrast, the STD and CV increased as inbreeding progresses. There was no significant difference in survival between the outcrossed, the half-sib and full-sib matings (see Table 3). But survival declined significantly as inbreeding progressed further (see Fig. 11).

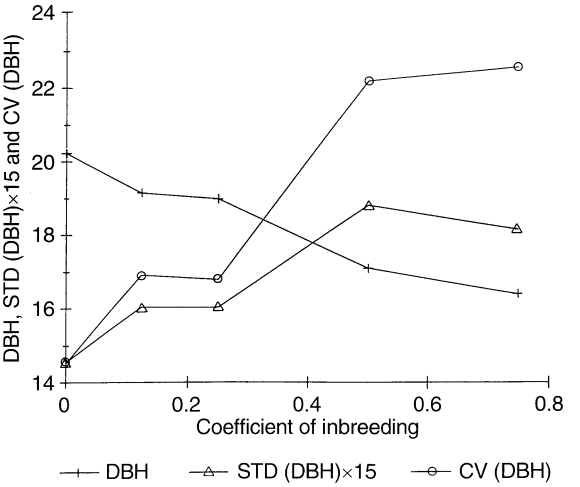


Fig. 7 Effect of inbreeding on DBH, the standard deviation [STD(DBH) × 15] and the coefficient of variation [CV(DBH)] in the 12-year-old inbreeding trial at Symmonds and Kilsbys

Differences between populations and replications were significant for all four traits whilst differences between founder clones were only significant for DBH (Table 2). When the population variation was further partitioned, only the linear effect of F was significant for DBH, survival, STD and CV at the population level. The founder clone by population interaction and the heterogeneity of regression were also significant for DBH, survival and CV, indicating that founder clones differed in their response to the F values (Table 2). Multiple-comparison tests indicated that there were no significant differences between outcross, half-sib, full-sib mated populations and between first-generation and second-generation selfs for these four traits (Table 3).

Relationship between inbreeding levels and growth, variance and survival

Due to significant heterogeneity of regression among founder clones, further regression analysis based on the whole population, as well as individual clones, was pursued. The regression of DBH on F based on populations indicated that only the linear effect of F was significant (Table 4). At the population level, the predicted depression for DBH was 0.55 cm (2.7%) for every 0.1 increase of F. However, the observed depression of DBH varied among F values and among individual founder clones (Figs. 7 and 8). At the population level, an increase of inbreeding level from F = 0.125 to 0.25 had little effect on DBH (1% reduction) while an increase of inbreeding from F = 0 to 0.125 and from F = 0.25 to 0.50 resulted in a 5% and 8% decline of

Table 2 Results of analysis of variance for DBH, survival, STD and CV of DBH for a 12-year-old radiata pine inbreeding trial. DBH – Diameter at breast height; STD and CV are the standard deviation and the coefficient of variation for diameter at breast height. *df* = Degree of freedom, M.S. = Mean square, F = F statistic

Sources of variation	DBH			Survival			STD			CV		
	df	M.S.	F	*df*	M.S.	F	*df*	M.S.	F	*df*	M.S.	F
Replication	3	33.5	6.62**	3	0.329	4.42**	3	9.39	3.90**	3	271.3	4.41**
Clone	7	60.9	6.02**	7	0.242	2.21 (0.06)	7	1.66	0.63	7	157.6	1.54
Population	4	238.2	18.73**	4	0.351	2.89*	4	11.60	4.30**	4	1188.7	9.68**
Linear	1	933.4	73.50**	1	0.944	7.78**	1	32.71	12.11**	1	3956.6	32.22**
Quadratic	1	1.6	0.13	1	0.069	0.57	1	2.06	0.76	1	5.1	0.04
Deviation	2	4.1	0.32	2	0.196	1.61	2	5.82	2.15	2	396.6	3.23
Clone × population	23	12.7	2.50**	23	0.122	1.63*	23	2.70	1.12	23	122.7	2.00**
Heterogeneity	7	35.7	7.05**	7	0.345	4.63**	7	4.93	1.89	7	240.8	3.92**
Deviation	16	2.6	0.52	16	0.024	0.32	16	1.72	0.72	16	71.0	1.15
Residual	529	5.1		528	0.075		517	2.41		517	61.5	
Total	566	8.1		564	0.082		554	2.49		554	72.87	

** $Pr < 0.01$ and * $Pr < 0.05$

Table 3 Means of DBH, survival and STD, CV of DBH and their standard error (SE) for five inbreeding levels in a 12-year-old radiata pine trial, inbreeding levels with the same letter in the Tukey – Kramer column are not significantly different. *SE based on original data and others based on plot mean. DBH – Diameter at breast height; STD and CV are the standard deviation and the coefficient of variation for diameter at breast height. Tukey–Kramer group – multiple comparison by the Tukey–Kramer criterion. Depression, calculated as $1 - (w_s/w_c)$ where w_s and w_c are measures of traits for inbred and outcrossed progeny

Traits	Inbreeding level	Mean (S.E.)	Tukey–Kramer groups	Depression
DBH	0	20.23 (1.5)	A	
	0.125	19.15 (1.7)	AB	5%
	0.25	19.00 (2.8)	AB	6%
	0.5	17.10 (2.2)	BC	15%
	0.75	16.35 (3.5)	C	19%
Survival	0	79.6 (19)*	AB	
	0.125	81.9 (17)	A	− 3%
	0.25	79.2 (20)	AB	1%
	0.5	74.0 (18)	AB	7%
	0.75	70.8 (27)	B	11%
STD	0	2.91 (1.1)	A	
	0.125	3.21 (1.1)	AB	− 10%
	0.25	3.21 (1.6)	AB	− 10%
	0.5	3.77 (1.0)	B	− 30%
	0.75	3.63 (2.2)	B	− 25%
CV	0	14.52 (5.7)	A	
	0.125	16.92 (6.1)	AB	− 17%
	0.25	16.81 (7.9)	AB	− 16%
	0.5	22.18 (7.0)	BC	− 53%
	0.75	22.56 (12.1)	C	− 55%

DBH respectively (Table 3). Founder clones had different responses to inbreeding (Figs. 8 and 9). Three response patterns of DBH to inbreeding were discernible. Inbreeding had no significant effect on DBH for founder clone 5 (Fig. 8 and Table 4). A continuous decline of DBH as F increases, as in founder clones 2, 3, 4 and 8, is characteristic for the second pattern. For founder clones 6 and 7, inbreeding reduced DBH up to $F = 0.5$, but the average DBH was higher at the higher inbreeding level of $F = 0.75$. The trend for founder clone 1 is not clear beyond $F = 0.5$ since it had only the first four inbreeding levels. Quadratic regression showed that only founder clones 3 and 6 had a curvilinear relation with F for DBH (Table 4). Founder clone 3 had a negative quadratic coefficient indicating an accelerated decline of DBH as F increases. Founder clone 6 had a positive quadratic coefficient indicating that DBH under higher levels of inbreeding is not much different from growth under moderate levels. The rest of the founder clones had a linear relation with inbreeding level, except for clone 5 which had no significant relation with inbreeding level.

Despite a rising trend for STD from $F = 0$ to $F = 0.5$ and a drop from $F = 0.5$ to $F = 0.75$ for populations, only the linear regression based on population means was significant (Table 4). A significant increase in CV along inbreeding levels (linear regression) was observed in this experiment (Fig. 7, Table 4). Similarly, different founder clones responded differently to the F values (Fig. 10). In general, there was a continuous increase in CV from $F = 0$ to 0.75 for four founder clones (3, 4, 7, and 8). However, founder clones 2 and 6 showed an increase from $F = 0$ to 0.50 and then a decline from $F = 0.5$ to 0.75. Clone 5 had a relatively stable CV at all inbreeding levels. Regression analysis revealed that seven founder clones had a significant linear relationship with F values.

Except at $F = 0$, depression of survival is more or less linear from $F = 0.125$ to 0.75 (Tables 3 and 4). Among the eight founder clones, only three (1, 2 and 4) had a significant relationship with F. Founder clone 1 had a quadratic relationship with F while the

Table 4 Linear and curvilinear regression of DBH, survival, STD and CV on inbreeding level in radiata pine based on population and individual founder clones, μ intercept, α- coefficient for linear effect of F, β – coefficient for quadratic effect of F, DBH – Diameter at breast height; STD and CV are the standard deviation and the coefficient of variation for DBH based on plot; n – indicates non-significant

Item	DBH			Survival			STD			CV		
	μ	α	β	μ	α	β	μ	α	β	μ	α	β
Quadratic												
Population		n	n		n	n		n	n		n	n
Clone 1		n	n	0.75	0.93*	− 2.24*					n	n
Clone 2	20.95	− 9.10*	1.95n		n	n				12.06	33.07*	−26.65n
Clone 3	19.82	− 0.68n	− 8.90*		n	n					n	n
Clone 4	20.16	− 9.86**	2.13n		n	n					n	n
Clone 5		n	n		n	n					n	n
Clone 6	20.75	− 11.42**	9.38*		n	n					n	n
Clone 7		n	n		n	n					n	n
Clone 8		n	n		n	n					n	n
Linear												
Population	20.17	− 5.52**		0.82	− 0.14**		2.98	1.03**		14.60	11.36**	
Clone 1	19.75	− 4.12*			n					14.33	17.65**	
Clone 2	20.79	− 7.55**		0.87	− 0.18*					14.21	12.50**	
Clone 3	20.43	− 6.99**			n					14.20	13.20**	
Clone 4	20.01	− 8.17**		0.829	− 0.44**					15.50	9.34**	
Clone 5		n			n						n	
Clone 6	20.04	− 4.05**			n					15.16	9.03*	
Clone 7	19.47	− 3.28*			n					14.11	12.81**	
Clone 8	19.33	− 7.43**			n					12.11	24.94**	

**Pr < 0.01 and *Pr < 0.05

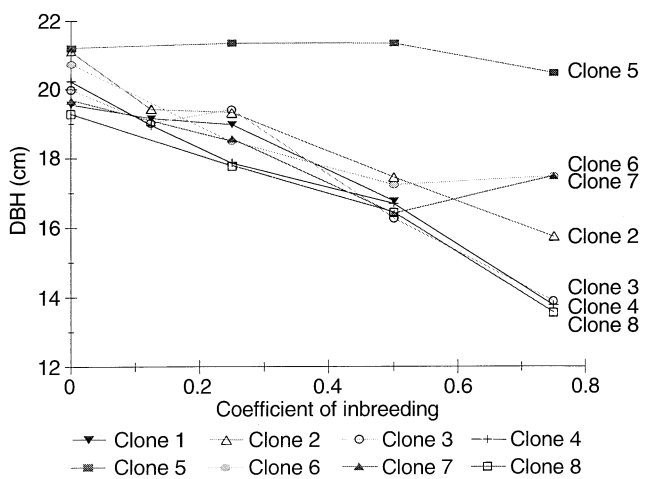


Fig. 8 Differential responses to inbreeding among eight radiata pine clones for DBH in the 12-year-old inbreeding trial

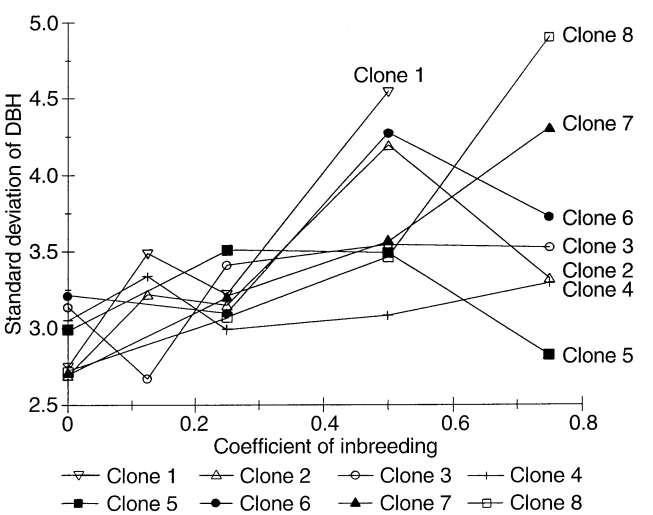


Fig. 9. Differential response to inbreeding among eight radiata pine clones for the standard deviation of DBH in the 12-year-old inbreeding trial

relationship for founder clones 2 and 4 was linear (Fig. 11).

Distribution of variance

Variances were estimated between clones, between-families, and within-families. Since there were only four clones in the half-sib mating (population 2) and one family per clone in population 4, the variances between clones for population 2 and within-families for population 4 were not used or calculated for the comparison. The change of variance with F values is depicted in Fig. 12. Between-clone variance as well as within-family variance increased as the inbreeding level increases, while between-family variance increased initially (at F = 0.125 and F = 0.25) and then declined at the highest inbreeding level (F = 0.75). The

within-family and between-family variance constitutes within-clone variance and this variance also increased as the inbreeding level increases, but at a slower pace than the increase for between-clone variance.

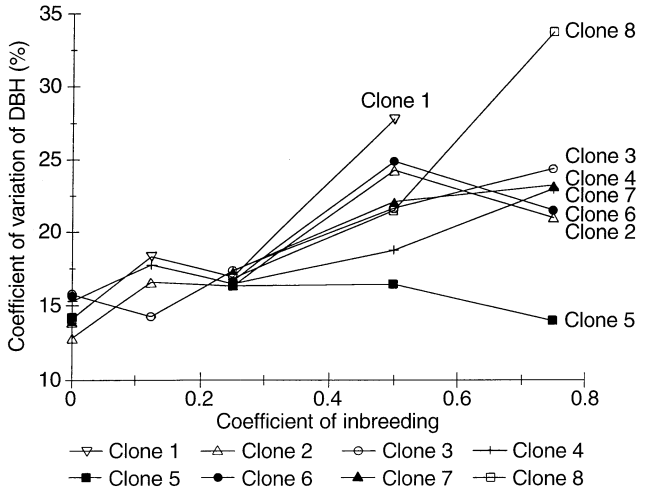


Fig. 10 Differential responses among eight clones for the coefficient of variation of DBH in the 12-year-old inbreeding trial

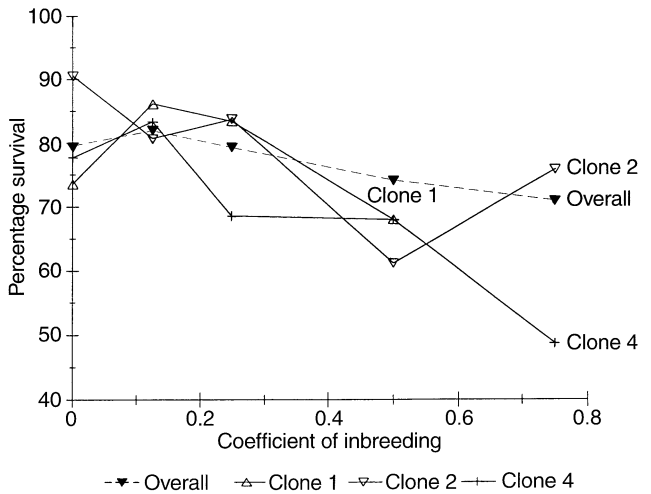


Fig. 11 Effect on inbreeding on survival for three clones and overall in the 12-year-old inbreeding trial. Clones with non-significant regressions are not shown

Relationship with DBH breeding values

The estimated DBH breeding values were not significantly correlated with the mean DBH of outcrossed families (Table 5), full-sib mated families and first-generation selfed (S_1) families, based on the eight founder clones. However, the DBH breeding values were very closely correlated with the mean DBH of second-generation selfs (S_2) based on the seven available clones; the Pearson linear correlation was 0.96 (Pr = 0.01) and the rank of breeding values among the seven clones was similar to the rank of DBH of the S_2 families. The coefficient of linear regression of DBH on F (α for DBH, Table 5) was also positively correlated with GCA, S_1 and S_2 family means (Table 5). Thus, there was less inbreeding-depression in performance for clones with higher breeding values.

Discussion

Inbreeding depression on survival and growth

Inbreeding depression in radiata pine survival was relatively low; the depression of selfed progenies was only

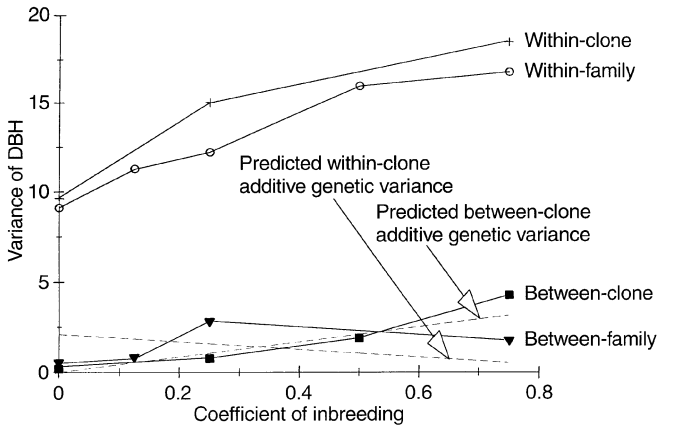


Fig. 12 Effect of inbreeding on between-clone and within-clone (between-family plus within-family) variances of DBH in the 12-year-old inbreeding trial at Symons and Kilsbys

Table 5 Correlations of DBH breeding values (GCA), means of outcrossed, full-sib, S1 and S2 families, coefficient of linear regression of inbreeding effect forDBH (α_{DBH}) and CV (α_{CV}) among eight clones (there were only seven clones for S2; the figure in parenthesis is the significance level)

Item	GCA	Outcross	Full-sib	S1	S2	α_{DBH}
Outcross	0.63 (0.09)					
Full-sib	0.53 (0.17)	0.63 (0.09)				
S1	0.58 (0.13)	0.45 (0.25)	0.74 (0.03)			
S2	0.96 (0.00)	0.60 (0.15)	0.72 (0.06)	0.76 (0.04)		
α_{DBH}	0.73 (0.04)	0.30 (0.47)	0.69 (0.06)	0.80 (0.01)	0.95 (0.00)	
α_{CV}	− 0.63 (0.09)	− 0.81 (0.02)	− 0.69 (0.06)	− 0.70 (0.05)	− 0.75 (0.05)	− 0.58 (0.13)

7%, not significantly different from that of outcrossed progenies. Milder inbreeding (half-sib and full-sib mating) did not reduce survival at all. Survival in population 5 (F = 0.75) was only 11% less than in outcrossed progenies. Low depression of selfed progeny for survival was also observed in *P. pinaster* (7% in S_1 and 20% in S_2, Durel et al. 1996). In contrast, much larger inbreeding depression for survival was reported in *P. elliotii* (Snyder 1972), *P. monticola* (Barnes 1964) and *P. sylvestris* (Dengler 1939; also see Table 4 in Sorenson and Miles 1982). The low depression for the survival rate of radiata pine may indicate that many of the lethal or semilethal alleles have been eliminated in the seed-production and nursery stages. In the same set of material Griffin and Lindgren (1985) observed that S_1 and S_2 had a much lower seed yield (43% and 42%) compared with populations from outcrossing, half-sib and full-sib mating. Also, because there were 60 seeds sown for most families and only the best 24 seedlings were used, some selection must have occurred in the nursery stage. Thus, it is possible that most lethal or more deleterious alleles may have been purged in the early seedling stage as well as in the seed-production stage.

The depression of DBH at various inbreeding levels observed in this experiment is in agreement with earlier observations from the radiata pine selfing experiments of Pawsey (1964) and Wilcox (1983), but had the lowest inbreeding depression for selfing in DBH (15%) and height (8.5%, Wilcox 1983) than for the depression observed in other conifers (Gansel 1971; Erikkson et al. 1973; Libby et al. 1981; Matheson et al. 1995; Durel et al. 1996). Effective purging of deleterious alleles for growth in the seed-production and nursery stage, and possibly the species biology, may be major reasons for this low inbreeding depression in radiata pine. The low inbreeding depression in radiata pine may be related to the species' mating history, its small natural distribution and strong geographic subdivision, as observed in maritime pine (Durel et al. 1996). Natural stands of radiata pine are limited to only three disjunct coastal populations in California and two island populations in Mexico with a total distribution of about 8400 ha (Moran and Bell 1987). The largest population at Monterey is only about 3 million trees (4700 ha) and smallest population on Guadalupe Island consists of only 368 trees in 1978 (80 ha). The genetic diversity in *P. radiata* ($H_T = 0.117$) is low compared with that of other tree species and conifers (the average H_T was 0.207 for 20 conifer species, Hamrick et al. 1981; Moran et al. 1988). Furthermore, a relatively large portion of genetic diversity in radiata pine was distributed between populations (16.2% compared with the average of 6.8%, Loveless and Hamrick 1984) and this between-population diversity is among the highest recorded for conifers (Moran et al. 1988).

The eight grandparents in this study were selections from first-generation plantations in Australia and New Zealand and have been used in Australian seed orchards. Isozyme study revealed that genetic diversity in Australian breeding populations was somewhat less than the natural stands and they were derived mainly from the Monterey and Año Nuevo (1.2 million trees in 1100 ha) populations (Moran and Bell 1987). In a study of levels of inbreeding in a *P. radiata* seed orchard, a selfing rate of about 10% was detected (Moran et al. 1980). It has also been observed that selfing did not show much adverse effect on adult fecundity in radiata pine (Pawsey 1964), and that natural selection against selfs and other inbreeding progenies was effective in radiata pine (Matheson 1980; Plessas and Strauss 1986). Thus, it is likely that the rather small geographic populations with a high degree of population subdivision in the natural distribution, combined with the relatively high fecundity of inbred progenies, could have increased inbreeding in natural populations of radiata pine and created an opportunity to purge deleterious alleles by natural selection.

A quadratic regression of DBH on F was significant for two founder clones (3 and 6). A quadratic regression usually suggests that there is a directional interlocus interaction or epistasis (Crow and Kimura 1970). For founder clone 3, the depression of DBH was more than expected from the predicted linear decrease at the higher inbreeding levels, indicating a reinforcing effect from inter-locus interaction deviations (Crow and Kimura 1970). In marked contrast, founder clone 6 had less depression for DBH at the higher inbreeding levels than expected from linear regression, demonstrating a collectively diminishing effect from the interaction deviations. These two individual clones showed quite different epistatic effects. The existence of a directional epistatic effect for the individual clone and an absence at the population level may indicate that these directional epistatic effects were cancelled out when all clones were pooled together. Similar linearity at the population level and quadratics at the individual clone were observed. These events indicate that the absence of any significant deviation from linear regression at the population level is not sufficient to rule out epistatic effects (Hallauer and Miranda 1981). Indeed, only epistatic gene effects could account for the severe reduction of seed production in the S_1 and S_2 populations for the same clones (Griffin and Lindgren 1985).

Inbreeding effect on variance

The genetic variance is a quadratic function of the inbreeding coefficient (Crow and Kimura 1970). If there are only additive gene effects, inbreeding would increase genetic variance in a linear way since $V_g = V_0(1 + F)$, where V_g is the genetic variance at inbreeding level F and V_0 is the genetic variance in the base population (F = 0). If inbreeding results in a subdivision of the population, this genetic variance would be

distributed between lines ($2FV_0$) and within lines $[(1 - F)V_0]$. With dominance, the trends of genetic variance between and within lines are dependent on underlying gene frequencies (Falconer 1981). Empirical evidence of a variance increase under inbreeding is widespread in tree species (Rudolph 1981; Wilcox 1983; Sniezko and Zobel 1988). We were able to partition total variance into between-clone, between-family and within-family variances in this study. The increasing between-clone variance with F observed in radiata pine is very similar to the theoretical prediction for between-line variance.

Inbreeding also affected within-clone variance, between-family, within-plot STD and CV. The explanation for within-clone, within-family and within-plot variance trends is more difficult to accomodate since it consists of both genetic and environmental variances. If there is only additive variance, within-line genetic variance should decline with the progress of inbreeding. However, all the experiments in conifers indicate that within-family or within-plot variance increases with increasing inbreeding (Rudolph 1981; Geburek 1986; Lundkvist et al. 1987; Sniezko and Zobel 1988; Matheson et al. 1995). In radiata pine, we also observed an increase for within-family variance as well as within-clone variance. There may be genetic reasons for the increase of within-plot, within-family, or within-clone variance (Robertson 1952; Goodnight 1988) as well as environmental reasons. Environmental variance may increase as inbred plants become less buffered against environmental stress (Robertson 1952; Falconer 1981; Matheson et al. 1995; Williams and Savolainen 1996) offsetting the decrease of genetic variance within a line. With the estimated additive genetic variance (2.1) for the base population (estimated by between-clone variances for $F = 0.25$, 0.5 and 0.75 under the assumption of a complete additive genetic model), and assuming only additive genetic effects, between-clone and within-clone genetic variance can be predicted at various inbreeding levels (Fig. 12). There is a large difference between the observed within-clone variance and the predicted within-clone additive genetic variance. If the difference between the observed within-clone variance and the predicted within-clone additive genetic variance is predominantly due to environmental variance, then this variance increases as inbreeding progresses (Fig. 12).

Variation of inbreeding depression among clones

Founder clones had significant differences on inbreeding depression in growth and variance. Founder clone 5 had no inbreeding depression for DBH. In addition, variance (STD and CV) and survival were not affected by inbreeding in this same founder clone (Table 4). There are two possible explanations for this phenomenon. First, it is possible that clone 5 might have few lethal or other deleterious recessive genes. Second, this clone might have more genetic homogeneity. Indeed, founder clone 5 had the highest breeding value in DBH and had the best DBH in the outcrossed progenies. This could mean that founder clone 5 has many desirable alleles fixed and should be highly prized for advanced-generation breeding for growth rates.

Relationship with breeding values

There should be a perfect correlation between selfed and outcrossed breeding values if there is no non-additive genetic variance (Matzinger and Cockerham 1963). Thus, Wright (1980) advocated the use of selfs for progeny testing to predict the general combining ability (GCA) for outcrosses. Barker and Libby (1974) showed that the performance of self families can be a reliable indicator of general combining ability and could, therefore, be useful for progeny testing and family selection. However, reported S_1-GCA correlations in conifers are mixed. There was a nearly zero correlation for loblolly pine (Sniezko and Zobel 1988). Wilcox (1983) observed that correlations between outcrossed and S_1 family means were moderately strong for several traits, including height, diameter and volume at age 4 in radiata pine, and recommended that screening for needle resistance from selfing would be effective. But a zero correlation between S_1 and the 7-year-old diameter was reported. We observed that the means of S_1 families were not significantly correlated with either DBH breeding values or the mean DBH of outcrossed progenies. However, the means of S_2 families were highly correlated with DBH breeding value ($r = 0.96$). The close correlation between second-generation selfs and breeding values suggests that selfing could be an effective breeding tool even in the early generations, if selection intensity in the first generation is strong enough to improve the correlation between breeding values and S_2, or with later selfed generations. Presumably, in this experiment, selection in the first generation was strong enough to eliminate many deleterious alleles thus improving the correlation between inbred performance (S_2) and breeding values. The decline of standard deviation from $F = 0.5$ to 0.75 and the non-significant difference between S_1 and S_2 for DBH observed in our study may also indicate that purging of deleterious alleles has taken place in the S_1. The high seed mortality in S_1 and S_2 observed in this experiment (Griffin and Lindgren 1986) and the selection of S_1 from early flowering trees (presumably more vigorous as well) for second-generation selfing may have exerted some purging effect. For most conifers, selfing is usually not encouraged for reducing inbreeding depression in small subpopulations or elite lines of deleterious alleles (Williams and Savolainen 1996); sib- or random-mating was recommended as a better option in the early generations of conifer domestication

(Namkoong and Kang 1990; Matheson et al. 1995). For radiata pine, with its low genetic load and inbreeding depression, many deleterious allele may already have been purged through natural selfing and selection. Also, radiata pine was reported as having no severe inbreeding depression on adult fecundity in selfs (Pawsey 1964; Wilcox 1983). With potential short generations in radiata pine (Cotterill et al. 1988; Matheson et al. 1994) and the early demonstration of inbreeding depression, the species may be ideal to capture the theoretical advantages of inbreeding through intensive early purging. A difference between breeding trees from breeding animals and some crops is that large progenies can be derived from a single mating (selfing); thus, very high selection intensities could be exercised at an early age following selfing. If early selection for the purging of deleterious alleles is effective, generating superior allele combinations from selfing may be a far quicker way to obtain genetic gain than an outcrossed breeding strategy.

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References

Andersson E, Jansson R, Lindgren D (1974) Some results from second crossings involving inbreeding in Norway spruce (*Picea abies*). Silvae Genet 23 : 34–42

Barker JE, Libby WJ (1974) The use of selfing in selection of forest trees. J Genet 61 : 152–168

Barnes BV (1964) Self- and cross-pollination of western white pine: a comparison of height growth of progeny. USDA For Ser Res Note INT-22

Bingham ET (1973) Possibilities for improvement of western white pine by inbreeding. USDA For Ser Res Pap INT-44

Bramlett DL, Popham TW (1971) Model relating unsound seed and embryonic lethal alleles in self-pollinated pines. Silvae Genet 20 : 192–193

Buijtenen JP van, Lowe WJ (1979) The use of breeding groups in advanced generation breeding. In: Proc 15th Conf on Southern Forest Tree Improvement, pp 56–65

Bulmer MG (1980) The mathematical theory of quantitative genetics. Oxford University Press, UK

Burdon RD, Namkoong G (1983) Multiple populations and sublines. Silvae Genet 32 : 221–222

Burrows PM, Askew GR (1982) Experimental inbreeding depression. For Sci 28 : 148–156

Cotterill PP, Dean CA, Cameron J, Brindbergs M (1988) Nucleus breeding: a new strategy for rapid improvement under clonal forestry. In: Proc IUFRO Meet On Breeding Tropical Trees, Pattaya, Thailand, pp 180–223

Crow JF, Kimura M (1970) An introduction to population genetics theory. Harper and Row, New York

Dengler A (1939) Über die Entwicklung künstlicher Kiefernkreuzungen. Zeitschrift f Forst- u Jagdwesen 71 : 457–485

Durel CE, Bertin P, Kremer A (1996) Relationship between inbreeding depression and inbreeding coefficient in maritime pine (*Pinus pinaster*). Theor Appl Genet 92 : 347–356

Eriksson G, Schelander B, Akebrand V (1973) Inbreeding depression in an old experimental plantation of *Picea abies*. Hereditas 73 : 185–194

Falconer DS (1981) Introduction to quantitative genetics, 2nd edn. Longman, UK

Fowler DP (1965) Effects of inbreeding in red pine, *Pinus resinosa* Ait. Silvae Genet 12 : 12–23

Franklin EC (1970) Survey of mutant forms. USDA For Serv Res Pap SE-61

Freeman GH, Perkins JM (1971) Environmental and genotype-environmental components of variability. VIII. Relations between genotypes grown in different environments and measures of these environments. Heredity 27 : 15–23

Gansel CR (1971) Effects of several levels of inbreeding on growth and oleoresin yield in slash pine. In Proc 11th Conf On Southern Forest Tree Improvement, Atlanta, Georgia, USA, pp 173–177

Geburek T (1986) Some results of inbreeding depression in Serbian spruce [*Picea omorika* (Panc.) Purk.]. Silvae Genet 35 : 169–172

Goodnight CJ (1988) Epistasis and the effect of founder events on additive genetic variance. Evolution 42 : 441–454

Griffin AR, Lindgren D (1985) Effect of inbreeding on production of filled seed in *Pinus radiata* – experimental results and a model of gene action. Theor Appl Genet 71 : 334–343

Hallauer AR, Miranda JB (1981) Quantitative genetics in maize breeding. Iowa State University Press, Ames, Iowa, USA

Hamrick JL, Mitton JB, Linhart YB (1981) Levels of genetic variation in trees: influence of life history characteristics. In: Conkle MT (ed) Proc Symp On Isozymes of North American Forest Trees and Forest Insects. USDA For Ser Gen Tech Rep, PSW-48, pp 35–41

Koski V (1971) Embryonic lethal of *Picea abies* and *Pinus sylvestris*. Commun Inst For Fenn 75 : 1–30

Layton PA, Goddard RE (1983) Low-level inbreeding effects on germination, survival, and early height growth of slash pine. In: Proc 17th Conf On Southern Forest Tree Improvement, Athens, Georgia, USA, pp 106–115

Libby WJ, McCutchan BG, Millar CI (1981) Inbreeding depression in selfs of redwood. Silvae Genet 30 : 15–25

Loveless MD, Hamrick JL (1984) Ecological determinants of genetic structure in plant populations. Annu Rev Ecol Syst 15 : 65–95

Lundkvist K, Eriksson G, Norrel L, Ekberg I (1987) Inbreeding depression in two field trials of young *Pinus sylvestris* (L). Scand J For Res 2 : 281–290

Matzinger DF, Cockerham CC (1963) Simultaneous selfing and partial diallel test crossing. I. Estimation of genetic and environmental parameters. Crop Sci 3 : 309–314

Matheson AC (1980) Unexpectedly high frequencies of outcrossed seedlings among offspring from mixtures of self and cross pollen in *Pinus radiata* D. Don. Aust For Res 10 : 21–27

Matheson AC, Brown AG (1983) Radiata pine breeding manual. CSIRO Division of Forest Research, Canberra, Australia

Matheson AC, Spencer DJ, Magnussen D (1994) Optimum age for selection in *Pinus radiata* basal area under bark for age : age correlations. Silvae Genet 43 : 352–357

Matheson AC, White TL, Powell GR (1995) Effects of inbreeding on growth, stem form and rust resistance in *Pinus elliottii*. Silvae Genet 44 : 37–46

McKeand SE, Bridgwater FE (1993) Third-generation breeding strategy for the North Carolina State University-Industry Cooperative tree improvement program. In: Proc IUFRO Conf S2.02.-08 On Breeding Tropical Trees, pp 223–233

Moran GF, Bell JC (1987) The origin and genetic diversity of *Pinus radiata* in Australia. Theor Appl Genet 73 : 616–622

Moran GF, Bell JC, Matheson AC (1980) The genetic structure and levels of inbreeding in a *Pinus radiata* D. Don seed orchard. Silvae Genet 29 : 190–193

Moran GF, Bell JC, Eldridge KG (1988) The genetic structure and the conservation of five natural populations of *Pinus radiata*. Can J For Res 18 : 506–514

Namkoong G, Kang H (1990) Quantitative genetics of forest trees. Plant Breed Rev 8 : 139–169

Park YS, Fowler DF (1982) Effects of inbreeding and genetic variances in a natural population of tamarack [*Larix laricina* (Du Roi) K. Koch] in eastern Canada. Silvae Genet 31 : 21–26

Pawsey CK (1964) Inbreeding radiata pine. Dep Nat Dev For and Timber Bureau Leaflet No. 87

Plesas ME, Strauss SH (1986) Allozyme differentiation among populations, stands, and cohorts in Monterey pine. Can J For Res 16 : 1155–1164

Robertson A (1952) The effect of inbreeding on the variation due to recessive genes. Genetics 37 : 189–207

Rudolph TD (1981) Four-year height growth variation among and within S_0, $S_1 \times S_1$, S_1 open-pollinated and S_2 inbred jack pine families. Can J For Res 11 : 654–661

SAS Institute Inc (1989) SAS/STAT User's guide, version 6, 4th edn, Vols 1 and 2. Cary, North Carolina, USA

Satterthwaite FW (1946) An approximate distribution of estimates of variance components. Biometrics Bull 2 : 110–114

Searle SR (1971) Linear models. John Wiley and Sons, New York

Snedecor GW, Cochran WG (1967) Statistical methods, 6th edn. Iowa State University Press, Ames, Iowa, USA

Sniezko RA (1984) Inbreeding and outcrossing in loblolly pine. PhD thesis, North Carolina State Univ, Raleigh, North Carolina, USA

Sniezko RA, Zobel BJ (1988) Seedling height and diameter variation of various degrees of inbred and outcross progenies of loblolly pine. Silvae Genet 37 : 50–60

Snyder EB (1972) Five-year performance of self-pollinated slash pines. For Sci 18 : 246

Sorensen FC (1969) Embryonic genetic load in coastal Douglas-fir. Am Nat 103 : 389–398

Sorensen FC, Miles RS (1982) Inbreeding depression in height, height growth and survival of Douglas-fir, ponderosa pine and noble fir to 10 years of age. For Sci 28 : 283–292

Squillace AE, Kraus JF (1962) Effects of inbreeding on seed yield, germination, rate of germination, and seedling growth in slash pine. In: Proc For Genet Wkshp, Macon, Geogia, USA, pp 59–63

White TL (1993) Advanced-generation breeding populations: size and structure. In: Proc IUFRO Conf S2.02.-08 on Breeding Tropical Trees, pp 208–222

White TL, Matheson AC, Boomsma DB, Rout AF (1992 a) Logistics, costs and genetic gains of five options of nucleus breeding strategies. STBA Technical Report TR92-01

White TL, Rout AF, Boomsma DB, Dutkowski GW (1992 b) Predicted breeding values of 1213 first-generation parents. Confidential STBA Technical Report TR92-02

Wilcox MD (1983) Inbreeding depression and genetic variances estimated from self-and cross-pollinated families of *Pinus radiata*. Silvae Genet 32 : 89–96

Williams CG, Salvolainen O (1996) Inbreeding depression in conifers: implications for breeding strategy. For Sci 42 : 102–117

Wright AJ (1980) The expected efficiencies of half-sib, testcross and S1 progeny testing methods in single population improvement. Heredity 45 : 361–376