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Inbreeding Depression and Genetic Variances Estimated From Self- and Cross-pollinated Families of Pinus radiata

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Summary

Compared with artificially cross-pollinated progenies, self-pollinated progenies of 25 *Pinus radiata* D. DON plus-trees (seed orchard clones) grew more slowly in the nursery and, up to the age of 7 years on a fertile site in Kaingaroa Forest, grew more slowly in diameter (ratio of self: cross means = 0.88), had more crooked stems (0.88), had a less desirable branching habit (0.80), had poorer needle retention, i.e., were less resistant to *Naemacyclus* and/or *Dothistroma* needle diseases (0.84), and had slightly denser wood (1.01). There was a strong interaction between parent and inbreeding depression for diameter growth, giving a very weak correlation between the relative performance of selfs and their corresponding crosses. For other traits correlations between selfs and crosses were high, and self-pollinated families could be used to measure the general combining ability of the parents.

Estimates of family variance were higher in all traits for self- than for cross-pollinated families.

Additive, dominance, and additive $\times$ additive genetic variances were jointly estimated from separate and co-analyses of selfs and crosses. These estimates were not considered to be very reliable but nevertheless indicated that wood density, branching quality, and stem straightness were strictly under additive genetic control, diameter growth was under non-additive genetic control, whereas the genetic variance for needle retention was entirely additive and additive $\times$ additive.

It is concluded that selfing in *P. radiata* can be detrimental to growth, stem form, branching quality, and needle cast resistance, and that selfing and other severe forms of inbreeding must be minimised in seed orchards.

Key words: Pinus radiata, inbreeding, genetic variances, disease resistance.

Zusammenfassung

Verglichen mit Nachkommenschaften aus kontrollierter Kreuzung wuchsen Nachkommenschaften von 25 *Pinus radiata* D. DON Plusbäumen (Samenplantagen-Klone) aus Selbstung in der Baumschule und auf einem fruchtbaren Standort im Kaingaroa Forest bis zum Alter 7 langsamer, auch im Durchmesser (Verhältnis Selbstung: Kreuzung 0.88). Es wurden auch mehr krumme Stämme (0.88) und ein wenig wünschenswerter Zweighabitus (Aststellung (0.80) und eine schwächere Benadelung sowie geringere Resistenz gegen *Naemacyclus minor* BUTIN und/oder *Dothistroma*, jedoch eine geringfügig höhere Holzdichte (1.01) beobachtet. Im Durchmesserwachstum wurde eine Interaktion zwischen den Eltern und der Inzuchtdepression festgestellt, die eine sehr schwache Korrelation zwischen der relativen Leistung der Selbstungen und derjenigen ihrer korrespondierenden Kreuzungen erkennen ließ. Bei anderen Merkmalen waren die Korrelationen dagegen hoch, d. h. Selbstungsfamilien könnten verwendet werden, um die allgemeine Kombinationseignung von Elternbäumen zu ermitteln.

Die Varianzschätzungen für „Familien" waren bei allen Merkmalen für die Selbstungsfamilien größer als für die Fremdkreuzungsfamilien. Mit Ausnahme des Merkmals Nadelschütte waren bei den Selbstungen die Varianzen innerhalb der Parzellen größer. Die Heritabilitäten der Familien-Mittel waren bei den Selbstungen allgemein größer, und somit die Rangfolgebildung von Familien präziser. Phänotypische Korrelationen von Familien-Mitteln zeigten für verschiedene Kombinationen von Merkmalen vergleichbare Resultate bei Selbstungen und Kreuzungen; Ausnahme war die Kombination BHD-Holzdichte, wo die negative Korrelation bei den Selbstungen (−0,65) deutlich größer war als bei den Kreuzungen (−0,32).

Additive, Dominanz- und additiv $\times$ additive Varianzen wurden anhand separater und gemeinsamer Analyse von Selbstungen und Kreuzungen ermittelt. Obgleich diesen Schätzungen keine große Verläßlichkeit zugebilligt werden muß, deuteten sie doch an, daß Holzdichte, Aststellung und Schaftform unter ausschließlich additiver Genkontrolle stehen sowie Dickenwachstum unter nicht-additiver, währenddessen sich die genetische Varianz bei Nadelschütte gänzlich additiv und additiv $\times$ additiv zeigte.

Es wird gefolgert, daß Selbstung bei *P. radiata* nachteilig für Wüchsigkeit, Schaftform, Aststellung und Schütteresistenz sein kann und daß Selbstung sowie andere hohe Inzuchtgrade bei Samenplantagen so weit wie möglich verhindert werden sollten.

Introduction

Fear of inbreeding — the reduction in heterozygosity resulting from mating of close relatives — considerably influences the New Zealand Forest Service's breeding programme for *Pinus radiata*. In forest trees which are normally outcrossing, inbreeding by self-fertilisation (selfing) generally has deleterious effects including reduced seed set, poor seed germination, and slow seedling growth. One of the commonest phenomena associated with selfing is the appearance of rare recessive alleles normally hidden in the heterozygous state and often manifested as grossly abnormal seedlings. Some instances have been observed in *P. radiata* (Bannister 1965).

To minimise the opportunity for self-pollination between ramets of the same clone in seed orchards, it is usual to employ reasonably large numbers (24—80) of un-related clones in a design in which ramets of the same clone are planted far apart. Less severe forms of inbreeding such as mating between half-sibs, between full-sibs, and between parent and offspring, are minimised in second-generation clonal orchards and in seedling orchards (i.e., seed orchards planted from seedling progeny of selected parents) by initially selecting from a large base of unrelated families and then by spatially separating related trees within orchards; by maintaining separate first- and second-generation orchards (to minimise parent-offspring matings); and by strictly limiting the numbers of individuals in the orchard with common ancestors. Selfing *within* trees of a monoecious species such as *P. radiata* cannot be readily controlled in either clonal or seedling seed orchards, but may not be as prevalent as imagined (Matheson 1980).

The effect of one generation of selfing in *P. radiata* was examined with the objective of measuring the degree of inbreeding depression or other quantitative changes to progeny means associated with an inbreeding coefficient of F = 0.5. In addition, variances between and within self-pollinated families were compared with those of cross-pollinated families, genetic variances were estimated, and the prospects were examined for using selfs in a breeding programme for progeny testing and for other methods of selection.

Materials and Methods

Self- and Cross-pollinated Families

Twenty-five presumably unrelated and non-inbred (F = 0) *P. radiata* seed orchard clones of the N.Z. Forest Service's "850" series formed the parent material for this study. The parent ortets (plus-trees) were stringently selected (mainly in first-rotation plantations planted 1926—29 in Kaingaroa Forest) for straight stems, small-diameter multinodal branching, and fast diameter growth.

Self-pollinated seed was produced from each parent by controlled pollinations during 1964—68. Cross-pollinated seed was produced mostly by artificially crossing each parent, usually as a female, individually with four of five designated tester parents — No. 7, 19, 55, 97, and 121. These were considered to be among the best plus-trees, were well represented in clonal archives, and produced plenty of pollen. The five testers were also used as test parents in the crosses, with their own pollen excluded to eliminate any selfing. Equal numbers of seed from the separate crosses made on each female were composited to form the cross-pollinated seedlots. Twelve of the 100 crosses could not be made and for these was substituted open-pollinated seed from the parents involved, collected from ramets in the Kaingaroa seed orchard.

Mean 100-seed weight was recorded on each self- and cross-pollinated seedlot, but no accurate record was kept of the average number of filled seeds extracted per cone.

Experimental Design

Seed of the 50 seedlots (25 selfed and 25 cross-composites) was sown in randomised, unreplicated nursery plots at Rotorua in December 1972. No record was kept of subsequent seed germination or of frequency of abnormal seedlings.

Before planting out, obvious dwarfs, albinos, or other peculiar seedlings were culled from the plots of selfs.

Seedlings were planted in July 1974 in Compartment 1350, Kaingaroa Forest (Experiment No. R944/18), at a spacing of 4 × 4 m. A 5 × 5 balanced lattice design was used with six complete block replicates and five incomplete blocks per complete block. Within the incomplete blocks, selfs and crosses of the same parent were planted in adjacent 10-tree row plots, thus forming a split-plot lay-out.

Assessment Methods

Height of trees in nursery (1974)

Heights were measured on a sample of 20 self- and 20 cross-pollinated progeny of each parent in March 1974, 15 months after seed sowing.

Height 1 year after planting (1975)

Heights of all trees were measured in the field in June 1975.

Assessment 4 years after planting (1978)

An assessment of all trees was made in June 1978, as follows:

Height; in dm.

Diameter; at 1.4 m, in cm.

Volume; in dm³, calculated from height (h) and diameter (d) using paraboloid formula, $V = 0.4 \, (d/10)^2 h$.

Straightness; scored on a scale of 1 (very crooked) to 9 (very straight).

Stem malformation; scored on a scale of 1 (severely malformed with forks and multiple ramicorn branches) to 6 (free of any malformation).

Assessment 7 years after planting (1981)

In February 1981, the best four trees in each plot of 10 were selected on growth and stem form. Other things being equal, trees were selected to give the most even spacing in the plots after thinning. Measurements were made on the selected trees as follows:

Diameter; at 1.4 m, in cm.

Stem straightness; scored on a scale of 1—9.

Branching quality; scored on a scale of 1 (coarse uninodal branching with prevalent ramicorns) to 9 (small-diameter, horizontal, multinodal branching).

Malformation; scored on a scale of 1—6.

Needle cast; assessed on a scale of 0—100%, in 10% steps, representing percentage of green crown defoliated. The needle cast was associated with infection by *Naemacyclus minor* and/or *Dothistroma pini*. Re-expressed as "needle retention" measuring resistance

to *Naemacyclus* and/or *Dothistroma*, i.e., the complement (100- defoliation %) of "needle cast". [It is now not certain whether the needle cast was mainly caused by *Naemacyclus minor* or *Dothistroma pini*. At the assessment in February 1981, defoliation was generally severe and seemed to be associated with *Naemacyclus*. However, in August 1982 *Dothistroma* was by far the more obvious disease throughout the experiment.]

Wood penetration; assessed in units of 0.1 mm using the Pilodyn Wood Tester (Cown 1978). Two readings were taken per tree, both from the one sampling "window" at 1.4 m above ground, and averaged. Re-expressed as "basic wood density" estimated from Pilodyn penetration from DENSITY (kg/m³) = 436.1 — 0.6778 PENETRATION (mm/10).

This regression equation was derived from a sample of 60 trees, both selfs and crosses, covering the range of penetrations (140—265 mm/10) encountered in the experiment. Density (mean of rings 2—6) was determined from 2 × 5 mm increment cores per tree. The coefficient of determination (R²) was 0.49 — somewhat lower than the value obtained in some other studies (Cown 1978).

Data Analysis

Estimation of inbreeding depression

Means of selfs and crosses over the whole experiment, and the ratio of selfs/crosses (= "inbreeding ratio") were calculated.

Plot means of each trait for selfs and crosses assessed in 1975, 1978, and 1981 were subjected to a joint three-way crossed classification analysis of variance, the model for which is implicit in *Tables 2* and *3*. The lattice structure of the experiment was ignored, there being negligible gain in precision in a preliminary analysis of the 1978 assessment data. These analyses provided F-tests for parental effects, for inbreeding effects (i.e., contrast between selfs and crosses), and for parent × selfs v. crosses interaction. Replications and parents were assumed to be random effects, and selfs v. crosses a fixed effect.

Estimation of variances from selfs and crosses

Measurements from individual trees (1981) from the selfs and crosses treated as separate experiments were analysed in a two-way crossed classification random model, and family variance components (σ^2_f) were estimated by Henderson's Method 1 (Searle 1971).

As well as the separate analyses of selfs and crosses, a co-analysis was made of the plot mean data to give an estimate for each trait of the covariance between selfs and corresponding crosses of the same test clone (Matzinger and Cockerham 1963; Nasoetion *et al.* 1967). Correlation coefficients between selfs and crosses were also calculated.

Genetic interpretation of family variance components estimated from selfs and crosses and of the covariance between selfs crosses.

The genetic relationship between relatives (x and y) can be expressed in terms of coancestry and inbreeding according to S. Wright's classical concepts (Crow and Kimura 1970) as

$$r_{xy} = \frac{2\Theta_{xy}}{\sqrt{(1 + F_x)(1 + F_y)}} \quad \ldots \ldots \ldots \quad (1)$$

where r_{xy} is the coefficient of relationship or genetic correlation,

Θ_{xy} is Malécot's coancestry coefficient,

and $F_x(F_y)$ is the inbreeding coefficient of x (y).

(i) *Family variance component of crosses (σ^2_c)*

With only four testers used, the cross-pollinated families ($F_x = F_y = 0$) contain a mixture of half-sib ($\Theta_{xy} = 0.125$) and full-sib ($\Theta_{xy} = 0.25$) progeny, the expected proportions of each being a function of family size and the number of testers (Namkoong 1966; Squillace 1974). For the 1975 and 1978 assessments when all trees were included, families contained close to 40 progeny, and the proportion of offspring fathered or mothered by each of the four testers can be assumed to be 0.25. The proportion of full-sibs per cross-pollinated family is 0.23, and of half-sibs, 0.77, giving a mean coancestry coefficient of $\Theta_{xy} = 0.1538$, and a mean coefficient of relationship among offspring of $r_{xy} = 2\Theta_{xy} = 0.3075$.

When only the best four trees per plot were measured in 1981, family size reduces to 24, and it is assumed that the proportions of offspring from each tester are no longer equal but that selection will have heavily favoured one of them (e.g., Plus-Tree 55 because of its known exceptional vigour) at the expense of the others. Assuming the proportions of offspring from the four testers are 0.50, 0.20, 0.20, and 0.10, the proportion of full-sibs increases to 0.31, and that of half-sibs decrease to 0.69, giving a mean coancestry coefficient of $\Theta_{xy} = 0.1638$, and a mean coefficient of relationship of $r_{xy} = 0.3276$.

In each example, r_{xy} is the coefficient of the additive genetic variance (σ^2_A) in the genetic expectation of the family variance component, σ^2_c. When dominance (σ^2_D) and additive × additive (σ^2_{AA}) epistatic variances are included in the genetic model, their coefficients likewise have to be weighted according to the expected proportions of full-sibs and half-sibs within cross-pollinated families. The coefficient for σ^2_D is $1/4\ p_{fs}$ where p_{fs} is the proportion of full-sibs, and for σ^2_{AA} it is $1/16\ p_{hs} + 1/4\ p_{fs}$, where p_{hs} is the proportion of half-sibs. Thus, a genetic interpretation of σ^2_c for the 1975 and 1978 assessments can be expressed as

$$\sigma^2_c = 0.3075\ \sigma^2_A + 0.0575\ \sigma^2_D + 0.1056\ \sigma^2_{AA} \quad . \quad (2)$$

and for the 1981 assessment as

$$\sigma^2_c = 0.3276\ \sigma^2_A + 0.0775\ \sigma^2_D + 0.1206\ \sigma^2_{AA} \quad . \quad (3)$$

In neither (2) nor (3), however, are the coefficients for σ^2_D and σ^2_{AA} large enough for the Equations to be reliable for estimating non-additive genetic variances.

(ii) *Family variance component of selfs (σ^2_s)*

Inbreeding increases the amount of all types of genetic variances contained in the family variance (Cockerham 1961). In self-pollinated families from non-inbred parents, the coancestry coefficient of self-sibs is $\Theta_{xy} = 0.5$ (which is equivalent to the coancestry of a non-inbred individual with itself). Since the selfs are inbred to F = 0.5, the coefficient of relationship between self-sibs (r_{xy}) from (1) is 0.6667.

Now, the additive genetic variance estimated in an inbred population (σ^2_A*) is related to that in the non-inbred references population (σ^2_A) by $\sigma^2_A* = (1 + F)\sigma^2_A$, where F is the inbreeding coefficient of the inbred individuals. Thus, the total additive genetic variance among selfed individuals (F = 0.5) is $3/2\ \sigma^2_A$. The coefficient of the additive genetic variance (σ^2_A) in the genetic expectation of the family variance component (σ^2_s) is thus $r_{xy}\ 3/2\ \sigma^2_A$ or 1, leaving $1/2\ \sigma^2_A$ distributed *within* the selfed families.

When, and only when gene frequencies are a half, σ^2_s is a simple linear function of additive, dominance and ad-

ditive × additive (other epistatic components assumed to be zero) variances,

$$\sigma^2_s = \sigma^2_A + 1/4\ \sigma^2_D + \sigma^2_{AA} \quad \ldots \ldots \ldots \quad (4)$$

(MATZINGER and COCKERHAM 1963).

(iii) *Covariance between selfs and crosses* (Cov (S, C))

The inclusion of both selfs and corresponding crosses in the same experiment permits the estimation of a linear function of genetic variances from the covariance between the selfs and crosses. The phenotypic covariance between self-pollinated and cross-pollinated family means is also the estimator of the genetic covariance (MATZINGER and COCKERHAM 1963; NASOETION *et al.* 1967).

The coancestry coefficient of "self-half sibs" (SQUILLACE 1974), i.e., between an individual in a self family ($F_x = 0.5$) and an individual in a corresponding cross-pollinated family ($F_y = 0$) is $\Theta_{xy} = 0.25$, with a coefficient of relationship, r_{xy}, of 0.408.

Since self-half sibs have unrelated pollen parents, the covariance between selfs and crosses will be free of any dominance genetic variance, but there will be a contribution from additive × additive epistatic variance, equivalent to r_{xy}^2. Thus, the genetic interpretation of this covariance is

$$\text{Cov (S, C)} = 0.408\ \sigma^2_A + 0.1665\ \sigma^2_{AA} \quad \ldots \ldots \quad (5)$$

(iv) *Estimation of genetic variance*

For the 1981 data, additive, dominance, and additive × additive genetic variances were jointly estimated from Equations (3), (4), and (5) from
Formula 1.—

$$
\begin{bmatrix} \hat{\sigma}^2_A \\ \hat{\sigma}^2_D \\ \hat{\sigma}^2_{AA} \end{bmatrix} =
\begin{bmatrix} 0.3276 & 0.0775 & 0.1206 \\ 1.0000 & 0.2500 & 1.0000 \\ 0.4080 & 0 & 0.1665 \end{bmatrix}^{-1}
\begin{bmatrix} \hat{\sigma}^2_c \\ \hat{\sigma}^2_s \\ \hat{C}ov\ (S, C) \end{bmatrix}
$$

For the earlier assessments the coefficients for Equation (2) were substituted in the first row of the above matrix. The "hat" (⌃) signifies an estimate of the parameter.

Results

Initial effects of selfing

Seed size effects from selfing were examined only in the parental generation, not in the progeny generation. Selfing had no average effect on parental seed size, the mean 100-seed weights for self seed being 2.84 g, and for cross

Table 1. — Means of self- and cross-pollinated Pinus radiata progenies.

Trait	Selfs	Crosses	Inbreeding[1] ratio
Height – in nursery (cm)	45	55	0.82**
Height – 1 yr (cm)	76	83	0.92**
Height – 4 yr (dm)	43	47	0.91**
Diameter – 4 yr (cm)	6.9	8.0	0.87**
Volume – 4 yr (dm^3)	8.6	12.2	0.71**
Straightness – 4 yr (1-9)	5.65	5.88	0.96**
Malformation – 4 yr (1-6)	5.03	5.14	0.98*
Diameter – 7 yr (cm)	16.2	18.4	0.88**
Straightness – 7 yr (1-9)	5.23	5.96	0.88**
Branching quality – 7 yr (1-9)	4.24	5.29	0.80**
Needle retention – 7 yr (%)	53.4	63.8	0.84**
Wood density – 7 yr (kg/m^3)	302	298	1.01**

[1] Ratio of self/cross. *(**) = ratio is significantly different from 1.0, P = 0.05 (0.01).

seed, 2.81 g. The range in seed size averaged over selfs and crosses, for those 20 plus trees used in this study exclusively as female parents was 2.04 to 3.82 g, with mother tree effects highly significant (P ≤ 0.01).

Some trees were difficult to self, and produced very few filled seeds, though surprisingly, most produced about as many filled seeds per cone from self-pollination as from cross-pollination. Only a few self-pollinated seedlots germinated poorly and/or yielded a high frequency of abnormal seedlings. Unfortunately no measurements or counts were made.

Inbreeding depression on progeny height growth in the nursery was exhibited by all but two of the 25 parents, the average inbreeding ratio being 0.82 *(Table 1)*. The severest inbreeding depression ratio shown by an individual parent was 0.40 in the case of Plus Tree 97 (selfs 27 cm, crosses 68 cm).

Effects of selfing on progeny means

Means of selfs and crosses in the Kaingaroa test, averaged over all 25 parents, are shown in *Table 1*. The split-plot design enabled selfs and crosses to be compared very precisely so that even small average differences between

Table 2. — Analysis of variance of 25 pairs of Pinus radiata self- and cross-pollinated families at Kaingaroa Forest for height at 1 year (1975) and height and four other traits at age 4 years (1978).

Source	Df	Mean squares					
		Height (1975) (cm)	Height (1978) (dm)	Diameter (cm)	Volume (dm^3)	Straight (1-9)	Malform (1-6)
Replications	5	108.69	172.87	4.05	63.13	1.43	0.26
Parents	24	426.18	75.98	4.96	54.67	1.14	0.56
Selfs v. crosses	1	4006.90	1386.75	82.76	960.46	3.97	0.94
Parents x selfs v. crosses	24	357.92	42.61	2.75	26.45	0.39	0.21
Residual	245	41.53	18.11	0.69	10.52	0.31	0.17
F-test, parents		1.19 ns	1.78 ns	1.80 ns	2.07*	2.92**	2.67**
F-test, selfs v. crosses		11.19**	32.54**	30.09**	36.31**	10.18**	4.48*
F-test, parents x selfs v. crosses interaction		8.62**	2.35**	3.98**	2.51**	1.26ns	1.23ns

Table 3. — Analysis of variance of 25 pairs of *Pinus radiata* self- and cross-pollinated families at Kaingaroa Forest at age 7 years (1981).

Source	Df	Mean squares				
		Diameter (cm)	Straight-ness (1-9)	Branching Quality (1-9)	Needle retention (%)	Wood density (kg/m³)
Replications	5	8.23	7.25	1.91	5480.80	199.84
Parents	24	14.85	7.32	8.11	1369.73	427.73
Selfs v. crosses	1	402.56	39.85	82.25	8398.75	1637.11
Parents x selfs v. crosses	24	13.83	1.39	1.76	475.26	60.60
Residual	245	2.05	1.17	1.83	299.75	31.81
F-test, parents		1.07ns	5.26**	4.61**	2.88**	7.06**
F-test, selfs v. crosses		29.11**	28.67**	46.73**	17.67**	27.01**
F-test, parents x selfs v. crosses interaction		6.73**	1.19ns	0.96ns	1.58**	1.91**

selfs and crosses were statistically significant. Inbreeding depression, as measured by a loss in economic value (inbreeding ratio < 1) was shown by all traits except wood density. Inbreeding depression was especially marked in branching quality (0.80) and needle retention (0.84), i.e., resistance to fungal needle cast. Selfing produced a 12% average loss in mean diameter growth by age 7 years, which, for trees of this size, represents a loss in volume of about 30%.

Responses to selfing in individual clones

The self- and cross-pollinated progeny means of the 25 individual parents are too bulky to be listed here. The associated analyses of variance are summarised in *Tables 2* and *3*.

At age 4 years, progeny of about half of the parents showed significant inbreeding depression for growth. By age 7 years, 15 of the 25 parents showed significant depression in diameter growth in the selfs. The depression measured in four of the other ten parents was too small to be

Fig. 1. — Four-year-old cross- (0) and self-pollinated progenies (S) of *Pinus radiata* Plus Tree 850-119 at Rotorua. The pronounced inbreeding depression showed by this parent was already evident in the nursery. (H. G. Hemming, Neg. 40049).

Table 4. — Phenotypic correlations between 25 self- and cross-pollinated family means of *Pinus radiata* at Kaingaroa Forest.

Trait[1]	Correlation
Nursery height	0.21 ns
Height — 1 yr	0.11 ns
Height — 4 yr	0.44 **
Diameter — 4 yr	0.42 **
Volume — 4 yr	0.43 **
Straightness — 4 yr	0.59 **
Malformation — 4 yr	0.47 **
Diameter — 7 yr	0.06 ns
Straightness — 7 yr	0.72 **
Branching quality — 7 yr	0.69 **
Needle retention — 7 yr	0.54 **
Wood density — 7 yr	0.75 **

[1] 4 yr data based on 10 trees per plot (before thinning); 7 yr data based on 4 trees per plot (after thinning).

statistically significant under these experimental conditions, though in six parents, no difference or an actual (but not significant) increase in diameter growth from selfing was recorded. Plus-Tree 117 had the lowest inbreeding ratio, 0.63. Inbreeding depression in progenies of one of the plus-trees planted in a supplementary test at Rotorua is shown in *Figure 1*.

At age 7 years, selfs were significantly poorer than crosses for stem straightness (7 parents), branching quality (6 parents), and needle retention (6 parents). In 9 parents, selfs had higher wood density than crosses, probably just as a correlated response to the change in diameter since all these 9 parents showed depressed diameter growth (BANNISTER and VINE 1981).

Plus Trees 7, 89, 96, 99, and 111 showed no detectable inbreeding effects from selfing in any trait.

Not only did the plus trees differ from each other in the degree of inbreeding depression suffered in the progenies, as evidenced by the F-tests in *Table 2* and *3* for parent $\times$ selfs v. crosses interaction, but it was apparent that the performance of the selfs was not always well correlated with that of the crosses. The phenotypic correlation be-

Table 5. — Estimates of family variance components and covariances between selfs and crosses from 25 self- and cross-pollinated families of *Pinus radiata*.

Trait	Families $(\hat{\sigma}_f^2)$ [1]		$\hat{Cov}(S,C)$ [2]
	Crosses $(\hat{\sigma}_c^2)$	Selfs $(\hat{\sigma}_s^2)$	
Height - 4 yr (dm)	- 1.10 ns	14.4 **	3.2 **
Diameter - 4 yr (cm)	0.078 ns	0.978 **	0.196 **
Straightness - 4 yr (1-9)	0.004 ns	0.146 **	0.062 **
Malformation - 4 yr (1-6)	0.017 ns	0.056 *	0.030 **
Diameter - 7 yr (cm)	0.13 ns	3.94 **	0.08 ns
Straightness - 7 yr (1-9)	0.29 **	0.76 **	0.50 **
Branching quality - 7 yr (1-9)	0.33 **	0.72 **	0.53 **
Needle retention - 7 yr (%)	35 *	171 **	72 **
Wood density - 7 yr (kg/m³)	25.3 **	45.6 **	29.4 **

[1]) F-tests for H_o: $\sigma_f^2 = 0$; ns = not significant, * = significant at 0.05 level; ** = significant at 0.01 level.
[2]) t-tests for H_σ: Cov (S,C) = $r_{s,c}$ = 0.

tween self and cross means *(Table 4)* was close to zero for diameter growth at age 7 years (truncated sample), though for the other traits was strong enough to indicate that self-pollinated families could provide a reasonably reliable measure of general combining ability.

Estimates of variance components from selfs and crosses

Family variance components

Estimates of the family variance components obtained from crosses $(\hat{\sigma}_c^2)$ and selfs $(\hat{\sigma}_s^2)$, and of the covariance between crosses and selfs, Côv (S, C), obtained from the co-analysis, are shown in *Table 5*. In every trait, $\hat{\sigma}_s^2$ was greater than $\hat{\sigma}_c^2$ with $\hat{\sigma}_c^2$ with Côv (S, C) usually somewhere in between. Furthermore, F-tests showed that $\hat{\sigma}_s^2$ was significantly greater than zero for every trait, whereas $\hat{\sigma}_c^2$ was not significantly greater than zero in several cases, most notably for diameter at age 7 years, based on a subsample of the better progenies. Needle retention and growth traits showed the greatest increases in family variance from selfing.

Table 6. — Estimates of genetic variances from 25 self- and cross-pollinated families of *Pinus radiata*.

Trait	Additive $(\hat{\sigma}_A^2)$	Dominance $(\hat{\sigma}_D^2)$	Additive x additive $(\hat{\sigma}_{AA}^2)$
Height - 4 yr (dm)	-2.41	-33.25	25.12
Diameter - 4 yr (cm)	-0.002	-0.804	1.180
Straightness - 4 yr (1-9)	0.044	-0.651	0.265
Malformation - 4 yr (1-6)	0.009	-0.094	0.010
Diameter - 7 yr (cm)	-2.08	1.79	5.57
Straightness - 7 yr (1-9)	1.29	-1.47	-0.17
Branching quality - 7 yr (1-9)	1.47	-1.31	-0.43
Needle retention - 7 yr (%)	132.64	-276.20	107.41
Wood density - 7 yr (kg/m³)	92.60	13.35	-50.34

Genetic variances

Estimates of additive, dominance, and additive × additive genetic variances are shown in *Table 6*. Though subject to large sampling errors and possible biases from violations of several underlying assumptions about the nature of the genetic model, these estimates have some noteworthy features. Firstly, genetic variance appears to be entirely additive for wood density, branching quality, and stem straightness; secondly, additive × additive epistatic variance seems important in needle retention and also in growth traits; and thirdly, dominance genetic variance was absent (negative estimates) in all traits except 7-year diameter. The blatant negative estimate of σ_D^2 for needle retention is probably symptomatic of some peculiarities in the data or of a mis-specified model, but was not further explored.

Discussion

Inbreeding depression of diameter and height growth resulted in an estimated net loss in volume of about 30% at age 7 years in these *P. radiata* families. Selfing also caused losses in economic value in stem straightness, branching quality, and needle retention, but a small gain in wood density. It is suggested that this latter effect of selfing may be a correlated response to depressed diameter growth rather than a direct genetic consequence of inbreeding. Most of the 25 parents tested showed some ill-effects from selfing, though the magnitude of the effects varied from tree to tree. Self-pollination, and probably inbreeding in general, is thus likely to be undesirable in *P. radiata* seed orchards.

The test site in the northern part of Kaingaroa Forest is considered excellent for the growth of *P. radiata,* and therefore the selfs may not have been subjected to undue nutrient or moisture stresses. Furthermore, with the wide spacing used, inter-row competition for light, moisture and nutrients was not obviously severe. The degree of inbreeding depression exhibited thus probably represents a minimum, and, on poorer sites, the selfs would probably have fared comparatively worse.

Inbreeding depression from selfing, and the lack of a correlation between the performance of selfs and crosses, especially after culling the worst trees fro meach family (see BARKER and LIBBY 1974), could signify the importance of dominance and possibly epistatic variances in the genetic control of growth in *P. radiata*. In contrast, the high correlation between the performance of selfs and crosses in wood density, stem straightness, and branching quality, is strong evidence for additive variance being the major genetic component in these traits.

As well as these indirect clues about the kind of gene action controlling quantitative genetic variation in these families, some direct estimates of genetic variances were obtained. The use of inbred material for estimating genetic variances introduces many problems, among which may be mentioned the correlation between additive and dominance effects and the dependence of the contribution of individual genetic variance components in the covariance between relatives on gene frequencies (STUBER 1970). The assumption that gene frequencies were a half (p = q = 1/2) was made, but with no real justification or conviction except that it allowed the selfs and crosses in the experiment to be used in estimating additive, dominance and additive × additive genetic variances. It must be stressed that the estimates themselves are not reliable. Neverthe-

less, they do give some indications as to the types of gene action likely to be most important in the different traits. The strong additive inheritance of stem straightness, branching quality, and wood density implied by reportedly moderate to high heritabilities (BANNISTER and VINE 1981; COTTERILL and ZED 1980) and general combining ability effects (WILCOX et al. 1975) is confirmed. Needle retention was unusual in that the variance among selfs was nearly 5 times greater than the variance among crosses, resulting in a sizable additive $\times$ additive component of epistatic genetic variance. If this is really the nature of the gene action controlling resistance to fungal needle cast, it means that additive effects at some loci depend on what genes are present at other loci. One possible consequence is that the realised heritability of this trait in an actual selection programme could be greater than expected from additive variance alone since the covariance between parent and offspring or between half-sibs will be boosted by additive $\times$ additive effects.

Additive $\times$ additive variance was also important in growth traits, whereas dominance variance was not evident in any trait except in diameter at age 7 years.

The additive genetic variance for wood density requires some comment as the estimate from this experiment ($\hat{\sigma}^2{}_A = 89$) is 2 to 3 times lower than some other values obtained in juvenile wood of *P. radiata* (e.g., BANNISTER and VINE 1981). The most likely reason for the small variance in this experiment is that density was indirectly estimated from Pilodyn penetration, and the relative insensitivity of the instrument, and the rather weak regression ($R^2 = 0.49$) obtained, effectively reduced the family-to-family variance.

The use of selfs for progeny testing in normally-outcrossing crop species can be highly efficient, and has been advocated by WRIGHT (1980). Some possible applications of self-pollinated families in selection programmes with forest trees have been explored by BARKER and LIBBY (1974). They have shown that the performance of self families can be a reliable indicator of general combining ability under outcrossing, and therefore useful for progeny testing or in family selection. But first, the selfed families should be culled of weak individuals obviously homozygous for deleterious recessive genes. Removal of these inbreeding effects, which are assumed to be largely non-additive, thus allows effective selection between the selfed families for general combining ability. Within-family selection for the strictly additive traits would also be effective. In *P. radiata*, progeny tests using selfs seem promising particularly for screening parents for resistance to *Dothistroma* and *Naemacyclus*.

Other possibilities for using selfed material in trees are discussed by LINDGREN (1975). The most important is the use of inbred (selfed) clones in seed orchards. Such a system might be worth trying in *P. radiata* to utilise the non-additive genetic variance in growth traits; no technical difficulties are forseen, since PAWSEY (1964) showed that the flowering and cone production of selfs was more or less normal, though whether *P. radiata* selfs can be readily propagated by cuttings is not known. A further possible use of the selfed material in this expriment is in the production and testing of a series of full-sib families from crossing betwen inbred, unrelated, plus-trees selected within the self families. Some of these highly heterozygous non-inbred families may prove to be superior in vigour, and more uniform than equivalent families from con-ventional non-inbred second-generation plus trees selected in the cross families, and thus be of particular value for mass multiplication via tissue culture and cuttings.

Conclusions

The main conclusions from this study are as follows:
1. Selfing in the normally-outcrossing *Pinus radiata* often resulted in inbreeding depression in vigour, with associated deterioration in stem straightness, branching quality, and needle retention. Wood density was usually slightly increased by selfing. Several parents exhibited no ill effects from selfing.
2. For wood density, straightness, branching quality, and needle retention in which genetic variance was largely additive (additive $\times$ additive also important in needle retention), the performances of selfs and corresponding crosses were highly correlated. For growth traits, in which non-additive genetic variance (mainly additive $\times$ additive) seemed to be important, there was a poor correlation between the performance of selfs and crosses.
3. Genetic variances estimated from self- and cross-pollinated families generally corroborated the indirect evidence about gene action obtained from effects of inbreeding on means, and from the correlation between selfs and crosses, namely, that non-additive genetic variance was most important in growth traits and that additive genetic variance was most important in other traits.

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A provenance test of Japanese larch in eastern Canada, including comparative data on European larch and tamarack

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Summary

Japanese larch (Larix leptolepis (Sieb. et Zucc.) Gord.) of 20 provenances, European larch (L. decidua Mill.) of 3 provenances and tamarack (L. laricina (Du Roi) K. Koch of 2 provenances were tested in central New Brunswick, Canada. Geographic variation among the Japanese larch was high for most characters studied i.e., height and diameter at ages 4, 8, 12, and 19, stem straightness, damaging agents, survival, volume, and flower production. Geographic variables of the provenances, e.g., latitude, longitude, altitude, temperature, and precipitation were not related to phenotypic variables, i.e., the genetic variation pattern appears to be random.

Although tamarack had a height advantage over the other species up to age 8, Japanese larch was clearly superior in both diameter and height growth by age 12 and increased its superiority through age 19. In general, Japanese larch was straighter and less damaged by porcupines (Erethizon dorsatum L.) than the other species at age 19. Despite somewhat poorer survival than tamarack, volume of Japanese larch for the best five provenances averaged 156 m³/ha and was more than double that of tamarack. It is suggested that Japanese larch of good provenance could be used as a short rotation species for fibre production in eastern Canada.

Key words: Provenance test, Larix leptolepis, L. decidua, L. laricina, random genetic variation.

Zusammenfassung

Es wurden 20 Provenienzen von Larix leptolepis (Sieb. et Zucc.) Gord., 3 Provenienzen von L. decidua Mill. und 2 Provenienzen von Larix laricina (Du Roi) K. Koch in Zentral-New Brunswick (Kanada) untersucht. Die geographische Variation zwischen den Japanlärchen war für die meisten untersuchten Merkmale hoch, beispielsweise für Höhe und Durchmesser im Alter 4, 8, 12 und 19, für Geradschäftigkeit, Krankheitsursachen, Überlebensprozent, Volumen und Blütenproduktion. Geographische Variablen der Herkünfte, wie Längengrad, Breitengrad, Höhenlage, Temperatur und Niederschlagsmenge standen nicht mit phänotypischen Variablen in Beziehung. So war z. B. das genetische Variationsmuster zufällig. Obwohl Larix laricina bis zum Alter 8 einen Höhenvorteil gegenüber den anderen beiden Arten hatte, war Larix leptolepis im Höhen- und Durchmesserwachstum im Alter 12 klar überlegen. Die Überlegenheit nahm im Alter 12 noch zu. Im allgemei-
nen war Japanlärche geradschäftiger und im Alter 19 weniger durch Erethizon dorsatum L. geschädigt als die anderen Arten. Trotz eines etwas geringeren Überlebensprozents betrug das Volumen der Japanlärche für die 5 besten Provenienzen 156 m³/ha und war mehr als doppelt so hoch wie das von Larix laricina. Es wird vorgeschlagen, daß Japanlärchen guter Herkunft als Art für den Kurzumtrieb zur Faserholzproduktion in Ostkanada in Frage kommen.

Introduction

Japanese larch (Larix leptolepis (Sieb. et Zucc.) Gord.) is edemic to the central part of the island of Honshu, Japan, where it is found as geographically isolated populations at elevations of 900 to 2500 m. All natural stands of the species are located in an area of about 200 km square (Farnsworth et al. 1972). Japanese larch is of economic importance within its natural range and as an exotic elsewhere.

Cook (1971) expounds the virtues of Larix species, emphasizing such things as ease of vegetative propagation, early flowering, stem and branch characteristics as they relate to harvesting, rapid growth and a large amount of genetic variation. In eastern North America, Japanese larch is considered to be the fastest growing of the larches (Littlefield and Eliason 1956, Schober 1958, Eliason and Carlson 1963, Stairs 1965, MacGillivray 1969). Cook (1971) considers that Japanese larch of good provenance will outgrow other conifers commonly planted in the Northeast by more than two to one.

Most provenance studies of Japanese larch have reported highly significant differences among populations and that these differences are not closely associated with altitude, latitude, or any other recognized environmental characteristic of the seed source (Langner 1961, Wright 1962, Lester 1964, Pauley et al. 1965, Farnsworth et al. 1972). In contrast, Krusche and Reck (1982) found that nearly half of the variation between provenances, grown at 11 locations in Germany, could be explained by climatic differences at the place of origin. In addition Langner (1961) reported a relationship between elevation and fall needle color, and Genys (1971) suggested that stem quality and susceptibility to larch sawfly Pristophora erichsonnii (Hartig) were correlated with longitude.