EFFECTS OF SITE ON EXPRESSION OF CONE CHARACTERS IN RADIATA PINE

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ABSTRACT

Cone samples were collected from a trial with 18 clones of radiata pine (**Pinus radiata** D. Don) replicated within and between four contrasting sites, of which one was deficient in soil phosphate. The cones were assessed for length, breadth, volume, length : breadth ratio, number of scales, and mean seed weight. One objective was to evaluate the influence of site on cone characters and on taxonomic information that they afford.

Cone dimensions, number of scales, and mean seed weight all showed significant clonal repeatabilities within sites (0.35 to 0.85, $P < 0.001$), confirming the genetic control of these characters. Cone volume and mean seed weight were about one-third less at the phosphate-deficient site than elsewhere. Number of scales varied little between sites. The only important clone-site interactions appeared to reflect differences between clones in response to the phosphate deficiency.

Cone size and seed weight, although differing widely between natural populations, are of restricted diagnostic value as a guide to the ancestry of stands in New Zealand, owing to their environmental plasticity.

INTRODUCTION

In radiata pine, studies of clones have shown that cone characters vary greatly among genotypes, but are comparatively consistent within clones (Fielding, 1964). This consistency (or repeatability) indicates strong genetic control. Large differences in cone size have also been recognised among the natural populations in mainland California, even though the tree-to-tree variation causes much overlap (Fielding, 1953; Forde, 1964a, 1964b). Similar differences have been found in mean seed weight (Fielding, *loc. cit.*; Burdon and Bannister, 1970). However, some other characters which vary greatly among trees were not found to differ significantly between populations (Ford, *loc. cit.*). Although there is no direct proof, the major population differences are presumably of mainly genetic origin.

With these large differences among natural populations it might be expected that mean values for cone dimensions or seed weight could indicate the ancestry of stands where the ultimate seed sources are unrecorded. However, if measurements of any single character are to be used as a clue to genetic origins, we need to ensure that comparisons are not disturbed by environmental effects. This can be done by growing the various populations in a common environment; alternatively it may be possible to show that environmental effects are unimportant. Even if any genetic differences are confounded with appreciable environmental effects, it may still be possible to allow for the latter in interpreting the comparisons.

Practically no New Zealand radiata pine is growing alongside material of known Californian origin. Furthermore, the records of seed origins within New Zealand of existing stands are generally sketchy. This makes it desirable to ascertain the influence of site on the phenotype, especially because of the circumstances surrounding the importation of the species. The New Zealand gene pool of radiata pine has apparently originated mainly from the Ano Nuevo population, with an appreciable admixture from Monterey, and very little, if anything, from Cambria (Bannister *et al.,* 1962; Blight *et al.,* 1964; Burdon and Bannister, 1970). However, the representation of Monterey genes could be greater in the Auckland region, because at least one significant planting made during the 1860s was judged on morphological and historical evidence to be of pure Monterey origin (M. H. Bannister, pers. comm.). Small cones and small seeds would normally indicate Monterey ancestry, but in the Auckland region many radiata pine stands suffer from severe phosphate deficiency, which was believed to depress cone and seed size. Direct evidence concerning the effect of this site factor was available from a trial in which clones were replicated within and between four sites, one of which was a typical phosphate-deficient clay of the Auckland region. This paper is concerned primarily with the effect of site on the expression of cone characters and on the taxonomic information that they afford. Another paper (Burdon and Low, in press) is concerned with production of seed within the clones.

MATERIALS AND METHODS

The Experiment

The experiment is described elsewhere (Burdon, 1971), so only a brief account will be given here. Eighteen clones were replicated as cuttings within and between four sites, at Glenbervie, Whakarewarewa (Whaka), Gwavas and Berwick State Forests respectively. Not all clones were represented on all four sites, and the number of surviving cuttings (ramets) within clone-site subclasses ranged from one to six. The cones were collected 12 years after planting.

At Glenbervie the trees showed symptoms that are normally associated with acute phosphate deficiency. Foliar analyses (Burdon, in prep.) have revealed phosphorus levels which are typical of this condition. At Whaka two clones showed signs of an unidentified nutrient deficiency; otherwise growth was very vigorous. There were no indications of appreciable nutrient deficiencies at either Gwavas or Berwick. At Berwick the latitude was appreciably higher and the rainfall markedly lower than at the other sites.

Sampling Procedure

Up to three stem cones and three branch cones were collected from each tree (ramet), if acceptable specimens were available. Cones were only chosen if they appeared to be properly developed, were unopened and were in no way misshapen or damaged. As far as possible only one cone was taken from a cluster, and any that were distorted through crowding within the cluster were avoided. The sampling was inevitably biased towards the larger cones but strictly random sampling was impracticable because of some intergradation between normal cones and occasional ones that were obviously underdeveloped.

The collection procedure led to very unequal representation among the clones within each site. Accordingly, a second-stage sampling was adopted in choosing cones for measurement. From the collection for each clone-site subclass normally five stem cones and five branch cones were chosen at random. If only five cones or less were available within a category all were accepted. However, after a preliminary study, it was decided to pool the stem and branch cones so chosen; hence all cones within a clone-site subclass were treated as a homogeneous sample. The imbalance of the classification, although reduced, was still considerable (Table 1).

TABLE 1—Numbers of individual cones in clone-site subclasses

Clone	Site				Total
	Glenbervie	Whaka	Gwavas	Berwick	
57	6	—	7	4	17
58	10	—	8	—	18
59	4	—	4	3	11
60	5	—	6	7	18
61	2	—	7	5	14
62	5	—	9	7	21
63	5	—	7	9	21
64	5	4	8	—	17
65	9	6	9	—	24
66	4	10	12	10	36
67	1	10	10	10	31
68	—	2	7	3	12
69	10	10	6	10	36
70	8	7	3	9	27
71	5	10	3	5	23
72	8	10	2	—	20
73	10	10	10	10	40
74	1	8	10	—	19
Totals	98	87	128	92	405

Measurements

For each cone finally chosen the following data were recorded:

(1) Total length along geometric axis (cf. Fielding, 1953; *see* Forde, 1964a), hereafter denoted length, to nearest 0.025 cm

(2) Maximum breadth, perpendicular to plane of bilateral symmetry, hereafter denoted breadth, to nearest 0.025 cm

(3) Volume

(4) Total number of cone scales (scale number)

(5) Number of sound seeds

(6) Total weight of sound seeds

In addition, length : breadth ratio and mean weight per sound seed were calculated. The first four measurements were made on unopened cones, after which the cones were opened by warming in an oven and then the seed extracted and dewinged. In obtaining mean seed weight the empty seeds were separated off using an adjustable updraught: the draught was regulated to ensure removal of all unfilled seeds, although this did entail losing a few of the smaller full seeds, causing some upward bias in the estimate of mean seed weight.

In addition to the main measurements, a small sample of the cones was studied for asymmetry of apophysis thickness (cf. Forde, 1964a). For seven clones a total of 81 opened cones was chosen from the Gwavas and Glenbervie sites. Using calipers, the maximum thickness of the following scales was measured:

(1) The thickest scale on the cone, which was automatically on the outside

(2) One scale, on the inside of the cone half way along the axis

The ratio of these two values represents the asymmetry.

Analysis

For each character, at each site, the clonal repeatability and within-clone and between-clone coefficients of variation were calculated from analysis of variance. Because of the sampling procedure these parameters were subject to bias, so the main interest lies in the comparative magnitudes, between characters and to a lesser extent between sites, of the coefficients of variation.

Repeatability is given by the ratio $V_C/(V_C + V_E)$, where V_C is the between-clones variance component, and V_E is the cones-within-clones (error) component (cf. Burdon, 1971). The test for significance of clonal differences is automatically a test for the significance of repeatability. Coefficients of variation were calculated as:

$$\sqrt{V_E} \div S \text{ and } \sqrt{V_C} \div S \text{ respectively,}$$

where S is the overall mean for the site in question. V_C is, of course, a parameter of the particular clones present on a site.

Testing the significance of site differences and the study of clone-site interactions was complicated by the imbalance of the classification (Table 1), and by the enforced departures from random sampling. The approach adopted was to consider sites in all possible combinations of pairs. The tests used are necessarily approximate.

Site means were calculated on the basis of the averages of available clonal means. For clones common to both Gwavas and a site in question, the average difference in clonal means between the two sites was calculated. To give the site mean this average difference was then added to (or subtracted from) the Gwavas mean.

The difference between any two sites was studied by means of a paired t-test, the pairs of values representing the means, on the respective sites, for each clone that was common to both sites. With these numerous tests $P < 0.01$ was adopted as the criterion of statistical significance in each case.

The possibility of clone-site interactions was studied indirectly. Initially, correlations between clonal means were calculated for each character, in all possible pairs of sites. Very high correlations suggest that true interactions were minor. Moderate to low correlations, however, could result either from appreciable interactions, or from poor representation in some of the clone-site subclasses in conjunction with low repeatabilities. In such cases the clonal means for one site were plotted against the corresponding means for the other site, and the situation could be interpreted by inspection of the graph.

RESULTS

Clonal Differences

Calculated values for clonal repeatabilities are listed in Table 2, and coefficients of variation, expressed as percentages, in Table 3.

TABLE 2—Calculated clonal repeatabilities for cone characters, by sites

Character	Site: Glenbervie	Whaka	Gwavas	Berwick
Length	0.80	0.85	0.60	0.56
Breadth	0.48	0.35	0.52	0.39
Length : breadth	0.82	0.73	0.68	0.53
Volume	0.63	0.66	0.49	0.48
Mean seed weight	0.54	0.75	0.44	0.53
Scale number	0.45	0.81	0.53	0.67

TABLE 3—Coefficients of variation (%) for cone characters, by sites

Character	Coefficient	Site: Glenbervie	Whaka	Gwavas	Berwick
Length	between-clones	17	16	14	11
	within-clones	9	7	11	9
Breadth	between-clones	10	7	9	6
	within-clones	10	9	9	7
Length : breadth	between-clones	14	12	10	9
	within-clones	7	7	7	8
Volume	between-clones	30	27	26	21
	within-clones	23	20	27	21
Mean seed wt	between-clones	24	18	17	15
	within-clones	22	10	19	14
Scale number	between-clones	9	15	12	13
	within-clones	10	7	11	9

The repeatabilities are all very highly significant ($P < 0.001$). Length showed higher repeatabilities than breadth, because of greater variability among clones which is reflected in the clonal coefficients of variation. Cone volume and mean seed weight were both more variable than length and breadth, but this can be related to the fact that the former are three-dimensional characters. Length : breadth ratio was highly repeatable, with rather low within-clone coefficients of variation. Scale number showed appreciably lower coefficients of variation than cone volume, indicating that considerable variation must exist in mean scale size.

Site Differences

Calculated site means for different characters are listed in Table 4.

TABLE 4—Site means for cone characters

Site	Character					
	Length cm	Breadth cm	Length : breadth	Volume cc	Mean seed weight mg	Scale number
Glenbervie	9.88 b	5.56 c	1.80 a	128 c	23.4 c	196 ab
Whaka	11.02 a	6.20 b	1.77 a	179 b	33.0 a	209 a
Gwavas	11.76 a	6.43 a	1.82 a	207 a	35.4 a	195 ab
Berwick	11.28 a	6.58 a	1.71 b	208 ab	30.5 b	189 b

Reading down columns, values with a letter in common do not differ significantly at the 1% probability level.

At Glenbervie the cones were clearly much smaller and the seeds lighter than elsewhere. At Whaka the cones were smaller than at Gwavas, and possibly Berwick ($P < 0.05$). However, although the cones were large at Berwick, seed weight was less there than at Gwavas and Whaka. Scale number varied little between sites, but was significantly less at Berwick than at Whaka. At this level scale number and cone volume varied quite independently. Length : breadth ratio also varied little between sites but, like scale number, was lowest at Berwick.

In the cones studied for asymmetry of apophysis thickness the mean of clonal means at Gwavas was 2.61 compared with 2.27 at Glenbervie. This difference is significant ($t_7 = 2.75$, $P < 0.05$). Pilot plottings of asymmetry of apophysis thickness against axis length, for stem cones within clone-site subclasses, gave further indications of a positive non-genetic association between these two characters.

Clone-Site Interactions

All correlations, for clonal means, among the sites Whaka, Gwavas, and Berwick, were high, ranging from 0.78 to 0.97. This indicates a lack of important clone-site interactions among these sites. The correlations between Glenbervie and the other sites were mostly weaker (Table 5), except for scale number.

TABLE 5—Correlations between Glenbervie and other sites for clonal means

Character	Other Site		
	Whaka	Gwavas	Berwick
Length	0.82**	0.65**	0.62NS
Breadth	0.37NS	0.47NS	0.71*
Length : breadth	0.65*	0.67**	0.54NS
Volume	0.69*	0.49*	0.67*
Mean seed weight	0.84**	0.54**	0.72*
Scale number	0.88***	0.80***	0.76*

NS denotes not significant, $P > 0.05$

* denotes significant, $P < 0.05$

** denotes highly significant, $P < 0.01$

*** denotes very highly significant, $P < 0.001$

Analyses of variance, which were admittedly subject to bias, indicated that in most cases there were significant interactions between Glenbervie and the other sites. Moreover, graphical analyses (e.g., Figs. 1 and 2) further indicated that the low correlations were not generally attributable to sampling error effects. Overall, it appears that almost all the important interactions are with respect to Glenbervie and elsewhere.

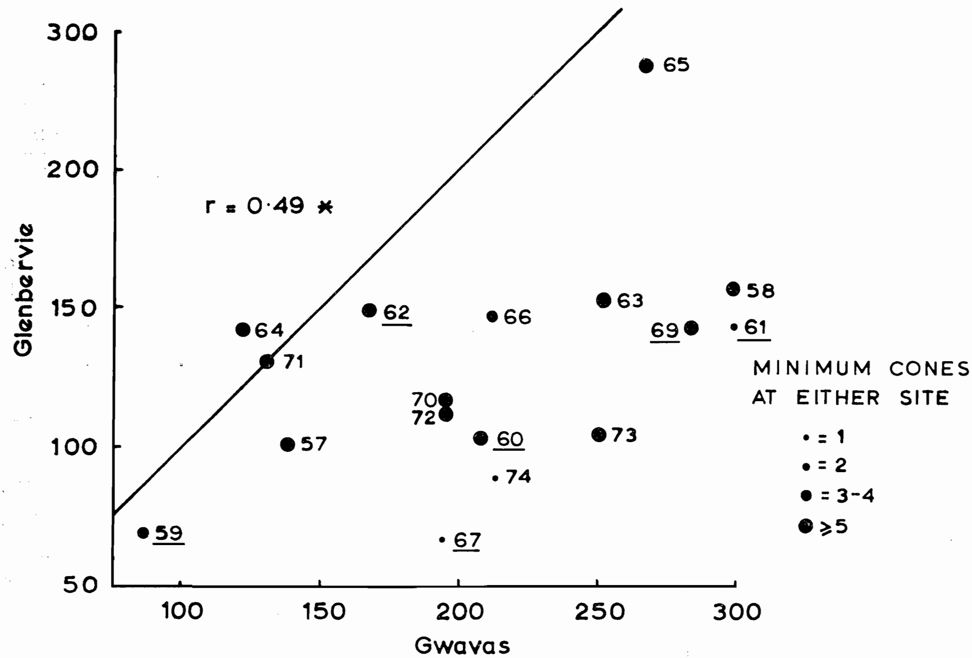

FIG. 1—Mean cone volumes (cc), by clones, Glenbervie v. Gwavas. Diagonal represents 1 : 1 ratio between the sites. Underlining denotes clones of very poor vigour at Glenbervie. Size of dot represents site with fewer cones.

Fig. 1 shows, for cone volume, the clonal means at Glenbervie against those at Gwavas. Fig. 2 represents the corresponding graph for mean seed weights. Individual points are labelled with the clone numbers, and those clones which grew very poorly at Glenbervie have the numbers underlined.

Although some clones, such as 67 and 74, were poorly represented at Glenbervie, it is evident that much of the scatter of points reflects interactions rather than sampling error. For instance Clones 65 and 73, which were well represented on both sites, behaved very differently from each other at Glenbervie. The clones which maintained vegetative vigour there did not maintain cone size or seed weight consistently better than the others. However, clones 64 and 65, which were not only vigorous but very free from dieback at Glenbervie, maintained both cone size and seed size particularly well there.

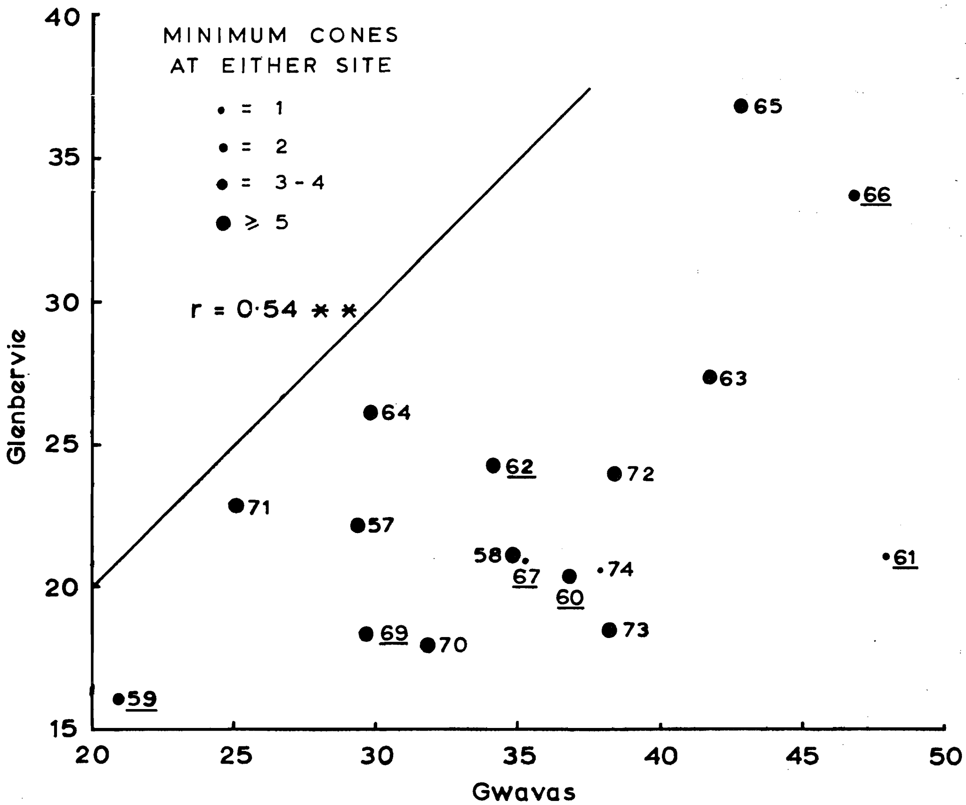

FIG. 2—Mean seed weight (mg), by clones, Glenbervie v. Gwavas. Diagonal represents 1 : 1 ratio between the sites. Underlining denotes clones of very poor vigour at Glenbervie. Size of dot represents site with fewer cones.

DISCUSSION

Clonal Repeatabilities, Gross Site Effects, and Clone-Site Interactions

The generally high clonal repeatabilities for cone characters were expected, and accord with values obtained by Fielding (1964).

The main site effect was the depression of cone dimensions and seed weight at Glenbervie. This almost certainly resulted directly or indirectly from the phosphate deficiency there. The pattern of site differences in scale number, however, was different, and it may be that this character is influenced by length of growing season. Mean seed weight did not always respond to site in the same way as cone volume, but since final cone volume is attained several months before the seed is fully mature (Sweet and Bollmann, 1971), the two characters could be subject to different environmental influences on the same site. At Berwick, seed weight may well have been influenced by drought during summer and autumn.

The main cause of clone-site interactions appears to have been the very poor cone development among certain clones in response to the phosphate deficiency at Glenbervie.

This effect resembles certain vegetative disorders (Burdon, 1971) in being largely unrelated to the growth rates shown there by individual clones.

Taxonomic Implications

Cone dimensions and seed weight can clearly be much influenced by site. The potential taxonomic significance of these site effects can be gauged from Table 6, which shows mean cone length and mean seed weight respectively for different populations growing at different locations.

TABLE 6—Means for cone length (cm) and seed weight (mg) observed in different populations on different sites

Character	Current Experiment		Population studies			
	Glenbervie	Other sites	(N.Z.)	Natural stands		
			(two stands)	Ano Nuevo	Monterey	Cambria
Length	9.9	11.0-11.8	—	11.8	9.5	14.3[1]
Axis length*	—	—	—	10.1	8.5	11.5[2]
Seed weight	23.4	30.5-35.4	37.7	41.4	23.6	47.9[3]
			—	33.8	24.2	39.9[1]

* Values for this measurement typically slightly smaller than for length as measured in current study.

[1] Fielding (1953); [2] Forde (1964a).

[3] Unpublished, quoted by Burdon and Bannister (1970). Seeds extracted from Forde's cone samples.

Even allowing for sampling error (presumably greatest for site effects and for Fielding's seed weight determinations) and various types of bias, there can be little doubt that site effects may be large in relation to observed differences between natural populations. On the available evidence differences between sites can be almost if not fully as large as those between the Monterey and Ano Neuvo populations growing in their respective natural habitats.

The severe reduction of cone size and seed weight on the phosphate-deficient Glenbervie site suggests that these characters are of restricted taxonomic value in New Zealand, at least as diagnostic criteria, because this effect will be widespread precisely where the growing stock is suspected of being largely of Monterey ancestry instead of mainly Ano Nuevo. Nevertheless, useful information may still be available if sampling is done from pockets of fertile soil or from topdressed sites in the region, but further study of local site effects would be needed. Scale number, although varying little between sites, affords little taxonomic information, because it appears not to differ between the Ano Nuevo and Monterey populations (Forde, 1964a). Asymmetry of apophysis thickness, although differing slightly between these natural populations, appears to show non-heritable variation in parallel with cone dimensions.

CONCLUSIONS

1. Significant clonal repeatabilities (mainly 0.4 to 0.8 within sites) confirm the genetic control of cone length, breadth, length : breadth ratio and volume, and of number of scales and mean seed weight.

2. Cone size and mean seed weight appear to be severely depressed by phosphate deficiency.
3. Scale number appears to vary little between the four sites which were represented.
4. Mean seed weight can vary with site somewhat independently of cone volume, presumably because seed development continues after final cone volume is attained.
5. It appears that phosphate deficiency affects cone development more severely in some genotypes than in others, and not always comparably with any effect on vegetative vigour.
6. Cone size and mean seed weight, although differing widely between natural populations, cannot be accepted as reliable indicators of ancestry on sites that are phosphate-deficient.

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REFERENCES

BANNISTER, M. H.; WILLIAMS, A. L., and McDONALD, I. R. C. 1962: Variation of turpentine composition in five population samples of **Pinus radiata. New Zealand Journal of Science 5:** 486-95.

BLIGHT, M. M.; BREWERTON, H. V.; McDONALD, I. R. C., and BANNISTER, M. H. 1964: Beta-pinene content of the **Pinus radiata** populations of Kaingaroa Forest. **New Zealand Journal of Science 7:** 457-9.

BURDON, R. D. 1971: Clonal repeatabilities and clone-site interactions in **Pinus radiata. Silvae Genetica 20:** 33-9.

——— (in prep.): Foliar macronutrient contents in **Pinus radiata** clones on four different sites.

BURDON, R. D., and BANNISTER, M. H. 1970: The natural populations of **Pinus radiata:** their early performance and a provisional evaluation of their silvicultural potential in New Zealand. **New Zealand Forest Service, Forest Research Institute, Genetics and Tree Improvement Report 49** (unpublished).

BURDON, R. D., and LOW, C. B. (in prep.): Seed production in **Pinus radiata** clones on four different sites.

FIELDING, J. M. 1953: Variations in Monterey pine. **Forest and Timber Bureau, Canberra, Bulletin 31.**

——— 1964: Some characteristics of the cones and seeds of **Pinus radiata. Forest and Timber Bureau, Canberra, Leaflet 89.**

FORDE, M. B. 1964a: Variation in natural populations of **Pinus radiata** in California. Part 3, Cone characters. **New Zealand Journal of Botany 2:** 459-85.

——— 1964b: Variation in natural populations of **Pinus radiata** in California, Part 4. Discussion. **New Zealand Journal of Botany 2:** 486-501.

SWEET, G. B., and BOLLMANN, M. P. 1971: Seasonal growth of the female strobilus in **Pinus radiata. New Zealand Journal of Forestry Science 1:** 15-21.