

GENERAL COMMENTS

It has been an eventful year for the Rooted Cutting Project. We had two companies join the project as of January 1, 1995. Welcome Federal Paper Board and James River! In addition, Packaging Corporation of America has officially notified us of their intention to join as of January 1, 1996. Thanks PCA! This brings the number of supporting companies to twelve for the beginning of the second phase of the project. We are supplementing industry funding with other grants. We recently obtained \$40,000 over 18 months from the North Carolina Biotechnology Center to study arabinogalactan proteins and rooting (see section on Root Initiation and Maturation).

The research team is undergoing some changes for the coming research phase. Barry Goldfarb, Bob Weir and Mike Greenwood continue to be principal investigators with Frank Blazich and Phil Dougherty playing advisory roles. Scott Surlles and Carmen Lanz-Garcia are central to the field/greenhouse and laboratory research studies, respectively. Alice Hatcher, Paula Otto and Chris Hunt of the Tree Improvement staff help us out as time permits. Brad Rowe (Ph.D.) and Lian Zhigang (M.S.) are doing their graduate research on project studies and Lin Xiaohong, a recent M.S. graduate from Anne Stomp's lab, has taken a technician position that is funded by the NCBC grant. Leslie Henry and Mack Thetford have moved on, but we are currently filling a post-doctoral research position in physiology of pine stem cuttings. We have an excellent group of applicants with substantial gas exchange and water relations expertise and we're confident that the selected applicant will be a big asset to the project.

One significant facility improvement was accomplished. With funding obtained from an internal University grant, we renovated the fan system in the propagation house in the Forestry greenhouses. This has resulted in significantly improved cooling capability which had been a real problem for us in the summer months. The mist delivery system was also redesigned to give a more even distribution pattern, although a perfectly uniform distribution remains elusive. We also continue to experiment with the outdoor, shaded, rooting bed with encouraging results.

A note for your calendars--we will try to schedule the next annual meeting for April instead of January. There seemed little point in meeting earlier, since we just met this past April for the Renewal Proposal meeting. We hope this is reasonably convenient for everyone. Barry will be fielding objections during his on-site visits. The exact date and location will be announced soon.

In the following pages, you will see highlights of recent research results. Feel free to contact us if you wish more information on a specific study and expect more details at the annual meeting. As always, we sincerely appreciate your support!

GENETIC CULLING TO IMPROVE ROOTING

Experiments were conducted to determine levels of variation for rooting ability among families and clones of slash pine in the winter and spring of 1995.

Family Performance--Winter and Spring, 1995

Overall rooting percentage of the 38 open-pollinated slash pine families was 55.7% in the winter trial. Families ranged from 23.2 to 86.7% rooted. Overall rooting in the spring trial was 46.6%. While this was not as high as we would like, it is a marked improvement over the 10% rooting we reported for last year's spring sticking. Families ranged from 0 to 76.7%. The family means from these studies were tested for their correlation with each other and the means from the winter, 1994 rooting trial (Table 1). The correlation between family rooting performance in winter, 1994 and spring, 1995 was ($r=0.56$). The family means from the winter, 1995 trial were weakly correlated with both the winter, 1994 ($r=0.33$) and spring, 1995 ($r=0.39$) trials. The low correlation of family means involving the winter, 1994 trial may have several explanations. Either: (1) there is a large amount of error in our estimates of family performance due to variation in the rooting bed, (2) randomly choosing 36 cuttings from the 15-20 hedges per family resulted in non-representative sampling of the families, or (3) the environments differed substantially and there is a family x rooting environment interaction. It was probably not due to the small number of hedges per family, because the same hedges were used for all the rooting trials. Results from other experiments (see early selection section below) leads us to believe that the results from winter, 1995 were not typical.

Clonal Performance--Winter and Spring, 1995

In the winter, the clonal performance trial was designed to determine the additional improvement in rooting ability to be gained from within-family culling. Eight families were chosen as having the best rooting performance, growth rate and rust resistance from among the 38 families in the overall study. In the spring, we also considered the question of whether there are good-rooting clones from poor-rooting, but otherwise desirable, families. In addition to four of the best families from the winter trial, two families were added that were classified as poor rooting, but excellent in growth and rust resistance. In both studies, twenty-four cuttings were taken from each of 15 hedges (clones) for each family and tested for rooting performance. The results of both clonal performance studies are currently being analyzed and will be reported at the annual meeting.

Early Selection--Mini-hedges

This trial was the next step in our efforts to test the feasibility of early selection of families for rooting ability. Seeds from the 38 slash pine families were sown on July 20, 1994 and the seedlings were topped approximately six months later on January 10, 1995. The resulting cuttings (one to five per seedling) were stuck in the outdoor, shaded, rooting bed on April 26, 1995 and scored for rooting on July 17, 1995. Sixty cuttings per family were taken

from the approximately 35 mini-hedges per family and divided between five replications in the rooting bed.

Overall rooting of the mini-hedge cuttings in the outdoor bed was excellent. The mean rooting percent across all the families was 81.8%. The best family rooted at 96.7%, while 60% of the cuttings from the worst family rooted. Family rooting percentages were correlated with hedge rooting at $r=0.54$ for winter, 1994, $r=0.53$ for spring, 1995, and $r=0.61$ for winter, 1994 and spring, 1995 combined (Table 1). The correlation with rooting in winter, 1995 was $r=0.34$, supporting the assessment of that rooting trial as atypical. The correlation of the mini-hedge rooting with hedge rooting was better than that between either hypocotyl or epicotyl cuttings and hedge rooting.

At the current time we are germinating seedlings for 25 families of loblolly pine for the next round of experiments. Effect of family and clonal culling will be assessed for impacts on improved rooting performance and growth.

Table 1. Family mean correlations (r values) for rooting percentages of different cutting types and different seasons of rooting.

	Hypocotyls	Epicotyls	Mini-hedges	Winter 1994	Winter 1995	Spring 1995	Winter 1994 and Spring 1995 ¹
Hypocotyls	---	0.27	0.17	-0.08	0.10	0.20	0.07
Epicotyls		---	0.38	0.16	0.30	0.31	0.27
Mini-hedges			---	0.54	0.34	0.53	0.61
Winter 1994				---	0.33	0.56	---
Winter 1995					---	0.39	0.41
Spring 1995						---	---

¹The combined winter, 1994 and spring, 1995 values may be the best estimate of repeatable rooting performance. The family means for the winter, 1995 trials are only weakly correlated with mini-hedges, winter, 94, and spring, 95.

ROOT INITIATION AND MATURATION

Hedge Maturation Study

The generation of plant material for this study continues on schedule. We now have a complete set of rooted cuttings for the serially propagated hedges from the first year seedlings. It appears that the serially propagated cycles will take two years as originally projected, rather than the one-year cycle we were trying for. The seedlings for the third-year hedges are grown to size and will be transplanted and hedged in April. As discussed previously, we are currently considering the idea of conducting preliminary rooting and field tests on the plant material from the first three years of the study, rather than waiting for the full seven years. The earliest that this could be done would be the summer of 1996 when the new seedling hedges have begun to produce an adequate number of cuttings.

Auxin-induced Gene Expression

We have used the previously cloned small gene fragments to isolate at least four full-length auxin-induced genes from pine hypocotyl cuttings. Their sequences match up in the expected places with the herbaceous plant genes. We are now sorting out whether some of the sequence variants we have identified represent different genes or just different alleles of the same genes. We are currently studying the expression of these genes. We have determined that one gene is turned on in hypocotyls 5-10 hours after auxin treatment and then is turned off after 24 hours; we are currently testing this, and the other three genes, in both hypocotyl and epicotyl cuttings. The next step will be to characterize expression in different cell types to see if these genes are expressed in the cells that give rise to adventitious roots. If they are, we will compare their expression in stem cuttings from hedges of different ages to see if a specific auxin response is the limiting step in maturation-related loss of rooting ability.

Auxin-binding Proteins (ABPs)

We have just begun a project to clone auxin-binding proteins from pine using an approach analogous to the one we used for the auxin-induced genes. We have compared known auxin-binding protein genes from herbaceous plants to determine regions of conserved sequences. We then designed polymerase chain reaction (PCR) primers complementary to these regions and used them to amplify DNA from pine hypocotyls. We are currently cloning the amplified DNA fragments and will use them to identify full-length ABP genes from pine.

QTLs for Rooting Ability

This study is testing for quantitative trait loci (QTLs) that determine genetic variation for rooting ability. The molecular mapping of the 400 progeny from family 9-1020 is 85% complete. We are now in the process of obtaining reliable estimates of the rooting ability of all 400 hedges. We have conducted two rooting trials--15 cuttings per hedge were stuck in both the winter and spring of 1995. Overall rooting in the winter was 42%, with individuals ranging from 0 to 100%. Results from the spring are currently being analyzed. Rooting trials will continue until we are confident we have accurate estimates of the hedges' genetic ability to root. At that

time, the rooting performance of the hedges will be correlated with the molecular markers to determine if there are any that are closely linked to rooting ability.

Arabinogalactan Proteins (AGPs) and Rooting

This is a new project that is funded by a grant from the North Carolina Biotechnology Center. AGPs are glycoproteins that are correlated with plant development. Some AGPs have been shown to cause non-embryogenic carrot cultures to undergo somatic embryogenesis, so we are interested in determining if there are others that play a similar role in root formation from pine stem cuttings. Basically our approach has two facets: (1) to determine if there are unique AGPs associated with rooting vs non-rooting cuttings, (e.g. pulsed vs. non-pulsed hypocotyls cuttings) and (2) to determine if the application of AGPs purified from rooting cuttings will enhance rooting in cuttings that normally root poorly (e.g. young vs old hedge stem cuttings). To accomplish these objectives we have purified AGPs from various cutting types and will generate monoclonal antibodies to AGPs from hypocotyl cuttings during rooting. The antibodies will be useful for finding unique AGPs and also for purifying AGPs for rooting experiments.

University of Maine Research Team (Greenwood, Diaz-Sala, and Hutchison)

While both hypocotyl and epicotyl cuttings respond to auxin by exhibiting cambial dedifferentiation, epicotyls do not organize root meristems within 30 days of auxin application. Because localized areas of cell division are associated with the formation of root meristems, we have tested the hypothesis that epicotyls will be less responsive to combined auxin and cytokinin treatments that are commonly used to induce callus formation in plant tissues. However, epicotyl explants formed callus just as readily as explants from hypocotyls, so there is no obvious difference in ability to exhibit rapid cell division in response to auxin. Because cytokinins were not needed to induce callus, we are currently testing whether there will be differences in response to lower concentrations of several auxins.

We are continuing to compare gene expression in hypocotyl and epicotyl cuttings using differential display, which permits detection of genes that produce relatively small quantities of mRNA. We have found and sequenced parts of several genes that are differentially expressed. One of them appears to be tenascin, an extracellular matrix protein, which is only expressed in hypocotyls within 24 hours of hormone application. We are attempting to validate these results using northern blot assays for mRNA abundance.

STOCK PLANT NUTRITION

In our previous reports, we reported on experiments stuck in May and July, 1994. We had found that over the range of concentrations tested (0, 5, 10, 20 and 40 ppm N) rooting success was greatest in either the 20 or 40 ppm N treatments, depending on the season and the family tested. Rooting success of the "horticultural control" (Osmocote fertilizer, 8-month formulation, 18-6-12 NPK, one teaspoon twice a year) was lower, even though the nitrogen availability was higher than in any of the other treatments. This year, cuttings were taken in January, 1995 from hedges given those same N treatments. The same overall trend was observed, with the best rooting from hedges fertilized with 20 ppm N (Figure 1). In addition, rooting of the "horticultural control" cuttings was much poorer than cuttings from hedges receiving either 20 or 40 ppm N.

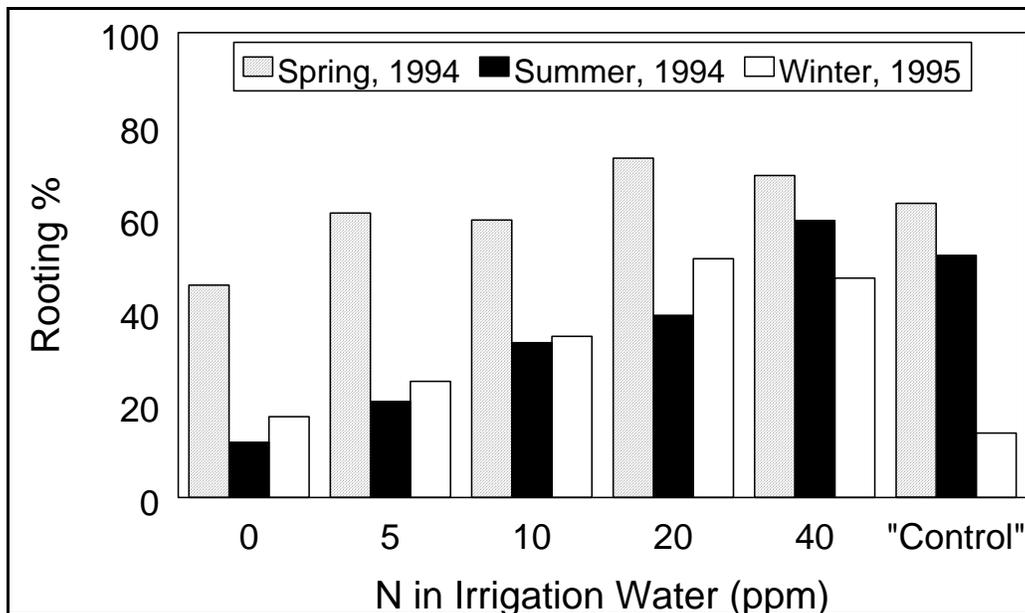


Figure 1. Effect of nitrogen fertilization of hedged stock plants on rooting of loblolly pine cuttings during three seasons in Summerville, SC.

Immediately after the January cuttings were removed from the hedges, N levels were adjusted (10, 25, 40, 55, and 70 ppm N and osmocote "control") to more precisely bracket optimal N treatment levels. Cuttings were stuck in May, 1995, both in the NCSU Horticulture Science greenhouse and Westvaco's greenhouse in Summerville, SC. Interestingly, optimal treatments appeared to differ according to rooting location (statistics are currently being run to determine significance levels). In Raleigh, the cuttings with the highest rooting percentage (4 families combined) came from hedges fertilized with 40 ppm N (46.1%), while 55 ppm N was optimal in Summerville (64.6%) (Figure 2). At both locations, osmocote-fertilized hedges

yielded the poorest rooting cuttings (15.3% in Raleigh, 0.8% in Summerville). There were substantial family x nitrogen and family x location interactions. One family (27-2 x 27-5), rooted better in Raleigh, ranging from 61-67% for the 25, 40 and 55 ppm N treatments, while the other three families (27-1 x 27-2, 27-1 x 27-3, 27-1 x 27-6) performed better in Summerville. One of these families rooted at 83% in the 40 ppm N treatment and another at 83% in the 55 ppm N treatment.

An additional experiment was stuck in July, 1995, but has not yet been scored, and the final rooting trial in this series will be stuck in January, 1996. That will complete two years of rooting experiments conducted at three times each year. The analyses to assess internal carbohydrate and nitrogen concentrations in the cuttings used for these experiments are currently underway.

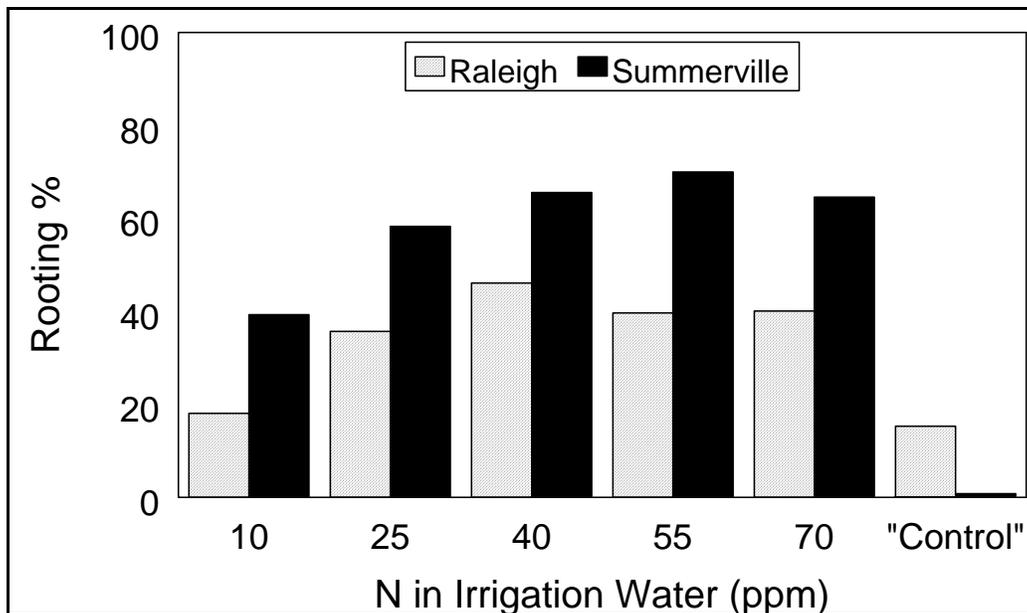


Figure 2. Effect of nitrogen fertilization of hedged stock plants on rooting of loblolly pine cuttings at two locations: Raleigh, NC and Summerville, SC.

REFINING THE ROOTING ENVIRONMENT

As mentioned above, the cooling system in the misthouse of the Forestry Greenhouse was renovated to yield better climate control in hot-weather conditions. Two years ago temperatures in the misthouse routinely exceeded 105°F with maximum temperatures at 108-110°. Last year, we increased the shade level on the misthouse roof and achieved some level of cooling, but temperatures still often exceeded 100° and occasionally reached 106°. This year replacing the single fan with two higher capacity fans resulted in substantially lower

temperatures. The daily maximum temperatures throughout most of the summer rooting period ranged from 90-95°, with the highest recorded temperature at 96°. In the coming year, we will revisit the question of the efficacy of shading for cooling. The new post-doctoral researcher in physiology will examine the effect of various shade levels on net photosynthesis and determine whether current photosynthetic accumulation is important for rooting success.

A similar approach will be taken with watering regimes. Our current misting treatments are based on our best estimates of whether the cutting leaf surfaces and rooting media are too wet or dry. These imprecise measures cause us to undershoot or overshoot the mark as far as keeping the cuttings ideally misted. The new physiologist will determine some basic parameters about the water relations of the cuttings. These will include: (1) how much water stress the cuttings experience under various environmental conditions, (2) what levels of stress are damaging to rooting success of the cuttings, (3) how successful different misting treatments are at mitigating water stress, and (4) whether a cutting's susceptibility to water stress changes over time in the rooting bed. We intend to have the new post-doc on board by Jan. 1, 1996. Expect more detailed study plans and maybe some preliminary results by the next meeting in April.

QUALITY OF ROOTED CUTTINGS

Field Test of Root Systems

In Winter, 1995, we stuck 7600 cuttings for this experiment. Six thousand of the cuttings were the same ones used to assess rooting ability of the 400 hedges of family 9-1020 for the QTL experiment. The additional 1600 cuttings were taken from the same hedges, but were treated with a 1-second dip in 5 mM NAA. After twelve weeks in the rooting bed, each cutting was scored for number of roots, orientation of roots and symmetry of root system. A total of 1833 cuttings were tagged and then transplanted into the G.H.W. Weyerhaeuser Nursery in Washington, NC in June, 1995. Survival in the nursery has been excellent, despite several tropical storms. While in the nursery, the cuttings have received two lateral root pruning treatments and one undercutting treatment. The cuttings will be lifted in December, scored again, and transplanted to two field locations. There they will be measured each year to determine if growth and form are dependent on the root system characteristics we measured.

Refining NAA Treatments

The objective of this experiment is to determine optimal NAA treatments for both rooting percentage and root system morphology. The first trial was first conducted in Winter, 1995 in the Forestry greenhouse, but extreme temperatures and water failures rendered the results meaningless, so it was repeated in Spring, 1995. Both liquid (1, 5, and 10 mM) and talc (931, 1862 and 3724 ppm [w/w]) treatments were tested. The experiment was conducted on four full-sib loblolly families, with 6 replications and 6 cuttings per treatment/family/rep combination for a total of 1008 cuttings.

Contrary to previous results, treating cuttings with NAA did result in higher rooting percentages than in the control cuttings (Table 2). As before, NAA treatment also resulted in more roots per cutting than in controls. From this experiment, the best treatments appears to be 5 mM NAA if applied as a liquid or 1862 or 3724 ppm NAA if applied as a powder, although higher concentrations of the latter cannot be ruled out. It is important to keep in mind that we have previously reported that cuttings in different stages (e.g. succulent vs. dormant) have different requirements for and sensitivities to auxin. We intend on repeating a similar experiment this coming winter for dormant cuttings and in the spring to achieve more confidence in the results.

Table 2. Effect of NAA application on percent of loblolly pine cuttings rooted and number of roots per cutting.

NAA Concentration	Method of Application	% Rooting	Number of Roots/ Rooted Cutting
0	---	18.8	1.37
1 mM	liquid	27.2	1.66
5 mM	liquid	37.8	1.90
10 mM	liquid	28.8	2.22
931 ppm (w/w)	talc	32.1	1.38
1862 ppm (w/w)	talc	34.5	1.65
3724 ppm (w/w)	talc	31.7	1.84

Recent Publications and Presentations

Diaz-Sala, C., K.W. Hutchison, B. Goldfarb, and M.S. Greenwood. Maturation-related loss in rooting competence by loblolly pine stem cuttings: the role of polar auxin transport and tissue sensitivity to auxin. Submitted to *Physiologia Plantarum*.

Diaz-Sala, C., M.S. Greenwood, and K.W. Hutchison. Loss of adventitious rooting ability during maturation in loblolly pine. Joint meeting of the IUFRO working groups on Molecular Genetics of Forest trees and Somatic Cell Genetics. Gent, Belgium. September 25-30, 1995.

Goldfarb, B., M.S. Greenwood, and K.W. Hutchison. 1995. Molecular and cellular events during adventitious root initiation in loblolly pine cuttings. Southern Forest Tree Improvement Conference. Asheville, NC. June 20-22, 1995.

Goldfarb, B., R. Whetten, and W.P. Hackett. 1995. Cloning of auxin-induced genes from loblolly pine. abstract in: Keystone Symposium: Frontiers of Plant Morphogenesis. Hilton Head Island, SC. March 29-April 4, 1995.

Greenwood, M.S. and R.J. Weir. 1995. Genetic variation in rooting ability of loblolly pine cuttings: effects of auxin and family on rooting by hypocotyl cuttings. *Tree Physiology* 15: 41-45.

Rowe, D.B., F.A. Blazich, F.C. Wise, and S.L. Warren. 1995. Effect of stock plant nitrogen nutrition on adventitious rooting of loblolly pine stem cuttings. Joint Congress of the American Society of Horticultural Science and the Canadian Horticulture Society. Montreal, Quebec. July 28-Aug. 3, 1995.

Related Publications

Rieckermann, H., B. Goldfarb, M.W. Cunningham, and R.C. Kellison. 1995. Root and shoot growth of sweetgum rooted cuttings. 1995. Proc. 23rd Southern Forest Tree Improvement Conference. Asheville, NC. June 20-22, 1995.

Goldfarb, B., G.M. Howe, W.P. Hackett, and O. Monteuis. Survival and growth of eastern white pine shoot apical meristems *in vitro*. Submitted to *Plant Cell, Tissue and Organ Culture*.