

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

**Annual Progress Report  
October 1, 2000**

**EXECUTIVE SUMMARY**

The NCSU Loblolly and Slash Pine Rooted Cutting Program is completing its ninth year of existence and the final year in the second phase of the program. Support by the members will allow the beginning of a new four-year phase beginning January 1, 2001 that is projected to end December 31, 2004. Our mission continues to be to conduct focused research and technology transfer activities to assist members in their deployment of rooted cuttings on an operationally meaningful scale.

As many of our members increase their internal rooted cutting efforts, two overall research priorities have emerged. The first is to provide information that will facilitate the production of high-quality rooted cutting stock in a more efficient and cost-effective manner. The second priority is to provide information that will be necessary to implement clonal forestry.

In the area of production efficiency, this report contains results from a new trial that is investigating the effects of different stock type production systems on rooted cutting quality. Initial results of a transplant system are encouraging and will be pursued this coming year. We also report results of two field trials using different container and peat pellet sizes that provide guidance for development and implementation of production systems. Ongoing studies using environmental and physiological measurements are providing useful information about effective ways of controlling the rooting environment.

Several lines of research are being conducted in support of clonal forestry. Our long-term hedge maturation study now encompasses material through seven years of age and results, though somewhat conflicting, continue to hold promise for the maintenance of juvenility through hedging and serial propagation. Through a clonal multiplication study, we are learning methods for rapidly multiplying selected clones and generating realistic estimates of multiplication rates. The field portion of our clonal selection study is completing its second growing season. The results will guide members in their clonal testing, selection, and multiplication programs and generate estimates of the genetic gain that can be realized through clonal deployment. Finally, we continue our efforts to understand the fundamental mechanisms controlling root formation and how it is affected by maturation. Substantial progress has been made in dissecting these processes using some of the most advanced technological tools.

Several member organizations are in the process of scaling up their rooted cutting production activities, including some who are beginning to test production on levels that are operationally meaningful. It is our intention that this report and the associated technology transfer activities of the program assist the members in their objectives.

## INTRODUCTION

The year 2000 marks the final year in the second phase of the Rooted Cutting Program. Looking back over the past 5 years and the four previous years of the first phase, the program staff has a great deal of pride in our accomplishments in rooted cutting research and assisting the members in their own propagation programs. This type of sustained and concentrated effort is made possible primarily by support from the member organizations, for which we are very grateful. As we look forward to 2001 and the third phase, we hope to continue this tradition of focused research and technology transfer in support of the members' goals of operational rooted cutting technology for the Southeast U.S.

The membership of the program continues to change due to industry forces. At the same time that more organizations are becoming interested in intensive silvicultural systems, including rooted cuttings, mergers and acquisitions have decreased our membership and the potential pool of new members. We are pleased to welcome Temple-Inland Forest, who joined as of the beginning of 2000, to our ranks. Champion International Corporation, purchased by International Paper Corporation, ceases its membership in 2000. The membership for the third phase stands at ten organizations and we will continue our efforts to recruit additional organizations.

A number of staff changes have occurred in the last year. A new M.S. student, Matt Gocke, is working with the program, splitting his time with the NCSU Hardwood Research Cooperative. Bernadette Cooney completed her M.S. degree and is now working in private industry. Rania Masri is writing her dissertation and should complete her degree this semester, while Victor Busov is on schedule to complete his PhD this academic year.

We have made two facilities improvements during the past year. An internal College of Natural Resources grant provided funding for a simulated nursery bed that was constructed at our facility at the NCSU Horticultural Research Laboratory. This bed is being used for our experiments on rooted cutting stock quality. In addition, we added a sub-irrigation system that is tied into our computerized environmental sensing and control system in the propagation greenhouse. This equipment is being used in the research seeking to understand how best to control the rooting environment.

This report includes summaries of experiments conducted or analyzed since the last progress report in October 1999. Additional details will be presented at the upcoming Annual Meeting in Raleigh. We in the program staff appreciate your support and the opportunity to continue working with you. This is an exciting time for rooted cutting technology as the fruits of all of our efforts begin to pay off in meaningful levels of deployment for many organizations.

## RESEARCH FOR OPERATIONAL PRODUCTION

### Stock Quality in Different Production Systems

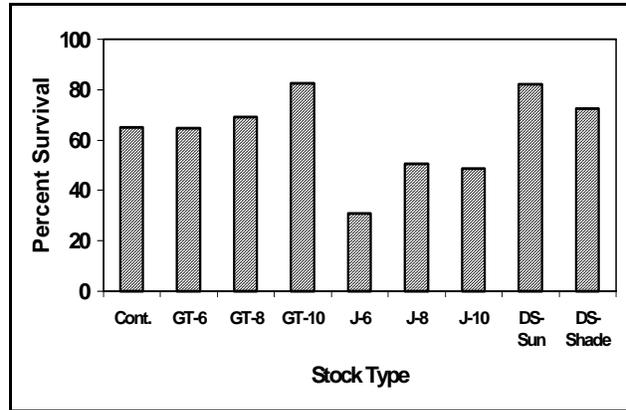
Inexpensive and efficient rooted-cutting propagation methods are necessary for the cost-effective production of rooted cuttings for reforestation. Besides high rooting percentages, producing a high proportion of rooted cuttings that are acceptable for planting and perform up to their genetic potential will be important components of a cost-effective system. Currently, there are three broad categories of potential production systems: (1) fully containerized cuttings, (2) cuttings stuck directly into outdoor nursery beds, and (3) cuttings rooted in small plugs or containers and then transplanted to a nursery bed for subsequent growth. A study, conducted by M.S. student Matt Gocke, was initiated in the winter of 2000 to evaluate the effects of these production systems on survival and stock quality of loblolly pine winter cuttings.

A 4 ft x 130 ft simulated nursery bed was constructed for the study. The sides of the bed were constructed with treated lumber. Weed mat was placed inside the structure to cover the ground and then a thin layer of gravel was placed on top of the weed mat. The bed was then filled with a loamy sand soil from the coastal plain of North Carolina and equipped with overhead sprinkler irrigation. Winter cuttings of three clones were collected between February 28 and March 4, 2000 for all of the treatments. The cuttings for the fully containerized treatment were stuck in Ray-Leach Super Cells filled with a 60% perlite : 40% peat medium mixture in the propagation house on March 10. After 12 weeks, the tubes were spaced one per every other hole (density=24/ft<sup>2</sup>) and the trays were moved to spaces within the nursery bed that had not been filled with soil. Two types of rooting plugs were used for the transplant treatment: Jiffy Forestry Pellets (18 mm) and Grow-Tech FlexiPlugs (#225). Both plug types were placed in the cavities of Winstrip trays (#162) to equalize the spacing (density=117/ft<sup>2</sup>). The cuttings for the transplant treatments were also stuck in the propagation house on March 10 and then transplanted by hand to the nursery bed (density=16/ft<sup>2</sup>) either 6, 8, or 10 weeks after sticking. Cuttings for the direct-stick treatment were stored in a walk-in cooler from the time of collection until April 25. They were then inserted one half their length (4 cm) into the nursery bed soil at a density of 16/ft<sup>2</sup>, either in full sun or under 50% shade cloth. All cuttings were treated with 10 mM NAA for 3 seconds before sticking. The design of the experiment was a randomized complete block, with nine stock type treatments, three clones, eight blocks, and 11 cuttings per plot, for a total of 2376 treatment cuttings, plus borders.

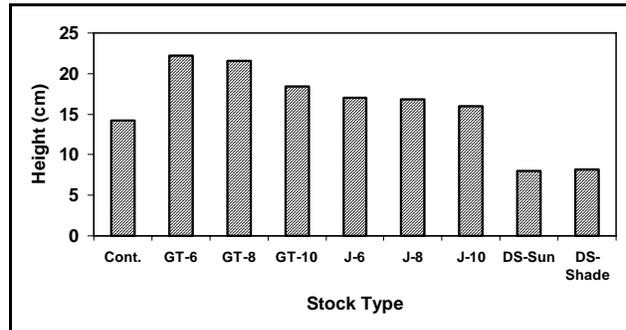
A fertilizer regime of Peters 20-20-20 was applied at 50 ppm N every 2 weeks to the containerized and transplanted cuttings from the eighth week to the 14<sup>th</sup> week after initial sticking. After the 14<sup>th</sup> week, the rate was increased to 100 ppm N every week for the remainder of the growing season. For the direct-stick cuttings, the 50 ppm N rate was begun four weeks after sticking and was increased to 100 ppm N/week at the same time as the cuttings that had been started indoors.

This coming December, the rooted cuttings will be destructively harvested and survival, shoot height, shoot dry weight, root collar diameter, root dry weight, and shoot:root ratio will be measured. For this report, shoot height and survival were recorded on September 7, 2000.

The highest percentages of surviving cuttings were found in the cuttings rooted in the Grow-Tech plugs and transplanted at 10 weeks (82.4%) and the cuttings stuck directly in the nursery bed under full sun (82.0%) (Figure 1). These were followed by direct-stuck cuttings under shade (72.6%), cuttings stuck in the Grow-Tech plugs and transplanted at 8 weeks (69.1%), containerized cuttings (65.1%), and the Grow-Tech plug cuttings transplanted at 6 weeks (64.7%). Survival was lowest for the cuttings stuck in Jiffy pellets (31.0% at 6 weeks, 50.7% at 8 weeks, and 48.8% at 10 weeks). These survival percentages largely reflect differences in rooting success, as all cuttings were transplanted to the nursery bed, regardless of rooting status. Too much importance should not be placed on the varying rooting percentages in the greenhouse, as misting conditions could be optimized for any of the treatments. For example, we previously reported high rooting percentages using Jiffy pellets, but under these conditions, they rooted at substantially lower percentages than the cuttings stuck in Grow-Tech plugs or containers. These preliminary results do demonstrate, however, that reasonably high survival percentages can be achieved with cuttings stuck directly in a nursery bed and cuttings stuck in small plugs in a greenhouse with a subsequent transplant to the nursery bed.



**Figure 1.** Effect of different stock type production methods on survival on Sept. 7 (Cont.=containerized, GT=Grow-Tech plugs, J=Jiffy pellets, DS=cuttings stuck directly in nursery bed.)



**Figure 2.** Effect of different stock type production methods on height on Sept. 7. (Cont.=containerized, GT=Grow-Tech plugs, J=Jiffy pellets, DS=cuttings stuck directly in nursery bed.)

The height of the cuttings varied considerably among the treatments. All of the transplanted cuttings had greater mean heights than the containerized cuttings (14.2 cm) (Figure 2). Among the transplanted cuttings, those stuck in the Grow-Tech plugs were taller than those stuck in Jiffy pellets. For both types of plugs, the cuttings transplanted at the earlier times were taller than those transplanted later. The cuttings stuck in Grow-Tech plugs and transplanted at 6 weeks had a mean height of 22.2 cm, followed by those transplanted at 8 weeks (21.6 cm) and 10 weeks (18.4 cm). The same trend was observed in the cuttings stuck in the Jiffy pellets, but to a lesser degree. The 6-week transplanted cuttings had a mean height of 17.0 cm, followed by the 8-week (16.8 cm) and 10-week (15.9 cm) transplants. The shortest cuttings were those stuck directly in the nursery bed. Those stuck under shade had a mean height of 8.2 cm, while those stuck in full sun had a height of 8.0 cm. This may have resulted from keeping these cuttings under mist for too long in the growing season. It was observed that height growth did not begin until the mist was discontinued and these

cuttings were irrigated using the same regimen as the other treatments.

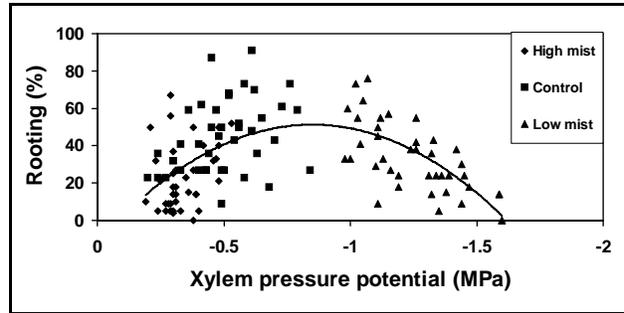
These preliminary results are encouraging about the potential survival and rooted cutting quality that can be achieved using these different propagation methods. High survival and vigorous growth was achieved in some of the treatments. However, given our relative inexperience with the non-containerized production methods, caution should be taken before interpreting these results as definitive. The study will be repeated next year with several modifications that should yield more reliable information. These include: (1) a reduced mist regime for the direct-stuck cuttings to encourage earlier rooting and growth; (2) a physical barrier between plots in the nursery bed to ensure that mist from the direct-stuck cuttings does not spray on to the transplanted or containerized cuttings; (3) a modified shade system to reduce the shading on the full-sun plots; (4) separate mist regimes in the greenhouse for the cuttings in containers, Grow-Tech plugs, and Jiffy pellets to maximize rooting and early root growth for each of the container types; and (5) rooting an excess of cuttings and only transplanting those that have rooted into the nursery bed, so that different densities will not confound growth. In addition, because of the large differences in the size of the cuttings in the different treatments this year, it is likely that these differences would persist for at least several years in the planned field test. Therefore, we will delay the field test for one year and establish it next year with cuttings produced during the next growing season.

### **Control of the Rooting Environment**

A study was conducted by Anthony LeBude, Research Assistant and PhD student, in June 1999 to determine the effects of soil water potential, xylem pressure potential, and sticking depth on rooting. A range of soil water potentials were created by applying three different amounts of mist to five different media (100:0, 75:25, 50:50, 25:75, and 0:100% peat:perlite v/v). Two amounts of mist were controlled by soil tensiometers that were preset at threshold levels of  $-1.3$  kPa (high mist) and  $-2.3$  kPa (low mist). When the control medium (1 peat:3 perlite) containing the tensiometer dried past the threshold, mist would be applied to that particular treatment. The third mist treatment was our normal computer controlled program using relative humidity and time of day to control frequency of mist application. Stem cuttings from 1 full-sib family were inserted either 2 cm or 4 cm into the medium. Each mist level contained 8 replications of five media. There were 16 cuttings per plot. Soil water potential and xylem pressure potential were measured weekly in each plot for five weeks. Data were then averaged over five weeks and rooting percentage was regressed against the values for each plot.

Rooting percentage was 45% in the control followed by 33% in the low mist, and 23% in the high mist. There was no difference between sticking depths for rooting percentage. There was only a weak relationship between soil water potential and rooting percentage. The highest rooting percentage occurred in a narrow range of soil water potentials ( $-1.3$  to  $-2.0$  kPa), but was accompanied by many plots, both among and within mist treatments, that rooted poorly in the same range of soil water potential. Thus, other factors, including mist level, made it difficult to detect the effect of soil water potential alone.

There was a moderate ( $R^2=0.33$ ), quadratic relationship between xylem pressure potential and rooting percentage (Figure 3). The highest rooting occurred between -0.5 and -1.1 MPa of xylem pressure. Outside this range of xylem pressure potential, cuttings rooted poorly. Previous research supports that poor rooting is highly correlated with water stress in stem cuttings, but very little research exists correlating too little water stress with poor rooting. It is possible that stem cuttings may need to experience some moderate water stress in order to initiate or elongate adventitious roots. At a minimum, these results show that keeping cuttings fully hydrated, which could be difficult to achieve in operational production systems, is not required for good rooting.



**Figure 3.** Correlation of rooting percentage with xylem pressure potential averaged over the first five weeks of the rooting period. Significant quadratic relationship:  $Rooting \% = 1.49 (XPP) - 0.88 (XPP^2) - 0.12$  ( $R^2=0.33$ ).

In May 2000, an experiment was set up to separate the effects of mist level, soil water potential, and their interaction on xylem pressure potential and rooting percentage. We inserted cuttings through a closed-cell sponge rubber mat and sealed them with non-silicone grease to prevent water seepage. Three levels of mist were applied to the aerial portion of the cuttings and three levels of soil water potential were maintained by drip irrigation controlled by soil tensiometers. Unfortunately, there was little or no rooting in any of the treatments. This could have been due to a number of factors, including low oxygen levels in the medium because of poor gas exchange between the air and the medium, disease buildup within the medium, and standing water on top of the rubber mats that may have created a low oxygen environment around the cutting bases. In addition, it became increasingly difficult to maintain the media at the desired negative (dry) water potentials, probably because the sealed mats limited evaporation.

We are revising the experimental system to allow us to test the effects of mist level and soil water potential separately and in combination. Preliminary tests using containers of various depths filled with a medium of fine sand have produced a range of soil water potentials within the same amount of mist. The differences between treatments are due to the distance from the top of the medium (where the cuttings are inserted) to the water table in the container. The water table of any container is a function of capillary action resulting from the water holding capacity of the medium and the depth of the container. For example, a shallow container will have a less negative (wetter) soil water potential than a deep container. By varying the height of containers used for rooting, we can create the desired soil water treatments and maintain integrity between treatments. In reduced mist treatments, supplemental water will be added to containers to maintain soil moisture at the appropriate treatment levels. The effect of these treatments on xylem pressure potential and rooting will be tested in winter 2001.

## Container Type and Growth of Rooted Cuttings

Previously, we reported on the effects of three sizes of Ray Leach tubes and six sizes of Jiffy Forestry Peat Pellets on rooting and rooted cutting morphology after the rooting period. In both of these studies, a sub-sample of cuttings was planted in Rayonier's Glennville nursery to determine if there were effects of container type and size on first-year field growth. The cuttings, which were originally stuck in June 1998, were planted in the nursery at a spacing of 1 ft. x 1 ft. in December 1998. They received only occasional watering and weed control and no fertilization to approximate field conditions. They were harvested in January 2000 and scored for shoot height, shoot dry weight, root dry weight and shoot:root ratio.

Tube size—Three sizes of Ray Leach tubes were obtained from Stuewe and Sons, Inc. (Corvallis, OR): Super Cells (10 in<sup>3</sup>, 164 ml), Super “Stubby” Cells (7 in<sup>3</sup>, 115 ml), and Pine Cells (4 in<sup>3</sup>, 66 ml). In the greenhouse, there were no significant differences among the tube sizes in rooting percentage, shoot height, shoot dry weight or root dry weight.

After one growing season, the cuttings that had been rooted in the Super Cells were taller than those from the other two tube sizes (Table 1). The means for shoot and root dry weight for the Super Cell cuttings appeared larger than for the other two tube sizes, however, these differences were not statistically significant. Based on this limited study, it appears that the size of container can influence early growth potential after outplanting.

*Table 1. Effect of container type on shoot height, shoot and root dry weight, and shoot:root ration after 1 year of growth in a nursery<sup>a</sup>.*

Ray Leach Tube Type	Shoot Height (cm)	Shoot Dry Wt. (g)	Root Dry Wt. (g)	Shoot:Root Ratio
Pine Cell	38.0 b	17.8 a	16.0 a	1.12 a
Super “Stubby” Cell	41.9 b	21.2 a	17.8 a	1.20 a
Super Cell	50.7 a	35.9 a	27.5 a	1.29 a

<sup>a</sup> Means within a column followed by the same letter are not significantly different at  $P < 0.05$ , as determined by Fischer's protected LSD.

Jiffy pellets—A companion study was conducted with six sizes of Jiffy Forestry Peat Pellets that were compared to a standard consisting of Ray Leach Super Cells filled with a medium of 60% perlite and 40% peat (v/v). In the greenhouse, rooting percentages in the smaller pellets were comparable to the standard, but percentages in the larger pellets were lower than the standard. Shoot heights and shoot dry weights were not affected by the treatments, but the root dry weights of all the pellets in the January experiment and the four smallest pellets in the June experiment were lower than the standard. The root dry weights of the cuttings rooted in the two largest pellets did not differ from the standard.

After one year in the nursery, the shoot heights of the cuttings in all the pellet sizes, except the largest, were less than the cuttings rooted in the standard (Table 2). The height of cuttings rooted in the 50 mm pellets did not differ from the control. Similarly, the shoot dry weights of the standard were greater than those of all the pellet sizes, except the 50 mm pellets, which were not significantly different. The root dry weights of all the pellet sizes were less than the standard.

*Table 2. Effects of rooting in Jiffy Forestry Peat Pellets on shoot height, shoot and root dry weight, and shoot:root ratio after 1 year of growth in a nursery.*

Pellet Size or Container	Shoot Height (cm)	Shoot Dry Wt. (g)	Root Dry Wt. (g)	Shoot:Root Ratio
25	35.0 *	16.1 *	12.0 *	1.34
30	33.8 *	15.7 *	11.2 *	1.40
36	36.4 *	18.0 *	12.7 *	1.42
42	37.7 *	18.5 *	12.9 *	1.43
42+	38.3 *	21.2 *	14.5 *	1.46
50	41.0	24.8	17.4 *	1.42
Super Cell	42.0	27.5	22.2	1.24

\* Means with an asterisk are significantly different from the standard (Ray Leach Super Cell) at  $P < 0.05$ , as determined by Fischer's protected LSD.

In general, all the measurements of rooted cutting size increased as the size of the pellet increased. It is likely that the smaller pellets limited root growth in the period between root formation and planting in the nursery. This translated into reduced growth in the next growing season. The only pellet that produced cuttings of comparable size as the Ray Leach Super Cell standard was the 50 mm pellet. However, in both rooting tests (winter and spring cuttings), cuttings in these pellets did not root at the same percentages as either the standard or the smaller pellets. Based on these results, it seems reasonable to conclude that if small pellets or containers are used for the rooting phase, a subsequent transplant to a larger container or to a nursery bed for the post-rooting culture phase could result in improved early field growth after plantation establishment.

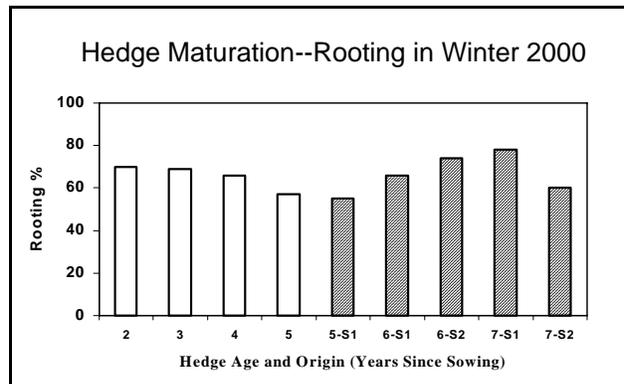
## **RESEARCH FOR CLONAL FORESTRY**

### **Hedge Maturation Study**

Our ongoing hedge maturation study, conducted by PhD student Rania Masri, is providing information critical to the utilization of rooted cuttings for both full-sib multiplication and clonal forestry. For full-sib multiplication, longevity of hedges will be an important operational consideration. For clonal forestry, identification and multiplication of superior clones will require

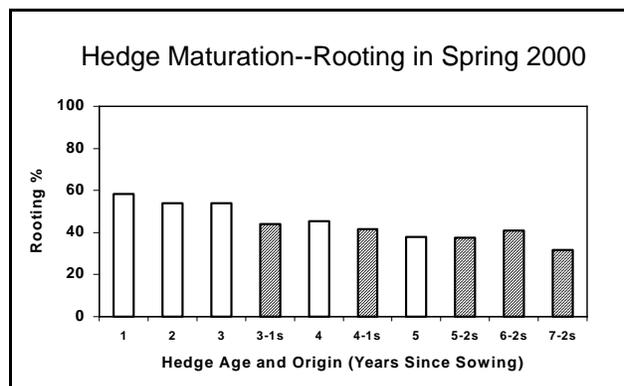
some period of time and the combination of hedging and serial propagation treatments must delay maturation sufficiently to maintain rooting ability and field performance of the selected clones. This study was begun in the spring of 1993. Each year, a new sample (16-20 clones per family) of hedges from seedlings are started from three open-pollinated loblolly pine families. In addition, every other year, serial propagation is conducted on the clones derived from the seedling hedges or the most recent cycle of serially propagated hedges (hedges from rooted cuttings). This report summarizes results from rooting experiments in winter and spring 2000 and growth of rooted cuttings in the field after two growing seasons. Previous reports summarized rooting in winter and spring of 1997 and 1999 and cutting morphology measurements in winter and spring 1999.

**Rooting Experiments**--In the winter 2000 rooting experiment, there was no significant effect of seedling hedge age on rooting percentage. However, a slight numerical decline was observed between three- and five-year-old seedling hedges (Figure 4). There was also no significant effect of serial propagation on rooting percentage. In fact, the highest rooting percentage for any treatment was observed in the seven-year-old clones that had been through one cycle of serial propagation (78%). The number of serial propagation cycles had an inconsistent effect. For six-year-old clones, hedges resulting from two cycles of propagation rooted at a higher percentage (74%) than hedges resulting from one cycle of propagation (66%). The trend was opposite for the seven-year-old clones, with the hedges resulting from two propagation cycles rooting at a lower percentage (60%) than those from one propagation cycle (78%).



**Figure 4.** Effect of clone age on rooting percentages of hedges derived from seedlings or rooted cuttings in winter 2000 (1s=one cycle of serial propagation, 2s=two cycles of serial propagation; white bars=seedling hedges, hatched bars=serially propagated hedges).

Overall, the rooting percentages were lower in the spring 2000 rooting experiment (Figure 5). Again, a slight decline was observed as the seedling hedges increased in age, especially after three years. The cuttings from serially propagated hedges, however, remained relatively constant, although at slightly lower percentages than the youngest seedling hedges.



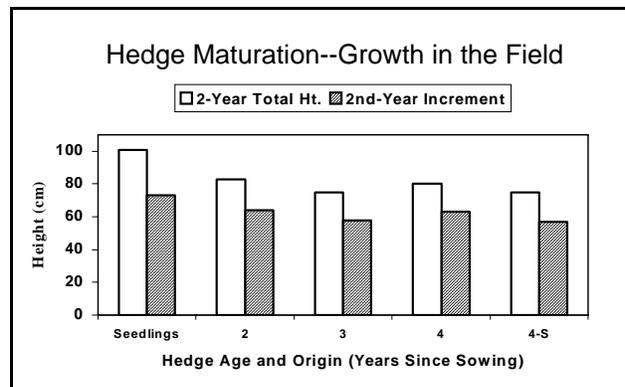
**Figure 5.** Effect of clone age on rooting percentages of hedges derived from seedlings or rooted cuttings in spring 2000 (1s=one cycle of serial propagation, 2s=two cycles of serial propagation; white bars=seedling hedges, hatched bars=serially propagated hedges).

The results of these experiments, as well as previous experiments, do not yet provide a definitive answer about the longevity

of acceptable rooting ability in loblolly pine clones. In some experiments, especially those in which overall rooting is low, an apparent decline with age is observed. Alternatively, in those experiments in which rooting percentages are higher, no decline is seen. These results could be interpreted as meaning that as loblolly pine clones age, they do become slightly more difficult to root. Thus, within the age span currently studied, seven years from seed, the clones have not yet “lost the ability to root,” but it may have become more difficult to root them at high percentages. While acceptable rooting can, in fact, be achieved, the increased difficulty may have ramifications for operational production. Moreover, if we are witnessing slowly creeping maturation in these hedges, the field performance of cuttings rooted from hedges of these advanced ages could also be in question. Further experiments will continue to test these materials, as well as additional age classes, to more clearly define the acceptable ages and treatments for propagation.

**Growth in the Field**--In February 1998, rooted cuttings from the spring 1997 rooted experiment were planted in a field test on Bowater Corporation land in South Carolina. Rooted cuttings from two-through four-year-old seedling hedges, cuttings from four-year-old serially propagated hedges, and seedlings of the same three open-pollinated families were planted in the field test. Height was measured at the time of planting and after the first and second growing seasons. Ground-line diameter was measured at the time of planting and after the second growing season.

At the end of the second growing season, the seedlings were taller than all the classes of the rooted cuttings (Figure 6). The difference in height growth between the seedlings and cuttings is most likely due to initial size and stock quality. The seedlings had been larger at the time of planting and even though their growth increment during the second growing season was also larger than the cuttings, the relative growth rates for the second growing season ( $2^{\text{nd}}$ -year height -  $1^{\text{st}}$ -year height /  $1^{\text{st}}$ -year height) of the seedlings (2.6) was actually smaller than all the cuttings (two-year-old seedling hedges=3.3, three-year-old seedling hedges=3.4, four-year-old seedling hedges=3.7, four-year-old seedling hedges=3.1). Moreover, analysis of covariance showed a significant effect of initial size on second-year height and when that effect was removed, there were no significant differences between seedlings and cuttings.



**Figure 6.** Growth of rooted cuttings and seedlings after two growing seasons in the field (three families combined). (s=cuttings from serially propagated hedges)

There were significant differences in height among the cuttings from the three ages of seedling hedges, but there was no apparent trend with age. Cuttings from three-year-old hedges were taller than those from both two- and four-year-old hedges. There was no significant difference between cuttings from four-year-old seedling hedges and cuttings of the same clones that had been serially propagated. Thus, after two seasons in the field, we have not detected a maturation effect on growth of rooted cuttings through four years-of-age, whether from seedling or serially propagated

hedges. The test will be measured again after the 2001 growing season, when the trees will have undergone four seasons in the field. In addition, a second field test is scheduled for fall 2002, which will include cuttings from hedges up to eight-years-old.

### Clonal Multiplication Study

Two studies have been conducted since February 1999 to test methods for multiplying selected clones and for generating realistic estimates of clonal multiplication rates. The 1999 study tested a two by two factorial of treatments. Hedges were grown indoors under high-intensity lights or outdoors under standard conditions. For this study, the indoor hedges were kept in the greenhouse under long days for the entire year. On half of the hedges in each group, cuttings were collected weekly, as they attained a minimum size of 7 mm. On the other half of the hedges in each group, cuttings were collected periodically when the majority of the cuttings on the hedges had reached the same minimum size. The number of cuttings collected and the rooting percentage of the collected cuttings were recorded and used to calculate a total cutting yield. Five clones were used in the study and two hedges (ramets) of each clone were included in each treatment. A progress report on the results for part of the year was included in last year's report. This is an update for the entire year.

*Table 3. Number of cuttings, rooting percentage and total yield of cuttings resulting from indoor vs. outdoor growth conditions and continual vs. periodic cutting collection. Values shown are for five clones combined on a per hedge basis.*

Growth and Collection Treatment	Number of cuttings collected	Rooting Percentage	Rooted Cutting Yield
Indoor-Continual	166	48.8	81
Indoor-Periodic	131	45.8	60
Outdoor-Continual	136	65.4	89
Outdoor-Periodic	136	54.4	74

The largest number of cuttings collected came from the indoor-continual collection (Table 3). However, because of the lower rooting percentage in both of the indoor treatments, the largest total yield of rooted cuttings came from the outdoor-continual treatment (89 rooted cuttings per hedge per year). As proposed in the previous report, the lack of substantially greater cutting production from the indoor treatments may be the result of keeping the hedges indoors throughout the growing season, where light intensity may not have been sufficient to maximize growth and, therefore, cutting production. The lower light levels in the greenhouse may have also contributed to the lower rooting percentages in the cuttings from hedges grown indoors.

The continual collection treatment resulted in a greater number of cuttings collected from the indoor, but not the outdoor, hedges. Whether or not this labor-intensive method will be worth the extra effort in an operational clonal multiplication program is questionable. However, it does

result in at least some of the cuttings being collected and rooted earlier and, when serial propagation is taken into account, could provide some benefit.

The 2000 clonal multiplication study made several modifications. First, the continual collection treatment was omitted. Second, a serial propagation component was added. After each collection and rooting, a sub-sample of the rooted cuttings were transplanted, returned to the appropriate growing environment, and pruned to produce additional cuttings. Third, the indoor hedges were only kept indoors during the cold part of the year. The study was begun in February. The indoor hedges were moved outside in April and returned to the greenhouse, under a long photoperiod, in September.

Under this regime, the indoor hedges produced the first crop of cuttings approximately three weeks before the outdoor hedges. The second crop was collected approximately four weeks earlier in the indoor hedges. As of this writing, the number of cuttings collected in the two treatments is similar. However, the indoor hedges have another crop almost ready for collection, but it is unlikely that the outdoor hedges will produce another crop before spring. It remains to be seen whether the indoor hedges continue to grow and produce cuttings all through the winter. In addition, the serially propagated cuttings in the indoor environment have also produced a small crop of cuttings that is ready for collection. The study will continue through the end of 2001, which should allow us to determine: (1) whether there is a significant benefit to the indoor treatment through the winter, (2) whether growing the indoor hedges continuously through the winter results in a loss of vigor during the second year, and (3) reasonable estimates of clonal multiplication rates over a two-year period, including serial propagation.

### **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. From this study, we will generate quantitative estimates of: (1) the ideal number of clones per cross to begin selection (2) number of ramets per cross necessary to characterize growth on one site, (3) efficiency of selection at different ages, (4) multiplication rates for a large number of clones, and (5) magnitude of predicted genetic gain for the best clones in each cross.

The study began with approximately 100 clones of each cross. After hedge production from the seedlings, rooting and sorting, 450 clones were planted in two field tests: 168 clones on International Paper land (formerly Champion International) near Jay, Florida in December 1998 and 282 clones on Westvaco land in South Carolina in November 1998. The experimental design is a randomized complete block, with 9 blocks and one ramet per clone per block.

Measurements on survival and height after two growing seasons have just been received. Analysis is underway and the results will be presented at the Annual Meeting. In general, survival seems to be adequate, but test uniformity could be problematic. Most likely, this is due to marginal stock quality at the time of planting. We will be examining the data, with particular attention to the variation within clones across the blocks (clone x block coefficient of variation). If this measure is not at a sufficiently low level after two growing seasons, it could impact our estimates of the optimal selection age. In that case, we would consider a set of duplicate tests with the same clones in 2001.

Concurrently, we have begun the clonal multiplication phase of the project. In February 1999, cuttings from all the clones in the field tests were stuck and four cuttings per clone were transplanted to 3-gallon pots. These rooted cuttings have received their first pruning and are now producing a small number of cuttings. If the field tests show acceptable uniformity, we will begin to cull clones based on the second-year measurements and continue to multiply the remaining clones this winter. If we are not satisfied with the current field tests, all clones will be retained and multiplication will be delayed.

### **Mechanisms of Root Formation and Maturation**

This research is studying the molecular and biochemical steps in rooting and how they are affected by maturation. Previously, we cloned 5 genes from loblolly pine (*LPEAs*: Loblolly Pine Early Auxin-induced) that belong to a large family of auxin-induced plant genes known as the Aux/IAA genes. Previous research has demonstrated a correlation between the expression of these genes and the onset of adventitious root formation. We are now studying the function and regulation of the *LPEA* genes to determine if any are directly involved in the process of adventitious root formation.

Promoter analysis of *LPEA1*--Carmen Lanz-Garcia previously cloned the DNA containing the *LPEA1* gene and its promoter, or regulatory region. The nucleotide marking the start of transcription (formation of the active gene) has now been identified. This piece of information is important in defining the gene and its promoter and it appears that one of the three proposed auxin-responsive elements (AREs) actually lies within the transcribed region of the gene.

Protein-DNA binding (gel shift) experiments are being used to further characterize the *LPEA1* promoter. We have found that nuclear proteins (DNA regulating proteins) from pine hypocotyls have a specific binding affinity for the *LPEA1* promoter DNA. This specific binding is stronger in proteins from auxin treated than from non-auxin treated hypocotyls. Additionally, we have tested individual pieces from within the promoter and are beginning to identify elements that affect protein binding. Two elements, called TGA boxes, seem to be responsible for a high proportion of the auxin-induced binding in hypocotyls.

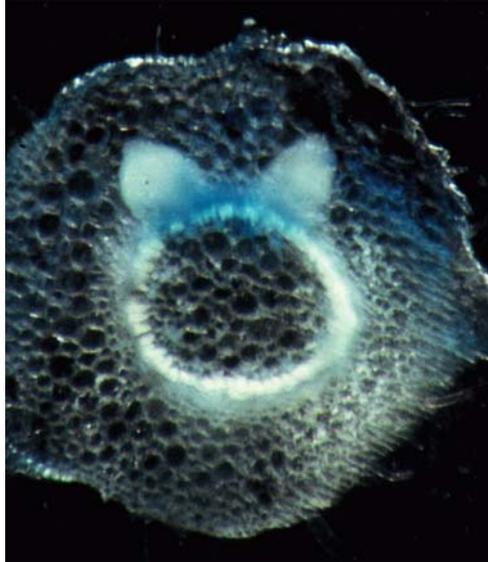
In gel-shift experiments with nuclear proteins from pine roots, we do not observe the same specific binding affinity. These experiments have shown that, although the promoter is bound by root nuclear proteins, the binding is both specific and non-specific—that is, the bound proteins also bind to random pieces of DNA. This experimental system is providing a method of comparison of

the function of this promoter in various tissues and will allow us to determine specific elements and interactions that control auxin-induced genes during the rooting process.

Transgenic tobacco plants are also being used to examine the function of the *LPEAI* promoter. These plants contain an inserted gene constructed from the *LPEAI* promoter and the GUS reporter gene. We induced adventitious roots on tobacco stem cuttings on lines containing both the full promoter and on lines containing a shortened promoter that deletes one TGA box and two of the three AREs. With both constructs, the majority of newly forming root primordia lack GUS expression (Figure 7), although with higher levels of auxin than are necessary for root initiation, GUS expression is observed. This suggests that *LPEAI* is functioning in an auxin-responsive manner, but not directly in the early stages of adventitious root formation. This interpretation is supported by the examination of these same cuttings and adventitious roots later in the experiment. In this case, tobacco cuttings containing the full promoter strongly express GUS a short distance behind the meristem in elongating roots (Figure 8). The cuttings containing the shortened promoter express a much reduced level of GUS (Figure 9). Thus, it appears that *LPEAI* functions more in the elongation of adventitious roots than in their initiation and, that in shortening the promoter, we may have eliminated a root-specific element.

Transgenic tobacco over-expressing the *LPEAs*--To get additional information about the function of the *LPEAs*, Victor Busov fused the coding regions of three of the genes (*LPEAs 1, 2 and 5*) with a constitutive promoter (CMV 35s) and introduced them into tobacco plants. We are in the process of screening the transgenic plants for phenotypes that could give clues to each gene's function. One experiment is testing each *LPEAs*' effect on germination and early root formation, two auxin related growth processes. At the end of two weeks, the root systems of two of these lines showed a striking abnormality. Tobacco lines containing over-expressed *LPEA2* or *LPEA5* had root systems that were distinctly agravitropic, with roots growing along the surface of the agar and even curling into the air. Plants of these lines are currently being grown to a size sufficient to produce cuttings for adventitious rooting experiments to test whether over-expression of these genes enhances or inhibits root formation.

Additional Auxin and Rooting Genes--Research on the *Aux/IAA* gene family in *Arabidopsis* is rapidly advancing and demonstrating that the family members are involved in complex interactions during all auxin-related processes. Because we have now demonstrated that the *LPEAs* are inducible both in mature and juvenile pine cuttings, it is likely that these genes, though an integral part of the pathway, are not the metabolic blocks to root formation. Therefore, we are expanding our search to other genes that are involved directly in root formation. Victor Busov, in collaboration with the NCSU Forest Biotechnology Group, used microarray technology to screen for additional auxin-regulated genes. An array containing 3456 genes was screened with mRNA from auxin-treated and control hedge cuttings. To date, 46 genes have been tentatively identified as being induced by the



**Figure 7.** Cross section through transgenic (full LPEA1 promoter-GUS fusion) tobacco stem cuttings during adventitious root formation. Note lack of GUS expression in tips of root primordia, but presence of the GUS protein (blue color) at the base of the primordia.



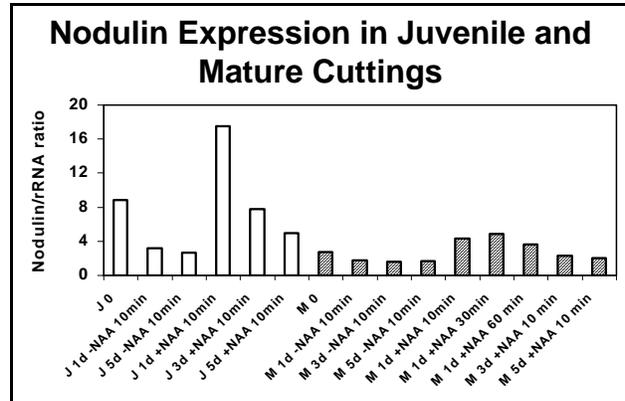
**Figure 8.** Adventitious roots from a transgenic (full LPEA1 promoter-GUS fusion) tobacco stem cutting. Note strong GUS expression (blue color).



**Figure 9.** Adventitious roots from a transgenic (truncated LPEA1 promoter-GUS fusion) tobacco stem cutting. Note lack of strong GUS expression (blue color).

auxin treatment. The genes fall into a number of functional categories based on sequence similarities to genes from other organisms. There are also several genes for which no presumptive function can be identified. We selected 13 genes that are representatives of the different functional groups and performed northern blots to confirm auxin inducibility. Then, a second set of experiments tested whether expression differed between juvenile and mature cuttings. One gene, a presumptive aldehyde dehydrogenase, was found to be more highly expressed in mature cuttings. This enzyme converts highly reactive aldehydes to less reactive compounds and, at this point, its connection to adventitious root formation seems tenuous.

A second gene was more highly induced by auxin in juvenile than in mature cuttings (Figure 10). Even longer durations of treatment with auxin did not result in mature expression levels that equal juvenile cuttings. Sequence searches in gene sequence databases revealed a high degree of similarity with a gene first identified during nodulation in alfalfa (*Medicago trunculata*). There are also genes related to this so-called nodulin in *Arabidopsis* and rice, although these species (like pine) do not undergo symbiotic nitrogen fixation. Thus, it appears that this gene, though first identified during the nodulation process, may have a more generalized, auxin-related function. Further studies with this gene show that it is induced by the other auxins IAA, IBA and 2,4-D, but not by gibberellic acid or the cytokinin BA. It is present at low levels in the needles of stem cuttings, but not induced by auxin. In the roots of young seedlings, it is both present and induced by auxin treatment.



**Figure 10.** mRNA abundance of the putative nodulin transmembrane transport gene after auxin (+NAA) or control (-NAA) treatment of juvenile (white bars) or mature (hatched bars) cuttings. Labels: age, time of sampling after treatment, auxin treatment, duration of auxin treatment.

A protein structure prediction program predicted a protein with 10 membrane-spanning domains and a central cytoplasmic region that is predicted to form a pore in the membrane. Proteins with this type of structure often serve as transporters of molecules across the cell membrane. This transport is usually highly regulated and can be part of signaling events. Experiments are underway to localize expression within cuttings and to determine the function of this very interesting gene and whether it is related to adventitious root formation.

## SUPPORTING COMPANIES IN 2000

Boise Cascade Corporation	Rayonier
Bowater, Inc.	Temple-Inland Forest
Champion International Corporation	The Timber Company
Gulf States Paper Corporation	Westvaco Corporation
International Paper Corporation	Weyerhaeuser Company
Mead Coated Board	

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Victor Busov, PhD student  
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Frank Blazich, Collaborating faculty

## PUBLICATIONS OF INTEREST TO THE MEMBERS

- Busov, V.B., Y.-H. Sun, B. Goldfarb, R. Whetten, R. Sederoff. Microarray for cloning auxin-induced genes from loblolly pine. Poster and abstract in: Annual Meeting of The American Society of Plant Physiologists. San Diego, California, July 15-19, 2000.
- Dubois, J.-J.B., F.A. Blazich, S.L. Warren, and B. Goldfarb. 2000. Propagation of *Anemone x hybrida* by rooted cuttings. *J. of Env. Hort.* 18: 79-83.
- Frampton, L.J., Jr., B. Goldfarb, S.E. Surles, and C.C. Lambeth. 1999. Nursery Rooting and Growth of Loblolly Pine Cuttings: Effect of Rooting Solutions and Full-Sib Family. *South. J. of Appl. For.* 23: 108-115.
- Frampton, L.J., B. Li, and B. Goldfarb. 2000. Early field growth of loblolly pine rooted cuttings and seedlings. *South. J. of Appl. For.* 24: 98-105.
- Goldfarb, B., V.B. Busov and C. Lanz-Garcia. In press. Auxin-induced genes and adventitious root formation in loblolly pine. *in Proc. of the Third International Symposium on Adventitious Root Formation.* 27 June - 1 July, 2000. Veldhoven, Netherlands.

- Goldfarb, B., S.E. Surles, M. Thetford, and F.A. Blazich. 1998. Effects of root morphology on nursery and first-year field growth of rooted cuttings of loblolly pine. *South. J. of Appl. For.* 22: 231-234.
- Lanz-Garcia, C., V.B. Busov, and B. Goldfarb. 2000. A promoter of an auxin-response gene from loblolly pine and its possible role in root formation. Poster and abstract in: Third International Symposium on Adventitious Root Formation. 27 June - 1 July, 2000. Veldhoven, Netherlands.
- LeBude, A.V., F.A. Blazich, and B. Goldfarb. 1999. Effects of Jiffy forestry peat pellets on rooting stem cuttings of loblolly pine. *Proc. Southern Nursery Association Research Conference, 44<sup>th</sup> Annual Report.* pp. 335-338.
- LeBude, A.V., F.A. Blazich and B. Goldfarb. 2000. Effects of Jiffy forestry peat pellets on rooting and subsequent field performance of stem cuttings of loblolly pine. Oral presentation and abstract in: 16<sup>th</sup> North American Forest Biology Workshop. July 17-21, 2000. Merida, Mexico.
- LeBude, A.V., F.A. Blazich and B. Goldfarb. 2000. Effects of Jiffy forestry peat pellets on rooting and subsequent field performance of stem cuttings of loblolly pine. Poster and abstract in: *HortScience* 35: 451.
- Rieckermann, H., B. Goldfarb, M. Cunningham, and R. Kellison,. 1999. Influence of nitrogen and photoperiod on root and shoot growth of rooted sweetgum stem cuttings. *New Forests* 18:231-244.
- Rowe, D.B, F.A. Blazich, and R. J. Weir. 1999. Mineral nutrient and carbohydrate status of loblolly pine during mist propagation as influenced by stock plant nitrogen fertility. *HortScience* 34: 1279-1285.