GENERAL COMMENTS

The first half of 1994 has been very productive for the Rooted Cutting Project. Because of industrial sponsorship and university backing we have been able to assemble a critical research mass that is now generating additional enthusiasm and support. We recently received confirmation that Georgia-Pacific has agreed to support the project as of July 1, 1994. In addition, the USFS, Southern Forest Experiment Station Research Work Unit on the Biotechnology of the Southern Pines and Hardwoods (RWU #4410) has initiated a cooperative research agreement to sponsor basic research on rooting that will bring $7,000 into the project.

The necessary physical facilities are now in place, although we continue to make improvements. The over-wintering structure on the container pad at the NCSU Horticultural Field Laboratory will be doubled in size this fall to accommodate an increasing number of stock plants. The propagation house in the Forestry greenhouse is now functional. The bottom heat has been installed, additional shading has resulted in a significant reduction in summer temperatures, and the timer-controlled mist delivery system is in place. We will reconfigure the mist nozzles this fall to improve within-bench uniformity. Due to limited capacity in the one bay, we may need to expand into another bay in the future. This spring a small "shadehouse" was constructed over a nursery-type bed next to the Forestry Greenhouse and equipped with a mist delivery system. Goldfarb's laboratory is now fully functional and humming with activity. To make it even busier, a new M.S. student, Lian Zhigang from the People's Republic of China, joined the project on Aug. 1, 1994. Lian will be studying the molecular biology of root initiation. We were also fortunate to have Ms. Sandy Bishop, a high school biology teacher, as a summer intern. She helped out quite a bit on a number of projects in the laboratory and greenhouse. Sandy's stipend was provided by the College of Forest Resources and the Research Triangle Science and Math Partnership.

In May, Greenwood and Goldfarb visited spruce rooted-cutting operations at J.D. Irving in New Brunswick and at the Quebec provincial nursery at Ste. Modeste. Those groups are quite far along and we gained some significant insights. Similarly, Mike Menzies of the New Zealand Forest Research Institute visited here in June with John Frampton of Weyerhaeuser. Mike told us a great deal about the *Pinus radiata* rooted-cutting experience and after seeing our operation, made a number of helpful suggestions. More details on what we've learned will be discussed at the next annual meeting.

On the research front, the following pages demonstrate that the wait for development of facilities and plant material is over. Data are rolling in from all phases of the project. In this report we have presented progress updates and brief summaries of the data in hand. More detailed results from all the studies will be presented at the annual meeting.

GENETIC CULLING TO IMPROVE ROOTING - WEIR

Hardwood ("dormant") cuttings were collected from the hedges of 38 slash pine wind-pollinated families in February, cold stored for 2 weeks, and stuck in the forestry greenhouse in early March. The cuttings were not treated with any auxin or root promoting hormone. The rooting was done in a peat/perlite (50:50 by volume) media. Bottom heat was used to maintain the rooting zone at a temperature between 65 and 70°F (18-21°C). Intermittent mist was applied every 4 minutes for 8 seconds between 7 am. and 6 pm. daily. The rooting experiment was a randomized complete block design with 6 blocks and 10 cuttings per family per block. Thus the family means or percentages were based on 60 total cuttings. Estimates of breeding values for the parents (families) are available from the University of Florida Cooperative Forest Genetics Research Program.

Preliminary results are as follows:

Overall rooting	= 54.3 %
Rooting of Best Family	= 86.7 %
Rooting of Worst Family	= 18.3 %

Family Mean Correlations:

Percent Rooting with Volume	= - 0.16 ns
Percent Rooting with Rust Resistance	= - 0.25 ns

While overall rooting was lower than hoped for (see discussion below) we did observe a very wide range of rooting percentage among families (18 to 87 %). Correlations of rooting percentage with breeding values for volume growth and rust resistance were low, negative (unfavorable), and statistically nonsignificant. This suggests we can cull poor rooting families without any major adverse impact on our objective of improving target traits.

During the course of the rooting experiment we began to suspect that our water distribution was not uniform across the bench. Subsequent to lifting and scoring the experiment we determined that the center of the bench was receiving nearly twice as much water as the edges. The water distribution pattern was associated with rooting success. Cuttings on the edge of the bench rooted at 68% while cuttings in the center rooted at less than 40%. This indicated that in addition to the uneven distribution, the cuttings on the whole were receiving too much water. In the current experiments we have reduced the frequency and duration of misting and in the fall we will reconfigure the water distribution system (mist nozzle spacing) to improve the distribution.

Last summer we hedged the trees that produced the cuttings for this experiment too early in the season and this resulted in some cuttings that had a second flush of growth. We were concerned that these double flush cuttings would behave differently, so they were not used in the

experiment. We did collect a bulk sample of both single- and double-flush cuttings and Ben Cazell of Rayonier conducted a comparison trial in his propagation greenhouse near Yulee, Fl. Ben reports that there was little difference in rooting percentage between the two cutting types.

A second experiment with an identical design was started in June 1994 with softwood (succulent) cuttings. Cuttings were harvested and stuck on June 13 and will be lifted on or about September 1 (twelve weeks after sticking). Once the rooting data for the softwood cutting experiment are collected, they will be compared and, if appropriate, combined with the results of the hardwood cutting experiment. With both data sets in hand a more detailed analysis of culling strategies will be completed. We will assess the affect of culling poor rooting families on overall rooting expectations and the associated impact on genetic gain for volume and rust resistance. These same experiments will be repeated in the winter and summer of 1995 and in addition we will begin to assess the variation in rooting among hedges within individual families.

EARLY PREDICTORS OF ROOTABILITY - SURLES/WEIR/GOLDFARB

Hypocotyl/Epicotyl Cutting Screening Experiment

The ability to predict rooting performance in families and individuals would greatly facilitate selecting genotypes for use in a vegetative propagation deployment program. Because screening genotypes as hedged stock plants could be time-consuming and expensive, we are exploring various methods of predicting rooting ability at an early stage. Work by Mike Greenwood suggested that the number of roots per plant formed on 21-day-old hypocotyl cuttings (rooted in an IBA bath) might be associated with rooting of stem cuttings from hedges. On a limited sample of 9 full-sib loblolly families, Mike estimated a family rank correlation of approximately +0.70. With this in mind, we have been exploring using this technique on both hypocotyl and epicotyl cuttings to predict rooting in slash pine.

The same families that were grown into hedges for Bob Weir's Family Culling experiments were used for the hypocotyl/epicotyl screening work. For hypocotyl cuttings, we severed the roots on seedlings that were 21 days old and placed the cuttings in styrofoam rafts which were floating in 10 μM IBA baths in a growth chamber. After 10 days, the IBA was removed and replaced with distilled water. After 21 days, each cuttings were scored for root number and the family means for percent rooting and number of roots per cutting were determined. Seedlings for the epicotyl experiment were grown for 50 days in the growth chamber. Epicotyls were then severed and placed in an identical IBA bath treatment as for the hypocotyl cuttings. Because the epicotyl cuttings root more slowly than the hypocotyl cuttings, they were scored at 70 days and 90 days after sticking. Rooting data from hedge cuttings from the Family Culling experiment reported above were used for purposes of correlation analysis.

After 21 days, 79% of the hypocotyl cuttings had rooted. Initial analyses indicate that hypocotyl cutting rooting is not correlated with rooting of "hardwood" stem cuttings from hedges. All correlations of root numbers and rooting percents were zero or very close to zero.

Epicotyl cuttings rooted at 19% after 70 days and 37% after 90 days. Epicotyl rooting after 90 days was positively correlated with hedge rooting. The correlation of percent rooting on a family mean basis was +0.46 after 90 days. The family mean correlation for number of roots (epicotyls vs. hedge cuttings) was +0.59 after 90 days. These preliminary correlation estimates were all statistically significant, although additional analyses may be necessary, including evaluation of possible variable transformations.

These results are not entirely unexpected. Since epicotyls have the same internal anatomy as do stem cuttings, a strong association of epicotyl cutting rooting with rooting of stem cuttings may be reasonable. Apparently, rooting of hypocotyl cuttings, which have a more root-like anatomy, is a poorer indicator of family hedge rooting capacity in slash pine. We plan to combine the rooting data from hedge cuttings taken in June (softwood cuttings) with the hardwood hedge cutting rooting data reported above to do an overall analyses.

Additional work focusing on a screening system for rootability with "mini-hedges" is planned and described below.

Mini-Hedge Cutting Screening Experiment

Background: We now have data that suggests that there is little correlation between hypocotyl cutting and hedge cutting rooting, on a family basis in slash pine, however, rooting of fifty-day-old epicotyl cuttings appears to correlate somewhat with hedge rooting. It is possible that this family-mean correlation may be even higher when using cuttings from 3-month-old "mini-hedges." If so, this intermediate screening method could still represent considerable time and cost savings over screening full-grown hedges.

Objective: To determine if 3-month-old mini-hedges can be used to screen open-pollinated slash pine families for rooting ability.

Experimental Design: This study employs the same 38 open-pollinated families of slash pine used in the Family Culling experiment described above. Three cuttings will be taken from each of 20 individuals per family (60 cuttings/family) and stuck in a mist bed in the forestry greenhouse.

Status: Seedlings from the 38 slash pine families have been sown in the greenhouse and will be de-capitated in mid-October. The resulting cuttings will be stuck in the mist bed in December and scored for rooting percent and root number in March. This data will be compared to full-grown hedge rooting data previously measured on the same families.

BASIC RESEARCH ON ROOT INITIATION - GOLDFARB/SURLES

Gene Expression During Adventitious Root Initiation

The general approach in this line of research remains to identify the component molecular events in the root initiation pathway and then to compare the pathways in juvenile (rooting) and mature (non-rooting) cuttings. We are actively studying two parts of the pathway at the present time.

1) Early Auxin Genes. A gene which is turned on very rapidly by auxin treatment in elongating pea epicotyls has been isolated by Theologis and co-workers. The rapid timing of gene regulation and sequence information indicates that the gene may be an auxin-responsive transcriptional regulator. That is, in response to auxin it turns on other genes which are responsible for the morphological changes such as cell elongation, cell division, or changes in developmental patterns. We are using the published sequence of this gene to identify analogous genes in loblolly pine cuttings. We treated hypocotyl cuttings with NAA and sampled them over time. After purifying RNA from the hypocotyls, we synthesized cDNA and used sequence-specific primers to amplify DNA fragments using the polymerase chain reaction (PCR). We obtained fragments that were of the expected size and have cloned them into bacterial vectors. They are being sequenced and we will use the sequence information to determine if they are similar to the pea genes. The next step will be to test for the specificity and timing of expression in cuttings treated with auxin and undergoing the very early stages of root initiation.

2) Unknown Pine Genes. We are collaborating with Alan Jones (UNC-Chapel Hill), and his graduate student Andrew Groover, to isolate previously unidentified genes important in root initiation in pines. Using NAA- and control-treated hypocotyl cuttings of eastern white pine four days after treatment (remaining from Goldfarb's Minnesota days), and a new PCR-based technique called differential display, we have tentatively identified 10 gene fragments which may be differentially expressed. We are currently cloning these fragments, and will have them sequenced in the near future. The sequence data will be used to search gene databases to determine if similar genes have been identified in other species. As with the early auxin genes, the next step will be to test for the specificity and timing of expression in relation to root initiation. This will be done concurrently in white and loblolly pines.

QTLs for Rooting

Our objective is to identify DNA markers associated with the rootability of cuttings in loblolly pine. We are collaborating with Dr. Dave O'Malley and Ms. Barbara Crane in the Forest Biotechnology Group who are simultaneously investigating markers for height growth in family 9-1020. QTL analyses consists of: 1) screening available primers for polymorphic bands, 2) constructing a saturated linkage map using polymorphic primers and a subset of progeny, 3) developing a framework map (selecting a subset of the saturated map bands that are evenly spaced across the genome to improve efficiency of locating QTL's), and 4) conducting the QTL

analysis (testing whether the presence or absence of particular bands or markers is associated with high or low rooting ability) on phenotypically scored progeny. At the current time the saturated map is nearly complete and we are selecting framework markers and performing DNA extractions on our progeny for the QTL analysis.

We began gathering the phenotypic rooting data for this study last fall when we tested 1400 hypocotyl cuttings for rooting. With the hypocotyl rooting data in hand, we will begin the first QTL analyses this fall and we anticipate that results from this analyses will be presented at the 1995 annual meeting. In addition, the rooted hypocotyl cuttings were transplanted to pots and are now nearly one-year old. They were hedged for the first time in late July and a subset will be tested for rooting as soon as possible. As soon as the hedge rooting data are collected, QTL analyses for this trait can be performed.

Hedge Maturation Study

Field installation of the hedge maturation study has begun. Seedlings grown for one year, first in the greenhouse and then on the container pad were transplanted to the field on April 28, 1994. The plants were decapitated on June 2 and on Aug. 3, 20 cuttings per hedge were collected and stuck in the propagation house. We will get a rough estimate of initial (juvenile) rooting ability for each plant and one rooted cutting per plant will be grown to size and transplanted to the field as the first of the serial propagation treatments. Seedlings for the second year were sown in the greenhouse on April 15, 1994.

***Agrobacterium rhizogenes* and Pine Cuttings**

Objective: To determine the effect of *Agrobacterium rhizogenes* (agro) treatments on rooting of loblolly pine (agro is the bacterium which produces "hairy root" disease).

Background: Four agro treatments (2 virulent strains, 1 avirulent strain, and a no bacterium control) were tested on hypocotyl cuttings of loblolly and slash pines. We observed no agro treatment effects. Because hypocotyl cuttings readily produce roots, we decided to test agro on hedge cuttings which are more difficult to root.

Experimental Design/Treatments: Four "genotypes" of loblolly pine (juvenile cuttings of 9-1019, 11-1103, 7-1037, and bulk-lot cuttings from 7-year-old hedges obtained from Rayonier {thanks Ben!}) were treated with the same 4 agro treatments as above. Two auxin treatments (control, 1500 ppm IBA dip for 5 seconds) were also imposed, creating a 4 x 2 factorial. Ten cuttings were stuck from each genotype/treatment combination in each of 4 replications (4 genotypes x 4 agro treatments x 2 auxin treatments x 10 cuttings x 4 replications = 1280 total cuttings stuck).

Status: The cuttings were stuck in late May and were scored in late July. The results of this study will be reported at the annual meeting, although preliminary analysis revealed no dramatic agro effects.

Arabinogalactan Proteins and Pine Cuttings

Although we came very close, the grant proposal we submitted with Ross Whetten of the Forest Biotechnology Group to the North Carolina Biotechnology Center to study these proteins in pine root initiation was not funded. As resources permit, we may attempt to gather sufficient preliminary results on this topic to submit proposals to other agencies.

BASIC RESEARCH ON ROOT INITIATION - GREENWOOD

The auxins IAA, IBA and NAA all promote rooting in loblolly pine hypocotyls with IAA being the least effective and NAA the most. NAA is much less rapidly metabolized than IAA. Following 6 hours of uptake of radioactive auxin, about 20% of extracted radioactivity is free IAA, while over 80% of the NAA is in its original form. There is considerable evidence that a high concentration of free auxin occurs at the base of the cutting during the early (within the first 2 to 3 days) phases of adventitious root formation (Blakesley 1994). The fact that NAA is not rapidly metabolized in the loblolly pine hypocotyl presents the opportunity to examine the distribution of label within the hypocotyl during the early phases of root formation, to see if it is localized in groups of cells where roots will form. In addition, NAA is an effective promoter of rooting if applied in pulses as short as 5 minutes, in contrast to IBA which must be supplied continuously. Using a 1h pulse of 160 μM NAA (carboxyl labeled with ^{14}C) applied to hypocotyls of both 24 and 50 day old seedlings, we have followed the uptake and distribution of label over a 24h period using autoradiography of tissue frozen over dry ice. A similar application of cold NAA at the same concentration resulted in over 70% of the cuttings forming roots along the length of the hypocotyls of both types of cuttings. Histological examination of NAA-treated hypocotyls reveals that root meristem organization is localized in vascular parenchyma, immediately centrifugal to the resin canals. We have tested the hypothesis that root formation in this tissue is preceded by localized accumulation of auxin in the cells which give rise to the root meristem.

Autoradiography of longitudinal and cross sections of hypocotyls pulsed with C-NAA for 1h were made following 0, 3, 6, and 24h chases in distilled water. Following development, the autoradiographs and the corresponding frozen tissue were viewed at 40x magnification. The label from the NAA is taken up in through the xylem (the cuttings continue to transpire) and the epidermis; with 0h chase, the highest concentration of label is in the basal 2 mm of the cutting, and in the xylem itself. In cross section, label could be seen initially concentrated in the column of primary xylem, which are only 3-4 cells in diameter (the diameters of xylem cells are similar to those of parenchyma cells which will forms roots). With increasing chase time, the label moves from the xylem and epidermis into the cortex, which becomes uniformly labeled. The

density of label remains highest at the base of the cutting, but there is no evidence of label being concentrated at potential sites of root formation. Instead, it appears to be uniformly distributed throughout the cortex. The resolution of this technique may not be adequate to resolve increased label within a single cell. We can conclude that auxin is not preferentially bound within areas of several cells in diameter where cell divisions, marking the beginning of meristem formation, occur beginning about 2 days after exposure to auxin.

Blakesley, D. 1994. Auxin metabolism and adventitious root initiation. *in* Biology of Adventitious Root Formation, T.D. Davis and B.E. Haissig eds., 343 pp. Plenum Press, New York.

STOCK PLANT PHYSIOLOGY, HEDGE NUTRITION STUDY - HENRY/ROWE

Objectives of the hedge nutrition study are to determine: 1) the effect of nitrogen availability on the number of orthotropic shoots produced by hedged stock plants, 2) the effect of nitrogen availability on stock plant carbohydrate/nitrogen (C/N) ratios, 3) if C/N ratios influence subsequent adventitious rooting of cuttings severed from these stock plants, 4) changes in C/N ratios of stock plants and differences in rooting responses of cuttings severed at different times of the year, and 5) changes in C/N ratio of cuttings over the 12 to 14 week period they are in the rooting bed.

Hedges from potted seedlings have been growing at the container pad since May, 1993. The initial hedging was performed during the first week of February, 1994. Seedlings were decapitated 20 cm. above the rim of the pot and terminal buds of all remaining branches were removed such that half of any individual branch remained intact. This cutting material was not used in the study, but needle samples were collected and are now being utilized to refine the analytical procedures for enzymatic carbohydrate analyses.

During April, the automated irrigation system was reactivated, N treatments were initiated for the trees growing in the perlite/sand culture, and trees growing in the standard peat mix were fertilized with Osmocote. The six N treatments consist of the Osmocote control and five levels of N (650 ml of 0, 5, 10, 20 and 40 ppm N per day) supplied as NH_4NO_3. By growing trees under a range of N availabilities, we hope to establish hedged stock plants with differing C/N ratios.

Throughout the growing season, foliar samples will be collected monthly and analyzed to monitor the nutrient levels within the tissues. Adjustments to the nutrient solutions will be made if necessary. In addition, a subsample of the soil solutions are being analyzed monthly utilizing the Virginia Tech Extraction Method.

The first crop of cuttings was collected for tissue samples and rooting studies the week of May 29th. The second crop was collected in late July, 94, and subsequent crops will be collected in January, 1995 and May, 1995. The number of cuttings $\geq$10 cm. will be counted for each tree

at each hedging. From these, three cuttings from each tree will be used for tissue analyses. Tissue samples will be freeze-dried and stored in desiccators pending nutrient and carbohydrate analyses. Total non-structural carbohydrates (glucose, fructose, sucrose, and starch), total nitrogen, and macro- and micronutrients will be determined for needles and stems separately.

The other nine cuttings from each tree will be utilized for rooting experiments. The rooting experiments will take place simultaneously at three locations. The Horticultural Science greenhouse (HSGH), the Forestry greenhouse (FGH), and in Summerville, SC in cooperation with Westvaco Forest Research. There will be a total of 864 cuttings at each location with the exception of HSGH which will have 1728. The extra cuttings at HSGH will be pulled up in pairs every 3 weeks and analyzed for carbohydrate and mineral nutrient content and will provide information on the changes in carbohydrates and nutrients from the time the cutting is stuck until the end of the rooting period. Cuttings will be sampled at 0, 3, 6, 9, and 12 weeks.

After 12 to 14 weeks, all cuttings will be harvested from the mist beds and the number of roots per cutting, root lengths, root areas, shoot diameters, percent rooting, and a symmetry rating will be recorded. Dry weight data will then be generated to calculate cutting dry weight (sum of leaf, stem, and root dry weight), shoot dry weight (sum of leaf and stem dry weight), and root:shoot ratio (root dry weight:shoot dry weight). The first set of cuttings will be harvested from the mist beds in late August or early September. Procedures for the enzymatic carbohydrate analyses should be worked out shortly so the lab analyses can commence. If all goes as planned, we should have both rooting and nutrient analysis data from the first harvest available by January, 1995.

ROOTING ENVIRONMENT AND THE QUALITY OF ROOT SYSTEMS - BLAZICH/THETFORD

Experiment 1 was conducted in February, 1994 and was designed to investigate three new auxin formulations (Hormodin 3, Hare's Powder, and NAA) and compare them to the previous treatments of IBA and PITB. Auxin treatments included NAA (20, 30, 40 mM), IBA (20, 40 mM), P-ITB (20, 40 mM), Hormodin 3, and Hare's powder (0.5 + 1.0 X). Dormant cuttings were stuck February 17, 1994 and were evaluated May 12 and 13, 1994. Rooting data from this experiment are currently being analyzed and will be available soon. One interesting observation from this experiment is that treating with NAA produced a morphologically different root system. Root systems of NAA-treated cuttings were very coarse with roots shorter than those of other treatments. These roots had little or no secondary root development and were oriented downward regardless of the number of roots per cutting. The morphology of NAA-induced roots is similar to that found on roots induced on *in vitro* shoots using NAA in experiments conducted by the Tissue Culture Cooperative.

The effects on plant performance of root systems induced by the different auxin treatments is currently being assessed. Rooted cuttings that graded as commercially acceptable were out-planted in the seedling nursery of Westvaco Corp., Summerville S.C. and will be lifted

and evaluated this fall. Initial measurements of cutting height, root collar diameter, stem diameter, root length, and root number will be used to evaluate rooted cutting performance when these same variables are measured following several months of growth in the nursery.

Several experiments were initiated in June 1994 utilizing softwood cuttings and will soon be ready for evaluation. **Experiment 2** utilizes the same experimental design as the February '94 experiment, except that softwood cuttings were used and auxin concentrations were reduced.

Auxin	ppm	mM
NONE	0	0
IPA (50%)	0	0
IBA	1016	5
IBA	2032	10
PITB	1477	5
PITB	2954	10
NAA	931	5
NAA	1862	10
NAA	3724	15
Hormodin 3	8000	39.4
Hare's	1X	
Hare's	1/2X	

Experiment 3 was initiated to provide additional cuttings for evaluation of root system parameters. Auxin sources and concentrations were selected from the previous list of treatments and cuttings are being rooted in an operational system utilizing Rootrainer trays. Primary objectives are to 1) quantify root:shoot dry weight ratios 10 and 20 weeks after initiation and 2) evaluate root system characteristics as in the previous experiment. Data to be collected will include: shoot length, shoot dry weight (needles and stems) total root area, root number, root diameter, root collar diameter, total root length, and root dry weight. A total of 1728 cuttings were stuck for this experiment and treatments were as follows:

Auxin	ppm	mM
Nontreated	0	0
IBA	1016	5
PITB	1477	5
NAA	931	5
Hormodin 3	8000	39.4
Hare's powder	1X	

Experiment 4 is designed to evaluate the influence of several rooting media on rooting and subsequent root growth of softwood cuttings of loblolly pine treated with IBA or NAA. Auxin sources and concentrations and media were as follows:

Auxin	ppm	mM
IBA	1016	5
NAA	931	5

Medium	Ratio (v/v)
Peat:perlite	1:1
Peat:perlite	1:2
Peat:perlite:vermiculite-with fertility added	2:2:1
Peat:perlite:vermiculite-no fertility added	2:2:1
Pine bark	100%
Pine bark:sand	6:1
Pine bark:perlite:vermiculite-with fertility added	2:2:1
Pine bark:perlite:vermiculite-no fertility added	2:2:1

Experiment 5 is a preliminary evaluation of the effects of uniconazole on rooting and subsequent root growth of softwood cuttings of loblolly pine treated with (NAA or IBA) or without auxin. Several researchers have reported increased root initiation for adult phase cuttings of English ivy (*Hedera helix*) treated with triazole compounds and attribute this response to inhibition of gibberellin biosynthesis within the cuttings.

Cuttings were treated with auxin and/or uniconazole following the same procedures as previous experiments. Uniconazole was prepared using a 500 ppm stock solution of uniconazole and distilled water. Cuttings were treated with the appropriate auxin solution on 9 June and allowed to air dry for 20 minutes prior to treatment with uniconazole. After uniconazole treatments had air dried for 20 minutes the cuttings were stuck into a perlite:vermiculite (1:1 v/v) medium and then placed under mist. Auxin sources and concentrations and uniconazole concentrations were as follows:

Auxin	ppm	mM
Nontreated	0	0
IBA	1016	5
NAA	931	5

Uniconazole	ppm	µM
	0.00	0.00
	0.05	0.17

1.00	3.43
5.00	17.10
10.00	34.30

Experiment 6 is still in the planning stage and will investigate effects of rooting medium pH and auxin sources on rooting cuttings of loblolly pine. Cuttings will be obtained from a single family and treated with the following auxins utilizing the same procedures as previous experiments.

Auxin	ppm	mM
Nontreated	0	0
IBA	1016	5
NAA	931	5

Cuttings will be rooted in the standard peat:perlite (1:1 v/v) medium which has been adjusted to varying pH levels.

The quantities of dolomitic lime required to adjust pH of this medium will are being determined empirically. A sample of the propagation medium was amended on 19 May with dolomitic lime at the following rates per cubic yard: 0, 1, 5, 10, and 15 lbs. These media were placed in quart pots and incubated for 1 week at room temperature. The initial pH of the non-amended rooting medium was 5.2 and the tap water pH was 6.7. The media were placed under intermittent mist on May 16. Since these media will require a period of incubation for the pH to equilibrate it is likely this experiment will be conducted in the winter of 1995.

ROOTING ENVIRONMENT RESEARCH - TOM GILMOUR/WEIR

During the late winter and early spring a preliminary study was conducted using cuttings from the slash pine hedges being maintained for the Family Culling experiments. A bulked sample of cuttings randomly chosen from 38 families were harvested in early February and stuck in late March in the forestry greenhouse. Five media types and two container sizes were used. The misting regime provided 8 seconds of mist every 4 minutes between 7 am and 6 pm daily. The results of the exploratory work are as follows:

Media Types	Volume Mixture %	% Rooting - 4.0 Cubic Inch Tubes	% Rooting - 9.6 Cubic Inch Tubes

Bark	100	47	68
Perlite	100	17	20
Bark / Perlite	50 / 50	38	41
Peat / Perlite	50 / 50	57	56
Peat / Sand	50 / 50	37	67

The rooted cuttings were also scored for the quality of the "root ball" keeping in mind how difficult it would be to transplant the rooted cuttings to a nursery for growing to field planting size. With this watering regime some interaction among container size and media is apparent. Clearly the perlite media and bark/perlite media were inferior.

An experiment was established on June 13, 1994 again using bulked cuttings from the 38 families of slash pine hedges stuck in the same two container types and with the same 5 media mixes. The study is a randomized split plot design with the container size serving as the main plots. The study was installed in both the forestry greenhouse and the adjacent, mist-equipped shade house. Cuttings will be lifted and scored in mid-September for rooting percentages and "root ball" quality.