

THE CLONAL TEST: AN AID TO PROGENY TESTING
AND A WAY TO SPEED UP GENETIC GAINS

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ABSTRACT.--Phenotypic measurements of loblolly pine (Pinus taeda L.) and Virginia pine (Pinus virginiana Mill.) were obtained from parent trees in wild stands, from ramets in clonal orchards, and from seedlings in control-pollinated progeny tests. Total tree height, stem diameter, crown form, bole straightness, and wood density were the characteristics assessed. The degree of resemblance between ortets and ramets, ramets and progeny, and parent trees and progeny was determined by estimating variances and heritabilities. Broad- and narrow-sense heritabilities were estimated from clonal and progeny populations. Ortet-clone, ortet-seedling, and clone-seedling regression and correlation analyses were used to measure the likeness between parent and progeny. These analyses revealed greater similarities between progeny and ramets than between progeny and mature parent trees in the forest indicating the possibility of improving the efficiency of selection through clonal testing. Calculations of expected genetic gains confirmed the importance of roguing inferior clones from the seed orchards as a step toward maximizing gain in a single generation. Clonal tests that can be converted into seed production orchards are recommended as aids to the more standard and expensive progeny tests and to speed up genetic gains both for Virginia and loblolly pines.

In Canada 300 million seedlings are produced annually in forest nurseries. With such a large program, even small genetic improvements in the planting stock will result in appreciable returns at time of harvest.

Selection of elite trees is the basis of a forest tree breeding program and much effort has been expended on this activity during the

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past 15 years. Tree breeders now recognize a large amount of variation in growth, form, and wood characteristics within most tree species. How much of this variation is caused by the genetic make-up of the tree and how much represents a response to the environment is a function of the degree of inheritance; this is usually expressed through the concept of heritability.

The demand for genetically improved trees is great, but the time and costs involved in setting up a tree improvement program to produce large quantities of genetically improved seed are important. The task involves tree selection, seed orchard establishment and maintenance, progeny testing, plus many more activities such as disease and insect control. In order to progeny test selected clones to determine their breeding value, a "tester" system is often adopted. However, despite the fact that clonal orchards produce seed early, it is difficult to obtain sufficient seeds of the crosses needed. The result is that after 5 or more years of effort, seeds of only a portion of the planned crosses may be available for the test. Because any delay in an applied program costs money, evaluations of the general combining ability of the clones must be based on an insufficient number of crosses, or tests must be delayed until all crosses are available. Therefore, a method that could reduce the costly procedure of progeny testing would be of great value to tree improvement programs.

Faster testing may be possible through the use of the resemblance between ortets^{2/} and ramets,^{3/} between ortets and seedling progeny or between ramets and progeny. A greater similarity between clones^{4/} and seedling progeny than between ortets and seedling progeny would suggest the possibility of selection, using a clonal test in a clonal seed orchard or a clone bank; this would be more effective than selection based upon the parent tree in the forest. Clonal testing would be of value for preselection of suitable material for use in the breeding program and could reduce the cost of the very expensive progeny tests.

This paper reports the results of an investigation of the resemblances between ortets and grafts, grafts and seedling progeny, and between ortets and progeny of loblolly pine (Pinus taeda L.) and Virginia pine (Pinus virginiana Mill.) and suggests a technique to speed up genetic gains.

^{2/} The original plant from which a clone has been derived.
^{3/} An individual member of a clone.
^{4/} A group of genetically identical plants derived asexually from a single individual.

MATERIAL AND METHODS

Plant Material

Three distinct groups of genetically related material were used in the study: parent trees, grafts of the parents, and control-pollinated progeny. The loblolly pine population consisted of 20 mature trees growing in wild forest stands, grafted clones of first and second propagation, and 57 control-pollinated families of the clones. Loblolly pine ortet ages were between 29 and 75 years and averaged 52 years.

The Virginia pine population was composed of 21 parents, their 10-^{5/} year-old grafts, and 105 progeny families obtained from the diallel mating of the clones. The complete failure of certain crosses or an insufficient number of seeds produced resulted in the reduced number of families available. In addition, the selfs were excluded from the study. The Virginia pine ortets averaged 23 years old, their age varied between 17 and 36 years. For both species progenies were 4 years old at the time of measurement.

Vigorous scions were collected in the lower portion of the ortet crowns and pot grafted on 2-year-old rootstocks. The first propagation clones were 9-years old at the time of the second propagation.

Measurements

Measurements of stem diameter were taken to the nearest 0.1 inch at breast height on the ortets, at 2.5 feet above the scion-rootstock union on grafts, and at 2.5 feet above the root collar on the seedlings.

Total heights of both parents and grafts were determined to the nearest foot using a Blumes-Leiss altimeter. The progeny heights were measured with a graded height pole.

Crown form and stem straightness were subjectively evaluated using a scoring system based on crown radius, crown density, uniformity, dominance of the main leader, and branch characteristics such as branch diameter and length, branch angle, and sinuosity. Trees with the best crown scored 6 and trees with the poorest scored 1. Subjective stem grading was similar using a 1 to 6 scale depending on the trees' stem straightness. In all instances measurements were taken on 10 ramets per clone and on a maximum of 60 seedlings per full-sib family.

Unextracted wood specific gravity was determined for wood samples obtained at breast height from each ortet using a 3/8-inch increment borer. In the orchards, three grafts per clone were chosen at random

^{5/} Mating design resulting from the crossing of the parents in all possible combinations.

and a wood sample obtained at 2.5 feet above the graft union, also using the increment borer. In the progeny test, 2 trees per plot, for a maximum of 20 per full-sib family, were cut and a disk 2 inches thick was taken at 2.5 feet above the ground level.

Statistical Procedures

A general least squares analysis for a diallel experiment was done on the progeny population (Schaffer and Usanis 1969). A nested analysis of variance assuming a random model was used to separate the genetic and environmental sources of variation from the clonal population.

Narrow-sense heritabilities were estimated using the formula appropriate to the diallel mating:

$$h^2 = \frac{4\sigma_{gca}^2}{\sigma_{gca}^2 + \sigma_{sca}^2 + \sigma_b^2 + \sigma_\omega^2}$$

where h^2 = narrow sense heritability,

σ_{gca}^2 = variance due to difference in general combining ability,

σ_{sca}^2 = variance due to difference in specific combining ability,

σ_b^2 = plot to plot variance, and

σ_ω^2 = within plot variance.

Broad-sense heritability was calculated as the ratio of the among clone variance to the total variance and may be presented as follows:

$$H = \frac{\sigma_C^2}{\sigma_C^2 + \sigma_r^2}$$

where: H = broad sense heritability,

σ_C^2 = among clone variance, and

σ_r^2 = within clone variance.

Heritability estimates have also been calculated from the regression of progeny on ortet and on the grafted clone. The appropriate formula is:

$$b = \frac{\text{Cov (O-P)}}{\sigma_p^2} = \frac{1}{2} h^2$$

where: b = regression coefficient of progeny on parent,

Cov (O-P) = covariance of progeny mean on one parent,

σ_p^2 = phenotypic variance of parent, and

h^2 = narrow-sense heritability.

To assess the reliability of the heritability estimates, standard errors of the variance components were calculated using the equation given by Anderson and Bancroft (1952) and by Falconer (1960) (Corriveau 1974).

RESULTS

Nested analyses of variance of clonal populations revealed highly significant genetic differences among clones for all characteristics studied. Results obtained from first and second propagation grafts of loblolly pine indicated that cloning effects^{6/} (topophytic and cyclophytic effects) biased estimates of total genetic variance upward for growth but had little effect on variances in the cases of stem straightness and crown form (table 1). Differences among ramets of the same clones are less than among clones because they share the same genotype as well as a common nongenetic component caused by physiological and morphological properties of the mother tree. When scions or cuttings are taken from ramets adapted to different micro-environments, this common nongenetic component may be partially or totally lost. Two-stage cloning has been proposed by Libby and Jund (1962) to reduce cloning effects. Virginia pine clone height and diameter were negatively correlated with ortet age ($r = -0.62^{**}$ and -0.56^{**} , respectively), reflecting strong cyclophytic effects. Environmental components were found to be less affected and broad-sense heritability values were less biased due to the presence of the confounded cloning component in both the numerator and denominator of the variance ratios.

Mean clone repeatabilities were moderate to strong. Due to large environmental variances, broad-sense heritabilities were smaller than were the repeatabilities (table 2).

^{6/} Combined effects of the scion position within the crown and of ortet age on the vegetative and reproductive development of the ramet.

Table 1.--Genotypic and environmental variance estimates of loblolly and Virginia pine height , diameter, stem straightness, and crown form from grafted clones 1/

Characteristic:	Among clones (σ_C^2) 2/			Within clones (σ_R^2) 3/		
	Loblolly		Virginia	Loblolly		Virginia
	First propagation	Second propagation	First propagation	First propagation	Second propagation	First propagation
Height	4.631±1.772	1.108±.653	4.782±2.263	9.638	9.333	26.705
Diameter	.795± .324	.494±.486	.091± .470	2.263	10.348	.649
Crown form	.426± .173	.496±.190	.376± .147	1.209	1.040	1.022
Straightness	.135± .038	.225±.102	.446± .165	.660	.954	1.010

1/ Variances are based on 10 ramets per clone.

2/ σ_C^2 = Among clone variance = total genetic variance plus a component due to cloning effects.

3/ σ_R^2 = Within clone variance = environmental variance.

Table 2.--Comparison of heritability estimates by variance components, regression, and correlation analysis

LOBLOLLY PINE								
Characteristic	1/		2/		3/		4/	
	Clonal population		Progeny population		Regression		Correlation	
	R_r	R_c	Family	Individual	Progeny- ortet	Progeny- clone	Ortet- progeny	Clone- progeny
Height	0.32	0.83	0.15	0.05	0.01	0.08	0.01	0.04
Diameter	.26	.78	--	--	--	--	--	--
Crown form	.26	.78	.23	.07	.02	.16	.01	.14
Straightness	.17	.67	.37	.13	.08	.25	.04	.04
VIRGINIA PINE								
M.A.H.B. ^{5/}	0.15	0.64	0.33	0.13	0.12	0.22	0.14	0.22
M.A.D.G.	.12	.58	.15	.06	.09	.12	.09	.08
Height	.15	.64	.33	.13	.04	.12	.01	.22
Diameter	.12	.58	.15	.06	.01	.04	.01	.15
Crown form	.27	.79	.43	.10	.04	.08	.09	.16
Straightness	.31	.82	.37	.10	.08	.06	.01	.12
Specific gravity	.77	.91	.47	.38	.53	.25	.24	.27

1/ R_r = repeatability of grafts of a clone or broad-sense heritability.

R_c = repeatability of a clone mean

2/ Family = family mean heritability, individual = individual heritability or narrow-sense heritability.

3/ Heritability estimates for loblolly pine are from the regression of seedling progeny on the female parent.

4/ Heritabilities obtained by the correlation of single parent and progeny and adjusted for the number of siblings per family (Franklin 1973).

5/ M.A.H.G. = mean annual height growth, M.A.D.G. = mean annual diameter growth.

In most instances, variance component analyses done on progeny data indicated statistically significant differences in general combining ability. Specific combining ability was an important source of genetic variation for growth characteristics while additive genetic variance was of prime importance in explaining genetic differences in crown form, straightness, and wood density. The same analyses also indicated that heritability values and efficiency of selection for advanced generations could be increased by the reduction of the environmental variance through careful planning of the field design.

Before progeny tests are available, clonal material is sometimes used to obtain estimates of heritability and to calculate expected genetic gains from selection and breeding. Results from analyses of clones and seedling progeny indicate that broad-sense heritabilities may be used with confidence as the upper limit for narrow-sense heritabilities. However, due to the variable importance of the first and second order genetic interaction from one characteristic to another, it would be unsafe to use a constant portion of the broad-sense heritabilities for any trait when calculating expected genetic gain. Too large or too small gains could be predicted leading to the acceptance or rejection of specific breeding programs. Narrow-sense heritabilities were 30 to 85 percent of the broad-sense heritabilities depending on the character involved (table 2).

Narrow-sense heritabilities calculated for growth and form characteristics from the regression of progeny on mature parent trees (table 2) were generally low and unreliable, reflecting the difficulty of predicting offspring juvenile performance on the basis of parental phenotypic measurement alone and vice versa. When growth of the parent trees are significantly correlated with age differences, the genotypic differences are partially or totally masked. Adjustments made to a common parental age were useful in revealing the presence of additive gene effects on the expression of the characters. The estimates obtained from the regression of seedling progeny on clonal parents were more precise and compared favorably to estimates obtained by variance analyses (table 2).

Correlations were also used to measure the degree of resemblance between ortet and graft, ortet and progeny, and between graft and progeny. Significant relations were found between mean annual growth of Virginia pine ortets and grafted clones ($r = 0.63^{**}$ for height and 0.52^{**} for diameter) and between clonal parents and seedling progeny ($r = 0.43^{**}$ for height and $r = 0.48^*$ for wood density). A significant correlation was also found between specific gravity of parent trees and their seedlings ($r = 0.45^*$). The correlation coefficient between parent and progeny is often preferred to the regression coefficient as an estimate of the heritability when large scale measurement differences exist between the relatives due to important age or environmental differences. Uncorrected heritabilities obtained by correlation

were larger than estimates obtained by regression and variance analyses. When adjustments were made for the number and degree of relation among members of a single family (Falconer 1960), the heritabilities were of the same magnitude as those calculated by regression analysis (table 2). However, correlation and regression methods can give exactly the same estimates only when the parent and progeny variances are equal.

DISCUSSION

Progeny testing is costly in time and effort. Even with a simple testing system, and despite the fact that clonal material reproduces early, complications still occur. Sometimes, after 5 or more years of effort, only a portion of the crosses necessary to obtain the required genetic information are available. An additional 5-year period is also required before reliable data can be obtained from the progeny tests for roguing. During the waiting period, seeds produced are improved by only one selection cycle. The longer the waiting period, the greater the proportional loss.

Greater similarities between progeny and clonal parent than between progeny and mature parent tree make possible the utilization of clonal testing as a tool to increase and speed up genetic gains through the roguing of seed orchards. Calculations have shown roguing^{7/} of the inferior clones in a seed orchard to be an important step toward maximizing gains in a single generation. Predicted genetic gains following two cycles of selection in loblolly and Virginia pine populations are presented in table 3. A 1 percent selection intensity was assumed for wild forest stands during the first cycle of selection. The second cycle represents the roguing of 50 or 70 percent of the clones in the seed orchard on the basis of either progeny testing or clonal testing. Results indicate that roguing of the loblolly pine orchard on the basis of the clones' own performance would result in substantially higher gains than roguing on the basis of the clones' 4-year-old progeny performance. More important gains are also predicted for Virginia pine height and diameter following roguing on the basis of clonal performance than on the basis of progeny performance.

Clonal testing has the advantage of not requiring time beyond the 5 or 6 years after clonal establishment. Clonal testing is a method of testing where the co-ancestry coefficient between parent and progeny (as well as among progeny of the same parent) is equal to one, and thus offers more reliable genetic information than any other type of relatives. However, clone performance includes effects due to cyclophysis and topophysis, which may give false information. To obtain

^{7/} Systematic removal of undesirable clones.

Table 3.--Expected genetic gains for growth and form characteristics and wood specific gravity of Virginia and loblolly pines from selection in wild stands followed by roguing of the clonal seed orchards on the basis of progeny testing and clonal testing

LOBLOLLY PINE															
	: Expected gain from : selection in natural : forest stands				: Expected gain from : roguing on the basis : of 4-year progeny : tests				: Expected gain from : roguing on the basis : of clonal : tests				: Unselec- : ted pop- : ulation : mean	: Total expected : gain from two : cycles of selec- : tion(percent)	
	$i_1^{1/}$	h_1^2	σ_p	G_1	$i_2^{2/}$	h_2^2	σ_f	G_2	$i_3^{2/}$	h_3^2	σ_c	G_3	\bar{X}	(G_1+G_2)	(G_1+G_3)
Height	2.66	0.01	6.12	1.9	0.80	0.15	0.726	2.0	0.80	0.08	3.74	3.5	8.73	3.9	5.4
					1.16	.15	.726	2.9	1.16	.08	3.74	8.0	8.73	4.8	9.9
M.A.H.G.	2.66	.07	0.31	2.6	.80	.15	.182	2.0	.80	.31	0.37	8.4	2.18	4.6	11.0
					1.16	.15	.182	2.9	1.16	.31	0.37	12.2	2.18	5.5	14.8
Straightness	2.66	.08	1.12	6.3	.80	.37	.276	4.3	.80	.25	1.26	13.3	3.78	10.6	19.6
					1.16	.37	.276	6.3	1.16	.25	1.26	19.3	3.78	12.6	25.6
Crown form	2.66	.02	1.03	1.6	.80	.23	.199	2.0	.80	.16	1.07	7.8	3.53	3.6	9.4
					1.16	.23	.199	3.0	1.16	.16	1.07	11.3	3.53	4.6	12.9
VIRGINIA PINE															
Height	2.66	0.04	5.24	8.1	0.80	0.33	0.426	3.3	0.80	0.12	2.30	6.5	6.85	11.4	14.6
					1.16	.33	.426	4.8	1.16	.12	2.30	9.4	6.85	12.9	17.5
Diameter	2.66	.01	1.14	2.4	.80	.15	.100	1.9	.80	.04	0.56	2.8	1.28	4.3	5.2
					1.16	.15	.100	2.7	1.16	.04	0.56	4.1	1.28	5.1	6.5
Straightness	2.66	.08	1.03	6.0	.80	.37	.224	3.6	.80	.06	1.09	2.9	3.66	9.6	8.9
					1.16	.37	.224	5.3	1.16	.06	1.09	4.1	3.66	11.3	10.1
Crown form	2.66	.04	1.02	2.6	.80	.43	.202	3.3	.80	.09	1.06	3.2	4.23	5.9	5.8
					1.16	.43	.202	4.8	1.16	.09	1.06	4.6	4.23	7.4	7.2
Wood specific gravity	2.66	.53	0.30	11.1	.80	.47	.015	3.0	.80	.25	0.30	3.1	.382	14.1	14.2
					1.16	.47	.015	4.3	1.16	.25	0.30	4.6	.382	15.4	15.6

Table 3 continued on next page

Table 3 (continued)

1/ i_1 = coefficient of selection intensity corresponding to a 1 percent intensity of selection in wild stands, i.e., $i_1 = 2.66$

2/ i_2 and i_3 = coefficients of selection intensity corresponding to the selection of 50 percent ($i_{(i)} = 0.80$) and 30 percent ($i_{(i)} = 1.16$) of the clones in the seed orchards.

h_1^2 = narrow-sense heritability estimated by regression of seedling progeny on parent trees,

h_2^2 = family mean heritability,

h_3^2 = narrow-sense heritability estimated by regression of seedling progeny on clonal parent,

σ_p = phenotypic variation of unselected natural loblolly pine population (Porterfield 1973) and phenotypic variation of 104 crop Virginia pine trees,

σ_c = phenotypic variation of clone means,

σ_f = phenotypic variation of half-sib family means,

$$G_1 \text{ percent} = (i_1 h_1^2 \sigma_p) \frac{100}{\bar{X}}, \quad G_2 \text{ percent} = 2(i_2 h_2^2 \sigma_f) \frac{100}{\bar{X}}, \quad G_3 \text{ percent} = 2(i_3 h_3^2 \sigma_c) \frac{100}{\bar{X}},$$

\bar{X} = means of the 4-year-old commercial check seedlings included in the progeny tests,

M.A.H.G. = mean annual height growth.

maximum gain and reliability, only trees of the same age class and growing in similar environmental conditions should be selected. In addition, scions should be collected at the same position within the crown of each tree.

On a clonal basis, a seed orchard could be rogued 5 years after establishment depending on its growth rate and precocity of seed production. From that time on, the seeds produced would be improved by two cycles of selection. In addition, the gain resulting from roguing the orchard can be increased by initially using a larger number of clones and closer spacing of the ramets. At 20-foot spacing, using the same number of ramets per clone, 100 clones require less area than 50 clones with 30-foot spacing between ramets. The difficulty of progeny testing large numbers of clones limits the number of parent trees that can be initially included in the seed orchard; this problem can be overcome if clonal tests are used for initial roguing.

A breeding program using clonal testing could be begun as follows. First, select 100 phenotypically superior trees in wild forest stands at a maximum intensity of selection. Second, establish the 100 clones in the orchard. If the final spacing required for maximum seed production is 30 feet, the initial spacing should be 16 feet between the ramets. Third, after 5 years, if the growth is rapid and the fructification is early, rogue 50 percent of the clones on the basis of their vegetative performance. As shown in table 3, the expected gain is twice as high as from roguing 50 percent of the clones on the basis of 4-year-old progeny performance for height in loblolly and Virginia pines and for stem straightness and crown form in loblolly pine. After the orchard is in production, control pollinate the clones left to provide seedlings for advanced generation selection. A modified diallel type of mating design would provide reliable information about the mode of gene action and would produce the maximum number of unrelated families. On the basis of progeny performance, inferior clones that escaped the initial roguing could be eliminated.

SUMMARY

An investigation of the inheritance of growth, form characteristics, and wood density of Virginia and loblolly pine was undertaken with the objective of determining the possibility of using vegetative clonal performance of parent trees selected from wild stands as a measure of their breeding value. Measurements were taken from three distinct but genetically related tree groups: parent trees, grafts of the parents, and control-pollinated progeny.

Analyses were made of total tree height, diameter, bole straightness, and crown form. Virginia pine wood density was also investigated.

Analyses of variance were performed on clones and seedling progenies, while regression and correlation analyses were used as measures of the degree of resemblance between related groups and to estimate heritabilities. Nested analyses of variance of clonal populations revealed highly significant genetic differences among clones for all characters. Variance component analyses done on progeny data indicated statistically significant differences in general combining ability. Narrow-sense heritability on an individual tree basis was estimated to be 0.30 to 0.05 for height, 0.07 for crown form and 0.09 to 0.13 for stem straightness of loblolly pine. Virginia pine narrow-sense heritabilities were as follows: height = 0.13, diameter = 0.06, straightness = 0.10, crown form = 0.20, and wood density = 0.38.

Narrow-sense heritabilities estimated by regression of seedling progeny on the mature parent trees were generally low and unreliable, reflecting the difficulty of predicting offspring juvenile performance on the basis of parental phenotypic measurement alone. Higher and more accurate estimates were obtained by regression of progeny on the clonal parent than by regression on the ortet. Heritabilities equal to 0.22, 0.12, 0.06, 0.08, and 0.25 were found for mean annual height growth, diameter, straightness, crown form, and specific gravity of Virginia pine.

Greater similarities between progeny and clonal parent than between progeny and mature parent tree make possible the use of clonal testing as a tool to increase and speed up genetic gains. Clonal testing of loblolly and Virginia pine is proposed as a supplement or substitute to progeny testing, especially for early roguing. A gain of 11 percent in mean annual height growth, 20 percent in straightness, and 9 percent in crown form are expected for loblolly pine following a selection intensity of 1 in 100 applied to wild forest stands plus roguing of 50 percent of the clones in a specially designed clonal test. Gains of 15 percent in height, 9 percent in straightness, 6 percent in crown form, and 14 percent in wood specific gravity are expected for Virginia pine after initial selection and roguing.

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