RECIPROCAL CROSS EFFECTS IN PINUS RADIATA

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ABSTRACT

Twenty-nine pairs of reciprocal crosses of **Pinus radiata** D. Don were made by controlled pollination, incidental to producing full-sib families for progeny testing and third-generation selection.

Seed size of each cross was measured by the weight of 100 seeds. Of the two crosses of each full-sib family, the one with heavier seeds was classified as the "big-seeded" cross and that with the lighter seeds, the "small-seeded" cross. Seeds of the 58 crosses were sown in a replicated split-plot design in the nursery in October 1979. Heights of seedlings were measured in the nursery in March 1980, and in a field test in May 1981 and again in July 1982.

Significant reciprocal cross effects were detected in the nursery and field tests, with a general tendency for the "small-seeded" cross to be shorter than the "big-seeded" cross. Consequently, it was shown that selection among families at age 2 years after planting (mean height 228 cm) would result in one wrong selection (or culling) for every four correct ones.

INTRODUCTION

Pinus radiata is monoecious and the same tree can thus be used both as a male and a female parent in controlled matings. With normal chromosomal inheritance each parent equally contributes genes to the offspring, so the progeny of $m_i \times f_j$ are not expected to differ genetically on the average from those of $m_j \times f_i$. However, such reciprocal crosses may not necessarily grow the same owing to early phenotypic differences between them in the size and vitality of the embryos, associated with respective peculiarities in the maternal tissues of the seeds.

Seed size in *P. radiata,* as in other plants including pines (Righter 1945), is known to influence the growth rate of seedlings (Sweet & Wareing 1966). Thus, so-called "starting size" effects, or differences in height and diameter between progenies in the early phases of progeny tests, may largely reflect differences in seed size and germination behaviour rather than the genetic breeding value of the parents (Burdon & Sweet 1976).

But how large and persistent are these non-heritable or "acquired" reciprocal cross effects in comparison with genetic effects? Do they cause mistakes in the selection of superior families? And are they simply related to an obvious feature such as seed size? A set of reciprocal crosses was grown in the nursery and field to investigate these questions.

MATERIALS AND METHODS

Parent Trees

Forty-two *P. radiata* trees were involved as parents in this study. They had been planted in either Cpt 1350 Kaingaroa Forest or Cpt 767 Waimihia Forest in 1969 as part of large open-pollinated progeny tests and were selected in 1975, along with numerous other trees, as second-generation plus-trees of the N.Z. Forest Service "875"-series. This series of trees was crossed together in 1976–77 by controlled pollinations *in situ* using a disconnected diallel mating design. To get enough seed in some full-sib families it was necessary to make reciprocal matings. Resultant seed of 29 pairs of reciprocal crosses drawn from several individual diallels, but themselves not forming a diallel, was kept separate and provided 58 crosses for this study. The weight of 100 seeds was measured in each cross. In each full-sib family, the cross with the heavier seed was designated the "big-seeded" cross, while the cross with the lighter seed was designated the "small-seeded" cross (Fig. 1). This was done for separating in subsequent analyses of height growth the genetic effects of full-sib families from the gross maternal effects of seed size.

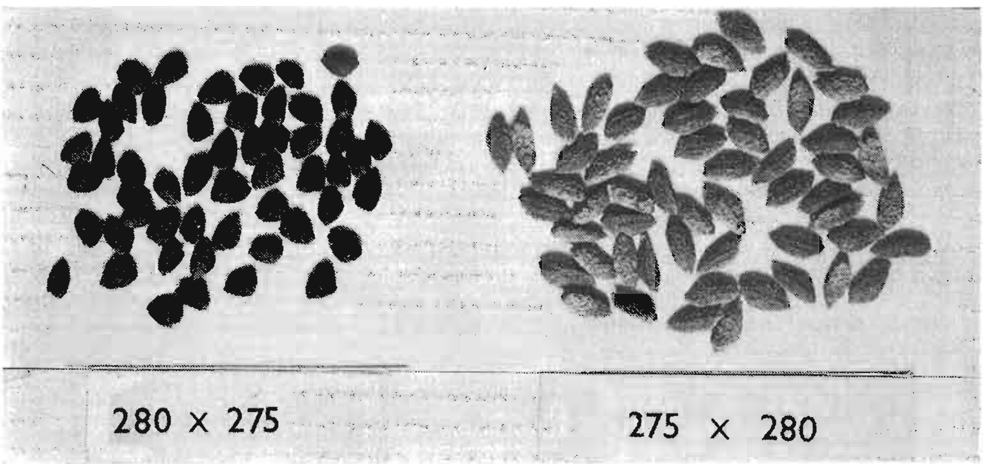

FIG. 1—Seed of reciprocal crosses between two **Pinus radiata** trees (875-280 and 875-275) showing large differences in seed size.

Nursery Study

Seeds of the 29 pairs of reciprocal crosses were sown in split-plots of 24 plants at 10 × 10-cm spacing in a nominal four-replicate randomised complete block design in the Forest Research Institute nursery at Rotorua in October 1979. Seed was soaked in cold water for 24 hours before sowing, but was not cold-stratified.

Up to the time of measurement, the seedlings were not subjected to any conditioning treatment, such as undercutting or lateral pruning of roots, which would restrain height growth (Fig. 2). The mean height of the 20 tallest seedlings (thus excluding runts) in each plot of each cross was measured in March 1980, and full-sib and individual cross means were calculated. Differences in mean height between big-seeded and small-seeded crosses in each full-sib family were measured (Fig. 2).

Plot-mean height data (there were several missing plots) were analysed in a three-way crossed classification by the method of fitting constants (Searle 1971) to test the effects of full-sib family, replicate, seed size (i.e., big-seeded *v.* small-seeded crosses), and full-sib family × seed size interaction.

FIG. 2—Seedling progenies of reciprocal crosses between two **Pinus radiata** trees (875-280 and 875-275) showing large differences in seedling height. The cross on the left, 280 × 275, had much smaller seed than the cross on the right, 275 × 280.

Field Study

Seedlings of the reciprocal crosses were planted in field tests at Kawerau and Kaingaroa in July and August 1980 as part of the "875"-series disconnected diallel tests for progeny testing and third-generation selection. Identity of the big-seeded and small-seeded crosses of each full-sib family was maintained in the field tests but reciprocals were not located close together in adjacent split-plots as was the case in the nursery. The trees grew much faster at Kawerau than at Kaingaroa, and results from only the former test are reported here. Details of the test are as follows:

Sample Plot R 1804, Onepu Block, Tasman Forestry Ltd, Kawerau; altitude – 20 m; soil – dark sandy loam over Tarawera Ash; vegetation – pasture grasses; treatment before planting – planting lines ripped, planting spots sprayed with paraquat; treatment after planting – glyphosate sprayed around trees individually to control paspalum and other grasses.

For the purposes of this reciprocal cross study, the design comprised 23 block replicates. One tree of each of the 58 crosses was planted at random in each replicate.

Heights were measured in May 1981 and July 1982. Dead trees and trees shorter than one-third the mean height of the three tallest trees in the cross were either not measured, or measured and subsequently not analysed. There were 76 such "missing" trees in 1981 and 81 in 1982 from a nominal total of 1334.

Height measurements of individual trees were subjected to a three-way crossed classification analysis of variance using the method of fitting constants to accommodate missing trees (Searle 1971). The statistical significance of differences in mean height between full-sib families, between big-seeded and small-seeded crosses, and of family $\times$ seed size interaction effects, was examined using F-tests. The proportion of variance in height among all the trees in the experiment accounted for by each source of variation was calculated using the w^2 statistic (Kirk 1968, p. 198).

The extent of serious mistakes from reciprocal effects in early selection for height growth was evaluated by a simple exercise of truncation selection of the 12 tallest and 12 shortest of the 58 crosses.

RESULTS

Within reciprocal full-sib families the big-seeded cross was nearly always the taller in the nursery, and also after 1 and 2 years in the field. Mean seed weights and progeny heights of individual crosses are listed in Table 1. Full-sib family means, and mean differences between big- and small-seeded reciprocal crosses, are shown in Table 2. The means of big- and small-seeded crosses over all 29 full-sib families are presented in Table 3. A feature of these results is the relatively small, and diminishing, margin in height growth between the big- and small-seeded groups despite the former having seeds 33% heavier.

The analyses of variance, summarised in Table 4, showed that progeny heights of the full-sib families averaged over big- and small-seeded reciprocal crosses differed in the nursery and field (Table 2). Furthermore, the relative importance of differences between full-sib families compared to non-genetic seed-size effects, as judged from the w^2 statistic (Table 4), increased as the trees got older. Seed-size effects were thus wearing off, there being only five statistically significant reciprocal differences in height by 1982, with all but one associated with above-average seed-size differences (Table 2).

In the 12 tallest of the 58 crosses in 1982, six were big-seeded and six were small-seeded reciprocals. However, in the 12 shortest crosses, 11 were small-seeded and just one was a big-seeded reciprocal. Culling from the bottom of the rankings resulted in a statistically significant ($\chi^2 = 8.42^{**}$) bias towards small-seeded crosses, whereas there was no bias in favour of large-seeded crosses by selecting from the top of the rankings ($\chi^2 = 0.08$). In 11 of the 29 full-sib families both reciprocals were taller than the experimental mean of 228 cm in 1982, in 11 both reciprocals were shorter than the mean, and in seven families one cross was taller and the other shorter than the mean. Thus early truncation selection for height growth wrongfully included or excluded about one in four of the families.

DISCUSSION

Reciprocal cross effects in progenies of forest trees are probably best studied in full diallel mating designs in which parental genetic effects (general and specific combining

TABLE 1—List of 29 pairs of **Pinus radiata** reciprocal crosses between "875"-series parents, with 100-seed weights and progeny heights

Code*	"Big-seeded" cross						"Small-seeded" cross					
	F × M	100-seed wt (g)	Height (cm)				F × M	100-seed wt (g)	Height (cm)			
			Nursery 1980	Field 1981	Field 1982				Nursery 1980	Field 1981	Field 1982	
28	220 × 213	2.5	23	83	210		213 × 220	1.7	18	68	204	
32	256 × 93	3.2	24	82	222		93 × 256	2.6	24	88	238	
36	93 × 225	2.9	25	88	225		225 × 93	2.4	23	79	212	
40	9 × 225	3.1	21	84	227		225 × 9	2.3	20	88	223	
43	241 × 71	3.1	26	95	239		71 × 241	2.8	23	87	235	
53	255 × 210	3.5	26	95	241		210 × 255	2.6	22	93	244	
59	44 × 204	3.1	21	81	224		204 × 44	2.7	21	75	214	
62	76 × 204	3.0	25	86	223		204 × 76	2.8	22	82	216	
73	62 × 235	2.8	23	89	246		235 × 62	2.0	22	88	235	
75	207 × 222	2.8	23	84	234		222 × 207	1.5	18	66	190	
76	222 × 218	2.5	23	88	236		218 × 222	1.9	21	91	245	
78	98 × 284	5.0	24	94	249		284 × 98	2.2	20	79	229	
79	98 × 207	3.8	22	75	221		207 × 98	2.2	19	78	211	
80	284 × 218	2.7	22	101	254		218 × 284	1.7	20	88	217	
82	207 × 218	2.7	20	89	239		218 × 207	1.7	20	89	246	
88	7 × 21	2.7	23	92	246		21 × 7	1.7	19	82	227	
95	20 × 257	2.7	22	89	233		257 × 20	2.7	23	85	223	
104	247 × 75	4.1	26	97	236		75 × 247	3.3	24	89	235	
107	203 × 75	3.3	23	87	231		75 × 203	3.1	23	81	205	
112	46 × 67	3.2	23	93	259		67 × 46	2.6	24	95	253	
124	239 × 205	3.6	25	91	237		205 × 239	3.4	25	91	235	
135	74 × 78	3.9	24	96	251		78 × 74	3.3	23	96	246	
138	253 × 78	4.5	25	82	224		78 × 253	2.0	20	76	206	
143	275 × 280	3.5	20	83	222		280 × 275	2.4	14	71	196	
144	275 × 289	3.2	19	79	221		289 × 275	2.4	18	72	213	
153	88 × 90	3.1	22	93	233		90 × 88	2.0	22	76	203	
155	43 × 88	4.6	25	84	220		88 × 43	2.6	20	85	216	
157	90 × 35	2.0	18	76	225		35 × 90	1.9	18	78	210	
159	293 × 90	3.2	23	88	230		90 × 293	2.0	23	92	249	
	MEAN	3.2	23	88	233			2.4	21	83	224	

* Code number of full-sib family in field tests

TABLE 2—Full-sib family means and mean differences between big- and small-seeded reciprocal crosses

Code	100-seed wt (g)	Full-sib family means† Height (cm) 1980	1981	1982	Differences between big- and small-seeded crosses‡ 100-seed wt (gm)	Progeny height (cm) 1980	1981	1982
28	2.1	21	76	205	0.9	4*	15*	6
32	2.9	24	85	229	0.6	0	–6	–16
36	2.7	24	83	219	0.5	2	9	13
40	2.7	20	86	225	0.8	1	–4	4
43	2.9	25	91	237	0.3	2	8	4
53	3.0	24	94	243	0.9	4*	2	–3
59	2.9	21	78	218	0.4	0	6	10
62	2.9	24	84	219	0.2	3*	4	7
73	2.4	23	89	240	0.9	1	1	11
75	2.1	21	76	212	1.3	5*	18*	44*
76	2.2	22	90	241	0.5	2	–3	–9
78	3.6	22	86	238	2.8	4*	15*	20
79	3.0	21	76	216	1.6	3*	–3	10
80	2.2	21	94	235	1.0	2	13*	37*
82	2.2	20	89	241	0.9	1	0	–7
88	2.2	21	87	235	0.9	4*	10	19
95	2.7	22	87	229	0.1	–1	4	10
104	3.7	25	93	235	0.8	2	8	1
107	3.2	23	84	217	0.2	0	6	26*
112	2.9	23	94	255	0.4	–1	–2	6
124	3.5	25	91	236	0.2	0	0	5
135	3.6	23	96	249	0.6	1	0	5
138	3.2	24	79	214	2.5	5*	6	18
143	2.9	19	77	207	1.1	5*	12*	26*
144	2.8	18	75	216	0.8	0	7	8
153	2.6	22	84	219	1.0	0	17*	30*
155	3.6	22	84	217	2.0	4*	–1	4
157	2.0	18	77	217	0.1	1	–2	15
159	2.6	23	90	240	1.2	0	–4	–19
MEAN	2.8	22	85	228	0.9	2	5	8

† Averaged over the two reciprocal crosses
‡ Differences marked * are significant ($p = 0.05$)
Least significant differences are 3 cm in 1980, 11 cm in 1981, and 22 cm in 1982

TABLE 3—Means of big- and small-seeded crosses

	Big seeds	Small seeds	Ratio (small : big)
Weight of 100 seeds (g)	3.2	2.4	0.75
Height in nursery, March 1980 (cm)	23	21	0.91
Height in field, May 1981 (cm)	88	83	0.94
Height in field, July 1982 (cm)	233	224	0.96

TABLE 4—Analyses of variance of height growth (cm) of big- and small-seeded reciprocal cross progenies of 29 full-sib families of **Pinus radiata**

Source	Nursery (1980)				Field (1981)				Field (1982)			
	df	MS	F	w^2†	df	MS	F	w^2	df	MS	F	w^2
Rep	3	376.3	60.69**	0.34	22	2582.5	75.73**	0.09	22	12428.4	9.41**	0.11
Full-sib family	28	26.2	3.97**	0.17	28	1771.3	3.63**	0.07	28	7280.0	2.67**	0.08
Seed size (big-seeded **v.** small-seeded crosses)	1	216.2	32.76**	0.06	1	7432.4	15.24**	0.01	1	17663.2	6.48**	0.01
Family × seed size	28	6.6	1.10NS	0.00	28	487.7	1.41NS	0.01	28	2724.5	2.06*	0.02
Residual	164	6.2			1178	346.1			1173	1320.9		

* significant at $p = 0.05$

** significant at $p = 0.01$

NS not significant

† w_i^2 = proportion of variation accounted for by ith source

$$= \frac{SS_i - df_i\,(MS_{res})}{SS_{total} + MS_{res}}$$

ability) can be separated from maternal or other non-genetic reciprocal effects (e.g., Fowler 1970). The *P. radiata* crosses investigated in this study were essentially a random assemblage of reciprocal single-pair crosses except for a few parents which were used in more than one full-sib family. Because general combining ability and maternal effects could not be estimated separately, one cross of each pair was qualitatively classified as "big-seeded" and the other as "small-seeded" to provide a basis for analysing reciprocal effects. The ensuing analyses of variance were informative in that full-sib family effects could be tested independently of the contrast between big- and small-seeded progenies.

Dissection of a few seeds showed that "big seeds" had much larger embryos and a greater bulk of endosperm than "small seeds". The maternal phenotype evidently plays a major role in the variability of seed coat characteristics and seed size, which in turn strongly influences the early growth of the embryo.

The mean weight of 100 seeds of the 58 crosses studied was 2.8 g, which is about average for unsorted seed collected in Kaingaroa Forest from ordinary plantations, and comparable to 100-seed weights in the N.Z. Forest Service's North Island clonal seed orchards (Waimihia 2.7 g, Kaingaroa 3.0 g, Gwavas 2.9 g; T. G. Vincent, unpubl. data). The magnitude of the seed-size effects in the study should therefore be typical of what would occur in commercial seedlots which are not graded by size.

The effects of seed size on height growth in the nursery might have been somewhat reduced if the seed had been cold-stratified for several weeks before sowing. Though not standard nursery practice with *P. radiata,* stratification generally reduces variation in germination rate between seedlots, which in turn tends to reduce differences in early seedling height. This point needs experimental verification, but it is likely that in progeny tests stratification would help to reduce biases from seed size differences.

The small but consistent influence of seed size evident in the nursery lingered in the field test, but with a diminishing trend.

CONCLUSIONS

A major generalisation suggested by this study is that initial differences in height growth between families of *P. radiata* can arise from differences in seed size. Such maternal influences may be largely non-genetic and therefore not heritable, and can give initial differences in height between progenies that will be meaningless in a genetic selection programme.

After 2 years' growth in the field the influence of reciprocal differences in seed size on height growth had weakened, though in several families was still strong enough to make early selection unreliable.

Thus in the various non-reciprocal mating designs used in the N.Z. Forest Service's breeding programme for progeny testing, individual and family selection, and for estimation of genetic parameters, early culling or selection for height could be partially ineffective.

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