INTRODUCTION

The focus of this report is on the activities of the NCSU Loblolly and Slash Pine Rooted Cutting Project since the April Annual Meeting in Jacksonville, FL. During the intervening period, activities included building the new propagation greenhouse (misthouse), scoring cuttings and analyzing data from the Winter, 1996 rooting experiments, growing and transplanting hedge material for new studies, maintaining existing hedges, installing new experiments in June and July, and proceeding with the laboratory and whole-plant physiology projects.

The current year has been the most active yet for the Rooted Cutting Project. In June, we stuck 25,000 cuttings in our new misthouse at the NCSU Horticultural Field Laboratory (HFL). This 60,000-cutting capacity research facility features a state-of-the-art computer system which continuously records environmental data and controls a variable cycle mist-boom, as well as the cooling and heating equipment. We will share the misthouse with the new NCSU Christmas tree genetics program. We express great appreciation to Georgia-Pacific, the Christmas tree program and the Department of Forestry for the funds to build this outstanding facility. We also owe a debt of gratitude to the Department of Horticultural Science for permission to locate it at the HFL and for the valuable assistance of their staff during construction.

By way of explanation, "winter" experiments refer to cuttings stuck in mid-February, "spring" experiments refer to cuttings stuck in late May/early June, and "summer" experiments to cuttings stuck in late July/early August. The Winter, 1996 cutting experiments were installed in the original Method Road propagation house. Overall, we had a fairly successful winter rooting season. Reducing the frequency of misting resulted in improved rooting success and greater precision for the research studies. All experiments for this spring were stuck in the new misthouse, except for the slash pine within-family genetic culling study, which was conducted in the Method Road house. The "spring" experiments will be scored beginning in September. We anticipate that this trend will continue in our new misthouse with its enhanced climate control and uniformity of mist application.

Due to improvements in root quality and percent rooting, a 3-second liquid NAA dip after re-cutting the cutting base is now standard practice for loblolly experiments, unless otherwise specified. We have an experiment underway to determine whether re-cutting is necessary. Winter and spring loblolly cuttings are treated with 10 mM and 5 mM NAA, respectively. Because slash pine cuttings are more sensitive to NAA, we are waiting for more experimental data before deciding on any standard NAA treatments.

The number of companies supporting the project currently stands at twelve. Current supporters are Boise Cascade Corp., Bowater (Coated Paper and Pulp and Newsprint Divisions, Champion International Corp., Georgia-Pacific Corp., International Paper Co., James River Corp., Jefferson Smurfit Corp., Rayonier, Tenneco Packaging, Union Camp Corp., Westvaco Corp., and Weyerhaeuser Co. Funds provided by these companies, along with other sources of support, provide resources for the research of two Post-Doctoral Research Associates, one Research Assistant, one Laboratory Research Technician, and two graduate students. In addition, the project benefits from the efforts of one full-time and two part-time faculty scientists.

We plan to hold our Annual Meeting in Raleigh during the month of April. In addition to updating research results and discussing the project's performance and future direction, we will

"show-off" the new propagation misthouse. We will be soliciting input on the exact dates and finalizing plans soon.

INFLUENCE OF GENETICS ON ROOTING

Slash Pine Among-Family Variation in Rooting Ability

The family genetics work with slash pine is complete. We now have family data from three winter experiments (1994, 1995, and 1996) and one spring experiment (1995). Overall rooting percentage of the 38 open-pollinated families was 64% for Winter 1996. This was the highest rooting success that we attained in any of the rooting trials (Table 1). On a family basis, rooting ranged from 32% to 94% in the most recent, Winter, 1996 trial. The slight improvement in rooting for the 1996 experiment seems to have resulted from attention to detail in managing the greenhouse rooting environment. Since there has been no decline in rooting through the four years these hedges were grown, it suggests that the rooting environment is as much or more influential on rooting than the age of the hedges.

Table 1. Overall mean percent rooting and range from poorest to best family in four rooting experiments conducted for 38 open-pollinated slash pine families.

Experiment	Mean % Rooting	% Rooting Poorest Family	% Rooting Best Family
Winter 1994	54.7	18.3	86.7
Winter 1995	55.7	23.2	86.7
Spring 1995	46.6	0.0	76.7
Winter 1996	63.6	32.2	94.0

Family means from the four rooting experiments were correlated with each other as shown in Table 2. The correlations of family means among experiments are only moderate in size, ranging from .33 to .56, although all are highly significant (probability of a greater r is less than 0.01). In each experiment, the family mean rooting percent resulted from bulking 3 to 4 cuttings from each of 15 to 20 hedge plants or individual seedlings from a family. Since there is a large amount of genetic variation in rooting % among hedges within a family, sampling variation could contribute to the moderate to low family mean correlations. It is also likely that the substantial variation in water (mist) distribution could cause differential family responses that were dependent on the random positioning on the greenhouse bench. A more uniform rooting environment would be expected to result in higher family mean correlations among experiments.

Table 2. Family mean correlations among four independent rooting trials of 38 open-pollinated slash pine families.

Experiment	Winter 1995	Spring 1995	Winter 1996
Winter 1994	.33	.56	.42
Winter 1995	---	.39	.52
Spring 1995		---	.46
Winter 1996			---

A primary focus of these family rooting experiments has been to determine the relationship of family rooting with breeding values (BV's) for volume growth and rust resistance. The idea is that by culling poor rooting families we can improve rooting efficiency but this can only be useful if we do not adversely impact the forest productivity gains to be derived from selection for volume and rust resistance. The 38 open-pollinated families used in these rooting experiments were chosen to represent a broad range of breeding values for volume growth and rust resistance. The family BV's for volume growth and rust resistance were determined in independent field progeny tests conducted by the University of Florida Cooperative Forest Genetics Research Program. Correlations for family mean rooting percent with BV's for volume and rust resistance are shown in Table 3. All correlations are near zero, indicating that rooting ability is independent from BV's for volume and rust resistance. The correlation of breeding values for rust resistance with rooting percentage are negative, which indicates there is a slight, but nonsignificant, tendency for better rooting families to be more rust resistant. With these correlations it is clear that it is possible to cull for poor rooting while still making good progress while selecting for volume and rust resistance.

Table 3. Family mean correlations for rooting percentages with breeding values for volume and rust resistance.(Probability of greater r)

Experiment	Breeding Value for Volume	Breeding Value for Rust Resistance
Winter 1994	-0.16 (prob.>0.34)	-0.25 (prob.>0.12)
Winter 1995	+0.20 (prob.>0.24)	-0.19 (prob.>0.25)
Spring 1995	-0.09 (prob.>0.61)	-0.21 (prob.>0.21)
Winter 1996	-0.14 (prob.>0.40)	-0.04 (prob.>0.83)

Slash Pine Within-Family Variation in Rooting Ability

Several rooting experiments have been conducted to determine the potential for additional gain in rooting ability by exploiting within-family variation. In the first experiment, during Winter, 1995, eight families were chosen as having the highest rooting abilities and breeding values for volume and rust resistance. Subsequent rooting experiments in Spring, 1995 and 1996 have employed 6 families, 4 good-rooting and 2 poor-rooting families. The goal of adding poor-rooting families was to determine if there are good-rooting individuals within poor-rooting families. Due to insufficient numbers of cuttings, the clonal performance study was not established in Winter, 1996. Results from the Spring, 1996 experiment and final conclusions will be reported at the next annual meeting.

We have added an experiment to examine the relationship between rooting ability and field growth within slash pine families. Cuttings from the Spring, 1996 experiment will be potted and grown in the greenhouse over the winter. In the spring of 1997, we will transplant the cuttings to a field test and follow growth over several years to correlate rooting ability and growth on a clonal basis.

Loblolly Pine Genetic Variation in Rooting Ability (New)

This study has similar objectives as the slash pine study, i.e., to examine the relationship between rooting and breeding values for growth and to explore among-family and within-family variation in rooting ability. We are using open-pollinated seed of 25 families from the Atlantic Coastal Plain region, with 25 individuals per family. Seeds were sown in October, 1995 and seedlings were hedged for the first time in June, 1996, 8 months after sowing. One-hundred cuttings from each family were stuck in July and will be scored shortly.

ROOT INITIATION AND MATURATION

Hedge Maturation

This study is testing the longevity of seedling and serially propagated (rooted cutting) hedges in terms of rooting ability and growth of rooted cuttings. Currently, we have seedling hedges in the field from seeds that were sown in 1993, 1994, and 1995. The 1996 seedling hedges are now growing in the greenhouse and will be transplanted to the field plot next spring. Cuttings from the 1993 seedling hedges that were rooted in summer of 1994 are now established in the field plot as serially propagated hedges. This year we are potting the rooted cuttings from the 1994 seedling hedges. These cuttings will also be transplanted to the field plot next spring, and grown into serially propagated hedges.

We continue to conduct rooting trials on all ages and hedges with sufficient cuttings as reported in Jacksonville. Unfortunately, during our unusually harsh winter, the cuttings on the maturation hedges suffered apparent cold damage. The cuttings were noticeably off-color when they were stuck in the greenhouse in February and exhibited very poor rooting. No such problems were observed on cuttings from potted hedges kept in our over-winterizing structure and rooted in the same environment. We will attempt to correct this problem this coming winter by applying a potassium-rich fertilizer to the hedges this fall.

In June, we stuck cuttings from the 1993, 1994, and 1995 seedling hedges, as well as hedges serially propagated from 1993 seedling hedges and will be scoring rooting shortly. In the coming winter, we plan on testing all the hedges for rooting ability and then establishing a field test with the rooted cuttings later in the year to measure the effect of hedge age and origin on growth rate and form of the rooted cuttings.

Loblolly Pine Early Auxin-Induced Gene Expression

To better understand the process of adventitious root initiation and how it is affected by maturation, we are studying a group of genes that we have cloned from loblolly pine called *Loblolly Pine Early Auxin-induced* (*LPEA*) genes. We reported in April that these genes are closely related to similar genes in angiosperms and that elements in their sequences suggest a role in an auxin signal transduction pathway. In addition, we reported that all five *LPEA*s are induced in hypocotyl cuttings when treated with NAA and that at least two of the *LPEA*s were induced more slowly in epicotyl and hedge cuttings than they were in hypocotyl cuttings.

To further characterize expression of the *LPEA*s in loblolly pine, we have conducted four additional experiments: (1) auxin induction in different organs, (2) induction by other plant growth regulators, (3) induction by other auxin types, and (4) auxin induction in juvenile vs. mature cuttings.

Auxin Induction in Different Organs. All five *LPEA*s have been tested for induction by NAA in hypocotyls, cotyledons, and roots from seedlings, as well as, needles and stems from mature trees. The analysis shows that all five are most strongly expressed in NAA-treated hypocotyls (Table 4). In addition, there is a complex expression pattern with some members of the gene family expressed and induced to varying levels in the various plant organs. For example, *LPEA*s1-4 are expressed at relatively high levels in NAA-treated cotyledons, but *LPEA*5 is only induced to a minimal level. *LPEA*s1 and 5 are induced in roots, but *LPEA*2 was not detected in roots. These data suggest that the *LPEA*s may play a role in the differential response of various plant organs to the auxin signal.

 Induction by Other Plant Growth Regulators. This experiment tested whether induction of the *LPEA*s is a specific auxin response or if it is a more generalized response to plant growth regulators. We tested hypocotyl cuttings exposed to cytokinin (BA), gibberellic acid (GA), and abscisic acid (ABA). In addition, we treated hypocotyl cuttings with the translation inhibitor, cycloheximide (CHX), with and without NAA. CHX has been reported to induce expression of the early auxin-induced genes in pea and *Arabidopsis*. We found that *LPEA*3 (the only *LPEA* so far

tested) was not induced above basal levels by BA, GA, or ABA. CHX, on the other hand, did induce *LPEA*3 expression and the effect was at least additive when the cuttings were also treated with NAA. CHX treatment may result in induction, because the genes are normally kept off by a labile repressor protein. We are currently carrying out this analysis for the other four *LPEA*s.

Table 4. Relative abundance levels of LPEA mRNAs in different organs of loblolly pine.

Organ	Hypocotyls		Cotyledons		Roots		Needles		Stems	
NAA	-	+	-	+	-	+	-	+	-	+
*LPEA*1	+	++++	+	+++	+	++	+	++	+	+
*LPEA*2	+++	++++	++	+++	-	-	+	+	+	+
*LPEA*3	+	+++	+	++	+	+	-	+	+	+
*LPEA*4	+	++++	+	+++	-	+	-	+	+	+
*LPEA*5	++	++++	-	+	+	++	-	-	-	-

Induction by Other Auxin Types. Three of the *LPEA*s have thus far been tested for induction by other forms of auxin to ensure that the response is not just limited to NAA. *LPEA*s2, 4 and 5 are all induced by IAA, IBA and 2,4-D. The analysis testing the other two *LPEA*s is in progress. In addition, we have begun experiments to more carefully characterize the dose response of *LPEA* induction to lower levels of NAA and correlate it with root induction in hypocotyl cuttings.

Auxin Induction in Juvenile vs. Mature Cuttings. Because we had previously observed slight differences in the timing of expression of *LPEA*s among hypocotyl, epicotyl and hedge cuttings, we wanted to determine whether the *LPEA*s are also induced in cuttings from mature trees which root only very infrequently. Last winter, we obtained scions from ramets of the mother tree of the open-pollinated seed we have been using for these experiments (11-1103, from International Paper Co.'s Lumberton breeding orchard, formerly Federal Paperboard). The scions were grafted onto seedling rootstock from open-pollinated seed from the same mother tree in February, 1996. Scions were also taken from the same seedlings and grafted onto half-sib rootstock for a juvenile, grafted control. Successful grafts were pruned back in early May and again in late July to approximate hedging treatments and the resulting shoots (cuttings) used to test for *LPEA* induction. Cuttings from mature and juvenile grafted hedges, one-year-old hedges and hypocotyls were treated with NAA or a control solution and analyzed for *LPEA* expression 24 or 72 hours later. As before, all five *LPEA*s were strongly induced by NAA treatment in hypocotyl cuttings (Table 5). *LPEA* induction was also evident in the juvenile hedge material regardless of whether the hedge originated from a graft. Cuttings from mature, grafted hedges, on the other hand, showed very limited induction by NAA. Only *LPEA*1 was induced significantly above background levels and this was at a lower level than any of the juvenile cutting types. Because of the small number of mature cuttings we had available, we regard this result as preliminary. We will be repeating this analysis with additional cuttings in the coming year, as well as expanding the number of sampling times and NAA treatments.

Nevertheless, this is a very promising result, as it may represent part of the auxin response pathway leading to root initiation that becomes defective in cuttings from mature trees.

Table 5. Relative abundance levels of LPEA mRNAs in loblolly pine cuttings of different ages.

Cutting source	Hypocotyls		1-year-old hedges		1-year-old grafted hedges		Mature grafted hedges	
NAA	-	+	-	+	-	+	-	+
*LPEA*1	++	++++	++	+++	++	+++	+	++
*LPEA*2	+	++++	+	+++	+	+++	+	+
*LPEA*3	+	+++	+	++	+	++	+	+
*LPEA*4	+	++++	+	++	+	++	+	+
*LPEA*5	+	++++	+	+++	+	+++	+	+

Current and Future Research on *LPEA*s. In addition to completing the experiments described above, we have begun a number of procedures to further elucidate the role of *LPEA*s in root initiation. These include: (1) cellular localization of *LPEA* expression using tissue prints, (2) analysis of *LPEA* gene copy number using Southern blot analysis, (3) construction of a genomic library to permit isolation of the genomic clones corresponding to *LPEA* cDNAs, (4) protein-DNA and protein-protein experiments to determine the biochemical functions of the *LPEA*s, and (5) construction of recombinant plant transformation vectors containing the *LPEA*s to permit generation of transgenic trees. We currently plan to introduce the modified *LPEA* genes into hybrid poplars until a reliable and routine loblolly pine transformation system is available. The transformation experiments will provide a direct test of the cause and effect relationships between *LPEA* expression and adventitious root initiation in trees.

QTL's for Rooting

Quantitative trait loci (QTL's) are regions within a genome that contribute to major variation in a trait. This study is testing for QTL's that control genetic variation for rooting percentage and root number in loblolly family 9-1020. The laboratory work of generating molecular markers and screening progeny is complete. At the annual meeting, we presented results from the first two rooting trials (Winter and Spring, 1995). The third trial was stuck in Winter, 1996 with the help of Ben Cazell at the Rayonier facility. Overall, the mean rooting percentage for clones in this trial was 53%. Currently, the final trial of the rooting experiment is in the new misthouse (360 clones x 24 cuttings/clone divided among 4 replications). None of the cuttings in the rooting experiments for this study were treated with NAA.

At the 1996 annual meeting, we showed several putative QTL's for rooting. Some of these markers overlapped between different rooting seasons and tissue type (i.e., hypocotyls and hedge

cuttings) and show promise as significant QTLs. The next step is to complete the final analyses of the marker-rotting associations including the two most recent trials.

University of Maine Research Team (Greenwood, Hutchison, et al)

Expansin. Using differential display we have demonstrated the expression of an auxin-induced gene called expansin, that is thought to be involved in loosening the primary cell wall. While only expressed in the presence of auxin, its expression is 4-fold stronger in hypocotyls which root than in epicotyls which root more slowly and at lower frequencies. Since cell wall loosening is probably a crucial event in the early stages of cellular dedifferentiation that precedes root meristem formation, the expression of this gene could play a key role in rooting. Expansin expression peaks at about 24 hours after auxin treatment. A visiting scholar from China, Xu Fuyu, has just arrived and will begin work on *in situ* hybridization studies to pinpoint the tissues where expansin is expressed. We also plan to test the specificity of the expansin response to auxin.

Cyclin-Dependent Kinases. A thorough analysis of the role of a cyclin-dependent kinase-like gene from loblolly pine during auxin-induced rooting in hypocotyls has been concluded by Antoinette Decker. She hypothesized that failure of epicotyls to root could be a function of the inability of their cells to resume cell division in response to auxin, which might be reflected in decreased expression of genes regulating the cell cycle. Using primers from the catalytic domain of cyclin-dependent kinases (cdks) of other organisms, Ann has cloned and characterized a cdk-like gene from loblolly pine (cdkpt-1), which Southern blot analysis has shown may be one of 8 similar genes. The gene is expressed in hypocotyls and epicotyls, with expression peaking 24 to 48 hours after the cutting is made. IBA treatment does not affect expression. Tissue prints have revealed that expression is observed in the cortex of hypocotyls earlier than in epicotyls, where expression is more pronounced in the epidermis and cambial region. Therefore, the earlier expression in the cortex of hypocotyls may reflect the competence of cells in this region to form roots. In conclusion, the expression of cdkpt-1 is not affected by auxin and is expressed in both hypocotyls and epicotyls, so differential expression of this gene is probably not directly associated with failure of epicotyls to root rapidly.

These results are consistent with the observation (by another graduate student, Cui Xiuyu) that hypocotyls and epicotyls readily form callus in response to auxin. There is no difference in response to auxin concentration; if anything, epicotyls are slightly more responsive to auxin. A histological study has shown that callus initially begins to form in the cambial region of both tissues after about 6 days, after which cell divisions begin throughout the cortex and epidermis. There is no evidence that the cells in the vascular parenchyma centrifugal to the resin canals, which are competent to form roots, respond more quickly to auxin. Thus we can conclude that cells from both epicotyls and hypocotyls can respond to auxin in terms of the capacity to dedifferentiate and divide to form callus. This reinforces the idea that the cells which are competent to form roots are localized and retain unique competence to organize root meristems. This ability is not reflected in an increased sensitivity to auxin in terms of a cell division response.

Arabinogalactan Proteins (AGP's)

AGPs are glycoproteins that are reported to stimulate somatic embryogenesis in nonembryogenic cultures of carrot and spruce. Our research on AGPs, funded by the North Carolina Biotechnology Center, is testing whether there are also AGPs in pine that affect adventitious root formation. To attempt to identify specific AGPs associated with rooting, we have raised monoclonal antibodies against AGPs purified from NAA-treated hypocotyl cuttings. Twenty-five antibody lines were tentatively identified as recognizing AGPs that were more abundant in hypocotyls than in needles, stems and xylem from mature trees. The two most promising lines have been screened further. One antibody line shows very strong recognition of hypocotyl AGPs and the abundance of the recognized AGP declines from 0 to 14 days after NAA treatment. This line also has been tested for recognition of AGPs in stems and needles of cuttings from 8-year-old hedges and stems and needles of mature trees. The antibody recognizes AGPs in both the stems and needles of the hedge cuttings, but shows very weak recognition of stems and needles from the mature tree. It is not yet clear whether the recognized AGP is more closely associated with rooting and juvenility than with growth differences between the materials tested. Future experiments will test this in greater detail.

As we continue our efforts to identify rooting-specific AGPs, we are also interested in testing whether there is a measurable effect on rooting from AGPs in general. We have installed a greenhouse study to address this question. Total AGPs were purified from hypocotyls and roots of 2-week-old seedlings and were applied to hedge cuttings. Cuttings from two families were placed in a solution containing three concentrations of AGPs (0, 12.5 and 25 mg/L, in a ratio of 1 part hypocotyl AGPs to 5.4 parts root AGPs) for 24 hours. The base of each cutting was then re-cut and placed in a 2.5 mM NAA solution for 3 seconds. The experiment consists of the three AGP treatments, two families, with 30 cuttings per family per AGP treatment divided among 6 replications. The experiment will be scored in September.

Brassinosteroids

This group of phytohormones has been reported to stimulate rooting in eucalyptus and Norway spruce. We had previously reported that in hypocotyl cutting rooting experiments, no significant effect was observed. Nevertheless, we wished to test for rooting stimulation in hedge cuttings which do not root as easily as hypocotyl cuttings. We soaked cutting bases in 0, 10^{-12}, 10^{-11}, 10^{-10} or 10^{-9} mM 24-epibrassinolide for either one or 24 hours. Half of the cuttings in each treatment then received an NAA treatment while the other half did not. As with the hypocotyl cuttings, there was no significant, consistent effect of the brassinosteroid treatments on either rooting percentage or the numbers of roots per rooted cutting. While we can not rule out any possible effect of brassinosteroids on rooting from these limited experiments, we do not anticipate further experiments at this time.

STOCK PLANT NUTRITION AND MANAGEMENT

Loblolly Pine Hedge Nitrogen Nutrition and Carbohydrates

This study is investigating the effects of different levels of nitrogen fertilization of hedges on internal carbohydrate status and rooting performance of cuttings. All rooting experiments and laboratory measurements for this study have been completed. We now have data for two complete years of rooting trials: Spring and Summer, 1994; Winter, Spring, and Summer, 1995; and, most recently, Winter, 1996. Summarized here are the data from the three trials stuck in Westvaco's greenhouse in Summerville, SC during the past year.

There were significant differences among seasons, families and nitrogen (N) treatments in regards to rooting percentages. When averaged over families and N treatments, greater rooting percentages occurred in spring softwood cuttings (59.5%) than in winter hardwood (40.5%) or summer softwood cuttings (34.7%). Across all seasons and N treatments, families 27-2 x 27-1 (green) and 27-6 x 27-1 (white) were the best rooters at 50.0%, followed by 27-2 x 27-5 (blue) at 44.0% and 27-3 x 27-1 (red) at 35.8%. Across all families and seasons, the highest rooting was obtained in cuttings from stock plants fertilized with 55 ppm N (58.1%), followed by 70 ppm (51.8%), 40 ppm (51.5%), 25 ppm (41.8%), and 10 ppm (21.3%), however, there was a significant family x N treatment interaction. To determine whether this interaction results from differential N uptake by families or from family-specific N requirements for rooting, we have plotted each family's rooting performance against the concentration of N in the tissue (% of dry weight for the entire cutting) for each season (Figure 1). In the spring and summer rooting trials, optimal rooting success for all families occurred when the cutting N concentration was generally in the range of 1.8 to 2.0%. In the winter cuttings, internal N concentrations were lower overall (1.5% or below), but rooting success tended to be better at the higher internal concentrations (1.3 to 1.5%) despite some unexplained variation.

Differences were also observed in the carbohydrate concentrations of cuttings by season. Total nonstructural carbohydrates (TNC) in winter cuttings (32.9%) were approximately twice those in spring (17.2%) and summer (16.3%). This was due primarily to higher soluble carbohydrate levels in winter (32.7%) than in spring (16.5%) and summer (15.8%). In contrast, starch levels were lower during winter (0.16%) than during spring (0.69%) and summer (0.58%). Seasonal differences in carbohydrate status do not appear to be a major determinant in rooting success. Family variation for TNC concentration was also observed, but was not well correlated with rooting success. Carbohydrate analysis also revealed that neither the TNC:N ratio nor the level of any individual sugar was closely correlated with rooting performance.
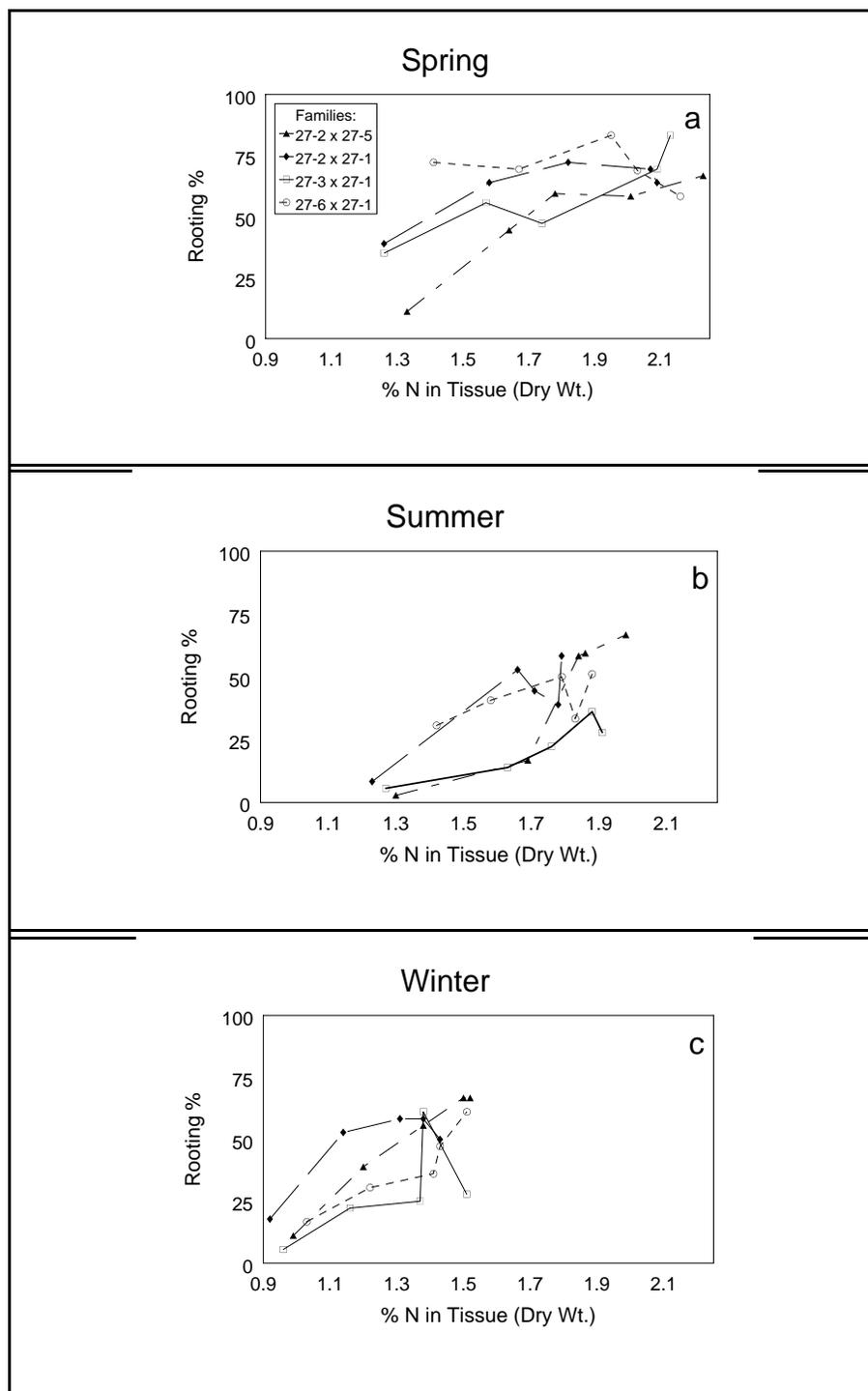
Figure 1.
Effect of tissue nitrogen concentration on rooting of hedge cuttings from four loblolly pine full-sib families in spring (a), summer (b), and winter (c).

Of the mineral nutrients other than N, boron (B) appeared to have an impact on rooting. There were no significant differences in B concentration among families or applied N treatments, however, an apparent B deficiency was observed during the spring in the "horticultural control" (Osmocote) treatment (8.7 ppm B) relative to the other five N treatments (21.7 ppm B). The control cuttings rooted only at 0.8% in the spring, but returned to 61.1% in cuttings from the same hedges the next winter following restoration of B levels to 15.9 ppm. It appears that a requirement for B at these levels may be relatively specific for rooting, because the B-deficient hedges produced the greatest number of shoots, had high TNC levels, and exhibited no obvious deficiency symptoms. We are investigating the specific B requirements of hedges for rooting in a new study (see below).

Slash Pine Hedge Nitrogen Nutrition (New)

We recently began an experiment to address nitrogen nutrition of slash pine hedges. Because slash pine may respond to fertilization differently than loblolly pine, we cannot use tissue concentration recommendations from the loblolly study. This experiment will be conducted using the same automated fertigation and experimental design as the loblolly nitrogen study. The genotypes are 4 full-sib families obtained from Rayonier. Seeds were sown in the greenhouse in January, 1996, hedged for the first time in late June, and transferred to the container pad in August when they received another hedging treatment.

Because the hedges are not yet full size, nitrogen treatments will be applied beginning early next spring. This study will initially employ the same N treatments as those used in the last year of the loblolly nitrogen study (10 to 70 ppm). We expect that the first cuttings from this study will be stuck in June, 1997. A companion study to investigate the effect of different osmocote treatments on foliar nitrogen levels was initiated this summer. Six Osmocote levels (3/4 to 2 tablespoons of Osmocote 18:6:12, 9-month release) were applied to 8 hedges in each of 3 slash pine families. Foliar nitrogen levels in these hedges will be compared to results from the larger study for use as a guide for operational fertilization of hedges to attain optimal rooting.

Loblolly Pine Hedge Boron Nutrition (New)

This experiment was initiated after literature reviews indicated that boron may influence rooting in both herbaceous and woody plants and because of our experience with the control hedges of the loblolly pine hedge nitrogen nutrition study. Despite the circumstantial evidence obtained from those hedges, we have not yet demonstrated a cause and effect relationship between boron and rooting and do not know the critical level for rooting.

Seedlings for this study were sown in December, 1995. Stock plants have now been hedged twice, in June and August, 1996. The experimental design is 6 boron levels, 4 full-sib families, and 4 hedges per family/boron level in each replication with 4 replications. The 4 full-sib families are South Atlantic Coastal Plain crosses obtained from International Paper Co. Boron treatments will be facilitated by either granular or foliar applications. Foliar boron concentrations will be closely monitored to assure proper levels are maintained. At the current time, the hedges have begun to show boron-deficiency and we will initiate the boron treatments during this fall. Nitrogen,

potassium, and phosphorus will be supplied in non-limiting levels via a twice per year Osmocote application. Micronutrients other than boron will be delivered with STEP micronutrient fertilizer (a slow-release mixture lacking boron).
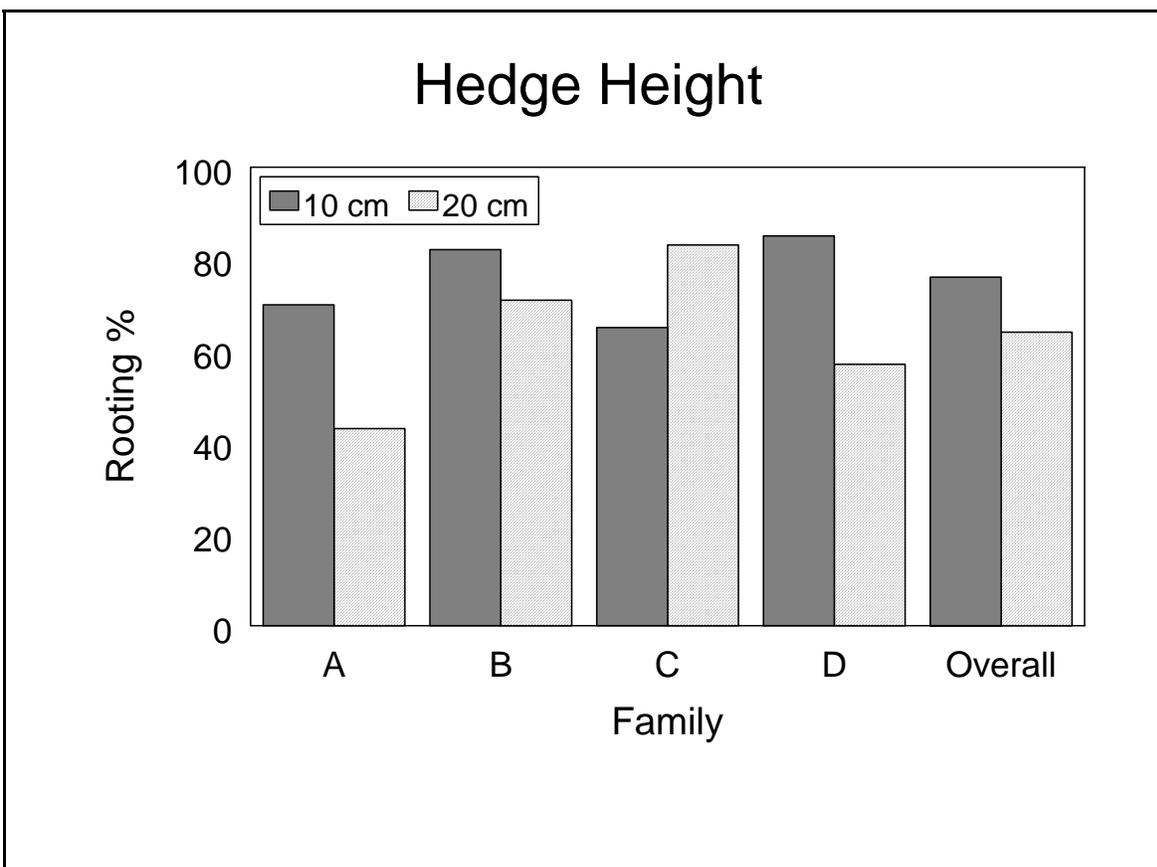
Hedge Height

The objective of this study is to determine the effect of the height at which hedges are pruned on rooting ability and shoot production. The seeds were sown in 1993 and the seedlings topped in 1994 at 10 or 20 cm above soil level. The four full-sib families used in this study are the same ones used previously in the studies testing the effects of different auxin types on root system morphology studies and were obtained from Westvaco. At the April meeting, we reported that in the June, 1995 rooting trial there was essentially no difference in rooting percentage between low and high hedges (41% and 39% respectively). The data from that particular experiment, however, were affected by a disease problem in the horticulture greenhouse and should not carry full merit. We repeated the rooting trial in February, 1996. The experimental design was 12 replications with 10 cuttings per hedge-height/family combination in each replication. These cuttings were stuck in the Method Road forestry misthouse and there were no disease problems.

This experiment revealed significant differences in rooting among the two hedge heights (76% in low hedges, 64% in high hedges). Standard errors for rooting percentage were about 2%. There were no significant differences in the number of roots per rooted cutting or the symmetry or orientation of cutting root systems between the hedge heights. There was, however, a significant family x hedge height interaction. Families A, B, and D had higher rooting in cuttings from low hedges, but family C had higher rooting in cuttings from high hedges (Figure 2). Thus, at this point, we cannot recommend that a 10-cm hedge height is preferable to 20 cm for every family. This experiment was repeated in June and the data will be available shortly. In June, we also conducted a shoot count on the hedge heights. Hedges pruned at 10 cm averaged 46 cuttings per hedge, while 20-cm hedges averaged 53 cuttings per hedge. We will continue to root cuttings from the hedges maintained at these heights to determine if the main effect and interaction are biologically significant.

Cutting Morphology (New)

Last winter we observed considerable variation in the needle morphology of cuttings growing on individual hedges of family 9-1020 (QTL hedges, etc.). Some hedges had cuttings with only primary needles (juvenile), while others had cuttings with both primary and fascicular (mature) needles. A third hedge type had a mix of cutting types. To test whether cutting needle morphology affects rooting performance, we selected 24 'juvenile' and 24 'mature' hedges (no mixed hedges were chosen). In the winter, we stuck 20 cuttings from each hedge with 5 cuttings in each of 4 replications.

Hedge Height

F
igure 2. Effect of the height at which hedges are pruned on rooting of loblolly pine cuttings.

The results show that "juvenile" cuttings rooted at 75%, whereas "mature" cuttings rooted at 57%. Standard errors for percent rooting were about 2%. Other rooting parameters (root number, symmetry, and orientation) were not significantly different between juvenile and mature cutting types. We planned to continue this test in the spring rooting season. However, the hedges did not develop the different needle morphologies as before. At the present time, as the hedges are nearing growth cessation for the year, the different needle morphologies have again be detected on the same clones. Thus, it appears that needle morphology is not necessarily a function of the internal maturation status of the hedge. It is possibly related to the timing of the onset of fascicular needle development or to the duration of cutting growth and development prior to bud set and growth cessation in the fall.

Nevertheless, the difference in rooting observed here could be significant in an operational production program. To further explore this importance we are looking at growth rate and form differences between the cutting types. Rooted cuttings from this study were transplanted into pots and grown in the greenhouse during the summer. In August, they were transferred to the arboretum container pad to be subject to natural growth cessation. We will measure total height, number of flushes, and degree of plagiotrophy in the fall. In the future, we may institute a study to test

different late summer hedging times and the effects on cutting needle morphology, rooting and growth of rooted cuttings.

ROOTING ENVIRONMENT AND PHYSIOLOGY OF CUTTINGS

Physiology of Cuttings and Hedges

This area of research is investigating the water relations and gas exchange of cuttings and hedges in order to provide guidance for optimizing rooting environments and stock plant management procedures. In April, we reported preliminary results on experiments determining the initial water stress levels that affect cutting survival and on diurnal trends in net photosynthesis and water status of cuttings at the beginning of the rooting period. Updates on these experiments and two new studies are described below.

Lethal Water Stress Experiment. Preliminary work suggests that loblolly pine stem cuttings can tolerate initial stress water potentials as high as -1.5 MPa (-15 bars) and still survive and produce roots. There was no major difference between the tolerance for stress levels between survival and rooting; thus if a cutting survived the initial stress period (before sticking) its subsequent rooting performance was not impaired relative to cuttings receiving less initial stress. This preliminary experiment with winter (dormant) cuttings is currently being repeated with spring cuttings.

Diurnal Trends in Xylem Water Potential. Very little is known about the water relations of loblolly pine stem cuttings during the period leading to root initiation. Basic information is needed on how cuttings respond to mist treatments in terms of water status. Xylem water potential (ψ_{XP}) and soil water content was measured once every 3 weeks during the 12-week rooting period.

Cuttings tended to remain under relatively high stress levels (approximately -1.2 MPa) until they formed roots. Although no distinct trend was observed in the water stress of cuttings during different portions of the rooting period (until rooting), daily and hourly fluctuations did occur. Cutting ψ_{XP} was sensitive to the vapor pressure deficit (VPD) which is a function of both temperature and humidity. On some measurement dates, cuttings did not regain full turgor during the night. Because of this observation we instituted a new infrequent mist treatment for all of the current experiments in the new propagation house. In addition, we are explicitly testing the effect of the night mist treatment on water status and rooting. The diurnal water status experiment was also conducted using winter cuttings and is now being repeated using spring cuttings.

Water Status of Cuttings During Handling and Storage. An experiment was conducted to determine the effect of harvesting, handling and short-term storage on the water status of loblolly pine stem cuttings. ψ_{XP} of hedges was measured at predawn (4:00 am) and 9:00 am on cuttings immediately after they had been removed from the hedges and on cuttings after they were wrapped in wet paper towels and subjected to several storage treatments. These included placing the wrapped cuttings in insulated coolers for 2 and 7 hours and in a walk-in refrigerated cooler for 21 hours.

Also, ψ_{XP} was measured on cuttings taken from either the top or side of the hedge to determine if position of the cutting on the hedge had any effect on water status of the cutting.

Mean predawn ψ_{XP} of the hedges was -0.44 ($\pm$ 0.05) MPa (Figure 3). Results did not indicate any significant difference between mean ψ_{XP} values of hedges measured on cuttings just prior to storage (9:00 am) (-1.07 $\pm$ 0.14 MPa) and cuttings stored for 2 hours in insulated coolers (-1.04 $\pm$ 0.22 MPa). However, cuttings stored either in an insulated container for 7 hours (-0.98 $\pm$ 0.21 MPa) or a refrigerated cooler for 21 hours (-0.82 $\pm$ 0.19 MPa) had significantly improved water status compared to cuttings at the time of removal from the stock plant. No significant difference in predawn ψ_{XP} values were observed between cuttings taken from the top or from the side of the hedge.

These results suggest that short-term (at least 7 hours) storage in insulated coolers under conditions of high humidity improves water status of cuttings and could, therefore, improve rooting performance. Improvement of water status of cuttings after wet and/or cold storage may be attributed to actual absorption of water or to reduced transpiration due to low VPD conditions in the coolers.

Basal Uptake of Water in Loblolly Pine Stem Cuttings. Water uptake of loblolly pine stem cuttings was estimated over a two-week period immediately after insertion in distilled water. The experiment was a 2 x 5 factorial split-plot design. Two greenhouses and five cutting procedures were the whole plot factors, and families was the sub-plot factor. The 5 treatments tested were cuttings: 1) with fascicles intact (normal sticking procedure), 2) with fascicles removed, 3) with bark and fascicles removed, 4) with the stem base re-cut just before insertion and the fascicles intact, and 5) with the base re-cut and the fascicles removed. Within each cutting group, three families were tested. Total uptake of water on a volume basis and uptake per unit of submerged stem surface area were estimated on the 6th, 8th and 12th day after insertion in the water. Total uptake on a weight basis was also estimated on the 6th and 12th day after insertion.

Average volume of water taken up across families was highest in cuttings that had the bark and fascicles removed. This was followed by cuttings with intact bark, re-cut bases and fascicles removed; then by cuttings that had been re-cut before insertion but had intact fascicles; and then by cuttings that had not been re-cut, but did have the fascicles removed. Uptake was lowest in the cuttings with the bark, stem base, and fascicles intact (normal sticking procedure). Across treatments, uptake was high for the first week of immersion, but was reduced drastically by the end of the second week. No significant difference was observed between the two greenhouses. Family differences did exist, but were not consistent from one week to another. Amount of surface area of stem exposed to water also had a significant effect on uptake and this varied by family.

Water uptake is very high in the initial couple of days and declines thereafter. Removal of bark appears to reduce resistance to water uptake. Similarly, removal of foliage also appears to decrease the resistance to water uptake, possibly by making additional entry points for water into the cuttings' xylem. These treatments will be applied to cuttings stuck in media this coming year to determine the effects on water status and rooting success of the cuttings.
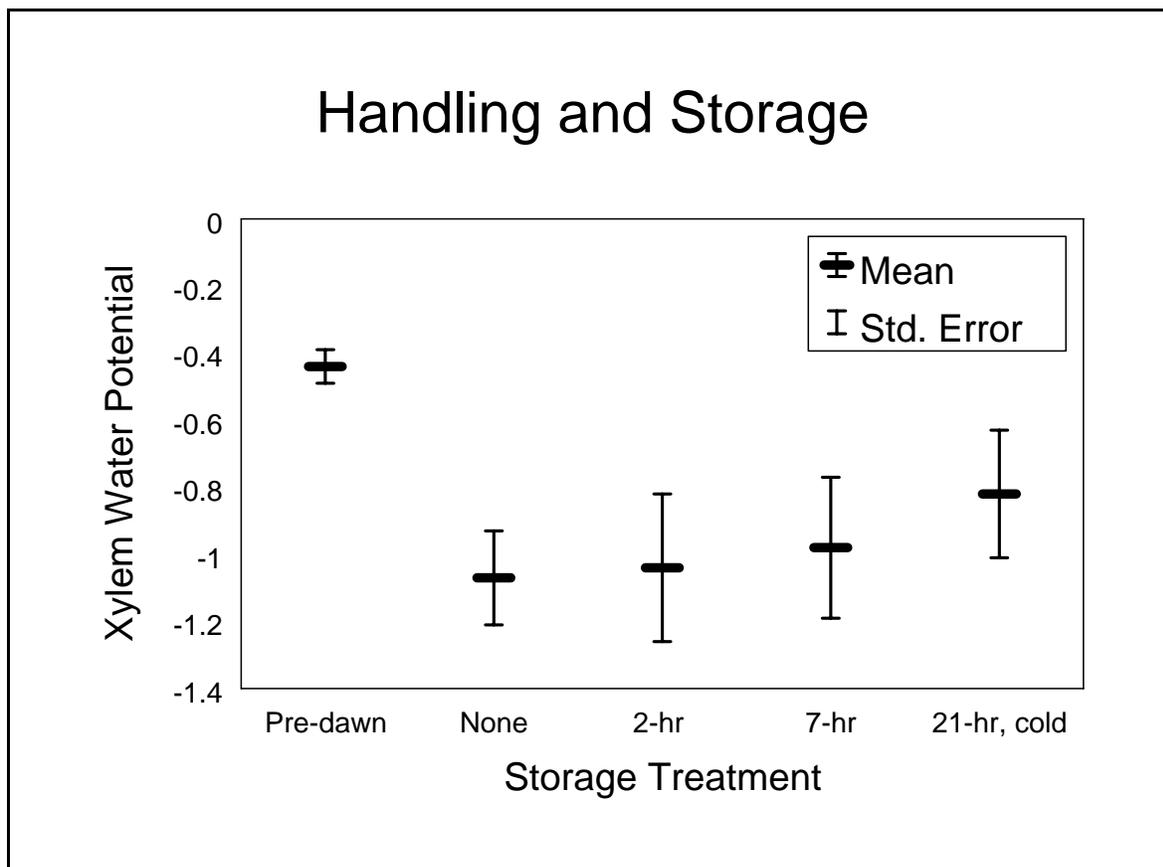
Handling and Storage

Xylem Water Potential vs Storage Treatment

Legend: Mean, Std. Error

Figure 3. Effect of handling and storage treatment on water status of loblolly pine cuttings.

Current and Future Research. We are planning a series of experiments that will be conducted this coming winter to more precisely define the effects of environmental factors on rooting. The studies will be carried out in controlled environment chambers at the NCSU Phytotron. Environmental factors to be tested include temperature, light intensity, photoperiod and CO_2 concentration. Ambient and elevated CO_2 treatments will be applied to both hedges while the cuttings are forming and cuttings during the rooting period.

Rooting Medium Composition (New)

This winter we conducted an experiment to test different ratios of peat and perlite in the rooting medium for the effects on rooting. The 50:50 mixture of peat and perlite that we had used previously appeared to hold too much water for optimal rooting. This experiment tested five medium compositions, including 70:30, 60:40, 50:50, 40:60 and 30:70 peat:perlite. The experimental design consisted of 8 replications, with each replication containing 20 tubes of all 5 media types in randomly arranged 'single tube' plots. Results from the Winter, 1996 experiment showed that a 40% peat and 60% perlite mixture had the highest rooting percentage (Figure 4). There were no significant differences in root symmetry or orientation among media. We are

repeating the same experiment this summer in our new misthouse. In the future, we may add different media components (e.g. bark, sand, vermiculite) to this series of studies.
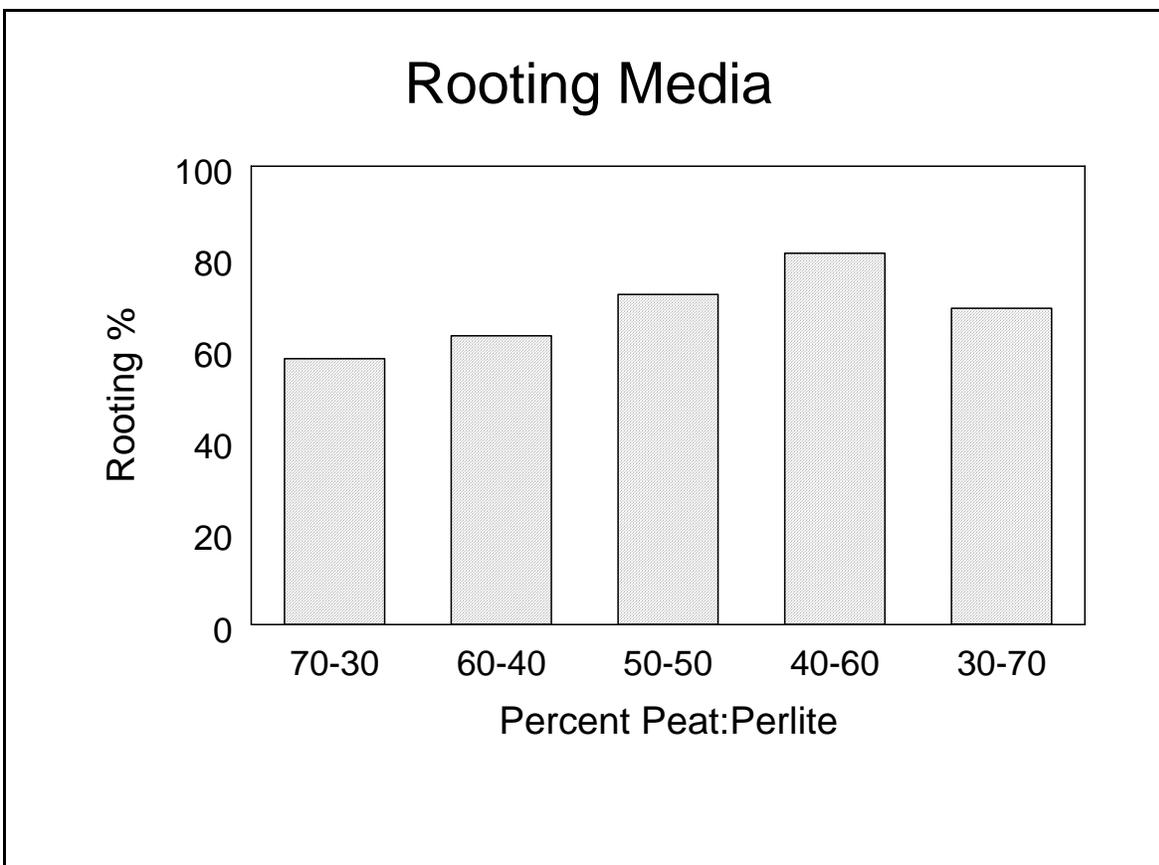
Rooting Media

Figure 4. Effect of medium composition (percent peat:perlite) on rooting of loblolly pine cuttings.

ROOT SYSTEM QUALITY

Root System Morphology Field Test

The main goal of this study is to determine the effects of variation in cutting root system morphology on field growth. The traits we have chosen for defining root system morphology are root number, symmetry, and orientation (number of roots with vertical orientation). As reported previously, cutting material for this experiment was obtained from the 400 QTL hedges (family 9-1020). After the Winter, 1995 rooting period, cuttings were scored for the root morphology traits and tagged to keep track of root system data by individual cutting. Approximately 1800 cuttings were transplanted into the G.H.W. Weyerhaeuser nursery (Washington, NC) in June, 1995. In late January, 1996, the cuttings were lifted and scored again for root morphology traits.

In February, 1000 cuttings were transplanted to a field site near Pineland, SC. The experimental layout for the Pineland site is four blocks containing 250 cuttings each (single tree plots). The entire experiment is surrounded by a double border row of seedlings. Staff from Georgia-Pacific are maintaining the site and will measure the cuttings for the next several years, beginning this fall. From these data, we will determine if cutting growth and form are dependent on root system morphology. First-year survival is nearly 100 percent.

NAA Treatments

We continue to test the effects of NAA on rooting success and root system morphology. Last year, we reported results of an experiment with 7 treatments: a control, 3 liquid NAA treatments with a 1-second dip (1, 5, and 10 mM), and 3 talc NAA treatments (931, 1862, and 3724 ppm). The highest rooting percentage was observed with the 5 mM NAA liquid treatment, but the 10 mM treatment had the highest root number. Compared to liquid NAA, rooting traits were not enhanced with the talc treatments. Overall, rooting percentage in this experiment was rather low.

This past winter, we began a series of new NAA experiments. We applied eight liquid NAA treatments to loblolly and slash pine cuttings to obtain full response curves. We also changed from a 1-second to a 3-second dip and increased the number of replications and number of cuttings per treatment to better control variation and get more precise estimates. The experimental design for loblolly pine cuttings included 8 NAA treatments (0, 5, 10, 15, 20, 30, 40, and 50 mM), 8 replications, 4 families, and 6 cuttings per family/treatment combination in each replication. The slash pine experimental design was the same as for loblolly, except that the genotypes consisted of two bulk samples, one from 5 poor-rooting and the other from 5 good-rooting families.

In loblolly pine winter cuttings, the percent rooting was highest for concentrations of 5 to 20 mM. Within this range, the rooting percentage varied (nonsignificantly) from 66 to 73% (Figure 5a). The number of roots per cutting increased from 1.6 (0 NAA) to 4.5 (20 mM NAA) and then appeared to level off. Similar increases in symmetry (% of cuttings with a symmetric root system) and orientation (number of vertical roots per cutting) were observed with the highest values of both variables observed in the 15 to 20 mM range (Figure 5b). NAA concentrations higher than 20 mM resulted in lower rooting success and root quality. It appears that benefit from increased root quality can be obtained by using a treatment in the 10 to 20 mM range without affecting rooting success. There was no significant family x NAA concentration interaction for any rooting trait.

Slash pine, however, responded quite differently. Rooting success was clearly inhibited by NAA treatment. The highest rooting was observed with no NAA (57%) and no rooting occurred with 50 mM NAA (Figure 6). Root numbers per rooted cutting did increase up to 10 mM NAA. Now that we have ascertained NAA response curves for winter cuttings of loblolly and slash pines, we will attempt to further refine the treatments next winter. For loblolly pine, we will focus on the
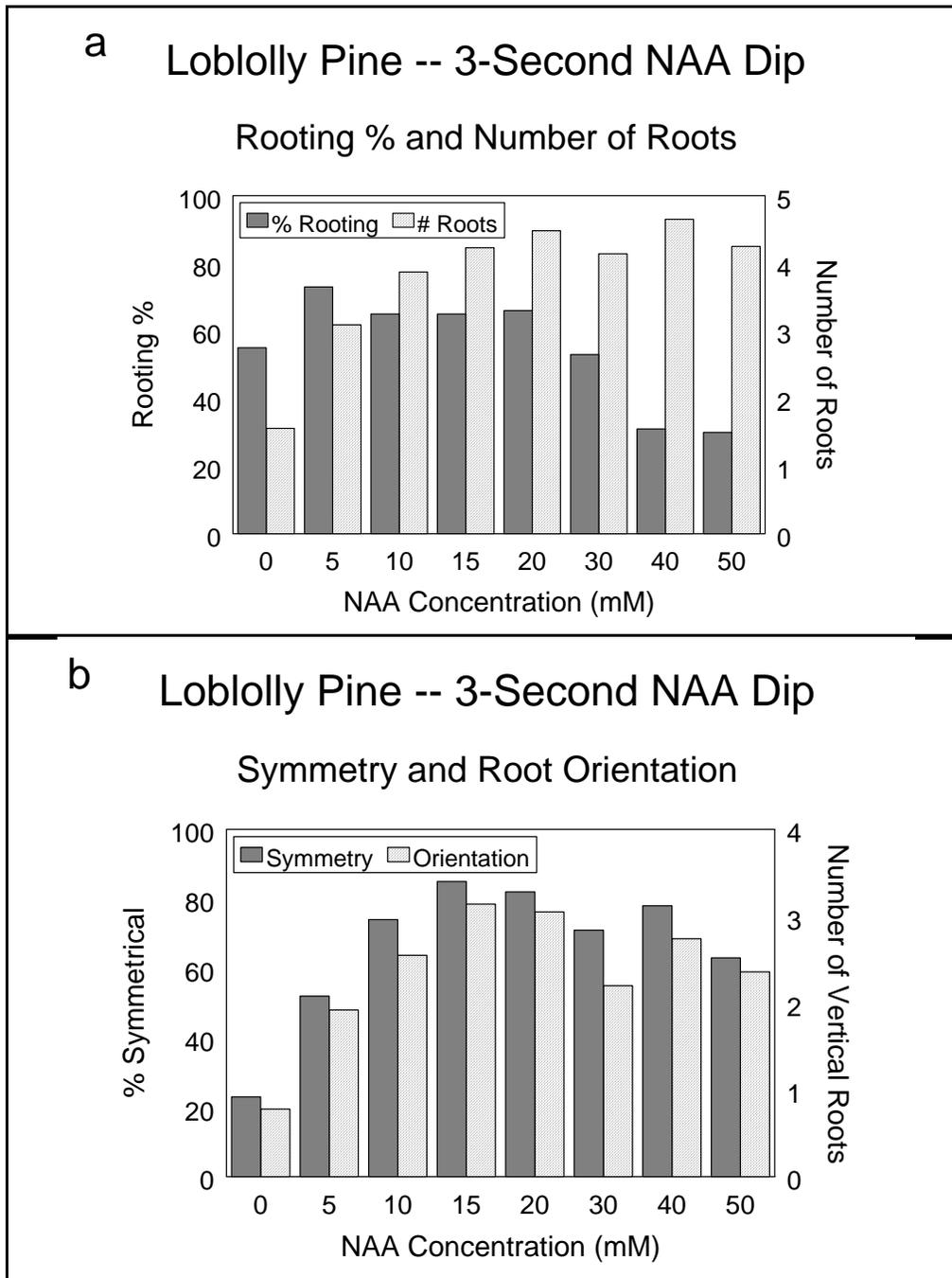
Figure 5. Effect of NAA concentration on rooting and root system morphology of loblolly pine cuttings (a, rooting percent and number of roots per rooted cutting; b, percent of cuttings with symmetrical root systems and number of vertical roots per rooted cutting).

range of 2.5 to 20 mM. For slash, we will focus on lower concentrations and/or other auxin types and methods of application.

We are continuing our NAA response experiments with spring cuttings of both species. We had determined previously that spring cuttings are more sensitive to NAA, so the treatments were revised accordingly. For loblolly cuttings, the NAA treatments used were 0, 0.5, 1, 2, 3, 4, 5, 7.5, and 10 mM. Given the apparent sensitivity of slash pine cuttings to NAA, the treatments were modified to even lower concentrations of 0, 0.5, 1, 1.5, 2.0, 2.5, 3.75, 5 mM.
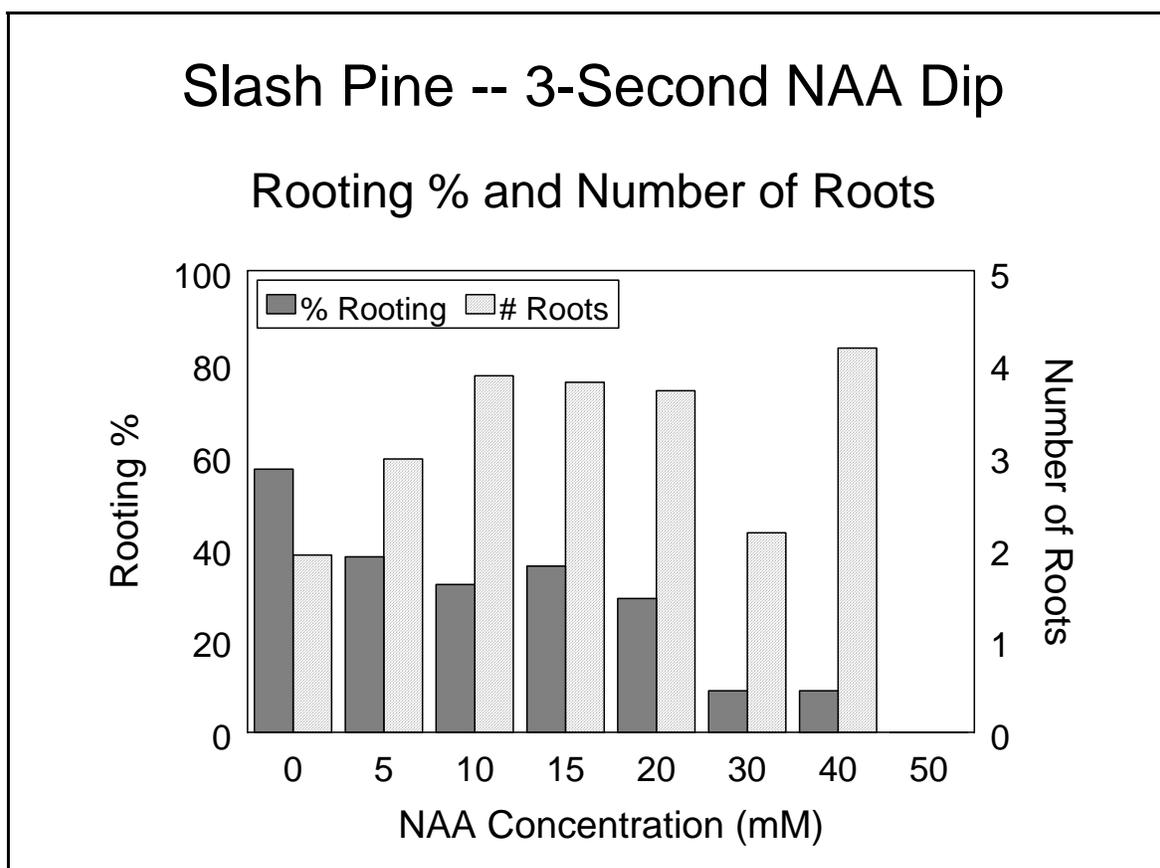

Figure 6. Effect of NAA concentration on rooting percent and number of roots per rooted slash pine cutting.

Effect of Re-Cutting Bases on Rooting

In the NAA response experiments conducted this year, the bases of cuttings have been re-cut immediately before applying NAA. Re-cutting may allow more uptake of NAA at the cutting base, but it also increases the workload and is thought to reduce rooting success in *Pinus radiata*. We installed an experiment this spring to determine if responses of loblolly pine cuttings to NAA are influenced by re-cutting just before sticking. The experiment is a 2 x 3 factorial with two cutting

treatments (re-cut, not re-cut) and three NAA treatments (0, 2.5, and 5.0 mM NAA). Seven cuttings were stuck for each of the six treatment combinations in each of 12 replications.   The experiment will be scored this September.

CONCLUSION

This report summarizes research progress by the NCSU Loblolly and Slash Pine Rooted Cutting Project since April. Studies in the areas of genetic variation in rooting, stock plant management, optimizing the rooting environment, and root system quality are providing information to assist supporting companies in their efforts to multiply outstanding full-sib families using rooted cuttings. The information reported here, including improved rooting percentages, demonstrates that this deployment strategy requires only greater reliability and cost-effectiveness to become operational. We look forward to continued improvements in the coming months and years.

All of the research areas, including that on root initiation and maturation, are providing information that will be helpful for the eventual implementation of clonal forestry using rooted cuttings. The large amount of genetic gain possible with this deployment strategy ensures that, as a project, we will continue our efforts to make this technology a reality.

NCSU LOBLOLLY AND SLASH PINE ROOTED CUTTING PROJECT

List of Current Studies

Influence of Genetics on Rooting
Slash pine among-family variation
Slash pine within-family variation
Loblolly pine genetic variation

Root Initiation and Maturation
Hedge maturation
Loblolly pine early auxin-induced gene expression
Quantitative trait loci for rooting
Expansin gene expression
Cyclin-dependent kinase gene expression
Arabinogalactan proteins and rooting
Brassinosteroids and rooting

Stock Plant Nutrition and Management
Loblolly pine hedge nitrogen nutrition and carbohydrates
Slash pine hedge nitrogen nutrition
Loblolly pine hedge boron nutrition
Effect of hedging height on rooting
Cutting morphology and rooting

Rooting Environment and Physiology of Cuttings
Lethal water stress of cuttings
Diurnal trends in xylem water potential and photosynthesis
Water status of cuttings during handling and storage
Basal uptake of water in cuttings
Rooting medium composition

Root System Quality
Root system morphology field test
NAA effects on loblolly pine cuttings
NAA effects on slash pine cuttings
Effect of re-cutting bases on rooting

Recent Publications and Presentations

Diaz-Sala, C., K.W. Hutchison, B. Goldfarb, and M.S. Greenwood. 1996. Maturation-related loss in rooting competence by loblolly pine stem cuttings: The role of polar auxin transport and tissue sensitivity to auxin. Physiologia Plantarum. 97:481-490.

Diaz-Sala, C., P.B. Singer, K.W. Hutchison, and M.S. Greenwood. 1996. Differential gene expression during maturation-caused decline in adventitious rooting ability in loblolly pine (*Pinus taeda* L.). Second International Symposium on the Biology of Root Formation and Development. Jerusalem, Israel. June 23-28.

Goldfarb, B. 1996. Status of vegetative propagation in the Southeast U.S. Western Gulf Tree Improvement Program Contact Representative's Meeting. Magnolia, AR. May 22-23.

Goldfarb, B. 1996. Rooted cutting research on loblolly and slash pines. Program on Southern Pine Tree Improvement in association with the NCSU-Industry Cooperative Tree Improvement Program Contact Meeting. Fredericton, New Brunswick. August 23.

Goldfarb, B., Z. Lian, C. Lanz-Garcia, and R. Whetten. 1996. Auxin-induced gene expression during rooting of loblolly pine stem cuttings. Second International Symposium on the Biology of Root Formation and Development. Jerusalem, Israel. June 23-28.

Murthy, R. and B. Goldfarb. 1996. Characterization of physiological parameters of loblolly pine stem cuttings during root initiation. 14th North American Forest Biology Workshop. June 16-20.

Thetford, M. and F. Blazich. 1995. Comparison of IBA and P-ITB for propagation of loblolly pine stem cuttings. Proc. South. Nurs. Assoc. Res. Conf., 40th Annual Report. p269-271.

Thetford, M. and F. Blazich. 1996. Auxin source influences root system quality of loblolly pine stem cuttings. Proc. South. Nurs. Assoc. Res. Conf., 41th Annual Report (In Press).

Related publications

Goldfarb, B., G.M. Howe, W.P. Hackett, and O. Monteuuis. 1996. Survival and growth of eastern white pine shoot apical meristems *in vitro*. Plant Cell, Tissue, and Organ Culture. (In Press)

Rieckermann, H., B. Goldfarb, M.W. Cunningham, and R.C. Kellison. Influence of nitrogen and photoperiod on root and shoot growth of rooted stem cuttings of sweetgum. Submitted to Tree Physiology.