NORTH CAROLINA STATE UNIVERSITY
LOBLOLLY AND SLASH PINE ROOTED CUTTING PROGRAM

Annual Progress Report
October 10, 2002

EXECUTIVE SUMMARY

The NCSU Loblolly and Slash Pine Rooted Cutting Program is completing its 11th year of existence. We are in the second year of a four-year phase that began on January 1, 2001 and is projected to end on December 31, 2004. Our mission is to conduct focused research and technology transfer activities to assist members in their deployment of rooted cuttings on an operationally meaningful scale. Our research topics are focused on providing information for the efficient, operational production of rooted cuttings and the implementation of clonal forestry.

In the area of operational production, this report contains results of studies that quantify the effects of different mist applications on **Cutting Water Potential and Rooting**. They add to our understanding of water relations in cuttings and are leading to simpler tools for using this understanding to control rooting environments. Another experiment is investigating the effects of different stock type production systems and treatments on **Rooted Cutting Quality**. Plants that rate highly according to existing seedling quality criteria have been produced using a variety of treatments and three production systems: bare-root, containerized, and transplanted rooted cuttings.

Several lines of research are being conducted in support of clonal forestry. Our long-term **Hedge and Clone Maturation Study** now encompasses material through nine years of age. We find that, even after nine years, we see little decline in rooting. Because nine years is approaching the amount of time that would be required to field test and multiply selected clones, this result makes it seem probable that at least a reasonable proportion of selected clones would retain sufficient juvenility for efficient propagation. A field test planted this year, of rooted cuttings from clones up to eight years old, will soon yield information about whether maturation is affecting growth in these clones. In our **Clonal Multiplication Study**, we have generated estimates of multiplication rates for five randomly selected clones in two environments. The field tests of our **Clonal Selection Study** are completing their fourth growing season. Clone mean heritabilities have steadily increased over the three years for which we currently have results. Data from year three have been used to estimate genetic gain obtained from selecting different numbers of clones and a simulation was conducted to determine the effect of number of ramets tested on gain. A **Wood Quality of Clones Study** found no differences in wood specific gravity between seedlings and rooted cuttings of the same families. Site had the largest effect on specific gravity, but there was considerable variation among clones. Moreover, ring by ring density analysis showed that, in cuttings as well as seedlings, overall core density was most strongly affected by the percentage of each core made up of latewood. Finally, in our research to understand the **Fundamental Mechanisms Controlling Root Formation**, we have begun to use poplar as a model tree species. We have cloned a poplar gene known to regulate root formation in *Arabidopsis*. In addition we are using a novel gene trapping system to identify other genes important for rooting. These new tools should allow us to make rapid progress in understanding rooting and maturation.

INTRODUCTION

The year 2002 is the second of four years in the third phase of the Rooted Cutting Program. We, on the program staff are proud of our accomplishments this year. We hope you find them substantive and are pleased with the results. We appreciate your support and look forward to continue working with you.

It has certainly been a challenging year for forestry and the forest products industry. A sluggish overall economy and weak prices for wood and forest products have put pressure on many organizations. Tight budgets, the continuing trend of mergers and acquisitions, and the divestment of timberlands by forest products companies has changed the organizational realities of some of our members. Despite the difficult current climate, many members are actively pursuing internal rooted cutting programs, as longer term forecasts predict shorter supply and higher wood prices. Economic forces have also resulted in cutbacks in many private and public sector research organizations. The Rooted Cutting Program lost two members during 2001/02–one to a merger and the other when a company dropped out to concentrate on other priorities. Fortunately, we have been able to maintain our overall level of activity by obtaining additional research funding from new sources.

A number of staff changes have occurred in the last year. Victor Busov graduated and began a post-doctoral position at Oregon State University. Patrick Cumbie is polishing his thesis and should graduate by December. Matt Gocke and Anthony LeBude are also in various stages of writing. This year we welcomed Qian Wu, a new MS student from China to the program. And last, but not least, Kelly Dougherty, an undergraduate who has worked with us for over three years, returned from a year-long internship at MeadWestvaco in January. Kelly graduated with a BS in Forest Management in May and continues to work with us while searching for a position with better compensation!

This report includes summaries of experiments conducted or analyzed since the last progress report in October 2001. Additional details will be presented at the upcoming Annual Meeting on October 24-25 in Raleigh. We hope to see many of you there.

Barry Goldfarb
Director

RESEARCH FOR OPERATIONAL PRODUCTION

Control of the Rooting Environment

Research on using cutting physiology to optimize environmental conditions during rooting is being conducted by Anthony LeBude, Research Assistant and Ph.D. candidate. Anthony has been determining the relationships between soil water potential (Ψ_{medium}), mist application, cutting water potential ($\Psi_{cutting}$), and rooting percentage. Last year, he showed that both aerial mist and Ψ_{medium} contributed to $\Psi_{cutting}$ and could influence rooting. While the level of mist was always an important factor, Ψ_{medium} affected $\Psi_{cutting}$ most strongly when the level of mist was sub-optimal. Moreover, last year's experiments suggested that the best rooting occurred when cuttings experienced mild to moderate moisture stress, while cuttings with no stress or extreme stress performed poorly. Because of the overall importance of mist application, it was decided that for this year's experiments, Ψ_{medium} would be kept constant and additional levels of mist would be examined to more fully determine the relationship between $\Psi_{cutting}$ and rooting percentage.

Six mist levels were applied by altering the speed of the traveling boom in the propagation greenhouse as it traveled over the cuttings. The mist levels were 4.2, 5.7, 6.8, 9.5, 13.6 and 28.8 ml/ft^2 of water each time the boom made a pass. The frequency of boom passes was constant across all treatments and was controlled using our standard computer program that varies frequency inversely with relative humidity and is modified according to time of day. Each mist level was replicated twice and Ψ_{medium} was held constant at -2.2 kPa (an average of the three treatments used in 2001) in all plots and maintained using tensiometers and sub-irrigation. Dormant (winter) cuttings of two full-sib families, also used in the 2001 experiments, were collected on February 14, 2002, stored at 37°F (4°C) for approximately 2 months, and then randomly mixed and placed into 2' x 3' x 10" tubs containing approximately 5" of coarse builders' sand on April 5, 2002. Six, 14, 21, and 28 days after sticking, $\Psi_{cutting}$ was measured, destructively, using a pressure bomb on two cuttings per plot at 5 am, 8 am, 11 am, 2 pm, 5 pm, 8 pm, and 11 pm. $\Psi_{cutting}$ measurements for each plot were averaged over each measurement day and then over the 4-week measurement period to provide a mean water stress experienced by the cuttings in each plot during the initial rooting period. Ψ_{medium} was measured each week for four weeks at 5 am and 2 pm only. Rooting percentage was recorded 10 weeks after sticking. Regression analyses were used to determine how $\Psi_{cutting}$ and rooting percentage varied with mist level and how rooting percentage varied with $\Psi_{cutting}$.

Mist level strongly affected $\Psi_{cutting}$. As the volume of mist per boom pass increased from 4 to 29 ml, $\Psi_{cutting}$ increased (became less negative) from -1.4 to -0.3 MPa (Figure 1). The rate of increase in $\Psi_{cutting}$ was greatest at the lower mist levels and tapered off at higher mist levels. Ψ_{medium} remained constant across all mist treatments so the differences in $\Psi_{cutting}$ were due strictly to the levels of mist. Across all mist levels except the wettest (29 ml/ft^2/pass), there was considerable fluctuation in $\Psi_{cutting}$ throughout the day (Figure 2). For example, in the 14 ml/ft^2/pass mist level, even though the mean $\Psi_{cutting}$ was -0.7 MPa, the mean daily minimum $\Psi_{cutting}$ (typically between 5 and 8 am) was -0.2 MPa and the mean daily maximum $\Psi_{cutting}$ (between 2 and 5 pm) was -1.3 MPa.

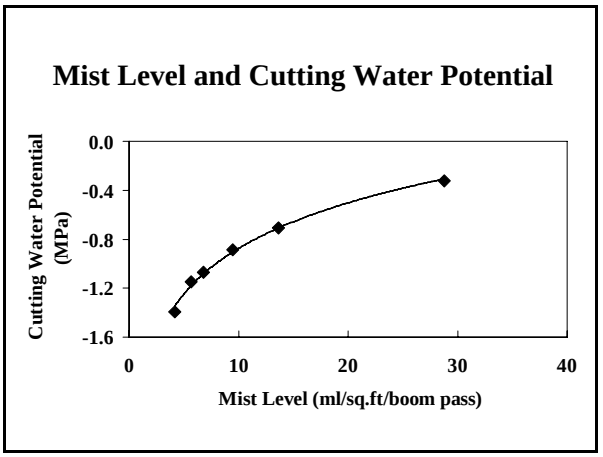

Figure 1. Effect of level of mist application on mean water potential of loblolly pine cuttings. Each value represents the mean of two plots, measured seven times of day over four weeks.

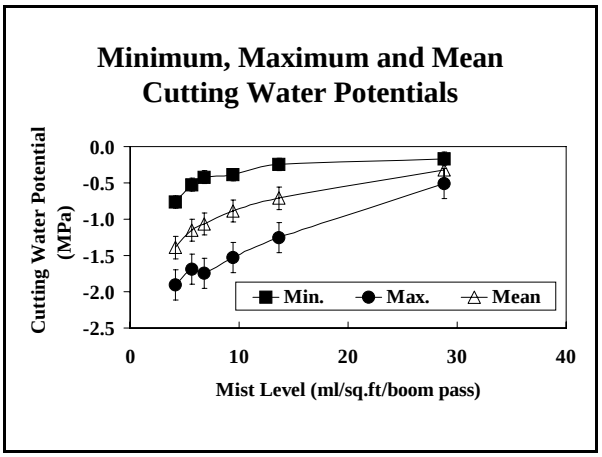

Figure 2. Mimimum, maximum and mean daily cutting water potentials of loblolly pine cuttings. Each value represents the mean of two plots measured over four weeks.

Mean rooting percentage across all mist levels in the experiment was 73%. Rooting percentage was highest at intermediate mist levels (93% at 14 ml/ft^2/pass, 90% at 9 ml/ft^2/pass), decreasing at both lower (46% at 4 ml/ft^2/pass, 71% at 6 ml/ft^2/pass, 65% at 7 ml/ft^2/pass) and higher (73% at 29 ml/ft^2/pass) mist levels (Figure 3). The effect of mist level on rooting percentage was largely explained by $\Psi_{cutting}$ in the mist level treatments (Figure 4). Cuttings with moderate mean levels of $\Psi_{cutting}$ rooted better than cuttings under more stress and those with little or no stress. This supports the results from last year which suggested that moderate water stress was, in fact, beneficial for stimulating rooting. It should also be emphasized that the cuttings receiving the most mist in this experiment did not show obvious signs of rot, as would be expected with overly saturated medium conditions. Because they were in medium that had the same Ψ_{medium} as the other treatments, they were green and healthy, even though they rooted at lower frequencies.

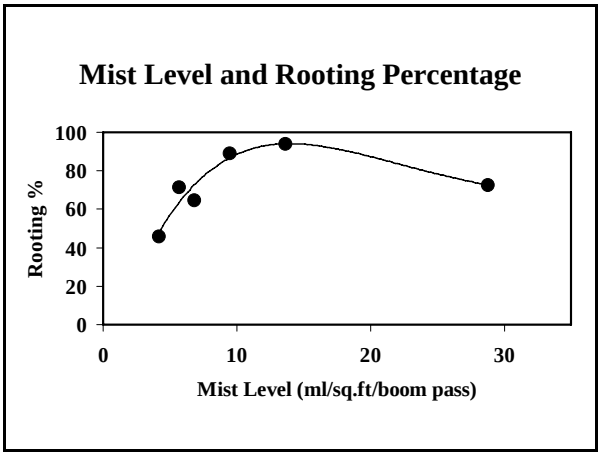

Figure 3. Effect of mist level on rooting percentage of loblolly pine cuttings. Each value is the mean of two plots.

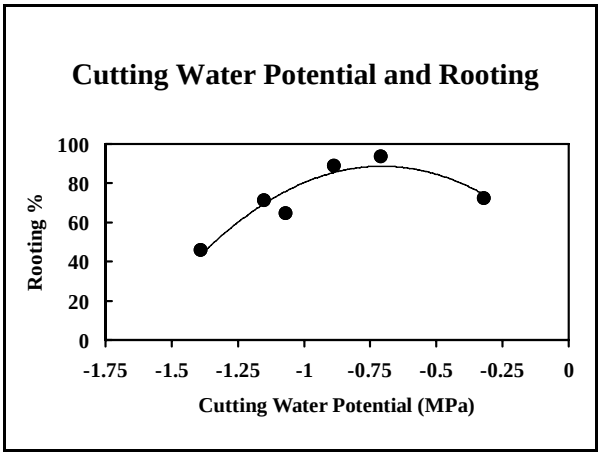

Figure 4. Effect of cutting water potential on rooting percentage of loblolly pine cuttings. Each value is the mean of two plots.

In order to regulate mist irrigation conditions to determine the proper amount of stress to impose on cuttings, growers have several options. First, a pressure bomb (Soil Moisture Equip. Corp., Santa Barbara, CA; Plant Moisture Stress, Corvallis, OR) may be used to sample cuttings during the rooting period. We recommend testing cuttings during the period of maximum stress (2-5 pm) on a typical sunny day. If $\Psi_{cutting}$ is more negative than -1.5 MPa, adjust your irrigation to either increase the frequency or duration of application. If the maximum $\Psi_{cutting}$ is less negative than -0.5 MPa, then cuttings are experiencing no water stress and irrigation should be decreased. Second, visual observations can help guide irrigation decisions. In this study, cuttings that received the intermediate mist levels and rooted the best, appeared moist, but not wet, just before the next irrigation cycle. Cuttings in the wettest mist treatment still had free surface water before the next mist application, while cuttings in the drier treatments took on a dull, gray-green appearance and were often completely dry between mist applications. Third, while not yet fully analyzed, continuous measurements of temperature and humidity were made in the cutting plots throughout this experiment. These may prove very instructive for guiding irrigation decisions and also be technologically straightforward to implement.

This entire experiment was repeated with spring cuttings this past season and the data are currently being analyzed. In general, similar trends were observed. In addition, measurements of net photosynthesis and transpiration were also taken during the spring experiment. These, the water potential readings, and the temperature and humidity readings will provide us with a much more complete understanding of how best to control the rooting environment.

Stock Quality in Different Production Systems

From February 2000 to December 2001, M.S. student Matt Gocke, conducted two experiments evaluating the effects of three potential rooted cutting production systems on stock quality of loblolly pine winter cuttings. The first experiment was conducted from February to December 2000, and tested the following production systems: (1) a direct-stick (DS) system in which cuttings were stuck directly into an outdoor nursery bed in full sun or under 50% shade cover; (2) a transplant system in which cuttings were rooted in the propagation greenhouse in Grow-Tech Rooting Sponges™ (GT) or Jiffy Forest Peat Pellets™ (Jiffy) and then transplanted at either 7, 9, or 11 weeks to an outdoor nursery bed; and (3) a fully containerized system in which cuttings were rooted in the greenhouse in Ray Leach SuperCells™ for 12 weeks and then transferred outdoors. For this experiment there were a total of 9 treatments: two direct-stick treatments (sun versus 50% shade), 6 transplant treatments (2 rooting plug types x 3 transplant times), and 1 containerized treatment.

Results of the 2000 experiment suggested that a transplant production system offered promise–cuttings produced using that system were among the largest of all the treatments (see Annual Report, 2001). In addition, while some of the cuttings in each treatment were of adequate size, an unacceptable proportion would have been rated as “cull” or “grade 2," based on root collar diameter criteria for bare-root seedlings (Figure 5). This may have been the result of sub-optimal fertilization of the containerized cuttings and slow rooting in the direct-stick cuttings.

Based on these results, a second experiment was conducted in 2001 with the following modifications: (1) two irrigation regimes were tested for the direct-stick system; (2) the Jiffy pellet treatments were dropped from the transplant system; (3) two, instead of three, transplant times were evaluated for the transplant system; and (4) an additional sticking date was evaluated for the transplant and containerized systems (Table 1). So, in this experiment, there were a total of 10 treatments: four direct-stick treatments (two irrigation regimes x full sun vs. 50% shade), four transplant treatments (two sticking dates [February and May] x two transplant times [9 and 12 weeks for the February sticking date and 8 and 10 weeks for the May sticking date]), and two containerized treatments (two sticking dates, the same as in the transplant treatments). The experiment was a split-plot design, with treatment as the whole plot factor and clone as the subplot factor. There were 10 stock type treatments, three clones, six blocks, and 15 cuttings per plot for a total of 2700 experimental cuttings, plus borders. Additional modifications from the first experiment included adapting an individual fertilization and irrigation regime for each treatment, transplanting only rooted cuttings for the transplant and containerized treatments, earlier sticking dates, and mulching the nursery bed surface to reduce weed growth and soil compaction. A variable-cycle control system (Davis Engineering Solar 6A Misting Controller) adjusted the frequency of the irrigation by measuring the accumulation of sunlight (1 solar unit = 2000 footcandles or 0.02 moles/m^2).

Table 1. Treatments used to test effects of propagation systems on stock quality in 2001.

Propagation System	Treatment	Sticking Date	Transplant Date	Density in Misthouse/Nursery bed (per ft^2)
Direct-Stick	High water–sun	Feb. 14	NA	25
	High water–shade			
	Low-water–sun			
	Low water–shade			
Transplant	Early stick, 9-wk transplant	Feb. 22	April 26	117 / 25
	Early stick, 12-wk transplant		May 17	
	Late stick, 8-wk transplant	May 4	July 3	
	Late stick, 10-wk transplant		July 24	
Containerized	Early stick	Feb. 22	May 17*	49 / 25
	Late stick	May 4	July 24*	

*Containerized cuttings were moved outdoors on the date indicated, but remained in the original containers.

Winter cuttings, 8 cm in length, were collected from hedged stock plants between January 25 and February 2, 2001, wrapped in moist paper towels, and stored in a walk-in cooler at 5°C until the time of sticking for all treatments. All cuttings were treated with 10 mM NAA for three seconds and inserted into the appropriate rooting medium. Cuttings for the direct-stick system were inserted one-half their length (4-cm) into the nursery bed soil. The transplant system cuttings were inserted 2-cm into the GT sponges in Winstrip™ trays (#162). The containerized system cuttings were inserted 2 cm into Ray Leach SuperCells filled with a medium consisting of peat, perlite and vermiculite (5:4:1, by volume). A fertilizer regime of Peter's™ 20-20-20 was applied at increasing rates from 50 to 150 ppm/N over the course of the growing season. Frequency and rate of application were tailored to individual treatments. An acid fertilizer (17-6-6) was used, when necessary, as a substitute for the Peter's mix to maintain medium and soil pH below 6 for the transplant and direct-stick treatments. Boron and iron chelate were applied to all treatments.

The February transplant treatments yielded the largest cuttings according to all the variables measured (Table 2). As expected, these cuttings were larger than the transplanted cuttings stuck in May, but they were also considerably larger than the February-stuck, containerized cuttings. There were no significant differences, however, between the cuttings transplanted after 9 weeks and those transplanted after 12 weeks. For the transplanted cuttings stuck in May, transplant time did affect shoot and root dry weight, but not root collar diameter. For both roots and shoots, the cuttings

Table 2. Characteristics of loblolly pine rooted cuttings produced by ten stock type treatments for three production systems in 2001.

Production Treatment	Root Collar Diam. (mm)	Shoot Height (cm)	Shoot Dry Wt. (g)	Root Dry Wt. (g)	Shoot:Root Ratio
DS–HW–Sun*	5.5 b**	25.6 bc	23.2 bcd	9.1 bcd	2.8 b
DS–HW--Shade	5.3 b	23.8 bcd	27.5 b	9.5 bc	2.9 b
DS–LW–Sun	5.0 bc	21.9 d	19.5 de	6.7 de	2.6 b
DS–LW–Shade	5.4 b	21.7 d	20.5 cde	7.9 cde	2.5 b
Feb--Trans--9-wk	7.2 a	43.2 a	73.9 a	23.3 a	3.4 a
Feb–Trans–12-wk	6.9 a	43.8 a	62.8 a	19.1 a	3.5 a
May–Trans–8-wk	5.5 b	27.2 b	26.3 bc	8.4 bcd	2.8 b
May–Trans–12-wk	5.2 bc	24.9 bcd	16.3 e	6.1 e	2.6 b
Feb–Cont	5.0 bc	22.8 cd	22.1 bcd	10.8 b	2.0 c
May–Cont	4.6 c	17.0 e	13.9 e	8.6 bcd	1.7 c

*DS=direct stick, HW=high water, LW=low water, Trans=transplants, Cont=containerized.

**Treatments with the same letter (within a column) had values that did not differ significantly ($p < 0.05$), SAS GLM Procedure, LSD means separation.

transplanted after 8 weeks were larger then those transplanted after 10 weeks. The cuttings stuck directly in the nursery bed, though stuck in February, were comparable in size to cuttings stuck in May using the other two propagation systems. There were few large differences among the direct-stick treatments, but the cuttings rooting under low water were shorter than those in the high water–sun threatment and had less shoot and root dry weight than the cuttings in the high water–shade treatment. Cuttings in these treatments also rooted at about the same percentage, ranging only between 44% and 55% (data not shown). The containerized cuttings (both stickings) had the lowest shoot:root ratios among the treatments. This may be a reflection of more complete recovery of roots for measurement. However, more intact root mass after lifting and transplanting could also translate into better performance during operational planting. The May-stuck, containerized cuttings were the shortest of all the treatments and had among the lowest shoot dry weights. However, the root dry weight was similar to the May-stuck, 8-week transplanted cuttings and was greater than the May-stuck, 12-week transplanted cuttings. Thus, timing and culture of cuttings stuck late in the season appears to be critical, although good results can be obtained with either the transplant or the containerized systems.

To evaluate the quality of the rooted cuttings produced, the existing, bare-root, seedling quality standards based on root collar diameter (RCD) were used to classify the rooted cuttings into the grades developed by Wakeley in 1954. In 2001 (Figure 6), a higher percentage of cuttings, in every treatment, were rated as Grade 1, as compared with 2000 (Figure 5). In addition, the percentage of cuttings rated as culls was lower in 2001 than in 2000 in all treatments, except for the early-stuck transplants that had few culls in both years. The February-stuck transplants yielded the highest percentage of cuttings rated as Grade 1, followed closely by the May-stuck, 8-week transplanted cuttings. The May-stuck, containerized cuttings yielded the lowest percentage of Grade 1 cuttings, but since these criteria were developed for bare-root seedlings, this does not necessarily predict poor field performance. All the other treatments had similar percentages of Grade 1 cuttings.

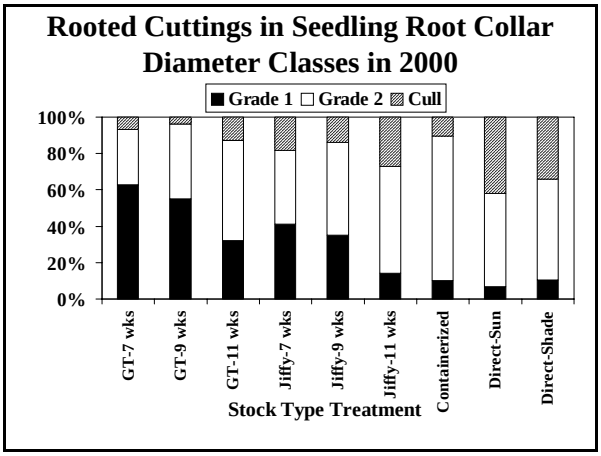

Figure 5. Percentage of rooted cuttings of each stock type treatment in 2000 rated as Grade 1 (>4.76 mm), Grade 2 (3.17 to 4.76 mm), or Cull (<3.17 mm), according to Wakeley's RCD criteria.

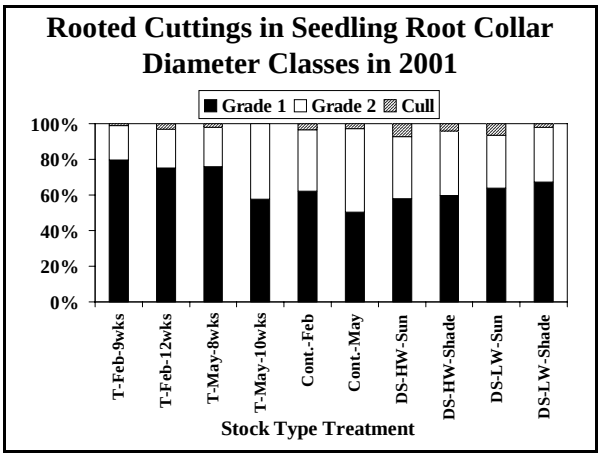

Figure 6. Percentage of rooted cuttings of each stock type treatment in 2001 rated as Grade 1 (>4.76 mm), Grade 2 (3.17 to 4.76 mm), or Cull (<3.17 mm) according to Wakeley's RCD criteria.

These results indicate that good quality rooted cuttings can be produced with all three of the propagation systems tested. Transplant treatments consistently yielded the largest cuttings, especially for those cuttings stuck early in the season. Achieving appropriate size standards is more challenging for cuttings stuck late in the season (May, in this experiment) and timing of sticking, transplanting (if appropriate), and fertilization becomes more critical. Different irrigation and sunlight regimes imposed on direct-stuck cuttings during rooting had only minor effects. These rooted and began to grow vigorously at about the same time as May-stuck cuttings. A sub-sample of rooted cuttings representing the three potential production systems and two sticking dates tested in this experiment were planted in a field test on January 14, 2002 in Stewart County, Georgia on MeadWestvaco land. The field results will help guide development of quality standards for rooted cuttings produced with different production systems.

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 of hedges (20 clones per family) are started from seed of three open-pollinated loblolly pine families. In addition to new seedling hedges, every other year, a new cycle of hedges is produced from serial propagation of cuttings of seedling hedges, or the most recent cycle of serially propagated hedges (hedges from rooted cuttings). For example, seedling hedges were started in 1993 and, in 1995, cuttings were taken from those hedges and the first serial cycle of hedges was produced. In 1999, the third serial cycle of hedges was produced from cuttings on the second serial cycle of hedges. Thus, the 2002 cuttings came from hedges that were only three years since the most recent propagation cycle, but these clones have been growing nine years, since 1993. This report summarizes results from rooting experiments conducted in Winter and Spring 2002. It is the first to containing cuttings from third-cycle serially propagated hedges in representative numbers. Unfortunately, due to an irrigation failure during summer 2001, seedling hedges from years 3 (1999) and 5 (1997) suffered mortality and were lost for the remainder of the experiments. These clones, however, had already been propagated to form serial hedges, so the long-term study is intact.

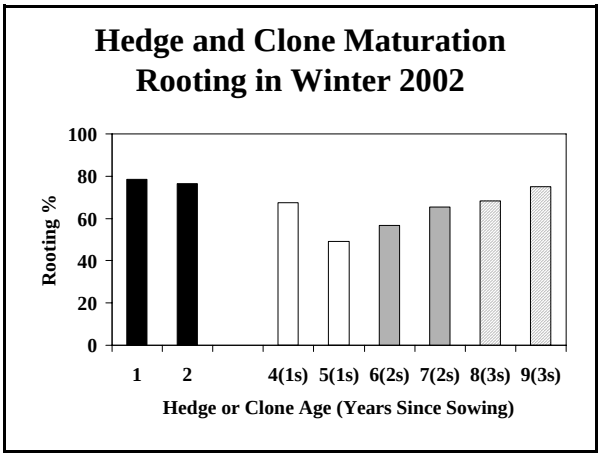

Figure 7. Rooting percentages of clones of different ages in winter 2002; black bars=seedling hedges and white, gray and hatched bars=hedges from one, two or three cycles of serial propagation., respectively.

In Winter 2002, rooting percentage across all ages and families was 66.3% (Figure 7).

While the highest percentage was obtained with the cuttings from 1- and 2-year-old seedling hedges (78.5 and 76.5%, respectively), serial propagation appeared to prevent large losses in rooting performance (Figure 7). Clones that were 8 and 9 years old, and had been serially propagated three times, rooted at 68.3 and 75.1%, respectively. The three families rooted at percentages that were consistent with their past performance. Family 7-1037 rooted at the highest percentage (79.3%) across all ages, family 11-1103 was intermediate (67.1%), and 9-1019 remained the poorest rooting family (50.0%).

The Spring 2002 experiments yielded a largely similar result. The overall rooting percentage was 66.1%. As with the winter experiment, the 1-year-old seedling hedges rooted at the highest percentage (88.4%) (Figure 8). Among all the other clone ages, however, there was little difference and no evidence of a trend with age. The range of rooting percentages found from clones 2 to 9 years old was 54.8% (6 years old) to 70.7% (5 years old) and the 9-year-old clones that had been serially propagated three times rooted at 64.3%. Each of the families performed almost exactly the same as in the winter: 79.9% for 7-1037, 65.2% for 11-1103, and 50.0% for 9-1019.

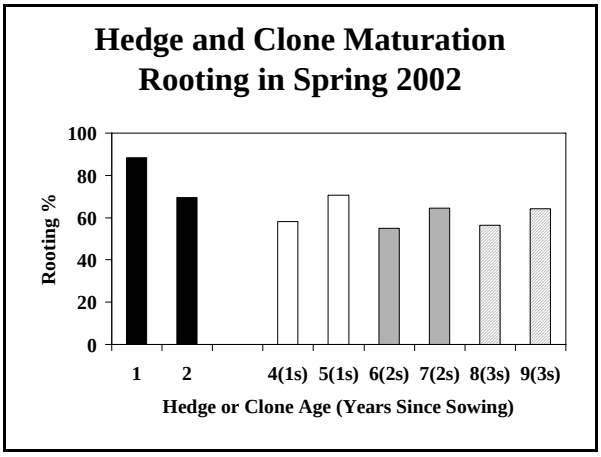

Figure 8. Rooting percentages of clones of different ages in spring 2002; black bars=seedling hedges and white, gray and hatched bars=hedges from one, two or three cycles of serial propagation, respectively.

It is possible that the surprisingly good rooting of older clones is a result of the gradual elimination of the poor rooting clones during serial propagation cycles. For example, while 60 clones from the three families were tested for the 1-year-old hedges, only 50 clones in the 8-year-old hedge class and 34 clones in the 9-year-old hedge class were tested. Thus, this inadvertent selection for better rooting clones could be counteracting gradual loss of rooting ability due to maturation. However, this level of selection for rooting is probably mild compared to what would be used for an operational clonal program. After nine years, over half of the original 60 clones are remaining, including those from a consistently poor rooting family, and they are rooting, on average, in the 65-75% range. This has to be considered a very encouraging result.

Besides the potential impact on rooting and rooted cutting production efficiency, another factor that will be important for determining the useful life of clones. If maturation substantially affects growth rates of rooted cuttings from older clones, then projected genetic gains will not be realized. On January 31, 2002, a field test was planted by Plum Creek Timber Company near Holly Hill, SC. The test contains four blocks of 296 trees each (total of 1184), including rooted cuttings from clones 2 through 8 years old and seedlings of the same three families. We are optimistic that this field test will provide a critical evaluation of the effect of clone age on growth.

Clonal Multiplication Study

The clonal multiplication study was started in 2000 to determine the effects of indoor and outdoor propagating environments clonal multiplication rates from existing hedges of five randomly selected clones. The study was begun in February 2000 with the harvest of existing cuttings from four hedges of each clone. After the cutting harvest, two hedges of each clone were either left outdoors or 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. Outdoor hedges remained in full sun during the growing season and in an unheated, plastic enclosure during the winter. There was also a serial propagation component. After the cuttings from each collection date were scored for rooting, a sub-sample of rooted cuttings were transplanted, returned to the appropriate growing environment, and pruned to produce additional cuttings. The final cutting harvest occurred in early March 2002 and these cuttings were then tested for rooting.

On average, 4326 rooted cuttings per original hedge were produced in the indoor treatment (Figure 9). This is substantially larger than the average of 2263 rooted cuttings per hedge in the outdoor treatment. There were two reasons for the greater yield from the indoor treatments. First, there were collections from the indoor hedges in November 2001, January 2002 and March 2002, but no corresponding cuttings were available at these times in the outdoor treatments (Figure 9). These late collections had a large influence on total yield because of the amount of serial multiplication that preceded it. For example, 53% of the rooted cuttings produced in the indoor treatment came from cuttings collected the final winter. Second, the indoor treatments had a greater contribution of cuttings from the second cycle of serially propagated hedges than the outdoor treatments. These indoor, second-cycle hedges came from cuttings that had been rooted earlier the year before than the corresponding cuttings in the outdoor treatments. Thus, the indoor hedges had developed further and produced more cuttings. Of all the rooted cuttings produced from serially propagated hedges between July 2000 and the end of the experiment (March 2002), second-cycle hedges made up 46% (1725 rooted cuttings per hedge) in the indoor treatment, but only 31% (562 rooted cuttings per hedge) in the outdoor treatment.

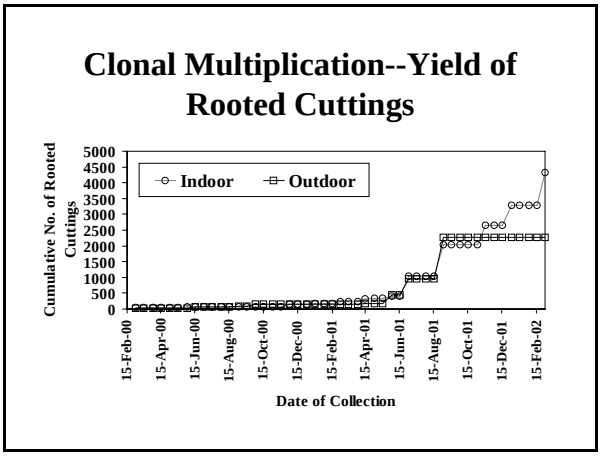

Figure 9. Cumulative yield of rooted cuttings per original hedge from serial propagation of indoor- and outdoor grown hedges from February 2000 to March 2002.

There was considerable variation in multiplication rate among clones. The clones ranged from B14 that only yielded 391 rooted cuttings in the indoor environment during the entire study to C66 that yielded 14,428 cuttings in the same environment and time period (Table 3). The differences in yield among the clones were largely caused by different rooting percentages. The rooting percentages and, thus, rooted cutting yields of clones were fairly similar in the indoor and

outdoor environments. These large differences in multiplication rates among clones suggests that, in addition to growth characteristics, rooting ability of clones should be considered, as it could substantially affect the time required to reach adequate hedge population sizes for operational deployment.

Table 3. Rooting percentage and yield of rooted cuttings of five clones from indoor and outdoor multiplication from February 2000 to March 2002.

	Indoor		Outdoor	
Clone	Rooting %	Yield of Rooted Cuttings	Rooting %	Yield of Rooted Cuttings
A3	43	2956	33	852
A45	31	754	19	367
B14	22	391	23	504
C66	75	14428	76	7531
D22	40	3101	43	2059
Mean	42	4326	39	2263

As would be expected for a serial propagation system, we observed a multiplicative increase in each of the two years of the study. After the first year of the study, a mean of 233 rooted cuttings was obtained from each original hedge using the indoor environment and 146 rooted cuttings with the outdoor environment. In the second year, the multiplication rate was 18.6X in the indoor environment and 15.5X in the outdoor environment. We would expect that similar multiplicative increases would occur in the next year and in future years, yielding an overall exponential increase. Clearly, however, the rate of exponential increase will depend on the rooting performance of the clone.

Clonal Selection Study

The objective of this study is to develop information that will facilitate efficient testing and selection of 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 lack of relatedness. 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, and (4) 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 MeadWestvaco 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, height and rust infection were taken after each of the first three growing seasons. Because of very low incidence in the FL test, rust infection was not analyzed for that site. Trees on both sites have undergone good growth and the initial problems with uneven stock quality have become less important. The Clone x Block CVs have fallen from approximately 35% after the first season to 13-14% after the third season (Table 4). Similarly, within-family, clone mean heritabilities for height were relatively low after the first season, but have increased steadily over the next two seasons (Figure 10).

Table 4. Third-year results for height and rust infection from the two sites of the Clonal Selection Study.

Trait and Test Site	Height-FL (ft)	Height-SC (ft)	Rust-SC (%)
Mean Height (±SE)	11.9 (±0.06)	13.6 (±0.04)	24 (±0.01)
Clone x Block CV (%)	13.9	13.1	--
Clone (Family) H^2 (±SE)	0.56 (±0.05)	0.69 (±0.03)	0.95 (±0.01)

Based on these early, third-year results, Research Associate Dr. Fikret Isik estimated genetic gain for height. Choosing the single best clone resulted in 26% gain over the check lot and 17% gain over the test mean in the SC test and 28% gain over the check lot and 15% gain over the test mean in the FL test (Figure 11). If the eight best clones were selected, gain was lowered somewhat to 23% and 13% at SC and 23% and 10% at FL. Fourth-season measurements will be taken this winter and diameter will be measured for the first time. This will allow estimates of the potential genetic gains in volume, which should be larger than those for height, but will also have to be adjusted for age-age correlations between selection and rotation ages.

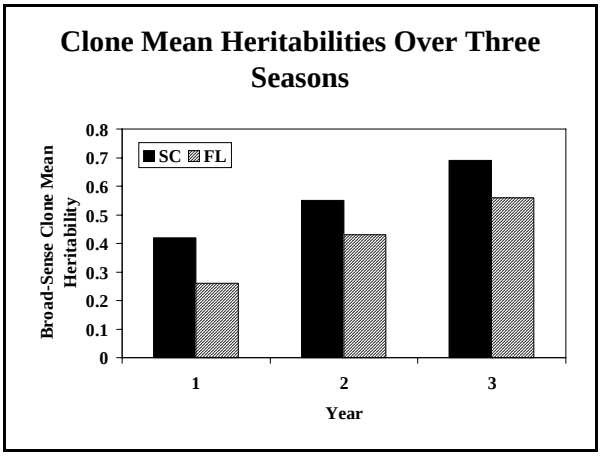

Figure 10. Clone mean broad-sense heritabilities for height after each of three growing seasons on the two sites of the clonal selection study.

The clonal test data were used to simulate the effect of number of ramets tested on genetic gain. For each clone, a Best Linear Unbiased

Predictor (BLUP) genetic value was estimated from the full data set. Then the data were resampled leaving out one block (ramet) at a time and the standard errors of the BLUP values were calculated. These were used as an indication of reliability of genetic values in a single gain equation. On both sites, gain increased sharply with an increasing number of ramets from two to approximately seven, but then increased only slightly with additional ramets (Figure 12). For example on the SC site, increasing the number of ramets from two to seven resulted in a 3.2% gain increase or 0.64% gain per additional ramet. Increasing the number of ramets from eight to 20, however, resulted in a gain increase of only 1.5% or 0.13% gain per additional ramet. Future analyses will examine how to optimize the numbers of clones vs. ramets for a fixed number of planted test trees to maximize gain.

With age three measurements, it is too early to conduct a definitive analysis on optimal selection age. As an example of the kind of information that will become available, however, an analysis is presented showing the age-age correlations and correlated responses between years 1 and 3 and years 2 and 3 (Figure 13). As expected, both the genetic correlations and the correlated responses to selection increased from year 1 to year 2. The genetic correlation in year 1 was higher in FL than SC, but the correlated response to selection was about the same on the two sites because of the higher clone mean H^2 in SC. Across both sites, selection in year 2 would have yielded approximately 70% of the gain obtained from direct selection at age three–a substantial increase from the approximately 40% that would have been obtained with selection in year 1. As data from future years become available, this kind of analysis should help us identify optimal selection ages.

Multiplication of the clones in the study is in its second cycle of serial propagation.

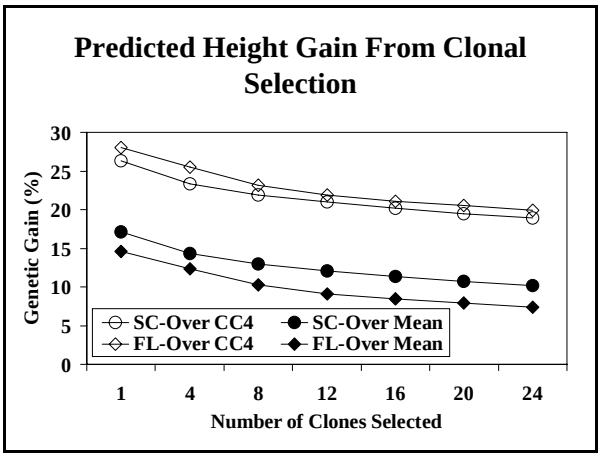

Figure 11. Predicted 3rd-year height gain from selecting various numbers of clones from among the 168 tested in FL and 282 tested in SC.

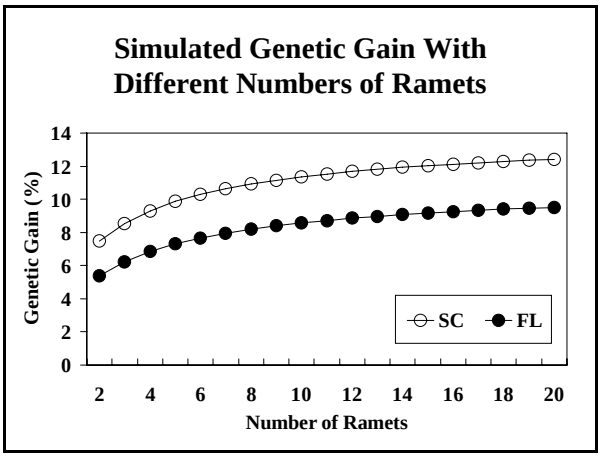

Figure 12. Simulated gain from testing different numbers of ramets, assuming that 10% of tested clones are selected.

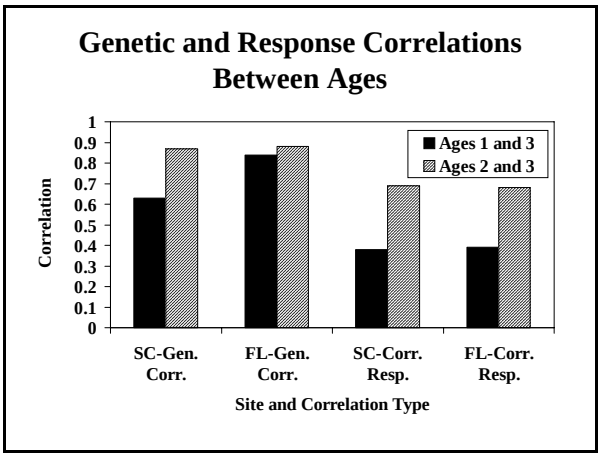

Figure 13. Genetic correlations and correlated responses to selection between years in the clonal selection study.

Decisions on which clones to retain and multiply and which to discard will be made shortly. The objectives and designs of future tests with these clones are the most important factors affecting this decision. We will discuss options at the annual meeting and eagerly await your input.

Wood Quality of Rooted Cuttings and Seedlings

Patrick Cumbie, an MS student working jointly with the NCSU Tree Improvement Program, has been studying wood density in rooted cuttings and seedlings. On a project funded by a USDA-IFAFS grant, Patrick sampled trees in field tests that were planted by the NCSU Tissue Culture Project in 1990 and 1991. 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, but for this report, we show results for whole-core specific gravity and individual ring density.

In last year's annual report, we reported no significant difference for whole-core specific gravity between rooted cuttings (0.430) and seedlings (0.427) across the two sites. However, there was a significant difference between the two sites. The Florida site had an average specific gravity of 0.444, while in Alabama, where growth was more rapid, the specific gravity was 0.411. The variance attributed to families was similar between rooted cuttings (20.2%) and seedlings (16.8%). However, in rooted cuttings, the clonal component accounted for 12.6% of the total variance. The site accounted for a large portion of the total variance in both seedlings (24%) and rooted cuttings (41%) and, although the clone by site interaction in rooted cuttings was significant, it made up only 1.5% of the total variance.

A ring by ring density analysis was conducted on wood samples from a sub-sample of rooted cuttings and seedlings from the two sites. Individual ring data were generated by an X-ray densitometer through collaboration with Dick Daniels of the Wood Quality Consortium at the University of Georgia and Alex Clark of the USFS, Athens, GA. Across the two-sites, approximately 7 rings were analyzed to compare seedlings and rooted cuttings for individual ring components. Earlywood density, latewood density, and the percentage of latewood were analyzed for their contribution to the individual ring density and overall density of the wood core. These three traits were weighted by ring to generate whole-core estimates for analysis. A fourth trait, LW40, was added to measure the ring in which latewood reached 40%, in order to determine the genetic and site factors influencing the timing of transition to a higher proportion of latewood.

In phenotypic individual tree and family mean correlations for both seedlings and cuttings, latewood percent was most strongly correlated with overall wood density, followed by LW40, earlywood density, and latewood density (Table 5). Analyses of variance for these wood traits revealed that latewood percentage ($p<0.044$) and LW40 ($p<0.0008$) differed significantly between sites, but latewood density and earlywood density did not. There were no significant differences between rooted cuttings and seedlings for any of the traits (Figures 14 and 15). In the separate analyses of cuttings and seedlings, there were no significant family effects, but clones varied

significantly for latewood percent ($p<0.005$) and latewood density ($p<0.035$).

Table 5. Phenotypic individual tree and family mean correlations (Prob. > |r| under H0) of earlywood density (EWD), latewood density (LWD), latewood percent (LW%), and age at which a tree's latewood reached 40% (LW40)with whole-core density of rooted cuttings and seedlings on two sites.

Correlation and Propagule Type	Correlations with Whole-Core Density			
	EWD	LWD	LW%	LW40
Indiv. Tree-Seedling	0.56 (<0.0001)	0.21 (<0.0001)	0.82 (<0.0001)	-0.58 (<0.0001)
Indiv. Tree-Cutting	0.40 (<0.0001)	0.24 (<0.0001)	0.86 (<0.0001)	-0.58 (<0.0001)
Family-Seedling	0.81 (0.0086)	0.60 (0.0847)	0.85 (<0.0001)	-0.89 (0.0014)
Family-Cutting	0.62 (0.0764)	0.37 (0.3261)	0.95 (<0.0001)	-0.88 (0.0014)

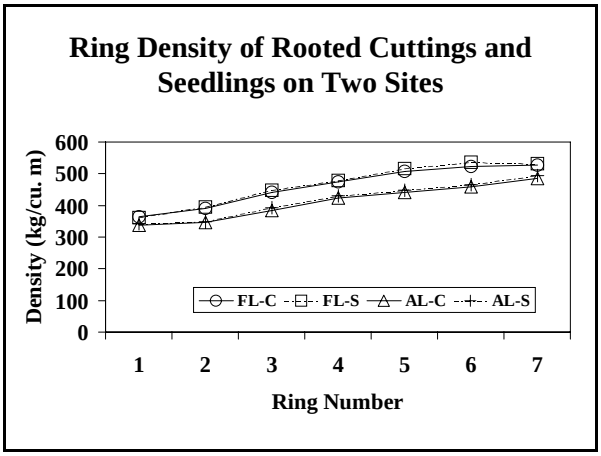

Figure 14. Wood density by ring for rooted cuttings and seedlings of the same families on two sites.

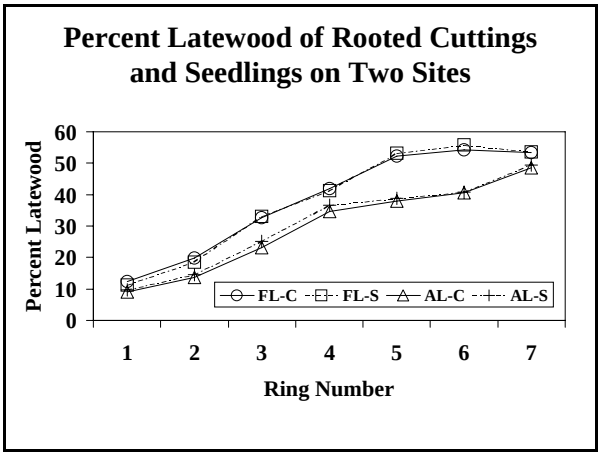

Figure 15. Percent latewood by ring for rooted cuttings and seedlings of the same families on two sites.

The lack of differences between rooted cuttings and seedlings in overall specific gravity and ring traits, along with the lack of differences in growth traits reported earlier, suggests that rooted cuttings could be deployed and deliver expected yields in growth and density, according to the genetics of the deployment population and the plantation site. Moreover, the proportion of variation associated with the clonal component suggests additional gain could be realized by deploying clones with specific desired wood properties.

Mechanisms of Root Formation and Maturation

Poplar as a model for studying root formation. Poplars have become a model tree species for studying genes during developmental and physiological processes, because they are easily propagated in tissue culture, have a small nuclear genome, and are easily transformed. Genetic transformation is a particularly useful tool, because it permits direct tests of gene function. In addition, the U.S. Department of Energy has undertaken the sequencing of an entire poplar genome. This information, which is scheduled to become available in the coming year, will prove to be an invaluable resource for understanding gene function in trees.

Rooting ability in poplars varies substantially among species. While some species easily form roots in branches from mature trees, in other species–such as cottonwood (*Populus deltoides*)–rooting ability is genotype-dependent. In the aspens, rooting is affected by maturation. Cuttings from seedlings root readily, but cuttings from older plants show markedly reduced rooting ability. (For propagating the aspens, maturation can be circumvented by taking cuttings from root suckers, which are juvenile.) Because of the ability to make rapid scientific progress, the fact that maturation affects adventitious root formation, and its poplar's potential as a system for obtaining grant funding, we have begun using it as a model system, with the intention of subsequently testing significant findings in pine.

Gene traps for studying rooting genes. Qian Wu, a new MS student from China, is using a technique called gene trapping to isolate and identify genes involved in root formation in poplar. This effort is a collaboration with Andrew Groover (Institute of Forest Genetics, USDA Forest Service, Davis, CA) and Rick Meilan and Steve Strauss (Oregon State University). An easy-to-transform hybrid poplar (*Populus alba x tremula*) clone was transformed using *Agrobacterium* and a vector containing the GUS reporter gene in a configuration so that it would only be expressed if it inserted in (gene trap) or near (enhancer trap) an expressed gene. Several hundred independent lines were recovered and were initially screened by taking micro-cuttings, placing them on root-forming tissue culture medium and staining them to look for GUS expression in newly formed roots. We received 23 lines from the initial screen at OSU. Qian is in the process of conducting more detailed tests to document the precise timing and location of the "trapped" genes. She is also using a variation of the polymerase chain reaction called TAIL-PCR to identify the trapped genes by amplifying and sequencing the chromosomal regions flanking the insertions.

To date, in-depth characterization of GUS expression has been conducted on seven of the 23 lines. Of these, one line showed expression in the stem base prior to root primordium formation (Figure 16a), five in the emerging rooting primordium (Figure 16b,c,d), and one in the vascular tissue during outgrowth of the root. Short pieces of flanking sequences have been isolated and sequenced for six of the seven lines (Table 6). These sequences will be used for searching sequence databases for *Arabidopsis*, poplar and other plant species. If matching sequences are found, they may allow us to hypothesize function. When the entire poplar genome sequence becomes available, the vast majority of these sequences should become informative. Tagged genes that are judged the most promising, based on expression patterns and sequence information, will be chosen for further study. First, the expression of the native gene will be tested to confirm that it matches the pattern

in the trap line. Then, transgenic lines can be produced to over-express the gene or abolish its expression, so its effect on root formation can be tested directly.

Table 6. GUS expression patterns and flanking region isolation results for seven gene and enhancer trap lines in poplar.

Transformed Line	GUS Expression Pattern	Flanking Region Sequenced
Ifg3-239	Emerging root primordium (ERP) and root tip	452 bp upstream
Ifg3-357	ERP, root tip and base of new root	167 bp upstream,
Ifg4-304	Stem base before root primordium formation, ERP, root tip and base	79 bp upstream, 151 bp downstream
Ifg4-29-1	ERP, root tip and base	318 bp upstream
Ifg4-537	ERP, root tip and base	None
Ifg4-511	ERP, root tip and base	126 bp upstream
Ifg4-30	Vascular tissue of new root	63 bp upstream

The role of NAC1 in root formation. Another approach to finding tree genes that function during adventitious root formation is to use information about homologous genes from other species. In *Arabidopsis*, a gene called NAC1 has been found to play a critical role in lateral root formation. NACs are a family of plant-specific transcriptional regulators and the NAC1 gene is root-specific. In mutant plants deficient in NAC1, lateral root formation is suppressed, and the phenotype is restored when the plant is transformed with a functional 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.

We have cloned a gene from poplar roots that has strong similarity to the *Arabidopsis* NAC1. The poplar gene contains the conserved "NAC domain" that is a feature of all the NACs (Figure 17). In addition it has strong similarity to the *Arabidopsis* NAC1 in the presumptive root-specific region outside of the NAC domain. In this region, 46% of the NAC1 amino acids are identical with the poplar NAC, as compared to only 19% identical amino acids with another NAC from Arabidopsis that is expressed in shoot meristems. This suggests that the poplar NAC may function in controlling root formation in poplar. Experiments to test the expression pattern of the poplar NAC are underway. If it is expressed in a manner consistent with a function in root formation, then transgenic poplars with altered NACs will be prepared to test its role in rooting of poplar.

```
            1                                                          60
POPNAC1     ~~MIEVDLNK CGPWDIPETA CVGGKEWYFY SQRDRKYATG LRTNRATASG YWKATGKDRH
 AtNAC1     LVLIQVDLNK CEPWDIPKMA CVGGKDWYFY SQRDRKYATG LRTNRATATG YWKATGKDRT
 AtNAMc     AAIGQADLNK NEPWDLPKIA KMGEKEFYFF CQRDRKYPTG MRTNRATVSG YWKATGKDKE

            61                                                        110
POPNAC1     ILR.KGTLVG MRKTLVFYQG RAPKGKKTDW VMHEFRLEG. .PVLGPPKTS LE..KEDWVL
 AtNAC1     ILR.KGKLVG MRKTLVFYQG RAPRGRKTDW VMHEFRLQG. .S.HHPPNHS LSSPKEDWVL
 AtNAMc     IFRGKGCLVG MKKTLVFYTG RAPKGEKTNW VMHEYRLDGK YSYHNLPKTA ....RDEWVV

            111   *                                                   160
POPNAC1     CRVFYKN...  .....TREVV AKPSIRSCYN DTGSSSLPAL MDSYITFDQT QPNL....DE
 AtNAC1     CRVFHKN...  .....TEGVI CRDNMGSCFD ETASASLPPL MDPYINFDQE PSSY..LSDD
 AtNAMc     CRVFHKNAPS TTITTTKQLS RIDSLDNIDH LLDFSSLPPL IDPGF.LGQP GPSFSGARQQ

            161                                                       210
POPNAC1     H.....EQVP CFSIF..SQI QT.NQNFPYI TQMEVPNLPT KGTGPF..GQ VPVNITTPSD
 AtNAC1     HHYIINEHVP CFSNL..SQN QTLNSNL..T NSVSELKIPC KNPNPLFTGG SASATLTGLD
 AtNAMc     HDLKPVLHHP TTAPVDNTYL PTQALNFPYH SVHNSGSDFG YGAGSGNNNK GMIKLEHSLV

            211                                                   266
POPNAC1     SF.SCDTKVL KAVLNHLNMM ESNANIKGSP SLGEGSSESY ..LSDVGMPN .LWNHY
 AtNAC1     SFCSSDQMVL RALLSQLTKI DGSLGPKESQ SYGEGSSESL ..LTDIGIPS TVWNC~
 AtNAMc     S.VSQETGLS SDVNTTATPE ISSYPMMMNP AMMDGSKSAC DGLDDLIFWE DLYTS~
```

Figure 17. Amino acid sequence comparison of poplar and Arabidopsis NAC-domain proteins. The highly conserved NAC-domain precedes the asterisk. NAC1 (AtNAC1) is expressed in roots and controls lateral and adventitious root initiation in Arabidopsis. The region following the asterisk is thought to be the root-specific portion of NAC1. The poplar NAC gene (POPNAC1) is more similar to NAC1 than either is to a shoot-meristem NAC gene (AtNAMc) from Arabidopsis. Identical amino acids are shaded in dark gray and similar amino acids shaded in light gray.

Regulation of pine auxin-induced genes. We have been continuing the study of the regulatory region (promoter) of the pine auxin-induced gene, LPEA1. Understanding the regulation of this gene should help us understand the different developmental programs found in roots that form lateral roots, juvenile shoots that form adventitious roots, and mature shoots that have lost the ability to form adventitious roots. Previous experiments have shown that there are elements in the far-distal region of the promoter that control differential expression in roots and shoots. Upon removal of this distal promoter region, gene expression was greatly diminished in roots, but unaffected in hypocotyls.

Carmen Lanz-Garcia, Research Analyst, is conducting experiments to further pinpoint the areas responsible for the interaction. Using the LPEA1 promoter sequence to search the Plant Cis-Acting Regulatory DNA Elements (PLACE) database (1999), she found that the distal promoter region contains a number of possible "root motif" elements. These are root-specific elements originally identified in the *rolD* promoter of *Agrobacterium rhizogenes*, the soil organism that causes hairy root disease–a proliferation of adventitious roots. We have engineered new gene constructs that contain various combinations of the identified promoter regions. These have been introduced into transgenic tobacco plants and will be used in GUS reporter assays to identify the mechanism of tissue-specific gene activation. It will be informative to test whether root-specific promoter elements continue to function in juvenile stems during adventitious root formation.

The same database identified numerous possible light-regulated elements in the promoter. Current research is beginning to draw a link between light- and auxin-responsive gene regulation. It appears that, in some cases, phytochrome and auxin signaling pathways are interrelated and it has been found that phytochrome can moderate the expression of some auxin-regulated genes. This is another example of how roots and shoots react differentially to stimuli. We are currently performing experiments to analyze the expression of LPEA1 in response to light in various plant parts, in coordination with elongation and rooting assays. These experiments should help us understand whether the light responsive and adventitious root formation pathways are antagonistic, complementary or unrelated and provide further clues as to what constitutes a shoot that is competent to form adventitious roots.

SUPPORTING COMPANIES IN 2001

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ROOTED CUTTING PROGRAM STAFF

Barry Goldfarb, Director
Carmen Lanz-Garcia, Laboratory Research Analyst
Anthony LeBude, Research Assistant and PhD student
Matt Gocke, MS student (joint with Hardwood Research Cooperative)
Patrick Cumbie (joint with Tree Improvement Program)
Qian Wu, MS student
Frank Blazich, Collaborating faculty
Steve McKeand, Collaborating faculty
Bailian Li, Collaborating faculty
Tim Mullin, Collaborating faculty

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