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Shoot Production and Rooting Ability of Cuttings From Juvenile Greenhouse Loblolly Pine Hedges

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ABSTRACT

Rooted cutting technology to produce loblolly pine (*Pinus taeda* L.) planting stock for reforestation is currently being explored as a complement to seedling propagation from a seed orchard program. Since both genetic and non-genetic factors are likely to influence rooting efficiency, an understanding of these influences is important in order to develop a successful rooted cutting program. The objective of this study was to determine the genetic control of shoot production and rooting ability of greenhouse-grown loblolly pine hedges over several rooting cycles. Results of this investigation indicate that genetic control of rooting ability is strong. Selection for high rooting percentages should prove to be a useful strategy for advancing the vegetative propagation of loblolly pine.

INTRODUCTION

Rooted cutting technology to produce loblolly pine (*Pinus taeda* L.) planting stock for reforestation is currently being explored as a complement to seedling propagation from a seed orchard program (Frampton and Hodges, 1989; Foster and Shaw, 1987; Hughes, 1987). Many potential benefits of this technology have been recognized including increased genetic quality and uniformity of the planting stock deployed (Frampton and Hodges, 1989; Libby, 1985; Zobel and Talbert, 1984).

Since both genetic and non-genetic factors are likely to influence rooting efficiency, an understanding of these influences is important in order to develop a successful rooted cutting program. Thus, the objective of this study was to examine the genetic control of shoot production and rooting ability of greenhouse-grown loblolly pine hedges over several rooting cycles. The results of this study are compared to those from similar studies previously reported (Foster, 1978; Foster, 1990).

MATERIALS AND METHODS

Production and Rooting of Cuttings

During December 1988, ten seeds from each of nine full-sib families arranged in a 3 x 3 factorial mating design were germinated. Throughout the duration of the study, these seedlings were grown in 5.36 l pots in the greenhouse and maintained as hedges in order to increase the number of shoots usable as cuttings and to delay the maturation process. Each tree was hedged 6 times (6 cycles, Table 1) to a height of approximately 45 cm. Six to eight weeks after each hedging, the number of shoots greater than 7 cm in length was assessed.

At the same time shoot numbers were assessed, cuttings approximately 7 cm in length were collected from shoots 10 cm or greater in length. Cuttings were selected at random from all available shoots on a tree. These cuttings were set into 3.1 x 3.1 x 6.3 cm cells of Styrofoam flats® (Todd Planter Flats) filled with a 5:3:1 mixture by volume of perlite, sand, and peat. Cuttings were not treated with auxin. Flats of cuttings were placed in an air-conditioned, polyethylene-covered mist bench maintained at 28 ± 5ºC. Cuttings were assessed for rooting at approximately two week intervals for 16 to 20 weeks starting with the first indications of rooting. Twelve to 16 weeks after setting, most rooting was completed and cuttings were transferred to an open mist bench.

Experimental Design and Data Analyses

In each of the rooting cycles, clonal plots were arranged in a completely randomized design within the rooting bench. Each plot consisted of 8 to 32 cuttings and each clone was represented by 2 to 4 plots per rooting cycle. Analyses of variance (Table 2) were performed on the number of shoots produced per hedge and on plot rooting percentages. Shoot production data were available for all 6 cycles. Rooting percentages were only available by family and clone for cycles 2 through 5. Cuttings produced after the first hedging were used in another study in which tree identification of cuttings was not maintained.

Prior to conducting the analysis of variance, data were checked to determine whether the assumptions for the analyses of variance were fulfilled. Untransformed data appeared to fulfill the assumptions as well as the transformed data. All further analyses were conducted using the untransformed data.

Variance components for all effects were estimated using the VARCOMP procedure of SAS (SAS Institute Inc., 1985). The percentage that each effect's variance component contributed to the sum of all the variance components was calculated. Total genetic variance (V_G), phenotypic variance (V_P), additive genetic variance (V_A), dominance genetic variance (V_D), and epistatic genetic variance (V_I) were calculated using methods described by Foster (1990):

$$V_G = \sigma^2_M + \sigma^2_F + \sigma^2_{MF} + \sigma^2_{C(MF)}$$

$$V_P = V_G + \sigma^2_{TF} + \sigma^2_{TM} + \sigma^2_{TFM} + \sigma^2_E$$

$$V_A = 2(\sigma_F^2 + \sigma_M^2)$$
$$V_D = 4(\sigma_{FM}^2)$$
$$V_I = \sigma_{C(MF)}^2 - (\sigma_F^2 + \sigma_M^2) - 3(\sigma_{MF}^2)$$

Narrow-sense heritability (h^2), narrow-sensed heritability based on full-sib family means (h^2_F), broad-sense heritability (H^2), and broad-sensed heritability based on clone means (H^2_C) were estimated using the following formulae:

$$h^2 = \frac{2(\sigma_M^2 + \sigma_F^2)}{V_P}$$
$$H^2 = \frac{V_G}{V_P}$$
$$h_F^2 = \frac{\sigma_F^2 + \sigma_M^2}{\sigma_M^2 + \sigma_F^2 + \sigma_{MF}^2 + \frac{\sigma_{C(MF)}^2}{c} + \frac{\sigma_{TF}^2}{t} + \frac{\sigma_{TM}^2}{t} + \frac{\sigma_{TMF}^2}{t} + \frac{\sigma_E^2}{tc}}$$
$$H_C^2 = \frac{V_G}{V_G + \frac{\sigma_{TF}^2}{t} + \frac{\sigma_{TM}^2}{t} + \frac{\sigma_{TMF}^2}{t} + \frac{\sigma_E^2}{tc}}$$

Table 2 provides definitions of formula components.

Total genetic correlation (r_G) and additive genetic correlation (r_A) between shoot production and rooting percentages of juvenile loblolly pine were calculated for Cycles 2 through 5 (Falconer; 1989).

RESULTS

Shoot Production

Shoot production of greenhouse hedges is illustrated in Figure 1. There was a large increase in the number of shoots produced after the first cycle with little change thereafter. Variance among hedging cycles accounted for 41.7% of the total variation in shoot production (Table 3).

Total shoot production over the first 6 cycles among half-sib and full-sib families ranged from 197 to 263 and 181 to 291, respectively (Table 4). Variation among female but not male parents was statistically significant. Male and female sources of variation accounted for 0.0% and 9.7% of the total variation, respectively. Male x female variation was not statistically significant and accounted for only 3.1% of the total variation. Figure 2 shows shoot production over time by family means.

Significant variation occurred among trees within families. Among-tree variation accounted for 11.9% of the total variation. Correlations between hedge cycles for shoot

production ranged from 0.36 to 0.61 and were statistically significant ($p<.05$) (Table 5). Family by hedging cycle variance (genotype by environment interaction) was significant, but only accounted for 2.1% of the total variation.

Heritability and variance estimates for shoot production of juvenile hedges are listed in Table 6. Additive genetic variance was 1.5 times as large as dominance genetic variance. Shoot production of loblolly pine was moderately controlled by additive genetic effects. Improved selection for shoot production is possible by selecting on family means or clone means.

Rooting Ability

Overall rooting percentages steadily decreased throughout the study from a high of 75% to 28% for cycles 1 through 5. Variance among rooting cycles accounted for 11.1% of the total variation in rooting (Table 3) for cycles 2 through 5.

Rooting percentages ranged from 19.4% to 40.8% among half-sib families and 11.5% to 55.3% among full sib-families (Table 4). Neither variation among female nor male parents was statistically significant. Male and female sources of variation accounted for 0.0% and 13.7% of the total variation, respectively. Male x female variation was statistically significant and accounted for 21.8% of the total variation. Figure 2 shows average percent rooting by family for rooting cycles 2 through 5.

Clonal rooting percentages ranged from 0% to 100%, 0% to 93%, 3.2% to 100%, and 0 to 75% within cycles 2 through 5, respectively. Significant variation occurred among clones within families. Among-clone variation accounted for 23.8% of the total variation. Correlations between rooting cycles for clonal rooting percentage ranged from 0.53 to 0.69 and were statistically significant ($p<.05$) (Table 5). Family by rooting cycle variance (genotype by environment interaction) was significant, but accounted for only 2.7% of the total variation. Variation in rooting among plots was the largest source of variation, accounting for 28.8% of the total variation. However, this proportion was considerably less than the 46% and 70% reported by Foster (1990) and Foster (1978).

Heritability and genetic effect estimates for rooting percentages are listed in Table 6. For purposes of comparison, results from Foster (1978) and Foster (1990) are also included in Table 6.

Additive genetic variation was only 1/3 of dominance genetic variation. This finding agrees with Foster's (1978) finding of nonadditive genetic variance 2.2 times as large as additive genetic variance for loblolly pine. However, Foster (1990) found additive genetic variance to account for almost all of the total genetic variance in loblolly pine.

Relationship between Rooting Ability and Shoot Production

Additive and total genetic correlation between shoot production and rooting of cuttings from juvenile hedges was -0.88 and -0.32 respectively. While the number of shoots produced and percent rooting of clones for the cycles 2 through 5 were negatively correlated, only the correlations in Cycles 2 and 3 cycles were significant. Based on the results of this study, selection for rooting may have an adverse impact on the shoot production of hedges.

DISCUSSION AND SUMMAY

Genetic control of rooting ability in loblolly pine appears to be strong. Selection for rooting ability both among and within families could provide substantial increases in rooting percentages. Screening and selection for rooting ability would be an obvious way to enhance the efficiency of a rooted cutting system. However, selection for rooting ability may constrain the improvement possible for growth and disease resistance.

Performance levels for productivity traits of the nine full-sib families used in this experiment are listed in table 7. Performance levels are used by the North Carolina State University Tree Improvement Cooperative to determine the relative worth of specific crosses in a tree improvement program (Hatcher et al, 1981). Family 27-5x27-6 is the best for growth, family 27-4x27-6 is the best for fusiform rust resistance, and family 27-4 x 27-2 is the best for both growth and rust resistance combined. However, none of these three families are among the best for rooting ability. Families 27-3x27-1 and 27-6x27-1 are the two best for rooting ability, but neither have exceptional performance levels for growth or rust resistance. With only six parents and nine full-sib families (3 x 3 factorial mating), it is not possible and was not the intent of this study to select full-sib families with outstanding performance levels for productivity traits (growth and disease resistance) that also have high rooting percentages. However, data from this study illustrates the potential reduction in selection intensity for growth traits associated with culling for rootability (Table 8).

Results of this investigation confirm that genetic control of rooting ability is strong. Selection for high rooting percentages should prove to be a useful strategy for advancing the vegetative propagation of loblolly pine.

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Table 1. Hedging and setting dates of loblolly pine hedges and cuttings.

Cycle	Hedging Date	Setting Date	Average Number Cuttings per Hedge
1	4/89	6/89	14.4
2	6/89	8/89	50.2
3	12/89	2/90	39.6
4	4/90	5/90	35.3
5	5/90	7/90	39.4
6	7/90	8/90	44.4

Table 2. Form of the analysis of variance for rooting percentages of loblolly pine cuttings. The clone(F*M) source becomes hedge(F*M) for the shoot production trait.

Source	Expected Mean Squares
Cycle (T)	$\sigma^2 + c\,\sigma^2_{TFM} + mc\,\sigma^2_{TF} + fc\,\sigma^2_{TM} + fmc\,\sigma^2_{T}$
Female (F)	$\sigma^2 + c\,\sigma^2_{TFM} + t\,\sigma^2_{C(FM)} + ct\,\sigma^2_{FM} + mc\,\sigma^2_{TF} + cmt\,\sigma^2_{F}$
Male (M)	$\sigma^2 + c\,\sigma^2_{TFM} + t\,\sigma^2_{C(FM)} + ct\,\sigma^2_{FM} + fc\,\sigma^2_{TM} + cft\,\sigma^2_{M}$
F*M	$\sigma^2 + c\,\sigma^2_{TFM} + t\,\sigma^2_{C(FM)} + ct\,\sigma^2_{FM}$
T*F	$\sigma^2 + c\,\sigma^2_{TFM} + cm\,\sigma^2_{TF}$
T*M	$\sigma^2 + c\,\sigma^2_{TFM} + cf\,\sigma^2_{TM}$
T*F*M	$\sigma^2 + c\,\sigma^2_{TFM}$
Clone(F*M)	$\sigma^2 + c\,\sigma^2_{C(FM)}$
Error	σ^2

where: c = # clonal plots per cycle, f = # females, m = # males, t = # cycles
σ^2_T = variance due to cycle effect
σ^2_F = variance due to female effect
σ^2_M = variance due to male effect
σ^2_{FM} = variance due to interaction of female and male effects
σ^2_{TF} = variance due to interaction of cycle and female effects
σ^2_{TM} = variance due to interaction of cycle and male effects
σ^2_{TFM} = variance due to interaction of cycle, female, and male effects
$\sigma^2_{C(FM)}$ = variance due to clone within female by male effect
σ^2 = error or plot to plot variance

Table 3. Degrees of freedom, mean squares, significance levels, variance components, and percent of total variation of shoot production and rooting percentages of loblolly pine cuttings.

Source	Degrees Freedom	Mean Squares	Variance Components	% of Total Variation
		Shoot Production		
Cycle (T)	5	13499.9*	142.4	41.7
Female (F)	2	7385.0*	33.1	9.7
Male (M)	2	1181.8	< 0.0	0.0
F*M	4	1033.4	10.7	3.1
T*F	10	621.9*	6.9	2.0
T*M	10	360.4	15.7	4.6
T*F*M	20	155.4*	7.1	2.1
Hedge(F*M)	80	329.7*	40.8	11.9
Error	400	85.2	85.2	24.9
Total	533			
		Rooting Percentage		
Cycle (T)	3	4541.0*	42.6	7.0
Female (F)	2	16529.2	834.0	13.7
Male (M)	2	5005.4	< 0.0	0.0
F*M	4	6205.4*	132.9	21.8
T*F	6	640.3	10.1	1.6
T*M	6	430.2	3.1	0.5
T*F*M	12	338.6*	16.3	2.7
Clone(F*M)	81	755.7*	145.8	23.8
Error	241	176.3	176.3	28.8
Total	356			

* = signficant at p<.05 level

Table 4. Average and clonal range (in parenthesis) of shoot production and rooting percentage of loblolly pine cuttings across rooting trials by family.

	Male Parent			
Female Parent	27-1	27-4	27-5	mean
	Shoot Production			
27-2	291[1]	217	281	263
	(210-400)[2]	(53-350)	(207-381)	(53-400)
27-3	207	186	198	197
	(172-239)	(134-238)	(139-260)	(134-260)
27-6	181	207	244	211
	(173-215)	(148-307)	(184-327)	(148-327)
mean	226	203	241	224
	(172-400)	(53-350)	(139-381	(53-400)
	Rooting Percentage			
27-2	11.5[3]	25.0	21.6	19.4
	(2-22)[4]	(11-55)	(6-44)	(2-55)
27-3	55.3	24.8	35.1	38.4
	(34-76)	(12-46)	(14-64)	(12-76)
27-6	54.0	37.9	30.4	40.8
	(32-80)	(18-70)	(9-41)	(9-80)
mean	40.3	29.2	29.0	32.9
	(2-80)	(11-70)	(6-64)	(2-80)

[1] Average of 10 clones summed over 6 hedging cycles.
[2] Range of 10 clones summed over 6 hedging cycles.
[3] Average of 10 clone means of 4 rooting cycles.
[4] Range of 10 clone means of 4 rooting cycles.

Table 5. Correlations between cycles for shoot production and rooting percentage. Correlations of family means are shown in the upper right corner of the table. Correlations of Clone means are shown in the lower left corner of the table. All correlations are significant at the <0.05 level.

	Cycle 1	Cycle 2	Cycle 3	Cycle 4	Cycle 5	Cycle 6
Cycle 1		0.56[1]	0.67	0.67	0.81	0.73
		-	-	-	-	-
Cycle 2	0.59		0.66	0.80	0.77	0.92
	-		0.83[2]	0.95	0.84	-
Cycle 3	0.51	0.56		0.62	0.90	0.52
	-	0.65		0.87	0.74	-
Cycle 4	0.37	0.45	0.37		0.46	0.75
	-	0.69	0.65		0.81	-
Cycle 5	0.36	0.52	0.57	0.54		0.74
	-	0.57	0.53	0.58		-
Cycle 6	0.48	0.65	0.49	0.47	0.61	
	-	-	-	-	-	

[1] Shoot production.
[2] Rooting percentage.

Table 6. Estimates of genetic parameters for shoot production and rooting percentage of juvenile loblolly pine. Values from other published data are included for comparison.

Parameter	Shoot Prod.	Rooting Percent			
	Anderson et al[1]	Anderson et al	Foster 1990[2]	Foster 1978[3]	McKeand Frampton 1982[4]
Narrow-sense heritability (h^2)	0.33	0.26	0.15	0.07	0.26
Narrow-sense heritability based on family means (h^2_F)	0.60	0.31	0.46	0.06	0.45
Broad-sense heritability (H^2)	0.42	0.63	0.13	0.23	0.42
Broad-sense heritability based on hedge (shoot) and clone (rooting) means (H^2_C)	0.82	0.87	0.40	0.38	0.54
Total genetic variance (V_G)	84.5	351.8	103.7		
Additive variance (V_A)	66.1	146.3	116.2		
Dominance variance (V_D)	42.7	531.7	18.8		
Epistatic variance (V_I)	-24.4	-375.1	-31.3		

[1] Data from this publication.

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Table 7. Rooting percent, performance level values for height growth, and fusiform rust resistance for each of the nine full-sib families.

Trait	Male Parent	Female Parent		
		27-2	27-3	27-6
Rooting[1]	27-1	11.5	55.3	54.0
	27-4	25.0	24.8	37.9
	27-5	21.6	35.1	30.4
Height growth[2]	27-1	44	53	47
	27-4	75	55	52
	27-5	69	71	79
Rust resistance[3]	27-1	44	31	60
	27-4	79	81	86
	27-5	47	39	70

[1] Rooting percentage.

[2] Performance value for height growth obtained from progeny tests conducted by North Carolina State Tree Improvement Cooperative, Raleigh, NC.

[3] Performance values for rust resistance obtained from progeny tests conducted by North Carolina State Tree Improvement Cooperative, Raleigh, NC.

Table 8. Percent of families and clones retained for propagation based on various rooting percentage culling intensities. The culling criteria was applied to the average of the current rooting cycle and all previous rooting cycles.

Hedging Cycle	Percent Root Culling Criteria			
	90	70	50	30
2	33[1]	56	89	89
	6[2]	17	39	68
3	11	33	78	89
	1	9	27	57
4	0	33	78	89
	0	6	22	53
5	0	33	56	89
	0	7	17	50

[1] Percent of families retained.
[2] Percent of clones retained.

Figure 1. Shoot production of juvenile loblolly pine greenhouse hedges by family.

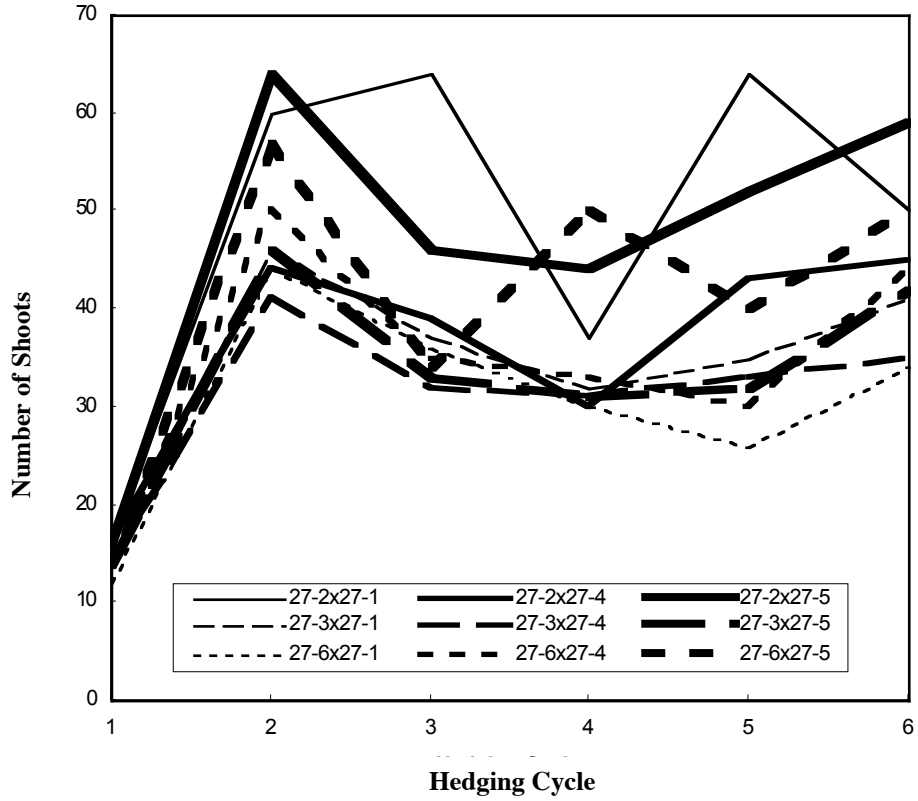

Figure 2. Percent rooting of juvenile loblolly pine cuttings by family.