Distribution of Testing Effort in Cloned Genetic Tests

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Summary

Forest tree populations were simulated to investigate the effects of GxE (5%, 25%, or 50% of genetic variances), number of breeding clones (40 or 64), and selection strategy on the optimum distribution of effort between number of sites (1 to 6), individuals per family (8 to 156), and ramets per individual (1 to 6) under a fixed resources clonal testing and production scenario. Breeding and population gains, and reliabilities of estimates of population additive genetic variance were compared among all combinations of factors to determine the optimum ranges.

Optimal distribution of testing effort was similar for maximizing production and breeding population gains and for accurately estimating true population additive genetic variance, with 1 to 2 ramets distributed over 2 to 6 sites being in the optimal range. The optimal distribution was sensitive to levels of GxE.

More families tested resulted in decreased gains in the breeding populations (lower within-family selection intensity, less precise half- and full-sib mean estimates). Higher levels of GxE resulted in less gain under all scenarios except when testing occurred on 4 or more sites, in which case there were no significant differences. Accuracy of estimates of additive genetic variance was improved with less families (more individuals per family) and lower levels of GxE when tested on less than 4 sites.

Key words: clonal testing, genotype x environment interaction, variance estimation, production gain, breeding population gain.

Introduction

When faced with limited resources, as most tree improvement programs are today, optimizing efficiencies in genetic tests will necessarily result in tradeoffs among numbers of test sites, genetic entries, and individuals per entry. The choices can be optimized with respect to statistical and operational efficiency (Libby, 1987). Optimization of statistical efficiency can refer to either the reduction of the variation of an estimated mean to some desired level (Libby, 1987), or the adequacy of a genetic test in accurately and precisely estimating population genetic variance components.

Optimization of statistical efficiency in progeny tests, with the goal of maximizing genetic gain through accurate evaluation of family means, has been investigated by a number of authors (see Cotterill and James, 1984). Optimization has either resulted in a tradeoff between the number of genetic entries (i. e. selection intensity) and number of individuals per genetic entry (i. e. accuracy of evaluating entry means) (Robertson, 1957), or, if the number of families are fixed, the optimum number of individuals per family and how they are deployed (e. g. plot size and shape, number of replications, test design) in genetic tests (Wright and Freeland, 1960; Lee, 1983; Lambeth et al., 1983; Cotterill and James, 1984).

Cloning seedlings for genetic tests results in another level of tradeoffs (i. e. number of ramets per clone) assuming a fixed resources program. Optimum allocation of resources with respect to maximizing genetic gain when clonal replicates are employed has been shown to be sensitive to heritability and selection intensity (Shaw and Hood, 1985; Russell and Libby, 1986), and to ratio of additive to nonadditive genetic variability (Shaw and Hood, 1985).

Operational efficiency involves the monetary costs of tradeoffs, for example, the costs of adding an additional site versus increasing the number of plants tested at each site. Lindgren (1985) investigated the tradeoffs between number of test sites and individuals per genetic entry in terms of evaluating genetic entries. Lindgren's model illustrated that variations in economic parameters are as important as biological parameters in influencing the optimal distribution of effort.

The objective of this study was to determine the optimum distribution of effort to sites, clones per family, and ramets per clone at different levels of genotype by environment interaction (GxE) and number of breeding clones with respect to i) production population gains, ii) breeding population gains, and iii) estimates of population additive genetic variance, using computer simulated populations.

Previous clonal population simulation studies (Shaw and Hood, 1985; Russell and Libby, 1986) assumed either testing on only one site or no GxE, an assumption which realistically will not be met. Shaw and Hood (1985) investigated breeding population gains in a recurrent selection program only, while Russell and Libby (1986) simulated production population gains with mass clonal selection and no family structure. As well, no simulation studies in the forestry literature that we are aware of, have addressed the consequences of varying the numbers of families and individuals per family on the estimation of population additive genetic variance.

Application of Simulation Results

In a breeding program involving recurrent selection for additive gene effects, the use of vegetative propagules can result in production population gains over seed orchards (Matheson and Lindgren, 1985; Foster, 1986; Shelbourne, 1990; King and Johnson, in prep) through clonal selection, and breeding population gains (Shaw and Hood, 1985; Shelbourne, 1990) through more efficient within-family selection. In order to realize these potential gain increases, individuals within families must be cloned for field testing (Matheson and Lindgren, 1985). Consequently, these genetic tests can serve the dual purpose of providing selections for advanced generation breeding populations based on additive gene effects and/or providing information for selection of improved clones for production (i. e. clonal forestry).

The objectives of a genetic testing program influence experimental design of the test. A number of tree improvement programs are proposing or are using separate tests for ranking genetic entries and for within genetic entry selection (van Buijtenen and Lowe, 1979; King and

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Table 1. — Summary of model parameters.

1. Total Number of test plants
CONSTANT — 12,500.

2. Genetic variances: (derived from 6.)
CONSTANT

male	0.5
female	0.5
male*female	0.17
clone	1.50

3. Site variance:
CONSTANT — 5.58

4. Error variance:
CONSTANT — 5.58

5. GxE variance
VARIES

i) 5% of σ_M^2, σ_F^2, σ_C^2

male*site	0.025
female*site	0.025
clone*site	0.075

$h^2_{n.s.} = 0.239$

ii) 25% of σ_M^2, σ_F^2, σ_C^2

male*site	0.125
female*site	0.125
clone*site	0.375

$h^2_{n.s.} = 0.225$

iii) 50% of σ_M^2, σ_F^2, σ_C^2

male*site	0.25
female*site	0.25
clone*site	0.75

$h^2_{n.s.} = 0.21$

6. σ_A^2:σ_D^2 ratio — 2.0:0.67 (3:1)
CONSTANT

$\sigma_M^2 = .25\sigma_A^2 = 0.5$
$\sigma_F^2 = .25\sigma_A^2 = 0.5$
$\sigma_{MF}^2 = .25\sigma_D^2 = 0.17$
$\sigma_C^2 = .50\sigma_A^2 + .75\sigma_D^2 = 1.5$
total = 2.67

7. Number of clones in breeding population
VARIES

i) 40 (80 families; 5 SNIFS)

ii) 64 (128 families; 8 SNIFS)

8. Selection strategies
VARIES

i) Production: top 25 clones (max. 3 clones/full-sib)

ii) Breeding: successive selection (3 levels) with the following numbers of families and individuals selected:

selection strategy	half-sib	full-sib	clones/full-sib
SS1[1])	4/8	1/4	2/C[2])
SS2	4/8	2/4	1/C
SS3	2/8	2/4	2/C

1. SS=selection stratgey
2. C=no. of clones/full-sib

9. # of clones, ramets, and sites (see Table 1)
VARIES

ramets = 1 to 6*
sites = 1 to 6
clones = 12,500/(# ramets * # sites * # families)

* combinations of ramets and sites which produced less than 8 clones/family not used.

JOHNSON, in prep.), using either single-tree or noncontiguous plots for ranking families and large family blocks for selecting within families. An alternative method would be to clone individual seedlings, thus each genotype can be observed over a number of environments improving accuracy of within-family selections (SHAW and HOOD, 1986). The use of clonal replicates as proposed above would not be feasible if sublining was an integral component of the advanced generation breeding strategy (KING and JOHNSON, in prep).

Increased gains from clonal forestry over the seed orchard option is highly dependent upon gain per unit time (MATHESON and LINDGREN, 1985). Thus, a tree improvement strategy which involves clonal testing as a separate, add-on component of the mainline recurrent selection program, may not be feasible, especially if generation turnover is relatively rapid. A dual-purpose test design as described above enables clonal forestry to become an integral part of the mainstream breeding program, thus potentially increasing gains per unit time (FOSTER and SHAW, 1987; RUSSELL, in press).

Materials and Methods

Populations of trees were simulated according to fixed and varying factors using SAS (SAS Institute Inc, 1985a) (*Table 1*). Normal deviates were generated by a pseudo-random normal function with a mean of 0 and variances presented in *table 1*. Male, female, clone, site and error variances were kept constant, while GxE variance varied as a percentage of genetic variance. Total additive variance was 2.0 and dominance variance was 0.67. Since genetic variances were constant, ratio of additive: dominance genetic variance was also. Narrow-sense heritability increased slightly with lower GxE (*Table 1*).

Two levels of GxE were tested with all combinations: 25% and 50% of male, female, and clone variances for the respective interactions with site. Thus, male x site and female x site variances would be 0.25 · 0.5 for 25% GxE, and the clone x site variance would be 0.25 · 1.50 (*Table 1*). In addition, a GxE variance equalling 5% of genetic variance was simulated for a subsample of combinations. Values of male x site and female x site variances were similar to those of 12 year to 15 year growth measurements of coastal Douglas-fir (*Pseudotsuga menziesii*) in British Columbia (J. WOODS, B. C. Ministry of Forests, pers. comm.), and clone x site variances were based on a survey of appropriate clonal trials (St. CLAIR and KLEINSCHMIT, 1986; PARK and FOWLER, 1987; BENTZER et al., 1988). Larger values of GxE within a breeding zone would most likely indicate inappropriate zone delineation.

The total number of test plants was fixed at 12,500. Populations were simulated with 40 or 64 breeding clones. Simulated individuals were mated using 4x4 SNIFs (small nonoverlapping independent factorials). Thus, for 40 breeding clones, 5 SNIFs with a total of 80 families were generated; 8 SNIFs and 128 families were generated for 64 breeding clones. The above level of testing represents a reasonable annual level of breeding and testing for a relatively small-scale tree improvement program, and overall would involve 4 series of breeding and testing, resulting in a total of 50,000 test plants with 320 to 500 full-sib families.

For each number of breeding clones and level of GxE, varying numbers of ramets per clone and sites (1 to 6) were used to generate individual breeding values and

Table 2. — Number of ramets, sites and clones per family, and selection intensities for production population and for within family selection (2 clones/full-sib) for breeding population.

	a. SNIFS = 5 (80 families)							b. SNIFS = 8 (128 families)						
sites	ramets						sites	ramets						
	1	2	3	4	5	6		1	2	3	4	5	6	
	156	78	52	39	31	26		98	49	33	24	20	16	*1
1	3.170	2.962	2.834	2.740	2.665	2.603	1	3.170	2.962	2.834	2.740	2.665	2.603	*2
	2.491	2.232	2.068	1.946	1.844	1.763		2.500	2.044	1.872	1.726	1.638	1.525	*3
	78	39	26	20	16	13		49	24	16	12	10	8	
2	2.962	2.740	2.603	2.502	2.440	2.353	2	2.962	2.740	2.603	2.502	2.440	2.353	
	2.232	1.946	1.763	1.638	1.525	1.416		2.044	1.726	1.525	1.372	1.270	1.138	
	52	26	17	13	10	9		33	16	11	8			
3	2.834	2.603	2.459	2.353	2.255	2.208	3	2.834	2.603	2.459	2.353			
	2.068	1.763	1.556	1.416	1.270	1.209		1.872	1.525	1.324	1.138			
	39	20	13	10	8			24	12	8				
4	2.740	2.502	2.353	2.255	2.169		4	2.740	2.502	2.353				
	1.946	1.638	1.416	1.270	1.138			1.726	1.372	1.138				
	31	16	10	8				20	10					
5	2.665	2.440	2.255	2.169			5	2.502	2.440					
	1.844	1.525	1.270	1.138				1.638	1.270					
	26	13	9					16	8					
6	2.603	2.353	2.208				6	2.603	2.353					
	1.763	1.416	1.209					1.525	1.138					

*1 = number of clones/full-sib family;
*2 = production selection intensity;
*3 = within family selection intensity (2 clones/full-sib).

phenotypes. Larger numbers of ramets per clone have consistently been shown to be inefficient (SHAW and HOOD, 1985. RUSSELL and LIBBY, 1986) as well as more than 6 test sites in coastal British Columbia (J. WOODS, B. C. Ministry of Forests, pers. comm.). The number of clones per full-sib family was determined by:

$C = 12\,500/(F \times R \times S)$ where;

C = number of clones/full-sib family;
F = total number of full-sib families;
R = number of ramets per clone; and,
S = number of sites.

The total number of test plants fluctuated slightly reflecting rounding off of number of clones per full-sib family to the nearest whole number. Combinations of sites and ramets which resulted in less than 8 clones per family were not used for simulations. *Table 2* outlines the number of ramets, sites, and clones used to simulate populations for the two numbers of breeding clones. In order to minimize environmental covariances, it is assumed that noncontiguous or single-tree plots were used for testing.

Two populations were selected: production and advanced generation breeding. The production populations were chosen based on mass clonal selection, with the top 25 clones selected based on phenotypic values. If 4 series of testing were done, this would eventually result in 100 clones in production. A maximum of 3 selected clones per full-sib family was imposed. Little theoretical work has been done to address the stability: diversity issue for forest trees. However, studies have indicated that 100 clones with the level of coancestry control as imposed above, distributed among 4 production sets, is appropriate to maintain adequate levels of genetic diversity and to minimize risk (LIBBY, 1982; HUEHN, 1986; BENTZER *et al.*, 1990).

The advanced generation breeding population was chosen using sequential selection at 3 levels: half-sib, full-sib and within full-sib. If the same full-sib family was selected from 2 half-sibs, than an alternative full-sib family was selected from the half-sib family with the lower breeding value. This type of selection imposes coancestry control, and although it may not result in the largest gains compared to index selection (SHAW and HOOD, 1985[5]), the results of the simulation are easier to interpret and more informative. The total number of clones selected was equal to the starting number of breeding clones (i. e.

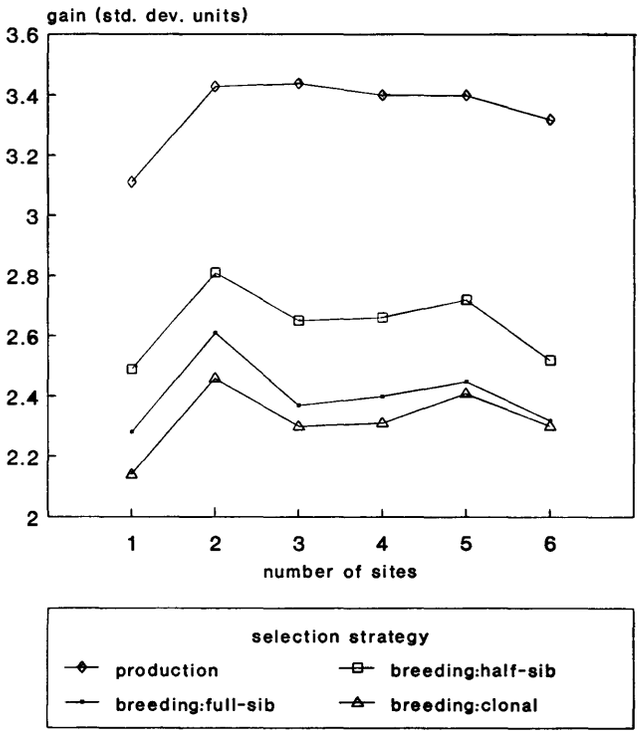

Figure 1. — Average genetic gain in standard deviation units for production population and 3 breeding populations with different selection strategies (GxE=25%, number of SNIF's=5, 2 ramets/clone).

Table 3. — Optimum numbers of sites and ramets, and predicted gain in phenotypic standard deviation units (in parenthesis) for 2 levels of GxE and 2 numbers of breeding clones for production population and for 3 different breeding population selection strategies.

optimum no. of sites, ramets (predicted gain)

% GxE	No. SNIF's	Production Population	Breeding Population SS1[1]	SS2	SS3
25	5	6,1 (3.45)[2]	2,2 (2.61)	6,1 (2.50)	2,2 (2.81)
		3,2 (3.44)	6,1 (2.59)	2,2 (2.46)	6,1 (2.78)
		2,2 (3.43)	3,1 (2.52)	5,2 (2.41)	3,1 (2.76)
25	8	5,2 (3.50)	4,1 (2.35)	4,1 (2.25)	4,1 (2.56)
		3,2 (3.48)	6,1 (2.33)	6,1 (2.22)	5,1 (2.54)
		6,1 (3.48)	5,1 (2.28)	5,1 (2.22)	2,2 (2,52)
50	5	5,1 (3.53)	5,1 (2.57)	5,1 (2.44)	5,1 (2.76)
		5,2 (3.42)	5,2 (2.54)	4,2 (2.42)	5,2 (2.73)
		4,2 (3.42)	4,2 (2.53)	5,2 (2.41)	4,2 (2.73)
50	8	6,1 (3.49)	4,1 (2.33)	6,1 (2.23)	4,1 (2.54)
		4,2 (3.43)	6,1 (2.32)	4,1 (2.21)	3,1 (2.53)
		3,2 (3.42)	3,1 (2.28)	3,1 (2.18)	6,1 (2.52)

[1]) see *Table 1* for explanation of selection strategies (SS1 to SS3)
[2]) top 3 optimum distributions of sites and ramets based on predicted gain

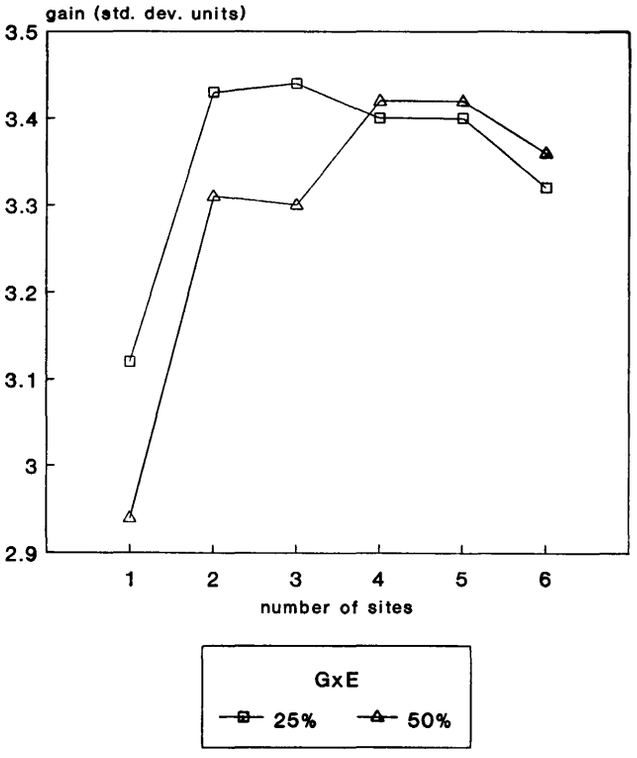

Figure 2. — Average genetic gain in standard deviation units for production population for two levels of GxE (number of SNIF's =5, 2 ramets/clone).

40 and 64). Selection was simulated within each SNIF according to 3 strategies involving different selection intensities at each stage (*Table 1*). *Table 2* outlines selection intensities under each simulation scenario for the production population and for selection within full-sib families (2 clones/full-sib family) for the breeding populations.

Gains were determined for each scenario by summing the breeding values of the selected clones for both the production and breeding population. Population additive genetic variance was estimated (true additive genetic variance = 2.0: see *Table 1*) for each scenario using SAS PROC VARCOMP, Method = Type 1 (SAS Institute Inc, 1985a), with all main effects considered random, such that:

$V_A = 2 (V_F + V_M)$, where

V_A = additive genetic variance;

V_F = female variance; and

V_M = male variance.

Each combination was repeated 24 times and average gains, additive genetic variances and standard errors were calculated.

The simulation model was based on the following assumptions:

1. only additive and dominance genetic variances are important for the traits that are being selected;
2. no variance is associated with cloning (i. e. no C-effects);
3. there is no significant male x female x site interaction; and,
4. there are no environmental covariances.

Results and Discussion

Production Population Gains

As expected, gains attributed to the production population were much greater than for any of the breeding population scenarios (*Figure 1, Table 3*). This is due to the combined effect of higher selection intensities (2.208 to 3.170) resulting from mass clonal selection, and less coancestry control among selected clones.

Gain was always greater with lower GxE for both numbers of breeding clones on 1 and 2 sites. Average gain over all numbers of ramets on 1 and 2 sites was 3.27, 3.23, and 3.12 for GxE levels of 5%, 25%, and 50%, respectively. However, predicted gain values were similar for all levels of GxE when clones were tested on 4 or more sites (*Figure 2*). Thus with higher levels of GxE, phenotypic selections are less reliable unless clones are tested on 4 or more sites.

Generally 1 to 2 ramets tested on each of 2 to 6 sites (total of 4 to 10 ramets per clone) optimized genetic gain. When GxE was small, gain optimization occured on less sites than with larger GxE. Considering the highest 3 gains (*Table 3*), the optimal distribution of effort was 2 ramets

Table 4. — Optimum number of sites and ramets for accuracy and precision of estimated additive genetic variance (true value = 2.0) for 2 levels of GxE and 2 numbers of breeding clones.

% GxE	No. of SNIF's	Accuracy			Precision		
		no. of sites	no. of ramets	estimated σ^2_A	no. of sites	no. of ramets	standard error of estimated σ^2_A
25	5	4	1	2.11[1]	3	4	.094[2]
		5	2	2.16	3	5	.099
		5	1	2.21	5	4	1.01
25	8	3	2	2.24	5	2	.08
		4	2	2.24	2	1	.084
		6	1	2.27	2	2	.086
50	5	3	1	2.17	6	1	.08
		5	3	2.17	5	3	.09
		4	2	2.23	4	2	.09
50	8	5	1	2.26	4	2	.071
		4	1	2.46	3	2	.084
		5	2	2.47	6	2	.087

[1]) top 3 optimum distributions of sites and ramets based on estimated σ^2_A

[2]) top 3 optimum distributions of sites and ramets based on standard error of estimated σ^2_A

on 2 to 5 sites or 1 ramet on 6 sites for 25% GxE (both levels of breeding clones) and 2 ramets on 3 to 5 sites or 1 ramet on 5 to 6 sites for 50% GxE. Two ramets on each of 3 sites resulted in highest gain among the limited number of combinations tested with 5% GxE.

Lowest gains were obtained when either all of the ramets were distributed on 1 site (GxE influence) or 1 ramet per site was tested on only 2 or 3 sites (poor estimates of clonal means). Low gains were also obtained when too many ramets were tested resulting in too few clones per family (< 9).

Breeding Population Gains

Increased gains were obtained for all ramet/site combinations and both numbers of breeding clones and levels of GxE when half-sib selection (selection strategy 3) was emphasized (2 out of 8 half-sibs selected versus 4 out of 8 per factorial) at the expense of selection intensity among full-sibs (selection strategy 2) and clones (selection strategy 1) (*Figure 1*). Estimates of half-sib breeding values are more reliable than full-sib or clone breeding values since more individuals are tested. SHAW and HOOD (1985) also found greater gains with more intensive half-sib selection. More intensive full-sib selection resulted in increased gains as compared to a selection strategy that emphasized within-family selection (*Figure 1*).

Within each selection strategy and level of GxE, gain was always higher with the lower number of breeding clones and consequent higher number of clones tested per family. This is due to greater selection intensity within full-sibs and more precise estimates of half- and full-sib breeding values. The total population size remained constant and number of selections equalled the original number of parents in each case. This provides insight into the tradeoffs between gain and breeding population size under a fixed resources model. The larger breeding population will allow lower coancestry levels and higher diversity for future generations, but at a cost in gain.

Within each selection strategy and number of breeding clones, less GxE resulted in greater gains only at the lower number of test sites, similar to the production population gains. Thus, the optimum number of test sites for a particular program depends upon the expected level of GxE, as discussed earlier.

Maximum gains occurred at similar combinations of ramets and sites as in the production population (*Table 3*). These results compare favourably to SHAW and HOOD (1985) when comparing similar model parameters on an individual site basis. The lowest gains, aside from 1 ramet tested on 1 site, occurred with small numbers of clones per family and large numbers of ramets. This results in poorer estimates of half- and full-sib family means. This is in contrast to production population gains where the lowest gains occurred with large numbers of clones and small numbers of ramets.

Estimations of Additive Genetic Variance Component

The estimated additive genetic variance was always over-estimated with the largest bias associated with greater GxE and low number of sites (*Table 4*). Only when GxE=5% did the estimates approach the true value.

Estimates of additive genetic variance were more accurate when 80 families were used (resulting in more clones per full-sib) than for 128 families (*Table 4*). Precision of estimation was improved by increasing the number of families tested.

Twenty-five % GxE resulted in more accurate estimates especially at low number of sites than 50% GxE. For those combinations tested with 5% GxE, accuracy was always better than with 25% or 50% GxE. The only estimate that could really be considered accurate was for 5% GxE with 1 ramet tested on each of 6 sites. Precision was better with lower GxE only at the low number of sites. It appeared that with a single site, the estimated additive variance included the GxE variance, and this effect was reduced with increased number of sites.

Accurate estimates of additive genetic variance occurred with similar distributions of effort as for optimizing production and breeding population gains (1 to 2 ramets tested on each of 3 to 5 sites). The most precise estimates, on the other hand, did not always correspond with the most accurate, and in most cases occurred with no apparent pattern (*Table 4*).

Conclusions

1. Overall, genetic gain ranked by selection strategy was: production population > breeding population (half-sib > full-sib > within-family).

2. Genetic gain was greater for lower levels of GxE only when clones were tested on 1 or 2 sites. Gain was similar among all levels of GxE when clones were tested on 4 or more sites.

3. Similar distributions of effort among sites, clones, and ramets were found for maximizing both production and breeding population gains, and for most accurately estimating additive genetic variance.

4. Optimal distribution of testing effort was sensitive to levels of GxE such that higher GxE variance shifted the distribution of effort by decreasing the total number of ramets per clone per site and increasing the number of sites.

In addition to the statistical considerations addressed by this study, the allocation of resources must be influenced by practical considerations such as expected survival rate and probability of site loss. We have also not considered the implications to the environmental design of using one or more ramets per site. For example, an incomplete block design may be necessary to optimize the allocation of effort, although analysis and interpretation of data is consequently more complicated than with a complete block design.

Literature Cited

BENTZER, B. G., FOSTER, G. S. and HELLBERG, A. R.: Impact of clone mixture composition on stability of 7th-year mean height in a series of Norway spruce clone tests. Can. J. For. Res. **20**: 757—763 (1990). — BENTZER, B. G., FOSTER, G. S., HELLBERG, A. R. and PODZORSKI, A. C.: Genotype x environment interaction in Norway spruce involving three levels of genetic control: seed source, clone mixture, and clone. Can. J. For. Res. **18**: 1172—1181 (1988). — COTTERILL, P. P. and JAMES, J. W.: Number of offspring and plot sizes required for progeny testing. Silvae Genetica **33**: 203—209 (1984). — FOSTER, G. S.: Making clonal forestry pay: breeding and selection for extreme genotypes. In: Proc. IUFRO Conf.: Joint Mtg. Working Parties on Breeding Theory, Progeny Testing, and Seed Orchards; Williamsburg, Va. (1986). — FOSTER, G. S. and SHAW, D. V.: A tree improvement program to develop clones of loblolly pine for reforestation. In: Proc 19th South. For. Tree Improve. Conf. pp. 17—21 (1987). — HUEHN, M.: Theoretical studies on the necessary number of components in mixtures. 3. Number of components and risk considerations. Theor. Appl. Genet. **72**: 211—218 (1986). — KING, J. N. and JOHNSON, G. R.: Computer simulations for seed production gain including clonal

selection options. (In prep). —— LAMBETH, C. C., GLADSTONE, W. T. and STONECYPHER, R. W.: Statistical efficiency of row and non-contiguous family plots in genetic tests of loblolly pine. Silvae Genetica 32: 24—28 (1983). —— LEE, C. H.: Statistical efficiency varies with plot size, number of replications and seedlots sampled. Silvae Genetica 32: 49—52 (1983). —— LIBBY, W. J.: What is a safe number of clones per plantation? In: HEYBROEK, H. M., STEPHAN, B. R. and VON WEISSENBERG, K. (Eds): Resistance to Diseases and Pests in Forests Trees. Proc. 3rd International Workshop on the Genetics of Host-Parasite Interactions in Forestry, Wageningen, the Netherlands, 14 to 21 September 1980. Centre for Agriculture Publishing and Documentations, Wageningen (1982). —— LIBBY, W. J.: Testing and deployment of genetically engineered trees. In: BONGA, J. M. and DURZAN, D. J.: Cell and Tissue Culture in Forestry. 2nd Ed. Martinus Nijhoff/Dr. W. Junk Publ., The Hague. Vol. 1, Chap. 11, pp 167—197 (1987). —— LINDGREN, D.: Cost-efficient number of test sites for ranking entries in field trials. Biometrics 41: 887—893 (1985). —— MATHESON, A. C. and LINDGREN, D.: Gains from the clonal and the clonal seed-orchard options compared for tree breeding programs. Theor. Appl. Genet. 71: 242—249 (1985). PARK, Y. S. and FOWLER, D. P.: Genetic variances among clonally propagated populations of tamarack and the implications for clonal forestry. Can. J. For. Res. 17: 1175—1180 (1987). —— ROBERTSON, A.: Optimum group size in progeny testing and family selection. Biometrics 13: 442—450 (1957). —— RUSSELL, J. H.: Clonal forestry in yellow-cedar. In: (Ed.) AHUJA, M. R. and LIBBY, W. J.: Clonal Forestry: Genetics, Biotechnology and Application. Springer-Verlag (in press). —— RUSSELL, J. H. and LIBBY, W. J. Clonal testing efficiency: the trade-offs between clones tested and ramets per clone. Can. J. For. Res. 16: 925—930 (1986). —— SAS Institute Inc.: Sas User's Guide. Cary, NC (1985). —— SHAW, D. V. and HOOD, J. V. Maximizing genetic gain per effort using clonal replications in genetic tests. Theor. Appl. Genet. 71: 392—399 (1985). —— SHELBOURNE, C. J. A.: Incorporating clonal forestry in the breeding strategy. In: Proc. Joint Meeting of W. F. G. A. and I. U. F. R. O. Working Parties S2.02-05, 06, 12, and 14. Aug. 20 to 24, 1990, Olympia, Wa. (1990). —— ST. CLAIR, J. B. and KLEINSCHMIT, J.: Genotype-environment interaction and stability in ten-year height growth of Norway spruce clones (*Picea abies* KARST.). Silvae Genetica 35: 177—186 (1986). —— VAN BUIJTENEN and LOWE: The use of breeding groups in advanced generation breeding. In: Proc. 15th Southern Forest Tree Improvement Conference, June 19 to 21. Mississippi State Univ. pp 59—65 (1979). —— WRIGHT, J. W. and FREELAND, F. D.: Plot size and experimental efficiency in forest genetics research. M. S. U. Agric. Exp. Stat. Tech Bull. No. 280. 27pp (1960).

Storage of Pollen of Norway Spruce and Different Pine Species

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Summary

The effect of 2 temperatures on pollen storage ability of the following conifers was investigated: *Picea abies* L., *Pinus nigra* ARNOLD, *Pinus pinea* L., *Pinus strobus* L., *Pinus sylvestris* L. and *Pinus uncinata* MIRB. Pollen was stored at —18° C and —196° C for 24 months and was assayed for viability every 2 months by means of *in vitro* germination tests, which are one of the most convenient methods for evaluating pollen reactivity to storage, although they may not necessarily provide the best indication of potential fertility.

The variability in germination response to the 2 storage temperatures was dependant on the species. At the end of the considered period, only the pollen germinability of *Picea abies* and *P. nigra*, in both storage conditions, did not show any decrease, while in the other species a decrease in pollen germination percentage was observed in freezer as well as in liquid nitrogen storage. The temperature of —196° C proved clearly to preserve the pollen better only in *P. uncinata*. In three of the species considered: *Picea abies*, *P. sylvestris* and *P. uncinata*, an increase in germination during the first months of storage at —196° C was observed.

Key words: Conifers, pollen storage.

Introduction

The preservation of viable pollen is very important for plant breeding. Successful pollen storage in the short term allows the hybridization of species which flower at different times or of populations which are separated geographically. The preservation of viable pollen for several years is one of the methods used for long term storage of plant germplasm. Pollen preservation for germplasm conservation is convenient, economical and space-saving. This is particularly evident for woody plants, which take several years to flower from the seedling stage.

The preservation of pollen viability presents problems which are quite similar to the problems with seeds. Optimal storage environments for pollen differ according to species, but common factors of importance are moisture content and storage temperature. Other factors, such as the composition and pressure of the gas phase around the pollen are known to affect longevity, but are rarely manipulated for optimum storage, except in storage under vacuum (TOWILL, 1985).

Unlike angiosperm pollen, whose reduction of moisture content below a certain level (20% to 30%) is usually fatal, in conifers the longevity of stored pollen increases with the decrease of its moisture content. High moisture content allows greater metabolic activity and also promotes the destructive activities of fungal and bacterial contaminants (MATTHEWS and KRAUS, 1981). For most conifers species pollen is best stored in a range of 10% to 20% moisture content and its viability is retained longer when a minimum of humidity fluctuation during the storage period is insured (HARRINGTON, 1970). Preservation at deep freeze temperatures (—18° C to —20° C) usually gives satisfactory results and is a common and practical way of storing pollen from one season to another, while pollen of several crop plants was successfully maintained in a viable state for prolonged periods in liquid nitrogen (FARMER and BARNET, 1974; NATH and ANDERSON, 1975; BARNABAS and RAJKI, 1976; TOWILL, 1981; TISSERAT *et al.*, 1983; COPES, 1985, 1987; GANESHAN, 1986). Nevertheless, the ideal storage conditions for short and long-term preservation of the pollen of many coniferous trees are not well-known and available data are approximate and incomplete.