Efficiency of Seedlings and Rooted Cuttings for Testing and Selection in *Pinus taeda*

Fikret Isik, Bailian Li, John Frampton, and Barry Goldfarb

**ABSTRACT.** Predicted genetic gains of polymix, cloned polymix, full-sib and cloned full-sib testing and selection options for a breeding population were estimated for *Pinus taeda* L. Heritabilities for volume from the clonal testing were considerably greater than the heritabilities from the seedling testing. Cloned options had higher expected genetic gains than seedling options, even after adjusting for their longer breeding cycles and higher cost of testing. This was mainly due to higher genetic gains from within-family selection of the cloned testing options. Adjusted genetic gain from within-family selection of polymix and cloned polymix were 0.27 and 0.36% per year, respectively. Increasing the number of ramets per clone did not increase the genetic gain, but gain was sensitive to the clone selection ratio within families. When the number of trees tested per family was fixed at 90 trees, all clone/ramet combinations gave greater within-family gains than the seedling option. A complementary breeding strategy consisting of polymix breeding for estimation of general combining ability and clonal selection from clonally replicated full-sib families appears to be more efficient for loblolly pine improvement than the currently used seedling-based testing system. For. Sci. 50(1):44–53.

**Key Words:** *Pinus taeda*, heritability, genetic gain, breeding strategies, clonal testing.

Currently, most forest tree improvement programs utilize seedling stock for testing and selection, even for relatively advanced cycles of improvement. With the progress of technology to vegetatively propagate forest trees, the potential benefits of clonally replicated genetic tests relative to seedling progeny tests need to be examined. In the case of loblolly pine (*Pinus taeda* L.), procedures for rooting stem cuttings at reasonable percentages have been developed (Goldfarb et al. 1997, Frampton et al. 1999) and could be used to clone individuals within families for evaluation and selection in genetic field trials. For this species, previous research has demonstrated that the growth of seedlings do not differ from that of rooted cuttings derived from juvenile stock plants, although seedlings have a significantly greater incidence of fusiform rust (caused by *Cronartium quercuum* [Berk.] Miyabe ex Shirai *f. sp. fusiforme* [Cumm.] Burds. *et* Snow) (Frampton et al. 2000). Further, similar family rankings between rooted cuttings and seedlings of loblolly pine have been reported. Correlations between seedling and rooted cutting full-sib family breeding values were 0.80 and 0.87 for height and fusiform rust incidence, respectively (Frampton et al. 2000).

In theory, vegetative propagules of a clone provide genetic information far more efficiently and precisely than a set of seedling progenies (Burdon and Shelbourne 1974). This is because, with clonal replication, micro-environmental variation is sampled for each genotype in a family. Smaller within-plot variances have been reported for clones than within-plot variances of seedlings of the same loblolly pine families tested at the same sites (Isik et al. 2003). With seedling
Rooted cuttings and full-sib seedlings of the same families were transplanted at two test sites, one in Florida and one in Alabama. There were six blocks at each test site. Each block was partitioned into two main plots in a split-plot design. Seedlings and rooted cuttings of the same families were the main plots. Each plot of seedlings was composed of two full-sib trees, whereas a plot of a clone had two ramets. Altogether, 74 clones representing nine full-sib families were used in the study. Tree height, diameter, and fusiform rust incidence were measured after six growing seasons.

**Statistical Analysis**

Seedlings and rooted cuttings were analyzed separately. Thus, the analyses of variance and covariance for volume and fusiform rust incidence were based on randomized complete block designs. Linear models and estimation of variance components were given by Isik et al. (2003). Fusiform rust incidence was observed as a binary variable (0 or 1) and had a binomial (η π) distribution where η is the conditional function g(μ) and μ is the conditional mean, π is the probability of a tree being not infected. In this study, a generalized linear mixed model was used to analyze fusiform rust incidence and estimate variance components.

\[ \eta = \log \left[ \frac{\pi}{(1 - \pi)} \right] = X\beta + Zu \]

where \( \beta \) is an unknown vector of fixed-effects (sites and replications within sites) parameters with known design matrix \( X \), \( u \) is vector of random-effects parameters (all other terms in the linear models) with known design matrix \( Z \). The random vector \( (u) \) was distributed normally with mean zero and had a variance-covariance matrix \( G = \text{Var}(u) \) (Littell et al. 1996). The genetic analysis was carried out using ASREML software, and the logit link function to transform the binary variable (Gilmour et al. 1999). Heritabilities and phenotypic variances were estimated for fusiform rust disease from the rooted cutting and seedling data sets. For volume, the variance components estimated by Isik et al. (2003) were utilized to estimate individual-tree, family, and within-family heritabilities at age six. The full formulae for calculating heritabilities, phenotypic variances and genetic gains are given in the Appendix. Standard errors of heritabilities were calculated using Taylor series approximations and applying the SAS IML Procedure (SAS Institute 1989).

The efficiency of seedlings and clones for testing and selection was compared by predicting genetic gains from four breeding options (Table 1). For simplicity, gain estimations were carried out for volume only. We assumed that the cost of testing for each option was largely dependent on the amount of time and number of trees planted into field tests. Thus, genetic gains from seedling and clonal testing options were adjusted for cycle length and for the number of trees tested per family (Table 1). For consistency, we assumed that all simulated breeding options had 200 mainline parents that were available for selection all at one time. These parent trees were assumed to have been selected from progeny tests and grafted into an archive or a seed orchard. We further assumed that all selections would reach sexual maturation and become ready for breeding in 5 yr. Seedling or clonally replicated...
Using a factorial mating design, each parent tree was mated to generate full-sib seedlings for progeny testing (Table 1). Genetic gain from selection among families was based on the clone mean within full-sib family heritability [Equation (14)] and gain from within half-sib family selection was based on the clone mean heritability within families [Equation (18)].

**Table 1. Breeding and testing options, cycle lengths, selection ratios and selection intensities used to estimate genetic gains for strategies based on seedling and clonal testing of loblolly pine.**

<table>
<thead>
<tr>
<th>Breeding and testing option</th>
<th>Cycle composition (yr)</th>
<th>Cycle length (yr)</th>
<th>No. of trees tested × 1000</th>
<th>Selection proportion</th>
<th>Selection intensity</th>
</tr>
</thead>
<tbody>
<tr>
<td>1. Polymix with seedling testing (PMX_S)</td>
<td>5+3+1+6</td>
<td>15</td>
<td>20</td>
<td>100:200</td>
<td>i₁ = 0.795</td>
</tr>
<tr>
<td>2. Polymix with clone testing (PMX_C)</td>
<td>5+3+1+2+6</td>
<td>17</td>
<td>80</td>
<td>100:200</td>
<td>i₁ = 0.795</td>
</tr>
<tr>
<td>3. Control-pollinated with seedling testing (CP_S)</td>
<td>5+3+1+6</td>
<td>15</td>
<td>20</td>
<td>100:200</td>
<td>i₁ = 0.795</td>
</tr>
<tr>
<td>4. Control-pollinated with clone testing (CP_C)</td>
<td>5+3+1+2+6</td>
<td>17</td>
<td>80</td>
<td>100:200</td>
<td>i₁ = 0.795</td>
</tr>
</tbody>
</table>

* Explanation of the cycle’s composition: Selection and grafting (5 yr), pollination and seed collection (3 yr), raising seedlings and planting (1 yr), cloning and rooted cutting production for clonal options (additional 2 yr), and progeny testing (6 yr).
† i₁ = Family selection and i₂ = within-family selection intensities.

Progeny trials of the breeding populations were set up at four test sites. The progeny tests (cuttings or seedlings) were measured 6 yr after planting for reliable early selection (McKeand and Bridgewater 1998). For all scenarios, we assumed that a selection ratio of 100:200 is practiced on the family level. Details of the breeding and testing scenarios are described below.

**Polymix Breeding with Seedling Testing (PMX_S)**

A polymix breeding strategy with seedling progeny tests was used as a baseline (Table 1). The parent trees in the archive were crossed with a pollen mix at yr 5. Half-sib seed was harvested at yr 8 allowing for 2 yr of pollinations. Progeny trials were planted at yr 9 using half-sib seedlings. Each family was represented by 25 seedlings per site and 100 seedlings over the four sites. Progeny trials were evaluated at yr 15 (age 6 of the progeny test). Half-sib families were ranked according to their breeding values. The best two trees from each of the top 100 families were selected to form the next cycle of the breeding population. Genetic gains were calculated on half-sib and within half-sib family selections [Equation (4)].

**Polymix Breeding with Clone Testing (PMX_C)**

Instead of using half-sib seedlings to test families, as in the PMX_S scenario, the polymix families were cloned and then tested in this scenario. For each family, 25 randomly selected seedlings were cloned, and 16 ramets were produced for each family (Table 1). Cloning 25 individuals from each of 200 families was estimated to require an additional 2 yr to produce enough rooted cuttings for testing compared to the PMX_S option. Each family was represented by 100 trees per site (25 clones/4 ramets per site), which was four times the number of trees per family as the PMX_S scenario. When genetic tests were evaluated, the best 2 of 25 clones from each of the top 100 families were selected to form the next breeding population. Genetic gain from selection among families was based on the half-sib family heritability [Equation (14)] and gain from within half-sib family selection was based on the clone mean heritability within families [Equation (18)].

**Control-Pollinated Breeding with Seedling Testing (CP_S)**

Another seedling-based scenario used control-pollination to generate full-sib seedlings for progeny testing (Table 1). Using a factorial mating design, each parent tree was mated to two other parents to generate 200 full-sib families. Control-pollinated seed was harvested at yr 8, allowing 2 yr for all pollinations. Full-sib seedlings were raised and planted at yr 9. For each cross (full-sib family) was represented by 25 trees at each test site. Progeny trials were evaluated at yr 15, and full-sib families were ranked according to the performance of their progeny. The best 100 full-sib families and the best two trees from each family were selected to form the breeding population for the next cycle. Selection intensities among and within families were the same as with the PMX_S option. Genetic gain was calculated based on full-sib family and within full-sib family selection [Equation (20)].

**Control-Pollinated Breeding with Clone Testing (CP_C)**

In this scenario, control-pollinated families were generated by the factorial mating designs as in the CP_S breeding option, but were then cloned and tested in the field (Table 1). Seed was harvested at yr 8, and full-sib seedlings were raised at yr 9. For each of the 200 full-sib families, 25 randomly selected seedlings were cloned, and 16 ramets were produced for each clone. Cloning and production of ramets was assumed to take an additional 2 yr. Cloned families were transferred to the field tests at year 11. The total number of trees tested was four times that of the CP_S breeding option. Families were ranked for their breeding values at yr 17. The best 100 of 200 full-sib families and the best two of 25 clones in these families are selected for the next generation. Full-sib family and clone mean within full-sib family heritabilities were used to estimate genetic gains [Equation (25)].

**Within-Family Selection Scenarios**

In addition to comparing seedling and clonal testing scenarios, genetic gains from different numbers of clone and ramet combinations were explored for the PMX_C and CP_C options. A simulation was carried out to determine the optimal number of ramets per clone for within-family gain estimation. In this scenario, within-family clone selection ratio was constant (2 out of 25), and number of ramets per clone increased by 2 from 4 to 14. Genetic gain for each option was adjusted by the cycle length (17 yr) and by the numbers of trees per family (number of ramets × 25 clones × 4 sites).

In the second within-family selection scenario, the number of ramets per clone was kept constant at 16, and clonal...
selection intensity increased from 7:25 to 2:25. The number of trees per family was the same (25 clones × 4 ramets × 4 sites = 400) for all within-family clonal selection intensity scenarios. Genetic gains for within-family selections were divided by the cycle length (17 yr) and by 4 to adjust for the requirement for planting more trees (×4) compared to the seedling testing option.

In the third within-family selection scenario, the number of trees per family that were planted in progeny tests was kept at about 400, but clone-ramet combinations varied. Within-family genetic gain was divided by the cycle length (17 yr) and by 4 to adjust for longer breeding cycle and for testing more trees per family. These adjustments make genetic gains from within-family selection comparable to seedling testing options, because in the seedling options, each family was represented by 100 trees (25 seedlings × 4 sites).

Results
Phenotypic Variances and Heritabilities

Half-sib and full-sib family phenotypic variances estimated from clones (rooted cuttings) were greater than the family phenotypic variances with seedlings (Table 2). For example, the half-sib family phenotypic variances from the clonal and seedling data for volume were 0.1133 and 0.0439, respectively. Conversely, within-family variances for clonal data were smaller than the within-family variances for seedlings. Greater family variances for the clones were also observed for fusiform rust incidence. Within-half-sib family variance for fusiform rust incidence in clones was greater than the within-half-sib family variance in seedlings. This was reverse for within-full-sib family variances for rust incidence in clones and in seedling.

A considerable proportion of the family and within-family phenotypic variances for volume estimated from clones was due to genetics. In contrast, environmental factors had larger effects on phenotypic values of family and within-family values when estimated from seedling tests. Family and within-family heritabilities for volume from clones were considerably greater than those based on seedlings (Table 2). Heritabilities of clonal means within half-sib and full-sib families were 0.35 and 0.70, respectively, whereas seedling-based within-half-sib (0.05) and within-full-sib family (0.03) heritabilities were low. Similar differences were also observed for individual-tree heritabilities from the clone and seedling data for volume (Table 2). For fusiform rust incidence, family heritabilities between seedlings and clones did not differ as much as they did for volume. Although the within-half-sib family heritability of clones (0.67) and seedlings (0.64) for fusiform rust incidence were similar, there was a considerable difference between the clonal (0.84) and seedling (0.55) within-full-sib family heritabilities. In contrast to volume, seedling individual-tree heritability for fusiform rust incidence was high (0.62).

Seedling and Clonal Testing

Clone-based testing and selection options yielded more expected volume gain than the seedling testing options (Figure 1a). Total expected genetic gains for volume from the clonal testing options (PMX_C, CP_C) were more than five times that of the seedling testing option (PMX_S, CP_S). Large percentages of the total gains for the clonal testing options were from within-family selection. For example, genetic gains from family and within-family selection of PMX_C were 6.8 and 24.3%, respectively. Similarly, gain from within-family selection of CP_C was more than two times that of family selection. On the other hand, gains from within-family selection of PMX and CP with seedling testing were less than one-sixth of the within-family selection of the clonal testing options. Clonal testing also increased the gain from family selection considerably. Genetic gain from family selection of PMX with seedling testing was 2.0%, but it was 6.8% with clonal testing. A similar difference in gain from family selection was also observed between the CP_S and CP_C testing strategies.

The clonal testing options maintained their superiority over the seedling-based options when adjusted for their longer breeding cycles and higher testing cost. Control-pollinated breeding with clonal testing had 0.460% total adjusted gain per year vs. 0.388% for the seedling testing option (Figure 1b). Again, the larger gains of the clonal testing and selection as compared to the seedling testing options were largely due to greater genetic gains from within-family selection. In fact, for family selection only, time and cost adjusted gain was greater in the seedling-based options (Figure 1b). Strikingly, gain from within-family selection of

![Table 2: Heritabilities (± SE) and phenotypic variances used for gain estimations for breeding with seedling and clonal testing options.](https://academic.oup.com/forestscience/article/50/1/44/4617253)

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Clonal Seedling</th>
<th>Clonal Seedling</th>
</tr>
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<tbody>
<tr>
<td>( h^2 )</td>
<td>0.30 ± 0.23</td>
<td>0.06 ± 0.14</td>
</tr>
<tr>
<td>( h^2_{HS} )</td>
<td>0.62 ± 0.13</td>
<td>0.28 ± 0.51</td>
</tr>
<tr>
<td>( h^2_w )</td>
<td>0.35 ± 0.12</td>
<td>0.05 ± 0.11</td>
</tr>
<tr>
<td>( h^2_{FS} )</td>
<td>0.62 ± 0.24</td>
<td>0.36 ± 0.67</td>
</tr>
<tr>
<td>( \sigma^2 )</td>
<td>0.70 ± 0.34</td>
<td>0.03 ± 0.08</td>
</tr>
<tr>
<td>( \sigma^2_{HS} )</td>
<td>0.9353</td>
<td>0.8421</td>
</tr>
<tr>
<td>( \sigma^2_{w} )</td>
<td>0.1133</td>
<td>0.0439</td>
</tr>
<tr>
<td>( \sigma^2_{FS} )</td>
<td>0.3540</td>
<td>0.7982</td>
</tr>
<tr>
<td>( \sigma^2_{w} )</td>
<td>0.2258</td>
<td>0.0695</td>
</tr>
<tr>
<td>( \sigma^2_{FS} )</td>
<td>0.1993</td>
<td>0.7649</td>
</tr>
</tbody>
</table>

**NOTE:** \( h^2 \) = Individual-tree heritability, \( h^2_{HS} \) = Half-sib family heritability, \( h^2_w \) = Within half-sib family heritability, \( h^2_{FS} \) = Full-sib family heritability, \( h^2_{wFS} \) = Within full-sib family heritability, \( \sigma^2 \) = Individual (i), half-sib (HS), within half-sib (w), full-sib (FS) and within full-sib (wFS) phenotypic variances. Within-family variances of clonal data are clone mean phenotypic variances.
CP_C was about 60% more than that of the gain from within-family selection of CP_S.

**Within Family Clone-Ramet Combinations**

Genetic gains from different clone-ramet selection scenarios showed that increasing the number of ramets per clone did not considerably increase the gain from within-family selection. When the number of ramets per clone increased from 4 to 14, expected genetic gain increased only slightly for within-half-sib family selection (data not presented). When the gains for different ramet numbers were adjusted by the cycle length and the cost (number of trees per family), gain decreased sharply (Figure 2a). However, changing the clonal selection intensity increased the gain considerably for both within-half-sib and within-full-sib selection (Figure 2b). When the number of ramets per clone was constant (16 ramets) and selection ratio for clones changed from 7:25 to 2:25, within-half-sib family gain increased from 0.28 to 0.41%. Similarly, increasing the clonal selection intensity within-full-sib families increased the gain. When the number of tested trees per family was kept constant at 100, the greatest gain was obtained for two ramets per clone and a 2:50 clone selection ratio (Figure 2c). As the number of ramets increased and the number of clones tested decreased, genetic gain from within-family selection decreased accordingly.

Comparison of expected genetic gains from seedling and clonal within-family selection scenarios on a comparable basis (the number of planted trees per family was kept 100)

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**Figure 1.** Expected genetic gains for volume from between and within-family selection from polymix (PMX) and control-pollinated (CP) breeding options with seedling (S) and clonal (C) testing. (a) Nonadjusted genetic gain. (b) Time and cost adjusted genetic gain.

**Figure 2.** Adjusted genetic gains for volume from within half-sib (HS) and full-sib (FS) family selection for various clone-ramet combinations. (a) The selection ratio for the clone was constant (2:25) and number of ramets per clone increased from 4 to 16. The gain was adjusted for the cycle length (17 yr) and for the number of trees tested per family (25 clones × number of ramets × sites). (b) The number of ramets was kept constant at 16, and clonal selection intensity increased from 7:25 to 2:25. Number of tested trees per family is the same (400) for all within-family clonal selection scenarios. Genetic gains were adjusted by the cycle length (17 yr) and by the number of trees tested per family. (c) The number of tested trees per family was fixed at 100 trees, and the number of clones and ramets was varied.
is presented in Figure 3. When the number of planted trees per family was limited, i.e., less than 20, gain from seedling testing was greater than from the clonal testing (Figure 3). However, as the number of planted trees per family increased, gain from clonal testing increased and exceeded the gain from seedling testing. In all classes, greater gain was obtained with more clones and fewer ramets/clone for the same number of planted trees. If 10 ramets per clone were assumed (9 clones), then about 90 planted trees would be needed to achieve greater gain than with the seedling option. However, with only 4 ramets per clone (9 clones), only 36 planted trees were required. All the clone-ramet combinations had greater genetic gains than seedling selection when the number of trees per family was 90 or greater.

**Discussion**

**Heritabilities**

In this study, heritabilities based on the seedling data set were consistent or within the range of other previous estimates. Balocchi et al. (1993) reported near zero (0.04) narrow-sense individual-tree heritability in loblolly pine for height growth until age 8 yr. Lambeth et al. (1983) and Li et al. (1996) also reported similar small individual-tree heritability estimates for loblolly pine at early ages. Gwaze et al. (2001) reported 0.04 to 0.61 individual-tree heritabilities for diameter for loblolly pine at different sites in the western Gulf region of the United States. In contrast, estimates from the clonal testing in this study were greater than previous reports. The narrow-sense individual-tree heritability for volume for clones in this study (0.30) was about two times that of the estimate (0.14) reported for a clonally replicated trial of loblolly pine at age 5 (Paul et al. 1997).

Similarly, family heritabilities based on clonal data in this study were considerably greater than the estimates reported by Paul et al. (1997).

The tendency of low individual-tree and within-family heritabilities for volume for young loblolly pine implies that the expected gain would be low if selection was applied at young ages, particularly from seedling progeny trials. However, with higher heritabilities from clonally replicated tests, greater genetic gains from within-family selection from young genetic field tests could be obtained. For fusiform rust incidence, heritabilities for the two propagule types did not differ greatly, except for within full-sib family heritability. Higher heritability for the clone means suggests that considerable gain could be realized if selection is based on clone means within families for fusiform rust incidence.

The higher heritability estimates from the clonal testing could be due to a better estimation of genetic variances and better control of environmental variance by clonal replication of families (Libby 1964, Stoneycypher and McCullough 1986, Isik et al. 2003). However, this also could be due to “c” effects common to certain clones, which might have inflated the additive genetic variance (Libby and Jund 1962). Sampling error and the limited number of parents included in the study could have also contributed to the different estimates of heritabilities and their high standard errors from the two material types.

**Efficiency of Seedling and Clonal Testing**

The magnitudes of heritabilities estimated from rooted cuttings and seedlings in this study were reflected in estimated genetic gains for different breeding and testing options. The results showed that, despite longer breeding cycles and higher costs of testing, clone-based testing substantially increased the expected gain for volume over the seedling testing options. The major difference in genetic gain between seedling and clonal testing options was from within-family selection. This is not surprising, because in clonal testing strategies, within-family selection is based on clone means and environmental variation is sampled by replicating the clones. Conversely, with seedling testing, within-family selection is based solely on phenotypes of individual trees. Cloning the families also increased the gain from among-family selection compared to the seedling testing and selection options. Greater genetic gains from cloned testing options could be due to better estimates of genetic parameters by reducing environmental noise. Using the data sets from these trials, Isik et al. (2003) reported smaller within-plot (error) variances for plots of clones than for seedling plots of the same families on the same sites.

A complementary breeding and testing strategy has been implemented for the third cycle of the North Carolina State University-Industry Cooperative Tree Improvement Program for loblolly pine (McKeand and Bridgewater 1998, Li et al. 1999). In this strategy, polymix breeding and testing for general combining ability estimation is followed by full-sib crosses for within-family selection of seedlings. Results from this study suggest that cloning the full-sib families and selecting in replicated clonal trials would be more efficient. With the development of efficient propagation methods (Goldfarb et al. 1997) the additional propagation and testing cost may be balanced by additional gains. When parental GCA values are estimated using polymix mating, the extra expense for propagation and testing could be minimized, because only the best families in the breeding population would be cloned and tested. Although genetic gains were not estimated for fusiform rust incidence in this study, a higher within-full-sib heritability for the clonal options showed the

![Figure 3. Time and cost-adjusted genetic gains from within-full-sib family selection with seedling (dashed line) and clonal (solid lines) testing, with the total number of tested trees held constant. The number of ramets (r) for clonal testing was fixed at 4, 7, or 10.](https://academic.oup.com/forestscience/article/50/1/44/4617253)
potential to improve resistance to fusiform rust disease in loblolly pine. Foster and Shaw (1987) proposed a breeding population with clonal testing and selection for improvement of resistance to fusiform rust disease.

Results from this study agree with results reported for other tree species. In radiata pine, the highest gain per year per cost was found for the cloned open-pollinated and cloned control-pollinated breeding populations (Shelbourne 1992). In a simulation study with Norway spruce (Picea abies L.), clonal testing was highly efficient through 10 generations, even for low heritabilities and a low number of ramets per clone (Rosvall et al. 1998). Mullin and Park (1994) reported higher genetic gain from clonal selection compared to mass and backward selection options in black spruce (Picea mariana [Mill] B.S.P). Jefferson et al. (1997) reported that when heritability is low, clonal testing could be more efficient than seedling testing for radiata pine.

When interpreting the results of this study, two additional factors should be considered. First, all of the clone-based strategies require cloning of families and testing more trees per family and consequently, more investment. Although genetic gains were adjusted for longer breeding cycles and number of trees tested, these adjustments did not include the extra cost of propagating rooted cuttings compared with container-grown seedlings. Though these costs are not completely known at present, one can assume that they will be relatively minor compared with the greater cost associated with progeny test establishment and maintenance. Second, variance components and genetic gain estimations in this study were based on a limited number of parent trees. Gain per year could, in reality, be either larger or smaller than that reported here. Thus, the results should be evaluated cautiously.

Clone-Ramet Combinations

When designing a clonal testing and selection program, the number of clones and ramets per clone should be optimized to maximize gain (Shaw and Hood 1985, Russel and Libby 1986, Russel and Loo-Dinkins 1993). When the number of ramets per clone is low, then clonal means may not be estimated precisely. In contrast, when the number of ramets increases and the number of clones decreases, then for a given testing effort, selection intensity within-family decreases. In this study, genetic gain from within cloned families was not highly sensitive to the number of ramets per clone. In contrast, increasing the within-family selection intensity increased the gain considerably. In a simulation study, Shelbourne (1992) suggested 10 ramets per clone to obtain optimal gain for radiata pine. In another simulation study on radiata pine, Jefferson et al. (1997) suggested that 9 to 12 clones per family would maximize genetic gain. The results in this study suggest that when a family is represented by a fixed number of trees, more gain is achieved by testing numerous clones with fewer ramets. In the clone-ramet scenarios we investigated, four ramets always resulted in greater gain than seven ramets with an equal number of test trees. However, considering that mortality or loss of blocks or sites can occur in progeny trials, the number of ramets per clone should perhaps be maintained above the theoretical optimum.

Conclusions

This study investigated the potential of clonally replicated progeny tests for achieving genetic gain in breeding programs of loblolly pine. Higher family and within-family heritabilities were estimated from the clonally replicated tests than seedling testing. Clonal testing and selection options yielded greater gain than seedling options, despite their longer cycles and higher cost. This was due to better control of environmental noise and greater genetic gains from within-family selection. Clonally replicated progeny trials should be considered for loblolly pine to increase the efficiency of current or future breeding strategies. Polymix mating for GCA estimation of parent trees and clonal selection in cloned control-pollinated families may be preferred. This option could be applicable for loblolly pine, if only the best families are control-pollinated and cloned. Strategies that test numerous clones with relatively few ramets per clone should provide the most genetic gain.

Literature Cited

APPENDIX

Equations for heritabilities and genetic gains for the polynmix and control-pollinated breeding options with seedling and clonal testing. Dominant and epistatic variances were negligible. Thus, only narrow-sense heritabilities were estimated. See Isik et al. (2003) for the linear mixed models, definition of the factors in the models and estimation of genetic variances.

Individual-tree narrow-sense heritabilities:

\[ h_i^2 = \frac{\sigma_i^2}{\sigma^2} \]  

Individual-tree phenotypic variance from the seedling dataset:

\[ \sigma^2 = \sigma_m^2 + \sigma_{sf}^2 + \sigma_{hf}^2 + \sigma_{rm}^2 + \sigma_{sfm}^2 + \sigma_{r(f)s}^2 + \sigma_{r(m)s}^2 \] 

Individual-tree phenotypic variance from the rooted cutting data set:

\[ \sigma_i^2 = \sigma_m^2 + \sigma_{fm}^2 + \sigma_{r(f)m}^2 + \sigma_{r(pm)}^2 + \sigma_{r(fm)}^2 + \sigma_{r(pm)}^2 + \sigma_{r(fm)}^2 + \sigma_{r(pm)}^2 + \sigma_{r(fm)}^2 \] 

**PMX_S: Polynmix Breeding with Seedling Testing**

Genetic gain from between and within half-sib families:

\[ \Delta G = i_1 h_{HS}^2 \sigma_{HS}^2 + i_2 h_{HS}^2 \sigma_m^2 = i_1 0.25 \sigma_A^2 / \sigma_{HS}^2 + i_2 0.75 \sigma_A^2 / \sigma_m^2 \] 

Half-sib family mean heritability:

\[ h_{HS}^2 = 0.25 \sigma_A^2 / \sigma_{HS}^2 \] 

Pooled estimate of HS family phenotypic variance:

\[ \sigma_{HS}^2 = (\sigma_{HS-S}^2 + \sigma_{HS-M}^2) / 2 \] 

Half-sib family mean phenotypic variance for females assuming random for a female parent across in males:

\[ \sigma_{HS-S}^2 = \sigma_f^2 + 1/m (\sigma_{sf}^2 + \sigma_{sm}^2) + \sigma_{sf}^2 / s \] 

\[ + 1/m (\sigma_{sf}^2 + \sigma_{sm}^2) + \sigma_{sf}^2 / s \] 

\[ + 1/m (\sigma_{sf}^2 + \sigma_{sm}^2) + \sigma_{sf}^2 / s \] 

\[ + 1/m (\sigma_{sf}^2 + \sigma_{sm}^2) + \sigma_{sf}^2 / s \] 

\[ + 1/m (\sigma_{sf}^2 + \sigma_{sm}^2) + \sigma_{sf}^2 / s \]
Half-sib family mean phenotypic variance for males assuming random for a male parent across in females:

\[
\sigma^2_{HS,M} = \sigma^2_m + \frac{1}{f} (\sigma^2_f + \sigma^2_{fm}) + \sigma^2_{im} / s + \frac{1}{f} \frac{s}{f} (\sigma^2_{ff} + \sigma^2_{ffm}) + \sigma^2_{(r)(im)} / sb + 1 / f sb \sigma^2_{(r)(imf)} + \sigma^2_{(r)(imf)} / f sbm (11)
\]

Within half-sib family heritability:

\[h^2_w = 0.75 \frac{\sigma^2_A}{\sigma^2_w} (9)\]

Pooled within half-sib family phenotypic variance:

\[
\sigma^2_w = (\sigma^2_{w,F} + \sigma^2_{w,M}) / 2
\]

or

\[
\sigma^2_w = (1 - 1/n)(0.75 \sigma^2_A + \sigma^2_D + \sigma^2_E) (10)
\]

Within half-sib family phenotypic variance for Female:

\[
\sigma^2_{w,F} = (m - 1) / m (\sigma^2_m + \sigma^2_{fm}) + (s - 1) / s \sigma^2_{sm} + (s - 1) / s \sigma^2_{sm} + (s - 1) / s \sigma^2_{sm} + (s - 1) / s \sigma^2_{sm} (11)
\]

Within half-sib family phenotypic variance for Male:

\[
\sigma^2_{w,M} = (f - 1) / f (\sigma^2_f + \sigma^2_{fm}) + (s - 1) / s \sigma^2_{sm} + (s - 1) / s \sigma^2_{sm} + (s - 1) / s \sigma^2_{sm} (12)
\]

PMX_C: Polymix Breeding with Clone Testing

Genetic gain from between half-sib families and clone mean within-half-sib family:

\[
\Delta G = i_1 h^2_{HS} \sigma_{HS} + (c - 1) / c i_2 h^2_{C(HS)} \sigma_{C(HS)}
\]

or

\[
\Delta G = i_1 0.25 \sigma^2_A / \sigma_{HS} + (c - 1) / c i_2 0.75 \sigma^2_A / \sigma_{C(HS)} (13)
\]

Half-sib family mean heritability from rooted cuttings:

\[h^2_{HS} = 0.25 \sigma^2_A / \sigma^2_{HS} (14)\]

Pooled phenotypic variance of half-sib family mean from rooted cuttings:

\[h^2_{HS} = (\sigma^2_{HS,M} + \sigma^2_{HS,M}) / 2 (15)\]

Phenotypic variance of HS family for Female:

\[
\sigma^2_{HS,F} = \sigma^2_f + 1 / m (\sigma^2_m + \sigma^2_{fm}) + 1 / cm \sigma^2_{(f,m)} + 1 / s (\sigma^2_{sf} + 1 / cm \sigma^2_{(f,m)}) (16)
\]

Phenotypic variance of HS family for Male:

\[
\sigma^2_{HS,M} = \sigma^2_m + 1 / f (\sigma^2_f + \sigma^2_{fm}) + 1 / f s (\sigma^2_{sf} + 1 / cm \sigma^2_{(f,m)}) + 1 / sb (\sigma^2_{(r)(f,m)}) + 1 / sbm (\sigma^2_{(r)(f,m)}) + 1 / sbm (\sigma^2_{(r)(f,m)}) (17)
\]

Clone mean heritability within half-sib families:

\[h^2_{C(HS)} = 0.75 \sigma^2_A / \sigma^2_{C(HS)} (18)\]

Phenotypic variance of clone mean within half-sib families:

\[
\sigma^2_{C(HS)} = (r - 1) / r (\sigma^2_{C(f,m)} + 1 / bt \sigma^2_{r(x)(f,m)}) + 1 / \sigma^2_{C(f,m)} (19)
\]

Assuming \(C(FM) = 1/2V_r + 3/4V_m, r = \) Harmonic mean # of ramets per clone \((r = 1.7), b = \) # of blocks \((b = 6), s = \) # of sites \((s = 2), c = \) # of clones per family \((c = 6.8)\)

CP_S: Control-Pollinated Breeding with Seedling Testing

Genetic gain from between and within full-sib families:

\[
\Delta G = i_1 h^2_{FS} \sigma_{FS} + i_2 h^2_{FS} \sigma_{sf} + i_1 0.5 \sigma^2_A / \sigma_{FS} + i_2 0.5 \sigma^2_A / \sigma_{sf} (20)
\]

Full-sib family mean heritability:

\[h^2_{FS} = 0.5 \sigma^2_A / \sigma^2_{FS} (21)\]
Phenotypic variance of full-sib family mean:

\[
\sigma_{FS}^2 = \sigma_f^2 + \sigma_m^2 + \sigma_{fm}^2 + 1/s(\sigma_{sf}^2 + \sigma_{sm}^2 + \sigma_{sfm}^2) \\
+ 1/sb(\sigma_{r(s)}^2 + \sigma_{r(s)m}^2 + \sigma_{r(s)fm}^2) \\
+ \sigma_{r(s)cf(fm)}^2 / sbt + \sigma_e^2 / sbtl
\] (22)

Within full-sib family heritability:

\[h_{uf}^2 = 0.5\sigma_A^2 / \sigma_{uf}^2\] (23)

Within full-sib family phenotypic variance:

\[
\sigma_{uf}^2 = (s-1)/s(\sigma_{sf}^2 + \sigma_{sm}^2 + \sigma_{sfm}^2) \\
+ (sb-1)/sb(\sigma_{r(s)}^2 + \sigma_{r(s)m}^2 + \sigma_{r(s)fm}^2) \\
+ \sigma_{r(s)f}^2 + (sbt-1)/sbt\sigma_p^2 + (sbm-1)/sbm\sigma_e^2
\] (24)

**CP_C: Control-Pollinated Breeding with Clone Testing**

Genetic gain from between full-sib family and clone mean within full-sib family selection:

\[
\Delta G = i_1h_{FS}^2\sigma_{FS} + (c-1)/c \cdot i_2h_{C(FS)}^2\sigma_{C(FS)} \\
= i_1 0.5\sigma_A^2 / \sigma_{FS} + (c-1)/c \cdot i_2 0.5\sigma_A^2 / \sigma_{C(FS)}
\] (25)

Full-sib family heritability:

\[h_{FS}^2 = 0.5\sigma_A^2 / \sigma_{FS}^2\] (26)

Phenotypic variance of full-sib family means:

\[
\sigma_{FS}^2 = \sigma_f^2 + \sigma_m^2 + \sigma_{fm}^2 \\
+ \sigma_{r(s)cf(fm)}^2 / c + 1/s(\sigma_{sf}^2 + \sigma_{sm}^2 + \sigma_{sfm}^2 + \sigma_{r(s)fm}^2) / c \\
+ 1/sb(\sigma_{r(s)}^2 + \sigma_{r(s)m}^2 + \sigma_{r(s)fm}^2) \\
+ \sigma_{r(s)cf(fm)}^2 / sbc + \sigma_e^2 / sbcl
\] (27)

Narrow sense clone mean heritability within full-sib families:

\[h_{C(FS)}^2 = 0.5\sigma_A^2 / \sigma_{C(FS)}^2\] (28)

Phenotypic variance of clone means within full-sib families

\[
\sigma_{C(FS)}^2 = \\
(c-1)/c(0.5\sigma_A^2 + 0.5\sigma_A^2 + c + 0.75\sigma_D^2 + \sigma_E^2 / rc)
\] or

\[
\sigma_{C(FS)}^2 = \\
(c-1)/c[\sigma_{C(fm)}^2 + 1/s\sigma_{sc(fm)}^2 + 1/sb\sigma_{r(s)cf(fm)}^2 + 1/sb\sigma_e^2]
\] (29)