NORTH CAROLINA STATE UNIVERSITY
LOBLOLLY AND SLASH PINE ROOTED CUTTING PROGRAM

Annual Progress Report
September 15, 2001

EXECUTIVE SUMMARY

The NCSU Loblolly and Slash Pine Rooted Cutting Program is completing its tenth year of existence and the first year in the third phase of the program. Support by the members allowed the beginning of a new four-year phase on January 1, 2001 that is projected to end on 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. While we research many individual topics, all fit within the framework of providing information important for either efficient operational production of rooted cuttings, the implementation of clonal forestry, or both.

In the area of production efficiency, this report contains results from ongoing studies using environmental and physiological measurements to provide information to propagators on controlling irrigation regimes in the rooting environment. An enhanced understanding of the effects of aerial mist and medium moisture on cutting water status and rooting is described. Another experiment is investigating the effects of different stock type production systems on rooted cutting quality. So far, results with a transplant system are most promising. We also report results of two field trials measuring performance of rooted cuttings with different root and shoot characteristics. These results will be useful for adopting grading criteria.

Several lines of research are being conducted in support of clonal forestry. Our long-term hedge and clone maturation study now encompasses material through eight years of age and rooting results are somewhat conflicting. Future experiments will help determine if we are seeing slight maturation in the older clones. 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 third growing season. In this report are descriptive statistics on the growth and uniformity of the two sites, as well as preliminary predicted gain estimates from clonal selection. We continue our efforts to understand the fundamental mechanisms controlling root formation and how it is affected by maturation. We are generating and studying numerous candidate genes and using information from model plants to help us dissect these processes. Finally, we began a new study on the wood quality of clones (rooted cuttings) and full-sib families. This is a collaboration with the NC State Tree Improvement Program and members of the NC State Wood and Paper Science faculty with substantial funding by the US Department of Agriculture. It will provide useful information about the potential for improvement and uniformity of juvenile pine wood quality using advanced regeneration technologies.

As always, we do our best to assist member organizations with their internal rooted cutting activities. As the level of activity increases among the members, it is gratifying to the staff in Raleigh to see our research results put to good use.

INTRODUCTION

The year 2001 marks the first year in the third phase of the Rooted Cutting Program. The program staff and students share my excitement at the task ahead. Through your support, we have the opportunity to do meaningful, focused research that, when implemented, will affect forestry in the Southeast U.S. For us, it is a unique opportunity to combine academic discovery and education with real-world problems and constraints. We feel it is a partnership that works.

Industry and economic forces continue to put stress on the membership of the program, as with most university-industry cooperative programs. There have been no membership changes during calendar year 2001, although at least two planned member ownership changes have been announced. The membership currently stands at 10 companies and we will continue our efforts to recruit additional organizations. With the slow-down in the economy, funding for intensive silvicultural investments, including rooted cuttings, may be more difficult for some to justify. However, many forward-looking companies and forest managers still realize the value of investing now in the quantity and quality of our future wood supply.

A number of staff changes have occurred in the last year. Victor Busov will be finishing his PhD program within the month. Rania Masri graduated with her PhD last December. Patrick Cumbie is a new MS student who is working jointly with the Tree Improvement Program. In addition, we are currently seeking a qualified PhD student to start in January and begin the new research on hedge nutrition and physiology.

This report includes summaries of experiments conducted or analyzed since the last progress report in October 2000. Additional details will be presented at the upcoming Annual Meeting in Lake Charles, LA. We hope to see many of you there and heartily thank Boise Cascade for hosting us. Good Rooting!

Barry Goldfarb
Director

RESEARCH FOR OPERATIONAL PRODUCTION

Control of the Rooting Environment

Two studies were conducted by Anthony LeBude, Research Assistant and Ph.D. student, to determine the effect of mist and medium water potential (Ψmedium) on cutting water potential (Ψcutting) and rooting percentage. One study used dormant cuttings (January) and one used succulent cuttings (June). The experimental design for both trials was a split-plot. Two mist regimes, a high ("normal") and a low regime consisting of 40% less mist, were the main plots and four Ψmedium treatments were the sub-plots. The Ψmedium treatments were -1.8 kPa (wet), - 2.6 kPa (intermediate), - 3.6 kPa (dry), and a control. They were created using containers of various heights filled with coarse builders' sand and maintained with sub-irrigation controlled by a tensiometer. The control medium consisted of 2 peat: 3 perlite (v/v) placed in a container with a height equal to the intermediate Ψmedium treatment, but sub-irrigation was not applied and the Ψmedium was not maintained. Stem cuttings were a random mix of 2 full-sib families consisting of approximately 30 clones from each family. Approximately 60 cuttings were placed in each plot. One, three, and five weeks after sticking the cuttings, Ψcutting was measured destructively on one cutting per plot at 5 am and 2 pm using a pressure bomb. Two and four weeks after sticking, Ψcutting was measured every three hours beginning at 5 am and continuing until 5 am the following morning (9 measurements). Ψmedium was also recorded for each plot at 5 am and 2 pm for each of the first five weeks. Rooting percentage was recorded after 10 weeks. Analyses of variance and regression were used to test the relationships between Ψmedium, Ψcutting and rooting percentage.

Table 1. ANOVA for effect of medium water potential and mist level on cutting water potential and rooting percentage in winter and spring stem cuttings of loblolly pine.

		Winter		Spring	
Source	Df	Ψcutting	Rooting %	Ψcutting	Rooting %
Mist	1	*	NS	*	*
Rep(Mist)	6	*	NS	*	NS
Ψmedium	3	*	NS	*	NS
Mist x Ψmedium	3	*	*	*	NS

*=significant, NS=not significant at $P = 0.05$.

Mean rooting percentage for the winter and spring trials was 23% and 47.5%, respectively. Based on the low rooting percentages and the levels of Ψmedium and Ψcutting obtained in the first experiment, the mist regime was decreased for the second experiment. Mist level, Ψmedium (averaged over the five-week period), and their interaction had a significant effect on Ψcutting in both the winter and spring trials (Table 1). In both experiments, cuttings receiving less mist and cuttings in drier media had lower (more negative) Ψcutting (Table 2). In the winter experiment, the effect of Ψmedium on Ψcutting was strongly dependent on mist level. The increase in stress with drier media was greater in cuttings receiving low mist than in those receiving high mist (Figure 1). This effect was less obvious in the spring experiment, where medium moisture effected Ψcutting equally in the two mist levels (Figure 2). These results demonstrate that uptake of water from rooting media contributes to the water status of non-rooted cuttings.

Table 2. Means of cutting water potential and rooting percentage by mist regime and soil water potential treatment for two rooting trials of stem cuttings of loblolly pine.

	Winter				Spring			
	Ψcutting (MPa)		Rooting %		Ψcutting (MPa)		Rooting %	
Medium Moisture Treatment	High Mist	Low Mist	High Mist	Low Mist	High Mist	Low Mist	High Mist	Low Mist
Control	-0.38	-0.62	21.0	33.6	-0.43	-1.13	51.1	31.1
Dry	-0.42	-0.81	31.5	22.1	-0.63	-1.33	62.4	35.2
Medium	-0.37	-0.85	18.3	14.7	-0.58	-1.24	62.2	32.9
Wet	-0.24	-0.36	5.0	41.7	-0.41	-0.84	64.1	41.3

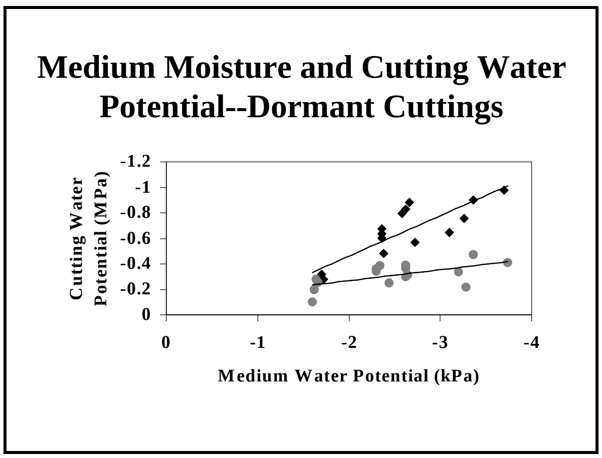

Figure 1. *Effect of medium water potential on cutting water potential in winter 2001, ◆=low mist, ●=high mist.*

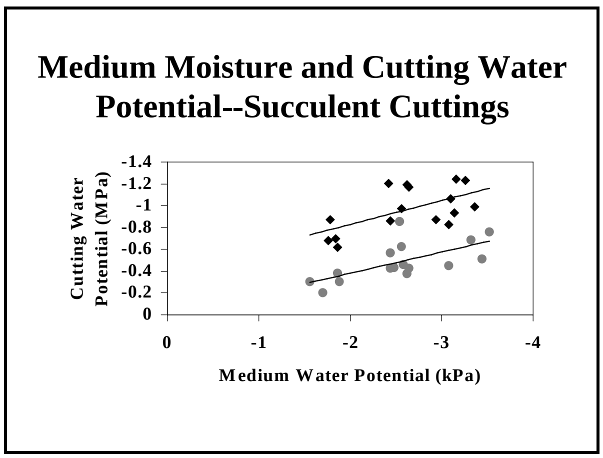

Figure 2. *Effect of medium water potential on cutting water potential in spring 2001, ◆=low mist, ●=high mist.*

In the winter experiment, the main effects of mist and Ψmedium did not significantly affect rooting percentage, however the interaction of the two was significant (Table 1). In the high mist treatment, rooting was highest in the dry medium, but in the low mist treatment, rooting was highest in the wet medium (Table 2). In contrast, in the spring experiment, only mist level significantly affected rooting. Rooting percentage was higher in the high mist treatment, regardless of medium moisture level.

The relationship of rooting percentage with Ψcutting depends on the level of moisture stress. Cuttings experiencing moderate to high stress (low mist in winter and both mists in spring) show an increase in rooting as their Ψ increases (decrease in stress) (Figure 3). In contrast, cuttings under little or no stress (high mist in winter) actually show an increase in rooting with decreased Ψ. This may be an indirect effect. For example, cuttings with no stress may also have been experiencing anaerobic medium conditions. However, no basal rotting was observed in the cuttings in these experiments. Thus, the propagator should not endeavor to eliminate all stress in cuttings. In these experiments, rooting was best between -0.4 and -0.65 MPa and was better in treatments in which the cutting surfaces dried between mist applications.

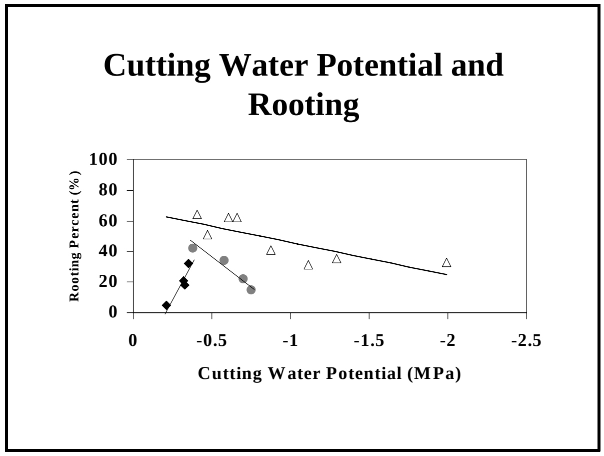

***Figure 3**. Effect of cutting water potential on rooting percentage, △=spring (high and low mist combined), ◆=winter–high mist, ●=winter–low mist.*

Stock Quality in Different Production Systems

In February 2000, an experiment was initiated by M.S. student, Matt Gocke, to evaluate the effects of three potential propagation systems on survival and stock quality of loblolly pine winter cuttings. The propagation systems tested in this experiment were: (1) a transplant system in which cuttings were rooted in a misthouse in Grow-Tech Rooting Sponges™ (GT) or Jiffy Forest Peat Pellets™ (Jiffy) and then transplanted to an outdoor nursery bed; (2) a fully containerized system in which cuttings were rooted in a misthouse in Ray Leach SuperCells™ and then transferred outdoors; and (3) a direct-stick system in which cuttings were stuck directly into an outdoor nursery bed.

A 4 ft. X 130 ft. simulated nursery bed was constructed for the study. The bed was filled with a sandy loam soil from the coastal plain of North Carolina and equipped with overhead sprinkler irrigation. Eight-cm winter cuttings were collected from three clones between February 28 and March 4, 2000, and stored in a cooler at 5^0 C until the time of sticking for all treatments. On March 10, 2000, cuttings were stuck for seven treatments representing the transplant and containerized treatments in the misthouse. Media used for the containerized treatments was a mix of 60% perlite : 40% peat. The GT sponges were placed in cavities of Winstrip trays (#162), while the Jiffy pellets remained in their plastic trays. The transplant treatments were transplanted to the nursery bed at seven, nine, or eleven weeks after sticking. Cuttings propagated in the containerized treatment were transferred outdoors twelve weeks after sticking. On April 22, 2000 cuttings for the direct-stick treatments were inserted one half their length (4 cm) into the nursery bed soil, either under full sun or partial shade (50%). All cuttings were treated with 10 mM NAA for three seconds before sticking. The study was a split plot design, with nine stock type treatments, three clones, eight blocks, and eleven cuttings per plot, for a total of 2376 treatment cuttings plus borders. A fertilizer regime of Peter’s 20-20-20 was applied at 50 ppm N every two weeks to the containerized and transplant cuttings from the eighth week to the 14th week after initial sticking. After the 14th week, the rate was increased to 100 ppm N every week for the remainder of the study. 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 started indoors. Preliminary survival and height measurements were presented in last year's report and annual meeting. This report presents final measurements of survival, number of primary roots, shoot height, shoot dry weight, root collar diameter, root dry weight, and root system symmetry, collected in December, 2000.

Table 3. Characteristics of loblolly pine rooted cuttings produced by nine treatments from three production systems in 2000.

Treatments	% Symmetrical	No. Roots	Survival (%)	Shoot Height (cm)	Shoot Weight (g)	Root Weight (g)	Root Collar Diam. (mm)
GT Transplant @ 7 weeks	70.6 ab*	3.2 b	63 b	28.8 a	48.3 a	14.1 a	5.3 a
GT Transplant @ 9 weeks	80.5 a	3.8 a	70 ab	27.4 ab	44.9 ab	14.8 a	5.1 ab
GT Transplant @ 11 weeks	74.1 ab	3.2 ab	80 a	23.4 c	38.9 b	12.2 ab	4.4 cd
Jiffy Transplant @ 7 weeks	65.7 ab	3.1 bc	29 e	24.8 bc	26.3 c	7.3 cd	4.6 bc
Jiffy Transplant @ 9 weeks	67.6 ab	3.1 bc	47 d	23 cd	23.3 cd	7.0 cde	4.3 cde
Jiffy Transplant @ 11 weeks	61.8 bc	2.8 bc	48 cd	19.9 d	16.6 de	6.0 def	3.9 efg
Containerized	64.4 ab	2.6 cd	64 b	15.9 e	14.3 de	9.4 bc	4.0 def
Direct-Stick Sun	41.3 d	2.0 de	63 bc	13.3 e	10.2 e	3.5 f	3.5 g
Direct-Stick Shade	44.7 cd	2.0 e	62 bc	13.8 e	9.9 e	4.1 ef	3.6 fg

*Values within a column that are followed by the same letter are not significantly different at p=0.05.

The GT transplanted cuttings for all three transplant times were among those with the highest values of all the variables measured (Table 3). In general, those that were transplanted earlier had slightly lower survival, but greater height, shoot and root weight, and root collar diameter. The Jiffy transplanted cuttings tended to be smaller than the GT cuttings, especially for shoot and root weight. The same opposite effects of transplant time on survival and growth were seen in the Jiffy cuttings. The containerized cuttings were shorter than all the transplanted cuttings, but had root weights intermediate between the GT and Jiffy cuttings. Their root collar diameters were comparable to the Jiffy cuttings, but smaller than the GT cuttings. The direct-stuck cuttings showed reasonable survival for both sun and shade (63 and 62%, respectively), but they had the lowest percentages of cuttings with symmetrical root systems. They also tended to be the smallest cuttings of all the treatments. This may have been due to our inexperience with this method of propagation and the fact that mist treatments may have been maintained too long into the growing season.

In order to rate the cuttings produced in terms of existing seedling quality standards, root collar diameter measurements were used to classify the rooted cuttings into the seedling grades developed by Wakeley in 1954. The GT cuttings had the highest percentages that were classified as Grade 1 and as acceptable (Grades 1 and 2 combined) (Figure 4). The containerized cuttings had only 10.6% culls, but few were classified as Grade 1. The direct-stick treatment produced the lowest percentage of acceptable cuttings, because of the small sizes noted above.

This study is being repeated in 2001 with a modification of some of the treatments. There are four transplant treatments (2 sticking times [February 22 and May 4] x 2 transplant times [8 and 11 weeks]), four direct-stick treatments (2 irrigation regimes x full sun vs. 50% shade), and 2 containerized treatments (early and late sticking, as in the transplants). All the transplants were stuck in GT Rooting Sponges placed in Winstrip trays. Other modifications from last year included adapting an individualized fertilization and irrigation regime for each treatment and transplanting only rooted cuttings for the transplant and containerized treatments. Preliminary measurements of height and survival for this report were collected on September 7.

Survival of the rooted cuttings in the transplant and containerized treatments was high (>90%) for both the February and May stickings (Figure 5). This was expected as only rooted cuttings were transplanted or moved outdoors (containerized). Survival was lower for all the direct-stick treatments. Of these the best survival was found in the Shade-High Mist treatment (59.2%), followed by the Shade-Low Mist (51.4%), Sun-Low Mist (48.0%), and Sun-High Mist (47.0%) treatments.

Shoot growth was greatest in the transplants that had been stuck in February. The

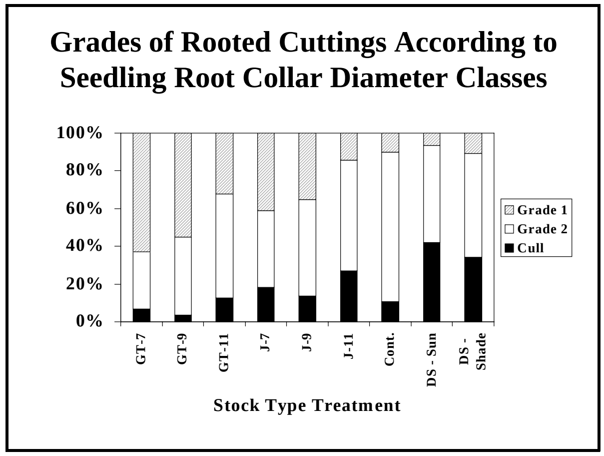

Figure 4. *Percentage of rooted cuttings in 2000 placed in grade classes according to seedling root collar diameter criteria.*

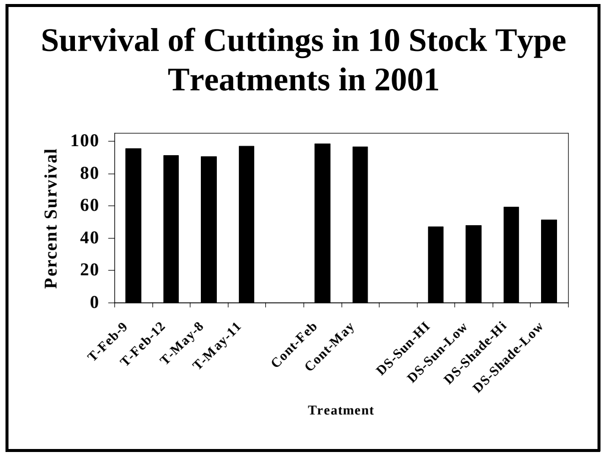

Figure 5. *Survival of rooted cuttings in 10 stock type treatments in September 2001.*

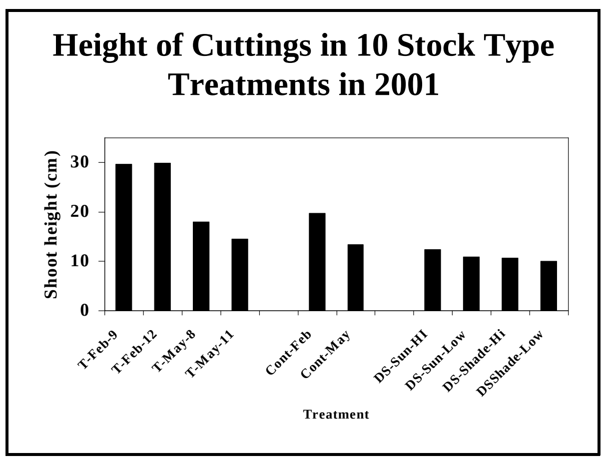

Figure 6. *Height of rooted cuttings in 10 stock type treatments in September 2001.*

cuttings transplanted at 9 and 12 weeks after sticking were approximately 30 cm tall (Figure 6). These were substantially taller than the transplanted cuttings that had been stuck in May. The containerized cuttings stuck in February, although not as tall as the transplants, were taller (20 cm) than the containerized cuttings stuck in May (13 cm). The direct-stuck cuttings were the shortest of the three propagation systems. The tallest cuttings were in the Sun-High Mist treatment (12 cm).

While further growth may yet occur this season, it seems likely that some of the treatments, particularly the cuttings stuck in May and those that were direct-stuck, will not produce rooted cuttings of an adequate size to meet Grade 1 seedling criteria. This may be a reflection of the relatively northerly climate in Raleigh. For example, the direct-stuck cuttings could not be set until mid-April, because of the possibility of frost-heaving. In a more southerly location, they could have been set two to six weeks earlier. In addition, the growing season at the end of summer would also be longer. This also holds true for the cuttings stuck in May in the transplant and containerized treatments. Over both years, the transplanted cuttings (early sticking) have performed very well. This treatment has excellent potential for producing high-quality rooted cuttings should it prove cost-effective.

Root System Quality Field Study

In 1995, we began a study to test the effects of root system morphology on field growth of loblolly pine rooted cuttings. Cuttings were rooted in open greenhouse benches and hand-transplanted to Weyerhaeuser Co.'s nursery in Washington, North Carolina. After finishing the growing season in the nursery, they were transplanted to a field test in the Coastal Plain of South Carolina on land belonging to The Timber Company in February 1996. After both the greenhouse and nursery phases, each cutting was scored for shoot height, the number of roots, root system symmetry, and the number of vertically oriented roots. Previous measurements showed that shoot height was correlated with the number of roots after the greenhouse and nursery periods, but not after one or two field seasons. Similarly, neither root system symmetry nor the number of vertical roots, as measured after both the greenhouse and nursery growing periods, significantly affected tree height in the field after two growing seasons. However, caution in interpreting those results was urged, because of the young age of the plantation. We now report results for height, diameter at breast height and tree volume after five field growing seasons.

The number of roots per cutting, as assessed after the greenhouse phase, did not significantly affect height, diameter or volume (Figure 7) after five seasons in the field. Root system symmetry also did not affect growth

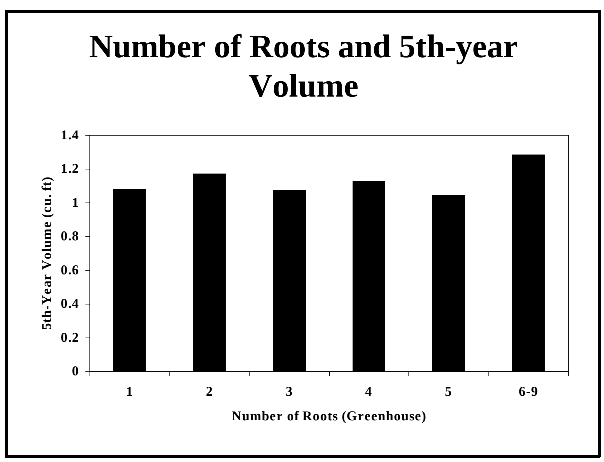

Figure 7*. Relationship between number of roots per rooted cutting (greenhouse) and fifth-year tree volume, no significant differences at p=0.05.*

(Table 4). Cuttings with the most extreme root systems (dog-legged), i.e. those having a single horizontal root, were not significantly different than those without this morphology for height, diameter or volume. Although the data are not shown here, there were also no significant effects of the number of roots, root system symmetry, or the number of vertical roots after the nursery phase on height, diameter or volume. These results, as well as those in the next section, indicate that additional stock quality standards for rooted cuttings, beyond those already employed for seedlings, are not necessary to ensure good field performance.

Table 4. Fifth-year growth measurements for loblolly pine cuttings with different root system morphologies.

Root System Trait	Symmetrical	Asymmetrical	Doglegged*	Not Doglegged
Height (ft.)	22.4a	22.4a	22.1a	22.4a
DBH (in.)	4.86a	4.83a	4.77a	4.86a
Volume (cu. ft.)	1.13a	1.11a	1.07a	1.12a

*Cuttings were rated as doglegged if they had only one root at the time of lifting from the greenhouse and that root was oriented in a horizontal direction.

Nursery Characteristics of Rooted Cuttings and Field Performance

In 1994, a study was begun by Weyerhaeuser to look at the effects of rooted cutting nursery characteristics on field performance. Bare-root cuttings of four open-pollinated loblolly pine families were produced from three-year-old seedling hedges. The cuttings were assigned to one of nine grading classes–a factorial of three root collar diameter classes (4-6 mm, 6-8 mm, and 8-10 mm) and three root quality classes (visual assessment as poor, fair or good). In addition, rooted cuttings that represented six putative cull classes were included ("runts," "jumbos," "dog-legged," "poor foliage cover," "multiple leaders," and "excessive sweep"). The rooted cuttings and commercial, bare-root seedlings of the same four families were planted in February 1995 in coastal North Carolina (Beaufort County). In March 1999, personnel from the Rooted Cutting and the Christmas Tree Genetics programs at NCSU measured survival, height, d.b.h, rust infection, and the presence or absence of stem sweep.

Survival for all the rooted cuttings (77%) was greater than for seedlings (42%). The low overall survival was due to an exceptionally dry spring immediately following planting. The cuttings probably had greater root mass than the commercial seedlings and, therefore, were better

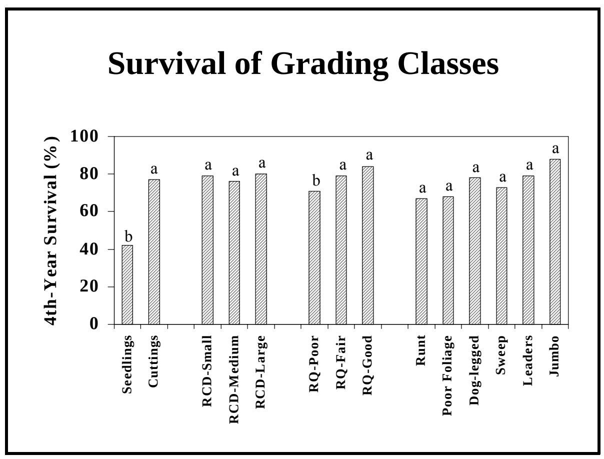

***Figure 8**. Fourth-year survival of rooted cuttings vs. seedlings and rooted cuttings in different grading and culling classes, values within a group with the same letter are not significantly different at p=0.05.*

able to establish in the dry soil. There were no significant differences in survival among root collar diameter classes, but root system quality did affect survival (Figure 8). Cuttings rated as having poor root systems survived at a lower percentage (71%) than cuttings with fair (79%) or good (84%) root systems. In addition, the number of primary roots per rooted cutting affected survival (Figure 9). Although numerical differences in survival were seen among the cull classes, none of these differences were statistically significant. Nor were any of the cull classes significantly different than the mean of all the rooted cuttings.

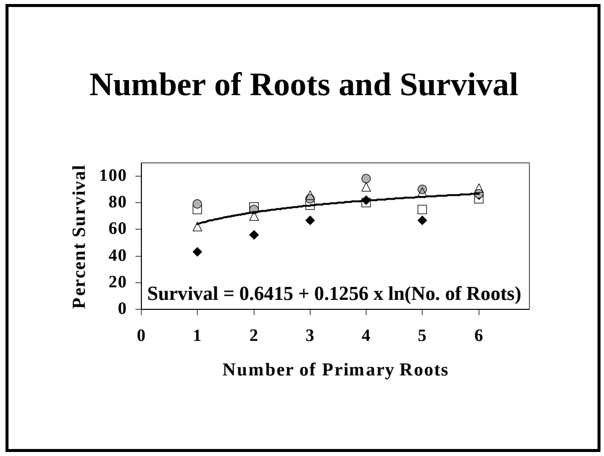

Figure 9. *Effect of number of primary roots on fourth-year survival of rooted cuttings, Symbols represent four open-pollinated families.*

In contrast, growth did not differ between the rooted cuttings and seedlings. After four growing seasons, mean tree volume was 0.259 m^3 for the cuttings and 0.285 m^3 for the seedlings (Figure 10). Cuttings with large root collar diameters grew more than those with small diameters. Similarly, cuttings rated as having good root systems had larger volumes than those with fair or poor root systems. Again, although numerical differences were observed among the cull classes, none were statistically significant.

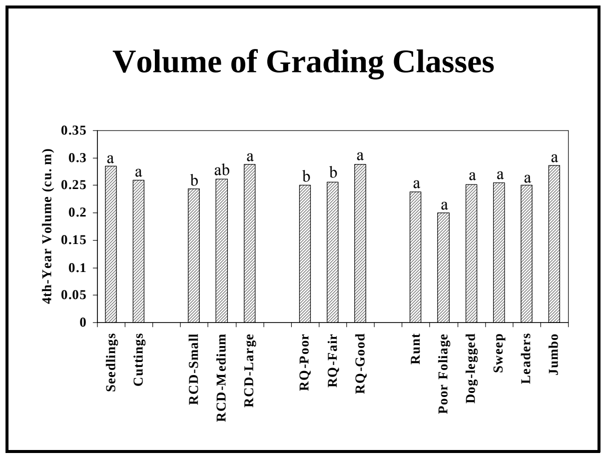

Figure 10. *Fourth-year tree volume of rooted cuttings vs. seedlings and rooted cuttings in different grading and culling classes, values within a group with the same letter are not significantly different at p=0.05.*

The results confirm other studies in that cuttings from relatively young hedges and seedlings grow equally well if they are from the same families and the same stock quality. The study also shows that survival of rooted cuttings in plantations could be improved if those with poorly developed root systems are culled. Alternatively, of course, production methods that minimize the number of cuttings with poor root systems would have the same effect. To maximize growth, the study suggests that both root collar diameter and root system quality are important. Thus, it continues to seem reasonable that existing seedling quality standards be applied to rooted cuttings. As with the previous root system quality study (see above), this study attempted to determine whether culling criteria unique to cuttings should be applied. Again we see no firm evidence for the necessity of additional culling criteria. Although we cannot exclude the possibility that a larger or more precise study would have detected reduced growth in the “runt” and “poor foliage cover” classes, it also seems likely that most of these cuttings would be culled with standard seedling culling procedures.

RESEARCH FOR CLONAL FORESTRY

Hedge and Clone Maturation Study

Our ongoing hedge and clone maturation study is testing the effectiveness of hedging and serial propagation for maintaining juvenility in loblolly pine. The study 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 2001. Previous reports summarized rooting in winter and spring of 1997, 1999 and 2000 and cutting morphology measurements in winter and spring 1999.

In the winter 2001 experiment, rooting percentage declined with the age of seedling hedges, particularly for hedges more than four years old (Figure 11). While rooting was relatively poor for the entire experiment (39.8% overall), serial propagation appeared to prevent the decline in rooting seen in the older seedling hedges. Serially propagated clones four through eight years old rooted at 39.4% (combined). This was not as high as two- through four-year-old seedling hedges (49.6% combined), but higher than five- and six-year-old hedges (26.1% combined). The seven-year-old clones, after two cycles of serial propagation, rooted at a percentage (49.1%) that was as high or higher than all other ages, except the two-year-old seedling hedges (54.7%).

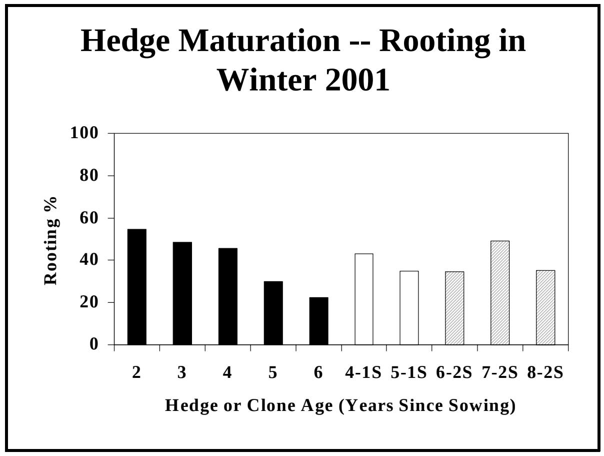

Figure 11. *Rooting of cuttings from hedges and clones of different ages in winter 2001; black bars=seedling hedges, white bars=1st-cycle serially propagated clones, hatched bar = 2nd-cycle serially propagated clones.*

In the Spring 2001 experiment, overall rooting percentage was somewhat higher (53.2%) than in the winter. There was less of a decline, both in older seedling hedges and in older serially propagated clones (Figure 12). Four- through six-year-old seedling hedges rooted at 55.9% (combined) and four- through eight-year-old serially propagated clones rooted at 47.2% (combined), compared with one- through three-year-old seedling hedges (60.5% combined). Oddly, the lowest rooting percentages were seen in the five- and six-year-old serially propagated clones (42.9 and 37.3%, respectively), but rooting

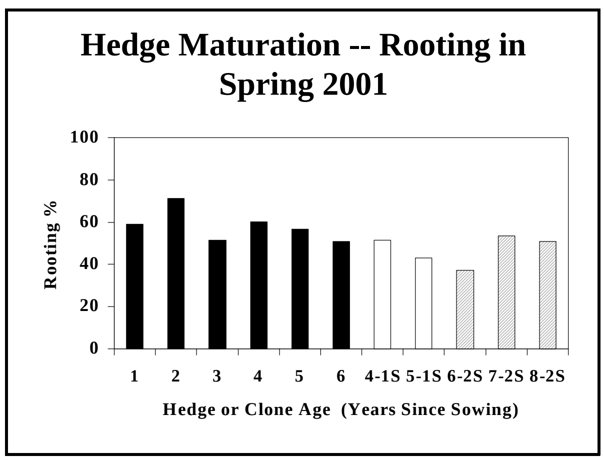

Figure 12. *Rooting of cuttings from hedges and clones of different ages in spring 2001; black bars=seedling hedges, white bars=1st-cycle serially propagated clones, hatched bars=2nd-cycle serially propagated clones.*

percentage in the seven- and eight-year-old clones (53.4 and 50.9%, respectively) was comparable to most of the other seedling hedge ages, with the exception of the two-year-old hedges (71.1%).

As in previous years, the rooting experiments do not provide a definitive answer regarding the precise degree of maturation of loblolly pine clones of different ages. In both experiments, the highest rooting percentages were seen in the youngest seedling hedges. Yet only a slight decline in rooting is seen in clones up to eight years old. Thus, it appears that a slight degree of maturation has occurred, but the operational significance of this amount of maturation is not yet clear. One factor that could influence this impact is the likelihood of finding clones or families that mature at different rates. While this study was not designed to follow individual clones over time, the three families show little interaction with age. In both experiments, and at nearly every age and treatment, family 7-1037 rooted the best, 11-1103 was intermediate, and 9-1019 performed the poorest. Besides the potential impact on rooting and rooted cutting production efficiency, a factor that will be important for determining the useful life of clones will be field performance. This coming winter, cuttings from the winter 2001 rooting experiment will be planted in eastern North Carolina on Weyerhaeuser Co. land to assess field performance. The test will include rooted cuttings from clones two through eight years old.

Clonal Multiplication Study

The clonal multiplication study that was started in 2000 is testing indoor vs. outdoor propagating environments and generating estimates of clonal multiplication rates from existing hedges. The study was begun in February 2000 with the harvest of existing cuttings from five clones and four hedges per clone. After the cutting harvest, two hedges of each clone were placed indoors under high-intensity discharge lights and a long photoperiod. The indoor hedges were kept in the greenhouse only during the cold part of the year. They were moved outside in April and returned to the greenhouse in September of 2000 and 2001. There is also a serial propagation component. After each collection and rooting, a sub-sample of the rooted cuttings was transplanted, returned to the appropriate growing environment, and pruned to produce additional cuttings. This report contains data on the number of cuttings collected and the yield of rooted cuttings through the May 2001 cutting collection. The study will be completed after the cuttings collected in February 2002 have been assessed for rooting.

In the outdoor treatment, 456 rooted cuttings per initial hedge have been obtained to date (Figure 13). This is more than the 415 obtained from the indoor treatment. However, these numbers reflect cuttings only from the original ortets and the first cycle of serial propagation. Currently, there are a large number of cuttings that were collected from those two

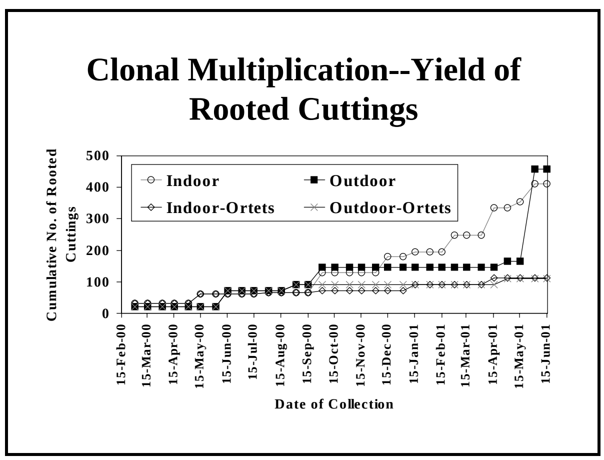

Figure 13. *Cumulative yield of rooted cuttings, including serial propagation, from indoor and outdoor-grown hedges from February 2000 to May 2001.*

sources and from the second cycle of propagation and these should have a large influence on the final totals. Because the indoor treatment yielded the first cycle of serial propagation earlier than the outdoor treatment, the number of cuttings produced by the second cycle may turn out to be larger. In addition, additional cuttings may be collected through the winter in the indoor, but not outdoor, treatment.

Serial propagation made a large contribution to the total number of rooted cuttings produced. If only the cuttings from the original ortet are counted, the indoor hedges produced a mean of 112 rooted cuttings per hedge and the outdoor hedges produced 109 cuttings (Figure 13). In addition, the rate of multiplication may increase as the cuttings from the second cycle of serial propagation becomes tallied.

Table 5. Number of rooted cuttings per hedge obtained from five loblolly pine clones between February 2000 and May 2001 in indoor and outdoor environments.

Clone	Indoor	Outdoor	Overall
A3	452	211	332
A45	226	103	165
B14	61	129	95
C66	1052	1453	1253
D22	262	391	327
Combined	411	457	434

There were large differences in the number of rooted cuttings produced among the five clones. Clone C66 yielded the most rooted cuttings per hedge with 1052 indoors and 1453 outdoors (Table 5). In contrast, clone B14 yielded the fewest cuttings (61 and 129 in indoor and outdoor treatments, respectively). These differences were largely due to rooting performance, especially during the initial rounds of rooting for serial propagation. Good or poor rooted cutting yields had a multiplicative effect on the number of cuttings that could have been harvested later on. In addition, in some cases (e.g. B14), mortality of one of the two hedges representing a clone-treatment combination reduced potential yield by 50% for all subsequent time points. Clearly, in addition to any possible treatments, clonal effects will be important for operational multiplication. Rooting success also

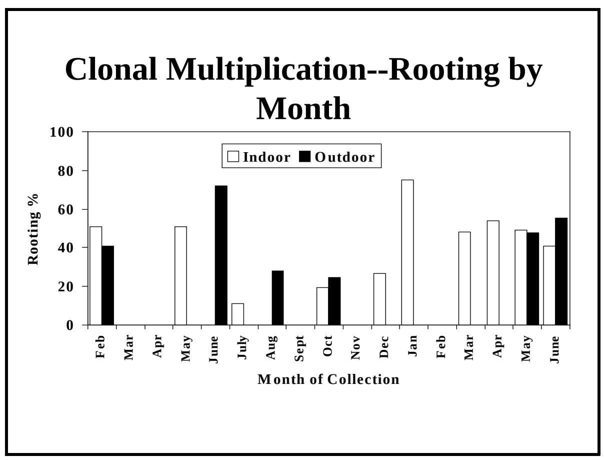

***Figure 14**. Rooting over time of cuttings from indoor and outdoor grown hedges during clonal multiplication.*

varied by season. Despite some differences, cuttings from both indoor and outdoor hedges rooted poorly in mid-summer and fall, but reasonably well from mid-winter through June (Figure 14). This study will be completed this winter and the next study is scheduled to test methods of rapidly initiating hedges from seedlings.

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 from four crosses on International Paper land near Jay, Florida in December 1998 and 282 clones from the other four crosses 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 were taken after each of the first two growing seasons. Third-season measurements will be taken this winter. After two growing seasons, survival is adequate on both sites–93% in Florida and 95% in South Carolina. Despite a slow start due to uneven stock quality, growth has also been good (Table 6). The initial variation is reflected in the relatively high Clone x Block coefficients of variation. These became considerably lower in the second year. Similarly, clone mean heritabilities were relatively low after the first season, but increased after the second season. We expect the genetic effects to continue to overtake the initial differences and the heritabilities to rise in the next couple of years.

Table 6. First and second-year results from the Clonal Selection Study.

Test Site	Florida		South Carolina	
Growing Season	First	Second	First	Second
Mean Height (ft)	1.6	5.5	1.9	6.0
Clone x Block CV (%)	34.8	24.3	34.9	25.0
Clone Mean h^2*	0.48	0.58	0.59	0.63

*Clone mean heritability calculated assuming no family structure.

Based on these preliminary results, it could be instructive to explore various clonal selection scenarios to estimate predicted genetic gain. It is important to keep in mind that this analysis does not consider the juvenile–mature correlation. On the other hand, the estimates in Table 7 are for height gains and it is reasonable to expect the gains in volume to be larger. The average breeding value for the four crosses was 18.5% above the commercial checklot (CC) for Florida and 13.9% above for South Carolina. Choosing only the best clone in each group would result in a height gain of 40.5% above the CC and 22.0% above the mean of the four familes for the Florida test and 37.8% above the CC and 23.9% above the family mean for the South Carolina test. The predicted gain decreases slightly if the best 8 clones are chosen, irrespective of family. Gain decreases further if the top two clones in each family are chosen. This is especially true for the Florida test, which contains one family with a breeding value considerable below the others. Interestingly, even at a low intensity of selection (best 25% of all clones), substantial gain could be achieved.

Table 7. Predicted genetic gain for height from several selection scenarios based on clone mean differences and clone mean heritabilities from the Clonal Selection Study.

Test Site	Florida		South Carolina	
Predicted Height Gain Compared to:	Mean of 4 Families	Commercial Checklot	Mean of 4 Families	Commercial Checklot
Best 1 Clone	22.0	40.5	23.9	37.8
Best 8 Clones	16.8	35.3	20.8	34.7
Best 2 Clones / Cross	13.9	32.4	18.9	32.8
Best 25% of All Clones	12.2	30.7	12.6	26.5
No Selection (Mean of 4 Crosses)	--	18.5	--	13.9

Wood Quality of Rooted Cuttings and Seedlings

In the Renewal Proposal of April 2000, we stated that we wished to study the wood properties of rooted cuttings and clones, but that the proposed budget did not include funding for that research. Through a collaboration with the NCSU Tree Improvement Program and a grant from USDA-IFAFS that work is now underway. Patrick Cumbie is a MS student who is sampling the 10-year-old study trees in the field tests that were planted by the NCSU Tissue Culture Project. That trial contains rooted cuttings and seedlings from the same nine full-sib families (3 x 3 factorial mating) and previous analyses indicated no differences between cuttings and seedlings for height, diameter, or volume at age six. The trial is planted on two sites, one on Rayonier land in Nassau Co., FL and the other on Joshua Management land in Monroe Co., AL. As part of the USDA grant, many wood and fiber traits will be analyzed. For this report, however, we show results for whole-core specific gravity.

There was no significant difference in mean specific gravity between rooted cuttings (0.430) and seedlings (0.427). However, there was a difference in specific gravity in the trees between the two sites. Trees at the Florida site had a mean specific gravity of 0.444, whereas in Alabama, where there was more rapid growth, the mean specific gravity was 0.411. The propagule type x site interaction for specific gravity was not statistically significant.

There were differences in the way that experimental variance was partitioned when the two propagule types were considered separately. Across both sites, the percentage of variance among families (female, male and female x male) was comparable for rooted cuttings (19.6%) and seedlings (18.0) (Table 8). However, the clonal component in the rooted cuttings explained 11.4% of the total variance. The variance percentage among clones within families was larger for each individual site (16.8% in Alabama and 35.6% in Florida), but was reduced when the two sites were combined, because of the large site effect. The presence of the clonal component resulted in a reduction of residual (within-plot) variance from 52.9% in the seedlings to 25.8% in the cuttings. A great deal of the variation occurred between sites for both types, but the replication within site (both types) and the site x clone interaction (for the rooted cuttings only) variation was minor.

Table 8. Percentages of experimental variance in whole-core wood specific gravity due to various sources in two field tests of 10-year-old, loblolly pine rooted cuttings and seedlings.

Variance Component*	Rooted Cuttings			Seedlings		
	Alabama	Florida	Combined	Alabama	Florida	Combined
Female	34.5	16.8	17.4	21.0	13.7	14.7
Male	5.5	2.1	2.2	2.6	3.0	2.6
Female x Male	0.0	0.0	0.0	0.0	1.9	0.7
Clone	16.8	35.6	11.4	--	--	--
Site	–	--	41.0	--	--	24.9
Rep (Site)	1.8	0.3	0.6	0.1	4.2	1.5
Site x Clone	–	--	1.5	--	--	--
Residual	38.2	45.2	25.8	72.9	75.3	52.9

*Replication x main effect, higher order replication x treatment, and site x family interactions were included in the full analysis, but are omitted here for brevity, because they were low or zero.

The lack of a difference in mean specific gravity between rooted cuttings and seedlings suggests that, as a reforestation alternative to seedlings, rooted cuttings would not have a negative effect on wood density. The proportions of the experimental variance found among clones suggests that deployment of clonal material shows promise for making wood properties from future plantations more uniform. In addition, using clones in progeny tests would allow more efficient selection for wood properties during breeding. Future results will provide more information on genetic parameters and uniformity of additional wood properties.

Mechanisms of Root Formation and Maturation

Screens for rooting genes--Previously, PhD student Victor Busov conducted a microarray screen of 3456 genes with RNA from auxin-treated and control hedge cuttings. Of the many genes that gave higher expression levels in auxin-treated cuttings, he selected nine for further study to determine if they were genes that could be involved in adventitious root formation (Table 9). All nine genes were tested by northern blot analysis and their induction by auxin was confirmed. To get information on their possible functions, about 600bp of the 5' region of each gene was sequenced and computerized database homology searches were performed to identify similar genes already characterized in other species and processes. We studied the timing of induction of the genes by wounding and auxin treatment, their expression in seedling roots and needles, and then compared their expression in auxin-treated cuttings from mature and juvenile grafted hedges in two separate experiments.

Of most interest for root formation are three genes whose function is tied to roots or root formation. Last year, we described a pine nodulin gene similar to one first identified in alfalfa undergoing nodulation with *Rhizobium*. A full search of gene databases revealed 38 highly similar genes from *Arabidopsis*, as well as one from rice and the one from alfalfa. Because alfalfa is the only one of these plants that forms nodules, it seems likely that the function of this gene is not restricted to nodulation. Because nodules, like lateral roots, are outgrowths of root tissues, the nodulin gene may be involved with organization and/or outgrowth of the root meristem. This is supported by the fact that the gene is induced later than some of the early auxin-induced genes and it is inhibited by the translation inhibitor, cycloheximide. Of all the genes tested, this one showed the greatest reduction in expression in mature cuttings (Table 9). Protein structure predictions suggested that this gene codes for a membrane transport protein, although it is not yet clear, which molecules are being transported. We fused the pine nodulin to a reporter gene, green fluorescent protein (GFP), and introduced the construct into tobacco leaf epidermal cells to visualize the cellular location of the nodulin protein. Confocal microscopy showed the protein located mostly in the periphery of the cell, as would be expected for a membrane protein. This supports the sequence-based prediction of membrane transport function, but further research will be necessary to determine the molecules that are being transported and the implications for root formation and development.

The second gene that may be involved in root formation shares sequence similarity with a subtilisin protease. In *Arabidopsis*, expression of this gene is repressed in a mutant (*NAC1*) that has a specific defect in the formation of lateral roots. The pine gene shows high similarity with the *Arabidopsis* subtilisin protease and it is also expressed at lower levels in mature cuttings. The third root-related gene is a proline-rich protein (PRP). Many of these cell wall protein genes have been isolated from plants undergoing developmental processes, including root formation. Our PRP is quite similar to an *Arabidopsis* gene first isolated in hypocotyls undergoing adventitious root formation. It shows only a modest reduction of expression in mature tissue, but research on juvenile and mature English ivy has shown it is the location of expression of cell wall protein genes that correlates with root formation. These three genes show promise as candidates for adventitious root formation functions and will be studied further.

Table 9. Loblolly pine genes identified as auxin-responsive by microarray screening, their putative functions based on sequence similarity, and the ratio of their expression level in auxin-treated cuttings from juvenile and mature grafted hedges.

Clone	Putative Function	Ratio of Expression Level in Auxin-Treated Juvenile:Mature Stem Cuttings		
		October	February	Combined
5ng4	Nodulin-membrane transport protein	1.53	4.27	2.90
2cd6	Caffeoyl CoA methyltransferase	1.48	1.90	1.69
6ca1	Subtilisin protease	1.53	1.40	1.46
5ng3	Methionine synthase	1.76	0.98	1.37
5ca7	Light inducible protein	1.30	1.39	1.34
9228	DNA binding protein	1.38	1.17	1.28
2cf5	Glycine hydroxymethyl transferase	1.64	0.93	1.28
7cg8	Proline-rich protein	1.27	1.13	1.20
2naa12	K+ channel	1.55	0.74	1.14
LPEA1*	Aux/IAA	0.83	1.13	0.98
LPEA2	Aux/IAA	0.58	0.72	0.65
LPEA3	Aux/IAA	1.20	0.92	1.06
LPEA4	Aux/IAA	1.29	0.76	1.02
LPEA5	Aux/IAA	1.14	1.13	1.13

*The LPEAs, identified as auxin-induced genes prior to microarray experiment, are included here for comparison.

Three other auxin-induced genes encode enzymes involved in methionine and lignin biosynthesis: glycine hydroxymethyl transferase (GHM), methionine synthase (MS) and caffeoyl-CoA-*O*-methyltransferase (CCoAM). Their induction by auxin is most likely related to lignification during vascular tissue (tracheid) differentiation–another process that is triggered by auxin. However, all three of these genes were also induced by wounding (control cuttings) and it may be that the pathways leading to vascular differentiation during root formation and wound healing are related.

The relationship of the last of the three microarray genes studied to root formation is less clear. Two of the three (DNA binding protein and K+ channel, but not light inducible protein) are more highly expressed in roots than needles of pine seedlings. The DNA binding protein inhibits photomorphogenesis (chloroplast development, etc.) in roots and etiolated seedlings of other plants. Thus, while its role in root formation is not clear, it may help to specify root tissue.

The genes identified in the microarray screening contain several promising candidates whose function may relate to root formation. With the complete sequencing of the *Arabidopsis* genome and continued mutant analysis by many laboratories, including those studying lateral root formation, the potential pathways leading to root formation are becoming much clearer. One of the most exciting developments is the identification of the NAC1 gene described above. NACs are a family of plant-specific transcriptional regulators and the NAC1 gene is root-specific. When it is mutated, the plant is deficient in lateral roots, and the phenotype is restored when the plant is transformed with a new NAC1. Moreover, when a normal plant is transformed so that it expresses NAC1 at a higher level, it increases lateral root formation without exhibiting many of the other phenotypes that are auxin-related. Thus, NAC1 seems to be a very specific controller of lateral root formation and we have begun efforts to clone the pine equivalent from roots. To date, we have only obtained a partial sequence and more work will be needed to obtain the full-length gene and ensure that it corresponds to NAC1.

<u>Regulation of auxin-induced genes</u>–One of the main differences between adventitious and lateral roots is that lateral roots are formed from divisions of predetermined root pericycle cells, while no such predetermined cells exist in stems. By studying the regulation of auxin-response genes in stems and roots, we will learn more about the mechanisms of these two types of root formation. We have been continuing the study of the promoter (regulatory) region of the pine auxin-induced gene, LPEA1. Previous experiments using deletions of the promoter indicated that removing distal portions of the promoter affected gene expression in roots, but not in hypocotyls. In addition, protein-DNA binding (gel-shift) experiments showed differences in the specific DNA binding affinities between proteins from hypocotyls and roots. The results of these experiments indicated that there were tissue-specific elements related to the expression of LPEA1 during treatments that led to rooting and that these elements were located in the distal region of the promoter.

Additional gel-shift experiments by Carmen Lanz-Garcia have further defined the operation of the LPEA1 promoter. We found a region within the distal 800 nucleotides of the promoter that binds proteins from both roots and hypocotyls, but is more specific to roots. The absence of this region vastly decreases gene expression in roots, but has no effect on expression in hypocotyls. While further experiments are underway to identify and characterize the smaller regulatory elements within this region, clues have been obtained from sequence analysis. This portion of the promoter shows a long “AT-rich” region. This is a region that contains an unusually high number of the nucleotides adenine and thymine. Stretches of DNA that are AT-rich are somewhat flexible, due to a weaker hydrogen bonding pattern. This flexibility, in itself, can serve as a mechanism of gene regulation by changing the conformation of the DNA strand, affecting access or positioning of proteins bound to the DNA. Another possible mechanism of gene regulation in an AT-rich region is control of expression by the AT-hook (HMGA) proteins from the High Mobility Group family of proteins. HMGA proteins bind to DNA and chromatin at certain functional motifs and act to modify the architecture of the DNA. In other systems, they are known to be tissue specific and to function in the perception of environmental and/or stress signals. We hope to determine if they are functioning to regulate genes during root formation.

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PUBLICATIONS OF INTEREST TO THE MEMBERS

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