EARLY RESULTS FROM A CLONAL SELECTION AND TESTING PROGRAMME WITH RADIATA PINE

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ABSTRACT

Selections were made of 216 trees of radiata pine (**Pinus radiata** D. Don) in 6-year-old stands, from which rooted cuttings were propagated and planted in a clonal test. Assessment of growth rates and morphological characters at age 6 years from setting has provided estimates of clonal differences, clonal and phenotypic correlations between characters, and predicted gains from selection on clone means.

Differences between clone means and clonal repeatabilities were substantial and therefore predicted gains from reselection on clone means were high for most characters, especially growth rate. Comparisons of rate of height growth between seedlings and cuttings gave some indication that cuttings have a lower growth potential than seedlings. The clonal correlation between growth rate and branch diameter was high, indicating the difficulty of selecting small-branched yet fast-growing clones.

Fifty of the clones were repropagated from ramets in the first test and a second clonal test was planted. Assessment of the second test at age 2 years from setting gave preliminary indications of poor repeatability of results between the two tests.

A possible way of maintaining high repeatability of growth rate in successive repropagations would be to maintain hedges of all clones. Before a clonal programme is applied to afforestation it is essential to verify that select clones retain their superiority through repeated repropagations.

INTRODUCTION

Radiata pine can be propagated by rooted cuttings more easily than most pine species. Commercial plantations of rooted cuttings of tested clones of this species are therefore a possible means of realising genetic improvement. During the last 7 years in New Zealand efforts have been made to select, propagate, and test clones, and then to mass-produce cuttings of those clones which prove to be the best. This paper reports the progress that has been made in one programme of this type. Results from clonal tests are reported, and the problems and the prospects for future use of this method are evaluated.

HISTORY

Good results from rooting experiments with cuttings of radiata pine led to the initiation of a programme of phenotypic selection and clonal testing in 1966 (Thulin and Faulds, 1968). Two hundred and sixty trees were selected in two compartments in

the northern part of Kaingaroa Forest, aged 6 to 7 years from seed (5 to 6 years from planting, mean height about 7 m). Phentoypic selection was non-intensive with the straightest and most vigorous trees in each group of 30 being chosen.

The select trees were felled and as many cuttings as possible were set, clone by clone, in nursery beds at the Forest Research Institute in May 1966. They were not randomised or replicated. All cuttings were transplanted in the nursery, again without replication or randomisation, at the end of the first growing season, and were given vertical and horizontal root pruning in the second summer. When assessed at time of lifting in June 1968, 243 clones out of 260 had rooted and the number of plantable ramets varied from 6 to 38 per clone. Clones with less than 12 plantable ramets were discarded for testing purposes, leaving 216 clones available for establishing a clonal test at two sites in June 1968. The field design was a randomised block with 2 to 5 single-tree plots per clone in each block, and three blocks totalling 726 trees each were planted at each site. Two seedling control lots were also included, with thirty or more of each lot in each block, individually randomised with the cuttings. Spacing between trees was 2.7 × 2.7 m.

Cuttings were collected in 1971 from the upper crown of ramets of most clones in the 1968-planted test (aged 5 years from setting) and six ramets from 51 clones were planted as single tree plots in a randomised complete block design during June 1972.

Long-term preservation of most of the original 216 clones has been effected by repropagation from the original R_1 ramets planted in 1968. Cuttings from these were set at ages 4 years, 5 years and 7 years from setting of the R_1 ramets. These cuttings have been used to establish rows of ramets which are being pruned to develop hedges. These hedges will, it is hoped, fulfil the dual role of maintaining juvenility and rooting ability (Libby *et al.,* 1972) and of forming a source of large quantities of easily collected cuttings.

ASSESSMENT AND ANALYSIS OF 1968 CLONAL TEST (R_1 GENERATION)

Assessment was confined to one location, Compartment (Cpt) 1350 Kaingaroa Forest, as weed growth at the other site caused very erratic growth and survival. On the average there was a total of about 9 ramets per clone at the site assessed. Heights were measured at time of planning (referred to as "age 2 years" from setting) and at age 4 years. At age 6 years a complete assessment of growth and morphological characters was carried out. Characters measured and assessed were height, diameter at breast height (over bark), bole straightness (subjective scores, 1 being crooked, and 9 being straight), total number of branches on last two years' leader growth, diameter of largest branch in second and third annual height increments from time of planting, branch angle (from the stem) of these two branches, total number of branch clusters on stem, branching pattern (1-6 scale, 1 being uninodal, 6 being quadrinodal), and crown diameter.

The data on all characters were subjected to balanced analyses of variance and covariance, as a randomised complete block design with three replications. The analyses were made at the individual tree level on the 182 clones that had at least two trees per block, the first two trees listed being systematically chosen for analysis. From these analyses estimates of variance components and covariance components for all characters

were obtained which enabled clonal repeatability, clone mean repeatability and clonal and phenotypic correlations between characters to be calculated.

Volume was not included in the analysis of variance and covariance but clone mean volumes were estimated roughly from clone mean heights and diameters as

$$3.142 \times \left(\frac{\text{Diameter}}{2}\right)^2 \times \frac{1}{3} \times \text{Height.}$$

Clonal repeatability, clone mean repeatability, phenotypic and clonal correlations were calculated as follows, assuming nine ramets per clone and not 6 as in the analysis of variance:

$$\text{Clonal repeatability} = \frac{\sigma^2 c}{\sigma^2 c + \sigma^2 bc + \sigma^2 w}$$

$$\text{Repeatability of clonal means} = \frac{\sigma^2 c}{\sigma^2 c + \sigma^2 bc/3 + \sigma^2 w/9}$$

Clonal correlation between characters x and y

$$= \frac{\sigma c_{xy}}{\sqrt{\sigma^2 c_x \cdot \sigma^2 c_y}}$$

Phenotypic correlation between characters x and y

$$= \frac{\sigma c_{xy} + \sigma bc_{xy} + \sigma w_{xy}}{\sqrt{(\sigma^2 c_x + \sigma^2 bc_x + \sigma^2 w_x) \cdot (\sigma^2 c_y + \sigma^2 bc_y + \sigma^2 w_y)}}$$

where $\sigma^2 c$ = component of variance between clones
$\sigma^2 bc$ = component of variance due to interaction of clones and blocks
$\sigma^2 w$ = component of variance within blocks, within clones
σc_{xy} = component of covariance between clones, between characters characters x and y
etc.

Selection differentials were calculated for clonal selection for single characters. The overall mean of the 216 clones was subtracted from the mean of the best 22 for each character and this differential was expressed as a percentage of the overall mean. Predicted gains from this clonal mean selection were calculated as (*actual selection differential*) × (*heritability of clone means*) assuming 9 ramets per clone.

RESULTS OF 1968 CLONAL TEST (R_1 GENERATION)

Highly significant differences ($P < 0.01$) between clones and also between replications were shown in the analysis of variance for all characters but the interaction of clones and replications was in all cases not significant ($P > 0.05$). The relative percentages of the total variance for each component in each character are shown in Table 1, with the clonal repeatability and repeatability of clone means. Although statistically significant the amount of variation accounted for by blocking was small and most of the variation

TABLE 1 - Variance components and clonal repeatabilities

Character	Components of variance(1)					Components as percent of total				Clonal repeatability	Repeatability of clone means (9 ramets/clone)
	σ_c^2	σ_r^2	σ_{rc}^2	σ_w^2	Total	σ_c^2	σ_r^2	σ_{rc}^2	σ_w^2		
Height age 2 yrs (dm)	0.46	0.01	0.13	0.79	1.38	33	1	9	57	.33	.78
Height age 4 yrs (dm)	2.64	0.33	0.06	9.32	12.35	21	3	1	75	.22	.71
Height age 6 yrs (dm)	22.49	1.56	1.54	32.49	38.08	39	3	3	55	.40	.85
DBHOB (mm)	80.1	5.6	0	149.2	234.9	34	2	0	64	.35	.83
Bole straightness (1-9)	0.87	0.02	0.24	2.23	3.37	26	1	7	66	.26	.73
Total branches (2 yrs)	15.65	9.27	0.47	38.86	64.25	24	14	1	61	.29	.78
Branching type (1-6)	0.38	0.01	0	0.61	1.00	38	1	0	61	.38	.85
Branch diameter (mm)	7.40	0.15	0	17.74	25.28	29	1	0	70	.29	.79
Branch angle (deg.)	10.73	0.21	1.23	32.93	45.10	23	1	3	73	.24	.73
No.of branch clusters	1.74	0.18	0	2.56	4.48	39	4	0	57	.41	.86
Crown diameter (dm)	2.96	0.14	0	4.65	7.75	38	2	0	60	.39	.85

(1) σ_c^2 = variance due to differences between clones

σ_r^2 = variance due to differences between replications

σ_{rc}^2 = variance due to interaction of replications and clones

σ_w^2 = variance between trees within plots

was found between clones and between ramets of a clone within a block. Clonal repeatability for different characters varied from 0.22 for height at age 4 years to 0.41 for total number of branch clusters. The somewhat surprising feature of these results was the considerable amount of within-clone variation, amounting to from 61 to 79% of the total.

Repeatabilities of clone means, assuming 9 ramets per clone, were naturally much higher than those based on individual tree values, and ranged from 0.71 for height at age 4 to 0.86 for number of branch clusters.

The ranges of variation about the overall means for different characters are shown in Table 2 as well as the mean of the two seedling controls. Also shown in this Table are the standard errors of the difference between two clonal means assuming 9 ramets per clone. For instance, to discriminate between clones for height at age 6 measuring 9 ramets allows detection of differences between clone means exceeding 5.8 dm, this being twice the standard error of the difference. It is probable that more precise discrimination may be required and this will entail planting more ramets of each clone.

TABLE 2—Variation among clones and differences between clones and seedlings

Character	Clone means			Seedling means	S.E. (1) Difference between clone means
	Lowest	Highest	Mean		
Height age 2 yrs (dm)	2.5	6.5	4.6	2.8	0.51
Height age 4 yrs (dm)	9.3	20.1	15.0	12.7	1.45
Height age 6 yrs (dm)	35.8	63.9	51.8	45.6	2.87
DBHOB (mm)	45.1	106.0	79.9	72.8	5.76
Bole straightness	2.8	7.7	5.3	4.3	0.81
Total branches (2 yrs)	43.7	67.6	53.6	59.2	2.99
Branching type	2.0	6.0	4.8	3.7	0.37
Branch diameter (mm)	13.6	30.2	22.2	22.6	1.99
Branch angle (deg.)	50	76	61	56	2.85
No. of branch clusters	7.2	16.3	10.6	8.0	0.75
Crown diameter (dm)	7.1	18.2	11.8	12.0	1.02
Volume(2) (dm^3)	19.1	188.0	89.5	62.2	

(1) Standard error of difference between clone means, 9 ramets/clone

(2) Volume derived from clone mean height and DBH — not analysed

The mean height of the seedlings (based on 235 trees) was considerably less at time of planting (age 2 years) than that of the cuttings (2.8 versus 4.6 dm), but by age 6 years the difference was relatively much smaller. If mean heights of all cuttings and of all seedlings at ages 2, 4 and 6 years are transformed to their logarithms and plotted

against age, as shown in Fig. 1, it is apparent that the rate of height growth of the cuttings is somewhat less than that of the seedlings. If the mean heights of the tallest 22 clones and the tallest 20 (10%) of the seedlings are similarly plotted, the growth of the tallest clones appears considerably slower.

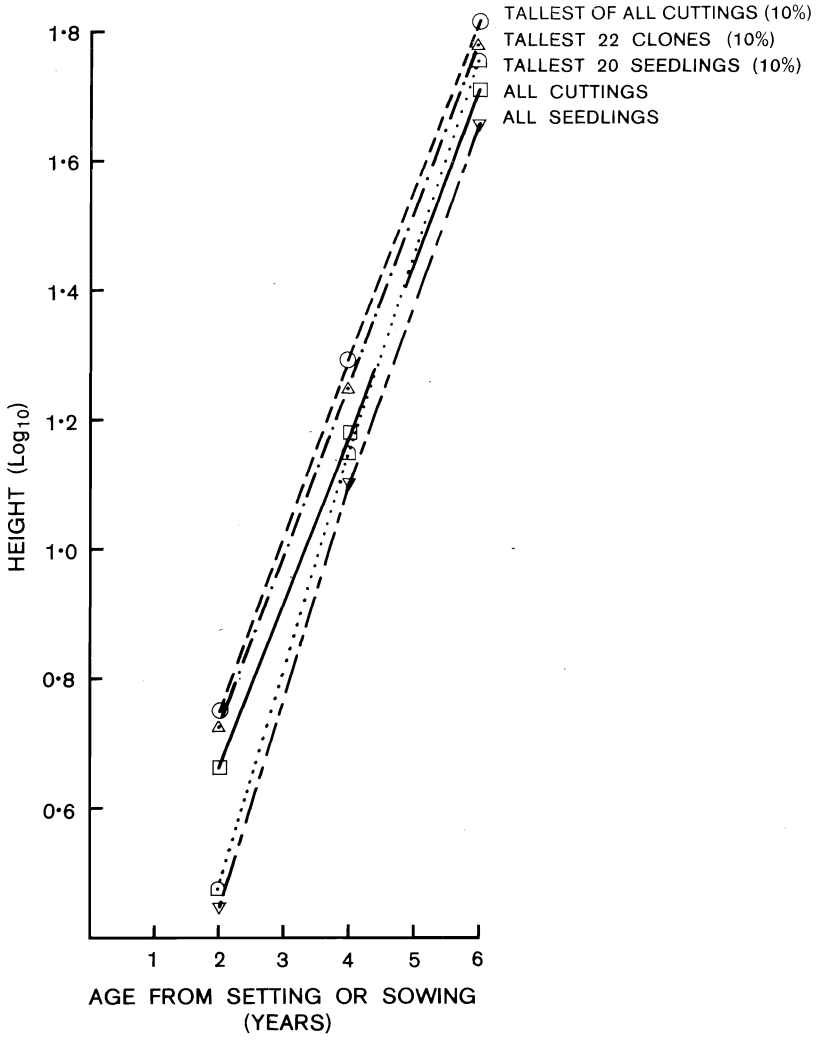

FIG. 1—Comparison of rate of height growth between seedlings and rooted cuttings.

Relative height growth rates were calculated according to the method discussed by Sweet and Wells (1974) for the two periods 2 to 4 years, and 4 to 6 years, from setting as $RHGR = \frac{Log_e\ H_2 - Log_e\ H_1}{t_2 - t_1}$ where H_1 is the height at time t_1 and H_2 is

the height at time t_2. Relative height growth rates were computed for the following types of material:

All cuttings
All seedlings
Tallest 10% of cuttings at age 6 years
Tallest 10% of seedlings at age 6 years
Tallest 22 clones (out of 216) at age 6 years

Predicted heights at age 6 years were also calculated for those types of material assuming a common height at time of planting of 40 dm, by solving the equation above for H_2 after calculating RHGRs for the two periods.

These data are given in Table 3. Seedlings show higher relative growth rates than cuttings and this is particularly marked in the period from 2 to 4 years from setting, i.e., in the first 2 years after planting. The RHGR values for seedlings and cuttings agree quite closely with those expected from interpolation of Sweet and Wells (1974) Fig. 3; their data derive from an experiment planted on the same site at the same time.

TABLE 3—Relative height growth rates in the 1968 R_1 generation test with predicted heights at age 6 years

Period	All cuttings	All seedlings	Tallest 10% of cuttings	Tallest 10% of clones	Tallest 10% of seedlings
Age 2 to 4 years dm/dm/yr	0.597	0.756	0.630	0.555	0.798
Age 4 to 6 years dm/dm/yr	0.619	0.639	0.593	0.603	0.687
Predicted height at age 6 (dm)	45.0	65.2	46.2	40.5	77.9

The predicted heights at age 6 years for the different types of material, assuming a common height at planting, are much greater for the seedlings than the corresponding groups of cuttings. The predicted height for all seedlings was 65 dm compared with 45 dm for all the cuttings, an increase of 45%. The tallest seedlings showed an even greater superiority in predicted height to the tallest cuttings or tallest clones.

In general the seedlings grew somewhat less straight than cuttings, with a larger total number of branches, fewer branch clusters produced per year and slightly steeper-angled branching. Branch diameter of the seedlings was the same as the cuttings but would be bigger if seedlings were of the same diameter.

A clonal test, as such, is intended to provide a precise means of selecting between clones. The selection differentials from selecting the best 22 clones are shown in Table 4. The predicted gains from this clonal selection are given by the selection differential × the heritability of clone means, and are shown also in Table 4. Predicted gains were likely to be of greatest economic importance in height and diameter and thus in volume. Variation between clones in mean volume per tree was extremely large (the smallest clone averaged 19.1 dm^3 and the biggest 188.0 dm^3 per tree), though sampling error

TABLE 4 - Selection differentials and predicted gains from selection of best 10% (22 clones) based on clone means - with resulting differentials in mean tree volume

Character	Overall clone mean	Mean of best 22 clones	Selection differential units	%	Predicted gain %	Correlated volume differential[2] dm^3	%
Height age 2 yrs (dm)	4.63	5.97	1.34	29	23	+27.7	+31
Height age 4 yrs (dm)	15.0	18.1	3.1	21	15	+35.8	+40
Height age 6 yrs (dm)	51.7	60.5	8.8	17	14	+44.3	+50
DBHOB (mm)	79.9	95.9	16.0	20	17	+50.5	+56
Volume (dm^3)	89.5	141.4	51.9	58	-		
Bole straightness (1-9)	5.3	7.1	1.8	34	25	+3.3	+4
Total branches(2 yrs)[1]	53.6	46.3	7.3	14	11	-7.0	-8
Branching type (1-6)[1]	4.8	5.8	1.0	21	18	+32.6	+36
Branch diameter (mm)	22.2	16.8	5.4	24	19	-35.4	-40
Branch angle (deg.)	61.4	68.4	7.0	11	8	-2.2	-2
No.of branch clusters[1]	10.5	13.2	2.7	26	22	+33.0	+37
Crown diameter (dm)	11.8	8.7	3.1	26	22	-33.7	-38

1) A tree with the lowest total number of branches but with multinodal branching (type 6) or large number of branch clusters was assumed to be the most desirable.

2) Calculated as the mean volume of the selected 22 clones (for their character) minus the mean volume of all clones.

in estimating clone means from their mean height and diameter is large also. The selection differential for volume from selecting the biggest 22 clones out of 216 was 58% of the overall mean. Predicted gains for height and diameter were 14% and 17% respectively but are not available for volume. Meaningful gains in straightness and number of branch clusters could also be made by a 10% clonal selection.

Also shown in Table 4 are the resulting selection differentials for volume from selecting for each of the other characters individually. Among the 22 clones which were tallest at age 2, the average estimated volume at age 6 was 31% above the overall mean—somewhat over half the differential obtained by selecting for volume *per se*. Selecting simply for diameter at age 6 was almost as effective as selecting directly for volume, giving a 56% differential in volume. This means that the time-consuming task of measuring tree height is unnecessary in selecting for volume, at least at this particular age. Selecting the best clones for bole straightness, total number of branches

and branch angle, has very little effect on their mean volume. However, selecting for a large number of branch clusters (the highly multinodal type of tree) resulted in a +36% volume differential, whereas selecting for small branch diameter or for narrow crowns showed a −40% volume differential. This means that branch diameter, crown diameter and growth rate are so closely inter-related that it will be very difficult to select narrow crowned, small branched, vigorous trees.

Clonal and phenotypic correlations are another means of describing the pattern of relationships between characters, and are shown in Table 5. Height at age 2 years shows strong clonal correlations with heights at 4 years and 6 years, as well as with diameter at age 6 years which is the main determinant of volume at that particular age. Stem diameter was strongly correlated with branch diameter and crown diameter (clonal correlations 0.785 and 0.743) and moderately correlated with branching type and number of branch clusters (0.225 and 0.465). This means that any selection for uninodal clones will tend to be at the expense of stem volume.

ASSESSMENT, ANALYSIS AND RESULTS FROM THE 1972 CLONAL TEST (R_2 GENERATION)

Cuttings were collected in 1971 from ramets of all clones in the 1968 test and six ramets from 51 clones were planted as single tree plots, in a randomised complete block design, during June 1972. The test site was in Cpt 905, Kaingaroa Forest. These cuttings were measured in September 1973 for height and diameter at half height. Volumes were estimated for individual plants as $3.142 \times (\text{diameter}/2)^2 \times \text{height}$. There were highly significant differences between clones for height, diameter and volume, and also significant block differences in volume. Clonal repeatability for height was estimated as 0.46 (compared with 0.33 in the 1968 test at the same age), 0.22 for diameter and 0.26 for volume, and repeatabilities of clone means were 0.84, 0.63 and 0.68 respectively. There was extreme variation between clones in volume; the biggest clone had a mean volume per tree of 1.19 dm^3 and the smallest averaged 0.16 dm^3, with an overall mean of 0.57dm^3. Selection differentials from selecting for single characters the best 20% (10 clones) in the test were 26% for height, 18% for diameter and 64% for volume, and predicted gains for this clonal selection, assuming 6 ramets/clone, were 22%, 11% and 44% respectively.

The average volume of these same 10 clones in the 1968 test at age 6 years was 103.1 dm^3, 12% above the mean (92.4 dm^3) of the 51 clones. In the 1968 test the average volume of the best 20% (44 clones) of clones was 45% above the overall mean, which was 89.3 dm^3. The identification of high-volume clones in the 1968 test therefore does not agree well with the early results from repropagation of these clones in the 1972 test.

These results should be viewed with some caution for several reasons: repeatabilities of clone means and thus precision of estimates are lower in the 1971 test than in the 1968 test, and there is the possibility that clone × site interraction is confounded in comparisons between the two sites. Also contributing to the lack of correspondence between results in the two tests are the effects of increasing maturation, interaction between clones and maturation, and the effects of different physiological and nutritional status between cuttings from the original ortet and from the R_1 ramets. These factors,

TABLE 5 - Phenotypic and clonal correlations between characters

	Height age 2 yr	Height age 4 yr	Height age 6 yr	DBHOB	Bole straight-ness	Total branches 2 yr	Branch-ing type	Branch diameter	Branch angle	No.of branch clusters	Crown diameter
Height age 2 yr	-	.844[1]	.667	.736	-.020	.091	.110	.672	.085	.310	.626
Height age 4 yr	.500	-	.847	.831	-.012	-.042	.214	.622	-.175	.399	.614
Height age 6 yr	.435	.768	-	.863	.009	.023	.269	.569	.022	.501	.643
DBHOB	.465	.770	.839	-	.151	.011	.225	.785	-.133	.465	.743
Bole straightness	-.007	.072	.131	.167	-	-.179	.194	.109	.046	.188	-.089
Total branches (2 yr)	.081	.057	.104	.100	-.025	-	-.324	.073	-.076	-.203	.183
Branching type	.098	.235	.312	.280	.150	-.149	-	-.117	.277	.867	.066
Branch diameter	.323	.477	.500	.607	.077	-.045	.043	-	-.465	.044	.751
Branch angle	-.024	-.048	-.024	-.085	.005	-.028	.107	-.264	-	.251	-.013
No.of branch clusters	.218	.349	.470	.427	.102	-.090	.577	.148	.101	-	.280
Crown diameter	.381	.525	.607	.665	.051	.086	.116	.546	-.013	.236	-

[1] Important correlations underlined.
The significance of various correlations is not readily tested and depends on the size of the correlation, the clonal repeatability of the two characters, and the precision of the estimates of clonal variance and covariance components (good, in this case, with 181 degrees of freedom).

however, are difficult to avoid in a clonal selection programme involving repeated repropagation of selected clones.

For this group of 51 clones, correlation coefficients were computed between clonal means in the R_1 generation (1968 clonal test) and the means in the R_2 generation (1972 clonal test).

Independent Variable X	Dependent Variable Y	Correlation
Height R_1, age 2 years	Height R_1, age 6 years	0.62
Height R_1, age 2 years	Volume R_1, age 6 years	0.65
Height R_1, age 2 years	Height R_2, age 2 years	0.41
Volume R_1, age 6 years	Volume R_2, age 2 years	0.20
Volume R_2, age 2 years	Height R_2, age 2 years	0.90
Volume R_1, age 6 years	Height R_2, age 2 years	0.11

Minimum r significant at P.05 level with 50 degrees of freedom = 0.27.

From these correlations between clone means, it appears that although heights in R_1 and R_2 generations at age 2 show some relationship, volume in R_1 at age 6 years shows a very weak relationship with volume at age 2 years in the R_2 generation ($r = 0.20$). Unfortunately no volume data are available for the R_1 generation at age 2 years. The low correlations in part reflect high within-clone variance and thus errors in estimation of clone means in both experiments, possible clone x site interaction as well as the ageing effects discussed above.

IMPLICATIONS OF RESULTS FOR THE CLONAL SELECTION PROGRAMME

The large variation between clones in most characters, particularly growth rate, together with the high repeatabilities of clone means shown in the R_1 generation indicated that a selection of the best clones would result in substantial gains in most characters over the mean of the clones in the test.

However, although similarly large variation between clones in height, diameter and volume was shown in the 1972 clonal test, the correspondence between the growth of clones in this test at age 2 years and that of the same clones in the 1968 test appears poor, although the 1972 test is still too young for the comparisons to be made with much confidence.

It is probable that the massive clonal variation in volume is not entirely genetic but contains a large non-genetic component which includes effects of the common physiological and nutritional status of the original ortets or "parent" ramets, as well as a common environment in the nursery beds, as clones were set without spatial replication. Some of these non-genetic clonal effects may differ in the two ramet generations and would contribute to differences in performance in the two tests.

Of particular concern is the effect that maturation of a clone may have on growth rate. It is evident from the growth rates of seedling and cutting material in the 1968 test (Fig. 1 and Table 3), that propagules from ortets aged 5 years are showing slower growth rates than seedlings. In the case of the 1972 test the clones were a further 5 years older from seed by the time they were repropagated, and interaction between clones and the expected decrease in growth rate caused by maturation (Sweet and Wells, 1974) might well account for the poor correlation between their performance at first and second

repropagation. This correlation could also have been depressed by clone x site interaction, as different sites were used for the two tests. No seedlings were included in the 1972 test so it is not possible to detect any further decrease in growth rate with maturation.

In an applied selection programme it is essential that select clones retain their superiority through repeated repropagations otherwise the viability of the whole scheme becomes questionable. It has been suggested that a possible way of maintaining high repeatability of performance in successive repropagations would be to set up repropagation hedges of all clones, and test cuttings from these. Cuttings from such hedges maintain rootability but it has yet to be established conclusively that hedging maintains growth vigour of the clones unchanged. The comparisons in growth rate between seedlings and cuttings of the R_1 generation indicate that cuttings from ortets aged 6 years are showing some decrease in vigour compared with seedlings. Initial selection and propagation should therefore be made on younger material and repropagation to form hedges must be done without delay. If these problems can be solved, clonal selection and mass vegetative propagation could become a powerful means of improvement of radiata pine.

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