NORTH CAROLINA STATE UNIVERSITY
LOBLOLLY AND SLASH PINE ROOTED CUTTING PROGRAM

Progress Report, October 1999

EXECUTIVE SUMMARY

The NCSU Loblolly and Slash Pine Rooted Cutting Program is an industry-sponsored research cooperative with a mission to conduct research and technology transfer in support of the operational use of rooted cuttings for the southern pines by its members. The program was formed in 1992 and renewed in 1996 for an additional five years. 2000 will be the last year in the current research phase and program staff and members have begun to identify research priorities for the next phase.

Since 1992, the program has conducted research into many facets of rooting and production of cuttings. Efforts in these areas have yielded a great deal of progress. In fact, loblolly and slash pines can no longer be considered "difficult-to-root" species. This report contains summaries of research progress in the last year. Emphasis has been on research to make production systems more efficient and cost-effective, and on developing the information necessary to implement clonal forestry.

One production efficiency study examined the hedging procedures necessary for operational production and measured cutting morphology to develop criteria for "target cuttings." Another study tested rooting and cutting growth in peat pellets and concluded that small pellets might be useful in a mechanized transplant system. We are also studying the importance of soil or medium water potential in rooting, as part of our ongoing effort to quantify the "art of rooting" and, therefore, assist cooperators to efficiently design rooting facilities and implement production procedures.

Efforts in support of clonal forestry include one study that is testing methods for rapid multiplication of clonal hedges once the superior clones have been identified in field trials. A second study is designed to provide quantitative estimates on the most efficient methods for selecting clones from populations, designing clonal field trials, and managing the clonal hedge populations. A third study continues our long-term effort to quantify maturation or juvenility over time in hedges derived from seedlings and those that have been serially propagated. A fourth study reports that there is no strong relationship between the rooting ability and growth rate of slash pine clones, so culling some clones on the basis of propagation ability will not bias selection for growth rate. The fifth area of study is investigating the fundamental mechanisms of root formation, auxin action and maturation. We are gaining insight into these processes that, ultimately, could lead to directed, rather than empirical, approaches to managing maturation.

We anticipate that in the near future, rooted cuttings will be an important tool for maximizing productivity in plantations in the southeastern U.S. Operational use of rooted cuttings to deploy outstanding full-sib seed from controlled crosses depends on continued development of efficient and cost-effective production systems. Using rooted cuttings to mass produce elite clones is now within our grasp and research continues to provide the necessary supporting information. Many of our members are increasing their internal rooted cutting efforts, with some approaching pilot-scale or operational levels.

INTRODUCTION

It has been a productive year for the program. Research progress has been made in a number of areas of interest to the members. We have completed the reorganization of research projects into two overall areas: (1) improving the efficiency of production systems to make rooted-cutting technology more reliable and cost-effective and (2) developing information necessary to implement clonal forestry using rooted cuttings. This report reflects the new organization and provides updates on a number of individual research projects in both areas.

The membership in the program continues to undergo changes. We began the year by welcoming two new companies–Gulf States Paper Corp. and Mead Coated Board–who joined the program effective January 1, 1999. During the course of the year, however, we learned that we will be losing two of our current members for 2000. Union Camp Corporation was acquired by International Paper Company and Smurfit-Stone Container Corporation recently sold its land base and has informed the program staff that they will not be able to continue membership in 2000. The restructuring of the forest products industry continues to be a challenge for the program, as we attempt to maintain stable financial support that will allow planning and execution of high-quality research.

1999 is the fourth year in the current five-year research phase. We are proud of our accomplishments so far. During the coming year we intend to submit a new proposal for the third phase of the program. We have begun consultations with the current members on research priorities. The current research focus on providing information on efficient production and clonal forestry will likely remain key elements in the new proposal. Plans for specific projects within these objectives are being developed. A renewal meeting is being planned for Spring 2000.

A number of personnel changes have occurred in the program since our last report. Carmen Lanz-Garcia has received a promotion from the university from Research Technician III to Research Analyst, a reflection of her outstanding work for the program. Anthony LeBude, a Research Assistant and PhD student, is capably replacing Scott Surles, who left the program to enter private business. Ramesh Murthy, a post-doctoral Research Associate, has taken a full-time research position with Columbia University. Three graduate students, Bernadette Cooney, Rania Masri, and Victor Busov, have made substantial contributions to the program and are making excellent progress toward their degrees.

Following is a series of research summaries on individual projects. Included are updates and new results since the last report in February 1998, with particular emphasis on results and progress realized in 1999.

RESEARCH FOR OPERATIONAL PRODUCTION

Shearing Height, Pruning Intensity, Shoot Origin, Shoot Morphology and Rooting

This research was conducted by Bernadette Cooney and sought to determine the specific hedging techniques that promote rooting at high percentages. Precise hedging treatments and documentation of cutting origin were employed to provide guidance on efficient hedge management techniques. All the hedging treatments tested fall within the definition of "intensively managed hedges." In addition, the effects of the hedging treatments on various cutting morphology traits were determined and the correlations between rooting and cutting morphology were tested to develop criteria for "target cuttings."

Treatments conducted on twenty hedges from each of three full-sib families consisted of two shearing heights, two pruning intensities (removal of all existing buds on the hedge or retention of a portion of existing buds on the hedge), and two cutting origins (newly formed cuttings became visible only after pruning and pre-formed cuttings were present prior to pruning). The cuttings resulting from the hedging treatments were collected and the following morphological traits were assessed: fascicle needle length, primary needle length, presence of cataphylls (sterile scales), shoot shear strength, and shoot basal caliper (diameter). The cuttings were then stuck in a rooting trial that was a randomized complete block design (10 cuttings per treatment combination per block with five blocks, total of 900 cuttings). The experiment was repeated twice, once in the spring of 1997 and the other in the spring of 1998.

Rooting percentage across all treatments in 1997 was 62.2% and this increased markedly in 1998 to 82.8%. The increase in rooting from 1997 to 1998 may have been due to root pruning treatments conducted on the hedges in 1997 or the increase in spacing between cuttings and reduction in sticking depth in 1998. Rooting percentage of each family increased in 1998, although there were rank changes among families between the two years (Table 1). The lower hedge height in 1997 and the newly formed cuttings in 1998 gave slightly higher rooting percentages than the comparison treatments (9.2% and 7.3%, respectively), although the trends were not consistent between years.

Cutting morphology was affected by the hedging treatments. In both years, hedges that had been sheared at the lower height, and the hedges with all the buds removed, produced cuttings with shorter fascicles, longer primary needles, a lower incidence of cataphylls, and lower shear strengths (Table 2). The same trends were observed for newly formed cuttings as compared to cuttings from pre-formed buds. The effects of the treatments on cutting caliper varied by year. In 1997, newly formed cuttings and those from the lower hedge height and more severe pruning intensity had larger diameters, while the opposite trend was seen in 1998. Thus, overall, the lower hedge height and more intensive pruning treatments yielded cuttings with a slightly more juvenile appearance, although this did not translate into large differences in rooting. Overall, the cuttings in 1998 had shorter primary needles and fascicles, fewer cataphylls, and smaller diameters. This suggests they may have been taken at an earlier stage of development and this could have also contributed to the

increased rooting percentage in 1998.

Table 1. Effects of family, hedge height, pruning intensity and shoot origin on rooting percentage in loblolly pine cuttings in 1997 and 1998.

Rooting %	Family			Hedge Height[1]		Pruning Intensity		Shoot Origin	
	A	B	C	Lower	Higher	Buds removed	Buds intact	Newly formed	Pre-formed
1997	48.4c[2]	63.4b	74.6a	66.8a	57.6b	66.1	60.2	60.0	60.6
1998	77.7b	88.5a	82.2b	84.6	81.1	82.8	82.9	86.4a	79.1b

[1]Hedge heights were 18 and 23 cm in 1997 and 15 and 26 cm in 1998.
[2]Means within a treatment and year with the same letters are not significantly different ($p \geq 0.05$)

Table 2. Effects of family, hedge height, pruning intensity and shoot origin on loblolly pine cutting morphology in 1997 and 1998.

Trait	Family			Hedge Height[1]		Pruning Intensity		Shoot Origin	
	A	B	C	Lower	Higher	Buds removed	Buds intact	Newly formed	Pre-formed
Primary needle length(cm)-1997	3.15a[2]	2.57b	2.59b	3.14a	2.40b	3.04a	2.63b	2.69a	2.55b
1998	2.56a	2.10b	1.79c	2.46a	1.86b	2.39a	2.04b	2.31a	1.76b
Fascicle length(cm)-1997	7.43a	6.70b	7.18a	6.68b	7.51b	6.72b	7.30a	6.59b	8.05a
1998	6.06b	5.36c	6.43a	5.56b	6.34a	5.51b	6.17a	5.59b	6.79a
Presence of cataphylls (%)-1997	38.0	43.0	41.0	34.3b	47.1a	8.0b	57.6a	25.3b	91.8a
1998	27.0b	30.0b	46.0a	23.8b	44.7a	22.5b	40.2a	20.7b	60.6a
Diameter (mm)-1997	2.77	2.76	2.68	2.84a	2.64b	2.89a	2.66b	2.71a	2.60b
1998	2.46a	2.31b	2.31b	2.31b	2.41a	2.44a	2.32b	2.25b	2.39a
Shear strength (MPa)-1997	3.96a	3.54b	4.02a	3.26b	4.40a	3.37b	4.08a	3.72b	4.45a
1998	6.06	6.73	6.37	6.19b	6.58a	5.36b	6.90a	6.65	7.14

[1]Hedge heights were 18 and 23 cm in 1997 and 15 and 26 cm in 1998.
[2]Means within a treatment and year with the same letters are not significantly different ($p \geq 0.05$).

The correlations of rooting with shoot morphology were not consistent between years. In 1997, rooting percentage was positively correlated to cutting diameter, but in 1998, the correlation was negative. This indicates that diameter (within the ranges tested), by itself, may not be the most important determinant in rooting. The same hedging treatments (lower hedge height and disbudded hedges) gave cuttings with large diameters in 1997 and small diameters in 1998 and these cuttings tended to root at higher percentages. Rooting percentage was negatively correlated with shoot shear strength in 1997 (more succulent cuttings rooted at higher percentages) (Figure 1), but there was not a significant correlation in 1998. Across all treatments, diameters were larger and shear strengths were lower in 1997. Thus, shear strength may be an important factor for rooting for some stages and sizes of cuttings, but not for others. In 1998, but not in 1997, primary needle length was positively correlated to percent rooted, however, the correlation was weak.

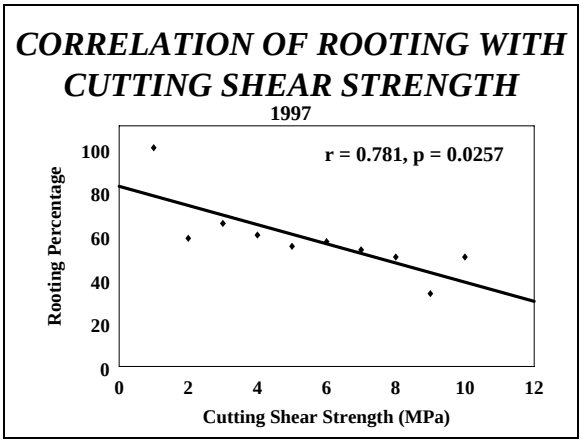

Figure 1. Correlation of rooting ability with cutting shear strength for loblolly pine cuttings in 1997.

While intensive hedge management is important for good rooting success, this research suggests that very intensive pruning procedures, such as removing all pre-formed buds, are not critical. Moreover, cuttings with specific morphological traits (within the ranges tested) do not need to be chosen from among those produced on intensively managed hedges. Both of these results are important for operational-scale rooted cutting programs, as very labor-intensive pruning and sorting would decrease cost-effectiveness.

Rooting and Rooted Cutting Development in Peat Pellets

In January and June of 1998, Anthony LeBude conducted two experiments to determine the effects of different sizes of Jiffy Forestry Pellets™ on rooting and subsequent rooted cutting development of loblolly pine. These experiments were partially funded by a research grant from Jiffy Products LTD. Three full-sib families were arranged in seven (January) or six (June) sizes of pellets. Ray-Leach Super Cells™ filled with 60% perlite and 40% peat served as the control. Pellet sizes used were 24-65 mm, 30-65 mm, 36-65 mm, 36-75 mm (January only), 42-65 mm, 42-75 mm, and 50-95 mm (dry diameters and expanded heights, respectively). The experiment was a split-plot design, with pellet size as the main plot and family as the sub-plot. There were ten cuttings per family per pellet size and eight replications. After twelve weeks in the propagation greenhouse, cuttings were scored for rooting percentage and rooted cuttings were scored for shoot height, shoot diameter, shoot dry weight, number of roots, symmetry of the root system, primary root dry weight, and secondary (and higher order) root dry weight. From the weight measurements, total root dry weight, the ratio of primary to secondary root dry weight, and the ratio of shoot to total root dry weight were calculated. In the January experiment, all the rooted cuttings were destructively

sampled for the weight measurements. In the June experiment, half the rooted cuttings in each plot were destructively sampled, while the other half were reserved for a field test, after nondestructive measurements were taken.

In the January experiment, pellet size 30-65 mm, had a higher rooting percentage (93%) than the control (83%) (Figure 2). The 24-65 mm and 36-75 mm pellets had rooting percentages (80% and 85%, respectively) that did not differ significantly from the control. The 36-65 mm and the larger pellets (42-65 mm, 42-75 mm and 50-95 mm), resulted in lower rooting percentages (74%, 73%, 35% and 57%, respectively) than the control. Total root dry weight was the morphological measurement that showed the greatest treatment response. Total root dry weights for all pellet sizes were less than the control (Figure 3). There was no clear relationship between total root dry weight and pellet size.

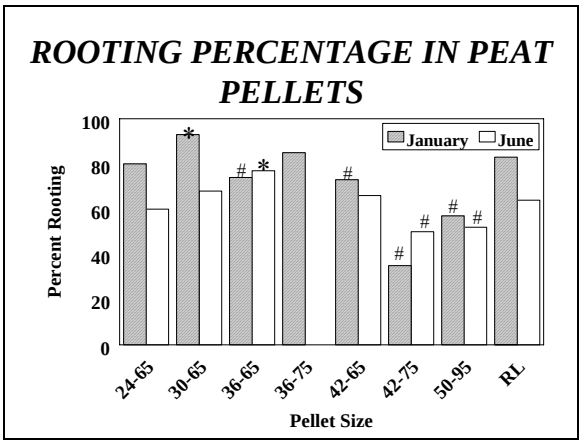

*Figure 2. Effect of peat pellet size on rooting percentage of loblolly pine cuttings in January and June. * = significantly greater than control for that season. # = significantly less than control for that season.*

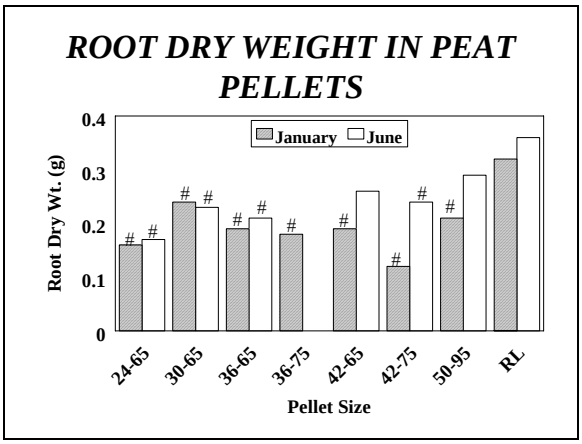

Figure 3. Effect of peat pellet size on total root dry weight of loblolly pine cuttings in January and June. # = significantly less than control for that season.

In the June experiment, rooting percentages were somewhat lower across all treatments. Rooting percentage for pellet size 36-65 mm (77%) was higher than the control (64%), whereas pellet sizes 42-75 mm (50%) and 50-95 mm (52%) were lower than the control (Figure 2). The 24-65 mm, 30-65 mm, and 42-65 mm pellets had rooting percentages (60%, 68% and 66%, respectively) that did not differ significantly from the control. There was a slight trend of greater total root dry weights in the larger pellets in the June experiment. Total root dry weights of cuttings rooted in pellet sizes 24-65, 30-65, 36-65, and 42-75 mm were lower than the control (Figure 3). Cuttings rooted in the 42-65 mm and 50-95 mm pellets produced total root dry weights that did not differ significantly from the control.

In assessing the biological significance of total root dry weight, it is important to note that twelve weeks from sticking is not the typical culture time for a cutting to become ready for field planting. Subsequent culture of the cuttings in the pellets (and in the control) in a non-misted environment may have increased the root dry weights to more acceptable levels. However, in our experience, longer culture of the rooted cuttings in the pellets could be problematic, due to the tendency of roots to grow into adjacent pellets. This tendency was most dramatic in the smaller

pellets. At a minimum, periodic root pruning would be necessary. In both experiments, cuttings in the smaller pellet sizes rooted well, but had lower total root dry weights than the control. Conversely, cuttings in the larger pellet sizes rooted poorly, yet had total root dry weights that were comparable to the control (at least, in the June experiment). A possible solution could involve rooting cuttings in pellets (or other small containers) for a brief period and then transplanting, either to another container or to a nursery bed.

The field test using rooted cuttings from the June experiment was established in Rayonier's Glenville Nursery in eastern Georgia in December 1998. It contains approximately 400 rooted cuttings planted at close spacing (1 ft x 1 ft). This fall, the cuttings will be lifted and measured to determine the effect of pellet size and cutting development on first-year field survival and growth.

Media Water Potential and Rooting

One explanation for the poor rooting of cuttings in the larger sizes of Jiffy pellets may have been the high level of moisture in the pellets. The cuttings in larger pellets were at wider spacing and, thus, less water was intercepted by the cutting foliage and more fell directly on the media. This illustrates the need for a better understanding of the importance of media moisture on rooting and subsequent root growth. Particularly when many are experimenting with different rooting environments (greenhouses, shadehouses, nursery beds) for operational production systems, a better understanding of the effect of media moisture would aid efforts in rooting facility design and maintenance of environmental conditions during and after rooting. The amount of moisture in media will vary with irrigation level, evaporation rate and media/soil physical properties. Media water potential is the measure of media/soil matric potential or the force necessary for plants to obtain available water from the surrounding media. Surprisingly, there is scant information for any plant species which directly and rigorously tests the effect of media moisture on propagation using stem cuttings.

In February 1999, Anthony LeBude began a series of experiments to study the effects of media moisture. The experiments are utilizing an equipment upgrade in the propagation house, in which electronic soil tensiometers (irrometers) were installed. The irrometers continuously measure the media water potential and send the information to the greenhouse computer via the Q-Com GEM3 software. In addition, the mist boom can be triggered by the media water potential readings. The first experiment sought to understand the basic relationships between mist level and different media. Five media, consisting of different ratios of peat:perlite (v/v): 0:100, 25:75, 50:50, 75:25, and 100:0 were placed under two different mist levels. The media were placed in square plastic tubs (15.75 in x 15.75 in x 6 in deep) with 60 cuttings per tub. There were 8 blocks of each medium in each mist treatment. In the high mist level, every time the media water potential in the 50:50 mixture became lower (more negative) than -3.0 kPa, the cuttings and media were misted, with the boom travelling at a speed that delivered 0.0047 gal/ft^2 (230 ml/m^2). The low mist treatment was triggered by the same irrometer probe and, therefore, occurred at the same frequency, except that the boom delivered 0.0030 gal/ft^2 (147 ml/m^2). In addition to triggering the boom, the irrometers were used to record the media moisture in each plot once per week. The stem (xylem) water potential of the cuttings was measured using a pressure bomb at the same time.

The media water potential differed between mist treatments across all media. Both mist treatments resulted in media water potentials and/or aerial mist applications that were too negative to support cutting survival. This occurred, despite the maintenance of media water potential in the 50:50 mix in one of the blocks at -3.0 kPa. There was a large amount of variation among blocks. Thus, while the triggering block of 50:50 mix was maintained at -3.0 kPa in the higher mist treatment, the mean of the 50:50 mixtures across all eight blocks was -6.7 kPa in the same mist treatment. Despite this variation, relationships of the different media to the mist treatments were apparent. Surprisingly, 100% perlite had the least negative water potential in both mist treatments (Figure 4). We expected that this medium, thought to have excellent drainage properties, would have had the most negative potential. Apparently, at these relatively moist levels of media moisture, there is always some free water and the water that is present is easily removed from the medium by the probe (and, by inference, by the cutting). The three mixtures were relatively similar to each other, but the 100% peat showed an interaction with mist level. In the higher mist treatment, peat had a less negative potential than the mixtures. In contrast, in the lower mist treatment, peat had a more negative potential than the mixtures. These observations are consistent with peat's reputation as a medium prone to both excessive wetness and dryness.

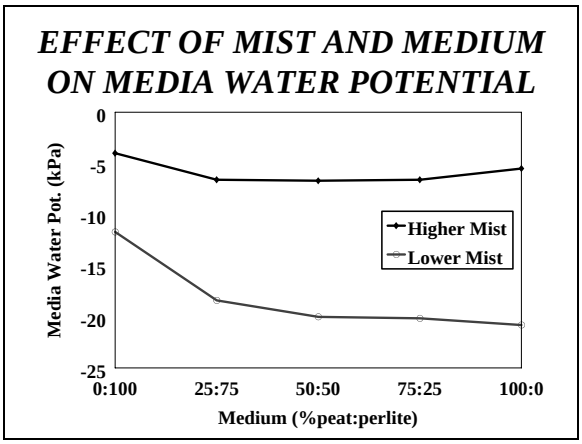

Figure 4. Effect of mist level and medium composition on media water potential.

During the first week after sticking, cutting water potential was strongly influenced by medium water potential (Figure 5). Regression analysis indicated that cutting water potential was related to media water potential and the square root of media water potential ($\psi_C = 0.71 - 0.07\ \psi_M + 0.70\ \psi_M^{½}$; $R^2 = 0.539$, $p = 0.0001$). The equation depicts a response in which cutting water potential becomes more negative rapidly at slightly negative media moisture levels and then continues to become more negative gradually, as media moisture decreases. The insufficient media moisture and/or mist levels prevented the documentation of this relationship past a few weeks into the rooting period and also did not allow for the correlation of rooting percentage with the cutting and medium water potentials. The information gained from this trial did, however, assist in the design of the second in this series of experiments.

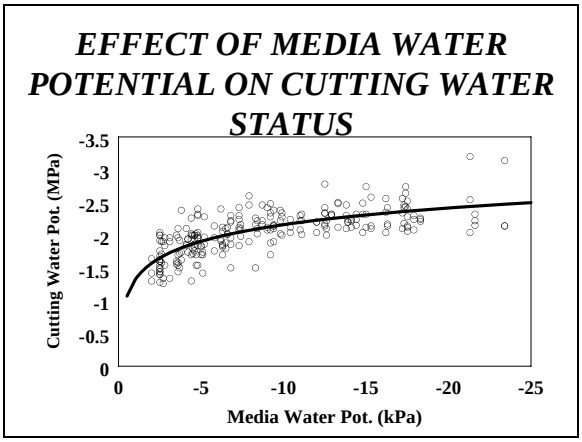

Figure 5. Regression of loblolly pine cutting water potential and media water potential one week after sticking.

The second experiment was begun in June 1999. It utilized the same five media to generate a range in media water potentials. There were three levels of mist. The first was a "wet" treatment in which the boom was triggered whenever the medium water potential (in the 75:25 mixture of one block) became more negative than -1.3 kPa.. The second mist level was a "dry" treatment, in which the boom was triggered whenever the medium water potential (in the 75:25 mixture of one block) became more negative than -2.3 kPa.. The third level was our standard mist regime, in which the boom is triggered at a variable frequency depending on the relative humidity in the greenhouse and the time of day. Thus, the media water potential was free to fluctuate. In addition, cuttings were stuck into the media at two depths, 2 and 4 cm. Media and cutting water potentials were measured at the two depths in each plot once per week.

After 12 weeks, remaining cuttings were scored for rooting percentage, number of roots, root system symmetry, root dry weight and shoot dry weight. Preliminary analyses of the results will be presented at the annual meeting. Future experiments will continue to explore the importance of soil/medium water potential and, if resources permit, will extend these experiments to outdoor rooting environments.

RESEARCH FOR CLONAL FORESTRY

Clonal Multiplication Study

The objective of this study is to test different methods for multiplication of clonal lines. Under most scenarios, clones with superior traits will be selected from field tests. Then, selected clones would be multiplied to achieve a sufficient hedge population for meaningful levels of operational deployment. An unmanageable number of hedges could arise if all clones from a field test are multiplied continually. Alternatively, if no multiplication is done until field results are available, a significant delay could result until individual, superior clones can be multiplied to sufficient levels. Ideally, clones could be maintained with a limited number of hedges until field results are meaningful and then rapidly multiplied for deployment.

The experiment is a two by two factorial, with two growing environments (the standard outdoor environment and an indoor environment with supplemental light) and two methods of cutting collection (the standard periodic collection when the majority of cuttings have reached a minimum size of 7 cm and a continual collection where all cuttings 7 cm in size are collected on a weekly basis). There are five clones in the study, with two ramets (hedges) of each clone in each of the four treatments. All hedges were derived from cuttings that were stuck in May, 1997 and had been pruned twice prior to the study. For each treatment and collection date, the number of harvested cuttings were recorded. All the cuttings were also stuck in the propagation greenhouse to determine the total yield of rooted cuttings per treatment and clone. Cutting collection began on February 26, 1999, when cuttings from all treatments were collected and stuck. At that time, all hedges received a standard pruning and the appropriate hedges were moved to an indoor greenhouse.

There appeared to be little or no advantage to continual, rather than periodic, cutting collection in either the indoor or the outdoor treatments (Figures 6 and 7). However, growing hedges in the two environments affected the timing of cutting production. Indoor hedges began flushing new shoots earlier than outdoor hedges. The first cuttings in the indoor-continual treatment were collected on April 9 (Figure 6). The first indoor-periodic cutting collection was on April 30 (Figure 7). The outdoor-continual and outdoor-periodic collections began on May 21 and May 28, respectively. By the last collection date currently being reported (September 10), the differences among treatments had become very small. The indoor-continual treatment yielded a total of 1138 cuttings, the indoor-periodic treatment yielded 1059 cuttings, the outdoor-continual treatment yielded 1187 cuttings, and the outdoor-periodic treatment yielded 1175 cuttings (all clones and ramets for a treatment combined). Thus, the earlier onset of collection in the indoor treatments was balanced, and even surpassed, by the greater number of cuttings produced in the outdoor treatments. This occurred, even in the periodic treatments, despite the fact that through the current time, three periodic collections were made from the indoor hedges and only two from the outdoor hedges. Of course, additional differences in cutting production between indoor and outdoor treatments are expected to show up this coming winter, when the outdoor hedges cease growth and the indoor hedges are expected to continue growth. Total cutting production (in the six-month period so far) from the original hedges is not the only important factor determining overall multiplication rate. Because cuttings were collected earlier in the indoor treatment, it is possible that the rooted cuttings from the early collections could be turned into a new round of hedges, yielding additional cuttings at an earlier time than the rooted cuttings from the outdoor treatment. This argument could also apply, to a lesser extent, to the continual, rather than periodic cutting collection treatment.

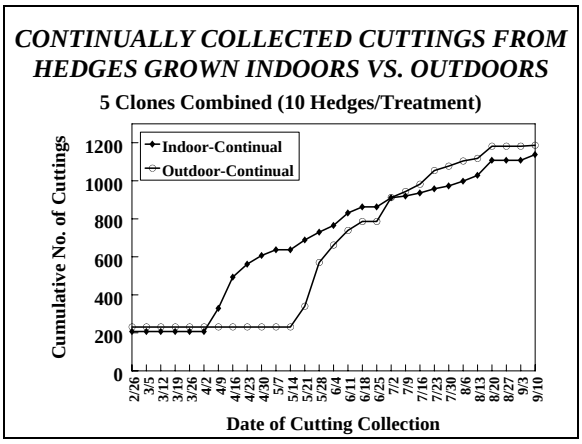

Figure 6. Cutting production from hedges grown indoors or outdoors. Cuttings were collected weekly when they reached a minimum size of 7 cm.

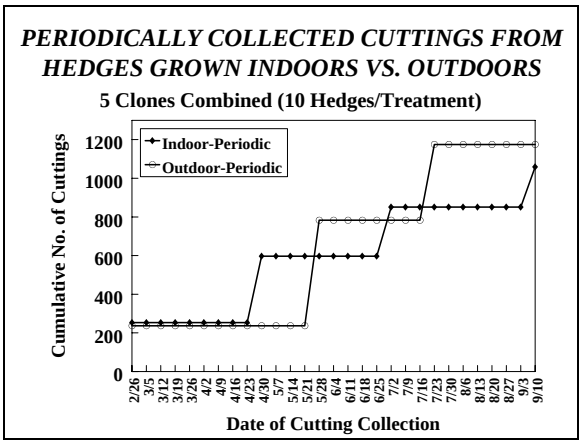

Figure 7. Cutting production from hedges grown indoors or outdoors. Cuttings were collected periodically when most reached a minimum size of 7 cm.

The greater rate of cutting production in the outdoor treatments may be related to the lower light intensity in the indoor treatments. If the overall vigor was reduced in the indoor treatments, we may also see lower rooting percentages in the indoor treatment. Rooting percentage data,

combined with cutting production information, will give us the total multiplication rate from the original hedges. One potentially useful treatment that could be tested in future iterations of this study is to start hedges indoors to take advantage of early collection, move them outdoors in early spring to take advantage of full light intensity, and then move them back indoors in the fall to prevent growth cessation. Within the current experimental design, as well as any new indoor/outdoor treatments, it will be important to determine whether hedges prevented from going through growth cessation maintain their cutting production levels and overall vigor. Another parameter worth investigating in more detail is the effect of the treatments on further rounds of serial multiplication. In the future, we may test fewer treatments, but place more emphasis on the timely transplant and hedging of rooted cuttings and the yield of cuttings from the serially propagated hedges.

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. In January 1998, 15 cuttings from each clone were stuck for rooting (single-cutting plots, 15 blocks, one cutting per clone per block). Overall rooting was estimated at 75-80%, however, difficulties in record keeping and sorting the rooted cuttings caused a reduction in the number of clones planted in the field tests. The crosses were divided geographically for the field sets. Four of the crosses were from Florida loblolly pine sources and were planted on Champion International land near Jay, FL in December 1998. The other four crosses were of Georgia and South Carolina Atlantic Coastal Plain origin and were planted on Westvaco land in South Carolina in November 1998. There are 450 clones in the field tests (Table 3). The experimental design was a randomized complete block, with 9 blocks and one ramet per clone per block.

The original plan called for culling 25% of the clones (25 clones) in each cross on the basis of rooting ability. A further culling of 25% was planned for Fall 1999 on the basis of first-year field results. Because the number of clones is smaller than planned, no culling will be conducted this year. Twenty-one cuttings (7 blocks, 3 cuttings per clone per block) from each clone that is in the field test were stuck in February 1999. This should begin to provide accurate rooting data for the clones in the field tests. Six rooted cuttings of each clone will be transplanted this winter to begin the clonal bulk-up process. Once superior clones have been selected, there will be opportunities to use them for other clonal field tests. Information on growth and yield dynamics for clonal blocks,

realized genetic gain, genotype x environment interactions for clones, and the degree of uniformity of wood properties in clonal stands could prove critical to the implementation of clonal forestry.

Table 3. Number of clones and location of clonal selection study field tests.

	Number of Clones per Full-Sib Cross								
Location	A	C	D	E	F	H	I	K	Total
FL	37	55	32	44	--	--	--	--	168
SC	--	--	--	--	69	67	66	80	282

Hedge Maturation Study

Our ongoing hedge maturation study is providing data critical to the utilization of rooted cuttings for producing planting stock for reforestation. For full-sib multiplication, these experiments will provide guidance on the expected longevity of a particular hedge crop. More important are the implications for clonal forestry. The identification and multiplication of superior clones will require some period of time (see clonal selection study) and the rooted cutting strategy will only be viable if hedging and/or serial propagation treatments delay maturation sufficiently to maintain rooting ability and field performance of the rooted cuttings.

This study was begun in the Spring of 1993 and is testing the juvenility of hedges derived from seedlings and rooted cuttings (serially propagated hedges) from three open-pollinated loblolly pine families (16-20 clones per family per age) over time. At the time of this report, the experimental population now includes seedling hedges two through six years-old, although substantial mortality has occurred in the five and six-year-old seedling hedges. In addition, serially propagated hedges have been produced from clones started three through six years ago. A second round of serial propagation has been conducted on clones started five and six years ago. The experiments in 1999 were conducted on seedling hedges started two through five years ago (insufficient hedges for the six-year-old seedling hedges) and on serially propagated hedges from clones started four through six years ago (first round of serial propagation).

Cuttings from all the hedges are being tested for cutting morphology traits and rooting ability. In Spring 1998, a field test was established using rooted cuttings from seedling hedges that had been started two through four years before and serially propagated hedges (1 cycle) from the clones started four years before. That field test is nearing the end of its second growing season. First-year height measurements indicated no age or hedge origin differences at that time.

Cutting morphology and rooting experiments were conducted in Winter and Spring 1999. Rooting percentages were poor overall in both tests (Figure 8), preventing definitive conclusions about the age and hedge origin effects. The largest decrease in rooting percentage occurred between two- and three- year-old seedling hedges. Other experience tells us this is probably not due to true maturation, as high rooting percentages are routinely obtained in hedges at least four years-old. There appeared to be a slight trend of declining rooting percentages with age in the seedling hedges, with the poorest rooting in the five-year-old hedges. This may be due more to the physiological condition of the older hedges than irreversible maturation, as shown by the rooting in all the age classes of serially propagated hedges. These cuttings rooted at comparable percentages to the three- and four-year-old seedling hedges. Intensive efforts are underway to improve hedge management practices and control of environmental conditions during rooting to ensure that future rooting trials have improved overall rooting percentages and, therefore, give a more realistic indication of juvenility or maturation.

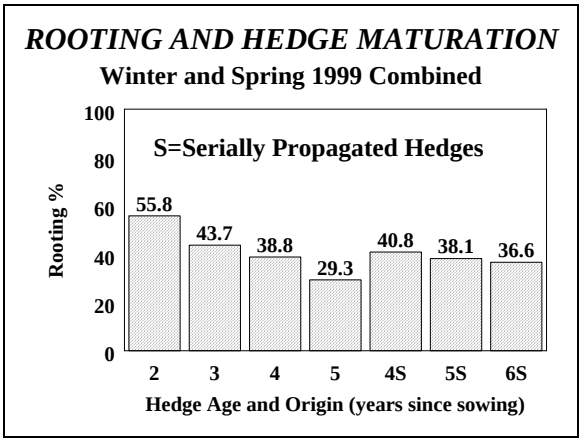

Figure 8. Effect of hedge age and origin (seedling or serially propagated hedges) on rooting percentage in Winter and Spring 1999 (combined).

Table 4. Morphological traits of cuttings from various ages of seedling and serially propagated hedges.

Hedge Age and Origin	Primary Needle Length (cm)	Fascicle Length (cm)	Diameter (mm)	Number of Primary Needles
2-yr-old seedling	2.0[1]	5.8	2.1	34.4
3-yr-old seedling	1.9	6.1	2.1	34.1
4-yr-old seedling	1.8	5.5	2.1	31.3
5-yr-old seedling	1.9	5.6	2.0	31.8
4-yr-old serial	1.8	5.5	2.1	31.3
5-yr-old serial	1.9	5.7	2.0	32.4
6-yr-old serial	1.7	5.4	1.9	25.6

[1]There were no significant differences ($p \geq 0.05$) among ages within seedling or serially propagated hedges for any trait.

Measurements on cutting morphology were also conducted in Winter and Spring. While Spring data are currently being analyzed, Winter measurements showed no clear trends in cutting morphology with age for either the seedling or serially propagated hedges. The largest changes were observed in the diameter and number of primary needles in the six-year-old serially propagated

hedges (Table 4). These differences were not statistically significant, but because they were seen in the oldest material in the study, they bear watching in the future to determine if they indicate the beginning of maturation. The frequency of cataphylls (sterile scales), another potential indicator of maturation, was so low in all the treatments that valid comparisons can not be made.

Relationship of Growth and Rooting for Clones of Slash Pine

We reported previously that growth rate is not correlated with rooting ability for open-pollinated families of both loblolly and slash pines. In March 1997, we established a field test to test the correlation of growth rate with rooting ability for clones of slash pine. Six open-pollinated slash pine families were chosen, as previously reported, for the within-family rooting tests. Rooting data were gathered in Spring, 1995 and 1996 (6 cuttings/clone/replication x 4 replications for each trial = 48 total cuttings per clone). From the Spring 1996 rooting, 10 ramets (rooted cuttings) from 65 randomly chosen clones were planted near Booker, FL on land belonging to Smurfit-Stone Container. The field test consisted of five replications with two rooted cuttings per clone per replication. Survival and total shoot height were measured after the 1997 and 1998 growing seasons. The height increment between the first and second field seasons was also calculated.

Overall survival of the test after the second growing season was 93.5%. Most of the mortality occurred in the first year. Overall height was 1.03 ft. after the first growing season and 2.78 ft. after the second season. Neither first-year height, second-year height, nor the growth increment during the second year was correlated with rooting ability ($p=0.2511$, $R^2=0.0205$; $p=0.7177$, $R^2=0.0021$; $p=0.5205$, $R^2=0.0065$; respectively) (Figure 9). These results indicate that culling clones on the basis of rooting ability should not bias selection for growth rate. Alternatively, clones chosen for rapid growth would have the same probability of having acceptable rooting as any other clones.

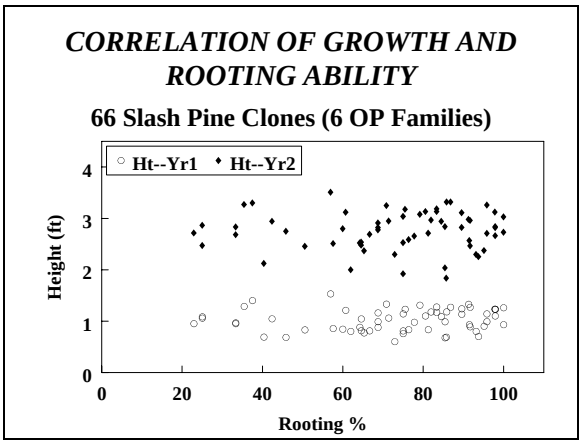

Figure 9. Correlation of first- and second-year height of slash pine clones with rooting percentage.

Gene Expression and Adventitious Root Initiation

This research seeks to understand the molecular and biochemical steps in rooting and how they are affected by maturation. Previously, we cloned 5 genes from loblolly pine (*LPEA*s: 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*s genes to test the working hypothesis that they are directly involved in the process of adventitious root formation.

Carmen Lanz-Garcia has completed the identification, cloning and sequence analysis of a complete genomic clone (the DNA containing the gene and surrounding sequences) corresponding to *LPEA1*. Work on cloning and sequencing the genomic clones corresponding to the other four *LPEA*s is ongoing. Since genomic clones contain promoters and other control elements that regulate gene expression, sequence analysis of all the *LPEA* genomic clones will allow us to make comparisons among the *LPEA*s and with genes from other plants. The *LPEA1* clone reveals that it has three introns (interruptions in the gene coding sequence) that occur at conserved locations with Aux/IAA genes from other plants. The promoter has several sequence patterns that suggest they function in auxin regulation. An auxin-response element, identified in angiosperms, is present in both forward and reverse orientation in the *LPEA1* promoter (Figure 10). Another area of the promoter shows similarity to the D4 element of the soybean *GH3* gene promoter. This element is also thought to contribute to regulation by auxin. It is interesting to note that the overall arrangement of sequences in the *LPEA1* promoter is most similar to the *GH3* gene. While GH3 is an auxin responsive gene, its coding sequence is not at all similar to the *LPEA*s or other Aux/IAA genes. We are taking two approaches to study the importance of these and other elements for auxin response and tissue specificity. The first is to conduct protein-DNA binding experiments. These use nuclear proteins purified from different tissues to test for interactions with specific small portions of the promoter. These experiments are underway.

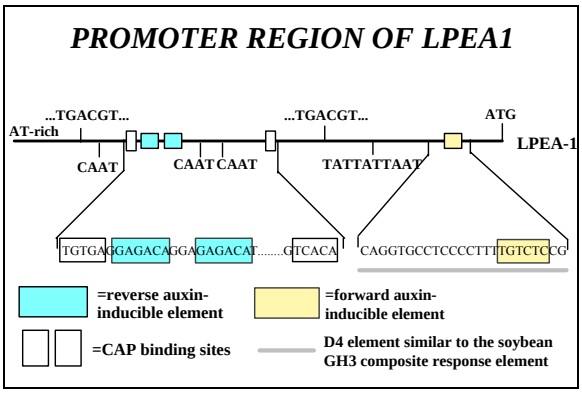

Figure 10. Sequence analysis of LPEA1 promoter with potential regulatory elements.

The second approach for studying the regulation of the *LPEA1* uses transgenic tobacco plants. Victor Busov has constructed four gene constructs (Figure 11) and introduced them into tobacco. One fusion contains a large fragment of the *LPEA1* promoter that is fused to the GUS reporter gene. The other three fusions contain progressively smaller fragments of the promoter fused with GUS. By measuring the expression of GUS, we can learn about how the promoter is regulating gene expression. All the constructs drive GUS expression predominantly in the vascular tissues of stems and leaves. Deleting portions of the promoter did not change the

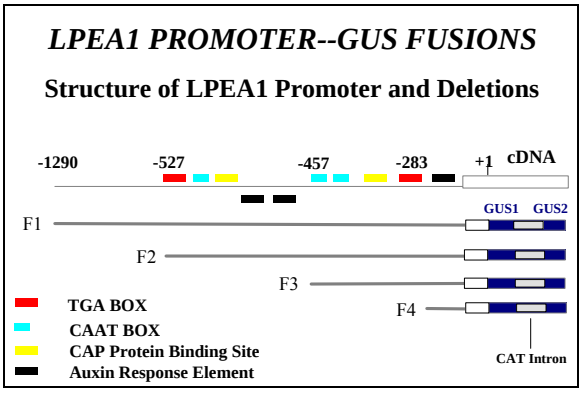

Figure 11. Four constructs using portions of the LPEA1 promoter fused with the GUS reporter gene and introduced into tobacco plants.

expression pattern in the above ground organs. In the roots, GUS expression was observed in the emerging lateral roots, but not in the existing roots (Figure 12). In the lateral roots, GUS expression was visible just behind the meristem (region of cell division) and further back in the developing root (region of cell maturation). However, no GUS expression was observed in the intervening region of cell elongation. This suggests that *LPEA1* may play a role in vascular cell fate specification in the meristematic zone and in the differentiation of these cells into vascular elements in the maturation zone. The two constructs containing the smallest promoter fragments changed the tissue specificity of expression in the roots. Tobacco plants containing these constructs had no GUS expression in the vascular elements of roots. Instead, GUS was visible in the root hairs of lateral roots (Figure 12). This suggests that the deleted portion of the promoter contains both positive and negative regulatory elements that control the tissue specificity of the promoter. Experiments are underway to study the auxin induction of these fragments in the transgenic tobacco plants. In addition, future experiments will test for GUS expression during adventitious root initiation in these lines. Patterns observed in the tobacco plant will be confirmed, to the extent possible, by measuring expression of the native *LPEA1* gene in pines.

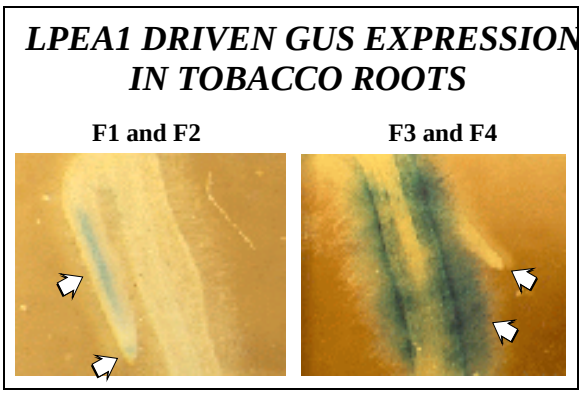

Figure 12. Expression of GUS reporter gene (blue color) under the control of the LPEA1 promoter in tobacco roots.

To study the function of the *LPEA*s, Victor fused the coding regions (full-length cDNAs) of *LPEA*s 1, 2 and 5 to the constitutive CMV 35S promoter and transformed the constructs into tobacco. The resulting plants were examined for growth and/or developmental abnormalities. The phenotypic characteristics varied among different lines. Most of the lines transformed with *LPEA2* did not show any abnormal phenotype. The most pronounced changes occurred in several lines transformed with *LPEA1* and *5*. These lines had slower growth, reduced stem elongation, low flower set and partial sterility. Some of these traits are opposite of what would be expected in a plant with an enhanced auxin response. To verify the successful integration and expression of the transgene, we used northern blot assays to measure expression. Interestingly, all the lines with aberrant phenotypes had a particular pattern of *LPEA* expression. The mRNA of these lines appeared as a smear on the blots. This has been known to occur during a phenomenon known as co-suppression or gene silencing. This is when introduction of a new gene causes both the new and native gene to become turned off. In contrast, lines without abnormalities, showed no signs of mRNA degradation. If these preliminary results are confirmed, these transgenic lines could be used as "knock-out" mutants to determine the functions of the genes in tobacco that are most closely related to the *LPEA*s.

To identify additional rooting genes, we are using a microarray approach in collaboration with the NC State Forest Biotechnology Group. We labeled total RNA extracted from cuttings treated and not treated by auxin, 1 and 3 days after treatment, and hybridized it to a microarray

containing 3456 expressed sequence tags (ESTs). The ESTs come from developing xylem and a variety of other loblolly pine tissues. We have identified several candidate genes from the microarray and are now in the process of confirming their induction by auxin and further characterizing their sequences and expression patterns. Preliminary results will be presented at the Annual Meeting.

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