

**NORTH CAROLINA STATE UNIVERSITY
LOBLOLLY AND SLASH PINE ROOTED CUTTING PROGRAM**

**Annual Progress Report
November 5, 2003**

EXECUTIVE SUMMARY

The NCSU Loblolly and Slash Pine Rooted Cutting Program is completing its 12th year of existence. We are in the third year of a four-year phase that began on January 1, 2001 and is scheduled to end on December 31, 2004. Our mission is to conduct focused research and technology transfer activities to assist members in their deployment of rooted cuttings on an operationally meaningful scale. Our research topics are focused on providing information for the efficient, operational production of rooted cuttings and the implementation of clonal forestry.

In the area of operational production, this report contains results of studies that extend our previous results on the importance of cutting water potential on rooting. Measurements of **Photosynthesis and Stomatal Conductance on Cuttings** receiving different mist treatments show that, while the driest cuttings have impaired gas exchange and root poorly, those with the highest rates of gas exchange do not root as well as cuttings with intermediate rates. These results support our previous reports that moderate water stress promotes rooting. In a related study, different mist regimes were applied in **Outdoor Rooting Beds** to loblolly and Virginia pine cuttings stuck in May and loblolly and slash pine cuttings stuck in June. While rooting was relatively poor for the loblolly and slash pine cuttings, the patterns again show that those receiving intermediate mist levels rooted at the highest percentages. We have grown seedlings for hedges and constructed a fertigation system for the upcoming **Hedge Nutrition Study**. This will test ratios of a suite of macronutrients to the elevated level of nitrogen found to be optimal in our previous studies. Results should become available next year. A field study testing the **Performance of Rooted Cuttings from Different Production Systems** shows no substantial differences in survival or growth among cuttings produced in containers, directly rooted in nursery beds, or rooted in a greenhouse and then transplanted to nursery beds.

Several lines of research are being conducted in support of clonal forestry. Our long-term **Hedge and Clone Maturation Study** again shows that a slight decline in rooting occurs after the first two years, but no further decline is observed with serial propagation through nine years from seed. A field test of rooted cuttings from clones up to eight years from seed was planted in January 2002. First-year results show a slight trend of decreasing height with age, but suggest this was due to initial planting size. Trees in our **Clonal Selection Study** are completing their fifth growing season. Analyses showed that gains at rotation could be substantial—on the order of 22%-27% increase in volume above outstanding full-sib families. Additional analyses provide insight into test design and selection age. Finally, in our research to understand the **Fundamental Mechanisms Controlling Root Formation**, we are using poplar as a model tree species. We have cloned three genes—two using a gene-trapping system and one based on similarity to an *Arabidopsis* gene that could be important for root formation. Ongoing studies will elucidate their roles.

INTRODUCTION

The year 2003 is the third of four years in the third phase of the Rooted Cutting Program. We, on the program staff, are focused on completing the studies outlined in the Renewal Proposal for this phase and on laying the initial groundwork for a potential fourth phase. We appreciate your support and look forward to continue working with you.

The challenges facing the forestry and the forest products industries over the last few years continued in 2002-03. Economic forces have resulted in cutbacks in many private companies. The Rooted Cutting Program lost two members during 2002-03 and a third company will no longer participate in 2004. Thus, membership stood at eight companies in January 2003, but will be reduced to five companies starting January 2004. To keep the program viable, we have actively pursued, with considerable success, additional research funding from new sources. Despite the difficult current climate, several members continue to actively pursue their internal rooted cutting programs. Recent data, including ours, on the gains that can be obtained from clonal forestry keep interest strong and the feasibility of producing clones, completely or partly, using rooted cuttings seems more possible than ever.

This report includes summaries of experiments conducted or analyzed since the last progress report in October 2002. Additional details will be presented at the upcoming Annual Meeting on November 13 in Atlanta. We hope to see you there.

Barry Goldfarb, Director

RESEARCH FOR OPERATIONAL PRODUCTION

Control of the Rooting Environment: Effect of Mist on Photosynthesis and Gas Exchange During Rooting

In June 2002, Anthony LeBude conducted a study to determine the effect of the rooting environment on rooting and physiology of stem cuttings. Two experiments were conducted on the same benches. The first determined the effect of mist on cutting water potential and rooting and the results were presented in the 2002 Annual Report. Briefly, the experiment suggested that loblolly pine cuttings might need a moderate amount of water deficit during the rooting period to stimulate adventitious root formation and/or development. In the second experiment, utilizing the same cuttings and experimental design, photosynthesis and stomatal conductance were measured on non-rooted stem cuttings. The objectives were to determine: (1) how gas exchange is affected by mist application and other aspects of the rooting environment and (2) whether gas exchange affects rooting. The results of this experiment are discussed here.

In June 2002, stem cuttings were collected from two full-sib families containing approximately 30 clones each, pooled, and inserted in 91 cm (length) x 61 cm (width) x 20 cm (height) rooting tubs filled with fine builders' sand. Mist treatments were 45, 61, 75, 102, 147, and 310 ml·m⁻² of mist applied during each pass of the mist boom. Mist frequency, which was

inversely related to relative humidity and modified according to time of day, was the same for all treatments. For comparison of gas exchange in non-rooted and rooted cuttings, cuttings of the same genetic origin, rooted in a previous experiment (April 2002), were potted in Ray Leach SuperCells filled with a medium of fine builder's sand and then grown for 2-3 weeks while being fertilized with a water soluble fertilizer solution. They received 1/8 tsp. Osmocote control-released fertilizer (18N-6P-12K) in each tube and were placed adjacent to the rooting tubs and given the same mist treatments. During the experiment, controls were watered twice daily (morning and evening) to keep the soil water potential at or near field capacity.

Ability to photosynthesize (A_{ability}) and stomatal conductance to water vapor (g_s) were measured destructively on two cuttings in each plot using a Li-Cor 6400 Photosynthesis Analysis System between 8:00 and 10:00 am and between 1:00 and 3:00 pm 2, 4, 6, 8, and 10 weeks after setting cuttings. All morning measurements were made under $250 \mu\text{mol}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$ photosynthetically active radiation (PAR), 75-80% relative humidity, $400 \mu\text{mol CO}_2\cdot\text{m}^{-2}\cdot\text{s}^{-1}$, and a temperature of 25°C (77°F) in the cuvette. For afternoon measurements, $450 \mu\text{mol}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$ PAR, 65-70% RH, $400 \mu\text{mol CO}_2\cdot\text{m}^{-2}\cdot\text{s}^{-1}$ and a cuvette temperature of 28°C (82.4°F) was used. These were the approximate ambient conditions averaged across all mist treatments. A_{ability} and g_s were measured for the controls, using the same regime, 4 and 10 weeks after setting. Data for any cuttings that had rooted during the experiment were excluded. Cutting water potential was also measured, using a pressure chamber, on the same plant material immediately following measurements using the Li-Cor.

Photosynthesis and conductance

A_{ability} of non-rooted stem cuttings was affected by mist volume applied, time of day of measurement (TOD) and week after setting cuttings (Table 1). Since TOD did not interact with other main effects, data for the effects of week and mist on A_{ability} and g_s were averaged over the AM/PM measurements. In general, A_{ability} decreased for cuttings in all mist treatments as the weeks after setting increased (Figure 1). Stomatal conductance was similarly affected by mist and weeks, however, stomatal conductance remained relatively equal, unchanged and lower for cuttings receiving the lowest mist volumes ($45, 61, \text{ and } 75 \text{ ml m}^{-2}$), throughout the measurement periods. In contrast to non-rooted cuttings, A_{ability} and g_s of rooted, control cuttings was not affected by mist volume applied (Table 1). A_{ability} and g_s of rooted control cuttings increased considerably between week 4 ($2.2 \mu\text{mol CO}_2\cdot\text{m}^{-2}\cdot\text{s}^{-1}$, $60 \text{ mmol H}_2\text{O}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$) and week 10 ($8.5 \mu\text{mol CO}_2\cdot\text{m}^{-2}\cdot\text{s}^{-1}$ and $330 \text{ mmol H}_2\text{O}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$). The low gas exchange values observed in the controls in week 4 may have been caused by transplant shock or the requirement of a period of acclimation to the high humidity environment.

The relationship between A_{ability} and g_s differed for non-rooted and rooted cuttings. In the non-rooted cuttings, A_{ability} increased with g_s until moderate g_s values were reached (Figure 2). As g_s increased further, however, no increase in A_{ability} was observed. The conductance of the non-rooted cuttings, and the photosynthetic responses, remained similar from 4 to 10 weeks. In contrast, while conductance and rates of A_{ability} were comparable to those of non-rooted cuttings at week 4, conductance values increased markedly in week 10 and rates of A_{ability} showed a linear increase with increases in g_s .

Table 1. ANOVA for effect of mist, weeks after setting, and time of day (AM/PM) on ability to photosynthesize (A_{ability}) and stomatal conductance to water vapor (g_s) for non-rooted and rooted stem cuttings (controls).

Source	Non-Rooted Cuttings			Rooted Controls		
	df	A_{ability}	g_s	df	A_{ability}	g_s
Replication (R)	1	NS ¹	NS	1	*	NS
Mist Volume (M)	5	*	*	5	NS	NS
Week (W)	4	*	*	1	*	*
AM/PM (TOD)	1	*	NS	1	NS	NS
W x M	20	NS	NS	5	NS	NS
TOD x M	5	NS	NS	5	NS	NS
W x TOD	5	NS	NS	1	NS	NS
W x TOD x M	18	NS	NS	5	NS	NS

¹NS, nonsignificant and *, significant at $P = 0.10$, respectively.

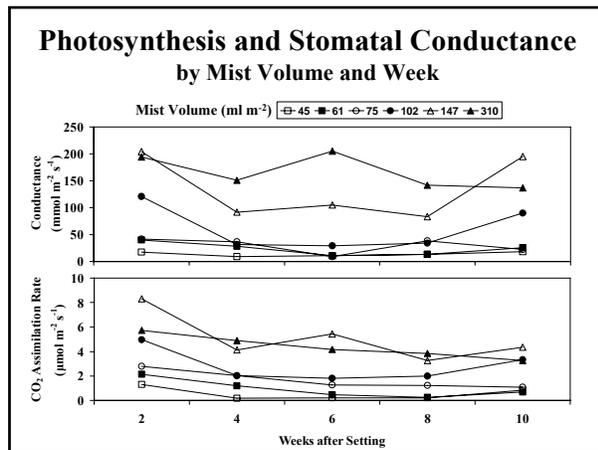


Figure 1. Conductance and photosynthetic rate for non-rooted stem cuttings by mist volume at two-week intervals after setting.

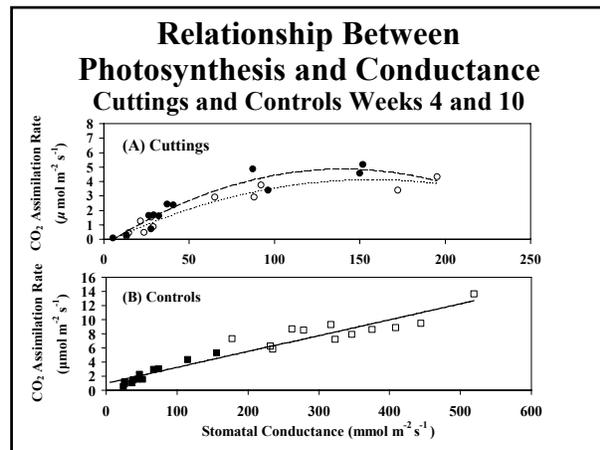


Figure 2. Photosynthesis as a function of conductance for non-rooted cuttings (A) and rooted cutting controls (B), four and ten weeks after setting. Filled and open symbols represent four-week and ten-week measurements, respectively.

Data for gas exchange in non-rooted cuttings during weeks 2, 4 and 6 were averaged together to represent the period during which adventitious root initiation and development would typically occur. A_{ability} and g_s were strongly related to the mist volume applied to non-rooted cuttings (Figure 3). A_{ability} and g_s increased until 200 ml m⁻² of mist volume, then A_{ability} declined while g_s remained relatively constant. The decline in A_{ability} in the highest mist

treatment, despite high conductance, could be due to leaching of mineral nutrients from the foliage or a waterlogged condition in the above-ground portion of cuttings.

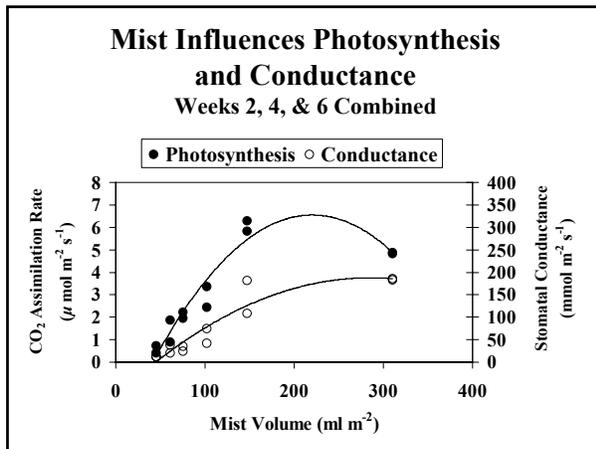


Figure 3. Effect of mist on conductance and photosynthetic rate for non-rooted cuttings over the rooting period.

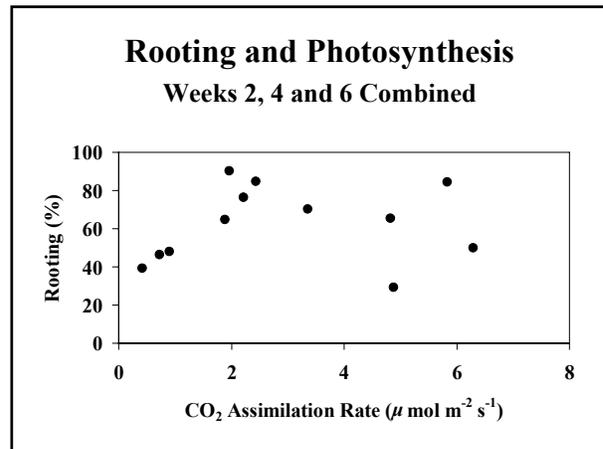


Figure 4. Relationship (non-significant) between photosynthetic rate and rooting percentage.

Relationship between photosynthesis, conductance and rooting percentage

Rooting percentage was not significantly related to A_{ability} of nonrooted stem cuttings (Figure 4). Rooting percentage was, however, related with g_s (Figure 5). Cuttings that had the lowest values of g_s (and low rates of A_{ability}) rooted poorly at 40%-50%. Rooting percentage was higher (65%-90%) throughout a relatively large range of conductance values. However, at the highest values of g_s , rooting declined (30%-65%). From these data, we cannot rule out a beneficial effect of at least minimal conductance and photosynthesis in cuttings during rooting. However, higher rates of gas exchange do not necessarily translate into better rooting. Taken together with the relationship between rooting percentage and cutting water potential reported last year (Annual Report 2002), these data support the finding that cuttings require a moderate amount of water deficit to stimulate adventitious root formation and/or development. Analysis of the *in situ* temperature and humidity measurements taken throughout the rooting period continues. This information may provide further guidelines for controlling the rooting environment.

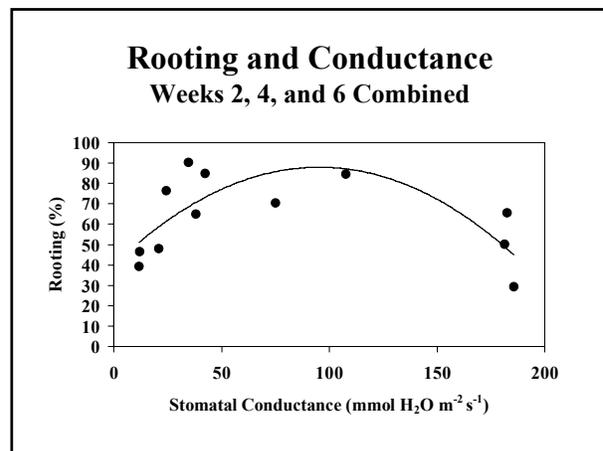


Figure 5. Relationship between stomatal conductance rate and rooting percentage.

Mist Applications During Rooting in Outdoor Beds

We conducted experiments in 2003 in collaboration with John Frampton, NCSU Christmas Tree Genetics Program, to test the effects of different misting regimes on rooting in

outdoor beds. These experiments built on those described above that tested the influence of mist and cutting water potential on rooting stem cuttings of loblolly pine in a greenhouse environment. Results of those experiments indicated that cuttings rooted better when subjected to moderate water deficit during the period of adventitious root initiation and development. These experiments tested whether this finding applied to cuttings rooting in outdoor beds and also compared these relationships in loblolly, slash, and Virginia pines.

In 2003, two studies, one in winter and one in spring, were conducted. The winter study used dormant stem cuttings of two full-sib families of loblolly pine (the same two families used in LeBude's previous studies) collected from hedges in February, pooled, and then stored in a cold room until setting in May 2003. Dormant cuttings of Virginia pine were collected from seedling tops of bare-root 1-0 seedlings donated by the NC Forest Service (seed orchard mix from the Virginia Division of Forestry). Virginia pine seedlings were stored in the same cold room for two months prior to collecting the seedling tops for rooting. The spring study utilized succulent (softwood) cuttings of loblolly pine collected in June from the same hedges as in winter, and slash pine cuttings were donated by Rayonier. The two studies overlapped for 7 weeks during the summer in adjacent rooting beds.

Five mist treatments were replicated three times in a split-plot design with mist treatment as the main plot and species as the sub-plots. The five mist treatments consisted of different irrigation regimes resulting from the following equation that specified the off-time between irrigation cycles,

$$OT = (75R) / ([10VPD]^{0.7}),$$

where: OT was the off-time (time between mistings) measured in minutes, R (mist regime factor) was a multiplication factor that varied among the treatments, and VPD was vapor pressure deficit calculated from temperature and humidity in millibars. The five values of R tested were 1, 4.5, 8.5, 13, and 18. So, for example, if the VPD was 1 kPa (10 mb), then the corresponding off-time in minutes for each treatment, respectively, would be 3, 13.4, 25.4, 38.8, or 53.7 minutes. The off-time equation was further modified by wind speed and rainfall. The OT was reduced by a factor of 0.05 x wind speed (mph) x the current OT calculation and 0.01" of rainfall reset the OT counter to 0. The duration of mist application (on-time) was held constant for all treatments at 20 seconds.

One hundred cuttings of each species were placed in each plot surrounded by two border rows. The rooting beds had previously been filled with coarse sand and the spacing within the plots was 3 in. x 3 in. In winter, stem cuttings were dipped in 10 mM NAA for loblolly pine and 7 mM NAA for Virginia pine cuttings. In spring, 2.5 mM NAA was used for loblolly pine and 0.3% IBA (talc) was used for slash pine. Cutting water potential was measured destructively, using a pressure chamber, on one cutting in each plot between 2:00 and 4:00 pm 7, 14, 21, 28, and 35 days after setting (DAS) in both experiments. Rooting percentage was scored 84 DAS.

Cutting water potential (Ψ_{cutting}) for all species in both experiments was strongly related to mist regime factor (Figure 6). Ψ_{cutting} decreased sharply from cuttings receiving the 1R regime to those receiving the 4.5R regime and then decreased more gradually after that. Calculations of

predicted mist applied during a typical 1-hour period at a VPD of 1 kPa (10 mB) showed that predicted mist was also closely related to Ψ_{cutting} (Figure 7). The data logger recorded the actual frequency and volume of mist applied during the entire rooting period and these values will be analyzed in the future.

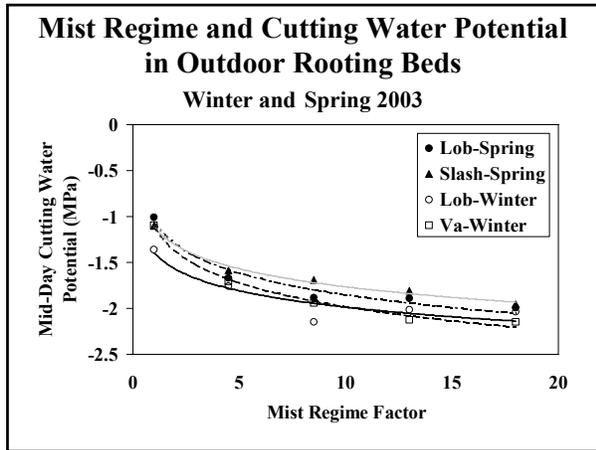


Figure 6. Effect of mist regime factor (inversely proportional to mist volume) on mid-day cutting water potential in outdoor rooting beds.

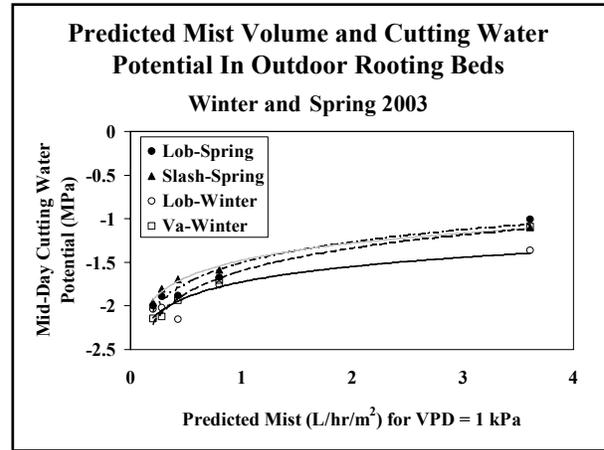


Figure 7. Effect of predicted mist volume on mid-day cutting water potential in outdoor rooting beds.

Overall rooting percentage was 50% for the winter experiment. Virginia pine rooted at 75%, whereas loblolly pine rooted at 25% (Figure 8). There are several possible reasons for the better rooting of the Virginia pine as compared with the loblolly pine cuttings. First, there may be intrinsic differences in the rooting ability of the two species. Second, the Virginia pine cuttings were derived from juvenile seedling tops, whereas the loblolly pine cuttings came from clones started in 1996 (nearly seven years old at the time of collection). Third, we experienced rainfall in the spring that was considerably above normal levels and the loblolly pine cuttings may have been more sensitive to saturated soil conditions. Fourth, because the set up of misting and monitoring equipment took longer than anticipated, cuttings were not stuck until the middle of May and optimal outdoor sticking time in the Raleigh area is more likely to be early to mid-April. The late sticking, however, did not adversely affect Virginia pine cuttings. Lastly, the winter loblolly pine cuttings may have been predisposed to disease, as the winter cuttings were in the clone maturation study (see below).

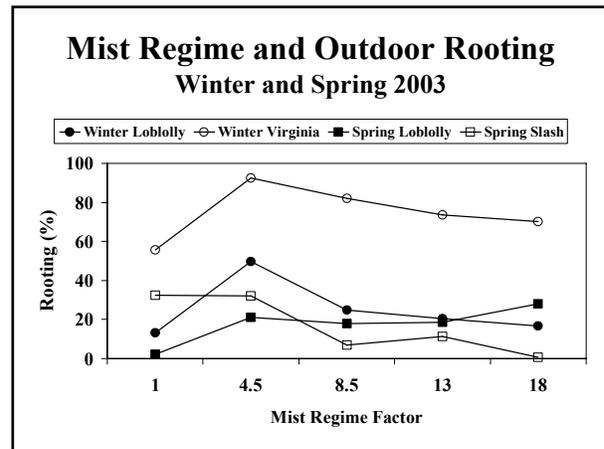


Figure 8. Effect of mist regime factor on rooting percentage of loblolly (winter and spring), slash (spring), and Virginia (winter) pine cuttings in outdoor rooting beds.

The highest rooting percentage occurred for both species under the intermediate mist treatment of R=4.5 and when cuttings had intermediate mid-day water potentials. In this treatment, the Ψ_{cutting} for loblolly pine was -1.75 MPa and 50% of the cuttings rooted (Figure 9). In the same treatment, the Virginia pine cuttings had a mid-day Ψ_{cutting} of -1.71 MPa and the cuttings rooted at 92%. This result is similar to the effects of cutting water potential on rooting of loblolly pine cuttings in a greenhouse reported by Anthony LeBude last year. In that study, the best rooting occurred at average water potentials of -0.5 MPa to -1.2 MPa, but the water potentials were averages of both mid-day (high stress) and pre-dawn (low stress) measurements. If one looked only at the high stress measurements, the best rooting occurred when cuttings had water potentials of -1.2 MPa to -1.6 MPa for winter cuttings and -1.4 MPa to -1.7 MPa for spring cuttings and these Ψ_{cutting} values for the best rooting are similar to those obtained in the current outdoor study. One difference between the studies is that, in the greenhouse, water potential of the rooting medium was controlled and was held constant across the different mist treatments. In the outdoor beds, however, soil water potential is potentially confounded with mist treatment. We recorded soil water potentials during the outdoor rooting experiments and it will be interesting to compare these values with those used in the greenhouse.

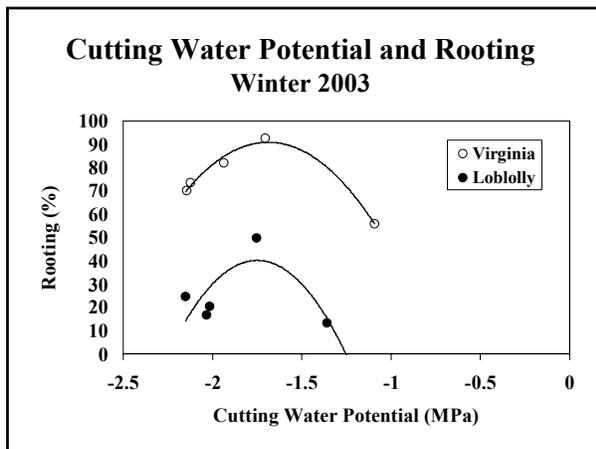


Figure 9. Effect of mid-day cutting water potential on rooting percentage of loblolly and Virginia pine winter cuttings in outdoor rooting beds.

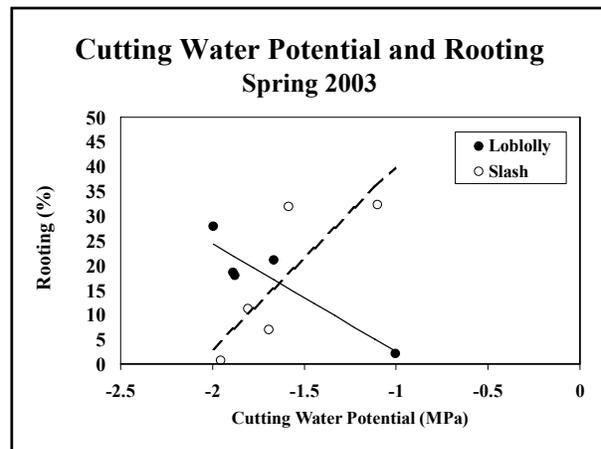


Figure 10. Effect of mid-day cutting water potential on rooting percentage of loblolly and slash pine spring cuttings in outdoor rooting beds.

In the spring experiment, both loblolly and slash pine cuttings rooted poorly. For each species, the average rooting percentage across all mist treatments was 17%. It may be that environmental conditions for a spring (June) outdoor sticking are too harsh for high rooting percentages, although it should be noted that the performance of the slash pine may have been hindered by damage in transit. Some cuttings had brown-tipped foliage and, while we tried to use only the best cuttings, we cannot rule out damage. Despite the low rooting, the two species appeared to exhibit opposite responses to mist treatment. The highest rooting percentage for loblolly pine (28%) was obtained in the driest treatment (R=18), while the best rooting in slash pine (32%) occurred in the wettest two treatments (R=1, 4.5) (Figure 8). These two species also seemed to have opposite responses to cutting water potential (Figure 10). Although the fit of the regression lines is marginal, there appeared to be a trend where slash pine rooted best when its

average mid-day water potential was between -1.1 MPa and -1.6 MPa, while loblolly pine showed better rooting between -1.7 MPa and -2.0 MPa.

Currently, we are planning to modify these experiments for next year. We will concentrate on the winter cuttings, stick the cuttings earlier in the season, include juvenile cuttings as controls, stick companion cuttings in the greenhouse, and adjust the mist regimes to provide more data points nearer to the projected optimal range. In addition, we may alter the irrigation to decrease misting as the cuttings begin root formation. This would allow us to determine whether the irrigation regimes have effects on plant growth after rooting.

Hedge Nutrition Study

A study was initiated in December 2002 to begin an investigation into hedge fertility and the subsequent effect on rooting of stem cuttings. Our past research has shown that tissue concentrations of nitrogen (N) at or above 2% in cuttings when they were removed from the hedge enhanced rooting. Interestingly, this optimal N tissue concentration is greater than for seedlings growing in the nursery, although hedge boron requirements for cuttings were similar to those for seedlings. To learn how fertility of the other macronutrients should be adjusted to the higher N levels, a study was initiated to investigate the ratio of a suite of macronutrients (P, K, S, Ca, Mg) to N in hedges during cutting production and how this might affect subsequent rooting.

Seeds of four unrelated full-sib crosses from three sources, slash pine, Atlantic Coast loblolly pine, and Western Gulf loblolly pine (Thanks!, Boise, Plum Creek, and Temple Inland, respectively) were germinated December 2002 in Ray Leach SuperCells. Seedlings were grown continuously over winter in a heated greenhouse and fertilized with Osmocote (18N-6P-12K, 8-9 Month, Controlled-Release). Eighty seedlings per cross were chosen randomly and potted in 3-gal. containers containing a medium of 3 perlite : 2 sand (v/v) in August 2003.

The fertigation system previously used by Brad Rowe in his N studies was redesigned to accommodate a larger number of hedges and rebuilt with the help of Mike Jett. Hedges were placed in a randomized, complete-block design containing four replications of five nutrient ratios. Four plants from each cross were placed in each treatment per replication for a total of 960 hedges for the experiment (12 crosses x 4 plants per cross x 5 nutrient ratios x 4 replications = 960 plants). Fertilizer treatments will be five ratios of macronutrients to the same concentration of N (2%). Macronutrients will be applied as an intermediate ratio of P=0.2%, K=1.0%, Ca=0.3%, Mg=0.1%, and S=0.08%. The remaining four treatments will consist of one lower and three higher ratios than this intermediate ratio. Micronutrients will remain constant for all treatments. All plants in the experiment currently are receiving the same standard fertilization containing macro- and micronutrients as described by Rowe et al. (2002) with a target foliage tissue concentration of 2% N. The initial pruning will be administered to hedges in February 2004 and the various fertilizer treatments will be applied with the resumption of growth in the spring. The first crop of cuttings will be collected Spring 2004 and tested for both nutrient concentrations and rooting.

Field Test of Rooted Cutting Stock Types

The objective of this field study is to compare the field performance of loblolly pine rooted cuttings originating from five different production systems/dates. The rooted cuttings in this field test originated from Matt Gocke's propagation study conducted from February to December 2001 that tested stock quality of cuttings rooted and grown in various production systems. Three clones were tested in the five rooted cutting production systems in the propagation study: 1) a direct-stick system (DS), in which cuttings were stuck directly into an outdoor nursery bed for rooting and growth, 2) a transplant system, in which cuttings were rooted in February in a greenhouse in Grow-Tech Rooting Sponges™ (Grow-Tech Inc., San Juan Bautista, Ca) and then transplanted after 9 or 12 weeks to an outdoor nursery bed for subsequent growth (Feb GT), 3) a containerized system, in which cuttings were rooted in February in a greenhouse in Ray Leach SuperCells™ for 12 weeks and then transferred outdoors in the same tubes for subsequent growth (Feb C), 4) a transplant system, in which cuttings were rooted in May in a greenhouse in Grow-Tech Rooting Sponges and then transplanted after 8 or 10 weeks to an outdoor nursery bed for subsequent growth (May GT), and 5) a containerized system, in which cuttings were rooted in May in a greenhouse in Ray Leach SuperCells for 10 weeks and then transferred outdoors in the same tubes for subsequent growth (May C). Additionally two mist levels and two light levels were tested in a 2 x 2 factorial combination for the DS system.

The ten treatments evaluated in the propagation study were reduced to five treatments in the field study by pooling treatments within the same production system. Only two of the three clones used in the propagation study were included in the field study, because of the poor rooting of one of the clones. Fifteen rooted cuttings per clone were selected at random from each of the five, pooled treatments, at the time the propagation study was lifted on December 17, 2001. The direct stick and transplant cuttings were stored after lifting at 5°C (40°F) in a cooler in seedling bags until the time of planting. The containerized cuttings were kept outdoors in a cold frame in their containers until the time of planting. On January 14, 2002 the cuttings were planted on land provided by MeadWestvaco in Stewart County, GA at a spacing of 8 feet within rows and 12 feet between rows. Each cutting was "deep-planted" by hand with flat shovels to a maximum depth of 50 cm, depending on the size of the plant. For all cuttings the terminal bud remained above the ground. The test had a split-plot design with clones as the whole plot factor and production system treatments as the sub-plot factor. Each replication consisted of a single-tree row plot of each clone with one ramet representing each of the five propagation treatments. There were fifteen replications, each containing one ramet per clone per production system, for a total of 150 experimental cuttings, plus seedling borders. First-year measurements were collected on January 30, 2003 and included survival, first-year total height, and first-year height growth increment (first year height minus height immediately after planting).

After one year, there was a marginally significant effect of production system on survival ($P=0.045$ in the ANOVA, but no significant differences in the means comparison). The lowest apparent survival was observed in the Feb GT cuttings (87%), as compared with 90% survival in the DS, 97% in the May GT, and 100% in the Feb C May C cuttings, when averaged over the two clones (Table 2). This potentially lower survival in the Feb GT cuttings may have been the result of root loss during lifting. In the propagation portion of the study, no lateral root pruning or undercutting treatments had been applied as the study was designed to test growth potential. Thus, by the time cuttings were lifted, the root systems were quite large and not very compact

and some loss of roots could not be prevented. If root-pruning treatments are applied during operational production, root loss could be minimized and may result in higher survival percentages from this production system. Across the production system treatments, 95% of the cuttings of each clone remained alive after the first growing season.

There were also no significant differences among the five treatments for total height after the first growing season or first-year height growth increment (Table 2). Averaged over the two clones, first-year height growth ranged from 65 cm (May C) to 77 cm (Feb C), corresponding to a range in total height of 66 cm to 79 cm, respectively. Thus, the deep-planting technique, where only the shoot tips remained above ground, appears to have successfully equalized growth in cuttings with different initial sizes. There were also no significant differences in height growth increment or total height between the two clones. Measurements in subsequent years will determine if this lack of growth differences persists, but the early results strongly suggest that good survival and growth can be obtained with rooted cuttings produced using all five production systems. Moreover, to date, production system does not seem to interact with clone (just two in this study) in determining field performance.

Table 2. Survival, first-year total height, and first-year height growth increment of rooted cuttings from two clones rooted and grown under five production systems.

Treatment	Survival (%)*	First-Year Total Height (cm)*	First-Year Height Growth Increment (cm)*
Direct-Stick	90	70.8	68.9
Feb. Transplant	87	74.8	71.0
Feb. Containerized	100	78.6	76.9
May Transplant	97	71.0	69.0
May Containerized	100	65.8	65.0
Clone C (all treatments)	95	69.5	67.4
Clone D (all treatments)	95	74.9	73.2

**There were no significant differences among production systems or between clones for any of the three variables using ANOVA and Tukey's multiple range test at $p = 0.05$.*

RESEARCH FOR CLONAL FORESTRY

Hedge and Clone Maturation Study

Our ongoing hedge and clone maturation study is testing the effectiveness of hedging and serial propagation for maintaining juvenility in loblolly pine. The study began in 1993 and 20 seeds each from three open pollinated families have been germinated each year to form hedged stock plants. In order to test the effect of serial propagation on the delay of hedge maturation, serial hedges were propagated from either the original seedling hedges two years after germination (first cycle serials) or from the previous serial cycle (second, third serial, etc.). For example, first cycle serial hedges were propagated in 1996 from seedling hedges germinated in 1994. In 1998 and 2000, second cycle and third cycle hedges were formed, respectively, from cuttings taken from the first and second cycle hedges. Only cuttings from the most recent serial cycle hedges are included in experiments. So, in 2003, the cuttings from these third cycle serial hedges were collected from hedges produced serially in 2000, yet these clones are from seed germinated nine years ago, in 1994. Rooting experiments were conducted in Winter and Spring 2003; however, because of disease problems during the Winter experiment, only results from the Spring experiment are reported. At the time of the rooting experiments, an insufficient number of hedges from the oldest clones (10 years old) were producing suitable cuttings. So, although these clones are omitted from the current analysis, they will be available for testing next year.

Rooting

In Summer 2003, rooting percentage across all ages and families was 60%. Rooting percentage was highest for cuttings from 1 or 2 yr old seedling hedges (approx. 80%) (Figure 11). Cuttings from clones aged 3-9 years old rooted approximately between 49% and 63%, with no apparent trend in age. This is the same result we have observed over the past three years. Thus, after the initial drop-off in rooting following age two, there appears to be no further decrease in rooting percentage with clone age, at least through 9 years of age. The three families rooted similarly across all ages as in previous experiments. Family 7 rooted highest (73%), family 11 was intermediate (65%) and family 9 rooted poorly (46%).

Field test

On January 31, 2002, a field test was planted by Plum Creek Timber Company near Holly Hill, SC. The test was a randomized, complete block with four blocks, seven clone ages (2 through 8 years old), three families, an average of 14 clones per family per age, one ramet per clone per block, and six seedlings of the same three families per block (total of 1184 trees). Initial height was measured immediately after planting and first-year height was measured after the first growing season.

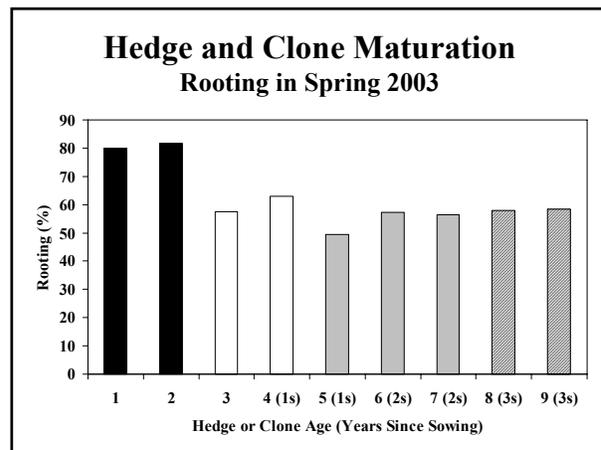


Figure 11. Rooting percentages of clones of different agea in spring 2003; black bars=seedling hedges and white, gray and hatched bars=hedges from one, two and three cycles of serial propagation, respectively.

Analysis by Robert Jetton showed that total height after the first growing season varied slightly, but significantly, with age. The tallest trees included rooted cuttings from the 2- and 3-year-old clones and the seedling checks (Figure 12). However, the only cuttings significantly shorter than the seedlings were those from 8-year-old clones. The differences in total height appear to be related to initial height after planting. First-year height growth increment was calculated and there were no significant differences (Figure 13). There was no apparent trend with age and even the cuttings from 8-year-old clones grew as much as the seedlings in the first year. This test will be re-measured in subsequent years and an undergraduate student, Scott Newkirk, has received an Undergraduate Research Grant from the College of Natural Resources to measure and analyze this test. In addition, Scott will be making measurements of morphology on cuttings as they are collected from hedges of the clones of various ages in the ongoing rooting study.

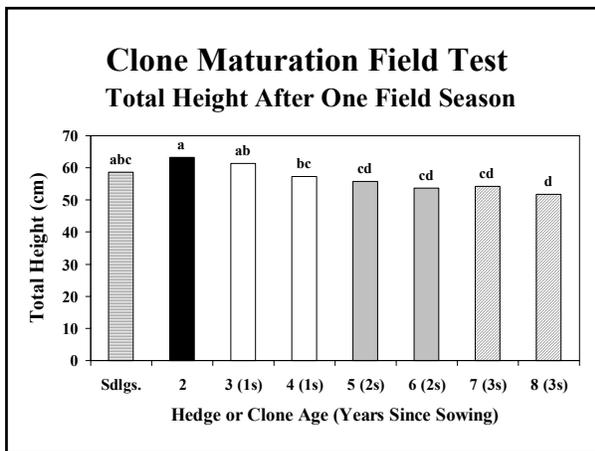


Figure 12. Total height after one growing season of seedlings (horizontal hatched bars) and rooted cuttings from seedling hedges (black bar) and hedges from one (white bars), two (gray bars) and three (diagonal hatched bars) of serial propagation. Bars with the same letter were not significantly different at $p = 0.05$.

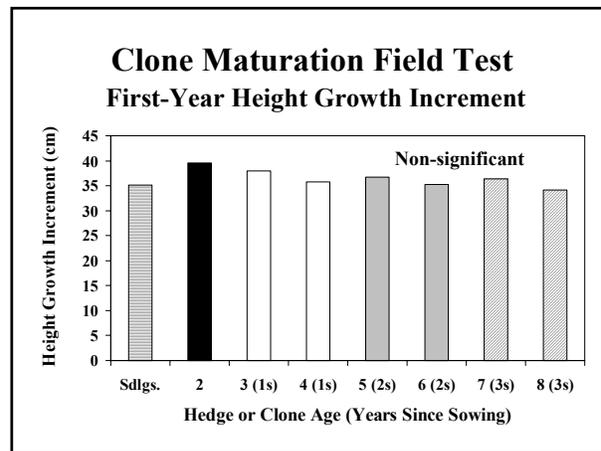


Figure 13. First-year height growth increment of seedlings (horizontal hatched bars) and rooted cuttings from seedling hedges (black bar) and hedges from one (white bars), two (gray bars) and three (diagonal hatched bars) of serial propagation. There were no significant differences at $p = 0.05$.

Clonal Selection Study

The objective of this study is to develop information that will facilitate efficient testing and selection of superior clones. The study is a joint project with the NCSU-Industry Cooperative Tree Improvement Program and was begun in October 1996 with the germination of seeds from eight full-sib crosses from the South Atlantic Coastal Plain region. The crosses were chosen from the Tree Improvement Program's diallel tests on the basis of rapid growth, good rust resistance, acceptable form, availability of seed, and lack of relatedness. From this study, we are generating quantitative estimates of: (1) predicted gain from selecting different numbers of clones, (2) the number of ramets per clone necessary to characterize growth on one site, (3) efficiency of selection at different ages, and (4) magnitude of predicted genetic gain for the best clones in each cross.

The study began with approximately 100 clones of each cross. After hedge production from the seedlings, rooting and sorting, 450 clones were planted in two field tests: 168 clones from four crosses on International Paper land near Jay, Florida in December 1998 and 282 clones from the other four crosses on MeadWestvaco land in South Carolina in November 1998. The experimental design is a randomized, complete block, with nine blocks and one ramet per clone per block.

Measurements on survival, height and rust infection were taken after each of the first four growing seasons and diameter was measured after the fourth season. Because of very low incidence in the FL test, rust infection was not analyzed for that site. Trees on both sites have undergone good growth and the initial problems with uneven stock quality appear to have become less important. Mean height was 5.6 m (18.3 ft) in FL and 5.5 m (18.1 ft) in SC (Table 3). Clone mean heritabilities for height increased between years three and four from 0.56 to 0.65 in FL and from 0.69 to 0.75 in SC. Heritabilities for diameter and volume in year four were similar to those for height. Clone mean heritability for rust infection on the SC site was quite high (0.94), indicating rust resistance to a particular rust population can be quite precisely estimated using clonal replication.

Table 3. Fourth-year test means for all clones (\pm SE) and clone mean heritabilities (\pm SE) for height, diameter, volume, rust infection and straightness from the two sites of the Clonal Selection Study.

Trait and Test Site	FL	SC
Mean Height (m)	5.55 (0.76)	5.51 (0.66)
Mean Diameter (cm)	9.05 (1.66)	9.25 (1.68)
Mean Volume (dm)	14.7 (5.82)	15.1 (5.93)
Mean Rust (%)	5.4	35 (47)
Clone Mean H^2 for Height	0.65 (0.04)	0.75 (0.02)
Clone Mean H^2 for Diameter	0.50 (0.06)	0.70 (0.03)
Clone Mean H^2 for Volume	0.60 (0.05)	0.66 (0.03)
Clone Mean H^2 for Rust	---	0.94 (0.01)
Clone Mean H^2 for Straightness	0.51 (0.06)	---

Clone genetic values were estimated by Fikret Isik using Best Linear Unbiased Predictors (BLUPs) and the genetic values were used to predict gain from selecting various numbers of clones from each test. Selecting the single best clone would result in 13.7% height gain above the average of all the clones in FL and 12.8% height gain in SC (Figure 14). Choosing the best four clones would result in 11.3% height gain in FL and 12.4% gain in SC. Height gain decreased with additional numbers of clones selected, but even with 24 clones (approximately 10% of all clones) height gain was 6.4% in FL and 9.2% in SC. Volume gain was considerably

larger than height gain. The best clone from each test is predicted to yield a gain of 42.2% in FL and 38.3% in SC (Figure 14). The best four clones resulted in 32.5% gain in FL and 32.6% gain in SC. Volume gains at these young ages tend to overestimate rotation age gains, while height gain tends to underestimate rotation age volume gains. A useful rule-of-thumb is to double height gains to predict rotation age volume gains. Based on this, one might expect rotation age volume gains of approximately 22%-27% from selecting the best one to four clones from 168 clones tested in FL and 282 in SC. It should be noted that these projected gains should be added to those obtained from using outstanding full-sib crosses to generate the clonal population. On the other hand, this analysis assumes a high correlation between clonal performance at age four and at rotation and no loss of growth rate with maturation due to clone age. The results of our clone maturation field test (described above) should soon provide information on whether maturation is an important issue in realizing projected gains from clones maintained as serially propagated hedges.

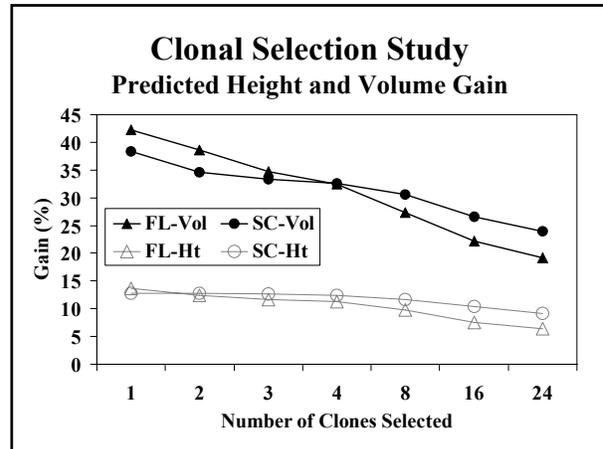


Figure 14. Effect of number of clones selected on predicted height and volume gain from fourth-year results of the Clonal Selection Studies in SC and FL.

The clonal test data were used to simulate the effect of number of ramets tested on genetic gain. The data were resampled leaving out one block (ramet) at a time and the standard errors of the BLUP clone genetic values were calculated. These were used as an indication of reliability of genetic values and to predict volume gain from choosing the best 10 clones from each test. On both sites, gain increased sharply with an increasing number of ramets from two to approximately six, but then increased only slightly with additional ramets (Figure 15). For example on the SC site, increasing the number of ramets from two to six resulted in an additional 7.0% volume gain or 1.7% gain per additional ramet. Increasing the number of ramets from six to 20, however, resulted in 5.0% more volume gain or only 0.4% gain per additional ramet. In an operational clonal testing program, the number of ramets tested will have to be balanced against the number of clones to comprise the total testing effort. These results suggest that (with good survival) approximately six ramets per clone are adequate to characterize clonal performance on one site.

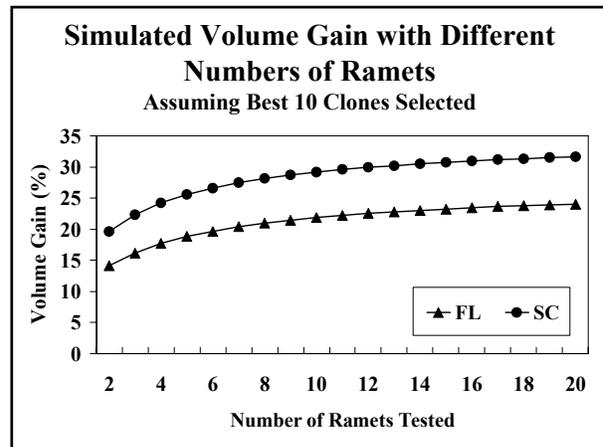
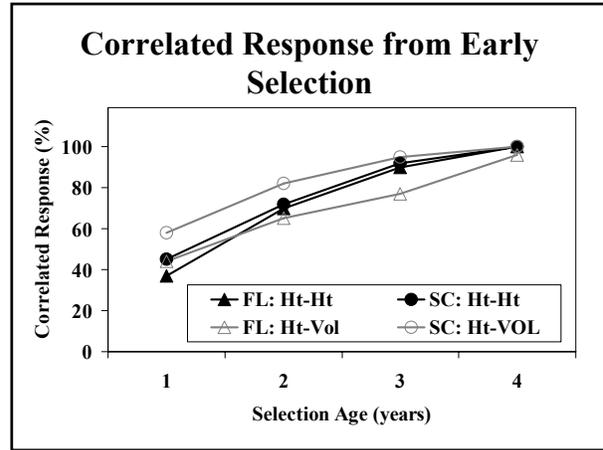


Figure 15. Simulated volume gain obtained from testing different numbers of ramets, assuming that the best 10 clones were selected.

Another important factor in designing efficient clonal testing programs is to determine how soon clones can reliably be selected. With only four-year results, it is too early to determine how efficient selection at any particular year would be for rotation age volume. However, in a preliminary analysis, we calculated genotypic correlations among the height measurements at the different ages and height and volume at age four. These correlations were used to estimate the correlated response to selection; that is, if we had selected



clones on the basis of height at ages one, two or three, how much of the fourth-year gains in height and volume would we have obtained. Gains from selection at age one would have been fairly low, ranging from 37% (of fourth-year selection) for height gain on the FL site to 58% for volume gain on the SC site (Figure 16). These increased in age two to a low of 65% of fourth-year volume gains in FL to a high of 82% for volume gain in SC. The gains were highest after selection at age three. Seventy-seven percent of volume gain in FL and 95% of volume gain in SC would have been obtained from selection for height at that age. The high correlation between ages three and four are to be expected and are not yet particularly meaningful, as we need to establish the correlated responses to selection at older ages. While it will not be possible to carry this test until rotation, we will continue these analyses and determine when the gains stabilize. When gains no longer increase dramatically with additional years, selection can be considered relatively efficient.

Figure 16. Correlated response of selecting for height at one, two or three years with gains in height and volume at age four.

We have conducted the second cycle of serial propagation on the clones in this study. As we multiply some clones, we are discarding others. Out of the 450 clones in the two field tests, we have saved over 100 clones. These were chosen by selecting those that were in the top 10% for height and/or volume from each of the eight full-sib crosses. In addition, representative clones from the 25th, 50th, 75th and 100th percentiles from each cross were retained. This collection of clones should allow us to proceed with the realized gain field test. Details will be discussed at the annual meeting.

Mechanisms of Root Formation

Last year, we began studies that use poplar (*Populus*) trees to study adventitious root formation. Poplars have become a model tree species for studying gene function during developmental and physiological processes, because they are easily propagated in tissue culture, have a small nuclear genome, and are easily transformed. Genetic transformation is a particularly useful tool, because it permits direct tests of gene function. In addition, the U.S. Department of Energy has now largely completed the sequencing of an entire poplar genome. While we are already using this sequence information as it is released, the full genome sequence is due to be published by the end of 2003.

M.S. student, Qian Wu, is using a technique called gene trapping to isolate and identify genes involved in root formation in poplar. This effort is a collaboration with Andrew Groover (Institute of Forest Genetics, USDA Forest Service, Davis, CA), Rick Meilan (currently at Purdue University) and Steve Strauss (Oregon State University). An easy-to-transform hybrid poplar (*Populus alba x tremula*) clone was transformed using *Agrobacterium* and a vector containing the GUS reporter gene in a configuration such that the reporter would only be expressed if it were inserted in (gene trap) or near (enhancer trap) an expressed plant gene. Several hundred independent lines were recovered and were initially screened by taking micro-cuttings, placing them on root-forming tissue culture medium, and staining to look for GUS expression during adventitious root formation. We received 23 lines from the initial screen at OSU. A variation of the polymerase chain reaction, called TAIL-PCR, was used to identify the trapped genes by amplifying and sequencing the chromosomal regions flanking the trap insertions. We have succeeded in amplifying flanking chromosomal sequences in 17 of the 23 lines tested so far. For each flanking sequence, a similarity search was conducted on the *Populus* genome database to identify nearby regions on the chromosome that appear to be functional genes. When candidate genes were identified, their sequences were used to search the Genbank database that contain all the known gene sequences, to possibly identify gene function. Finally, we labeled the sequences and used them as probes on northern blots to determine if the predicted genes are really expressed.

Qian Wu has found likely candidates genes in two enhancer-trap lines. In the earlier GUS staining experiments, both lines showed GUS expression in the bases of cuttings, prior to root meristem organization, and in the emerging root primordia. One gene, 4-304 is similar to genes that code for DNA-binding proteins from *Arabidopsis* and rice. DNA binding proteins regulate expression of other genes. We detected greater expression of the 4-304 transcript in (existing) roots than in leaves or stems (Figure 17). Expression was also increased slightly in auxin-treated roots, as compared with non-auxin-treated roots. The compiled gene sequence of 4-304 contains predicted auxin-response elements in the coding region of the protein and upstream of the gene.

The second candidate gene, 4-284, has a sequence that is similar to numerous predicted genes from *Arabidopsis* and rice, but, to date, the functions of these genes are not known. The 4-284 transcript was expressed most highly in roots, but was also present to a lesser extent in leaves (Figure 17). In both roots and leaves, expression was increased with auxin treatment. The predicted gene sequence for 4-284 reveals putative auxin-response elements, an abscisic acid-response element and several root elements.

In another approach, we previously reported using the sequence of an *Arabidopsis* gene called *NAC1* to clone a similar sequence from poplar. In *Arabidopsis*, *NAC1* plays a

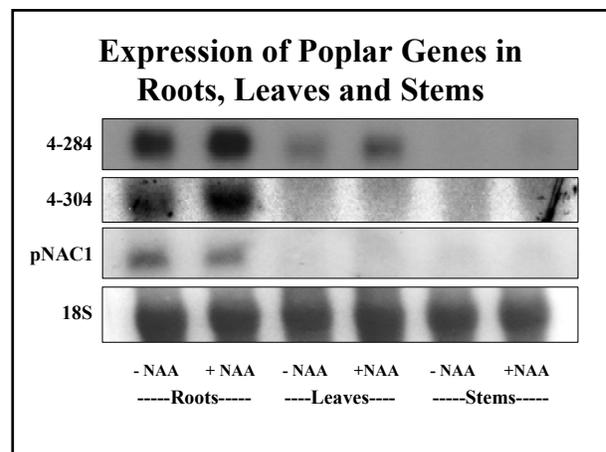


Figure 17. Northern blot showing abundance of mRNAs of three poplar genes that are candidates for functioning in adventitious root formation. 18S panel shows relatively equal loading of total RNA.

critical and specific role in lateral root formation. NACs are a family of plant-specific transcriptional regulators and the *NAC1* gene is root-specific. We used northern blotting to test whether the putative poplar *NAC1* was really expressed in a root-specific manner. Expression above background levels was detected in roots, but not in stems or leaves (Figure 17). In this case, however, auxin treatment did not appear to increase the expression level.

For all the candidate genes, we have not yet detected expression in stem bases during adventitious root formation, using northern blotting. Perhaps this is not surprising, because we would expect these genes to be expressed at low levels and only in a few cells. A much more sensitive method for measuring gene expression, Real-Time PCR, is now available. We recently were awarded an equipment grant with six other NC State scientists from the North Carolina Biotechnology Center to purchase the equipment for Real-Time PCR. The equipment should be delivered and ready to use in a few weeks. At the same time, we are developing protocols for *in situ* hybridization with root-forming tissues. This technique will allow us to localize expression to specific cell types. For all these genes, the final proof of function can be accomplished by modifying their expression in transgenic poplars. This will tell us whether under-expression or over-expression affects root formation and will provide clues to the specific functions of the genes.

Wood Quality of Clones

Last year, we reported on a study, conducted by Patrick Cumbie, that determined whole-core specific gravity and ring-by-ring density for seedlings and clones (rooted cuttings) of the same nine full-sib families growing on two sites. There were no differences between seedlings and cuttings for overall density, percent latewood, or transition age from juvenile to mature wood. Moreover, there were fairly large differences among clones for density and simulations showed that wood properties would be substantially more uniform in a stand made up of a single clone than one made up of seedlings from a full-sib family. Further research on the wood properties from this study is underway, as part of a USDA-IFAFS grant in collaboration with Bailian Li (Tree Improvement, Forestry), Ron Sederoff (Forest Biotechnology, Forestry) and Hou-min Chang and John Kadla (Wood and Paper Science). Approximately 300 cores from 45 clones have been analyzed with the SilvaScan system by CSIRO in Australia. They determined microfibril angle and modulus of elasticity using X-ray diffraction. Preliminary analysis of these data is underway and we will also analyze a sub-sample of the seedlings to test for differences between seedlings and rooted cuttings. In addition, fiber and chemical traits, such as fiber length, fiber coarseness, lignin content and cellulose content are being determined by the Wood and Paper Science group.

SUPPORTING COMPANIES IN 2002-2003

Boise Corporation

Bowater, Inc.*

Gulf States Paper Corporation**

International Paper Corporation***

MeadWestvaco Corporation

Plum Creek Timber Company

Rayonier, Inc.

Temple-Inland Forest

*Withdrew from membership in December 2002

**Withdrew from membership in March 2003

***Announced withdrawal from membership, effective December 2003

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Matt Gocke, Research Assistant and MS student (joint with Hardwood Research Cooperative)

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Frank Blazich, Collaborating faculty

Steve McKeand, Collaborating faculty

Bailian Li, Collaborating faculty

Tim Mullin, Collaborating faculty

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