GENETIC SURVEY OF PINUS RADIATA. 1: INTRODUCTION, DESCRIPTION OF EXPERIMENT, AND BASIC METHODOLOGY

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ABSTRACT

A provenance-progeny trial of *Pinus radiata* D.Don was planted in three stages on two sites in Kaingaroa Forest in the central North Island of New Zealand, to study the quantitative genetic architecture of the species and establish a gene resource. The trial contained basically 50 open-pollinated families (progenies) of each natural population (Año Nuevo, Monterey, Cambria, Guadalupe Island, and Cedros Island), representing the species' full natural range, plus two New Zealand populations (Kaingaroa and Nelson), with 20 seedlings per progeny per site. The seedlings were supplemented in the final planting by four juvenile clones from each of 30 progenies from each population except Cedros. Within each of the six site/year-of-planting blocks, randomisation was complete but for an interlocking block feature. This layout, a variation of non-contiguous plots, was designed for 50% systematic diagonal thinnings that would leave a balanced classification. The interlocking block layout showed some significant advantages. Its overall success, however, was limited through a combination of very adverse factors: the range of growth rates among populations; intense assertion of crown dominance and the associated stem mortality which were doubtless accentuated by needle-cast diseases; and prevalent stem malformation.

Keywords: variation; inheritance; genetic architecture; provenance; experimental layout; interlocking block; gene resource; *Pinus radiata*.

INTRODUCTION

Pinus radiata is a forest tree species that has become massively domesticated. This degree of domestication is reflected in its use as a plantation crop over an area about 500 times that of the native stands, the intensive management practices that are widely used, and the intensive genetic improvement that is being obtained.

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Serious efforts at genetic improvement began in the early 1950s in New Zealand, Australia, and South Africa, although preliminary genetic studies had begun before 1940 in both Australia and South Africa. The genetic improvement has come from intensive breeding programmes which began from local plantations within the countries concerned on the assumption that provenance variation would be unimportant in this species. This assumption was based largely on a belief that the species had an extremely limited range (1.5° lat.) on the Californian coast, while in a single trial in Australia (Fielding 1961a) two of the three Californian mainland populations (Año Nuevo and Monterey) performed similarly to local stock and better than the third Californian mainland population, Cambria. Also, the existing local stocks were evidently adapted to a range of sites and showed great variation which appeared to be largely genetic in nature. In the event, the decision to proceed with intensive breeding programmes was justified, although for long-term breeding the use of supplementary germplasm from some of the native populations appears to be well worthwhile.

After the intensive breeding began, several factors led to a re-examination of the provenance issue. It eventually became clear that the species also included two populations of pines on islands (Guadalupe and Cedros) offshore from the Baja California peninsula (Fielding 1953, 1961b; Bannister 1958, 1959; Axelrod 1980; Bannister & McDonald 1983; Millar 1986). Although belonging squarely within *P. radiata,* these island populations showed some obvious morphological differences from the mainland ones, which suggested that there would be many other genetic differences. Thus, the species shows a markedly wider geographic range and greater diversity than was originally thought. Fielding (1953) recommended that the Guadalupe population be introduced into Australia, accepting that it belongs to *P. radiata.* Bannister (1959), accepting that the Cedros Island population could also well belong to *P. radiata*, recommended that it be incorporated in a programme of experimental hybridisation between the various populations on the basis that wide genetic diversity and genetic recombination would offer both heterosis and long-term adaptability. In the meantime, a *P. radiata* provenance trial had been planted in 1955 on two sites in New Zealand but it was very limited in both provenance representation and within-site replication (Shelbourne *et al.* 1979).

Systematic seed collection from the entire natural range of the species was a natural means of securing the maximum genetic variability, and such a collection was duly accumulated. The immediate follow-up was to sow seed and grow the material in properly designed common-garden comparisons (Bannister 1963). Accordingly, a combined provenance-progeny trial was established, replicated on two contrasting sites in Kaingaroa Forest (Burdon & Bannister 1973b). These two plantings are collectively designated the *Pinus radiata* Genetic Survey experiment. In addition, spare seedlings were planted in subsidiary provenance trials on some other sites in New Zealand (Shelbourne *et al.* 1979), and some other minor experimental plantings included certain interpopulation hybrids.

The purpose of the Genetic Survey experiment involved both medium- to long-term breeding objectives and the underpinning genetic research. Breeding objectives included:

- Provision of a very broadly based gene resource;
- Preliminary evaluation of the genetic material contained therein;
- Provision for selection of superior trees that could be used for medium-term breeding work;

- Provision for spontaneous occurrence of a complex hybrid swarm, which would represent a very wide range of new gene combinations on which natural and artificial selection could operate.

The research objectives centred around studying the quantitative genetic architecture, for a wide range of traits. This entailed:

- Characterising the pattern of differences among natural populations;
- Comparing New Zealand populations among each other and with natural populations, to infer ancestry of the local populations and the genetic shifts that they may have undergone in New Zealand;
- Estimating the comparative magnitudes of genetic variation between natural populations, between local subdivisions of the same, and from tree-to-tree;
- Estimating heritabilities, with main emphasis on narrow-sense values, for individual traits;
- To a limited degree, studying genotype × site interaction;
- Studying patterns of association among traits, with main emphasis on genetic correlations.

Secondary research objectives which subsequently emerged included: investigating the breeding system both for its own interest and as an aid to estimating some genetic parameters; comparing performance of seedlings and cuttings; comparing the expression of gene effects in seedlings and cuttings respectively; and acquiring information relating to the appropriate timing and procedures for early selection in order to accelerate genetic gains in advanced-generation breeding.

Since the plantings were designed to serve as a long-term collection of genetic material in addition to being a genetic experiment, they embodied a new concept in experimental design (the "interlocking block") which is of interest in itself. In fact, it was not practicable to retain the Genetic Survey as a comprehensive genetic experiment for more than about 10 years after planting, but by that age a great deal of the key information was already available.

In the light of early results from the Genetic Survey and adjunct trials (including the 1955 plantings) further seed was imported from native populations for establishing more comprehensive provenance trials and specialised gene resource plantings in New Zealand (Burdon 1988).

Among preliminary publications of results from the Genetic Survey experiment, the main one was by Burdon & Bannister (1973b), which covered population differences during the first few years. Others included reports by Bannister (1966), Burdon & Bannister (1973a, b), Burdon & Low (1977, 1991a, b), Shelbourne *et al.* (1979), Harris (1989), and Burdon & Young (1991), in addition to notes in Forest Research Institute Annual Reports.

This series of nine papers serves to document the experiment and report and discuss the research findings. This one, the first of the series, documents the design and setting up of the experiment, its maintenance, the programme of assessment, and the principles of the data analysis. Reference is also made to subsidiary experiments. The development of the experiment is reviewed, with particular reference to experience gained in using the interlocking block design. The next seven papers cover different aspects of the research findings, addressing the original research objectives and most of the secondary ones that emerged. The final paper presents a discussion of more general issues that arose from the results.

EXPERIMENTAL

Population Sampling

The structure of the population sample is summarised in Table 1. Details are given below.

TABLE 1–Summary of population structure represented in experiment

Population	Subpopulations*	Families/ subpopulation	Total families
Mainland California			
Año Nuevo	5*	10	50
Monterey	5*	10	50
Cambria	5*	10	50
Guadalupe Island	Main	49	54
	High-altitude	5	
Cedros Island	North and South	25	50
New Zealand			
Kaingaroa	-	-	50
Nelson	-	-	50†

* *See* Forde 1964a.

† Plus 50 that were used in Stage I plantings only.

Californian mainland stands

The seed parents used were a 50% subsample of the trees used in the study reported by Forde (1964). Forde's sampling procedure began with choosing five localities within each of the three populations, based on the following considerations: covering as far as possible the range of ecological conditions within each population; vehicle access; and an open stand structure that allowed ready sampling from the ground (the main constraint).

From each locality, 20 trees were initially sampled, generally separated by 100 m or more, and four cones per tree (one per branch) were available for extracting seed.

Guadalupe Island

The pines were concentrated on the windward (north-west) side of a very exposed ridge which runs roughly north-east at the northern end of the island (Libby *et al.* 1968; Bannister unpubl.). Most of the extant pines were concentrated in a strip about 3.5 km long by about 300 m wide, ranging from about 400 m altitude in the north-east to about 950 m in the south-west. Higher up the ridge, 2–3 km to the south-east and at 1120–1160 m altitude, some relict trees were present as outliers. All trees were very old, there having been no effective regeneration for about 100 years because of the release of goats on the island. Surviving trees numbered about 400 at the time, but a recent report (Libby 1990) has put the current number of survivors at less than 50.

Seed was collected separately from the main stand and the high-altitude outliers. The main stand was sampled in 1958 by Dr Reid V. Moran who obtained viable seed from 49 trees, all within 100 m of the crest of the ridge. That population sample represented roughly an altitudinal cline with the seed parents as evenly spaced as was practicable. Choice of parents, however, was constrained by accessibility of cones, i.e., within 5 m from the ground.

From the high-altitude outliers, seed of five of the trees sampled by Libby *et al.* (1968) in 1964 was used for this experiment.

Cedros Island

The stands comprised two disjunct subpopulations ("North" and "South"), separated by about 13 km in a north-south direction, the North population beginning very close to the northern tip of the island. Both subpopulations were somewhat discontinuous, being concentrated on the windward (north-west) sides of ridges; North (including outliers) had a north-south extent of about 5 km and South about 6.5 km. A more detailed description of the stands has been given by Libby *et al.* (1968) who made the seed collection in 1964.

Seed was collected from 60 parents, 30 from each of the two subpopulations, 10 cones from each tree being used for the study. As far as was practicable the chosen parents were evenly distributed, generally being 200 m or so from each other, although they were concentrated near ridge tops. Although seed was collected mainly from trees with accessible branches, it seems unlikely that this caused significant genotypic bias in the sample.

For the field experiment, only 25 families were used from each of the North and South subpopulations, families being omitted first on grounds of low yields of seedlings and then on the basis of proximity of seed parents to each other.

New Zealand populations

The two New Zealand (NZ) control populations, Kaingaroa and Nelson, were known to have grown for at least two generations in the respective regions of the country. The Kaingaroa population was a 10-ha planting established in 1944 from a 140-kg seed collection (NZ Forest Service seedlot R41/337) made in Whakarewarewa State Forest, Rotorua, during felling of a stand in Cpt 22 (old numbering). The stand had other *P. radiata* immediately to the north of it, but *Pseudotsuga menziesii* (Mirb.) Franco and *Pinus ponderosa* P. et C.Laws. adjoined it elsewhere. Seed was collected at tree age 14 years from planting, shortly after a thinning and avoiding malformed trees. Sampling was done near the centre of the stand, away from outside pollen sources. The cones from each tree represented several annual cone crops and, when possible, several crops (clusters) within an annual growth stage. The pollen parents could thus have included some trees that were removed in the thinning.

The Nelson population was a plantation growing in Splash Gully, Cpt 301, of Tasman Forest (owned by H.Baigent & Sons). It was planted in 1945 from a large seedlot of unspecified local origin supplied commercially by H.G.Kingsland. The stand was 13 years old at collection, and it was unthinned. Trees sampled for seed were of co-dominant status or better and free from malformation. Other sampling details were as for the Kaingaroa population.

No special effort was made to avoid collecting from neighbouring trees within the respective plantations. At the time of seed collection oleoresin specimens and increment cores were taken from the Nelson seed parents (Bannister *et al.* 1962). Oleoresin specimens and increment cores were subsequently taken from many of the Kaingaroa seed parents.

Fifty families were chosen from the Kaingaroa population, and 100 from the Nelson; half the latter were used only in the first of the three stages of planting the experiment when no Cedros material was to hand.

Raising of Seedlings

Seed was extracted from the cones in New Zealand. All seed was stratified for 3 weeks or longer prior to sowing in late September to early October during the years 1963, 1964, and 1966 (Stages I, II, and III respectively). Sowing was done, unreplicated, in germinating boxes in a greenhouse. A few early losses resulted from damping off, but showed no evident pattern in relation to populations or families. Seedlings were pricked out in the early post-cotyledon stage into polythene tubes, roughly 50 cm diameter and 200 cm long, which were allocated at random within and among 35-tube boxes, subject to the restriction of one seedling per family per set. Obviously weak, deformed, or chlorophyll-defective seedlings were avoided; otherwise, the seedlings pricked out were essentially a random sample of the successful germinants. A few early losses after pricking out were made good by pricking out replacement seedlings. Material not represented in Stage I (namely, the Cedros population and the high-altitude Guadalupe progenies) was given supplementary representation in the seedlings pricked out from the 1964 and 1966 sowings for planting in 1965 and 1967 respectively (Table 2).

TABLE 2–Trees per progeny planted in each site/stage block (disregarding deficiencies and supplementary trees planted to make good deficiencies in earlier stages)

Propagules	Population(s)	Stage		
		I	II	III
Seedlings	Californian mainland	6	6	8
	New Zealand	6	6	8
	Main Guadalupe	6	6	8
	High-altitude Guadalupe	–	12	8
	Cedros	–	9	12
Cuttings	30 families of each population except Cedros	–	–	2 clones × 4 ramets

Stages I, II, and III were planted in 1964, 1965, and 1967 respectively.

Each seedling was labelled with an aluminium tag denoting the population and the progeny number, and this was pushed into the tube at pricking out.

Spare seedlings from the sowings were lined out in the nursery, by progenies, in unreplicated plots during the early autumn for providing replacement (or spare) 1/1 planting stock. These were conditioned for planting by undercutting and wrenching.

Production of Clonal Material

In Stage III of the experiment the seedlings were supplemented with a clonal component (Table 2) which was intended to allow estimation of certain variance components that could not be estimated just from the seedlings.

After a pilot trial, 40 families of each population (except Cedros) were chosen for clonal replication of seedlings or cuttings; six seedling genotypes per family were used to provide six cuttings each, in order to provide four plantable cuttings of each of four genotypes in 30 families. As reported by Burdon & Bannister (1985), cuttings were taken from lined-out seedlings, 3 years old from seed, which had been hedged to allow suitable cuttings to be taken from roughly 55 cm above the root collar, all with intact terminal buds or apical tufts.

The cuttings were 15–20 cm long, and were set with an unreplicated layout in polythene tubes like those used for pricking out seedlings. Randomisation of the tubes for the field layout was done shortly before planting. Choice of families and genotypes within families for the main experiment was governed by the aim to retain:

(1) Six families per subpopulation in the mainland populations to leave 30 families per population;

(2) Four genotypes (clones) per family;

(3) Four good cuttings per genotype.

The three-stage culling was based firstly on shortages of plantable cuttings and then on random choice. Of the four clones in each family two were allocated at random to one site and two to the other.

Each cutting was labelled with an aluminium tag denoting population, progeny number, and clone number, the tag being inserted in the rooting medium when the cutting was set.

Cuttings of spare genotypes were used for surround planting, while spare cuttings of chosen genotypes were lined out in the nursery and prepared for planting by undercutting and wrenching.

Sites

The two sites (A and B) were located in Kaingaroa Forest, but were markedly different.

Site A was in the Northern Boundary area: latitude 38°18'S, longitude 176°40'E; altitude c. 320 m; generally sheltered; undulating terrain; soil very deep mantle of various layers of rhyolitic ash (pumice) capped with 30 cm of raw, basaltic Tarawera scoria; rainfall c. 1600 mm; site index for *P. radiata* c. 34–35 m (mean height of 100 largest trees/ha at 20 years from establishment). Existing vegetation was light indigenous scrub, largely manuka (*Leptospermum scoparium* J.R. et G.Forst.) and bracken fern (*Pteridium esculentum* (Forst.f.) Kuhn.), plus some exotic tree lupin (*Lupinus arboreus* Sims.), much of which was crushed and burnt before planting.

Site B was on the Kaingaroa Plateau: latitude 38°24'S, longitude 176°32'E; altitude 525 m; moderately exposed; terrain flat except for a system of very small, shallow gullies dissecting one corner; soil about 3–4 m of various rhyolitic ash layers overlying partly welded ignimbrite and with a slight pan about 25 cm below the surface associated with the Taupo ash layer; rainfall c. 1550 mm; site index c. 29 m; existing vegetation low scrub, almost entirely manuka with a little bracken and some tussock (mainly *Poa caespitosa*). Site preparation consisted of ploughing a single furrow along each planting line in 1964.

Field Layout

Planting was carried out in 1964, 1965, and 1967 (Stages I, II, and III respectively), in separate but contiguous blocks on each site, which in the respective stages occupied roughly 2.3, 2.3, and 3.7 ha excluding surround planting. Spacing throughout was 2.74 × 2.74 m (9 × 9 ft) giving roughly 1330 stems/ha. Each site/stage block had effectively four rows of surround planting.

Allocation of genetic material to the respective site/stage blocks is shown in Table 2. Unavailability of Cedros Island and high-altitude Guadalupe material for the Stage I

planting in 1965 was a constraint. This was accommodated by adjusting numbers of seedlings per family per block in the later stages to give a total representation of 20 or 21 per family at each site.

Within each such block a form of "interlocking block" layout (cf. Libby & Cockerham 1980) was used. This was designed to leave a balanced genetic classification (in terms of numbers of genotypes per family) after 50% systematic thinnings, while achieving virtually complete randomisation of individuals from the 350 progenies within a single site/stage block. It was also designed to minimise imbalance in the classification if any part of the experiment was damaged or destroyed. Within each block there were eight sets designated as "colours" that were superimposed in a systematic grid arrangement, with basically one genotype per family per set.

The one restriction on complete randomisation within each site/stage block was the offsetting of colour replicates from each other by up to three rows and/or one column, which is minuscule in relation to block size. Application of the scheme to retain the balanced classification with the systematic thinnings is illustrated in Fig. 1. It varied from the (then unpublished) original proposal of Libby & Cockerham in that there was normally only one individual of a family represented per colour replicate per site/stage block. It paralleled the original proposal in that all the colour replicates that would be removed in one thinning could be treated as equivalent, but it was theoretically possible to conduct sample assessments of, say, every fourth row which would still represent a balanced classification.

In the first two stages the pink and orange replicates were not used, being occupied by routine nursery stock which served as "filler" trees.

Certain variations of the above scheme were adopted to make good the potential imbalances in representation within the total experiment. In addition to adjustments in the representation of Cedros and high-altitude Guadalupe families (Table 2) some other deficiencies that had arisen in Stage I were made good in later stages.

The clonal material, which was incorporated in Stage III (Table 2), was randomised in intimate mixture with the seedlings. The allocation to the colour replicates, however, differed from that used with the seedlings; of the two clones per family that were allocated exclusively to one site, the four ramets of one were allocated at random among the red and/or blue, yellow, and green replicates, while the four ramets of the other were allocated at random among the white, black, pink, and orange replicates.

Planting and Early Tending

Prior to planting, each position was marked with a 1-m-long, 25 × 25-mm stake which had the top painted according to the colour replicate. Planting was done in late winter to early spring, and after planting the identifying tag for each tree was nailed on to the marker stake. After the first year missing, dead, or sick trees were replaced with spare open-rooted stock from the nursery. Trees that could not be replaced with material of the same sub-classes were replaced with routine nursery stock which served as non-experimental filler trees.

At Site A it was necessary to hand-release many trees, mainly from tree lupin and bracken, but in 1968 Stage III was released from the lupin by aerial spraying with a selective herbicide which caused minimal lasting stem deformation in the trees. Some hand-releasing was needed on Site B to deal with a few small patches of lupin.

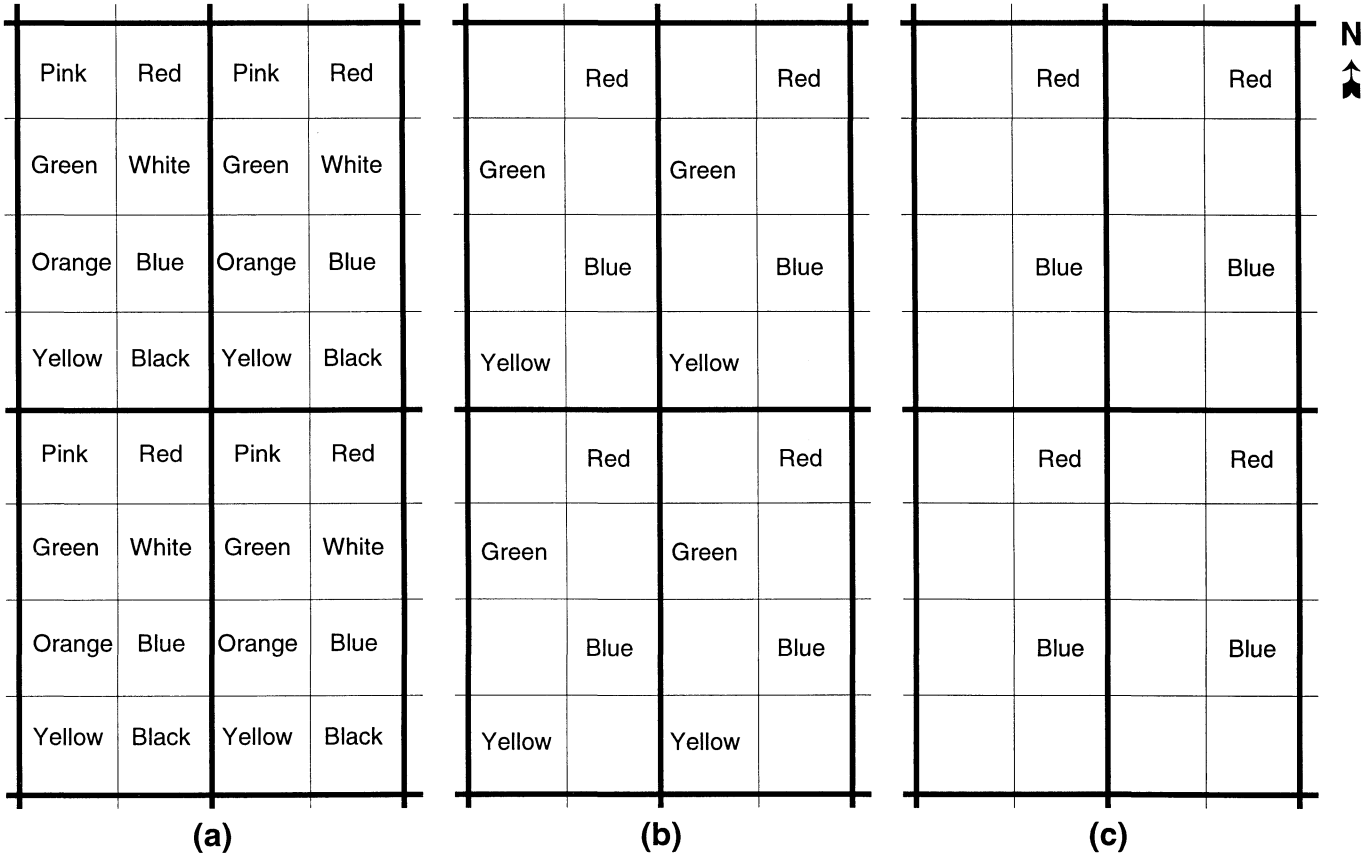

FIG. 1–Interlocking block layout, with basic modules of eight "colour" sets demarcated by bold lines (a) before thinning (2.74 × 2.74 m, i.e., 9 × 9 ft), (b) after first thinning (3.88 × 3.88 m), (c) after proposed second thinning (5.49 × 5.49 m). Columns run north–south; rows run east–west.

During 1968 the entire experiment was given an aerial topdressing with 317 kg borated sulphur superphosphate per hectare after the appearance of boron deficiency in many of the island-population trees. Spraying to control Dothistroma needle blight was carried out regularly from 1968 to 1977 according to the prevailing levels of infection, although control was incomplete.

Subsidiary and Follow-Up Experiments

Spare planting stock from Stage III was bulked by populations to establish provenance trials in 1968 at three other sites—Gwavas (Hawke's Bay foothills), Santoft (Manawatu coastal dunes), and Golden Downs (Moutere Gravels, Nelson)—and results have been reported by Shelbourne *et al.* (1979). Spare stock of the island populations from Stage II was planted as two unreplicated half-hectare blocks near Site A in 1968.

Follow-up seed collections were made in 1978 (Eldridge 1978). From these a country-wide series of provenance trials involving Californian mainland and New Zealand populations was established along with gene resource plantings of both mainland and Guadalupe origin (Burdon 1988). Plantings from those collections have also been made in Australia and several other countries (e.g., Eldridge 1986; Falkenhagen 1991).

Thinnings

Thinnings were carried out as summarised in Table 3. The systematic thinnings in Stages I and II actually removed only a nominal one-third of the experimental trees because two of the "colour replicates" were occupied by non-experimental filler trees. Deficiencies in the colour replicates to remain were made good by leaving trees in replicates that were otherwise due to be felled. Experimental trees that were felled in thinning provided wood specimens (Burdon & Low 1992) and the opportunity for height measurements and detailed assessment of branching pattern.

Eventually, the faster growth rate of the New Zealand material, which was contributing greatly to the progressive suppression of other material, led to a decision to remove the New Zealand trees in 1976–77, at which stage the trial was officially terminated as an intensive genetic experiment. However, it has been left standing as a gene resource and has already served as a base for selection of trees to include in the main breeding population (Burdon 1988); it has also remained available for follow-up assessments to meet limited objectives (e.g., Burdon & Young 1991).

TABLE 3–Schedule of thinnings, by site/stage blocks

Site	Stage	Planting year	Age at thinning (years)	
			50% systematic	Felling NZ trees
A	I	1964	7	12
	II	1965	8	11.5
	III	1967	–	9
B	I	1964	8	12
	II	1965	9	12
	III	1967	–	9

Assessments

All blocks were assessed in the first year after planting, primarily for survival and height growth. Between then and shortly before the first thinning each block was assessed two or three times (Table 4) mainly for height but also variously including counts of branch clusters on the stem, the transition to producing sealed adult buds, the presence or absence of boron deficiency, frost mortality, browsing damage by deer and/or rabbits, and needle-cast scores. Some of the records were taken as notes which were later coded numerically.

TABLE 4–Ages (approx.) at which 100% assessments were made in the respective site/stage blocks. All assessments included heights unless specified otherwise

Site	Stage	Ages (years) from planting
A	I	1, 2, 4, 6.5*, 9, 11*
	II	1†, 3, 4, 7*, 11.5*
	III	1, 2, 3, 7‡, 8*
B	I	1, 2, 4, 5, 7.5*, 12*
	II	1, 3, 4, 9*, 12*
	III	1, 2, 3, 6, 9*

* Done immediately prior to thinning and including stem diameter.
† Height data not analysed because of animal damage.
‡ Including stem diameter but not height.

Shortly before thinning and after low pruning of all trees to c. 2 m, detailed assessments were made of height, stem diameter, bark thickness, tree form variables, and, where opportune, Cyclaneusma needle cast. For these assessments the policy was to exclude, in addition to dead trees, any that were suppressed, badly affected by top breakage, or grossly affected by initial toppling. Such rejection was generally done by field crews and involved relatively few trees, except in Stage III where for various reasons suppression was more serious. For Stage III on Site B an acceptance threshold of both 6 m height and 7 cm dbhob was adopted. In this block too few trees of the Cedros population met this criterion for worthwhile analysis.

The variables involved were measured as follows on each tree in the sample assessed. (The abbreviations are used subsequently only where brevity is important.)

Actual measurement

HT	Height (in centimetres during first 2 years, in decimetres thereafter)
DIAM	Dbhob (mm). For multi-stemmed trees finally recorded as square root of sum of squared diameters
BARK	Bark thickness (mm), using a bark gauge, and making an analogous adjustment with multi-stemmed trees.

Counts or qualitative records

BR CLUS	Number of branch clusters on main stem
RLDR	Retarded leader—1 = leader overtopped by lateral (except through dieback), 0 = otherwise
DBK	Diplodia-associated leader dieback—0 = absent, 1 = present

FORK	0 = no fork, 1 = forking (actual counts of forks made in early assessments)
ABORT	"Apical abortion" (putative thrip damage)—scoring 2 for each definite occurrence, 1 for each suspected occurrence
REJECT	0 = acceptable for pruning, otherwise numerically coded for prime cause for rejection out of crookedness, forking, lean, dieback/top breakage, poor health, inadequate crown status
FROST	Killed by frost or not
BROWSE	Browsed or not
B DEF	Boron deficiency symptoms present or absent

Visual scores

DOTHI (0–4)	Dothistroma needle blight—0 = much less than neighbours, 4 = much more
BUDS	Sealed bud score—0 = fully green apical tufts, 4 = fully sealed buds, 5 = fully sealed "multinodal" buds; scored in some assessments for leader only (0–5), in others separately for leader and for leader plus laterals and these scores summed (0–9) for each tree
CROWN (0–4)	Freedom from Cyclaneusma needle cast—0 = almost fully defoliated, 4 = considerable third-year foliage carried
BUTT (0–3)	Butt sweep—0 = unacceptable, 3 = negligible
STR (1–9)	Stem straightness—1 = very crooked, 9 = very straight (0–3 or 0–5 in some assessments)
BR QU (1–9)	Branch habit quality—1 = very rough, steep-angled, and irregular, 9 = very regular, light, and wide-angled; with emphasis on regularity such that a regular "uninodal" habit would rate as desirable (scored 0–3 in three early assessments)
BR FR (1–5 or 1–9)	Branch cluster frequency—1 = "uninodal", top score = very "multinodal"
BR ANG (1–5)	Branch angle—1 = almost flat, 5 = very steep.

Where several assessment crews operated within a block, each would normally cover particular colour replicates to allow for observer effects.

The ages at which particular traits were assessed in each block are given in Table 5.

Data Preparation and Preliminary Analysis

For each site/stage block, data files from various assessments were eventually merged and converted into the current Forest Research Institute Statistical Package format.

Missing data for individual variables could not be handled satisfactorily with the available statistical package. Accordingly, trees that did not survive to provide meaningful data for all variables assessed up to a thinning date were omitted from the main data analysis. Separate merged data files were actually created for all the trees in a block remaining until first thinning and for just those remaining until second thinning. Data from assessments of subsamples of the experiment (e.g., particular blocks or colour replicates) were thus omitted from the main data analysis, but were subjected to subsidiary data analyses. Some preliminary

TABLE 5–Ages (years) at which traits, other than height and stem diameter (*see* Table 4), were assessed. In addition, samples were taken for wood property determinations (Burdon & Low (1992) and oleoresin analysis (Burdon, Gaskin, Zabkiewicz & Low 1992; Burdon, Zabkiewicz & Andrew 1992), and a needle study (Burdon & Low 1977)

Trait	Site/Stage Block					
	A I	A II	A III	B I	B II	B III
BR CLUS*	1, 2, 7	4, 7	1,3	1, 2, 5	1, 4	1
BUDS	2	4	2	2	4	2
RLDR	2	–	1, 2, 3	2, 5	4	1, 2, 3
ABORT†	2	–	–	2	–	–
FROST‡	1	1	–	–	–	–
B DEF‡	2, 3	2	–	2, 3	2	–
BROWSE‡	–	2	–	–	–	–
PINEUS‡	–	3	1	4	3	1
DOTHI	3‡	–	–	6‡	6‡	8
CROWN	10	7	7	12	12	–
DBK	6.5‡	6.5	7	7.5‡	9	–
BUTT	6.5, 11	7, 11.5	7	7.5, 12	9, 12	9
STR	6.5, 11	7, 11.5	7	7.5, 12	9, 12	9
BR QU	6.5, 11	7, 11.5	7	7.5, 12	9, 12	9
BR FR	11	7, 11.5	7	12	9, 12	9
BR ANG	–	7	7	7.5	9, 12	9
FORK	6.5, 11	7, 11.5	7	7.5, 12	9, 12	9
BARK	12	11.5	7	12	9, 12	9

* Plus counts on trees felled in systematic thinning (Table 3).
† Covered by Burdon & Bannister (1973a)
‡ Covered by Burdon & Bannister (1973b)

results (Burdon & Bannister 1973b) were, however, based on the larger samples of trees within particular blocks that survived to earlier ages.

For seven variables assessed in all blocks, combined multi-block data files were created. This often entailed treating heights or diameters at different ages but at the same stages of stand development as equivalent variables. Sometimes visual scores had to be recoded to give common ranges, e.g., converting some 0–3 stem straightness scores to the usual 1–9 scale.

Feasibility checks, based on absolute or likely bounds for individual variables, were supplemented for height and diameter by calculating periodic increments for individual trees, and by field checks where necessary. Some preliminary analyses (details not reported) indicated a need to allow for colour-replicate (effectively observer) effects, in which event adjustments were made before the main analysis.

Main Data Analysis

Analyses of variance and cross-products proceeded on the following sets or subsets of data:

(1) Block by block
  (a) Trees surviving to first thinning (six blocks)
    • All populations individually

- Californian mainland populations together
- New Zealand populations together.

(b) Trees surviving to second thinning (four blocks), as for (a).

(2) Over all six blocks (Method of Unweighted Means)
- All populations individually except Cedros
- Californian mainland populations together
- New Zealand populations together.

The main body of analysis thus involved about 100 sets or subsets of data, with up to 30 variables (original and derived). Data analysis over all populations was not attempted for several reasons—namely, limitations of computer capacity, differences between population groups in both the structures and degree of imbalance in the classifications, and the fact that most of the critical comparisons were between populations within groups (i.e., between Año Nuevo, Monterey, and Cambria and between Kaingaroa and Nelson). It was still possible, where needed, to construct ancillary tests for comparing populations of different groups. Analyses involving the tree samples for wood properties (Burdon & Low 1992) involved about 40 additional sets with 3–6 variables per set, while further analyses were conducted on the oleoresin data.

Some basic models for analysis of variance (ANOVA) are set out in Table 6, from which estimation of variance components and approximate F-tests is self-evident.

TABLE 6–Some alternative analysis of variance (ANOVA) models for within-block analyses

Effect	Degrees of freedom	Expected mean square (e.m.s.) in terms of variance components
ANOVA 1 Mainland populations together, full model, random except for populations		
Populations (p)	2	$\sigma^2_w + n_1 \sigma^2_{f(sp(p))} + 10n_4 \sigma^2_{sp(p)} + 50n_6 V_p$
Subpopulations within p (sp(p))	12	$\sigma^2_w + n_2 \sigma^2_{f(sp(p))} + 10n_5 \sigma^2_{sp(p)}$
Families within sp(p) (f(sp(p)))	135	$\sigma^2_w + n_3 \sigma^2_{f(sp(p))}$
Trees within families (w)	balance	σ^2_w
$n_1 \approx n_2 \ldots \approx n_6$, ≈arithmetic mean of trees per family		
ANOVA 2 As for ANOVA 1, but assuming no differences between subpopulations within populations		
Populations (p)	2	$\sigma^2_w + n_7 \sigma^2_{f(p)} + 50n_9 V_p$
Families within populations (f(p))	147	$\sigma^2_w + n_8 \sigma^2_{f(p)}$
Trees with families (w)	balance	σ^2_w
n_7, n_8, n_9 as for n_1 etc. in ANOVA 1		
ANOVA 3 Individual mainland populations, assuming subpopulations random		
Subpopulations (sp)	4	$\sigma^2_w + n_{10} \sigma^2_{f(sp)} + 10n_{12} \sigma^2_{sp}$
Families within sp (f(sp))	45	$\sigma^2_w + n_{11} \sigma^2_{f(sp)}$
Trees within families (w)	balance	σ^2_w
n_{10}, n_{11}, n_{12} as for n_1 etc. in ANOVA 1		

In ANOVA 1 the inclusion of $\sigma^2_{sp(p)}$ in the populations e.m.s. treats subpopulations within populations as a random effect, whereas they have an undetermined intermediate

status between a random effect and a fixed one. The test thus implied for populations was therefore over-stringent (but it was normally used, unless F<1 for subpopulations); the alternative test, assuming a fixed effect and therefore omitting $\sigma^2_{sp(p)}$ from the populations e.m.s., would be under-stringent.

For considering variation specifically within each individual mainland population, the populations effect was dropped and the listed degrees of freedom (d.f.) in ANOVAs 1 and 2 for the other effects were divided by three.

For the New Zealand populations together, ANOVA 2 was applicable, except that there were 1 and 98 d.f. for populations and families within populations respectively. For studying variation within these populations individually, a one-way ANOVA (designated ANOVA 4 but not tabulated because it is self-evident) with 49 families d.f. was appropriate.

For Cedros Island, the model was ANOVA 3 except that there were 1 and 48 d.f. for subpopulations and families within subpopulations respectively, if the subpopulations differed.

For Guadalupe Island, the basic model was a one-way ANOVA (cf. ANOVA 4). An alternative model (cf. ANOVA 3), which was used for some supplementary analyses, entailed designating the five high-altitude families as a separate subpopulation from the rest.

Analyses over all site/stage blocks employed the approximate Method of Unweighted (family/block subclass) Means to cope with the imbalance in the classifications. The analyses were also complicated by the question of whether sites and stages were fixed or random effects. Preliminary analyses showed that while second-order interactions between sites, stages, and genotypic effects were prevalent, particularly in the early assessments, the first-order interactions between genotypic effects and either sites or stages appeared to be generally minor or non-existent. Accordingly, it was decided that, except for wood properties (Burdon & Low 1992), the sites and stages effects would be combined into blocks, with all block interactions deemed to be random. The incorporation of blocks as a main effect is illustrated, for the two New Zealand populations together, in Table 7. In this analysis there is no direct F-test for populations, necessitating a Satterthwaite-type approximation (Henderson 1959).

TABLE 7–Analysis of variance for the two New Zealand populations pooling sites and years into a single random effect, namely, blocks

	Effect	d.f.	Coefficient of variance component in e.m.s.						F-test
			σ^2_w	$\sigma^2_{bf(p)}$	σ^2_{bp}	$\sigma^2_{f(p)}$	V_p	V_b	
1	Blocks (b)	5	$1/n$	1	50	–	–	100	$1/4$
2	Pops (p)	1	$1/n$	1	50	6	300		$2+5/3+4$
3	Families within p (f(p))	98	$1/n$	1	–	6			$3/5$
4	b × p (bp)	5	$1/n$	1	50				$4/5$
5	b × f(p) (bf(p))	490	$1/n$	1					$5/6$
6	Within subclasses (w)*	balance	$1/n$						

n = harmonic mean of subclass sizes

* original within-subclass s.s./m.s. divided by n

RESULTS AND DISCUSSION

Field Designs

The interlocking block design showed some of the expected advantages, but overall it was not very successful in this situation. It is appropriate to consider the lessons to be drawn from its use.

Advantages of the layout included convenience for assessing essentially balanced, individually randomised, population samples by taking any sample of one or more colour replicates or any strip or patch of experiment. This latter feature afforded highly efficient population comparisons for traits (e.g., Dothistroma needle blight, browsing damage) that occurred patchily. The layout also allowed convenient partitioning, if needed, of observer effects as each observer could be sent along every second or every fourth row. It has undoubtedly favoured the creation of a complex hybrid swarm of wind-pollinated offspring, except for the widespread suppression of Cedros trees.

The layout certainly failed in respect of preserving a balanced genetic classification for more than a few years, but this resulted largely from several features of the particular trial. There were substantial differences between populations in growth rates (Burdon & Bannister 1973b); Cedros was very much the slowest, with Guadalupe appreciably slower than the remainder, and Kaingaroa and Nelson appreciably faster than any of the native populations. Superimposed upon these population differences in growth rate was the strong assertion of crown dominance and high natural stem mortality that typifies *P. radiata* stands on most of the North Island volcanic plateau. This assertion of dominance was accentuated by the appearance of Dothistroma needle blight, which affected some populations far more than local material, and by a high incidence of stem malformation in native-population material, which tends to be associated with loss of crown status. The population differences in growth rate led to considerable suppression, which in the slower-growing populations prevented realistic evaluation of longer-term growth rate and of tree habit—the squat habit of Cedros was effectively masked in this experiment. Earlier thinning would have pre-empted some of the suppression, but by itself it would have accelerated the run-down of residual degrees of freedom and may not have materially prolonged the life of the experiment as it had been conceived. Indeed, the thinnings were done earlier than originally envisaged. In retrospect, the interlocking block design seems appropriate for situations where very good and even establishment is assured, growth rate differences are subtle, assertion of dominance is weak, and mortality, wind breakage, and general malformation are minimal.

Alternative designs would have had their own problems. Contiguous multi-tree plots, while allowing a balanced classification to remain after selective thinning, would reduce the precision of the experiment by reducing effective replication. A split-plot layout, grouping populations into sub-blocks within which trees were individually randomised, would presumably have sufficed to reveal many population differences and avoided much of the suppression, but there would still have been edge effects, and it would probably have reduced the interpopulation hybridisation that was an intended outcome. In retrospect, however, this layout might have been the best option. A lattice (incomplete block) design could not even have been contemplated at the time because of its complexity, and in retrospect appears to have fewer major advantages than the split-plot design. Use of wider spacing and/or more filler trees would have reduced the problems associated with thinning, but the larger areas of land needed would have increased costs and probably inflated the error variation.

Micro-environmental variation was a problem with the large block units, particularly at Site A where the undulating terrain made it very difficult to partition microsite variation using conventional block layouts. An alternative approach would have been to correct for immediate environmental effects. Tackling this with a matrix of plots of a standard control throughout the trial would have considerably increased the size of the experiment. Corrections that are internal to the main body of data (reviewed by Magnussen 1989) are potentially intricate but could offer substantially improved gains in precision. Such gains, however, would be more important in respect of genotypic rankings than in respect of the parameter estimates that were of main interest. Any adjustment for local environmental effects would have been complicated, in this trial, by certain population differences in response to microsite effects (e.g., some populations suffering more than others in frost hollows).

Other Aspects

Some of the problems were partly independent of the experimental layout. The high incidence of stem malformation, which arose from various causes, made it very difficult to rate stems for certain tree-form variables, notably stem straightness and branching habit. Further problems arose from the toppling of many stems which occurred in Stage 1 on Site A, while a combination of frost injury and animal damage in Stage II on Site A caused some uneven growth there.

The clonal adjunct certainly made a very important contribution (Burdon & Bannister 1985; Burdon, Bannister & Low 1992) to the total information provided by the experiment, and was very efficient. However, it brought a significant complication through considerable suppression of seedlings which arose from the size difference between the cuttings and the seedlings at planting. The cross-referencing of variance component estimates between cuttings and seedlings, which was the prime reason for growing the two categories in intimate mixture, was only a partial success (Burdon, Bannister & Low 1992). On the other hand, mixing cuttings and seedlings did allow an excellent comparison of these two categories (Burdon & Bannister 1985).

The use of tubed planting stock was a practical necessity, given the chosen layout and the manpower available. However, using tubes that were small enough to be manageable meant that the stock either would be prone to toppling through getting too big for the tubes (Stage I), or would be rather too small for the standard of site preparation that could be achieved (Stage III).

The large hierarchical genetic classification is clearly difficult to accommodate satisfactorily in an experimental layout in order to obtain precise genetic information.

The staggering of establishment over 4 years, with three planting seasons, was a practical necessity, offered an element of insurance, allowed a start to be made without all material being to hand, and afforded slightly greater generality to results. It did, however, mean an undesirable time lag between site preparation and the planting of Stages II and III, which undid some of the site preparation. It also entailed small numbers of genotypes per family per block (Table 2). Where data for a variable could be combined satisfactorily over blocks this was not a serious problem. For other variables, however, that could not be done, which meant that there were often far too few individuals per family for efficient estimation of most genetic parameters (Robertson 1959; Brown 1969).

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