NORTH CAROLINA STATE UNIVERSITY
LOBLOLLY AND SLASH PINE ROOTED CUTTING PROGRAM

Annual Progress Report
October 16, 2004

EXECUTIVE SUMMARY

The NC State University Loblolly and Slash Pine Rooted Cutting Program is completing its 13th year of existence. We are in the fourth year of a four-year term that will conclude the third phase of research that began January 1, 2001. The fourth renewal phase of research will begin in 2005 with great potential for advancement in the implementation of clonal forestry. Our mission is to conduct research and technology transfer activity to assist members in the production and deployment of rooted cuttings on an operational scale.

Operational production: the **Vapor Pressure Deficit** study is both a culmination of the last phase of research and commencement of the new phase. Results support our earlier findings that moderate amounts of water deficit stimulate adventitious root formation. On a real-time basis, vapor pressure deficit can be used to signal the frequency of mist application that manages the necessary moderate water deficits for adventitious root formation, thus freeing the propagator from daily adjustments to mist application due to weather fluctuations. This is the basis of the **Multi-Cooperator Vapor Pressure Deficit Study** beginning in 2005. Vapor pressure deficit will be used to signal mist application frequency in a number of rooting environments in the southeast. This will allow more control of the rooting environment over a larger scale and has the potential to provide a propagation “cook book” that will be applicable across production systems. The **Hedge Nutrition Study** will provide critical foliar nutrient concentrations for a suite of macronutrients as well as determine the optimal ratios between these macronutrients and one, elevated, optimal nitrogen concentration. In the initial round of cutting harvests and rooting trials, treatments were administered for approximately four weeks prior to collecting stem cuttings, but this was not enough time to induce large differences in foliar nutrient concentrations. Stem cuttings of all three sources, Atlantic and Western Gulf loblolly pine, and slash pine, had moderately high rooting percentages after receiving all of the nutrient concentrations. Nutrient treatments were applied beginning July 2004 and will continue until stem cuttings are collected for the next experiment in February 2005.

Research for clonal forestry: The **hedge and clone maturation study** is testing the effect of age and serial propagation on maintaining juvenility in loblolly pine. As reported in previous years, rooting declined slightly after the first two to three years, but no further decline in rooting occurred through 11 years of age. There was a similar trend for primary needle length of stem cuttings, that is, needle length declined after age 2 or 3, but did not shorten further through age 11. A field test containing rooted cuttings, from clones aged 2 through 8 years old, and seedling checks was planted January 2002. After two years of field growth, there was a slight difference in total height that probably reflects differences in initial planting sizes. Height growth increment, however, did not differ between seedlings and

rooted cuttings at any age. Trees in the **Clonal Selection Study** are finishing their sixth growing season. Similar to previous analyses, heritabilities for growth characteristics and rust resistance remain high for both field sites. Form traits were measured this year and heritabilities were moderately high for some of these traits as well. These results suggest that form traits should be considered in addition to growth traits when selecting clones, and considerable improvement in growth and form can be achieved through selection and deployment. After the third growing season, the top 10% of all clones in each family were selected for height and volume. Stem cuttings from these clones were rooted in Winter 2004 and will be planted in the **Clonal Rank Verification Study** this fall to determine the robustness of clonal rank in two independent field tests containing all eight families.

INTRODUCTION

This is the last year of the four-year third phase of the Rooted Cutting Program. In 2004, we concluded a number of research studies necessary to fulfill the third phase of research while simultaneously preparing for the fourth phase. The renewal proposal for the fourth phase of research was presented in Atlanta last spring and has been accepted; the research required to meet those goals has begun. The next phase of the Rooted Cutting Program is marked by a cooperative spirit among the members. Four field tests, critical to the development of clonal forestry, will be planted in 2004/2005 on land from four separate members. At the annual meeting this year, we will visit the nursery and rooted cutting production system of MeadWestvaco.

The year began with five members and, at the current time, five members remain committed to the next phase of the program. The largest change occurred in Raleigh in the Rooted Cutting Program staff. Barry Goldfarb, Lead Scientist and Director since 1993, was promoted to Professor and Head of the Department of Forestry. All of us are grateful for his long-term commitment to the Rooted Cutting Program, and his ability to analyze complex problems, discuss the concerns and consequences with interested stakeholders, and then articulate plausible, comprehensive solutions underscored by scientific principle. Barry conceived completely, or in part, many of the experiments that continue today; the maturation and serial propagation study, for example, is the most comprehensive long-term study of its kind with any species anywhere in the world. Additionally, his keen sense of observation, ability to assess various situations, and knack for implementing workable solutions are remarkable. Barry has also shown a deep commitment to the cooperators and to the development of the people that work in the program. These qualities coupled with a devotion to the solutions he has implemented, bind him to this program for the foreseeable future. He will be missed, and we wish him continued success, but frankly, he will not be able to stay away.

The Department of Forestry continues to support the efforts of the program and is actively seeking a new faculty member to assume the duties of Director of the Rooted Cutting Program. Meanwhile Anthony LeBude, Research Assistant for the program since 1998, has assumed the role of Interim Director.

This report includes summaries of experiments conducted or analyzed since the last progress report in November 2003. Additional details will be presented and discussed at the Annual Meeting on October 26-27, 2004 in Summerville, SC, hosted by MeadWestvaco. Hope to see you there!

Anthony LeBude
Interim Director

RESEARCH FOR OPERATIONAL PRODUCTION

Vapor pressure deficit for controlling the rooting environment

Producing stem cuttings for scale up will require cost-efficient, effective control of the rooting environment. Our goal has been to identify the critical environmental factors that affect adventitious root formation and use those factors to aid in the design of suitable rooting environments. Previously, Anthony LeBude reported that rooting percentage was related to mean daily cutting water potential (ψ_{cut}) (Annual Report 2002). Additionally, it was reported that vapor pressure deficit (VPD) in the rooting environment was correlated with rooting success (Annual Report 2003). Because VPD can be automatically monitored by measuring temperature and relative humidity (RH), it is possible that VPD could be used to trigger mist application frequency and maintain the levels of ψ_{cut} necessary to increase rooting in stem cuttings Therefore, a new experiment was initiated in Spring 2004 to use VPD as a mechanism for controlling the frequency of mist application.

Four VPD threshold treatments were created using four separate temperature and relative humidity (RH) probes (50Y QCOM, Corp.) connected to an environmental software program (GEM3, QCOM, Corp.). Air temperature and RH were measured in one replication of each treatment and VPD was calculated by the program. By utilizing some grower information specific to the crop, the software program calculated the amount of predicted water loss as a function of VPD level within each treatment. Treatment thresholds were set to 8.1 mL m^{-2} (0.0018 gal/yd^2), 21.7 mL m^{-2} (0.0048 gal/yd^2), 35.3 mL m^{-2} (0.0078 gal/yd^2), or 67.9 mL m^{-2} (0.0150 gal/yd^2) of accumulated water loss. When accumulated water loss reached the threshold specific for each treatment, mist was delivered and the accumulation was reset to zero. Therefore, higher VPDs within a treatment caused more rapid accumulation of predicted water loss from stem cuttings and triggered more frequent misting events. When mist application was triggered for a specific treatment, the boom was sent through the greenhouse to deliver 120 mL m^{-2} (0.0076 gal ft^{-2}) of mist per pass to that treatment plot in each replication. Threshold treatments were created based on the timing between misting events utilized by our last mist frequency program, which was based solely on RH (for details see LeBude et al., 2004). Treatments with the lowest thresholds of accumulated water loss (i.e. 8.1 mL m^{-2}) received more frequent mist application than those with higher thresholds.

Spring cuttings from two full-sib families [used previously by LeBude (Annual Reports 2001, 2002, and 2003)] were collected on June 30, 2004 and stored at 39 F (4 C) in insulated coolers in a cold room until setting on July 11, 2004. Cuttings were re-cut to 9 cm and dipped for 3 s in 2.5 mM NAA. Cuttings were set in a rooting medium of either fine silica sand in large rooting tubs (91.4 cm L x 61.0 cm W x 30.5 cm D) or in peat: perlite (2:3, v/v) in Ray Leach Super Cells (169 ml) that served as the control. All rooting tubs were watered once every 90 minutes during the day or once per 2 hours at night using a sub-irrigation system placed on the surface of the sand within the tubs. Soil water potential was not measured in tubs during rooting nor was it used to trigger subirrigation in this experiment. The controls were hand watered once or twice during rooting to apply a pesticide drench to control fungus gnats. The experimental design was a split plot with VPD threshold level as the whole plot factor and rooting container as the sub-plot factor. The design contained three replications.

Cutting water potential was measured destructively on one cutting per rooting container per plot (24 cuttings total at each measurement period) between 2 and 4 PM each week for 5 weeks after setting. Border cuttings were used to replace sampled cuttings to maintain canopy dynamics during rooting. Rooting was recorded 10 weeks after setting. VPD was recorded every 15 minutes (although the computer monitored it continuously) and averaged for the first five weeks after setting for each hour to show mean hourly VPD. In addition, VPD was averaged only for the hours that provided the most meaningful relationships between VPD and other variables (as indicated in the figures).

Table 1. ANOVA for cutting water potential (Ψ_{cut}) and rooting percentage of spring stem cuttings rooted under four VPD treatments in July 2004. Ψ_{cut} was averaged over the first five weeks and rooting was recorded 10 weeks after setting. Values in bold are significant at $P \leq 0.10$.

	Ψ_{cut}		Rooting (%)	
Source	df	P>F	df	P>F
Date	4	**0.06**	--	--
Replication	2	**0.04**	2	**0.06**
Treatment	3	**0.01**	3	**0.06**
Container	1	**0.10**	1	0.55
Treatment X Container	3	0.28	3	0.11

The largest differences in VPD among threshold treatments occurred during late morning and afternoon. For example, between 1000 HR and 1800 HR, VPD was lowest for treatments 1 and 2 (0.30 kPa) and highest for treatment 4 (0.75 kPa) (Fig. 1). VPD in treatments 1 and 2 were very similar, so treatment thresholds will be adjusted in future experiments. There were only minor VPD differences among threshold treatments before 1000 HR and after 2000 HR. Ψ_{cut} was affected by VPD treatment and container type, but not by their interaction (Table 1). Mean afternoon Ψ_{cut} in both container treatments was strongly related to the mean VPD averaged for 1300 to 1700 HR (Fig. 2), although, on average, cuttings in the Ray Leach tubes experienced slightly more water deficit at a given VPD. Perhaps the differences in available substrate moisture between the rooting tubs and the Ray Leach tubes account for the differences in Ψ_{cut}.

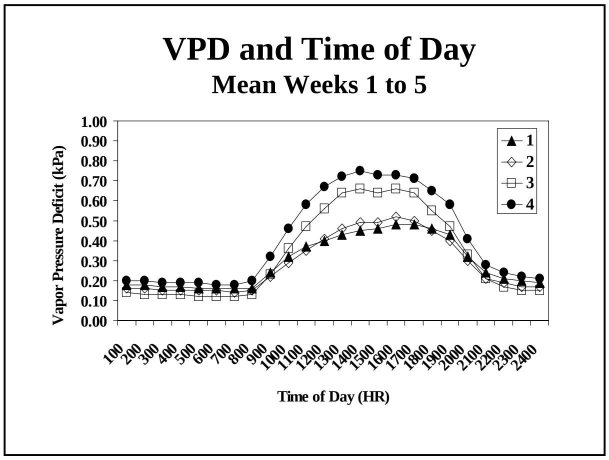

Figure 1. Mean vapor pressure deficit (VPD) by treatment for each hour averaged over the first five weeks after setting spring stem cuttings July 2004.

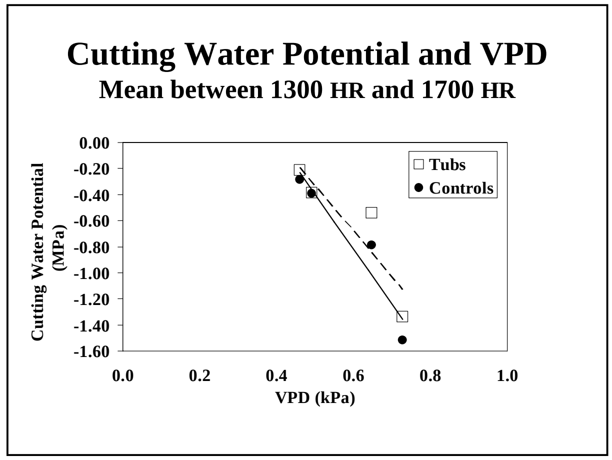

Figure 2. Relationship between mean afternoon cutting water potential (Ψcut) and mean vapor pressure deficit (VPD) between 1300 and 1700 HR over the first five weeks after setting stem cuttings in tubs (open symbols and dashed line) or Ray Leach tubes (control) (solid symbols and solid line). The regression equations are Ψ_{cut} (control) = -4.19VPD + 1.70, P=0.01, r^2 = 0.91, Ψ_{cut} (open tubs) = -3.50VPD + 1.42, P=0.01, r^2 = 0.80.

Rooting percentage over all treatments and containers was 35.7%. The relatively low rooting percentage was most likely due to the late sticking date, caused by the time required to reconfigure hardware and software for the VPD-based mist application system. Nevertheless, the relationship between rooting percentage of stem cuttings in tubs and mean ψ_{cut} was similar to previous studies. Rooting was highest (60%) for cuttings with

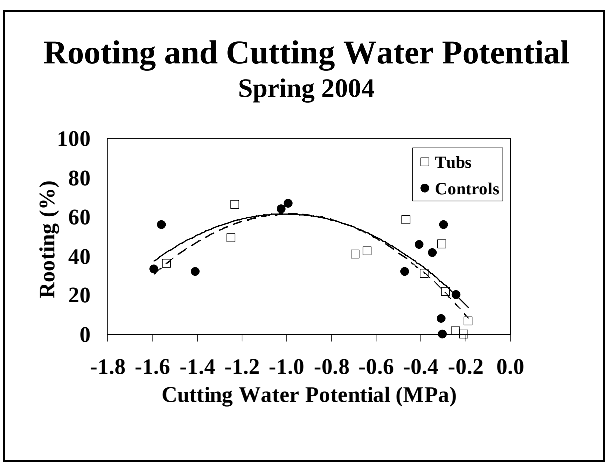

Figure 3. Relationship between rooting percentage and Ψ_{cut} for spring stem cuttings rooted in tubs (open symbols – dashed line) or Ray Leach tubes (control) (solid symbols – solid line) rooted under four vapor pressure deficit treatments. Regressions equations are Rooting % (tubs – dashed line) = - 27.60 - 202.44(Ψ_{cut}) – 100.33 (Ψ_{cut}^2), P=0.01, r^2=.071, and Rooting % (control – solid line) = - 12.07 – 155. 94 (Ψ_{cut}) – 76.70 (Ψ_{cut}^2), P=0.14, r^2=0.35.

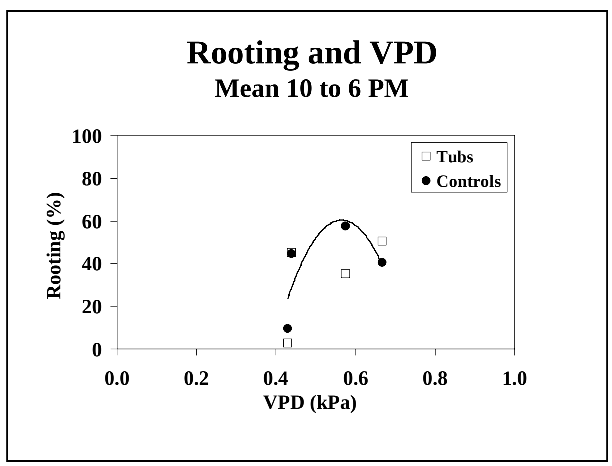

Figure 4. Relationship between rooting percentage and vapor pressure deficit (VPD) for spring stem cuttings rooted under four VPD treatments in tubs (open symbols) or Ray Leach tubes (control) (solid symbols – solid line). Regression equations are Rooting (%) (control – solid line) = - 578.54 + 2262.9VPD - 2003.6VPD^2 P = 0.01, r^2 = 0.63, no significant regression for rooting tubs.

intermediate Ψ_{cut} values between -0.8 and -1.1 MPa (Fig. 3). Interestingly, a fairly similar relationship was observed in cuttings rooted in tubs and Ray Leach tubes. Rooting of stem cuttings in Ray Leach Tubes was moderately related to VPD, but this relationship was not significant for cuttings rooted in tubs (Fig. 4). The nearly significant threshold treatment by container interaction for rooting percentage (Table 1) suggests that the ideal VPD might depend on the particular rooting substrate and type of propagation system used to root cuttings. Taken together, these data suggest that VPD can be used to determine the frequency of mist application, however, this principle needs to be tested in various propagation systems and rooting environments to test broad applicability.

Multi-cooperator vapor pressure deficit study

A voluntary, multi-cooperator rooting experiment is being planned for 2005 to test VPD control of mist application in a number of propagation systems and seasons. The objectives of the experiment are to: (1) test the feasibility of VPD-based irrigation control, (2) determine the optimal range of VPD accumulation threshold values, and (3) determine if optimal thresholds vary across propagation environments and systems. Each participating cooperator will use the same VPD threshold values to control mist application in their individual propagation environment. To ensure that results can be meaningfully compared across sites, stem cuttings from one full-sib family will be provided by MeadWestvaco for use in all propagation sites. Cooperators will also root their own material under the same irrigation conditions. The details of the experiment will be discussed at the Annual Meeting.

Hedge Nutrition

A study was initiated in December 2002 to determine the effects of stock plant fertility on stem cutting production and subsequent rooting. Sara Millar, MS student in Horticultural Science (advised by Frank Blazich), is taking lead on this study. Previously, we found that rooting in stem cuttings was enhanced when foliage concentrations of nitrogen (N) in stem cuttings were at 2% or above at the time they were severed from the hedges. Initially, it was thought that all stem cutting nutrient concentrations should be higher than recommended for nursery grown seedlings, however, the requirements for boron were found to be similar to those of seedlings. Therefore, the current experiment is testing the effect of five ratios of a suite of the other macronutrients [phosphorous (P), potassium (K), magnesium (Mg), calcium (Ca), and sulfur (S)] to 2% N on stem cutting production of hedges and the subsequent rooting of stem cuttings.

Seeds of four unrelated full-sib crosses from each of three sources of pine – slash, Atlantic Coast loblolly, and Western Gulf loblolly (provided by Boise, Plum Creek, and Temple Inland, respectively) were germinated in December 2002. In August 2003, 80 healthy seedlings per cross were chosen randomly and potted in 3 gal. containers containing a medium of perlite:sand (3/2, v/v). Hedges were placed in a randomized complete block design containing four replications of five nutrient ratios. Four plants from each cross were placed in each treatment per replication for a total of 960 hedges for the experiment (12 crosses x 4 plants per cross x 5 nutrient treatments x 4 replications = 960).

Fertilizer treatments are five ratios of macronutrients to the one level of N. The "standard," intermediate treatment (X) will apply macronutrients to obtain levels of tissue concentrations of P= 0.2%, K=1.0%, Ca=0.3%, Mg=0.1%, and S=0.08%. The remaining four ratios will be 0.5X, 1.5X, 2.0X, and 2.5X, that is, one lower ratio and three higher ratios. Micronutrients will remain constant for all treatments. Hedges received the standard treatment from August 2003 until approximately April 1, 2004 (six weeks after hedging in Feb. 2004), when the individual treatment applications began.

The total number of stem cuttings produced by each hedge that were suitable for rooting were counted prior to collection. Then, nine stem cuttings were collected from each hedge May 7, 2004. Three stem cuttings per hedge per cross per treatment per replication (3 cuttings x 4 hedges = 12 cuttings per plot) were randomly selected for nutrient analysis. These twelve cuttings were oven-dried at 70°C (150°F) for 72 hours and then ground to a mesh size < 1 mm. A sub-sample of this tissue from the twelve crosses was pooled for each source across replications (15 total samples, 3 sources x 5 treatments = 15) and sent to A&L Laboratories to determine treatment efficacy, and to provide early feedback for treatment calibration during the summer/fall growing season, prior to collecting stem cuttings again in February 2005. The rest of the tissue was stored for subsequent full nutrient analysis according to the experimental design. The remaining 24 cuttings for each cross [(6 cuttings per hedge x 4 hedges per cross per plot = 24)(5760 total)] were set in Ray Leach Super Cells containing a medium of peat:perlite (2:3 v/v) and misted intermittently using our boom system and standard misting regime as controlled by QCOM. Rooting percentage, number of new roots, and root system symmetry were recorded after 12 weeks.

Preliminary nutrient analysis from A&L showed no large differences in nutrient concentrations among the treatments. Foliar concentrations of nutrients reflected the standard treatment hedges had received during the previous eight months. Apparently, applying the treatments for only one month before cutting harvest was not sufficient to cause differences in foliar nutrient concentrations. Based on this result, we decided not to conduct the full nutrient analysis on the remaining tissue and to apply treatments earlier in the summer/fall growing season of 2004.

Table 2. ANOVA for stem cutting production, rooting percentage, and root number per rooted cutting for spring stem cuttings collected from hedges receiving five mineral nutrient solution treatments and then rooted in June 2004. Values in bold are significant at $P \leq 0.10$.

		No. Cuttings	Rooting (%)	No. Roots
Source	df	P > F	P > F	P > F
Rep	3	**0.01**	**0.01**	**0.01**
Treatment	4	0.71	0.97	0.93
Source	2	**0.01**	**0.01**	**0.01**
Fam(Source)	3	**0.01**	**0.07**	**0.01**
Treatment X Source	8	0.92	0.92	0.73
Treatment X Fam(Source)	12	0.85	0.83	0.89

Nevertheless, we have a baseline on cutting production and rooting of the three sources in response to the standard treatment. Slash pine hedges produced more cuttings per hedge, 16.6, than Atlantic Coast loblolly, 11.7, or Western loblolly, 12.8, which were similar (Table 2). There was more family variation in the number of cuttings produced in the slash and Western Gulf sources than in the Atlantic source (Fig. 5). As this was the first meaningful harvest from these hedges, cutting productivity by families and sources could change and we expect the overall cutting production to increase in subsequent cutting harvests.

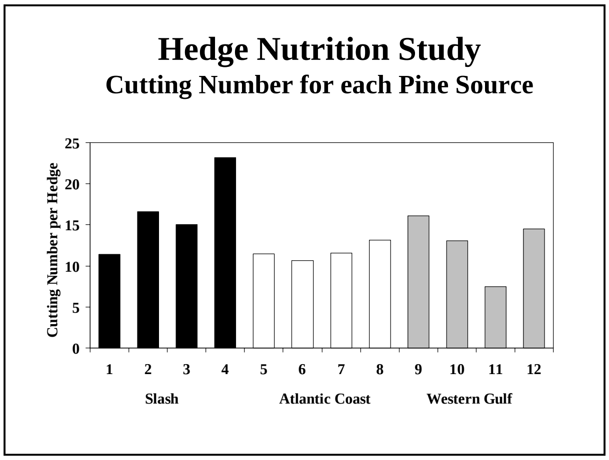

Figure 5. Number of stem cuttings produced per hedge for four crosses each of Slash pine (black bars), Atlantic Coast loblolly pine (white bars) and Western Gulf loblolly pine (grey bars). Cuttings were counted prior to collection in May 2004.

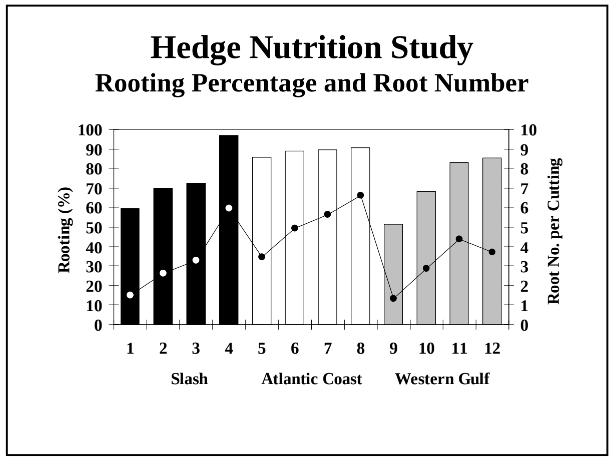

Figure 6. Rooting percentage and number of roots per rooted cutting for spring cuttings of four crosses each of Slash pine (black bars), Atlantic Coast loblolly pine (white bars) and Western Gulf loblolly pine (grey bars). Cuttings were rooted in May 2004.

Overall rooting percentage for the experiment was 78.1%. Stem cuttings of Atlantic Coast loblolly pine rooted consistently higher (88.9%) than slash pine (74.6%) or Western Gulf loblolly pine (70.9%), which rooted similarly (Table 2). Families of the Atlantic Coast source all rooted between 85% and 90%, whereas the range was between 50% and 96% for the other two sources (Fig. 6). Stem cuttings of one family of slash pine, however, rooted consistently higher than all families of the Atlantic Coast source (Fig. 6). Root number per cutting followed a similar pattern as rooting percentage (Table 2 and Fig. 6). Rooted cuttings from the Atlantic Coast source had 5.2 roots per cutting, which was higher than either the slash source, 3.4, or the Western Gulf source, 3.1, which were similar.

Hedges were pruned in mid-July 2004 and nutrient treatments were applied immediately and will continue for the remainder of the experiment. Stem cuttings will be collected again in February 2005 and the same procedures for stem cutting production measurement, nutrient analyses and rooting will be followed.

RESEARCH FOR CLONAL FORESTRY

Hedge and clone maturation study

The hedge and clone maturation study was initiated in 1993 to test the effectiveness of hedging and serial propagation for maintaining juvenility in stock plants of loblolly pine. Each year since its inception, 20 seedlings from three open-pollinated families were produced and maintained as containerized, hedged stock plants. Two years after germination and hedging, when stock plants first became established, stem cuttings were rooted from these stock plants to form more recent, serially propagated stock plants. These are referred to as the first-cycle serial hedges (1s). Two years after first-cycle serial hedges were established, stem cuttings were rooted from those plants to form the second-cycle serial hedges (2s). Subsequently, serial propagation has been performed every two years. In all rooting experiments, cuttings from only the latest cycle of serially propagated hedges were included and compared with those from the most recent 1-, 2- or 3-year-old seedling hedges. This year, we tested cuttings from clones ranging from 2 (seedling hedges) to 11 years old (fourth-cycle serial hedges, 4s). Primary needle length of stem cuttings, another indication of the phase change from juvenile to mature, was also measured this year on winter cuttings. We also report on a field test that was planted to test the effect of clone age on growth of the derived rooted cuttings. The field test includes rooted cuttings from clones 2 through 8 years old, as well as seedlings from the same three families. Results on height growth after two seasons are presented.

Winter 2004

Rooting percentage across all ages and families was 67%. Rooting percentage was highest for cuttings from 2-year-old seedling hedges (78%) and 9- (79%) or 10- (79%) year-old, fourth-cycle serially propagated hedges (Fig. 7). Cuttings from hedges aged 4, 6, or 8 years old rooted higher (~ 70%) than cuttings from 3-year-old seedling hedges or 5-, 7-, or 9-year-old (~ 50%), serially propagated hedges. This is not an age trend, per se, but appears to be a production cycle anomaly that caused variation in plant vigor. Serially propagated hedges for seeds germinated in odd numbered years, for example 1995, 1997, 1999, etc., and the original seedling hedges for 2001 were all potted or germinated in 2001. This corresponds to hedge ages 3, 5, 7, and 9 in Fig. 7. That year, we potted everything, mistakenly, in relatively non-composted shredded pine bark. Last year, we lost an entire experiment with those cuttings (Winter 2003) and many of those hedges died this year after hedging in winter and spring. The even-numbered years on this graph and the 11-year-old hedges that were potted in 2002, instead of on schedule in 2001, were all potted in fully composted pine bark and represent a more realistic rooting percentage. The overall age trend in this year's experiment is consistent with trends we have seen in previous years. That is, after an initial drop in rooting percentage after age 2, there appears to be no further decrease in rooting percentage with clone age, at least through 11 years of age. In fact, in this experiment, the cuttings from clones 10 and 11 years of age rooted as well as the youngest seedling hedges. This continues to be surprisingly good news for the maintenance of rooting ability by hedging and serial propagation. The three families rooted similarly as in years past. Stem cuttings from family 7 rooted the highest at 77%, family 3 rooted intermediately at 66%, and family 9 rooted the lowest at 55%.

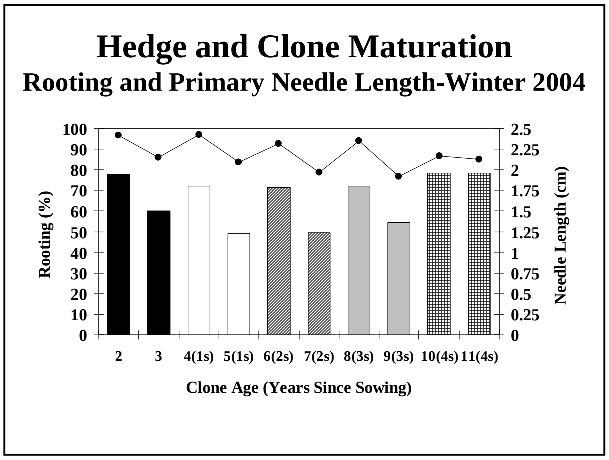

Figure 7. Rooting percentage and primary needle length of stem cuttings collected from 2-11 year old hedges in Winter 2004. Black bars=seedling hedges, and white, diagonal hatch, grey, and cross hatch bars= hedges from one, two, three, or four cycles of serial propagation, respectively.

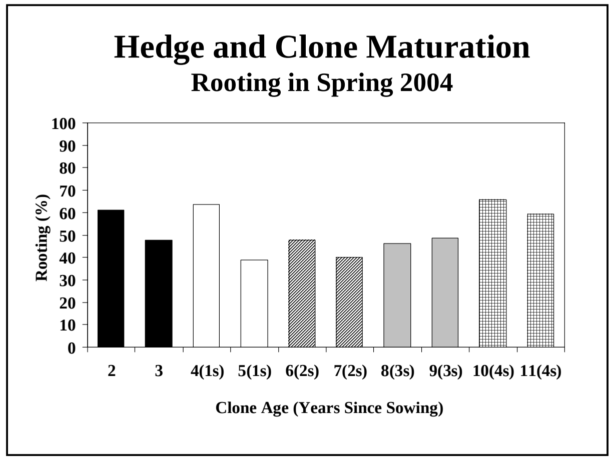

Figure 8. Rooting percentage and primary needle length of stem cuttings collected from 2-11 year old hedges in Winter 2004. Black bars=seedling hedges, and white, diagonal hatch, grey, and cross hatch bars= hedges from one, two, three, or four cycles of serial propagation, respectively.

Primary needle length

Prior to setting cuttings in Winter 2004, two stem cuttings from each clone were randomly selected from those that were to be set. These cuttings were stored in insulated coolers for two weeks. Two recently expanded needles from each stem cutting were measured and averaged to provide the mean length of primary needles for each clone. Length of primary needles ranged from 1.9 to 2.5 cm. As age increased, there was a slight decline in needle length; however, the amount of decrease between 2 and 11 year old hedges was less than 0.5 cm (Fig. 8). Interestingly, needle length variation in relation to odd-numbered hedges followed the same general trend as rooting percentage. This underscores the significance of production practices to produce vigorous plants and the subsequent effect on morphology and rooting percentage of stem cuttings.

Spring 2004

Rooting percentage across ages and families was 54.9%. Rooting percentage was highest for stem cuttings from seedling hedges aged 2 (60%) and serially propagated hedges aged 4 (65%), 10 (65%) and 11 (60%) (Fig. 8). Cuttings from seedling hedges aged 3, and serially propagated hedges aged 5-9 all rooted approximately 45%. Although the variation in rooting percentage between hedges from even and odd numbered years in winter cuttings decreased, this was due largely to a 10-20% drop in rooting for hedges aged 4, 6, and 8 years, rather than an improvement in rooting for odd aged hedges. The initial drop in rooting percentage after age 3 is evident here again; however, this drop was not as pronounced as in previous years and there seems to be a slight increase in rooting with older aged hedges. Family 7 rooted the highest at 65%, however for the first time recently, family 9 rooted higher (54%) than family 3 (44%). Overall, cuttings in winter and spring rooted 60% or higher in hedges aged 10 and 11. The importance of hedge vigor is apparent in these data and the ongoing hedge nutrition study will provide necessary recommendations for preserving good vigor and maintaining high rooting over long periods.

Field Test 1

A field test was planted by Plum Creek Timber Company near Holly Hill, SC., on January 31, 2002, to test the effect of clone age on growth of rooted cuttings compared to seedlings. The test was a randomized complete block design with four blocks, seven ages (2 through 8 years old), and three families (3, 7, and 9). Each family contained approximately 14 clones per age represented by one ramet, per clone, per block (single tree plots). Six seedlings of the same three families were planted as comparisons (1184 total trees planted). Initial height at planting and height after years 1 and 2 were measured. At the time of planting, cuttings from older-aged hedges were shorter than cuttings from younger hedges. This trend continued after the first growing season, however, height growth increment was the same for all ages (Annual Report 2003). After the second growing season, rooted cuttings from 5- (137 cm), 7- (132 cm), and 8- (129 cm) year-old hedges were smaller than the seedlings (152 cm) (Fig 9). Once again, however, height growth increment was not significantly different among ages (Fig. 10). Whether or not this trend persists will be determined with future measurements and two new field tests.

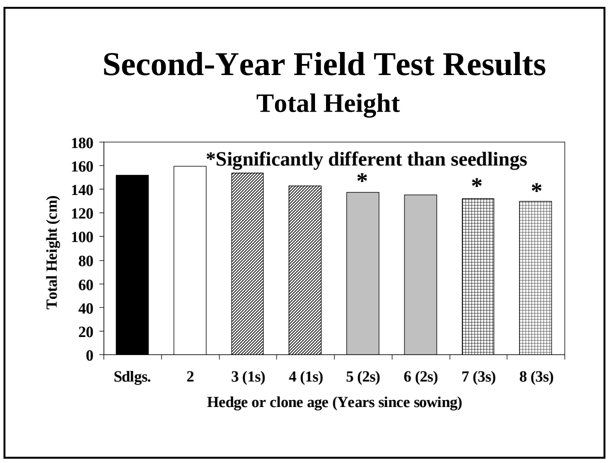

Figure 9. Total height after two growing seasons of seedlings (black bar) or rooted cuttings of seedling hedges (white bar) or hedges that have undergone one (diagonally hatched bars), two (grey bars) or three (cross hatched bars) cycles of serial propagation. Bars with an asterisk are significantly different from seedlings at P≤ 0.05.

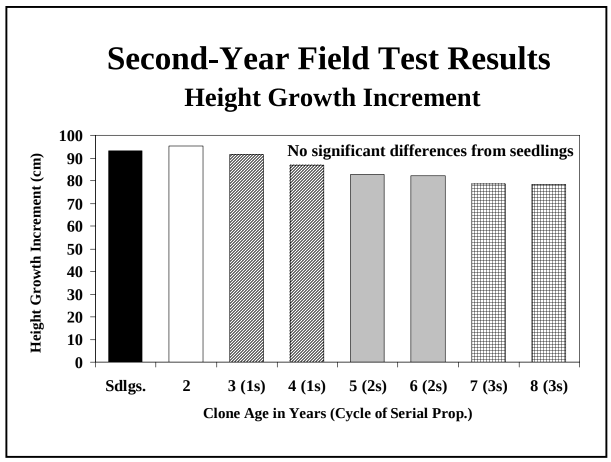

Figure 10. Height growth increment for year two of seedlings (black bar) or rooted cuttings of seedling hedges (white bar) or hedges that have undergone one (diagonally hatched bars), two (grey bars) or three (cross hatched bars) cycles of serial propagation.

Field Tests 2 and 3

Two field tests will be planted in Fall 2004 or Winter 2005 to test the effect of clone age on growth of rooted cuttings compared to seedlings on two independent planting sites. Both tests will contain the same plant material, or very similar plant material depending on availability. Tests are randomized complete block designs with four blocks, 10 ages (2 through 11 years old), and three families (3, 7, and 9). Each family will contain approximately 10 clones per age represented by one ramet, per clone, per block (single tree plots). Six seedlings of the same three families will be used as comparisons (total 1200 trees planted). Test 2 was planted October 2004 on MeadWestvaco land in a bedded lower coastal plain site in South Carolina, quite near field test 1. Field test 3 will be planted on Rayonier land. Height, survival, and rust infection will be measured annually.

Clonal Selection Study

Since its inception in 1996, this study remains a joint project with the NCSU-Industry Cooperative Tree Improvement Program. The objective of the study is to develop information that will facilitate efficient testing and selection of superior clones. From this study we are generating quantitative estimates of: (1) predicted gain from selecting different numbers of clones, (2) the number of ramets per clone 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.

Eight families of the South Atlantic Coastal Plain region were chosen from the Tree Improvement Program's diallel tests based on rapid growth, good rust resistance, acceptable form, availability of seed, and lack of relatedness. Seeds were germinated to produce hedged stock plants of one hundred clones per family. Four-hundred fifty clones remained after initial hedge establishment and subsequent production of sufficient quantities of rooted cuttings for field tests. Rooted cuttings of 168 clones from four crosses were planted December 1998 on International Paper land near Jay, Florida. Rooted cuttings of 282 clones from the remaining four families were planted November 1998 on MeadWestvaco land in South Carolina. Both tests were a randomized complete block design with nine blocks each containing a single ramet per block. Measurements were made on survival, height, and rust infection for the first five growing seasons, diameter after growing seasons four and five, and form traits after the fifth growing season.

To assess form traits, we measured the number of forks and ramicorn branches per tree, average branch angle, and bole straightness. Ramicorn branches were scored if a branch grew at an acute angle to the main stem while penetrating three subsequent whorls. Average branch angle was estimated in the butt log by assessing 3 branches in the same general ordinate of each tree. A branch whorl at approximately 2 meters was selected, and a prominent branch pointing towards the north was selected. A prominent branch in the closest whorl above and the closest whorl below this first branch whorl was also selected. A clear protractor with lines marked at 15° intervals was held next to the branch, and each of the three branches was given one of the following scores: 1 = 90° flat branch or perpendicular from the stem, 2 = 75°, 3 = 60°, 4 = 45°, 5 = 30°, 6 = 15°. The sum of the 3 branch scores was recorded, and the average branch angle was calculated. Straightness was measured on a scale of 1 to 6, with one being straight and 6 being crooked. Prior to measuring straightness, data recorders walked the entire test site and visually ranked trees based on this scale. Then, during individual scoring of trees, recorders assessed tree straightness relative to the average straightness of the entire site. By this process, straightness values would be normally distributed over the site and comparable across sites.

Previously, we used clone genetic values to predict height gain based on selecting various numbers of clones from each test after age four (Annual Report 2003). Selecting the best one to four clones at both sites would result in approximately 12.5% gain over all the clones planted at each site. Selecting up to 24 clones (approximately 10% of all clones) would result in height gains of 6.4% at FL and 9.2% at SC. If we doubled height gains to predict rotation age volume gains, selecting one to four clones would result in 25% gain in volume at both sites, while selecting up to 24 clones would result in 12% to 20% gain at FL and SC, respectively. Additionally, for the top 10 clones at each site, we used the standard error of the clone genetic values to simulate the gain in volume as the number of ramets increased for each set of clones. Over both sites, gains in volume increased as the number of ramets increased from two to six, suggesting that with good survival, approximately six ramets are adequate to characterize growth of a clone on one site.

After the fifth growing season, mean height was 7.24 m (23.8 ft.) in Fl and 7.37 m (24.2 ft.) in SC (Tables 3 and 4). The height growth increment for the fifth growing season was 1.64 m (5.4 ft.) in FL and 1.86 m (6.1 ft.) in SC. Clone mean heritabilities for height, diameter, and volume at age 5 at both sites were slightly higher than at age 4 (Tables 3 and 4). Heritabilities for growth traits were somewhat higher in SC than FL, as in previous years.

Table 3. Variance components explained by each factor in the model (±SE), repeatability of clone means (H^2_c±SE), coefficient of variation (CV_c) and the site mean (±SD) for growth, rust and form traits at the Florida site at age five.

Source	Height (m)	Diameter (cm)	Volume (dm3)	Rust (0-1)
Family	0.13 ± 0.11	0.25 ± 0.22	12 ± 10.7	1.31 ± 0.030
Clone (F)	0.11 ± 0.02	0.43 ± 0.09	18 ± 3.1	1.62 ± 0.384
Residual	0.44 ± 0.02	2.67 ± 0.11	77 ± 3.3	1.00 ± 3.63
H^2_c ±SE	0.65 ± 0.041	0.56 ± 0.052	0.64 ± 0.043	0.92 ± 0.016
^bCV_c	12	16	36	2.63
Mean ±Std	7.24 ± 0.86	11.6 ± 1.87	29.7 ± 10.7	0.10 ± 0.0083

Source	Straightness (1-6)	Branch Angle (0-100)	No. of Ram. Branches	No. of Forks
Family	0.03 ± 0.03	1.72 ± 1.75	0.00 ± 0.00	0.05 ± 0.07
Clone (F)	0.19 ± 0.03	12.99 ± 2.05	0.12 ± 0.09	0.01 ± 0.12
Residual	77.60 ± 3.3	41.21 ± 1.75	1.00 ± 0.00	1.00 ± 0.00
H^2_c ±SE	0.71 ± 0.035	0.71 ± 0.035	0.48 ± 0.20	0.43 ± 0.31
^bCV_c	26	10	2.44	2.56
Mean ±Std	3.45 ± 0.91	76 ± 7.86	0.22 ± 0.0116	0.15 ± 0.099

Table 4. Variance components explained by each factor in the model (±SE), repeatability of clone means (H^2_c±SE), coefficient of variation (CV_c) and the site mean (±SD) for growth and form traits at the South Carolina site at age five.

Source	Height (m)	Diameter (cm)	Volume (dm3)	Rust (0-1)
Family	0.03 ± 0.025	0.56 ± 0.48	12.5 ± 10.8	0.38 ± 0.35
Clone (F)	0.29 ± 0.031	1.13 ± 0.13	37.9 ± 4.55	2.81 ± 0.33
Residual	0.50 ± 0.016	2.72 ± 0.09	112.4 ± 3.63	1.00 ± 0.00
H^2_c ±SE	0.83 ± 0.017	0.78 ± 0.022	0.74 ± 0.025	0.96 ± 0.005
^bCV_c	12	16	36	1.54
Mean ±Std	7.37 ± 0.90	12.5 ± 0.20	35.3 ± 12.67	0.385 ± 0.009

Source	Straightness (1-6)	Branch Angle (0-100)	No. Ram. Branches	No. Forks
Family	0.12 ± 0.10	1.11 ± 1.00	0.00 ± 0.000	0.02 ± 0.02
Clone (F)	0.21 ± 0.03	5.24 ± 0.74	0.02 ± 0.004	0.05 ± 0.01
Residual	0.60 ± 0.02	27.30 ± 0.86	0.24 ± 0.007	0.16 ± 0.01
H^2_c ±SE	0.75 ± 0.02	0.62 ± 0.04	0.40 ± 0.054	0.71 ± 0.003
^bCV_c	27	10	1.79	1.85
Mean ±Std	3.55 ± 0.97	62.2 ± 6.05	0.23 ± 0.0086	0.18 ± 0.0078

Heritability for rust at SC remained high, suggesting considerable gains for rust resistance from selection of individual clones (Table 3).

Clone mean heritabilities for form traits were moderately high on both sites. The lowest heritability was observed for the number of ramicorn branches per tree (0.40 in FL, 0.48 in SC) (Tables 3 and 4). Bole straightness and branch angle heritabilities were consistent across sites and of similar magnitudes as growth traits. The heritability for the number of forks per tree was higher in SC (0.71) (Table 4) than in FL (0.43) (Table 3). These results suggest that form traits should be considered in addition to growth when selecting clones, and that considerable improvement in form can be achieved. Future analyses will explore various selection indices combining growth and form traits.

Clonal Rank Verification Study

Results from the clonal selection study suggest that considerable gains can be made from selecting and deploying elite clones from the eight full-sib crosses tested. However, these estimates are based on a single test for each clone and it would be useful to verify clonal rankings in independent tests. Based on the data from age three, 112 of the 450 clones in the field tests were selected. Included in this group were the 10% of clones ranking highest for both height and volume from each of the eight crosses. In addition, to obtain a representative sample of clones with different growth rates, clones from the 25th, 50th, 75th, and 100th percentiles from each cross were selected. Hedges of all of these clones were multiplied by serial propagation and cuttings from these hedges were rooted in Winter 2004 for two field tests that will comprise the Clonal Rank Verification Study. One test will be planted on Plum Creek land and the other on Temple-Inland land in Fall 2004 and or Winter 2005. Unlike the previous tests (Clonal Selection Study), all clones will be planted on both sites, allowing us to estimate clone by environment interactions, as well as the correlation of clonal rankings with those in the original tests. The design of the tests will be a randomized complete block, with each clone represented by one ramet in each block (single-tree plots) and *n* blocks per test. Details on the number of blocks, timing of planting, care of the tests, and measurement schedule will be discussed at the annual meeting.

SUPPORTING COMPANIES IN 2003-2004

Boise Corporation
Plum Creek Timber Company
Temple-Inland Forest
MeadWestvaco Corporation
Rayonier, Inc.

ROOTED CUTTING PROGRAM STAFF

Anthony LeBude, Interim Director
Barry Goldfarb, Past Director and Advisor
Carmen Lanz-Garcia, Laboratory Research Analyst
Matt Gocke, Research Assistant and MS student (joint with Hardwood Research Cooperative)
Qian Wu, MS student
Sara Millar, MS student
Frank Blazich, Collaborating faculty
Charles Davey, Collaborating faculty
Steve McKeand, Collaborating faculty
Bailian Li, Collaborating faculty
Fikret Isik, Collaborating faculty
Tim Mullin, Collaborating faculty

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