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**GENETIC VARIATION IN NUTRITION AND
EDAPHIC REQUIREMENTS IN *PINUS*
RADIATA: THE EVIDENCE, IMPLICATIONS
AND RESEARCH PROPOSALS**

by

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EXECUTIVE SUMMARY

Substantial genetic variation exists within *Pinus radiata* in respect of its edaphic requirements, notably its mineral nutrient demands. The understanding of these differences is still limited, but they have potentially important implications for genetic improvement, nutrition research and, above all for forest management. The issues are discussed, the potentially suitable classes of genetic material for the research are reviewed, and guidelines are proposed for a research programme involving the Forest Nutrition Cooperative and possibly other FRI/Industry Cooperatives.

GENETIC VARIATION IN NUTRITION AND EDAPHIC REQUIREMENTS IN *PINUS RADIATA*: THE EVIDENCE, IMPLICATIONS AND RESEARCH PROPOSALS

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ABSTRACT

The forest grower can meet the problem of infertile sites in one or more of several ways: use of fertilisers, avoiding the worse sites, and using better adapted genetic material and, to some extent, weed control. Using genetic material that tolerates lower nutrient status has to date been the least used of these approaches in New Zealand. Yet there is important genetic variation in the edaphic requirements of *P. radiata*, both among provenances and from tree to tree; and the wider ramifications of this variation could be considerable for the forest grower. Two key areas of research are (1) the relevant patterns of genotype-environment interaction, which relates to both whether it is necessary to select certain genotypes for specific site categories and the choice of screening environments for the selection and (2) the use of genotypes with divergent nutritional characteristics to obtain a better understanding of various nutrient deficiencies. In many respects clones would be the ideal research material, but for maturation effects and the lack of clones that are already characterised for certain site tolerances. A major hurdle exists in establishing that the genotype-environment interactions shown by clonal material are comparable in magnitude and patterns to those shown by seedlings. The categories of potentially suitable genetic material that would be available for future research on nutritional requirements are reviewed. Various experiments are proposed, as studies primarily involving the Forest Nutrition Cooperative, but prospectively involving the Radiata Breeding Cooperative and the Stand Management Cooperatives as well. In particular, it is foreseen that the programme could in large measure be integrated with development work on clonal forestry.

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INTRODUCTION

On many sites mineral nutrients and/or other edaphic factors can limit tree growth, or affect tree form or wood properties. Should the limitations be significant, forest managers can respond in three main directions:

- (i) Add fertilisers and/or carry out other site amelioration.
- (ii) Avoid certain sites for afforestation.
- (iii) Use genotypes that are more tolerant of the conditions. Given the greatly preferred status of *P. radiata*, alternative species will not be considered.

In addition, weed control may be important in making mineral nutrients available to the trees, along with water and light.

These options are not mutually exclusive, except in that a decision to avoid sites, when once taken, will preclude the other two options. In fact, the options are highly interactive with each other. Cheap and effective correction of deficiencies etc. can obviously make certain marginal sites worthwhile using, as could the use of better-adapted genetic material. This availability of better-adapted genotypes could alternatively allow a reduction in fertiliser budgets on sites that are afforested. Conversely, if highly cost-effective site amelioration can be achieved it might avert the need to pursue special genetic adaptation to problem soils. Also, a decision (or a need) to afforest poorer sites will create a need for site amelioration and/or genetic improvement in tolerances. While fertilisers may mitigate competition from weeds for nutrients they may exacerbate competition for other resources (light and water).

A choice of sites for afforestation must always be made, although it has often been severely constrained by availability of land at a price that is acceptable. In fact, a recent trend has been for companies to avoid many of the poorer sites, whatever the cost of the land. Use of fertilisers and intensive site preparation, and even sustained weed control, have become common practice in New Zealand. By comparison, the specific pursuit of genotypes that are better adapted to problem soils has received much less attention, at least at an operational level.

Of the various ways of addressing the problem of poor soils genetic improvement in adaptation has received very little attention to date.

Within *P. radiata* there is clearly substantial genetic variation in certain edaphic tolerances which include resistance to certain specific nutrient deficiencies. This report will focus upon the nature of such genetic differences and their implications for research in both genetic improvement and mineral nutrition. Initially the evidence for genetic variation will be reviewed, at both the provenance level and the tree-to-tree level, then the potential implications for forest growers will be spelled out in more detail. Research issues will be considered in terms of the information needed, and thence the appropriate areas of study. The problem of choosing optimal genetic material for the research, and the available categories of genetic material are reviewed. Finally, some experiments are proposed, with an analysis of options to be considered in drawing up an overall programme of investigations.

GENETIC VARIATION

Provenances

P. radiata occurs naturally in five discrete natural populations (Figure 1), which span a range of 9° latitude, although the New Zealand stock is derived only from the two northernmost ones, and predominantly from Año Nuevo. The geological formations and soil types vary greatly within the natural range of the species (Table 1), largely between populations but also within the Monterey population. It is hardly surprising, then, that provenance differences have been observed in edaphic or nutritional requirements. There are four lines of evidence for such differences:

1. *Boron deficiency in island provenances.* In a provenance-progeny trial planted on two sites in Kaingaroa Forest (Burdon and Bannister 1973) the five native provenances and two New Zealand ones were in intimate mixture. By 2-3 years after planting roughly one-quarter of the island population trees were showing pronounced symptoms of boron deficiency which were associated with some very low levels of foliar B (down to 2 ppm). No such symptoms were evident in the mainland Californian and New Zealand material. In 1968 the trial was topdressed at both sites with borated sulphur superphosphate, and no further boron deficiency symptoms were observed.

This evidence is not totally rigorous in that no untopdressed control areas were retained. On the other hand, the fact that the plantings were staggered over 1964, -65 and -67 would argue against an age-related peak in incidence leading to a spurious effect of the topdressing. The incidence of B deficiency could result in part from neighbourhood inbreeding and consequent inbreeding depression in the island populations, but that can be only a partial explanation; there was also appreciable

inbreeding in the unaffected mainland material and, more convincingly, three-way outcrosses involving both island populations have also shown boron deficiency in Kaingaroa Forest (Burdon and Low 1977).

TABLE 1: Features of the habitats of the different natural populations

Location	Latitude (°N)	Altitude (m)	Exposure (mm)	Rainfall (mm)	Geology	Soil
Año Nuevo	37	0-330	Varied often sheltered	675-900?	Argillites mildly calcareous	Fine loams, depth variable
Monterey	36.5	0-420	Generally moderate to sheltered	375-700?	Variable, sediments and some granites	Very varied fertility and base status
Cambria	35.5	0-180	Varied	450-575?	Sandstone	Sandy loam, localised poor drainage
Guadalupe	29	330-1220	Severe	300-900??	Tholeitic basalt	Rocky loam
Cedros	28	290-640	Locally severe	200-400??	Metamorphics and ancient sediments	Skeletal

Note: Rainfall figures for higher altitudes in mainland populations are tentative, while all rainfall figures for island populations are conjectural.

2. *Differences in performance on infertile clay soils.* In 1980 a countrywide series of provenance trials was planted, representing the three Californian mainland provenances, plus three regional seedlots of climbing select status, which collectively represented a New Zealand provenance. For convenience, the planting sites could be categorised as follows:

- 3 infertile clay (2 'gumland', 1 Nelson)
- 3 coastal dune
- 3 pumiceland
- 15 miscellaneous.

The comparative performance of provenances, in terms of growth and survival, was markedly different among the site categories (Table 2). The outstanding feature was how Monterey and to a lesser extent Cambria fared better, relative to Año Nuevo and New Zealand material, on the infertile clays than elsewhere (remembering that the New Zealand material is predominantly of Año Nuevo ancestry). Expressing the

performance in terms relative basal areas at Riverhead, which was the key trial on the infertile clays, the Año Nuevo, Monterey and Cambria provenances showed values of 75, 134 and 120 m²/ha respectively versus 100 m²/ha for New Zealand. This pronounced superiority of Monterey was despite possible adaptive responses to local selective pressures in the New Zealand material and the fact that the New Zealand material does not show the neighbourhood inbreeding that occurs in natural stands. The Monterey provenance is broadly adapted to New Zealand conditions except for certain cooler and more southerly sites, and it appears that an infusion of Monterey selections into the breeding population could boost the performance of future improved seedlots on many of the inherently less fertile clay sites. The Cambria population has the attraction of apparently similar soil tolerances, and comparatively good tree form, but it poses problems of susceptibility to needlecast diseases and to shoot dieback associated with infection by *Diplodia pinea*.

TABLE 2: Comparative performance of different native populations in 1980 provenance plantings (NZ controls = 100) (from Burdon *et al.* 1986, and unpubl.).

Variable	Site type (no. of sites)	Native population		
		Año Nuevo (37°N)	Monterey (36.5°N)	Cambria (35.5°N)
Dbhob	Infertile clays (3)	93	106	102
	Coastal dunes (3)	92	98	95
	Pumicelands (3)	92	97	87
	Others (15)	94	96	93
Height	Infertile clays (3)	95	103	97
	Coastal dunes (3)	97	97	94
	Pumicelands (3)	94	94	88
	Others (15)	94	94	90
Survival	Infertile clays (3)	98	106	102
	Coastal dunes (3)	98	97	96
	Pumicelands (3)	100	97	97
	Others (15)	100	97	88

The exact basis for such provenance differences in soil tolerance has not been clearly established, but the really distinctive feature of the Riverhead and Waitangi sites is

phosphorus deficiency, while the Nelson site is low in phosphorus as well as some other nutrients. Provenance differences in resistance to the root pathogen *Phytophthora cinnamomi* (Butcher *et al.* 1984), which can be troublesome in Northland, may have been a factor, but this explanation is open to objections.

3. *Incidence of needle fusion.* In the Genetic Survey experiment (Burdon and Bannister 1973), planted in Kaingaroa Forest, a sprinkling of Guadalupe provenance trees showed needle fusion, albeit often transiently. The evidence is anecdotal, in that systematic counts of affected trees were not made, while an effect of differential inbreeding among provenances cannot be ruled out. On the other hand, needle fusion evidently reflects a nutrient deficiency, since it can be readily corrected by fertiliser treatment, although it does not appear to be as specific to P deficiency as was once thought; and a requirement for a higher soil fertility fits well with the basaltic nature of Guadalupe Island.
4. *Tolerance of soil salinity.* Marked provenance differences in tolerance of salinity in culture solutions were observed by Cromer *et al.* (1982), Cambria showing the greatest tolerance and Guadalupe the least. While not bearing directly on the issue addressed in this report it illustrates the pervasive differences among provenances for almost whatever trait is studied. A unique finding was the evidence of marked differences among ecological subdivisions of the native populations; paradoxically, the coastal subpopulations showed less tolerance than the more inland ones, which was attributed to greater dilution of salinity by the more abundant fog drip near the coast.

Tree-to-tree variation

1. *Responses to P-deficient clays.* Just as the comparative performance of provenances are anomalous on such sites relative to elsewhere, so are the comparative performances of individual clones and seedling progenies. Fielding and Brown (1961) observed that certain clones fell away dramatically in growth rate on the less fertile ridges on a site that was generally low in phosphorus, while other clones held their performance much better. Burdon (1971, 1976) observed, in a clonal trial, replicated on four very different sites, that certain clones performed consistently very poorly on an infertile (P-deficient) gumland clay, but showed essentially normal vigour on more fertile sites of both sandy and greasy textures.

A common feature of stands on such gumland clay sites is the tree-to-tree variability in growth, and such results indicate that it has a true genetic basis. Uncorroborated results with clonal material are suspect, in that clonal differences may be generated by differential responses of genotypes to the process of vegetative propagation, but the

clonal results have been corroborated with seedling material. Much the most precise seedling study has been reported by Johnson *et al.* (1987) and Johnson and Burdon (1990), who found that progeny rankings were very different between a pair of Northland clay sites and a pair of pumice plateau sites, but extremely similar between the sites within each region even though the sites within the regions were contrasting (Table 3). Two other studies, by Shelbourne (1985) and Carson (1990), also showed family rankings on a P-deficient clay at Maramarua to be well out of line with rankings on other sites; but no really coherent patterns were evident in the degree to which family rankings concurred among the remaining sites. Matheson and Raymond (1984) also failed to discern any clear pattern of rank changes among sites in Australia. Among these three latter studies, however, those by Shelbourne and Carson were plagued by the poor quality of the experiments at the (common) P-deficient site, and Carson's had a limited number of parents involved. The Matheson and Raymond study involved imprecise experiments which were therefore inherently unlikely to identify any coherent patterns of rank change.

TABLE 3: Estimates of genetic correlations between sites for stem volume, which reflect the progenies for rankings to remain the same among sites, from '880'-series open-pollinated progeny trial (from Johnson and Burdon 1990)

	Pumiceland		Northland Clays	
	Rotoehu 1	Taupo 2	Good 3	Poor 4
1	1.00	0.96	0.29	0.16
2		1.00	0.52	0.55
3			1.00	0.84
4				1.00

2. *Occurrence of boron deficiency.* Where boron deficiency occurs it is common for adjacent trees to be affected to greatly differing degrees. While not a rigorous demonstration this strongly suggests genetic differences which would parallel those that are evident among provenances.
3. *Clonal differences in foliar nutrient contents.* Several studies have revealed clonal differences in foliar nutrient contents (e.g. Forrest and Ovington 1971, Burdon 1976, Knight 1978). These differences are not always consistent with season, and can vary

markedly according to the nutrient and position in the crown. Moreover, they may not show straightforward relationships to nutritional status. For instance, a genotype that is more affected than others by a particular nutrient deficiency may not show lower contents of the nutrient; indeed, its contents may be anomalously high on account of inefficient remobilisation within the plant. Nutrient concentrations in tissues, therefore, are less a reflection of straightforward genotypic differences in nutrient requirements than of genotypic differences in various aspects of nutrient metabolism. As such they are potentially a valuable tool for studying the mechanisms of genetic differences in nutritional requirements rather than for establishing the existence of the differences.

4. *Family differences in response to added nutrients.* Some family differences have been observed in response to added fertiliser (superphosphate) (Boomsma *et al.* 1981). While the data are not voluminous they do relate to an experimental modification of nutrient status, rather than just to differences in natural soil fertility.
5. *Clonal differences in upper mid-crown yellowing.* Tree-to-tree differences in upper mid-crown yellowing have always been evident, and the repeatability of such differences in replicated clones (M. Skinner, unpubl.) supports the presumption of genetic differences. Clonal differences in symptoms are matched by clonal differences in distribution of foliar Mg concentrations within different parts of the crowns, which strengthen the evidence both for a nutritional basis for the disorder (Mg deficiency) and for genetic variation in that basis.

Within *P. radiata* there is considerable genetic variation in tolerance of adverse soil conditions, which reflects at least in part varying tolerances of certain nutrient deficiencies. The variation is evident both within and between provenances.

IMPLICATIONS FOR THE FOREST GROWER

The potential implications for the forest grower of any genetic improvement in nutrient demands are various, and they include:

- Potential reduction in fertiliser costs and/or fewer nutrient deficiency problems.
- Changed threshold of acceptability of sites for commercial forestry.
- Reduced weed problems.
- Reduced water quality problems.
- Additional decisions to be made in purchases of seedlots.

- The potential to host genetic experiments and even gene resources.

The potential for reducing in fertiliser costs is obvious enough. At the same time better growth might be obtainable for a given level of expenditure on fertiliser. In practice both benefits may be obtained in some degree, and with more tolerant genotypes a better performance might be obtainable even with optimal fertiliser treatment. Uneven distribution of fertiliser is likely to matter less with more tolerant genotypes.

As mentioned earlier, the availability of seedlots with better tolerance of nutrient deficiencies or other adverse soil conditions could lower the land-quality threshold for worthwhile afforestation. While no quantitative calculations have been carried out this may have major strategic advantages as well as direct-cost advantages.

The use of more tolerant crops could mitigate weed problems in two possible ways: by improving the competitive ability of the trees under given site conditions, and by raising the vigour of the trees to a given level with less of a boost to weed growth.

Reduced requirements for fertilisers should reduce the nutrient loadings in streamwaters and, perhaps more importantly, in any lakes fed by the streams. This may not be of direct concern to the forest manager, but there are situations where it could be a very thorny public relations issue.

The choice of seedlots is an area where the forest manager should eventually be able to exercise considerable control over the situation. In the past many opportunities were probably lost, at present the opportunities seem to be limited, but in future new and exciting opportunities should open up. There is little doubt that natural selection would operate for tolerance to 'gumland clay' conditions, if regeneration were based on seed collected from final crop trees on such sites. A strong suspicion exists that the surviving old trees in that part of the country contain a higher proportion of Monterey ancestry than is typical for the rest of the country. This stems not only from the *a priori* consideration that Monterey material enjoys a selective advantage on such sites, but also from the appearance of the tree crowns and from anecdotal reports of highly resinous heartwood being prevalent in Northland *P. radiata* (Monterey material has recently proved to have more resinous heartwood than Año Nuevo material (Burdon and Young 1991)). Small cones, which are a feature of the Monterey provenance, are also prevalent in Northland, but they could be a phenotypic effect of the sites (Burdon and Low 1973), as to some extent could various other features of trees growing on gumland clays.

Despite the scope for capitalising on natural selective forces on such sites, and over twenty years of knowledge of its potential importance, very little was done to capitalise on it. Yields of cones, and of seeds per cone, are evidently poor on such sites. Climbing trees would have been very difficult, and clearfellings were often limited. Thus any locally collected seed would have been both inconvenient and expensive to obtain. These factors seem to have effectively deterred forest managers (probably wrongly) from making local collections, in favour of using the abundant seed that was readily collected (and extracted) from the Volcanic Plateau.

Currently, any choice would be between essentially unimproved, strictly local seedlots (with all the problems of collecting them) and improved seedlots from the main breeding programme which has essentially been based in the Volcanic Plateau. While these improved seedlots may show far less superiority in gumland clays than elsewhere, through the parents not having been screened on such sites, they can still be expected to show significant superiority on such clays. On present evidence, therefore, it is not recommended that local collections should be used in preference to improved lots of high GF rating. Collections of seed of Monterey origin are hardly a practical proposition (unless subject to vegetative multiplication of seedlings), nor have they been shown to be superior to local lots of high GF rating on gumland clays.

For future reference, it should be possible to produce seedlots with specific advantages for such sites (subject to qualifications, which shall be discussed later, concerning whether such sites do represent a reasonably discrete category with respect to patterns of genotype-environment interaction). The control-pollinated (CP) seed orchard technology makes it possible to produce, at little if any extra cost, a range of seedlots for specialised situations. In future there will be seed parents available which will have been evaluated on such sites, and some such parents will eventually have known Monterey ancestry.

To obtain the various benefits, the forest growers will need to make various inputs. These will include direct funding of the requisite research and breeding work, and the various in-kind contributions. The latter include making sites available, preparing them, providing labour for establishing the trials, and carrying out the maintenance and tending. Most importantly, genetic gains can only be obtained efficiently for sites that generate anomalous rankings for performance if those sites are represented in the screening programme. Thus, if gumland clays represent a reasonably distinct site category for genotype-site interaction they will need to be included in the testing programme, despite the considerable difficulties of obtaining good sites for progeny tests and of maintaining the trials in condition for satisfactory assessment. They also include opportunity costs through departing from preferred tending regimes in the interests of obtaining maximal genetic information, and from including in trials genetic material that may be less than

optimal for certain sites. For long-term breeding needs there are potential opportunity costs from hosting gene resource stands, which in the short term will be largely unimproved, and which could represent appreciable areas. However, the deployment of gene resource plantings is based on planting material where selective pressures should reinforce the adaptive advantages of the respective natural populations involved (Burdon 1986, 1988), which should therefore minimise such opportunity costs.

Improved edaphic tolerances could offer a range of potential benefits to certain forest growers, including a wider choice of seedlots, but some inputs would be needed from the growers to achieve the benefits.

IMPLICATIONS FOR THE BREEDER

The implications of genetic differences in nutrient demands for operational tree breeding are also severalfold. They include:

- Potential for improved soil tolerances.
- Side effects on breeding goals.
- Potential for producing regionalised breeds.
- Choice of sites for screening.
- Roles of gene resource material.

Genetic improvement in tolerances while evidently obtainable, is likely to be only partly interchangeable with site amelioration, for reasons that have been discussed earlier, viz. practical and economic limitations on the feasibility of site amelioration and the potential effects of site amelioration on weed growth and water quality.

There are also the interactions with other breeding goals. Improved soil tolerances will tend to be pursued at the expense of some potential genetic gain in other breeding goals, although wood properties and tree form are often no problem on poor soils. However, as growth becomes more vigorous, either through site amelioration or improved site tolerances, tree-form traits, say, may become more relevant as breeding goals.

Improving specific site tolerances would seem to form an obvious basis for producing regionalised breeds, but the argument is not entirely clear-cut. It was shown by Johnson and Burdon (1990) that, even though gumland clay sites produced markedly different family rankings from Volcanic Plateau sites (Table 3), it was possible to select a group of families for all sites studied that gave very nearly as much genetic gain as selecting families for

specific site categories. With CP orchards, however, the selection and production of different sets of families for different situations should cost little if any extra, which would make even marginal gains very attractive. In practice, differences in relative economic worth of traits, between sites or wood processors/users, could be a far more important basis for producing regionalised breeds than the sort of ranking changes that seem to exist between site categories for individual traits. For example, growth rate may be paramount as a breeding goal on warm sites of inherently low soil fertility, while on the more fertile sites tree form must always be important, and wood density may also require close attention depending on the intended processing and end uses. For some sites disease resistance can become a very important breeding goal. Such specialisation of breeding goals is likely to be of importance even for creating subdivisions of the breeding population and not just for producing specialised commercial seedlots.

Choice of sites for screening genotypes is an issue that always arises in breeding. If there is important genotype-site interaction, of the type that generates changes in rankings, it becomes crucial to identify an appropriate set of screening environments. As Johnson and Burdon (1990) showed, it was important to screen genotypes on both of two distinct site categories even though it was apparently possible to select a single set of genotypes of near-optimal growth performance for growing throughout. As Burdon (1977) pointed out the urgent need is to characterise various environments both for patterns of rank change and for the precision with which they resolve genetic differences. The ideal is to identify a subset to screening environments which give sharp and early resolution of genetic differences, and where good performance by genotypes is a guarantee of success in a wide range of other sites. For instance, it was shown by Carson (1990), in a study involving 12 sites, that progeny tests confined to the three 'best' sites could between them give more genetic gain than tests over the eight or nine 'worst' sites.

The pursuit of improved edaphic tolerances could have several implications, in the breeding goals, the production of separate breeds, and in where the breeding material is deployed.

An important underpinning of a breeding programme is the gene resource material, which will need to be maintained as growing trees (rather than just cool-stored seed) if the tree breeder is going to be able to use it (Burdon 1986). It is considered best to deploy such material as far as possible, on sites that impose pressures of natural selection that would reinforce the adaptive advantages of the respective provenances.

RESEARCH IMPLICATIONS

The implications for research involve: obtaining information needed for breeding decisions; defining the optimal roles of genetic improvement, fertiliser applications and other forms of site amelioration respectively; and providing pointers to the most appropriate site amelioration measures.

For the breeder there are the questions of whether regionalisation (not necessarily into contiguous geographic units) is needed, particularly for the breeding population, and what subsets of sites should be chosen as screening environments. These questions are closely intertwined with the issue of the comparative roles of genetic improvement and site amelioration.

Characterising the extent and the patterns of genotype-site interaction is potentially rewarding, and yet can be very difficult to achieve satisfactorily. The state of knowledge concerning soil-related genotype-site interaction in *Pinus radiata* is reviewed in a separate section, and while there are indications that gumland clays represent a distinctive and reasonably discrete category with respect to genotypic rankings the evidence is very incomplete. Site amelioration is of interest not just as an alternative or complementary means of improving crop performance, but also as a potential way of changing the patterns of, or even virtually eliminating, certain troublesome interactions.

For the researcher in mineral nutrition it is clearly desirable to know the appropriate roles of genetic improvement and site amelioration. At the same time the presence of genetic differences in mineral nutrition, or other in edaphic tolerances, can allow developmental studies that can help to define the nature of the nutritional problem and even provide pointers to appropriate site-amelioration measures (e.g., form of nutrient needed and timing of application).

For studying the patterns of genotype-site interaction the problem is how to achieve adequate site coverage with experiments that are powerful enough to give conclusive results, without requiring excessive resources. Choice of appropriate genetic material for the research is potentially crucial. Clonal trials appear promising for this; such is their signal-to-noise ratio that a clonal experiment can typically be much smaller than an open-pollinated progeny trial and still give genetic information of comparable precision (Table 4). In theory, the clonal experiment needs to be only one-fifth of the size, and in practice it may provide equally precise information when much smaller still. Not only do clonal experiments stand to provide empirical information on genotype-site interaction, but the good signal-to-noise ratio should also help in obtaining precise developmental information. For example, clonal material should be particularly favourable for studying

details of the nutrient economy through the patterns of variation in contents and concentrations of mineral nutrients in various parts of the tree.

TABLE 4: Approximate 'signal' and 'noise' variances respectively for different classes of genetic experiment, assuming (for simplicity) complete randomisation of individuals and ignoring 'C-effects' in clones and maternal effects

Items	Clones	Pair-crosses	Open-pollinated or polycross progenies
Signal	V_g^*	$\frac{1}{2}V_A$	$\frac{1}{4}V_A$
Noise	V_e/n	$\geq \frac{1}{4}V_{NA}^\dagger + (V_e + \frac{1}{2}V_g)/n$	$\geq (V_e + \frac{3}{4}V_g)/n$

where V_g = total genetic variance
 = V_A (additive genetic variance) + V_{NA} (non-additive genetic variance)
 V_e = tree-to-tree environmental variances (ignoring probable increases with size of experiment)
 n = number of trees per clone or family
 * for screening candidates for clonal forestry
 † this term belongs to 'signal' if particular pair-crosses are being evaluated for mass propagation

Despite the theoretical advantage of clones there are potential drawbacks. There seems to be no suitable clonal material of *P. radiata* available which has an existing 'track record' for genotype-site interaction as a basis for 'retrospective' studies. All work with clonal material is haunted by the prospect of maturation effects. This can eventually sap the vigour of clones and make them hard to propagate. More insidiously, however, maturation is liable to distort the expression of differences among clones. The challenge remains to halt maturation, or at least contain it to levels that do not matter, and while there are hopes that this can be achieved for a number of years in individual clones it is not yet assured. Even if maturation does not cause problems there are potential complications through clones reflecting non-additive gene effects, in addition to the additive gene effects that are captured in seed orchards and represent the cumulative genetic gain from generations of breeding. Present indications, though, are that non-additive gene effects would be of only secondary importance.

It is to be hoped that adequate control of maturation can be achieved, and that could be tested by repeating some clonal plantings on some essentially identical sites after the

elapse of some years. If rankings remain stable, despite the time that the material is being held for further propagation, it would seem unlikely that maturation effects are generating any 'nuisance' interactions. It is not known, either, whether a uniformly more advanced maturation state makes material more interactive with site, but it is suspected that it does in respect of growth performance; that would mean that cuttings with some maturation would offer an over-sensitive test for interaction, which may have some advantages.

Overall, it would be extremely desirable to establish that clonal material can exhibit essentially the same pattern of genotype-site interaction as seedlings, preferably with continued propagation of the same clones, and to use clones as the routine 'probe' for the detailed patterns of interaction. Achieving such a cross-reference between clones and seedlings, however will be a major hurdle. The clonal material that is readily available is not closely comparable to the most appropriate seedling material in terms of relatedness, breadth of genetic base or history of selection. To make good this deficiency, it appears essential to start with an appropriate set of seedling families. These should be either very numerous and essentially a random sample of an appropriate base population, or they should represent a wide spread of interactive behaviour. If the families are numerous and not intensively select the between-site 'genetic correlations' for performance between sites are likely to be estimated with good precision and little bias, and would provide a good basis for genetic gain prediction provided the patterns of interaction are well characterised. If, on the other hand, the families are chosen for their interactive behaviour, they should provide a more sensitive test for the existence of interaction and a better characterisation of the patterns of interaction, but would give an exaggerated picture of the extent of interaction with consequent bias in genetic gain predictions for certain breeding options. This approach also assumes that the known interactions shown by the families essentially covers the full spectrum of interactions, in other words, that families that have hitherto shown stable or non-interactive performance will not prove to be interactive when challenged by further environments.

Characterising patterns of genotype-site interaction is a crucial task. Another task is developmental analysis of nutritional disorders. A choice of experimental material is needed. Seedlings will give less precise information, but material is available with a 'track record' to build on. Clones will give far more precise information, but are prone to maturation etc., and have not been proven to show the same interactions as seedlings. A major challenge will be to cross-reference the behaviour of clones with that of seedlings, so that clones could be safely used as a routine 'probe' for interactions.

PRESENT UNDERSTANDING OF GENOTYPE-SITE INTERACTION

There is no doubt that some genotypes are markedly more tolerant than others of certain difficult, infertile clay sites. What is not certain, however, is whether these represent clearly definable (if intergrading) categories of sites for which specific adaptational features can be sought and within which it is possible to use just one or two sites for screening genotypes for all sites within such a category.

There are various indications that phosphate-retentive 'gumland' clay sites stand apart from other sites in New Zealand, in that growth on such clay sites shows poor genetic correlations with growth on other New Zealand sites; that is to say, the rankings of genotypes ($r \leq 0.5$) for growth performance on such sites tend to be markedly different compared with elsewhere. While it is possible to find genotypes that are almost optimal on both a gumland clay site and elsewhere in New Zealand it appears impossible to select efficiently for certain gumland clay sites without screening on one or more of those clay sites. The question, then, is whether some or all of the gumland clay sites represent a clearly definable category of sites within which between-site genetic correlations for growth rate are very high. There is only one piece of evidence on this point: the high genetic correlation estimate (0.84) for $4\frac{1}{2}$ -year stem volume between two clay sites of very differing fertility at Moerewa (Table 3), along with their much poorer genetic correlations with pumice plateau sites. While encouraging, this is far from conclusive. Point estimates of genetic correlations were seldom very precise, although the study concerned ('880'-series progeny tests) was relatively powerful. Moreover, the Northland clays are infamous for both their variability and the fine-grained patterns of variation, while much of the afforestation in Northland is moving on to sites that are even better than the more fertile Moerewa site. The proximity of the two Moerewa sites is also a concern, although it is doubtful whether the variations in climate in Northland (outside coastal dune sites) are sufficient to cause substantial genotype-site interaction.

Another concern stems from the difficulty that has been experienced elsewhere in recognising clearly definable site categories with respect to genotype-site interaction in *P. radiata*, with the notable exception of pumice plateau sites within which interactions seem consistently minor. The lack of clear patterns of interaction was evident in the studies of Shelbourne and Low (1985), Carson (1991) and Matheson and Raymond (1984), as reflected in between-site genetic correlations for growth rate. At the same time those studies do not of themselves establish the lack of coherent patterns of interaction, partly because the sampling of sites was necessarily coarse-grained, even when many sites were represented (the Carson study, for instance, only included one gumland clay out of 12 sites), and partly because some of the studies lack power in being based on limited population samples and/or in having low experimental precision at individual sites.

Gumland clays appear to be the one really discrete site category with respect to genotype-site interaction, but this is not firmly established.

AVAILABLE GENETIC MATERIAL FOR RESEARCH STUDIES

Several promising categories of material are available for a more detailed study of soil-related genotype-environment interaction in *P. radiata*. They are:

- Seedlings:** Spare seed of open-pollinated '880'-series progenies which were used in the study described by Johnson and Burdon (1990). Most progenies are still represented by at least 500 uncommitted seeds, and many by at least 1000. In many cases, though, much of the seed comes from a repeat collection, and the extent of consequent genetic differences from the original collection is unknown, although it is assumed to be minor.
- Clones:**
- (i) Tissue-cultured clones produced by Tasman Forestry Ltd, which have been placed on offer for research studies by John Gleed.
 - (ii) Stool-bed clones already in the FRI nursery, representing nine polycross progenies of top-ranked '268' clones, plus a control seedlot and a polycross with clone 850.55. This material is to be kept in the FRI Nursery expressly for sustained repropagation (Work Plan 2030).
 - (iii) 'Puruki' clones, numbering six, which have already been used in the clonal study at Puruki.

Pros and cons of the different categories are as follows:

- Seedlings:** In their favour are the number of families and the degree to which families have already been characterised for interactions with sites. Adverse factors are the inherently weak signal-to-noise ratio for half-sib families, and possible complications arising from the fact that much of the available seed represents repeat collections.

Clones: Clones, as mentioned earlier, should give a much better signal-to-noise ratio in experiments, but there remains the question of how well maturation state can be held, particularly over a long period of time. Considering the two categories separately:

- (i) Tissue cultured (Tasman Forestry Ltd). Large numbers of propagules will be readily available, but there will be questions as to whether *in vitro* propagation has caused maturation effects, or other forms of cultivar decline, that might cause exaggerated site interactions. The greatly accelerated maturation that was observed in early studies with plantlets should be a thing of the past, but uncertainties remain, particularly if clones are held in culture over long periods. Cool storage under refrigeration is subject to problems, while cryopreservation (using liquid nitrogen) has not yet been developed for *P. radiata*.
- (ii) Stool beds (FRI Nursery). Adequate multiplication factors should be obtainable. Staggered plantings over several years may be needed for the logistics of multiplication, the convenience of cooperators and for getting more reliable research information. It is not yet known how long the clones can be maintained satisfactorily as stool beds, or how much maturation they will undergo over time. A few of the clones are tissue cultured, in cold storage. The value of this material, however, has been significantly reduced by some losses that have left limited representation of some families and by appreciable maturation that apparently occurred in the early staged.
- (iii) Puruki clones. These have a very well documented track record for growth rates, crown characteristics and nutritional characteristics, but they will be in an advanced maturation state which must relegate them to an adjunct role in studying genotype-site interactions.

Several classes of material are available for the research, but all have their own advantages and disadvantages. They will need to be used complementarily.

EXISTING STUDIES

One study, designated a Sustainable Forestry Trial (Workplan in progress), is already underway with field layout completed and planting stock in the nursery. This involves seedlings of 47 open-pollinated '880'-series progenies plus three controls, which are to be planted in Kinleith Forest this year. Within the core study, four site treatments, simulating different practices for harvesting and preparation for replanting, are involved, with two fertiliser regimes per site treatment; giving eight treatments in all with 4 seedlings per family per treatment, i.e., a total of 32 seedlings/family. Supplementary studies will involve soil compaction, weed control and 'maximum stress' (a combination of adverse factors).

In the main trial there will be only four seedlings per family per treatment which, with the families being half-sib in nature, will not be conducive to high precision unless differences are absent between certain pairs of treatments. However, the genetic design of the study is not so much for studying detailed information as to ensure that comparable genetic samples are used for all treatments.

The 850-series diallel experiment is of interest since it may provide a unique opportunity to study genotype-site interaction in the second half of the rotation.

Also relevant is the clonal material at Puruki, which has been used to advantage in the study of upper mid-crown yellowing.

RESEARCH PROPOSALS

Objectives

The proposed studies are designed to address a range of questions:

- (i) Characterising, if possible the detailed patterns of genotype-site interaction with prime emphasis initially on sites varying in nutrient status, which will probably require edaphic studies to help identify the main site factors that generate interactions.
- (ii) Ascertaining the degree to which such interaction can be reduced or otherwise modified by site amelioration.
- (iii) Empirical evaluation of the comparative precision of seedling and clonal trials for revealing interaction.

- (iv) Cross-referencing seedling and clonal trials for whether they show parallel patterns of interaction in respect of variance structures and/or performance rankings of genotypes (the latter aspect being much the more difficult to achieve).
- (v) Developmental studies, using clones/families known to vary in edaphic tolerances, to elucidate the nature of disorders and the detailed basis for genetic differences.
- (vi) In some degree incidental to addressing (iii) and (iv), further exploring the feasibility of true clonal forestry (large-scale and continued commercial propagation of a limited number of known and intensively select clones).

The execution of the research programme will need, on both technical and logistical grounds, to be staggered over a number of years.

General considerations

Testing clones for maturation effects, or any other forms of cultivar decline, will necessarily take considerable time. In addition, the full programme of trial establishment would overstretch resources if attempted all at once and would presumably be impossible to arrange simultaneously among all cooperators.

The large majority of the work entailed will be of considerable interest to all cooperatives involved, although the relative importance to the respective cooperatives may vary between trials.

The appropriate complementarity, in using different classes of material, seems to be as follows:

Use of families ('880'-series): Further exploration of the main patterns of interaction, using a limited number of sites that vary widely (noting that data from existing experiments can be used).

Use of clones: This shall proceed under two headings:

- (i) Study of GE interaction, embracing a range of sites/site modifications. In a small subsample of cases the clonal trial will be located on the same site as a seedling trial (above). These clonal trials, in particular, can be staggered over a period of time.

- (ii) Developmental studies following in some degree the Puruki clonal study, using a limited number of sites, handy to existing trials and/or laboratory facilities.

Requisite linkages

The following linkages would need to be established between different trials or groups of trials:

1. Between seedling progeny trials and a sample of clonal trials, by location on the same sites.
2. Between clonal material of a range of maturation states, by either or both of:
 - including stool-bed and field cuttings of the same clones in single trials
 - replicating trials in time on the same sites with the same clones
 using several contrasting sites in each case.
3. Between routine field trials of clones and detailed tissue/biomass analysis in relation to fertiliser treatments (e.g., Mg applications).
4. Between clones planted in individually randomised trials and clones planted in solid blocks, using, for the latter, a subset of clones with distinctive features and planting the blocks on a limited number of sites initially.
5. Between clonal experiments in this general connection and routine tests of new candidate clones, by juxtaposition on the same site(s).
6. Between core genetic material for probing interactions (probably a set of clones after some years) and supplementary genetic material. Augmentations of that basic set of clones would include:
 - (i) Clones from native-provenance families, which need not necessarily be purebred.
 - (ii) New material generated in the main breeding population.

7. Between main series of clonal trials in this area and experiments designed to verify expectations of genetic parameters (between and within families) in vegetative propagules.

Experimental design parameters

Seedling trials

Not less than 50 open-pollinated families (planted in two sets within replicates) x ≥ 25 replicates/environment.

Clonal trials

Not less than 50 'core' clones (with typically up to 30 supplementary clones) x 5-8 replicates/environment, using sets in reps design with ca 25 clones per set.

Choice of genetic material

Seedlings

Core material: Open-pollinated progenies of '880'-series parents which have been extensively field tested, chosen to deliberately include the following categories:

- | | |
|--|-------|
| - families of very stable performance | ca 15 |
| - families of average or randomly chosen stability | ca 20 |
| - families with unstable performance | ca 15 |

Supplementary: Wind-pollinated progenies of Monterey and Cambria selections in NZ ca 30

Clones

Core material: One from each of the families represented in the core seedling material (two/family to be kept in Nursery).

Supplementary (including interim material):

- | | |
|--|----------|
| (i) Clones of Monterey and Cambria parentage | ca 30 |
| (ii) Existing FRI stoolbed clones | up to 60 |
| (iii) Puruki clones | 6 |

Responsibilities

It is proposed that the research be initiated the aegis of the Forest Nutrition Cooperative with prospective involvement on the part of the Radiata Pine Breeding and Stand Management Cooperative as well.

More immediate responsibilities would be as follows:

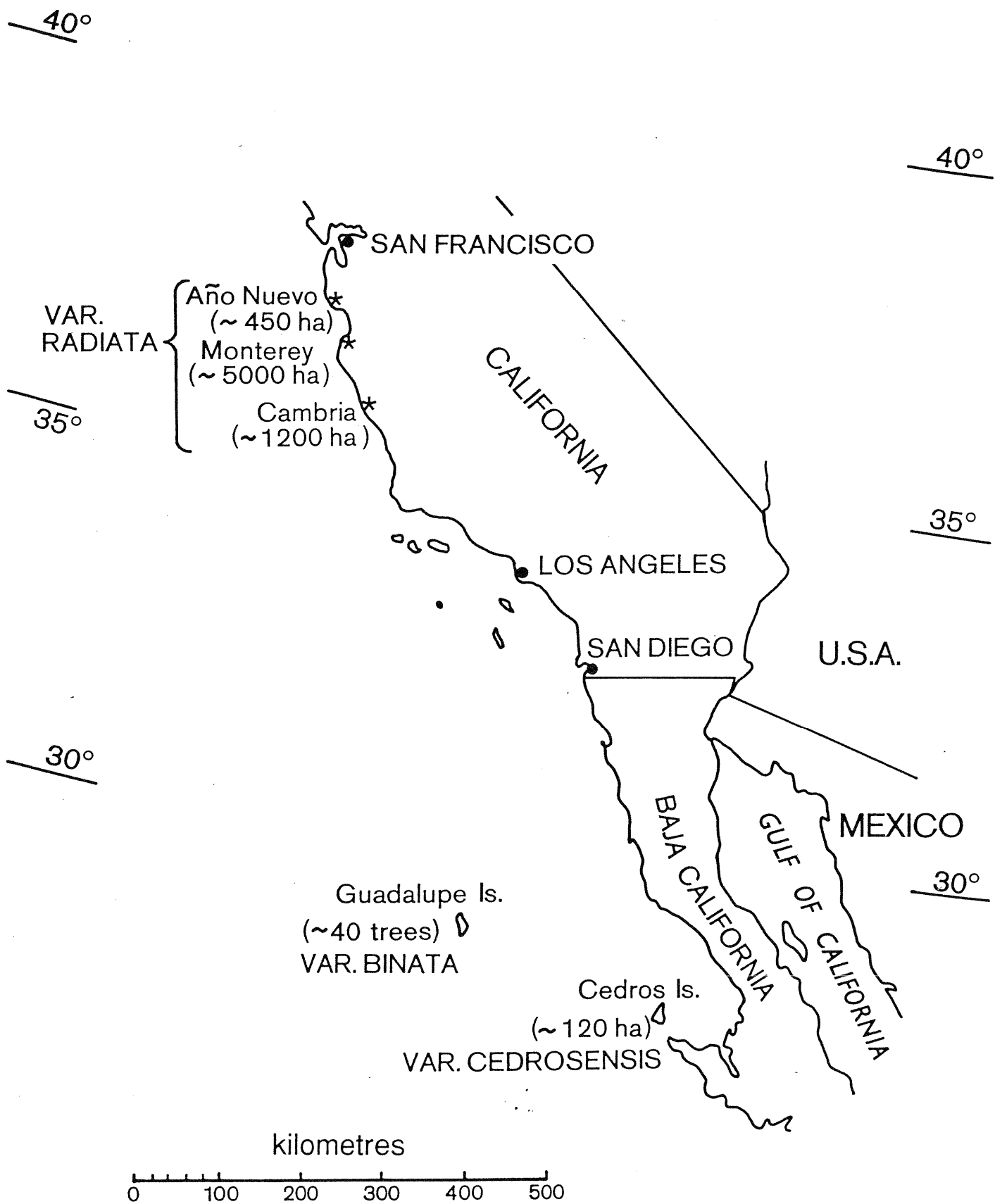
Choice of genetic material	GTI
Choice of sites	GTI, Soils
Choice of site modifications	Soils
Site characterisation, soil profile, soil analysis	Soils
Design	GTI, Soils
Physical layout	GTI
Measurements	GTI (Soils)
Tissue sampling and analysis	Soils
Data analysis	GTI (Soils)
Write-up	GTI, Soils
Site preparation, planting labour, protection, tending	Industry

An appropriate research programme would entail plantings over a number of years and involve at least two Cooperatives, to meet a range of interrelated objectives and to make complementary use of the respective advantages of different classes of genetic material.

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FIGURE 1



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