NORTH CAROLINA STATE UNIVERSITY
LOBLOLLY AND SLASH PINE ROOTED CUTTING PROGRAM

Annual Progress Report
December 2, 2005

EXECUTIVE SUMMARY

The NC State University Loblolly and Slash Pine Rooted Cutting Program is completing its 14th year of existence. 2005 was the first year in a four-year phase that was scheduled to conclude in 2008. However, changes in forest industry make it unlikely that the phase will be completed as planned. Nevertheless, we have completed many of the key research objectives and established field tests to address others. Our mission has been to conduct research and technology transfer activity to assist members in the production and deployment of rooted cuttings on an operational scale. While the use of rooted cuttings to implement clonal forestry is uncertain, we have developed much information to provide that option for our members and generated additional insights into clonal forestry.

Operational production: the **Vapor Pressure Deficit** study is building on the research findings from previous years that VPD during the critical rooting period was correlated with rooting success. In this year's study we attempted to capitalize on this finding by using VPD as a control mechanism for mist irrigation. As with previous studies, we found that intermediate levels of mist resulted in the best rooting. Thus, VPD control appears to be a viable method for controlling irrigation on a mass scale. However, because the optimal VPD control point differed in cuttings in two types of containers, it appears that this control mechanism will have to calibrated for each specific propagation environment. The **Hedge Nutrition Study** is attempting to determine optimal foliar nutrient concentrations for macronutrients in relation to the previously determined optimal nitrogen concentration. During the first year, despite a wide range of fertilization treatments, foliar analysis on cuttings from all three sources, Atlantic Coast and Western Gulf loblolly pine and slash pine, showed no differences in nutrient concentration. Thus, it appears to be unlikely that macronutrients other than nitrogen can be pre-loaded with nutrients to enhance rooting. Because of this result, we modified the study to implement a range of lowered nutrient concentrations to determine minimum threshold values. Slight differences are beginning to appear, with potential correlations with rooting performance.

Research for clonal forestry: The **Hedge and Clone Maturation Study** is testing the effect of clone age and the efficacy of hedging and serial propagation on maintaining juvenility in loblolly pine. 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. In 2005, rooting success appeared to be more related to cultural practices than clone age. Thus, although we cannot rule out some degree of maturated-related decline, we continue to be surprised at the relatively good rooting of clones up to eleven years of age. A field test containing rooted cuttings, from clones aged 2 through 8 years old, and seedling checks was planted January 2002. After three years of field growth, there was a slight difference in total height between seedlings and rooted cuttings from clones greater than three years of age. While some of that difference appears to be related to initial planting

size, a slight trend of declining height growth increment with increasing clone age was detected. Two additional field tests containing rooted cuttings from clones up to eleven years of age were established this year. The **Clonal Rank Verification Study** was initiated this year to determine if similar clonal performance can be expected in independent tests in a range of environments. Rooted cuttings from a subset of approximately 100 clones from the Clonal Selection Study were planted on three coastal plain sites, including North Carolina, Georgia and Texas. Research continued on the **Wood Quality of Clones** on a grant funded by the USDA. A variety of wood property measurements were made on trees from a seedling/rooted cutting (clonal) test established in 1990. All variables measured were strongly influenced by sited differences. However, there was substantial genetic variation for microfibril angle and modulus of elasticity at the clonal level, with high heritabilities. This contrasted with cellulose and lignin contents, and fiber length and coarseness, which showed little meaningful genetic variation in this test. Finally, a new research grant from NSF, **Association Genetics of Wood Properties and Disease Resistance in Loblolly Pine**, was obtained in conjunction with scientists at three other universities. This new project will study the allelic variation underlying economically important traits in loblolly pine. This success of this effort, which holds great promise for molecular breeding and selection, was partially dependent on our ability to clonally propagate 500 unrelated clones from across the natural range of loblolly pine.

INTRODUCTION

In 1992, a group of forest products companies pooled resources to sponsor research into using rooted cutting technology for vegetative propagation of loblolly and slash pines. We at NC State were fortunate to have been chosen to lead this effort. At that time, loblolly and slash pines were considered very difficult to propagate by rooted cuttings. Fourteen years of focused research have shown that many of the innate obstacles could be overcome with research-generated knowledge and sound management techniques.

This time period has also been one of dramatic change in the forestry industry. Most of the original sponsoring companies no longer exist, have undergone mergers, or have divested themselves of their forest land base. Moreover, progress on alternative cloning technologies, such as somatic embryogenesis, has resulted in decreased interest in rooted cutting technology in other companies. Reflecting these changes, membership in the program, which reached its maximum at 13 companies, stood at only four members during 2005 (see list of all members at the end of this report).

2005 was the first-year of a research phase that was scheduled to last for four years. It is unlikely that there is sufficient support remaining to complete the phase, as was originally envisioned. However, many of the research objectives have already been addressed and, for others, key field tests have been established. Thus, we are proud of our record of accomplishment throughout the fourteen years of the life of the program, as well as during the last year. We have always prided ourselves on being focused on the research priorities of our members and in providing technology transfer assistance to their internal programs. We hope you agree. While doing this, we have been able to discover and disseminate new knowledge about how to root loblolly and slash pine cuttings and more general knowledge about clonal forestry with these species. In addition, we have trained numerous undergraduate and graduate students and other research personnel, some of whom now work for forest industry.

We are pleased to report that Anthony LeBude, who served admirably for many years as graduate student, Research Assistant, Assistant Director and Interim Director of the program has been hired as a faculty member in the Department of Horticultural Science at NC State. We wish Anthony continued success. In his absence, we owe a special debt of gratitude to Lela Walker, an undergraduate student in horticulture, who has ably filled in, supervising our greenhouse activities and making sure that studies were continued and completed on time.

None of us, at the beginning of this program, knew where it would lead. Our goal was to explore rooted cutting and clonal forestry research, so that companies would have the option of utilizing them in their forest management activities. While the immediate future of the implementation of these technologies is unclear, we all know a lot more about these things than when we started. Thus, the goal of providing options to members has largely been met. Along the way, it has been a truly gratifying experience to work with all the fine people at member companies, who supported, cajoled and, yes, even sometimes badgered us to produce more and better research. Please accept our heartfelt thanks for all the years of support.

Barry Goldfarb, Director

RESEARCH FOR OPERATIONAL PRODUCTION

Vapor Pressure Deficit for Controlling 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. Over the past 3 or 4 years we have reported that rooting percentage was related to mean daily cutting water potential (ψ_{cut}) (Annual Report 2001 and 2002), and vapor pressure deficit (VPD) in the rooting environment (Annual Report 2003 and 2004). In Spring 2004, VPD was used as a mechanism for controlling the frequency of mist application. Rooting results were promising, but less than expected overall. In 2005, we repeated this study, with minor modifications. The original plan was to implement similar control mechanisms in cooperator propagation environments. However, these were not installed, so the scope of our results is limited to a greenhouse environment. The objectives of the experiment were to: (1) test the feasibility of VPD-based, dynamic irrigation control, (2) determine the optimal range of VPD accumulation threshold values, and (3) determine if optimal thresholds vary when different containers and rooting media are used.

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. The software program calculated the amount of predicted water loss as a function of VPD level within each treatment. Treatment thresholds were set to 10.9 mL m^{-2} (0.0024 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. Thus, treatments with lower thresholds of accumulated water loss (i.e. Treatment 1 - 10.9 mL m^{-2}) received more frequent mist application than those with higher thresholds. 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.

Two sources of winter cuttings were tested in two container types. Winter cuttings from two full-sib families (RCP) [used previously by LeBude (Annual Reports 2001, 2002, 2003, and 2004)] were collected February 2005, pooled randomly, and stored at 39 F (4 C) in insulated coolers in a cold room. Winter cuttings from a mass controlled-pollinated family (MCP) were supplied by MeadWestvaco in February and stored similarly until setting on April 25, 2005. The bases of all cuttings were re-cut to a cutting length of 9 cm and dipped for 3 s in 10 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). 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. The tubes 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 cutting source and rooting container as the sub-plot factors. The design contained three replications. Rooting was recorded 10 weeks after setting. VPD was recorded every 10 minutes

(although the computer monitored it continuously) and averaged for the first five weeks after setting for the hours between 10 a.m. and 6 p.m.

Mean VPD (10 a.m. to 6 p.m.) was 0.61, 0.66, 0.80, and 0.82 kPa for treatments 1, 2, 3, and 4 respectively. Between 10 a.m and 6 p.m., treatments 1 and 2 were very similar but distinct from treatments 3 and 4, which were also similar to one another (Figure 1). The objective was to create the optimal range for rooting of 0.60 to 0.85 kPa as suggested by LeBude et al. (2005) and the 2004 Annual Report. The mean of each treatment was within this range.

Overall rooting percentage was 35.9%. VPD, stem cutting source and the VPD by container interaction affected rooting percentage (Table 1). Rooting was higher for RCP cuttings (46%) than MCP cuttings (26%). This was true for every VPD treatment and container type; however, the magnitude of difference was not consistent. For RCP cuttings only, rooting was highest in the intermediate VPD treatments—64% in treatment 3 and 58% in treatment 2. Wetter (treatment 1) and drier (treatment 4) treatments yielded lower rooting percentages. The significant VPD treatment x container interaction showed a consistent trend. Cuttings in RayLeach tubes performed relatively better in the wetter treatments, while those in tubs performed relatively better in the drier treatments (Figure 2). These results indicate that, while VPD-controlled mist frequency offers promise for dynamic control of irrigation according to varying conditions, the exact settings need to be calibrated for container and substrate types. We can expect that the same would be true for different propagation environments.

Table 1. ANOVA for rooting percentage of winter cuttings from two sources rooted in two container types under four VPD treatments in April 2005. Values in bold are significant at $P \leq 0.05$.

Source	df	*P*>F (Rooting %)
Replication	2	0.26
Vapor Pressure Deficit Treatment	3	**0.01**
Cutting Source	1	**0.01**
Container	1	0.34
Container x VPD Trt	3	**0.01**
Cutting Source x Container (VPD Treatment)	10	**0.03**

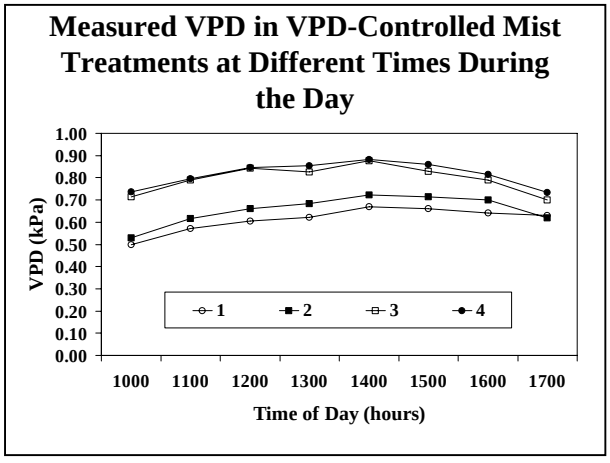

Figure 1. Mean VPD (kPa) in VPD-controlled mist treatments at different times of day averaged over 5 weeks.

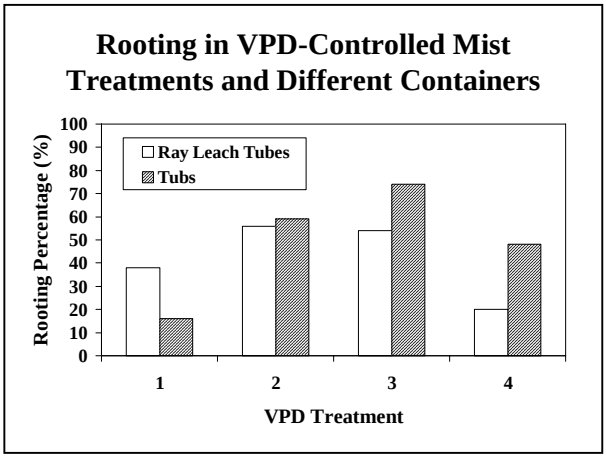

Figure 2. Rooting percentage of winter cuttings (only RCP cuttings shown) in different mist treatments and container types.

Hedge Nutrition Study

A study was initiated in December 2002 to determine the effects of stock plant fertility on stem cutting production and subsequent rooting. Previous results showed that rooting in stem cuttings was enhanced when foliage concentrations of nitrogen (N) in stem cuttings were at 2% or higher at the time of severance from the hedge. 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 macronutrients [phosphorus (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).

Fertilizer treatments were five ratios of macronutrients to the one level of N. The "standard" intermediate treatment (1X) applied macronutrients targeted 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 were 0.5X, 1.5X, 2.0X, and 2.5X, that is, one lower ratio and three higher ratios. Micronutrients remained constant for all treatments. Hedges received the 1X treatment from August 2003 until approximately April 1, 2004 (six weeks after hedging in Feb. 2004), when the individual treatment applications began. The different treatments were applied from April 2004 to February 2005. At that time, treatments were revised, based on results of foliar analyses.

The total number of stem cuttings produced by each hedge that were suitable for rooting were counted prior to collection. In May 2004 (experiment 1), thirty-six stem cuttings were collected per hedge. 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 70ºC (150 ºF) for 72 hours and then ground to a mesh size <1mm. 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 a subsequent full nutrient analysis according to the experimental design. The remaining 24 cuttings 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 twelve weeks. Immediately following stem cutting collected, hedges were pruned to maintain tissue juvenility. All of the above procedures were repeated in February 2005 (experiment 2) and May 2005 (experiment 3).

Preliminary results from A&L in Winter 2004 showed no large differences in shoot nutrient concentrations among the treatments. Foliar concentrations of nutrients reflected the standard treatment hedges had received during the previous eight months. Evidently, 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.

Results from A&L in Winter 2005 once again showed no substantial variation in shoot nutrient concentrations among treatments higher than the standard. Apparently, nutrient uptake in the hedges was maximized at the standard treatment and the higher nutrient ratios were not being utilized. Based on this result, we decided to modify the experiment to examine nutrient ratios lower than the standard, with the goal of determining minimum acceptable tissue concentrations. In spring 2005, the treatment ratios were adjusted to 0X, 0.125X, 0.25X, 0.5X, and 1X, with nitrogen and micronutrients still held constant across treatments. In addition, based on the foliar analyses, N was decreased and Ca was increased to more closely approach the Davey and Jett targets. Results from A&L in Spring 2005 showed slight differences in shoot nutrient concentrations among the treatments, most notably in K concentration (Figure 3). A full nutrient analysis on the remaining tissue is being conducted in Fall 2005 in the NCSU Forestry lab.

Experiment 1

Overall, the number of cuttings per hedge for the experiment was 13.6. Western Gulf loblolly pine produced more cuttings per hedge (14.8) than slash pine (13.8) and Atlantic Coast loblolly (12.4). Overall, rooting percentage and number of roots per cutting for the experiment were 78% and 3.8, respectively. Stem cuttings of slash pine had both higher rooting percentages (90.6) and number of roots per cutting (5.7) than Atlantic Coast loblolly (81.9% and 3.7) and Western Gulf loblolly (63.2% and 2.2). At the time of cutting collection, the actual treatments had only been administered for approximately one month, and these results reflect the standard baseline treatment.

Experiment 2

Overall, the number of cuttings per hedge for the experiment was 15.0. Western Gulf loblolly pine produced slightly more cuttings per hedge (15.8) than slash pine (14.8) and Atlantic Coast loblolly (14.5). Overall, rooting percentage and number of roots per cutting for the experiment was 76.5% and 6.4, respectively. Stem cuttings of Atlantic Coast loblolly had both higher rooting percentages (87.7) and number of roots per cutting (7.2) than slash pine (68.3% and 6.9) and Western Gulf loblolly (73.1% and 5.2). Overall stem cutting production and rooting percentage, and root production increased slightly from experiment 1. Prior to stem cutting collection, preliminary nutrient analysis results indicated that the hedges were not taking up the extra nutrients we were applying (treatments higher that the standard), so we reduced the nutrient ratios and made other minor modifications at this time.

Experiment 3

Overall, the number of cuttings per hedge for the experiment was 27.9. Again, Western Gulf loblolly pine produced more cuttings per hedge (29.7) than slash pine (26.4) and Atlantic

Coast loblolly (26.9) (Figure 4). Overall, rooting percentage and number of roots per cutting for the experiment was 70.8% and 2.3, respectively. Stem cuttings of slash pine had both higher rooting percentages (84.1%) and number of roots per cutting (3.5) than Atlantic Coast loblolly (75.8% and 2.2) and Western Gulf loblolly (52.6% and 1.2) (Figure 5). Stem cutting production dramatically increased from experiment 2, whereas rooting percentage and root number declined somewhat.

Future analyses will examine the statistical significance of treatment differences and correlate cutting production, rooting percentage and root number with actual sample tissue nutrient concentrations. The nutrient treatments that were imposed beginning in Spring 2005 were continued throughout the growing season and, if resources permit, another set of nutrient concentration determinations and rooting results could be conducted in Winter 2006.

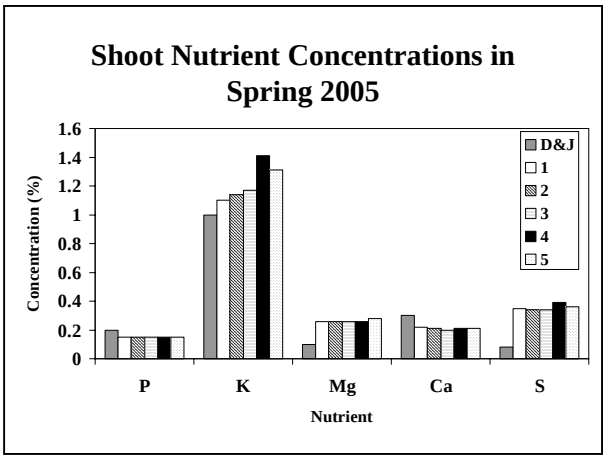

Figure 3. Concentrations of phosphorus (P), potassium (K), magnesium (Mg), calcium (Ca) and sulfur (S) in cuttings receiving different fertilization treatments, collected in June 2005.

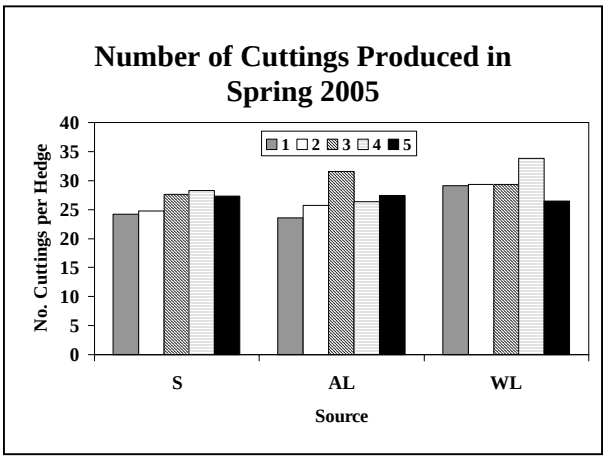

Figure 4. The number of cuttings produced on hedges receiving different fertilization treatments in June 2005.

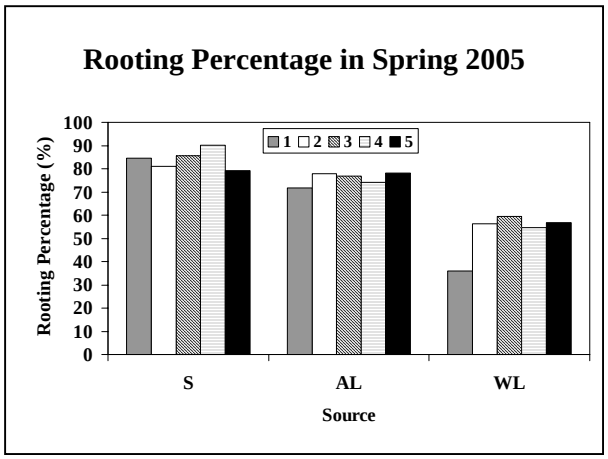

Figure 5. Rooting percentage of cuttings from hedges receiving different fertilization treatments in June 2005.

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 12 years old (fourth-cycle serial hedges, 4s). We also report on three field tests that were planted to test the effect of clone age on growth of the derived rooted cuttings. The first 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 three growing seasons are presented. The other two field tests contain ages 2 through 11 years old and were planted in 2004 in two locations.

Rooting in Winter 2005

Rooting percentage across all ages and families was 63%. Rooting percentage was highest for cuttings from 4 (82%), 8 (79%), or 10 (72%) year-old clones (Figure 6). Surprisingly, cuttings from 2-year-old-seedling hedges rooted only at 45%. A low rooting percentage for 2-year-old clones has not been seen in any of the previous trials. Family rooting performance was consistent with earlier trials. Stem cuttings from family 7 rooted the highest at 75%, family 3 rooted intermediately at 59%, and family 9 rooted the lowest at 49%.

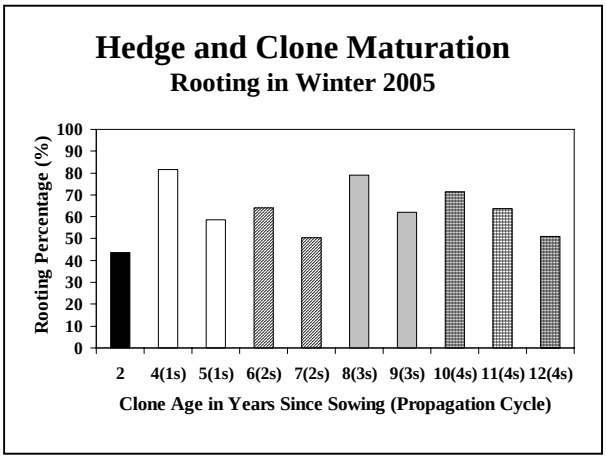

Figure 6. Rooting percentage of cuttings obtained from clones 2-12 years old in February 2005.

Overall, the decline in rooting found in this long series of experiments (9 trials over six years) has to be considered surprisingly small. This contradicts most commonly held beliefs about the useful life of clones maintained as hedges by serial propagation. It should be noted, however, that these results were obtained under controlled experimental conditions. That is, clones were regularly serially propagated on a schedule (once every two years) that few propagators would be likely to adhere to. Moreover, rooting trials were conducted in a reasonably optimized rooting environment, rather than in a large-scale operational scenario. Finally, only three families were studied and, while there are a fairly large number of clones per

family (approximately 20 per family per year), we cannot exclude the possibility that significantly decreased rooting performance of some clones could be seen under operational conditions.

Field Tests

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 comparison checks (1184 total trees planted). Initial height at planting and height after years 1, 2, and 3 were measured.

We previously reported that, at the time of planting, cuttings from older-aged hedges were shorter than cuttings from younger hedges. This trend continued after the first and second growing season, however, height growth increment was the same for all ages (Annual Report 2004). After the third growing season, rooted cuttings from 4 (2.66 m), 5 (2.56 m), 6 (2.54 m), 7 (2.53 m), and 8 (2.42 m) year-old hedges were shorter than the seedlings (2.83 m) (Figure 7). Although the differences in third-year height growth increment were barely non-significant (P = 0.08) (Figure 8), regression analysis that excluded seedlings did show a slight trend of decreasing growth increment with increasing clone age (Figure 9). These results confirm previous rooted cutting-seedling comparison studies that showed no growth depression when cuttings were obtained from fairly juvenile (two to three year-old) hedges. However, they do suggest a slight depression in growth of rooted cuttings obtained from older clones.

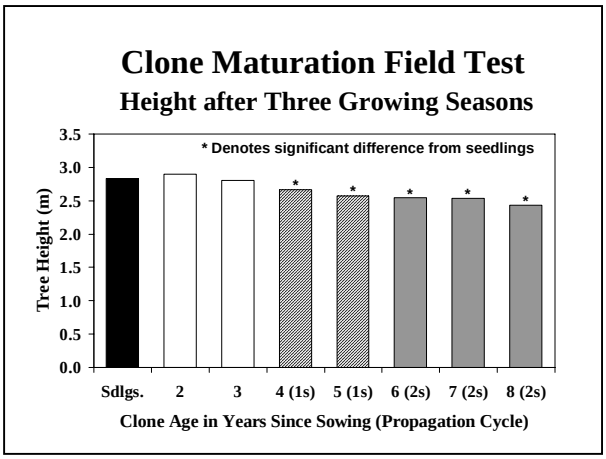

Figure 7. Tree Height of rooted cuttings from clones 2 through 8 years old and seedlings after three growing seasons.

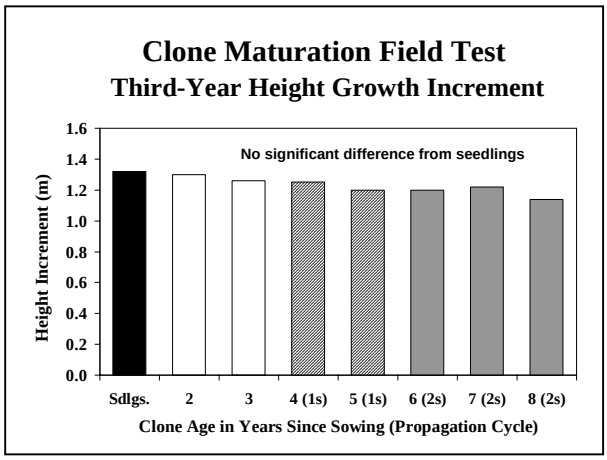

Figure 8. Third-year height growth increment of rooted cuttings from clones 2 through 8 years old and seedlings.

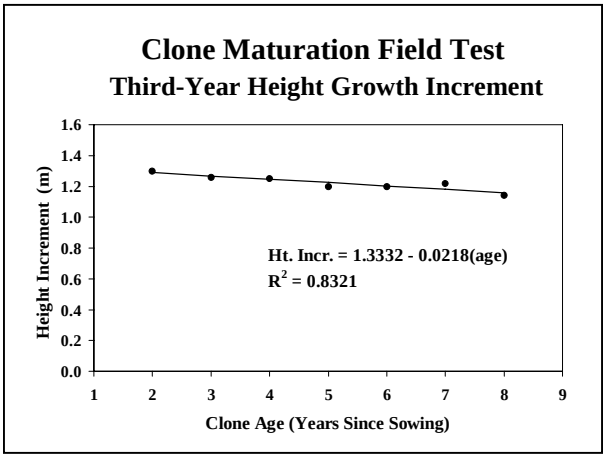

Figure 9. Regression of 3rd-year height growth increment against clone age for rooted cuttings from clones 2 through 8 years old.

Last winter, two additional field tests were established to gain additional certainty on this question and to extend the range of clone ages tested. Rooted cuttings from clones 2 through eleven years-old, with appropriate seedling controls, were established in a similar design as the first field test on land belonging to MeadWestvaco in South Carolina and Rayonier in Florida.

Clonal Rank Verification Study

In 1996, we undertook the Clonal Selection Study jointly with the NCSU-Industry Cooperative Tree Improvement Program. The objective of the study was to develop information that will facilitate efficient testing and selection of superior clones. From this study we generated 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. That information is summarized is a recent paper (Isik et al 2005, Canadian Journal of Forest Research, 35:1754-1766, and in several previous annual reports. In addition, in last year's report, we provided clonal variation and heritability data on form traits, including stem straightness, branch angle, forking, and ramicorn branching.

This year, we initiated a follow-up study, called the Clonal Rank Verification Study. Its objectives are to: (1) ascertain the level of confidence in clonal rankings and gain estimates from a single test and (2) determine the extent of clonal rank changes across widely varying sites (clone x environment interaction). A subset of clones from the original 450 in the Clonal Selection Study were established in three locations, including Jasper Co., TX (Temple-Inland), Telfair Co., GA (Plum Creek), and Onslow Co., NC (NCSU Hofmann Forest). Each test has a similar design, with 95-99 clones (depending on the test) and three seedling checklots: polymix 7-56, a coastal seed orchard mix, and CC4 (unimproved). In each test, there are nine blocks with each clone represented in each block once, in a single-tree plot, in a randomized complete block design. Each seedling checklot is represented by six seedlings per block. The clones were selected to represent a range of growth rates and approximate balance among the original eight full-sib crosses. While most of the clones are planted in all three tests, there was some substitution where ramet numbers were not sufficient for all three tests. Cuttings for the test were obtained from hedges that had previously been serially propagated and were rooted in Winter 2004. The tests were established during the 2004-05 planting season and have now gone through one growing season. We hope to continue to obtain measurements from these tests to increase our understanding of clonal testing and to publish this information in the public domain to increase the overall level of confidence in clonal forestry.

Wood Quality of Clones

For several years, we have been studying wood properties in loblolly pine clones as part of a $3 million USDA grant with scientists from the Department of Wood and Paper Science, the Tree improvement Program, and the Forest Biotechnology Program at NC State. We previously reported on whole-core specific gravity and x-ray densitometry of cores from trees in a seedling—rooted cutting (clonal) comparison test planted on two sites—in Alabama and Florida. Previous results indicated no significant difference in density between seedlings and cuttings, but large differences between sites. Despite the large site effect, clone mean heritability for density

was extremely high and there was negligible clone x site interaction. We now have data on additional traits measured on the cores from a subset of the full tests. The new traits include cellulose content, lignin content, fiber length, fiber coarseness, microfibril angle (MFA) and modulus of elasticity (MOE, computed from density and MFA).

Cellulose content, lignin content, fiber length, and fiber coarseness all showed very low levels of genetic variation in these samples. There were significant site effects, with trees on the site with rapid growth (Alabama) having lower cellulose content, higher lignin content, shorter fibers, and coarser fibers than trees on the site with slower growth (Florida). MFA and MOE also differed between the two sites. Trees in Alabama had larger MFAs and lower MOEs than trees on the Florida site. In addition, rooted cuttings had larger MFAs and lower MOEs than seedlings on the same sites. Both MFA and MOE showed a reasonable amount of genetic variation. Most of this variation was among clones, as opposed to among families (Table 2). Despite the genetic variation attributable to clones, there was still substantial variation among ramets of a clone (error). Clonal heritabilties were strong for both traits, while half-sib heritabilites were moderate.

These data, combined with our earlier results from the Clonal Selection Study, suggest that considerable improvement in form and strength traits can be made by selection and deployment of clones with desirable properties. Chemical traits, such as cellulose and lignin contents appear to show more limited potential for exploitation, at the clonal level.

Table 2. Source of genetic variation (site effects removed) and heritabilities (SE) of MFA and MOE in a clonal test of loblolly pine rooted cuttings.

Parameter	MFA	MOE
Female parent	4	12
Male parent	0	0
Full-sib cross	2	0
Clone within cross	24	20
Error	70	68
H^2_{clone}	0.74 (0.06)	0.71 (0.07)
$h^2_{half\text{-}sib}$	0.20 (0.27)	0.40 (0.09)

Association Genetics of Wood Properties and Disease Resistance in Loblolly Pine

Recently, a group of investigators from four universities—University of California-Davis, North Carolina State University, University of Florida and Texas A&M University--was awarded a $5.9 million grant from the National Science Foundation to study the allelic basis for economically important traits in loblolly pine. Association genetics is an emerging technique that correlates phenotype determinations with gene sequence variation in the accompanying

genotypes. These "associations" represent candidates for alleles in genes that control the trait of interest. A key component of this project, called ADEPT2, is the availability of cloned genotypes for precise phenotype determination and two populations will be utilized in this research. One is the CCLONES population of The Forest Biology Research Cooperative. The other is a newly assembled, range-wide, loblolly pine population consisting of seed from the NCSU-Industry Cooperative Tree Improvement Program and the members of the Western Gulf Forest Genetics Cooperative. This population is made up of approximately 500 unrelated individuals—the ideal constitution for an association population. The early work on this grant resulted in the cloning, by rooted cuttings, of each individual. This research demonstrates the value of rooted cutting propagation technology for research purposes and promises to yield fundamental insights into the genetic variation that underlies all tree improvement activities.

SUPPORTING COMPANIES IN 2004-2005

MeadWestvaco Corporation
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SUPPORTING COMPANIES THROUGHOUT THE EXISTENCE OF THE ROOTED CUTTING PROGRAM

Boise (Boise Cascade)
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Container Corp. (Jefferson Smurfit, Smurfit-Stone)
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Fort James Corp (James River)
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Lela Walker, Greenhouse technician and manager
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Charles Davey, Collaborating faculty
Fikret Isik, Collaborating faculty
John King, Collaborating faculty
Bailian Li, Collaborating faculty
Steve McKeand, Collaborating faculty

PUBLICATIONS OF INTEREST TO THE MEMBERS
(2002 - 2005)

Baltunis, B.S, D.A. Huber, T.L. White, B. Goldfarb, and H.E. Stelzer. 2005. Genetic effects of rooting of loblolly pine stem cuttings from a partial diallel mating design. Can. J. For. Res. 35:1098–1108.

Brunner, A.M., Goldfarb, B., Busov, V.B., and S.H. Strauss. 2003. Controlling maturation and flowering for forest tree domestication. Pp. 9-44 In Stewart, C.N. (ed.) Transgenic Plants: Current Innovations and Future Trends. Horizon Scientific Press, Wymondham, England. 296p.

Busov, V.B., E. Johannes, R.W. Whetten, R.R. Sederoff, S.L. Spiker, C. Lanz-Garcia and B. Goldfarb. 2004. An auxin-inducible gene from loblolly pine (*Pinus taeda* L.) is differentially expressed in mature and juvenile phase shoots and encodes a putative transmembrane protein. Planta 218: 916-927.

Frampton, J., F. Isik, and B. Goldfarb. 2002. Effects of nursery characteristics on field survival and growth of loblolly pine rooted cuttings. South. J. of Appl. For. 26:207-213.

Goldfarb, B., C. Lanz-Garcia, Z. Lian, R. Whetten. 2003. *Aux/IAA* gene family is conserved in the gymnosperm, loblolly pine (*Pinus taeda* L.). Tree Physiology 23:1181-1192.

Isik, F., B. Goldfarb, A. LeBude, B. Li and S. McKeand. 2005. Predicted genetic gains and testing efficiency from two loblolly pine clonal trials. Can. J. For. Res. 35:1754–1766.

Isik, F., B. Li, J. Frampton, and B. Goldfarb. 2004. Efficiency of seedlings and rooted cuttings for testing and selection in *Pinus taeda*. Forest Science 50: 44-53.

LeBude, A.V., B. Goldfarb, F. A. Blazich, J. Frampton, and F. C. Wise. 2005. Mist Level Influences Vapor Pressure Deficit and Gas Exchange During Rooting of Stem Cuttings of Loblolly Pine. HortScience 40:1448-1456.

LeBude, A.V., B. Goldfarb, F.A. Blazich, F.C. Wise, and J. Frampton. 2004. Mist, substrate water potential and cutting water potential influence rooting of stem cuttings of loblolly pine. Tree Physiol. 24:823-831.

Rosier, C.L., J. Frampton, B. Goldfarb, F.C. Wise, and F.A. Blazich. 2004. Growth stage, auxin type, and concentration influence rooting of Virginia pine stem cuttings. HortScience 39:1392-1396.

Rosier, C.L., J. Frampton, B. Goldfarb, F.A. Blazich, and F.C. Wise. 2004. Growth stage, auxin type, and concentration influence rooting stem cuttings of Fraser fir. HortScience 39:1397-1402.

Rosier, C.L., J. Frampton, B. Goldfarb, F.C. Wise, and F.A. Blazich. 2004. Stumping height, crown position and age of parent tree influence rooting of stem cuttings of Fraser fir. HortScience 40:771-777.

Rowe, D.B., F.A. Blazich, and C.D. Raper. 2002. Nitrogen nutrition of hedged stock plants of loblolly pine. I. Tissue nitrogen concentrations and carbohydrate status. New Forests 24:39-51.

Rowe, D.B., F.A. Blazich, F.C. Wise, and B. Goldfarb. 2002. Nitrogen nutrition of hedged stock plants of loblolly pine. II. Influence of carbohydrate and nitrogen status on adventitious rooting of stem cuttings. New Forests 24:53-65.

Sykes, R., B. Li, G. Hodge, B. Goldfarb, J.F. Kadla and H-M. Chang. 2005. Rapid prediction of wood properties of loblolly pine using transmittance near infrared spectroscopy. Can. J. For. Res. 35:1098–1108.