NORTH CAROLINA STATE UNIVERSITY
LOBLOLLY AND SLASH PINE ROOTED CUTTING PROGRAM

Progress Report for 1997

EXECUTIVE SUMMARY

Rooted stem cuttings of forest trees are increasingly being used around the world to deploy elite genotypes in intensive forest plantations. In 1992, the NCSU Loblolly and Slash Pine Rooted Cutting Program was formed as an industrially sponsored research cooperative. The primary mission of the program is to conduct research to facilitate the operational use of rooted cuttings for the pines of the southeastern United States.

Since 1992, the program has conducted research into many facets of rooting and production of cuttings. Primary research areas include stock plant management and physiology, the rooting environment, genetic variation in rooting ability, fundamental mechanisms of root formation and maturation, and the quality of cutting root systems. Efforts in these areas have yielded a great deal of progress. In fact, loblolly and slash pines can no longer be considered "difficult-to-root" species. Research continues on many fronts, including optimizing procedures to make production reliable and cost effective, developing supporting information for the implementation of clonal forestry, as well as the continued elucidation of the mechanisms of maturation and developing techniques for maintaining juvenility.

The future of rooted cuttings is bright. Operational use of rooted cuttings to deploy outstanding full-sib seed from controlled crosses depends only on cost effectiveness and designing production systems that are efficient and complementary to seedling systems. Using rooted cuttings to mass produce elite clones is now within our grasp and research continues to provide the necessary supporting information. We anticipate that in the near future, rooted cuttings will be an important silvicultural tool for maximizing productivity in plantations in the southeastern U.S.

INTRODUCTION

It has been a very productive year for the NCSU Loblolly and Slash Pine Rooted Cutting Program. Much research progress has been made, rooting percentages continue to increase, and the quality and performance of the rooted cuttings has improved. While research activities continue on a broad array of topics, they can be grouped into two overall areas of focus: (1) improving the efficiency of production systems to make rooted-cutting technology more cost-effective and (2) developing information necessary to implement clonal forestry using rooted cuttings. The latter is a new area of emphasis and we anticipate its continued importance in the future.

There were 12 industrial sponsors of the program in 1997. They were: Boise Cascade Corp., Bowater, Inc., Champion International Corp., Fort James Corp., International Paper Co., Jefferson Smurfit Corp., Rayonier, Tenneco-Packaging, The Timber Company (GP), Union Camp Corp., Westvaco Corp., and Weyerhaeuser Co. For 1998, membership will be reduced to 10 companies, with the loss of Tenneco-Packaging and Fort James Corp. (formerly James River), due to economic circumstances. We are actively recruiting new members. As always, the Raleigh staff is appreciative of your support.

The staff of the program includes one full-time and two part-time faculty scientists, two post-doctoral research associates, one technician, and three graduate students. In addition, administrative contributions from additional personnel are instrumental to our efficient functioning.

The focus of this report is primarily on studies that were carried out during 1997. Rooting experiments were conducted in the Winter (January-February) and Spring (May-June). The past Winter was our first Winter rooting in the new propagation greenhouse. Success was excellent in this season as it had been for Spring rooting in 1996. Spring 1997 experiments were even more successful as we continue to optimize our procedures and conditions in the greenhouse.

INFLUENCE OF GENETICS ON ROOTING

These experiments report on the variation in rooting ability among 25 open-pollinated loblolly pine families, the correlations of rooting ability with breeding value for growth, and the correlations among three different rooting trials. In addition, the results of one within-family rooting experiment are summarized.

Among-Family Rooting Variation

The three family variation rooting experiments were conducted in Summer 1996, Winter 1997 and Spring 1997. Rooting success for the experiments reflects the improvements made by the program in hedge management and the rooting environment, with the overall study means increasing from 67 to 78% (Table 1). In each study, the 10 best rooting families all rooted at greater than 80%. Family mean correlations of rooting percentage with breeding values for height and diameter are near zero and nonsignificant. The independence of these traits means that simultaneous culling for growth and rooting ability should yield fast-growing families that also root well. Family mean correlations for rooting percentages among the three different rooting experiments are substantially higher than we had previously obtained for open-pollinated slash pine families. In addition to possible differences between the species, it is likely that the higher correlations reflect the more uniform rooting environment achieved in the new propagation greenhouse. These higher correlations indicate that, with an adequate sample, one or two well carried out rooting trials may be sufficient to characterize rooting ability in loblolly pine families. In addition, this type of screening can take place at a relatively early age. The rooting trial conducted in Summer 1996 that occurred nine months after seed were sown was highly correlated with the later trials.

Table 1. Rooting results, family mean correlations of rooting % with breeding values, and correlations among rooting experiments for 25 open-pollinated loblolly pine families.

Rooting Study	Summer 1996	Winter 1997	Spring 1997
Overall Rooting %	67.6	74.1	77.6
Family Range (Rooting %)	50.0 -- 93.0	53.8 -- 89.3	53.1 -- 93.5
Mean of Best 10 Rooting Families (Rooting %)	81%	82%	89%
BV for Height[1]	0.078	0.183	0.114
BV for Volume[1]	0.039	0.079	-0.023
Summer 1996[1]	----	0.757	0.735
Winter 1997[1]	----	----	0.765

[1]Family mean correlations

Within-Family Rooting Variation

An additional study was conducted in Spring 1997 that quantified the variation for rooting within loblolly pine families. The 10 families with the highest breeding values were selected from the 25 families used in the previous experiment and 12 cuttings were collected from each available hedge (clone). The 10 families had rooted at a mean percentage of 77% in the previous experiment as compared with 74% for all 25 families. In the Spring 1997 experiment, the overall mean rooting percentage was 83%. The families ranged from 59% to 98% and the 198 clones within all the families ranged from 0% to 100%. All the families had at least one clone that rooted at 100%. The family with the highest breeding value for height and volume rooted at 97% with 11 of the 17 clones rooting at 100%. These results indicate that within-family selection can be carried out simultaneously for growth and rooting ability to identify superior clones.

ROOT INITIATION AND MATURATION

This research area encompasses efforts to quantify the extent to which juvenility is maintained by hedging and serial propagation and to understand the basic mechanisms of adventitious root formation and how they are affected by maturation.

Hedge Maturation Study

Our ongoing hedge maturation study is providing data critical to the utilization of rooted cuttings for producing planting stock for clonal forestry. The identification and multiplication of superior clones will require some period of time and the rooted cutting strategy will only be viable if hedging and/or serial propagation treatments delay maturation sufficiently to maintain rooting ability and field performance of the rooted cuttings. This study was begun in the Spring of 1993 and is testing the juvenility of hedges derived from seedlings and rooted cuttings (serially propagated hedges) from three open-pollinated loblolly pine families (16-20 clones per family per age) over time.

During the past year, two rooting experiments were conducted. In Winter 1997, rooting was tested on cuttings from seedling hedges from seed germinated in 1993-1995 (2- to 4-year-old seedling hedges) and from serially propagated hedges, derived from rooted cuttings from the 1993 seedling hedges (4-year-old serially propagated hedges). Rooting percentage was highest in the youngest (2-year-old) hedges for all three families (Figure 1). Cuttings from serially propagated hedges rooted better than those from seedling hedges of the same age. However, in only one of the three families (7-1037) did the rooting percentage of serially propagated hedges reach a level comparable with that of the 2-year-old hedges. Because of the low overall rooting percentage (58%), poor appearance of the cuttings on the hedges growing in the field, and the rapid rate of rooting decline compared to what we have observed in our other potted hedges, we embarked on an intensive fertilization program to restore vigor to the field hedges for the Spring 1997 rooting experiment. In the Spring experiment, we tested serially propagated hedges that were maintained in pots rather than the field as an additional comparison.

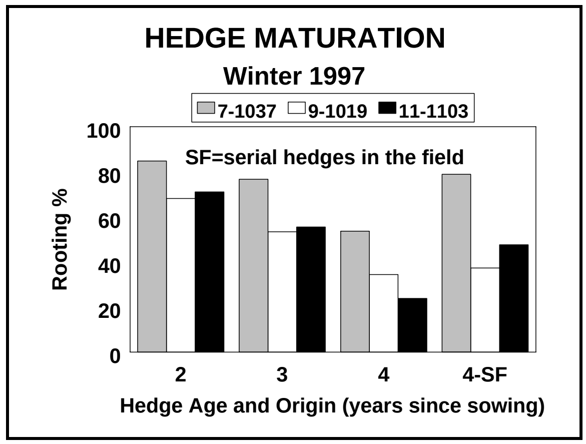

Figure 1. Effect of hedge age and origin on rooting percentage in Winter 1997.

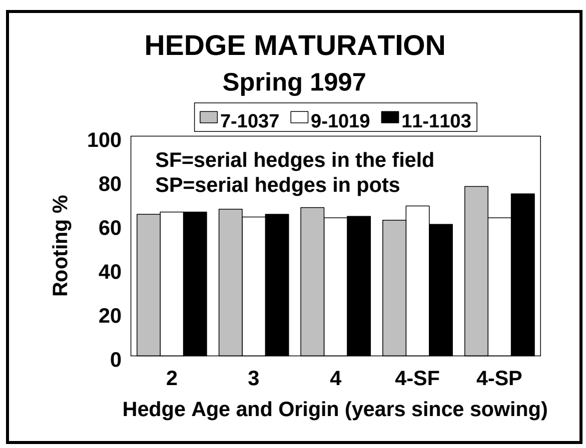

Figure 2. Effect of hedge age and origin on rooting percentage in Spring 1997

The rooting experiment in the Spring gave a markedly different result. There was no decline in rootability with age in any of the three families (Figure 2). Overall, the rooting percentage was higher than in the previous experiment, ranging from 63-65% in the cuttings from the different ages of field hedges and was 71% in the serial hedges maintained in pots. Thus, our most recent data indicate that there is no decline in rooting ability in these three families through four years of age. Moreover, cultural conditions for the stock plants may be more important than maturation and poor conditions may present symptoms resembling maturation.

Due to the further deterioration of all the hedges in the field that occurred last summer after the experiment, we have transplanted all the hedges to pots and will continue the study with better control of irrigation and fertilization and without the poor drainage conditions present in our field setting.

In addition to the rooting experiments, morphological measurements were taken on the cuttings from the different families and hedge origins. Characteristics measured included cutting diameter, length of primary needles, length of fascicles, number of fascicles, and occurrence of sterile scales. We are currently analyzing the data sets from both Winter and Spring to determine if morphology is affected by hedge age and origin and whether rooting ability is correlated with any of the morphological characteristics.

In February 1998, rooted cuttings from the Spring rooting experiment will be planted in a field test on the land of Bowater Corp. in South Carolina. The test will determine whether growth rate and other morphological traits vary in rooted cuttings from hedges of different ages and origins (through 4-year-old hedges).

Gene Expression and Adventitious Root formation

We have proceeded on several fronts to determine the basic developmental processes controlling root formation and how they are affected by maturation. Previously, we reported on the cloning and initial characterization of a family of auxin-induced genes from loblolly pine cuttings (***L***oblolly ***P***ine ***E***arly ***A***uxin-induced genes [LPEAs]). This year, we have conducted several further analyses of these genes and their role in root formation.

Last year, we reported that, in a preliminary experiment, the LPEAs showed only limited induction by auxin in cuttings from mature trees. This year, Victor Busov, a Ph.D. student working with the program conducted a more comprehensive experiment comparing auxin induction in cuttings from mature trees vs. seedling hedges at 1, 3, and 5 days after treatment. All five LPEAs were induced to lower levels in the mature cuttings than in the juvenile cuttings (Figure 3). In addition, expression of the LPEAs was more short-lived in the mature cuttings. Maximal expression was observed 1 day after auxin treatment and progressively declined on the third and fifth day. Expression remained high through 5 days after auxin treatment in the juvenile cuttings. This raises the possibility that these genes may be prematurely turned off in mature (nonrooting) cuttings. One possible explanation of this phenomenon would be an overactive wound response in the mature cuttings. We will pursue this hypothesis in future experiments by testing the timing and magnitude of expression of two genes known to be activated by wounding. The first gene is phenylalanine ammonia lyase (PAL, provided by Ross Whetten, NCSU Forest Biotechnology Group), which is a regulatory branch point between primary metabolism and the wound pathway. The second gene, chitinase, is more specifically regulated by wounding in numerous plant species. Chitinase (pine chitinase provided by John Davis, University of Florida) is thought to protect plants against fungal and insect attack. This experiment should provide information about root formation in juvenile vs. mature cuttings and possibly on nonrooting of less than optimal juvenile cuttings.

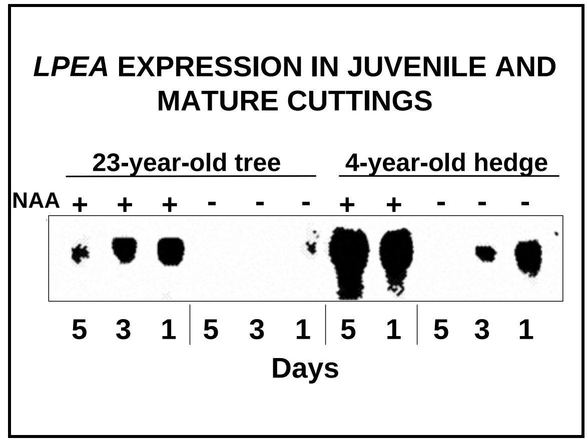

Figure 3. LPEA1 mRNA abundance in juvenile and mature loblolly pine cuttings 1, 3 and 5 days after auxin treatment.

In order to fully understand how genes are regulated, it is necessary to clone, identify and study the nucleotide sequences surrounding the actual coding region of the genes. These flanking sequences contain the promoters and other control elements and are the locations where other molecules interact with the genes to turn them on or off. We have begun to identify genomic clones, which contain both the coding and surrounding sequences, from a genomic library made from loblolly pine DNA. To date, we have identified a genomic clone that appears to correspond to LPEA1 and contains substantial sequence on both sides of the coding region. We are in the process of sub-cloning this very large DNA fragment into several more manageable pieces and then will sequence the entire strand. The sequence information obtained and the cloned DNA itself will be instrumental for testing hypotheses about the mechanisms by which expression is turned on or off, for example, in juvenile vs. mature cuttings. Cloning of the flanking sequences for the other LPEAs will proceed as time permits.

A direct way to determine whether the LPEA genes play a role in root formation is to manipulate their expression in transgenic plants and determine if root formation is affected. Victor Busov has initiated such experiments with Allan Wenck (NCSU Forest Biotechnology Group). The first step is to over-express the LPEAs in tobacco plants. This species was chosen because of the ease of transformation. It will allow us to determine whether over-expression interferes with recovery of transformed plants, give us a quick screen for the phenotypes of transformed plants, and guide our decisions on the genes and constructs to be introduced into trees. To date, we have recovered a large number of putatively transformed tobacco lines carrying LPEA5 fused to a CMV-35s (constitutive) promoter. Experiments with these plants will be conducted to quantify their morphological characteristics, with special focus on adventitious root formation. In addition, we have initiated transformation of tobacco with three other LPEAs and have promising looking plant lines. When these experiments are concluded, we will introduce a subset of genes and constructs into hybrid poplar and eventually into a conifer, either spruce or pine, depending on the availability of suitable transformation systems.

STOCK PLANT MANAGEMENT

The overall thrust in this research area is to continue to make improvements in rooting percentage and to determine the factors that influence reliability of rooting performance. Three areas were pursued in 1997.

Boron Levels in Hedges

Two experiments were conducted to determine optimal levels of boron (B) in hedges prior to harvesting cuttings. The first experiment was conducted in Winter 1997 and targeted B concentrations in the hedges ranging from 8 to 35 ppm. The experiment was conducted using 2-year-old hedges of four full-sib loblolly pine crosses. Rooting percentages, averaged across the four crosses, ranged from 80 to 87% for the B concentrations at 10 ppm or above. There were no large differences among these treatments. At 8 ppm B, however, rooting percentage was significantly reduced (67%) (Figure 4).

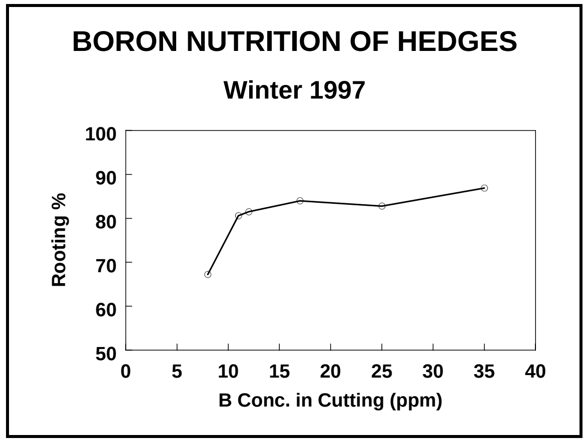

Figure 4. Effect of internal boron concentration on rooting of four full-sib loblolly pine families in Winter 1997.

Based on these results we conducted a second experiment in Spring 1997 to examine the lower portion of the B concentration range in greater detail. The hedges from the four crosses were the same as in the previous experiment and the B concentration ranged from 5 to 23 ppm B. Overall, the rooting percentages in this experiment were lower than in the Winter experiment, apparently due to the larger number of treatments below the critical B concentration. A concentration of 10 ppm B again appeared to be the critical level. Below this concentration, rooting decreased sharply, while above 10 ppm, rooting was 81 to 86 % for three of the crosses and 59% for the fourth cross (Figure 5). Thus, we recommend that hedges be maintained at a concentration of at least 15 ppm B. To date, we have not observed B toxicity at high concentrations, but may pursue this in future experiments.

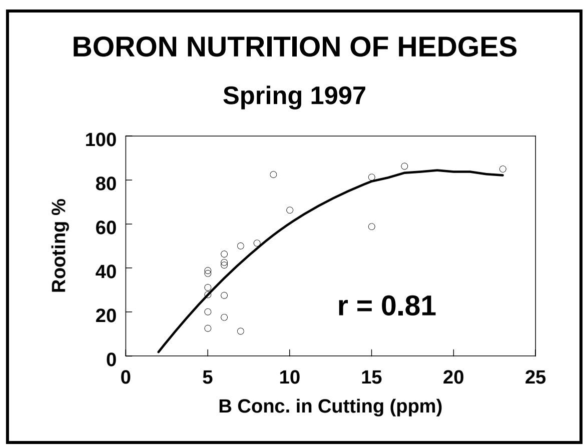

Figure 5. Effect of internal boron concentration on rooting of four full-sib loblolly pine families in Spring 1997.

Timing of Cutting Harvest

One factor that may affect rooting performance of Spring cuttings is the time that they are harvested and whether the cuttings are actively elongating or have a transient resting bud. To assess this, we sampled cuttings from hedges from four full-sib loblolly pine crosses weekly from June 6 to July 11. At each sampling time, the stage of growth of each cutting was rated as: (1) no bud set (first elongation phase), (2) first resting bud, (3) second elongation, or (4) second resting bud and rooting percentage of the cuttings was determined.

Overall rooting percentage was very high for the experiment. Over the first five weeks, rooting percentage ranged from 89 to 95%. At the sixth sampling time (July 11), rooting percentage decreased to 77% (Table 2). There was a slight effect of the growth stage with cuttings having the second resting bud rooting slightly poorer (81%) than all other types (88-93%). It is not clear whether this was causal, because there was a preponderance of second resting buds in the last sampling time and all three growth stages (first flush was not present) rooted at slightly lower percentages than the other sampling times (Table 2).

Table 2. Effect of collection date and growth stage on rooting of loblolly pine cuttings (240 cuttings set at each date).

Collection Date	First Flush		First Bud		Second Flush		Second Bud		All
	% of Cuttings	Rooting %	% of Cuttings	Rooting %	% of Cuttings	Rooting %	% of Cuttings	Rooting %	Rooting %
June 6	85	92	15	77	0	--	0	--	90
June 13	31	93	68	87	1	100	0	--	87
June 20	10	100	80	92	10	88	0	--	92
June 27	3	100	38	91	59	90	0	--	91
July 4	0	--	22	94	58	95	20	95	95
July 11	0	--	13	68	38	82	49	75	77
All		93		88		90		81	89

Hedging Height, Hedging Intensity, Shoot Origin, and Cutting Morphology

To further understand sources of variation in cutting performance, Bernadette Cooney, an M.S. student, initiated a study to test the effects of hedging height, hedging intensity and shoot origin on cutting morphology and rooting. Twenty hedges from each of three full-sib loblolly pine families were used and ten each were hedged at 18 and 23 cm above the soil surface. On five of the hedges from each group, all the pre-existing fascicular shoots were removed. The remaining hedges were left intact and all the pre-existing shoots were marked with a plastic ring. The hedging treatments were conducted in March 1997 and the resulting shoots were sampled in June. Morphological measurements were made on 10 cuttings of each shoot origin (newly formed and pre-existing, where present), including cutting basal diameter, length of the longest primary needle, length of the longest fascicle, presence or absence of sterile scales, and the extent of stem lignification. The latter was estimated using a device called an Instron (kindly made available by the Dept. of Agricultural Engineering, NCSU) that measures the force necessary to cut through the stem. After measurements, all the cuttings were placed in the propagation greenhouse to test for rooting.

Overall, rooting percentages in this experiment were lower than for others conducted at the same time. This could be due to the handling of the cuttings necessitated by the morphological measurements or by the placement in the rooting beds that was deeper and denser than normal. A future repeat of the experiment will utilize standard setting protocols and include unmeasured cuttings as controls. Only small differences in rooting were observed as a result of the treatments. Across all families, 67% of cuttings from hedges sheared at 18 cm rooted, compared with 58% of cuttings from hedges sheared at 23 cm. Sixty-six percent of cuttings that had all the preformed shoots removed rooted, compared with 60% of cuttings from hedges that had both pre-existing and newly formed shoots. Surprisingly, only a small difference was observed for shoot origin. Sixty-three percent of newly formed cuttings rooted, compared with 61% of cuttings from pre-existing buds. Analyses of the effect of the hedging treatments on cutting morphology and the effect of cutting morphology on rooting is underway. The experiment will be repeated this coming spring with the modifications noted above and other possible adjustments of the treatments.

ROOTING ENVIRONMENT

In this area of research, we are using physiological techniques to determine the factors of the rooting environment that influence rooting and growth of rooted cuttings. Four research approaches are described.

Cutting Dehydration, Water Stress, and Rooting

Experiments conducted in 1996 and analyzed in 1997 yielded interesting results about the rate of dehydration and effects on rooting. Cuttings placed in dry medium under low humidity (high vapor pressure deficit) conditions lost turgor rapidly, as expected. These cuttings exhibited a linear decline in rooting percentage with increased drying time. Cuttings placed in the same medium when humidity was higher dried out much more gradually. In fact, cuttings left in unwatered medium for over 20 hours still rooted at over 50% after subsequent placement under mist. Measurements of xylem pressure potential indicated that a critical value for subsequent rooting was -1.7 MPa. Cuttings with less water stress exhibited no substantial decline in rooting,

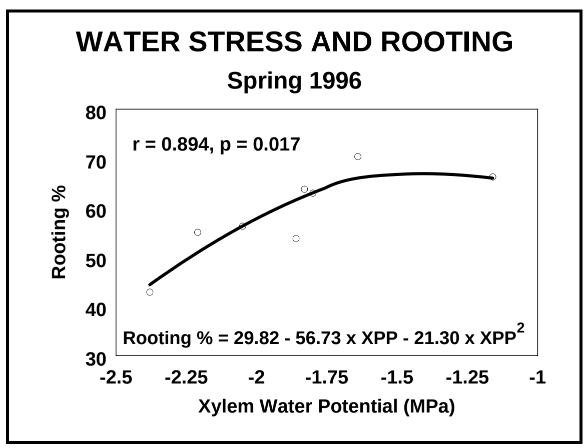

Figure 6. Effect of drying and xylem water potential on subsequent rooting of loblolly pine cuttings.

but in cuttings with greater water stress, rooting began to decline (Figure 6).

Basal Uptake of Water and Rooting

Previous experiments had shown that various wounding treatments applied to the bases of cuttings influenced the amount of water taken up by cuttings (placed in water) over a 12-day period. In this experiment, these wounding treatments were applied to cuttings to determine if basal water uptake is important for subsequent rooting. Six treatments were applied to cuttings of three loblolly pine full-sib crosses: (1) bases left intact, (2) basal fascicles removed, (3) basal bark and fascicles removed, (4) bases recut, (5) bases recut and fascicles removed, and (6) bases wounded with a needle. No auxin treatment was used in this experiment.

Cuttings that had the bases recut but intact fascicles rooted at the highest percentage (88%). Control cuttings rooted at 73% (Figure 7). This effect is similar in magnitude to that reported below (see Recutting Bases and Liquid NAA). Cuttings wounded with a needle did not differ from controls. Removing the fascicles had a detrimental effect on rooting, with the lowest rooting obtained in the treatment in which both the bark and fascicles were removed (50%). These rooting results do not correspond to those reported previously for water uptake.

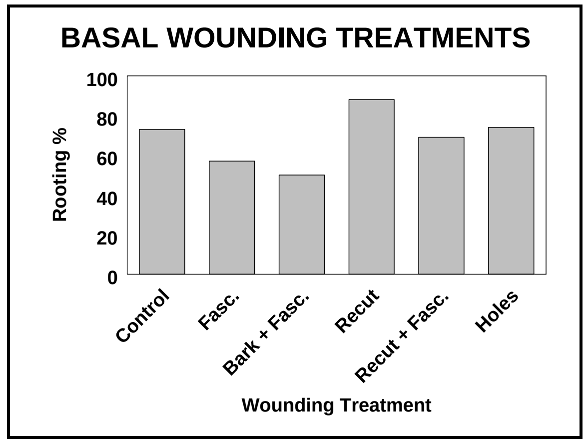

Figure 7. Effect of wounding treatments on rooting of loblolly pine cuttings (see text for treatment descriptions).

Misting Frequency and Nitrogen and Phosphorus Fertilization During Rooting

This experiment quantified biomass accumulation in cuttings under two misting regimes and four fertilization treatments. Cuttings were placed in Ray-Leach tubes with the standard 60% perlite:40% peat mixture and subjected to the normal misting regime for 6 weeks. In the seventh week, all the cuttings were moved to the Method Road misthouse, where half the cuttings were placed under a standard misting frequency and half under a reduced misting frequency. Further, one fourth of the cuttings in each misting regime were treated with weekly applications of nitrogen, phosphorus, nitrogen and phosphorus, or no fertilizer. Samples were collected weekly until 12 weeks after sticking and analyzed for rooting percentage, root biomass, and shoot biomass. Results reported here are from the final measurements as weekly measurements are currently being analyzed.

Rooting percentages were higher for cuttings in the standard misting regime across all fertilization treatments (69% in standard mist and 53% in reduced mist). The lower rooting percentages in the experiment relative to other studies probably reflect non-uniform mist distribution in the fixed spray nozzle setup at the Method Road greenhouse. In addition, misting frequencies may have been on the low side for both treatments, resulting in substantially lower rooting in the reduced mist regime. Rooting percentage was highest in cuttings fertilized with both N and P under both misting regimes, although these data are contradictory to the mycorrhizal inoculation and fertilization experiment (see below) in which no effect of fertilization on rooting was observed. No clear trends in biomass accumulation at 12 weeks were observed for the misting and fertilization treatments. Thus, the substantial differences in growth due to fertilization reported below probably occur after the 12 week rooting period.

Carbon Dioxide, Temperature and Rooting

These two experiments were conducted in separate facilities. A CO_2 enrichment experiment was conducted in growth chambers in the greenhouse of the USDA Agricultural Research Service at the Method Road complex. Cuttings were rooted in either ambient (375 ppm) or elevated (1000 ppm) CO_2 in growth chambers set up with mist nozzles. Cuttings in the elevated CO_2 rooted at a slightly higher percentage (54%) than cuttings in ambient CO_2 (42%). Cuttings in the elevated CO_2 also had greater numbers of roots and total root length. The low overall rooting percentages for the study reflect the difficulty of applying even, optimal mist in the chamber environment.

A study to test the effect of temperature on rooting was conducted in the temperature-controlled greenhouses at the NCSU Phytotron. The three temperature regimes were 20/18, 26/22, and 30/26 (day/night temperature, °C). Mist was applied from spray nozzles set up on greenhouse carts that were surrounded on all sides by plastic sheets. Rooting was best in the lowest temperature regime (40%), followed by the intermediate temperature regime (17%) and lowest in the highest temperature regime (3%). Again, the low overall rooting success in the experiment reflects the sub-optimal misting uniformity and frequency and limits the conclusions that can be drawn.

Because of the technical difficulties described in setting up ad hoc rooting systems, research for this year will concentrate on testing and characterizing conditions in the propagation greenhouse used for our other studies. One experiment will transfer cuttings from standard mist to reduced mist at various times during the rooting period. Water stress, net photosynthesis, and transpiration will be measured. A second experiment will measure gas exchange in unrooted cuttings under different light intensity, vapor pressure deficit, and temperature conditions and use comparisons with seedlings to make inferences about cutting physiology.

QUALITY OF ROOTED CUTTINGS

This research area reflects our efforts to understand the factors that influence the quality of cutting root systems and field performance of rooted cuttings. Experiments testing auxin treatments, field performance of cuttings with different root systems, mycorrhizal inoculation and fertilization, and different containers and media are described.

Auxin Treatment and Root System Morphology

This past winter we conducted two auxin treatment experiments that complete a series of studies testing the effect of various auxin treatments on rooting percentage and root system morphology of pine cuttings.

Experiment 1--Auxins Applied as a Powder. This experiment tested NAA, IBA and both auxins applied as powders. The individual treatments included a control, 3 levels of NAA (0.25, 0.5, and 0.75%), 3 levels of IBA (0.5, 1.0, and 1.5%) and 3 levels of the NAA/IBA combination (0.25/0.5%, 0.5/1.0%, and 0.75/1.5%). The experimental design consisted of the 10 hormone treatments, 4 families, 8 blocks, and 6 cuttings per family/treatment combination in each block. The cutting bases were re-cut immediately prior to hormone treatment.

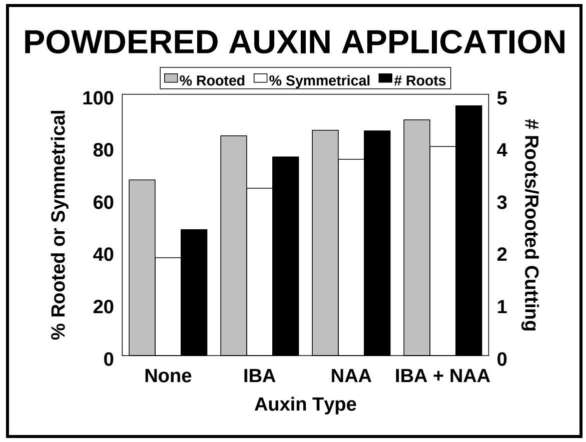

Figure 8. Effect of powdered NAA and IBA on rooting percentage, percent symmetrical root systems, and number of roots per rooted cutting.

Rooting percentages (averaged across the 3 levels) were high across all the auxin powder treatments (86, 84, and 90% for NAA, IBA, and both auxins, respectively) and substantially higher than the control (67%) (Figure 8). A similar trend was seen for the number of roots per rooted cutting and the percentage of cuttings with symmetrical root systems, with the highest values obtained from the combination of both auxins and the lowest from the controls. No strong or consistent differences were observed among the three levels of the auxins for any of the three root variables measured.

Experiment 2--Recutting Bases and Liquid NAA. Previous experiments had shown a modest benefit for the practice of recutting the bases of cuttings prior to dipping in NAA. In this experiment, we tested recutting and used four NAA concentrations (5, 10, 20, and 40 mM) and a control to determine if the effects of this treatment depend on the level of NAA used. The design of this experiment consisted of 5 NAA treatments, 2 cutting treatments, 3 families, 8 blocks, and 6 cuttings per family/treatment combination in each block.

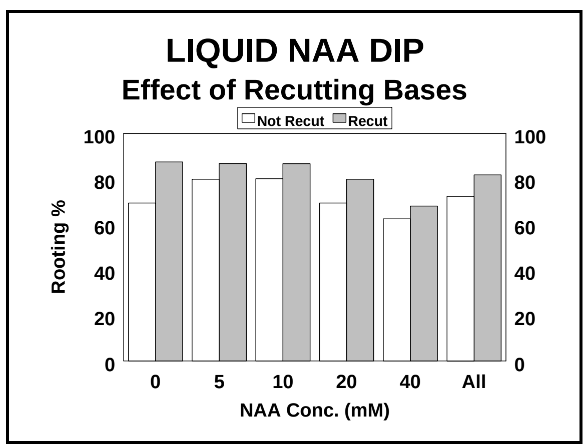

Figure 9. Effect of recutting bases prior to dipping in NAA on rooting percentage in loblolly pine cuttings.

Rooting percentage was slightly higher in cuttings that had been recut (82%) than in cuttings not receiving the recutting treatment (72%) when averaged over all NAA concentrations (Figure 9).

The effect was small, but consistent over all the NAA concentrations tested without evidence of an NAA concentration x recutting treatment interaction. As in previous experiments, rooting percentage was highest in the 0 to 20 mM NAA range. The number of roots per rooted cutting increased from 2.5 without NAA to 4.6 with 20 mM NAA. The number of roots was also slightly higher in cuttings that had been recut at all NAA concentrations, except for the control. The effect of recutting on root system symmetry was less clear, with no effect when cuttings were averaged over all NAA concentrations. Based on these and previous results, we use a 3-second dip in 10 mM NAA after recutting the bases as a standard treatment for loblolly pine winter cuttings.

Root System Morphology and Field Growth--Second Growing Season

This year, the field test of rooted cuttings that had been rated for root system morphology after rooting and after culture in a nursery bed was measured for height growth after the second growing season. Overall, the cuttings are growing rapidly and survival continues to be 100%. The mean 1997 growth increment was 114 cm (3.8 ft) and total height averaged 167 cm (5.6 ft). As with the previous year's measurements, there was no significant relationship between initial root number or root system symmetry and height growth. At this point in time, culling cuttings with presumed poor root morphology classes (e.g. cuttings with a single, horizontally oriented root) does not appear to be necessary. However, these data should be interpreted with caution and we will continue to measure growth for longer term effects.

Mycorrhizal Inoculation and Fertilization

Following rooting, rooted cuttings must be grown to optimize their performance in the field. The following experiments address various aspects of determining cultural practices that meet this overall objective.

In Winter 1997, an experiment was conducted to determine the effect of mycorrhizal inoculation and fertilization on rooting and subsequent growth of rooted cuttings. Cuttings from four full-sib loblolly pine crosses were stuck in Ray-Leach Super Cells in a medium of 60% perlite: 40% peat. A spore suspension of *Pisolithus tinctorius* was added to the tubes either before the cuttings were stuck, 6 weeks after sticking, or 12 weeks after sticking (at the end of the rooting period). A control treatment without the spores was also included. A 100 ppm (N) solution of Peter's 20-20-20 was added weekly to the tubes to the drip point beginning in the sixth or twelfth week after sticking. Again, there was a nonfertilized control. The experiment was a split-plot design with fertilization as the main plot factor. There were 12 replications of 7 cuttings per treatment combination for a total of 1008 cuttings. After 12 weeks, the cuttings were scored nondestructively for rooting percentage and then

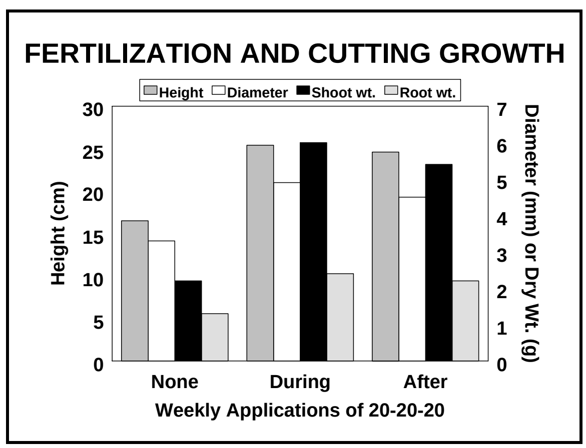

Figure 10. Effect of fertilization starting the sixth or twelfth week after sticking on growth of loblolly pine cuttings.

transferred to a greenhouse for further growth. Five months after transfer the cuttings were harvested and measured for: (1) shoot height, (2) stem diameter, (3) shoot dry weight, (4) root dry weight, and (5) an estimate of the percent of the fine roots containing mycorrhizal root tips.

Neither mycorrhizal inoculation nor fertilization, applied at any time, had a strong effect on rooting success. Overall, 79% of the cuttings in the experiment rooted. Mycorrhizal inoculation had a negligible effect on any of the morphological characteristics measured, including the percent of root tips classified as mycorrhizal. Fertilization with the balanced fertilizer, however, had marked effects on all characteristics measured (Figure 10). When fertilizer was applied either during or after the rooting period, there was approximately a 50% increase in shoot height, a 40% increase in stem diameter, a 150% increase in shoot dry weight, and a 75% increase in root dry weight relative to nonfertilized cuttings. Surprisingly, fertilization also resulted in approximately a 3-fold increase in the percent of fine roots classified as mycorrhizal, apparently as a result of more vigorous production of fine roots that could be colonized by ambient (not necessarily inoculated) mycorrhizae. In addition, fertilizing six weeks after sticking resulted in a 3% increase in shoot height, 7% increase in stem diameter, 12% increase in shoot dry weight, and 10% increase in root dry weight compared with cuttings receiving the fertilizer starting 12 weeks after sticking.

The experiment was repeated in Spring 1997 and again no marked effect on rooting percentage was observed as a result of the inoculation or fertilization treatments. Morphological characteristics are currently being analyzed.

Containers and Media for Rooting and Subsequent Growth

As part of our ongoing efforts to provide information for supporting companies to develop cost-effective operational systems, we are initiating two studies to examine the effect of various container and media combinations on rooting, root system development, and growth of rooted cuttings. The studies will be conducted by an M.S. student, Anthony Lebude, in the Department of Horticultural Science, NCSU through a continuation of our collaboration with Dr. Frank Blazich. The first study will be sponsored through a grant from Jiffy Products Ltd. Previously, we had tested Jiffy Forestry Pellets in a small preliminary screening, with encouraging rooting results. This study will test seven sizes of Jiffy Forestry Pellets and compare cutting performance in the pellets to our commonly used Ray-Leach Super Cells filled with 60% perlite: 40% peat. Rooting percentage, root and shoot size, and branching of the root system will be assessed. In addition, a small field test will be conducted to determine the optimal pellet size for subsequent growth of the rooted cuttings. A second study by the same student will test various combinations of containers and media and similar variables will be assessed. Look for results in the coming year.

CLONAL FORESTRY

Over the past two years, it has become apparent to the staff that we must increase the base of knowledge necessary for the implementation of clonal forestry using rooted cuttings. In addition to the results of our hedge maturation/serial propagation study, information is needed on how best to select and multiply clones and on the potential pest and other ecological consequences of planting pure clonal blocks. Our efforts in these areas are described below.

Clonal Selection Study

The objective of this study is to develop information that will enable individual organizations to efficiently select and propagate superior clones. The study is a joint project with the NCSU 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 nonrelatedness.

We currently have 100 seedling-derived hedges from each cross and rooting of the clones will begin this winter. Next planting season, a field test to allow selection for growth and other yield traits will be established with clones yielding an acceptable number of rooted cuttings. Clones will be further culled based on field measurements in successive years. Multiplication of remaining clones will occur simultaneously with culling. From this study, we will generate quantitative estimates of: (1) proportion of clones culled for rooting ability, (2) efficiency of selection at different ages, (3) multiplication rates for clones, and (4) magnitude of predicted genetic gain for the best clones in each cross. Once superior clones have been selected there will be opportunities to use them for other clonal field tests. Information on growth and yield dynamics for clonal blocks, realized genetic gain, and genotype x environment interactions for clones could prove critical to the implementation of clonal forestry.

Clonal Field Laboratory for Ecological Studies

Another critical area of research for the development of clonal forestry is the ecological and pest consequences of planting pure clonal blocks. At the current time, we have a proposal (joint with John Frampton, Forestry, NCSU) pending before the AF&PA/DOE Agenda 2020 program to determine these effects . If the proposal receives funding, we will plant four 36-acre tests, each with 18 2-acre plots consisting of pure blocks and mixtures of various numbers of clones. This field laboratory would be available for pathologists, entomologists, ecologists, and scientists from various other disciplines to study the effects of clonal plantations compared with mixed-clone plantations. Information from these studies would be valuable for guiding industrial forestry deployment decisions and also for providing sound scientific information to the public and potential government regulators. Four industrial sponsors of the Rooted Cutting Program have submitted letters stating their willingness to plant and maintain the test sites. If selected, the project will be entirely funded from the grant money. We will keep you posted on the status of this project.

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