CLONAL VARIATION IN ROOTING ABILITY OF VIRGINIA PINE

G.F. Brown[1], Q. Holifield[1], J.L. Ford-Logan[2], and G.S. Foster[2]

Abstract.--Twenty-four cuttings were taken from each of 25 clones of three-year-old Virginia pines (Pinus virginiana, Mill.) previously selected for superior qualities as Christmas trees. The cuttings were arranged in a randomized complete block design with four replications and six cuttings per replication. All cuttings were treated with Hare's rooting powder and placed in a mist greenhouse at International Forest Seed Company (IFSCO) in Odenville, AL on March 6, 1990. After 5 months, all cuttings were moved to Alabama A&M University, where they were evaluated. Rooting percentages ranged from 0 to 79 percent, with highly significant differences among the 25 clones. The overall rooting percentage was 40 percent, with nine clones having better than 50% rooting.

To test the effect of a different cutting date, cuttings from 25 clones (10 clones repeated from the first experiment and 15 new clones) were taken on 3 August, 1990 and placed in the greenhouse at IFSCO. After 4 months, the cuttings were evaluated. Results of this experiment were then compared to the first experiment. The final objective is to select those clones that maximize both quality for Christmas tree production and rooting success. Selections will be used for the establishment of a new cutting orchard.

Additional Keywords: Pinus virginiana, vegetative propagation, cutting dates

INTRODUCTION

Virginia pine (Pinus virginiana Mill.) comprises a large portion of the Christmas tree production in the southeastern U.S., with annual sales totalling over 20 million dollars, yet genetic improvement of the species is progressing slowly compared with the emphasis on other southern pines of timber value. Clonal propagation, a commonly used horticultural technique, provides an avenue to relatively rapid genetic improvement of a species as well as resulting in a much more uniform crop, a highly

[1] Department of Plant and Soil Science, Alabama A&M University, P.O. Box 1208, Normal, Alabama 35762

[2] USDA Forest Service Southern Forest Experiment Station, Alabama A&M University, P.O. Box 1208, Normal, Alabama 35762

The authors wish to thank Mr. Tom Caldwell, IFSCO for his assistance in rooting the cuttings and to the Alabama Universities/TVA Research Consortium for funding this project.

desirable feature in Christmas tree plantations. Production of genetically superior clones of Virginia pine emphasizing traits such as stem straightness, growth rate, and crown form, could represent a major advancement of the species for Christmas tree production.

Currently, cloning procedures which entail rooting of stem cuttings are the most cost effective methods available for forest tree species. An efficient rooting procedure is probably the most crucial factor in the entire production system. Genetic factors are known to control adventitious root formation in virtually all tested tree species (Foster 1990), and selection of superior clones must include rooting ability traits as well as growth traits (Foster et al. 1985). A single rooting trial with Virginia pine has been reported in the scientific literature, and it only assessed cultural factors governing adventitious root formation (Snow and May 1962). Knowledge is needed on which to base decisions regarding the strength of genetic control of rooting ability in Virginia pine in order to guide the design of clonal tree improvement programs.

The objectives of the current Virginia pine study were to:

(1) assess the degree of genetic control of rooting traits, and

(2) determine the reliability of clonal ranking for rooting ability.

MATERIALS AND METHODS

The clones tested in this study originated from within the Virginia pine tree improvement program being conducted at Alabama A&M University. Sample trees (ortets) were chosen at random from among second generation select trees in half-sib progeny tests of first generation select parents. No selection had been practiced for rooting ability. The ortets were located near Huntsville, Alabama.

Twenty-five trees were chosen on each of two dates during the second field season of the genetic test, hence the trees were 2 and 2-1/2 years old from seed, respectively. Trees selected at the second date included 10 of the same trees as in the first collection and 15 new trees. Rooting Trials 1 and 2 were initiated on March 6, 1990 and August 3, 1990, respectively.

The cuttings in both trials were rooted at International Forest Seed Co. (Odenville, Alabama) using their standard rooting procedure (Hughes 1987; Foster 1990). Stem cuttings were collected in the field, stored in ice chests, and transported to the greenhouse. Each cutting was standardized to a length of three inches, and each cutting had an intact tip bud. The basal 1 cm of the cutting was moistened with water and then dipped into Hare's (1974) talc-based rooting powder. Once treated, the cuttings were set 2-3 cm deep in 93 cm^3 plastic pots containing a 2:1 mixture of shredded peat and coarse perlite. Subsequently, the trays of cuttings were placed in a

greenhouse with intermittent fogging and irrigation (Hughes 1987). A randomized complete block design was used with clones as treatments and four replications of six cutting plots. All effects were considered to be random.

The cuttings were evaluated for rooting after four months in the greenhouse rooting environment. The number of rooted cuttings per plot was assessed on a plot basis.

Two separate analyses of variance were conducted. In the first, each rooting trial was analyzed separately using the form of analysis given in Table 1. The second analysis included only the ten clones common to both trials and used the form of analysis given in Table 2. Sums of squares were calculated using a least squares approach (PROC GLM, Type III, SAS Institute Inc. 1985) due to some imbalance in the data arising from unequal rooting among clones. Variance components were calculated by equating mean squares with expected mean squares (Kempthorne 1969). Coefficients of the variance components were adjusted for the data imbalance (Hartley 1967; Goodnight and Speed 1978).

Table 1. Form of the analysis of variance for the number of rooted cuttings of Virginia pine clones.

Source of variation	Degrees of freedom	Expected mean squares
Clones (C)	24	$\sigma^2_e + 4\,\sigma^2_C$
Reps (R)	3	$\sigma^2_e + 25\,\sigma^2_R$
Error	72	σ^2_e
Total	99	

Table 2. Form of the analysis of variance for the rooting of Virginia pine clones in a multiple trial study.

Source of variation	Degrees of freedom	Expected mean squares
Trials (T)	1	$\sigma^2_e + 4\,\sigma^2_{TC} + 40\,\sigma^2_T$
Clones (C)	9	$\sigma^2_e + 4\,\sigma^2_{TC} + 8\,\sigma^2_C$
T * C	9	$\sigma^2_e + 4\,\sigma^2_{TC}$
Reps (TC)	60	σ^2_e
Total	79	

Reliability of clone ranking for rooting traits was assessed in two ways. The magnitude and statistical significance level of the clone x trial interaction (Table 2) provided one assessment. Spearman's rank correlation (Sokal and Rohlf 1969) for clone means between trials provided the other evaluation.

Level of genetic control for each trait was determined by calculating broad-sense heritabilities. Broad-sense heritability was calculated for each trial separately on both an individual ramet basis (H^2) and also on a clone-mean basis (H Ty Expected genetic gain was calculated as the product of broad-sense heritability on a clone mean basis and the selection differential.

$$H2 = \frac{0.2c}{0^2c + ate} \qquad \frac{a2c}{G\ 2c + 02e}$$

where,

$o2,$ = variance among clones

$o2,$ = error variance

n = number of ramets per clone

RESULTS AND DISCUSSION

The effect of clones on the rooting of Virginia pine cuttings was highly significant for both trials, and the estimated variance components were very similar (Table 3). In the second trial, the effect of replications was highly significant. The rooting success for the first replication was only 27 percent, compared to 50 to 53 percent success for the other three replications. No obvious reason for this difference could be deduced. Otherwise the results of both trials were very similar.

The general statistics for the experiment are presented in Table 4. The mean number of cutfngs which rooted from groups of six is presented in the first column. It was assumed that a seven point (from 0 rooted to all six rooted) binomial distribution closely approximated a normal distribution. Therefore, this value was used for the analysis of variance. However, it is usually easier to comprehend and use average rooting percentages. Consequently, the rest of Table 4 (except the heritability estimates) is expressed in rooting percentages.

Table 3. Analysis of variance for the number of rooted cuttings from two trials of Virginia pine clones.

		Trial 1			Trial 2		
Source of variation	Degrees of freedom	Mean square	Variance component	% of total variance	Mean square	Variance component	% of total variance
Clones	24	5.36**	0.87	30.85	4.34**	0.78	31.84
Reps	3	3.65NS	0.07	2.48	14.70**	0.54	22.04
Error	72	1.88	1.88	66.67	1.13	1.13	46.12

** Significant at P<.01
NS Non-significant at P>.05

Table 4. Means[a], rooting percentages[b], ranges[b], heritabilities and select clone percentages[b] for two trials of Virginia pine clones.

	Average Rooting						Best 5 clones
Variable	Mean	Percentage	Max	Min	H^2	$H^2_{\bar{x}}$	Mean
Trial 1	2.56	42.67	75.00	12.50	0.32	0.65	69.17
Trial 2	2.75	45.83	87.50	16.67	0.41	0.73	70.83

[a]Mean per 6 cuttings
[b]Percentage of all 24 cuttings

Both trials had almost the same rooting percentage (43 and 46 percent, respectively). There was a large range in rooting percentage with relatively good rooting in some clones and poor in others. As stated above, the effect of clones was highly significant, and the relatively high heritability estimates indicate a strong genetic component. If the best five clones from either trial were used, then we should expect approximately 62 percent success on our rooting efforts. These results are very encouraging and lend support to our efforts to select the clones which will maximize rooting success. If select clones can be identified which will yield a 50 percent rooting success rate, then the establishment of cutting orchards may be a feasible way of producing improved plantlets.

The objective of this project was to determine the degree of genetic control of rooting and to determine the reliability of clonal ranks for rooting ability. Because of the need to screen as many clones as possible while providing some measure of repeatability, each trial only had 10 clones in common.

The relative ranks of rooted cuttings from the repeated 10 clones of Trial 2 were correlated with the results of Trial 1, resulting in a non-significant Spearman's rank correlation of -0.11. The results of Trial 2 were not correlated with Trial 1. In fact, if we use the results of Trial 1 to predict the outcome of Trial 2 under the assumption that the same clones should have the same rooting success rate, then a Chi-square test can be computed to compare the frequency of rooting success between the two trials. This test resulted in a Chi-square value of 35.14 at 9 degrees of freedom. There was a highly significant difference in the frequency of cuttings of the 10 clones between the two trials. This result is not encouraging. It was hoped that once a clone was selected based on superior rooting ability, that clone would maintain its superiority in future cutting trials.

The reason for the lack of repeatability is not known. It could be because the clones change relative rankings between trials. A clone that produces a high number of rooted cuttings in March may not do as well in August, whereas a clone with poor rooting success in March may do well in August. If this is true, a cutting orchard established based on one test may not yield good results if cuttings are taken at a different time of year or with different conditions. Another explanation for the lack of repeatability is that the 10 clones used for this part of the study are not representative of the entire population. We would then be observing random error.

If the first case is true, we would expect a fairly large clone by trial interaction term in our analysis of variance. However, from Table 5 it can be seen that this interaction was non-significant, along with the effects of the trial and the clones. The error term was large and accounted for 70 percent of the total variation. The clones are apparently not just shifting ranks. Because of this, the data from only the repeated clones were reanalyzed per trial in accordance with the form of the analysis of variance given in Table 1, except with only 10 clones instead of 25. In these analyses, the effect of the 10 clones was not significant in either trial. The probability of obtaining a larger F-tes value was 0.11 in the first trial and 0.29 in the second trial. The randomly repeated 10 clones did not yield the same results as the larger group of 25 clones. With all 25 clones we obtained highly significant clonal effects which accounted for approximately 45.8% of the total variance (sums of squares clones divided by total sums of squares). The effect of the 10 clones was non-significant and accounted for approximately 25.4% of the total variance. The evidence indicates that the 10 clones used for this part of the study were not representative of the rest of the clones.

Table 5. Analysis of variance for the rooting of Virginia pine clones in a multiple trial study.

Source of variation	Degrees of freedom	Mean square	Variance component	% of total variance
Trial	1	6.61	6.61 NS	3.68
Clone	9	25.56	2.84NS	14.22
T*C	9	**22.26**	2.47NS	12.39
Reps (TC)		125.25	2.09	69.70
Total	79			

This area obviously needs further study and more repeated trials. Another trial utilizing 30 clones with 20 repeat clones has already been set at IFSCO during the spring of 1991. Another trial is planned for later this year. The final goal is to screen for rooting ability at least 100 clones previously selected for Christmas tree characteristics and to obtain enough data to sufficiently judge the reliability of clonal ranking for rooting ability.

CONCLUSIONS

There appears to be a strong genetic component of the rooting success of juvenile Virginia pines. The overall rooting success of both trials was over 40 percent. Forty percent of all clones tested had rooting success of 50 percent or more. Selection of the top 5 clones would result in rooting success of approximately 50 percent. The selection of those clones with the highest rooting ability will greatly increase the efficiency of the clonal propagation process. This procedure appears to be a viable method of prcduction of genetically superior clones of Virginia pine for Christmas trees.

However, the reliability of these results from one trial to another is still in question. The rooting success of ten clones repeated over two cutting trials was not significantly correlated in these trials. Because of a non-significant clone by trial interaction term and a non-significant effect of clones in a re-analysis of only the ten repeated clones, it is believed that the ten clones may not be representative of all clones. This area should be further studied to obtain better conclusions.

LITERATURE CITED

Cochran, W.G. 1951. Testing a linear relation among variances. Biometrics 7:17-32.

Foster, G.S. 1990. Genetic control of rooting ability of stem cuttings from loblolly pine. Can. J. For. Res. 20:1361-1368.

Foster, G.S., R.K. Campbell, and W.T. Adams. 1985. Clonal selection prospects in western hemlock combining rooting traits with juvenile height growth. Can. J. For. Res. 15:488-493.

Goodnight, J.H., and F. M. Speed. 1978. Computing expected mean squares. SAS Institute Inc., Cary, NC. Tech. Rep. R-102.

Hare, R.C. 1974. Chemical and environmental treatments promote 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.

Hughes, H.F. 1987. Cutting propagation of rust resistant hedges of Pinus taeda. Plant Propag. 1:4-6.

SAS Institute Inc. 1985. SAS user's guide: statistics, version 5. SAS Institute Inc., Cary, NC.

Snow, Jr., A.G., and C. May. 1962. Rooting of Virginia pine cuttings. J. For. 60:257-**258.**

Sokal, R. R., and F. J. Rohlf. 1969. Biometry. W. H. Freeman Co., San Francisco.