LOBLOLLY AND SLASH PINE ROOTED CUTTING RESEARCH AT N.C. STATE UNIVERSITY

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Abstract. -- Through the efforts of several N.C. State University scientists and with support of nine forest product companies, a loblolly and slash pine rooted cutting research project was initiated in January of 1992. Focus of the research is on: (l) genetic selection (culling) for rooting ability, (2) physiological process research on the fundamental mechanisms for adventitious root initiation, (3) stock plant or hedge physiology and culture with emphasis on carbohydrate / nitrogen ratios in the stem cuttings produced, (4) rooting environment research, and (5) investigation of auxin treatments for stimulating rooting and their effect on root system quality.

Open-pollinated loblolly families (48) have been screened using a hypocotyl rooting system to find hard and easy to root families that are now being used in additional physiological mechanism studies. Open-pollinated slash pine families have been rooted using the hypocotyl system and the family mean correlation between the number of roots per hypocotyl and volume or rust are .055 (ns) and .148 (ns) respectively. Early rooting experiments with IBA and P-ITB (an aryl ester of IBA) show no strong response to auxin with cuttings from l-year old hedges, yet family rooting differences were substantial.

Keywords: Loblolly pine, slash pine, rooted cuttings

INTRODUCTION

Rooted cutting technology leading to clonal forestry offers enormous potential for increasing forest productivity and value. In many parts of the world, intensively managed forest plantations established with rooted cuttings of outstanding genotypes are producing high value / low cost wood for an increasingly competitive global forest products industry. Despite many years of research and development work, a reliable, cost-effective, rooted cutting system for difficult to root loblolly and slash pines is not available. We have organized, with the support of nine forest industries, a research initiative aimed at studying the

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fundamental processes of adventitious root initiation and development with complementary work aimed at developing a useful rooting technology for these species.

RESEARCH FOCUS and PROJECT ORGANIZATION

The project is organized with five primary areas of research emphasis. All five areas will contribute to an increased understanding of adventitious root initiation and development, and/or work toward refinement of a useful rooted cutting technology that will allow the full potential of clonal forestry to be realized for loblolly and slash pines. Significant interaction and interconnection exists between and among research areas. However, for purposes of description, the five primary areas of research are described separately below.

1. **Genetic Selection (culling) for rooting ability** is being evaluated for its potential to enhance rootability and the subsequent impact on genetic gain for growth and rust.

2. **Molecular biology** studies are concentrating on understanding the role of gene expression in the physiology and developmental process of adventitious root initiation. The work includes studies of polar auxin transport, auxin binding proteins and research on genetic control of maturation and the accompanying loss of rooting.

3. **Stock plant physiology** and culture with initial emphasis on managing the carbohydrate/nitrogen ratio in hedges and the stem cuttings produced is being evaluated.

4. **Rooting environment research** is focusing on the evaluation of a containerized "plug-nursery" system. This work will compare various rooting media, containers for rooting, seasons of the year for harvesting and sticking cuttings, as well as greenhouse versus shade house environments for rooting.

5. **Root system quality** developed on rooted propagules is being studied in relation to varying auxin treatments used to stimulate root initiation.

An N.C. State University research team comprised of physiology, molecular biology, genetics, and horticulture specialists has been assembled to conduct this research. Dr. Michael Greenwood, Ruth Hutchins Professor of Tree Physiology, University of Maine, served as lead visiting scientist for the project in year one. In year two physiologist / molecular biologist, Dr. Barry Goldfarb, Assistant Professor and Rooted Cutting Project Lead Scientist, N.C. State University joined the project. Work on rooting environment and propagule quality research is being done by Dr. Frank Blazich, stock plant nutrition studies by Dr. Leslie Henry, and genetic selection work and overall project coordination by Dr. Robert Weir. The research project duration is for four years with a decision to continue contingent on progress and additional research needs.

RESEARCH PROGRESS AND PLANS

Work has been underway on the rooted cutting research project for 18 months and to some extent we are still in the "getting started phase" of activity. However some results have been obtained and are reported below along with a description of study plans and additional work in progress.

Genetic Selection (Culling) for Rooting

Screening and selection of families for rooting ability is an obvious way to enhance the efficiency of a rooted cutting system. Genetic variation for rooting is substantial and the genetic control of rooting in loblolly pine appears to be strong. Broad sense heritability for rooting percent in loblolly pine was estimated from a small group of families (9) to range from .61 to .86 (Anderson et. al., in preparation). In a larger study involving 54 families, but with a small number of donor ortets per family, Foster (1990) reported broad sense heritabilities for rooting percent in the range of .20 to .40.

Selecting for rooting percentage may constrain the improvement possible for growth and productivity traits. Some hope can be derived, however, from the results of Foster (1985). In western hemlock, he found a positive genetic correlation (+.37) between rooting percent and subsequent growth of the rooted cuttings. While the literature abounds with evidence of family differences for rooting ability, little has been reported on the genetic gain consequences of applying this selection / culling to a population of fast growing families. This can be conceptually thought of as an independent culling problem. Select many outstanding families for growth superiority and then propagate only those families or individuals within family that have acceptable rooting percentages (75 + percent?). The question then becomes: what proportion of the good families must be discarded for poor rooting and how does the commensurate reduction in selection intensity impact growth and/or rust improvement?

Initial research efforts in this area are focused on establishing these relationships for slash pine. A reasonably good protocol (resulting in 70+ percent rooting) for rooting hedged slash pine currently exists. Seeds from 50 slash pine half-sib families from the University of Florida Cooperative Forest Genetics Research Program have been obtained. These families represent a wide range of good and poor volume and rust resistance performance. Cutting hedges, derived from 20 seedlings per family, have been started for each of the 50 families. A screening trial for rootability will be conducted to establish the relationship between culling for rooting percentage and the subsequent impact on gain for growth and/or rust resistance. Similar work will be done for loblolly pine, once physiology studies have provided information to improve the rooting protocol.

While we are developing hedges of the 50 slash pine families for rooting trials using stem cuttings, we have screened these same families for rooting ability using an experimental hypocotyl rooting system developed for loblolly pine by Michael Greenwood. In this experimental system, newly germinated slash pine seedlings (21 days from sowing) have their roots removed by severing the stem 2.5 cm below the cotyledons. Each hypocotyl cutting is placed in a hole drilled in a stryofoam sheet which is floated on a solution of 10 micro-molar IBA (Indole-3-butryic acid) and distilled water contained in an opaque plastic tray. Approximately 1 cm of hypocotyl stem is constantly bathed in the IBA solution. The cuttings are placed in a growth chamber and maintained at 80° F day, 68° F night, with a 16h photoperiod with 1000 to 3000 foot candles of incandescent light.

With IBA treatment, virtually all hypocotyl cuttings initiated new roots after 20 days. The most useful rooting response was the number of roots per hypocotyl at 15 days after sticking, as this trait displayed substantial family variation and, as discussed later for loblolly, correlated to some degree with cuttings from one to three year-old hedges. The family means for number of roots per hypocotyl ranged from a low of 4.1 to a high of 24.0. Narrow sense heritabilities for root number were .44 on an individual tree basis and .63 calculated on a family mean basis. Culling the lowest 50% of the families increased the mean number of roots per cutting from 12.4 to 16.0. The family mean correlation between root number and volume was .055 ns, and between root number and rust resistance was .148 ns. These results indicate that screening slash pine families for rooting ability could enhance the efficiency of a rooted cutting system without negatively impacting gains in volume and rust. These results should be viewed with caution, however, because the strength of the correlation between hypocotyl rooting and rooting of cuttings from hedges is still uncertain.

Molecular Genetics and Cell Biology of Root Initiation

Michael Greenwood developed a system for studying the importance of auxin in the rooting process using hypocotyl cuttings from loblolly pine seedlings placed in a solution containing 10 micro-molar IBA (Greenwood, 1992). He demonstrated that auxin treatment is necessary for root formation in these cuttings and when applied yields almost 100% rooted cuttings. Phytotropins (auxin transport inhibitors) applied at the same time as the IBA, inhibited root formation implicating auxin transport and possibly auxin binding as having critical roles in root initiation. A logical next step will be to examine a class of proteins known as auxin binding proteins. These have been hypothesized to be auxin receptors (Jones and Prasad, 1992). It will be informative to learn if these putative receptors differ in good and poor rooting genotypes, different tissues of the seedling, and stem tissues from trees of varying ages.

A major research focus is on gene expression during rooting and how expression of rooting genes changes during tree maturation. A hypocotyl cutting rooting system similar to the one described above for eastern white pine has been developed (Goldfarb et al, 1992) and is being adapted to loblolly pine. It differs from the previous system in that the hypocotyl cuttings are given a high-concentration (300 mg/L or l.6 mM) pulse with l-naphthaleneacetic acid (NAA) for 5 minutes and then transferred to water or moist sand for the root initiation period. This system is ideal for studying changes in gene expression during rooting, because a large number of roots are formed on each cutting (-20 for loblolly).

Gene expression will be studied by identifying genes expressed uniquely in root-forming pine cuttings using cDNA cloning and differential or subtractive hybridization. In addition to pine rooting genes identified in this manner, we can also use gene probes from other plants that may be involved in the rooting process. Candidate probes include a gene from tobacco that is expressed in root meristems (Conkling et al, 1990; Yamamoto et al, 1991), a cell-wall glycoprotein from English ivy whose expression is negatively correlated with adventitious root initiation (Wu et al, 1993), genes stimulated by auxin treatment in soybean hypocotyls (Li et al, 1991), and genes controlling transcriptional regulation during flower development in Arabidopsis (Coen and Meyerowitz ref) and leaf development in maize (Vollbrecht et al, 1991).

We are also collaborating with the Forest Biotechnology group at N.C. State to use RAPD mapping to analyze which portions of the genome contribute to variation in rooting success (quantitative trait loci or QTL analysis). One thousand seedlings from a single open-pollinated family have been screened for rootability in the IBA hypocotyl system and the DNA from the female gametophytes of those seedlings is being analyzed.

We intend that these approaches will provide us with a better understanding of the fundamental mechanisms of root initiation and ultimately with the information needed to design better rooted cutting systems.

Stock Plant or Hedge Physiology and Culture

Evidence reported in the literature suggests that the environmental history and the resulting physiology of a stock plant or hedge strongly influences subsequent rooting of stem cuttings. Two measures of physiological status which are strongly influenced by stock plant environment are carbohydrate and nitrogen contents (Moe and Anderson 1988). Changes in the relative amounts of either carbohydrates or nitrogen within cuttings have been shown to influence rootability (Haissig 1986) .

Although a regulatory role for carbohydrates in the rooting process has not been firmly established, it is understood that total non-structural carbohydrate (TNC = soluble sugars and starch) content within a cutting may influence rooting by establishing the amount of energy reserves and carbon skeletons available to support root initiation and growth (Haissig 1986; Veierskov 1988). Carbohydrate level may also influence mobilization, including loading and/or transport, of auxin from the leaves into the roots (Jarvis 1986). However, carbohydrate status alone does not necessarily determine rootability. Depending on the species, a high concentration of TNC at the time of severance may show a positive, negative or zero correlation with subsequent rooting (Veierskov 1988).

Nitrogen status of the stock plant may also influence rootability of cuttings (Moe and Andersen 1988; Haissig 1986). Nitrogen deficiency (not stress) within the stock plant generally promotes root formation of cuttings, possibly via its influence on carbohydrate metabolism (Haissig 1986). When nitrogen is deficient, further metabolism of the carbohydrates produced by the stock plant into organic compounds is restricted, and sink demand for carbohydrates to support shoot growth declines. Under these conditions, excess carbohydrates can be translocated as sucrose and stored within the stem tissues as a combination of sucrose and starch (Veierskov 1988). The greater levels of carbohydrates within the stem tissues would then be available to support root formation on cuttings. This indicates that it is not nitrogen status *per se* that influences subsequent rooting. It is rather the influence of nitrogen status on the amount and form of carbohydrate present within the stock plant prior to severance that influences root formation on cuttings. Once severed, nitrogen status within the cutting determines whether the available carbohydrate will be directed toward further shoot production or toward root formation: a high nitrogen status relative to available carbohydrate results in a tendency for stored carbohydrate and current photosynthate to be directed into shoot production.

In addition to the influence of environmental effects, stock plant physiology is influenced by the genetics of the individual plant. Correlations between carbohydrate / nitrogen ratio and rootability have been shown to have a genetic component (Struve 1981). Previous research with *Pinus rigida* has indicated that differences in rootability of clones may be related to differences in their carbohydrate / nitrogen ratios (Hyun and Hong 1968): a clone which rooted easily had a higher carbohydrate / nitrogen ratio throughout the year than did the clone which was difficult to root. Differences in the carbohydrate / nitrogen ratio were attributed to differences in the nitrogen content, with the easy-to-root clone having a lower nitrogen content than the difficult-to-root clone. The greatest differences in carbohydrate / nitrogen ratios between clones coincided with the time of year when rooting differences were at a maximum.

To determine the effects of stock plant nutrition on rooting of cuttings, hedge plants with differing carbohydrate / nitrogen status are being established by growing plants under a range of nitrogen availabilities (5, 10, 15, 20, and 50 ppm N supplied as NH_4NO_3; all other nutrients will be supplied at optimal levels). A sixth (control) treatment is also included that consists of a standard Osmocote fertilization regime. These treatments include an upper level of N which gives optimal growth of loblolly pine seedlings and a lower limit which causes a shift in carbohydrate partitioning from shoot to root growth. These treatments and the intermediate levels of N bracket the level of N (20 ppm) which has been shown to produce maximal rooting response in cuttings from red cedar stock plants (Henry *et al.* 1991). Hedges of two full-sib families of loblolly pine that have previously shown to have consistently high rootability (>50%) and two full-sib families that have shown consistently low rootability (<10%) are being used in the study.

A factorial design of families and nitrogen treatments is being utilized. Each family / treatment combination is represented by four hedges in each of four randomized complete blocks. The complete study is comprised of 384 hedges. Hedge plants for the five nitrogen treatments are being grown in a sand / perlite medium, while the seedlings in the osmocote control are growing in a medium of 2 parts peat, 2 parts vermiculite, and 1 part perlite. The first hedging and rooting experiments will be conducted in January 1994 with a second hedging to follow in May 1994.

The total non-structural carbohydrates (sucrose, reducing sugars, and starch) and total nitrogen (soluble and insoluble fractions) will be determined from analysis of the cuttings. These analyses will provide baseline information regarding the physiological status of the hedge plants at the time the hardened cuttings were removed. Rootability of cuttings from each nitrogen treatment will be determined under an "optimal" rooting environment in the greenhouse. Percent rootability will be compared between nitrogen treatments within a family. Correlations between rootability and carbohydrate and/or nitrogen status (including concentrations of each form of carbohydrate and/or nitrogen present) will be established for each treatment within a family. Since nitrogen availability will affect vegetative growth of the hedges, the effects of nitrogen treatment on the number of orthotropic shoots produced by hedged plants will also be determined.

Rooting Environment Research

Considerable research activity has been directed in recent years toward manipulation of the rooting environment. Some success has been achieved recently with trials that mimic the New Zealand approach to rooting radiata pine in an outdoor nursery bed environment (Frampton and Hodges 1989). Yet for every success, several failures have occurred. While the New Zealand system offers some promise in the southern U.S., the demands for excessively well drained rooting beds, high volumes of water applied as a high frequency fine mist, and the need for protection from mist disrupting and desiccating winds, may limit the usefulness and cost effectiveness of this rooting system in standard nursery beds. An alternative rooting system is being evaluated.

We have begun work on a containerized greenhouse / shadehouse "plug-nursery" system for producing rooted cuttings. This approach provides the following advantages:

1. Utilization of existing automated equipment developed for plug-one seedling production in the Pacific Northwest.

2. Improved control of environmental variables during the rooting period, especially temperature control.

3. The production of multiple rooted cutting crops per year thus providing cost benefits on facility production rates by increasing the utility of stock plant hedges overtime and by more evenly distributing personnel demands.

In developing a "plug-nursery" research effort we will examine the effect of container size, shadehouse / greenhouse environments, rooting in different seasons of the year, nutrition management, and rooting media. Rooting media effects could prove to be interesting in that the type of root system has a profound effect on lifting and outplanting of the rooted cuttings. Whether a coarse or fibrous root system develops on a cutting is strongly influenced by the rooting medium. For example, cuttings of some species, when rooted in sand, produce long, unbranched, coarse and brittle roots. However, when rooted in a mixture of medias, such as sand and peat moss or perlite and peat moss, the roots that are produced are well-branched, slender and flexible. The latter type is more suitable for digging and outplanting (Hartmann and Kester, 1983). If a standard protocol is to be developed for mass propagation of stem cuttings of pines, research is needed to determine which media will produce rooted cuttings of a quality that will reasonably assure survival and performance following outplanting. The criteria for evaluation will include: rooting percentage, plant quality, transplanting ease, and cost differences.

Quality of Rooted Propaqules

During vegetative propagation of various woody species by stem cuttings, treatment of the cuttings with root promoting compounds, most notably auxin(s), is a common practice. Although many species respond positively to auxin treatment, some do not, illustrating that a positive physiological response to auxin treatment is not universal. However, the benefit of auxin treatment is obvious in those species where rooting would not otherwise occur. In addition, four specific advantages associated with auxin treatment have long been recognized:

1. Increasing the percentage of cuttings that form roots.
2. Hastening root initiation.
3. Increasing the number and quality of roots produced per cutting.
4. Increasing the uniformity of rooting.

The first, second and fourth advantages need no explanation. However, the third, regarding the number and quality of roots per cutting, is intriguing because countless studies have noted it as a benefit of auxin treatment. Published reports on the influence of root numbers on establishment and subsequent cutting growth are conflicting yet indicate the possibility of a positive affect (Struve *et al.*, 1984; Struve and McKeand 1990; Wisniewski *et al.*, 1991).

When rooting stem cuttings of various pine species, root number per rooted cutting is usually quite variable within specific treatments regardless of percentage rooting for a treatment. Even though a particular treatment may yield a high rooting percentage, rooting percentage by itself is probably not a good indicator of a treatment because the number of roots on individual cuttings must also be considered. Although it appears logical that root number per rooting cutting is an important factor to consider when evaluating the relative effectiveness of particular treatments, the extent, as mentioned previously, to which root numbers influence establishment and subsequent cutting growth is unclear, especially for loblolly and slash pines.

To establish the relationships for rooting percent, root number and auxin treatment, a series of initial greenhouse rooting experiments are underway. The first experiment used cuttings from 100, one-year-old seedling hedges grown from four full-sib families. Harvested cuttings of 9 cm length were dipped (basal 1 cm) in auxin solutions, stuck in a raised greenhouse rooting bench, containing a media of 1 part peat and 1 part perlite. The cuttings received intermittent mist 6-8 seconds every five minutes for 13 hours each day to reduce desiccation. The auxin treatments used were 5, 10, 15, 20, and 25 mM indole-3-butyric acid (IBA) and an aryl ester of IBA [phenyl indole-3-thiolobutyrate (P-ITB)], plus a control. The aryl ester of IBA is a relatively new synthetic auxin and in some cases, has been superior to the free acid of the same compound in promoting root initiation (Blazich, 1988; Haissig, 1979; 1983). The experiment consisted of 6 replications of four families and 11 auxin treatments. There were six cuttings in each rep / family / treatment combination for a total of 1584 cuttings.

Overall rooting percentages were good, however, the auxin treatments did not appear to promote rooting, as the percent rooting for the control was always equal or higher than the auxin treatments (Figure 1). The aryl ester of IBA, P-ITB, at low concentrations, was associated with increased root numbers per cutting (Figure 2). Family differences for rooting percent were evident (Figure 3).

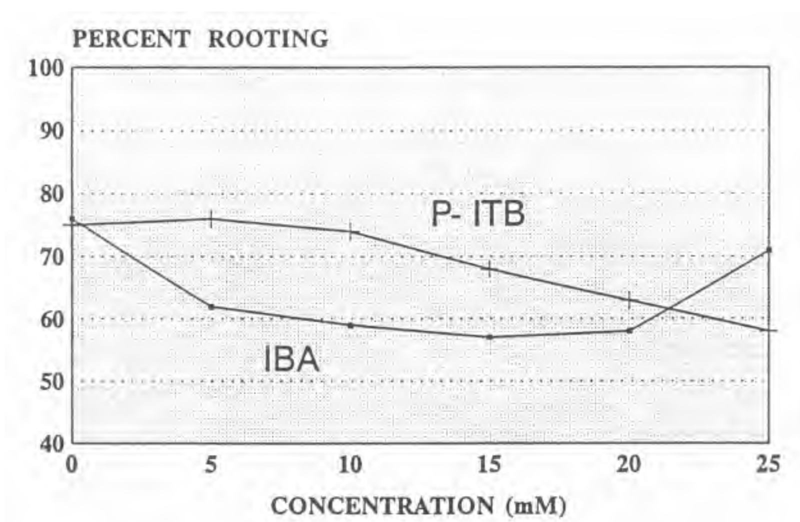

Figure 1. The effect of IBA and P-ITB on rooting percent of loblolly stem cuttings from 1-year-old hedges.

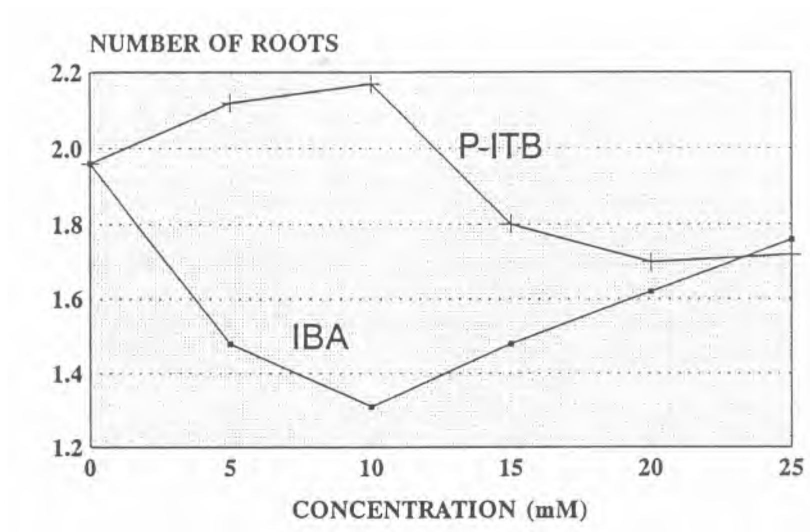

Figure 2. The effect of IBA and P-ITB on the number of roots on loblolly stem cuttings from 1-year-old hedges.

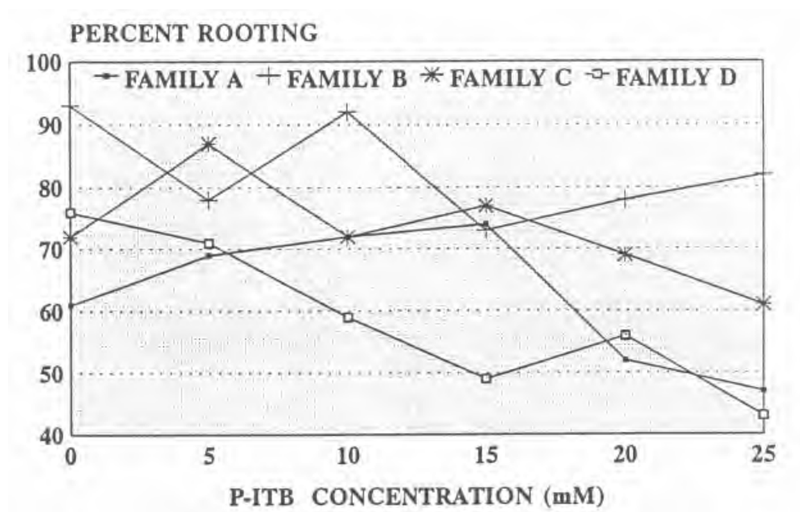

Figure 3. The effect of P-ITB on percent rooting for four families of loblolly stem cuttings from 1-year-old hedges.

Future work in this area will evaluate additional hormone treatments, including Hare's powder, and possibly other synthetic hormone compounds. It may be that as the hedges age and maturation commences, the hormone treatments will provide a greater root initiation stimulus. We also plan to evaluate the season of cutting harvest, especially comparing dormant season (hardwood) cuttings to succulent cuttings harvested in May and August during the growing season. A long term goal would be to develop quality standards for rooted cutting propagules, based on root numbers, or comparable criteria that may prove to be important. Outplantings will be established for subsequent survival and growth comparisons among quality classes.

SUMMARY

A research project focused on improving our basic knowledge concerning adventitious root initiation and development on loblolly and slash pine rooted cuttings has been initiated. The project encompasses a broad range of investigation from the very basic molecular biology studies, including gene expression, to more applied genetic selection and rooting environment studies. Some intriguing early results have been achieved, yet much remains to be done. The ultimate goal is to develop sufficient understanding about the fundamental process of adventitious root initiation and development to allow the development of a practical, cost effective rooted cutting system for loblolly and slash pines that will lead to the implementation of clonal forestry for these two regionally important pine species.

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