GENETIC TEST RESULTS FROM A TREE IMPROVEMENT PROGRAM TO DEVELOP CLONES OF LOBLOLLY PINE FOR REFORESTATION

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Abstract. Eleven years ago, International Forest Seed Company began a tree improvement program to develop a population of loblolly pine (Pinus taeda L.) to resist infection by fusiform rust [Cronartium quercum (Berk.) Miyabe ex Shirai F. sp. fusiformel and to improve volume production as well. One hundred and twenty-seven parent trees were selected from a pool of tested first-generation selections, then mated at random using small disconnected factorials. Seedlings emerging from rust screening with no rust galls were planted into cutting orchards, from which cuttings were taken, rooted, and planted into clonal field trials. Analysis of fifth-year data between seedlings and rooted cuttings of the same checklots reveals that there are no propagation effects. Further, rooted cuttings of the select clones performed significantly better than the commercial checks in terms of morphological traits and improved resistance to fusiform rust. Genetic analysis reveals a strong clonal effect and a possible G x E interaction for the morphological traits evaluated. Sensitivity analysis on the error variance of the clone mean suggests a reallocation of resources in designing future clonal studies.

Keywords: Pinus taeda L., fusiform rust, rooted cuttings, clonal propagation

INTRODUCTION

International Forest Seed Company (IFSCO) initiated a loblolly pine (Pinus taeda L.) tree improvement program in 1982 to specifically develop a population of highly rust resistant clones. Based on results of informal surveys and queries of forest land owners, fusiform rust [Cronartium quercum (Berk.) Miyabe ex Shirai F. sp. fusiformel was sufficiently serious at the time to justify a tree improvement program with the aim of improving the resistance to the pathogen. Joining the North Carolina State University-Industry Cooperative Tree Improvement Program, provided IFSCO with the base population of tested trees from which superior selections were chosen to breed. Rooted cutting procedures used by Hilleshog AB, Landskrona, Sweden were the basis of the system applied at 1FSCO to clonally propagate the offspring population. The purpose of using clonal propagation was to capitalize on both the time savings to large-scale implementation and the relatively larger genetic gains available through a clonal tree improvement program.

THE PROGRAM

One hundred and twenty-seven parent trees were selected from a pool of tested, first-generation selections from a combination of two sources: North Carolina State University-Industry Cooperative Tree Improvement Program, and the Cooperative Program between the USDA Forest Service and the Georgia Forestry Commission. Traits for parental selection include superior resistance to fusiform rust and superior height growth as evidenced in progeny tests. The select trees were mated in small disconnected factorials (generally 4 x 4) from 1983 through 1985.

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Seedlings from each successful cross were grown at the USDA Forest Service, Bent Creek Resistance Screening Center and screened for infection of fusiform rust using standard techniques (Anderson *et al.* 1983) Seedlings emerging from the screening with no rust galls became the base population for field testing. In some cases well over a hundred seedlings from a cross were rust free. All were planted in the cutting orchard, but in the later stages of field testing, a maximum of only 25 seedlings per cross were tested.

The cutting orchard was established by planting the six-month-old rust-free survivors in double rows with 6 ft^2 of growing space. Within six weeks after planting, the seedlings were initially hedged. Thereafter, the seedlings were hedged three times per year. Hedging served two purposes: (1) to maintain juvenility (Libby *et al.* 1972) and (2) to increase the number of potential cuttings (Foster *et* al. 1981). Other cultural practices included drip irrigation, pesticide applications, fertilizations, and weed control. Each practice was conducted on a very regimented basis, because we found that the hedge health had a tremendous influence on rooting success.

Initially, two populations of clones were to be maintained: (1) a breeding population and (2) a production population (Foster 1986). The purpose was to begin propagating the breeding population by selecting the best 300 clones after only three years of field testing. By age five only 160 were to be kept for breeding. This early selection procedure expends effort in maintaining some clones that would have never been included in subsequent generations, but shortens by two years the time required before crossing can be completed. The production population basically followed the same strategy of making more initial selections (in this case 200) than what ultimately was to be the final population (50 clones).

Currently, only the production population is being employed to any degree of work. A few thousand have been established in limited reforestation, primarily as demonstration studies. Some breeding population selections have been made, but propagating the clones and preparing them for mating has stalled. This is due to the apparent lack of market demand for this population.

PROPAGATION PROCESS

Since the program began, several improvements and modifications to our propagation procedures have been made. The objective always has been to produce large quantities of high quality rooted cuttings at reasonable costs.

Originally, after the seedlings were planted in the cutting orchard, the bud was nipped to retard height growth. Individual hedges were maintained at 1.5 feet via very selective hand pruning. When hedges are established now, the seedlings are allowed to grow up to three feet (12 months of growth) before they are hedged. This practice results in larger trees with many more acceptable cuttings (100 to 150 per plant). The hedge health is much easier to maintain as well. Good quality cuttings can be taken three times per year: in March, June, and October. After cuttings are taken, the plants must be hedged severely and the dead thatch removed.

Cuttings taken are best when they are three to four inches long with emerging needles at least 1.5 inches long. Only in October is a terminal bud necessary. They are cut to length during collection, placed in plastic bags and stood upright in cold storage approximately for three to seven days. Plant growth regulators involved in the rooting process are basipetally transported and accumulate at the base of the cutting. All cuttings are set directly to the cavity in which they will root and grow, after each cutting is dipped into IFSCO's modified Hare's rooting powder (Hare, 1974) that was developed to promote rooting. The cavities (5.6 $in.^3$) are filled with a peat:perlite (60:40) mixture.

The 40 cavity trays are set in a greenhouse equipped with specific equipment to maintain 95 to 100% relative humidity. This is primarily accomplished with a fogging machine. Air temperatures are held close to 85 degrees F, while soil temperatures are maintained at 85 degrees F. During the winter this is accomplished with infrared heat. It takes approximately 12 to 14 weeks for rooting to

occur. The goal during this period and the following three months is to lay down root growth, but inhibit shoot growth. We found our highest rooting percentages to occur in the fall.

IFSCO, during the course of producing cuttings for this program and other studies, has set in excess of 700,000 cuttings.

FIELD TESTING

Experimental Design

From 1986 through 1990, a total of ten clonal field tests were established across the Coastal Plain of the Southeastern U.S. For the purposes of this paper, we will focus on the five tests that are at least five years old. These tests are located near the towns of Blakely, Claxton, and Dublin in Georgia; Excell, Alabama; and Walnut Hill, Florida.

In order to further screen the greenhouse-test survivors for fusiform rust resistance, each test was located in a high rust hazard area (Anderson *et al.* 1986). A high hazard site was defined as an area where neighboring forest stands exhibited over 50 percent infection. Geographically diverse tests in high rust hazard areas are intended to maximize sampling of divergent rust strains and promote selection for generally resistant clones.

The field tests were designed to achieve a compromise between (1) the number of clones that could be tested given a fixed number of trees that could be planted and (2) efficiencies of genetic value estimation for families and clones within families (Shaw and Hood 1985). Each tested clone was planted at each location with two ramets per clone per location, providing a total of ten ramets per clone (five locations x two ramets per location). At each location, one ramet per clone was placed in each of two complete blocks with clones from a single family distributed among six blocks per location. This design achieved balance at the family level across replications and locations, but was only partially balanced at the clone within family level. Clones from single families were distributed randomly within blocks and treated statistically as a single, non-contiguous family plot (Lambeth *et al.* 1983).

Four commercial checklots from the North Carolina State University-Industry Cooperative Tree Improvement Program were planted at each test location. The checklots were (1) CC3, North Carolina Piedmont, (2) CC4, Georgia Coastal, (3) CC5, Lower Gulf, and (4) CC7, Georgia-South Carolina Piedmont. At each location, the checklots were rooted cuttings from hedged seedlings. In addition to the rooted cutting checklots, seedling checklots were established at all locations except Claxton and Dublin, Georgia.

Data Analyses

Five-year data were collected on survival, presence of rust galls, height (ft), and d.b.h. (in). Individual tree volume (ft^3) at age five was calculated using volume equations derived by Smalley and Bower (1968).

As a result of significant mortality during test establishment at the Claxton and Blakely test sites, we further restricted the dataset by including only those clones with at least one ramet surviving at each of the five locations at age five. Sixty-one clones out of the original pool of 375 met the criterion. In no case did the number of ramets per clone fall below nine. This reduced dataset precluded any detailed genetic analysis (i.e., subdivision of total genetic variance into additive, dominance, or epistatic variance). However, we did compare the different propagule types and estimate total genetic variance and the genotype x environment interaction.

From an earlier study with three of the same tests that are in this study, Paul (1993) learned that unequal error variances were present among the locations. To compensate for these unequal variances, all data in the current study were transformed to a standard normal variate with a mean of 0 and standard deviation of 1. This was accomplished by subtracting the replication mean from

the individual tree value and then dividing by the replication's standard deviation (Snedecor and Cochran 1980a).

For comparing fusiform rust resistance and morphological traits among the propagule types, we only included the three locations where all three propagule types were present. Fusiform rust assessment tallies among the propagule types were compared by using a series of Chi-square tests. The observed rust levels among each checklot propagule type generated the expected frequencies to which the observed frequencies of the superior clones were compared in separate Chi-square tests. We also used this procedure to test the difference between the rooted cuttings of the commercial checklots and their seedling counterparts. Simple t-tests were performed to test for a significant difference between the three propagule types with regard to height, d.b.h., and volume.

In the genetic analysis, the checklot propagules were excluded. Given some imbalance due to mortality, a least-squares analysis was used to calculate the sums of squares. The form of the analysis of variance is presented in Table 3. Variance components were calculated by equating the mean squares with the expected mean squares. The actual coefficients of the variance components were adjusted to compensate for some missing plots (Hartley 1967; Goodnight and Speed 1978). Standard errors for the variance components were calculated according to Becker (1984). All sources of variation were considered to be random.

The estimates of the variance components can be used to help improve the design of future field trials. Because the clone mean is the basis of selection, the error variance of a clone mean is of particular interest; it reflects the precision with which the means are estimated (Snedecor and Cochran 1980b; Foster *et al.* 1984). Also, the error variance of a clone mean, Vx, is used to calculate broad-sense heritability (H2x).

The theoretical error variance of a clone mean is

$$V_{\bar{x}} = \frac{\sigma^2 LC}{l} + \frac{\sigma^2}{nl}$$

and its broad-sense heritability is

$$H^2_{\bar{x}} = \frac{\sigma^2 C}{\sigma^2 C + V_{\bar{x}}}$$

where $V_{\bar{x}}$ = error variance of a clone mean

$H^2_{\bar{x}}$ = broad-sense heritability, clone-mean basis

σ^2 = error variance component

$\sigma^2 C$ = clonal variance component

$\sigma^2 LC$ = location x clonal variance component

l = number of locations, 4.7 (adjusted)

n = number of replications per location, 1.7 (adjusted)

To examine the influence of experimental design on results in future experiments, the variance components for height were assumed to be stable while the coefficients, n and I were varied. This is similar to the technique used by Schutz and Bernard (1967) to examine the sensitivity of the standard error of soybean strains to changing allocations of replications, locations and years.

propagation of loblolly pine via rooted cuttings and that via seedlings (Table 2). In other studies, Foster *et al.* (1987) and Foster (1988) have demonstrated that the growth habit for rooted cuttings of loblolly pine is quite comparable to that of seedlings when the cuttings are initially collected from seedlings less than 18 months of age.

Genetic analysis. The results from the genetic analysis revealed that the data transformation successfully reduced location and replication effects (Table 3). This allowed us to focus on the clone andlocation x clone components of the model.

Table 3. Analysis of variance for the standardized variables height (HT), d.b.h. (DBH5A), and volume (VOL5A) at age five for 61 superior clones of loblolly pine planted in five locations.

Source of Variation	df	Expected Mean Squares	Pr>F HT5A	Pr>F DBH5A	Pr>F VOL5A
Location (L)	4	$\sigma^2 + 1.64\sigma^2_{LC} + 100.27\sigma^2_L$	0.9921	0.9828	0.9781
Clone (C)	60	$\sigma^2 + 1.64\sigma^2_{LC} + 8.23\sigma^2_C$	0.0001	0.0001	0.0001
LxC	240	$\sigma^2 + 1.74\sigma^2_{LC}$	0.0497	0.0252	0.0103
Error	234	σ^2			

σ^2_L= variance among locations; σ^2_C= variance among clones; σ^2_{LC}= variance due to interaction of locations and clones; σ^2= error variance; Pr>F = the probability of obtaining an F-value larger than the one observed.

There were highly significant clone effects for the morphological traits evaluated (Table 3). This source of variation accounted for 14 to 23 percent of the total variation (Table 4). Selecting clones from the upper end of this normal distribution captures both the additive and non-additive genetic variance. This strategy yields a genetic gain greater than that of seedling propagation in which only a portion of the additive variance is exploited. Forest geneticists at N. C. State University estimate that full exploitation of the total genetic potential could increase gain by 4.8 and 9.5 percent for height and volume, respectively, over the expected gains from unrogued second generation seed orchards (Dr. Bob Weir, personal communication).

However, there was also a significant location x clone interaction effect observed for each trait. This source of variation accounted for 9 to 15 percent of the total variation (Table 4). The varying densities among the test plantings, as a result of differing mortality levels, may be one reason that this interaction was so significant, particularly with respect to the density-sensitive traits of d.b.h. and volume. For example, the Claxton test site had the highest mortality level (68%). When that location was removed from the analysis, the P-value of the interaction effect was 0.0498, 0.0713, and 0.0665 for height, d.b.h., and volume, respectively. Survival among the test sites ranged from 68% to 98%.

Another possible explanation for this interaction could be the confounding of ontogenetic age of the ortets with location. Loblolly pine does mature quite rapidly, with discernible expression of some maturation as early as age four years (Greenwood 1984). Repeated hedging appears to delay maturation in other pine species (Libby *et al.* 1972; Libby and Hood 1976), and loblolly is thought to behave in similar fashion. Since the hedges in the current study had been hedged repeatedly, beginning at six months of age, we assumed that the maturation effect would be negligible from 18 months (age of cuttings in the 1987 tests) to 30 months (age of cuttings in the 1988 tests). However, this may not be the case.

Table 4. Variance components (±SE), their percentage of total variation, and broad-sense heritability estimates (H^2x) for standardized height (HT5A), d.b.h. (DBH5A), and volume (VOL5A) at age five for 61 superior clones of loblolly pine planted in five locations.

	Trait					
	HT5A		DBH5A		VOL5A	
Source of Variation	Variance component +SE	% Total Variation	Variance component +SE	% Total Variation	Variance component +SE	% Total Variation
Location (L)	0	0	0	0	0	0
Clone (C)	0.22±0.05	22.7	0.13±0.04	13.5	0.16±0.05	16.5
LxC	0.09±0.07	9.3	0.12±0.06	12.5	0.14±0.06	14.5
Error	0.66±0.06	68.0	0.71±0.07	74.0	0.67±0.06	69.0
Total	0.97		0.96		0.97	
H^2_X[1]	0.68		0.54		0.59	

[1]The adjusted coefficients for n and I used to calculate broad-sense heritability were 1.7 and 4.7, respectively.

With respect to height, the interaction component is 42% of the clonal component of variance (Table 4). Shelbourne (1972) suggested that, as a rule of thumb, if the interaction component reaches 50% or more of the entry (clonal) component of variance, then the effects of the location x clone interaction are likely to cause problems for selection and testing. If the location x clone interaction is real and not the result of confounding with ontogenetic age, then one may have to consider establishing clonal testing zones in order to maximize gain from clonal selection.

Broad-sense heritability on a clone-mean basis was 0.68, 0.54, and 0.59 for height, d.b.h., and volume, respectively (Table 4). Unless clones are to be selected on the performance of a single rooted cutting or a single plot, the appropriate heritability estimate should be based on clone means. These heritabilities are lower than previously reported for this same population of clones. Paul (1993) estimated $_{H^2x}$ for height, d.b.h., and volume to be 0.90, 0.96, and 0.94, respectively. One possible explanation could be that there are more locations in this analysis compared to Paul's study.

Experimental design and the allocation of resources . The goal of *a* design for estimating clone means is to minimize the error variance of a clone mean ($V_{\bar{x}}$) for a given cost (Russell and Libby 1986). With respect to height growth, the greatest decrease in Vx occurs as the number of locations increases from two to four (Figure 1). Additional locations do not greatly reduce the error variance of a clone mean. Four locations should be adequate to sample divergent rust strains and promote selection for generally resistant clones. Given the constraint of four locations, we can decrease further if we increase the number of blocks from two to three.

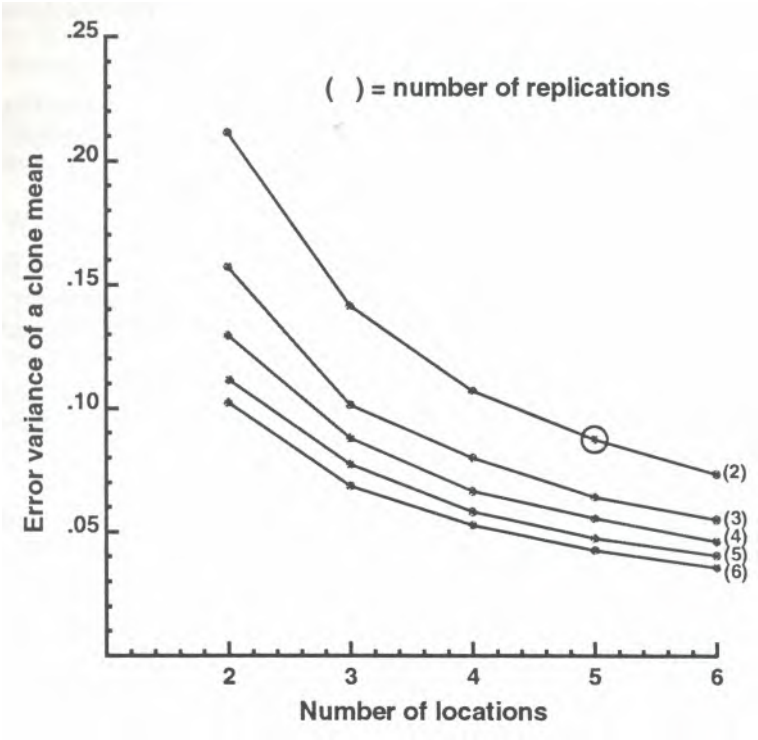

Figure 1. Error variance of a clone mean for height for different numbers of locations and replications per location. The circled data point indicates the experimental design of the current study.

While the current experimental design is good, the modified design would allow us to reduce both Vx and the cost of test establishment and maintenance by consolidating our efforts on fewer sites. The added cost of increasing the number of blocks at a given location from two to three should be offset by the reduction in the number of locations. It will also allow us to conserve a valuable resource, the industrial cooperator. Without them, a lot of these field trials would never be planted.

CONCLUDING REMARKS

Loblolly pine cuttings can be taken in March, June, and October of a given year. Juvenility, hedge health, and consistent environmental parameters are all critical to rooting success.

Five-year data reveal no significant difference between seedling and rooted-cutting propagules of similar genetic background, when the hedged donor plant is less than 30 months of age. Rooted cuttings of select clones perform better, on average, than the commercial checklots, both in terms of fusiform rust resistance and morphological traits.

There is a strong clonal effect for the morphological traits evaluated. The possible G x E interaction suggests that one may have to establish clonal testing zones in order to maximize gain from clonal selection. Sensitivity analysis on the error variance of the clone mean suggests that in future field trials, one could reallocate their resources to four test sites and three replications per site.

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LITERATURE CITED

Anderson, R.L., C.H. Young, and J.D. Triplett. 1983. Resistance screening center procedures manual: a step-by-step guide in the operational screening of Southern pines for resistance to fusiform rust. (Rev. June 1983). USDA For. Serv., State and Private For., Report #83-1-18.

Anderson, R.L., N.D. Cost, J.P. McClure, and C. Ryan. 1986. Predicting severity of fusiform rust in young loblolly and slash pine stands in Florida, Georgia, and the Carolinas. South. J. Appl. For. 10(1):38-41.

Becker, W.A. 1984. Estimation of Variance components and heritability: factorial design. In Manual of Quantitative Genetics (4th ed.). Academic Enterprises. Pullman, WA. pp. 74-78.

Foster, G.S. 1986. 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; Oct. 13-17, 1986. Pub. by N.C. State Univ. - Industry Coop. Tree Imp. Prg. pp. 582-590.

Foster, G.S. 1988. Growth and morphology of rooted cuttings and seedlings of loblolly pine and their genetic analysis. In Proc. 10th North American Forest Biology Workshop. University of British Columbia. Vancouver, B.C. pp 67-78.

Foster, G.S., F.E. Bridgwater, and S. E. McKeand. 1981. Mass vegetative propagation of loblolly pine - a reevaluation of direction. Proc. 16th South. For. Tree Imp. Conf. pp. 311-319.

Foster, G.S., R.K. Campbell, and W.T. Adams. 1984. Heritability, gain, and C effects in rooting of western hemlock cuttings. Can. J. For. Res. 14:628-638.

Foster, G.S., C. Lambeth, and M.S. Greenwood. 1987. Growth of loblolly pine rooted cuttings compared with seedlings. Can. J. For. Res. 17:157-164.

Goodnight, J.H. and F.M. Speed. 1978. Computing expected mean squares. SAS Instit. Tech. Report R-102.

Greenwood, M.S. 1984. Phase change in loblolly pine: shoot development as a function of age. Physiol. Plant. 61:518-522.

Hare, R.C. 1974. Chemical and environmental treatments promoting rooting of pine cuttings. Can. J. For. Res. 4:101-106.

Hartley, H.O. 1967. Expectation, variances and covariances of ANOVA mean squares by "synthesis". Biometrics, 23:105-114.

Lambeth, C.C., W.T. Gladstone, and R.W. Stonecypher. 1983. Statistical efficiency of row and noncontiguous family plots in genetic tests of loblolly pine. Silvae Genet. 32:24-28.

Libby, W.J., A.G. Brown, and J.M. Fielding. 1972. Effects of hedging radiata pine on production, rooting, and early growth of cuttings. N.Z. J. For. Sci. 2:263-283.

Libby, W.J. and J.V. Hood. 1976. Juvenility in hedged radiata pine. la Proceedings of Symposium on Juvenility in Woody Perennials. No. 56, Acta Horticulture Technical Committee, International Society of Horticultural Science. pp 91-98.

Paul, A.D. 1993. Trends in genetic and environmental parameters in a multilocation clonal study with loblolly pine. Alabama A&M University, Normal, AL. Ph.D. Dissertation. 57 p.

Russell, J.H. and W.J. Libby. 1986. Clonal testing efficiency: the trade-offs between clones tested and ramets per clone. Can. J. For. Res. 16:925-930.

Schutz, W.M. and R.L. Bernard. 1967. Genotype x environment interactions in the regional testing of soybean strains. Crop Sci. 7:125-130.

Shaw, D.V. and J.V. Hood. 1985. Maximizing gain per effort by using clonal replicates in genetic tests. Theor. Appl. Genet. 71:392-399.

Shelbourne, C.J.A. 1972. Genotype-environment interaction: its study and its implication in forest tree breeding. In Proceedings of Joint Symposia for the Advancement of Forest Tree Breeding of the Genetics Subject Group, IUFRO, and Section 5, Forest Trees, Society for the Advancement of Breeding Researchers in Asia and Oceania (SABRAO). Government Forest Experiment Station of Japan. Tokyo, October 5-20, 1972. pp. 1-28.

Smalley, G.W. and D.R. Bower. 1968. Volume tables and point-sampling factors for loblolly pines in plantations on abandoned fields in Tennessee, Alabama, and Georgia highlands. USDA For. Serv. Res. Paper SO-32. 13p.

Snedecor, G.W. and W.G. Cochran. 1980a. Normal distribution. In Statistical Methods. The Iowa State University Press. Ames, IA. pp 39-63.

Snedecor, G.W. and W.G. Cochran. 1980b. Two-stage sampling. in Statistical Methods. The Iowa State University Press. Ames, IA. pp 450-453.