NATURAL VARIATION IN ROOTING ABILITY OF WESTERN PROVENANCES OF SHORTLEAF PINE

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Abstract.--Thirteen trees from each of 22 geographic sources across the five western states of shortleaf pine's range were sampled. Percent of cuttings rooted ranged from 11 for an Oklahoma source to 33 for a Louisiana source. No significant difference was observed for percent rooted among sources, although differences among trees in source were significant. The broad-sense heritability estimate for rooting ability was .26. Four root characteristics, total root weight, total root length, tap root length, and total root number, were studied on the rooted cuttings. No significant differences among sources were found for these traits but differences among trees in source were significant. Root mass per unit root length was significant for sources, suggesting geographic differences in root structure. Results suggest considerable genetic gains in rooting ability are possible through selection, and that origin is probably not important in such a selection program.

Additional keywords: Vegetative propagation, geographic variation, root characteristics, clonal selection, broad-sense heritability, Pinus echinata.

Because of the interest in and usefulness of vegetative propagation as a tool for tree improvement, geographic and clonal variation in rooting ability need to be assessed on a species to species basis. Rooting ability is defined for this paper as the ability of vegetative cuttings to develop roots and is measured as percent rooted. This ability within a species may well be as variable as are the many other traits already described for various tree species. Effective selection for a plant character can be achieved only if genetic variation for that trait exists within the population of interest. If sufficient amounts of either geographic and/or tree to tree variation in rooting ability exists, and if rooting ability is sufficiently heritable, considerable improvement in rooting ability could be achieved through a selection program.

Since environmental conditions in the field are seldom optimum for establishment, favored clones would be those that produce fibrous, spreading root systems, assumed to be highly effective in water and nutrient uptake. In fruit trees it has been found that the unique structure and functioning of the understock of any given genotype has an influence on almost every characteristic of the composite plant (Rogers and Beakbane

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1957). If structural differences in root systems exist and are sufficiently heritable, these differences need to be assessed and procedures for evaluating them incorporated into selection programs.

To evaluate the rooting ability and physical characteristics of the roots of vegetative propagules in shortleaf pine (*Pinus echinata* Mill.) a study was initiated to examine both the geographic and tree to tree variation in those characteristics.

MATERIALS AND METHODS

Materials for the rooting trial were collected from 22 geographic sources across the five western states of shortleaf pine's natural range (Figure 1). At each location ten cuttings were collected from each of 13 ortets, making a total of 130 observations per source. For collection, cuttings had to be at least six inches long, and the ortet had to be five to eight years old. Collection began June 3 and lasted for 10 days. The stage of development of the first flush was deemed ideal for rooting at this date, as described by Reines and Bamping (1960).

During collection, the cuttings were stored and transported in an upright position in cold storage boxes. The boxes consisted of two horizontally placed, stripped-down refrigerator bodies. The inside of the refrigerators were fitted to support metal trays which held bags of ice four to six inches above the cuttings. Holes were drilled in each box to provide drainage and a six inch layer of perlite and vermiculite (50/50 mix) was added. The ice kept the air temperature around the cuttings near 40 degrees Fahrenheit, and the melting provided moisture for the cuttings.

At the study site the cuttings were placed in a mist room in a completely randomized design using a completely random planting sequence. Prior to potting, each cutting's basal one inch of needles were stripped off and the base was dipped in a rooting hormone (Hotmodin 3, 0.8 ppm IBA) mixed with 25 percent by wt. captan powder (50 percent active ingredient). The cuttings were then placed in 2.5 inch square, 4 inch deep pots which contained a 50-50 mix of perlite and vermiculite. The pots were placed on a single large table which had a 2.5 inch layer of the same medium, underlain with heating cables which provided a bottom heat of approximately 79 degrees Fahrenheit. The table was enclosed in a polyethylene covered room which was 15 x 26 feet.

Inside the mist room ten florescent lights (two 40 watt bulbs/light) were used to extend the daylength period to 16 hours. A minimum of 50 foot-candles was provided during the dark hours of the artificial photoperiod.

Cooling was provided by a 12 inch fan located in the center of one of the side walls and an air inlet was located in the center of the opposite wall. Cool air was provided by the greenhouse cooling system.

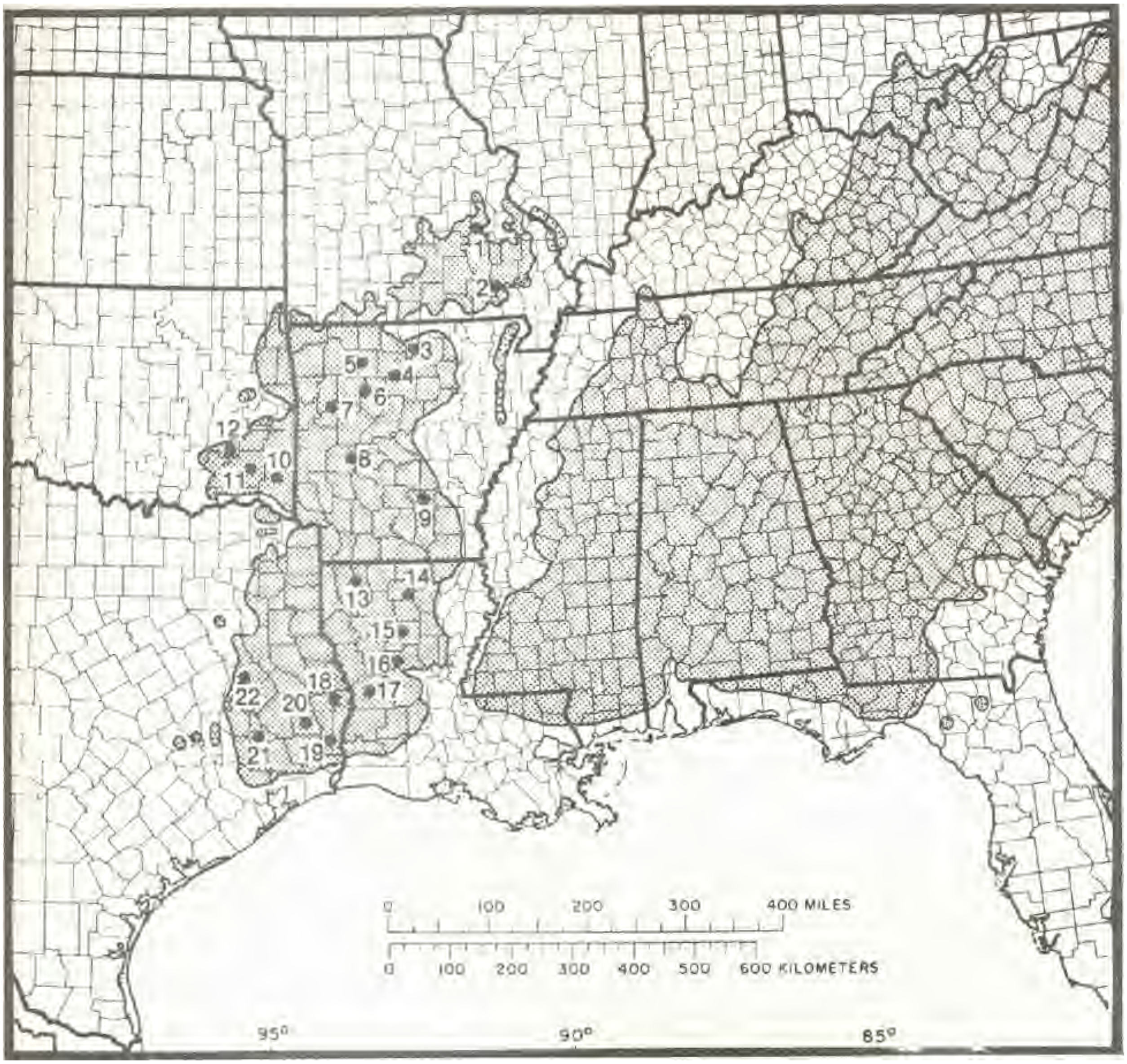

Figure 1.--Geographic location of collection points and western range of shortleaf pine.

The mist system consisted of 24 0.020 x 1/8 inch foggers. The nozzles were mounted on three lines spaced 47 inches apart, with the nozzles spaced at 32 inch intervals. The lines were suspended 20 inches above the cuttings. The nozzles were activated two ways: (1) When the thermostat turned on the chamber fan, it also activated the time clock that controlled the nozzles. The time clock activated the nozzles six seconds out of every six minutes (2.7 mm water per hour). (2) When the fan did not operate on cool days a separate time clock activated the spray for six seconds every two hours. These conditions provided an average relative humidity of 90 percent and an average daily temperature of 75 degrees Fahrenheit directly around the cuttings. After planting, a modified

Hoagland's solution (van Buijtenen, et al. 1975) was applied as a foliar drench nutrient additive once a day by hand spraying.

The following measurements and information were recorded during the planting phase:

(1) Initial cutting length (measured to nearest 0.1 centimeter)

(2) Initial cutting green weight (measured to nearest 0.1 gram)

(3) Presence of last years growth (yes or no)

(4) Time factor (number of days between collection and first day of planting)

The cuttings remained in the mist room for 106 days (June 14, 1980 to September 29, 1980). Spot checking the border row cuttings after 100 days indicated that the cuttings had been in the mist room long enough to allow sufficient root development for measurement of the characteristics of interest. Van Buijtenen, et al. (1975) reported that rooting percentages increased up to 14 weeks (98 days) then tapered off at an increasing rate.

After 106 days the propagules were lifted in the same order as potted, so as to equalize as best as possible, the number of days each cutting remained in the mist room. It took five days to pot the cuttings and four days to remove them. As each cutting was lifted it was classified according to its physiological state; dead, alive, callused or rooted. Additional data recorded following lifting were:

(5) Total number of roots (each root 0.5 centimeters or longer was tallied)

(6) Tap root length (measured to the nearest 0.1 centimeter)

(7) Total root length (measured to the nearest 0.1 centimeter and including tap root length)

(8) Root dry weight (measured to nearest .001 gram)

(9) Rooted cutting stem dry weight (measured to nearest .001 gram)

Measurements taken on the cuttings prior to planting were used to look for possible relationships between characteristics of the cuttings and percent rooted and root structure. Percent rooted and root characteristics measured were examined by analysis of variance to test for source and ortet in source differences and to estimate the broad-sense heritability of these traits (Table 1).

Table 1.--Method of analysis of variance and heritability estimation for percent rooted and root measurements.

Source of Variation	d.f.	Expected Mean Squares*
Source	$s-1$	$\hat{\sigma}^2_{c/o/s} + K_2\hat{\sigma}^2_{o/s} + K_3\hat{\sigma}^2_{s}$
Ortet in source	$\sum_{i=1}^{s} (o_i-1)$	$\hat{\sigma}^2_{c/o/s} + K_1\hat{\sigma}^2_{o/s}$
Cutting in ortet in source	$\sum_{i=1}^{s} \sum_{j=1}^{s_i} (n_{ij}-1)$	$\hat{\sigma}^2_{c/o/s}$

$$h^2_{bs} = \frac{\hat{\sigma}^2_{o/s}}{\hat{\sigma}^2_{c/o/s} + \hat{\sigma}^2_{o/s}}$$

* K_1, K_2, K_3 are respective coefficients of the expected mean squares.

RESULTS AND DISCUSSION

Of the 2,860 cuttings planted, 20.5 percent rooted, providing information on all 22 sources. More than half of the 286 ortets produced at least one rooted cutting. Figure 2 presents the percent and number of cuttings observed in each of the four physiological categories recognized at the termination of the study. The analysis of variance for percent of cuttings rooted and percent of ortets rooted showed no significant source differences. However, significant differences occured among ortets within sources for percent of cuttings rooted. Table 2 presents the percent of cuttings and ortets rooted, by source.

For comparison purposes, percent of cuttings rooted by state were computed. The order in which the states ranked, based on percent of cuttings rooted, was Oklahoma 14, Missouri 18.5, Arkansas 19.7, Texas 21, and Louisiana 25.8 percent. The order in which the states ranked based on percent of ortets producing at least one rooted cutting were Oklahoma 30.7, Arkansas 49.4, Missouri 50, Texas 55.5, and Louisiana 64.6. A trend from north to south seems to be evident when looking at these rooting values, but an analysis of rooting ability by state instead of source indicated that these differences were again not significant. One possible reason that this apparent geographic trend in rooting ability was nonsignificant is the hierarchical structure of the nested procedure of analysis. The important point, however, is that clonal variation in rooting ability is considerable, while source variation is relatively unimportant.

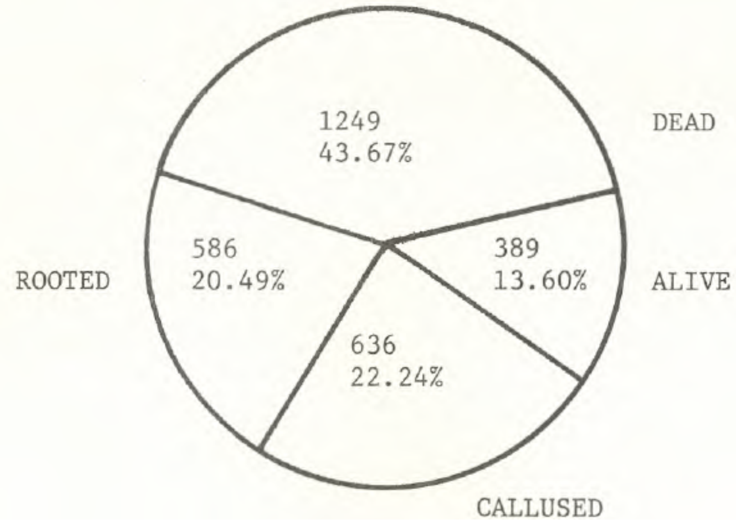

Figure 2.--Physiological state of cuttings at conclusion of study. (Percentages based on a total of 2860 cuttings).

The high proportion of alive and callused but unrooted cuttings (Figure 2) suggest the mist regime or some other unknown factor provided a suboptimal rooting environment. Under optimum rooting conditions, such a large number of living unrooted cuttings might not be expected.

Greenwood et al. (1980) reported, in an article published at the conclusion of this study, that the optimum mist regime for loblolly and shortleaf pine cuttings was .05 to .10 millimeters of water per hour. They reported that the amount of mist applied was the most critical factor of the environment affecting rooting. We applied approximately 2.7 millimeters per hour.

If an optimum environment had been present many of the callused cuttings might have rooted. An analysis of the combined data, cuttings callused plus cuttings rooted, showed that source differences were not significant and that tree in source differences were significant. This is the same conclusion as that reached when examining only the percent rooted data.

Table 3 presents the observed mean squares and F tests for the root characteristics measured. None of the four characteristics measured (tap root length, total root length, total root number, and root dry weight) were significantly different over sources. All four root characteristics were significantly different for ortets in sources. These results suggest that the root system and structure of pine cuttings can be modified through clonal selection for any of the four root characteristics measured.

The four stem characteristics recorded (initial cutting length, initial cutting green weight, presence of old growth, and rooted cutting stem dry weight) were of interest in terms of their relation to the

Table 2.--Percent of cuttings and ortets rooted, by source.

Source		Percent of Cuttings Rooted	Percent of Ortets Rooted
MISSOURI	1	20.8	53.9
MISSOURI	2	16.2	46.2
ARKANSAS	3	13.8	53.9
ARKANSAS	4	16.9	46.2
ARKANSAS	5	17.7	46.2
ARKANSAS	6	13.8	30.8
ARKANSAS	7	18.5	38.5
ARKANSAS	8	29.2	69.2
ARKANSAS	9	28.5	61.5
OKLAHOMA	10	11.5	30.8
OKLAHOMA	11	17.7	38.5
OKLAHOMA	12	12.3	23.1
LOUISIANA	13	16.9	53.9
LOUISIANA	14	20.8	46.2
LOUISIANA	15	33.1	92.3
LOUISIANA	16	26.2	61.5
LOUISIANA	17	31.5	69.2
TEXAS	18	23.1	53.9
TEXAS	19	16.2	38.5
TEXAS	20	20.0	53.9
TEXAS	21	23.1	69.2
TEXAS	22	23.1	61.5
Overall percent rooted		20.5 ± 3.5	51.8 ± 13.1

Table 3.--Observed mean squares and F tests for all characters over all sources.

SOURCE	d.f.	TOTAL ROOT LENGTH	F	TOTAL ROOT NUMBER	F	ROOT DRY WEIGHT	F
TOTAL	585	469.42		66.82		0.00248	
SOURCE	21	1505.67	1.39	199.34	1.38	0.00615	1.16
ORTET/SOURCE	127	1076.16	4.45*	144.07	3.79*	0.00528	3.54*
CUTTING/ORTET/SOURCE	437	241.95		37.99		0.00149	

SOURCE	d.f.	TAP ROOT LENGTH	F	ROOT UNIT WEIGHT	F
TOTAL	585	10.35		0.561×10^{-5}	
SOURCE	21	19.41	0.82	0.281×10^{-4}	3.07*
ORTET/SOURCE	127	23.71	3.93*	0.914×10^{-5}	2.60*
CUTTING/ORTET/SOURCE	437	6.03		0.351×10^{-5}	

SOURCE	d.f.	% ROOTING BY STATE	F	SOURCE	d.f.	% ROOTING BY LOCATION	F
TOTAL	2859	0.163		TOTAL	2859	0.163	
STATE	4	0.914	1.72	SOURCE	21	0.489	0.90
ORTET/STATE	281	0.531	4.36*	ORTET/SOURCE	264	0.540	4.44*
CUTTING/ORTET/STATE	2574	0.122		CUTTING/ORTET/SOURCE	2574	0.122	

*Significant at the .05 level of probability of lower.

percent of cuttings rooted and characteristics of the rooted cuttings. All correlations discussed except those including the variables percent rooted or presence of old growth are genetic correlations. Initial cutting green weight was not correlated with percent rooted. Initial cutting length was significantly and positively correlated with percent rooted (r = 0.59). Since the southern origins tended to provide longer cuttings, this correlation may explain the apparent slight geographic trend in percent rooted. The correlation also suggests that collection of longer cuttings could increase rooting success.

No significant correlations of initial cutting green weight and rooted cutting stem dry weight with the four root characteristics were found. Initial cutting length had a low but significant negative correlation (r = -0.15) with tap root length. Since most research with vegetative propagation utilizes a standardized cutting length that ranges from one to ten centimeters shorter than the cuttings used in this study, and since the correlation was so low, the effects of adjusting cutting length to favor tap root length would be minimal at best. Cutting length was not correlated with any other root characteristic examined.

All correlations between the presence of old growth and the four root characteristics measured were nonsignificant. Old growth was also uncorrelated with percent rooted, indicating that the inclusion of old growth as part of the cutting material was not important.

Seventy three percent of the cuttings that rooted ranged between .22 and .69 g/cm for cutting unit weight (cutting green weight per unit cutting length). The use of cuttings in this weight to length ratio range might facilitate mass production of rooted material.

The root mass per unit root length ratio was computed from total root weight and total root length. The root mass per unit length ratio was found to be significant both for sources and ortets within sources. Since it was apparent the ratio might reflect different developmental stages of the root systems among ortets, an analysis using simple linear regression was applied to the variables to examine the data further. Regression lines of root dry weight on total root length were estimated for each of the 22 sources. Had the slopes of the regression lines for each source been parallel (no significant slope differences), the variable (ratio) would have been interpreted as indicating differences in developmental stages of the roots. However, since significant differences in the slopes of the 22 regression lines were observed, the ratio suggest real source differences in structural characteristics of the roots. Some of the root systems formed tended to have small fibrous roots while others exhibited larger diameter, non-fibrous roots. If these root differences carry over into the field, both geographic and clonal selection might be used to develop clones with more fibrous root systems.

When the percent of rooted cuttings was correlated with the length of time in storage, an unexpected, high possitive correlation (r = 0.52) was observed. Cuttings that were stored longer rooted better, but length of storage may not have been the cause. There may have been phenological differences in the cuttings across the sample range at the time of collection. With the exception of two Oklahoma sources, which had time factor values of one day, the next shortest time factor values (three days to five days) were represented by the two Missouri sources and Arkansas sources three, four, and five, which were also the five northern most sources. Sources one through five would undoubtedly be composed of the least mature cutting material and if lack of maturity resulted in reduced rooting the observed correlation could result. The correlation could also reflect a slight geographic trend in rooting ability, or the relationship between initial cutting length (with a north to south difference) and rooting ability. However, since there is no conclusive data concerning the effects of storage on the rooting of cuttings, the possibility exists that a few days in storage increases rooting percentages.

Broad-sense heritability estimates for all of the root characteristics measured are given in Table 4.

Table 4.--Broad-sense heritability estimates for percent rooted and root characteristics.

TRAIT	ESTIMATE	STANDARD ERROR
Root Mass Per Unit Root Length	.26	±.049
Tap Root Length	.43	±.047
Total Root Length	.46	±.046
Root Dry Weight	.40	±.048
Total Root Number	.41	±.048
Percent Rooted	.26	±.022

All heritability estimates were generally high and it appears that some reasonable gains can be obtained by selecting within populations for more fibrous or spreading root systems. Development of such propagules should result in the production of individuals which have a greater ability to survive and utilize soil nutrients and moisture. The broad-sense heritability estimate for percent rooted was also reasonably high and suggests that rooting ability can be selected for and used as a tool in tree improvement.

CONCLUSIONS

Examination of geographic and clonal variation in percent of stem cuttings rooted of shortleaf pine indicates that genetic improvement in rooting ability is possible through clonal selection, and that origin is probably not important. However, analysis of the mass per unit length ratio of the roots suggested that there are differences in root structure from one location to the next which might make origin important in terms of root structure. The importance and/or usefulness of these differences has yet to be determined and only through field testing of selected clones can reliable information on the effects of these structural differences on survival be quantified.

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