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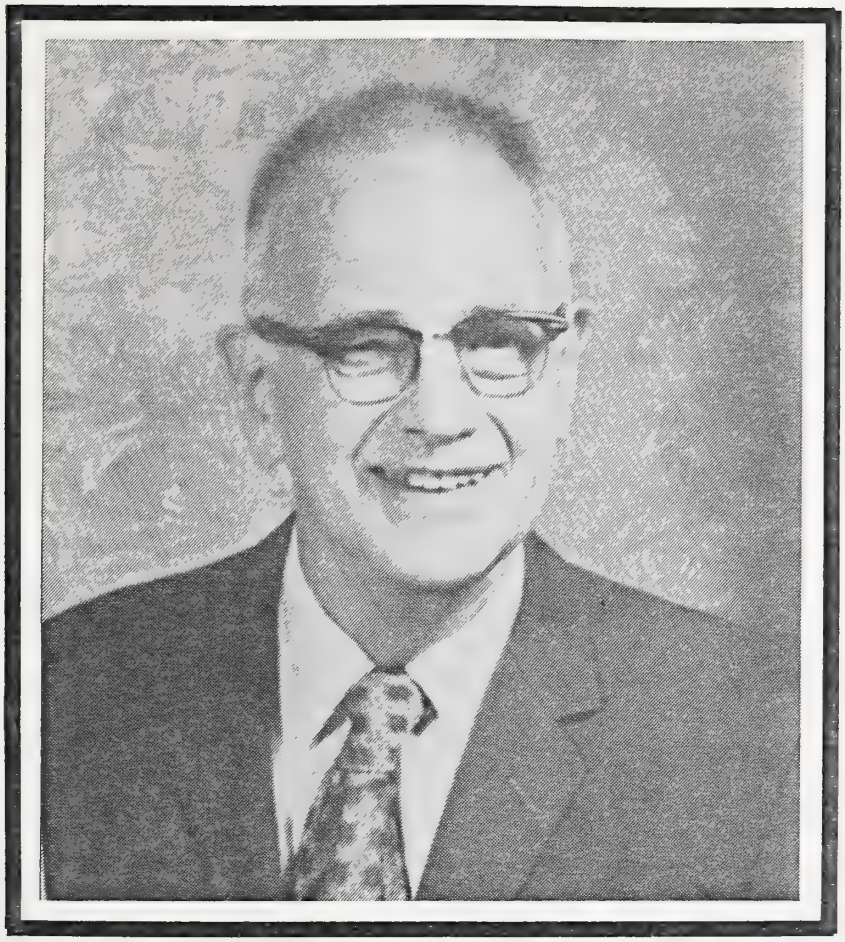

These proceedings are dedicated to Philip C. Wakeley who died August 28, 1983, at his home in Ithaca, New York. Phil was a charter member of the Southern Forest Tree Improvement Committee and was best known to most of us for his work in planning, establishing, and coordinating the Southwide Pine Seed Source Study. He was very proud of the Southwide Study but actually, it was only a capstone on a long career of silvicultural research with the Southern Forest Experiment Station. He and his wife Christine came to New Orleans in 1924 and left 40 years later when he retired. He worked part time for the Forest Service for about 5 years after that, publishing the 30 year results of regeneration studies established by himself, his Forest Service coworkers, and the Great Southern Lumber Company (now Crown Zellerbach) near Bogalusa, Louisiana.

He was author or coauthor of well over a hundred publications and the most famous of these, "Planting the Southern Pines" played an important part in the regeneration of the southern pinery as we know it today.

It was my privilege to work with Phil for 2 years before his retirement. He was truly dedicated to all aspects of his research from policy implications to the finest detail, and was widely regarded as one of the best technical writers in the profession. He received the USDA Superior Service Award and was a SAF Fellow. The SAF honored him with the Barrington Moore Award for Biological Research in 1956.

O. O. Wells

O. O. Wells
Southern Forest Experiment Station
USDA-Forest Service

Abstract: Biotechnology and the Future of Forest Genetics Research

Stanley L. Krugman[1/]

Forest genetics and tree improvement activities have now become an integral part of forest management in most parts of the U.S. In a sense our field of science has become of age, and we are no longer immune from changing societal pressures i.e., reduced research spending. In response to these changing priorities at least in the Forest Service we are placing a greater emphasis on basic genetics research. That is, research that would accelerate our understanding of the genetic variation found in trees and research that would increase our ability to capture such variation in improved tree material. These are among the major reasons for initiating a modest forest biotechnology effort in forest genetics at this time. We hope to identify or develop an appropriate array of techniques that will overcome or at least shorten the time to identify, capture and incorporate those characteristics into forest trees desired by our various user groups. Such a program to be successful and useful must be supported by a broad based conventional forest genetics program. There must be adequate known material to manipulate. We should continue our ongoing evaluation of current programs as to usefulness; but priority research in early selection, intraspecific and interspecific breeding, disease resistance and elements of basic physiology should be continued. Care will need to be taken to ensure that long-term conventional field evaluation studies are adequately supported. We can expect the overall research program to be leaner but with a sharper focus on the priority needs of the future.

[1/]Director, Timber Management Research, U.S. Forest Service, Washington, D.C.

BIOTECHNOLOGY I

MODERATED BY DR. BART THIELGES

University of Kentucky

GENETIC ENGINEERING IN FOREST TREES

F. Thomas Ledig and Ronald R. Sederoff[1)]

Abstract.--Gene transfer, using recombinant DNA technology, can be used to engineer new, improved trees in a fraction of the time required by traditional breeding methods. Genetic engineering requires isolation of genes, their multiplication in bacteria, their transfer to tree cells, and regeneration of the transformed cells into new trees. Success has already been achieved in cloning conifer genes and in developing a transfer system, and several genes of potential value to forestry have been isolated from bacteria. The inability to regenerate conifers from transformed cells is the major remaining barrier to application of genetic engineering in tree improvement.

Additional keywords: *Agrobacterium tumefaciens*, *Pinus lambertiana*, *Pinus taeda*, *Cronartium ribicola*, genetic transformation, isozymes, heterozygosity, microinjection, recombinant DNA, biotechnology.

INTRODUCTION

The long life and large size of trees have always been major barriers to progress in forestry, especially in forest genetics and tree breeding. To surmount these barriers, forest biologists tried to develop techniques to enable early evaluation of growth and disease resistance and to shorten the reproductive cycle (e.g., Kinloch and Comstock 1980, Ledig 1974). However, recent advances in molecular biology offer entirely new possibilities for tree improvement. Instead of devising techniques for early evaluation, it is now possible to direct genetic changes while bypassing the sexual cycle, at least in particular instances (Sederoff and Ledig 1985). Using new biotechnologies, improvements in forest trees can conceivably be made on the same time scale as those in agricultural crops, and the large size of trees, which presently restricts selection intensity, poses no difficulties for technologies that operate on the cellular or molecular level.

The new capability for biological manipulation using such tools as genetic transformation, parasexual hybridization by fusion of protoplasts, and multiplication of high value materials by cloning, have captured the public imagination like few other scientific developments. Our concepts of life are being changed as surely as they were by the public announcement of Darwin and Wallace's theory of evolution. If it is necessary to identify the beginning of the current revolution, then 1953 is a good candidate, when Watson and Crick published their classic paper on the structure of DNA. Since that time, knowledge of the genetic material and the ability to use that knowledge have been accelerating. The new genetic tools are much more powerful than the ones provided by Mendelism and its rediscoverers.

Application of the new technologies in forestry will require a major research effort. Our ignorance of the genetics, physiology, and biochemistry

1) Institute of Forest Genetics, Pacific Southwest Forest and Range Experiment Station, U.S.D.A. Forest Service, Berkeley, California 94701

of forest trees, their pests and pathogens is as deep as our opportunities are broad. Until recently, fundamental research, such as studies of biosynthetic pathways, photosynthesis, and stress metabolism, had little application because they only explained how things worked, without providing ways to modify the genetic controls. Now, basic studies have greater utility because they provide information that can be used to modify processes to advantage. Many aspects of biology, physiology, pathology, and biochemistry, have been integrated by the new genetics. To narrow the subject, we concentrated on the possibilities of direct genetic manipulation of forest trees (i.e., genetic engineering) and the research needed to apply the technology.

Genetic engineering implies directed genetic change in individuals, and subsequently, in populations. Directed change is not new. During prehistory, early agriculturists brought about desirable changes in plants and animals despite little formal knowledge of genetics. With the discovery of the statistical laws of inheritance in the nineteenth and twentieth centuries, breeders accelerated the rate of change in agriculturally important plants and animals. However, genetic engineering now implies manipulations at the cellular or molecular level, and one of the most powerful tools of genetic engineering is transformation, the ability to insert new genes. Transformation provides both an applied tool and a method of studying the nature of the gene.

TRANSFORMATION OF FOREST TREES

The Process

The insertion of a gene into a new host, thereby genetically "transforming" the host, has four components: a DNA fragment consisting of a single gene or a small block of genes must be identified and isolated; the block must be inserted into a vector where it is multiplied; the foreign DNA must be transferred to the host cell where it is incorporated and expressed; and the transformed cells must be regenerated into a plant. The process of DNA-directed transformation was first discovered in bacteria (Avery, MacLeod, and McCarty 1944), but in recent years has been applied to cells of higher plants (Goldsbrough et al. 1983, Murai et al. 1983), such as sunflower (Helianthus annuus L.) and tobacco (Nicotiana tabacum L.). Application to trees is not completely straightforward because of some unique aspects of their biology and because of the general lack of past effort in basic forest research.

The Genes

Single-gene traits in trees. Few simply inherited traits are known in forest trees, and most of these are of little or no economic interest. They can be divided into four major classes: isozymes, major visible aberrations, terpenes and other volatiles, and disease resistance factors. As many as 60 isozyme loci are known in some species (Conkle et al. 1982), but there seems to be no advantage in transferring them. Aberrations, such as albinism and dwarfism (Franklin 1970), are of negative value except perhaps for the narrow-crowned phenotype, considered to be inherited as a single gene in some European conifers (Karki 1983). Many volatiles are simply inherited and may have potential in conferring resistance to insects (Smith 1966), and in some cases, as valuable extractives. Disease resistance is the class of genes of obvious value for transfer among trees (Kinloch, Parks, and Fowler 1970).

Research at the Institute of Forest Genetics is directed toward transfer of genes that could improve yield or value of forest trees. One of these objectives is the eventual isolation and transfer of the major gene for white pine blister rust (Cronartium ribicola J.C. Fisch. ex Rabenh.) resistance from resistant sugar pine (Pinus lambertiana Dougl.) to susceptible individuals and species.

Determining the mode of inheritance of genetic characteristics (single genic or polygenic) is especially difficult in forest trees because it is plagued with one of the traditional barriers confronting forest genetics; i.e., the long generation time. One or two generations of crosses must be made to demonstrate Mendelian segregation, and even then the simultaneous segregation of genes with pleiotrophic effects may make it difficult to draw definitive conclusions. Trees are among the most heterozygous of organisms (Hamrick 1979), so the genetic background in most species is highly heterogeneous, obscuring the effects of segregation at individual loci.

On the other hand, conifers have some advantages for genetics. Many genes code for enzymes that are active in the megagametophyte, the nutritive tissue or "endosperm" of the seed. The megagametophyte is a haploid tissue that is derived from one of the four cells produced by meiosis (e.g. Allen and Owens 1972). Segregation can be detected as variation among seed (megagametophytes) from the same cone or from different cones on the same tree. A sample of several megagametophytes will show a 1:1 ratio of allelic types in a heterozygous individual. In classic Mendelian genetics, a 1:1 ratio is usually demonstrated by a "test cross", but use of the conifer megagametophyte eliminates the need for test-crossing (Conkle 1974). Therefore, for allozyme loci, conifers provide the advantages of haplogenetics, pioneered in fungi such as the bread mold (Neurospora crassa; Barratt et al. 1954).

Isolating genes. To isolate a gene, the DNA is cleaved with restriction enzymes and the fragments are spliced into the DNA of a self-replicating virus or plasmid, called a "vector", that infects bacterial cells. When the vector with its foreign DNA infects a bacteria, the fragment is multiplied, or "cloned", along with the vector's DNA. The colon bacteria (Escherichia coli) is a common organism used to clone DNA fragments, and a collection of bacterial colonies, each incorporating a different fragment, forms a "library" of the donor's DNA. There is often no way to tell which fragment carries the gene of interest unless a similar gene, previously isolated from another species, is available to "probe" for it with DNA-DNA hybridization techniques.

Isolation of genes in conifers would be difficult even if genes worthy of transfer were known. The conifer genome is very large, apparently 34.7 pg for 2C content in sugar pine (Dhillon 1980). By comparison, the genome of corn (Zea mays L.) is only about 11 pg (Bennett 1972), which itself is large compared to many animal species. The human genome is only 7.3 pg (Bachmannn 1972), and many insects have 2C contents that are another order of magnitude smaller, around 0.2 pg for fruit flies (Sparrow, Price, and Underbrink 1972).

Linkage mapping. Knowing where a gene is located is important if it is to be isolated. Linkage maps for conifers are very incomplete, and no genes have been associated with individual chromosomes. If genes could be identified to chromosome, it might be possible to rapidly sort out specific chromosomes with dual laser flow sorters (Dickson 1985). The task of constructing a fragment

library for a single chromosome would be less than one-tenth as difficult as constructing a library for the entire genome. Inserting entire chromosomes in plant cells, rather than fragments, is another possibility (Malmberg and Griesbach 1983), although aneuploids are unstable and usually aberrant in conifers (Mergen 1958, 1959). However, even isolating a chromosome would be difficult in forest trees, given present knowledge. For example, the 12 chromosomes of the haploid set that characterize most of the family Pinaceae are scarcely distinguishable with conventional stains (e.g., Saylor 1961). In most of the pines, spruces (Picea spp.), and firs (Abies spp.) only the smallest, heterobrachial chromosome can be identified with confidence. The others are all homobrachial and similar in size. Newer radiological and staining techniques employed in human cytogenetics may be fruitful. Recently, Hizume, Ohgiku, and Tanaka (1983) claimed to distinguish all of the chromosomes of Austrian pine (Pinus nigra Arnold) with fluorescent banding, but very little present research effort is focused on the conifer karyotype.

There is a chance of finding linkage between allozyme loci and genes controlling other characteristics, such as disease resistance. M.T. Conkle and B.B. Kinloch[2] (personal communication) have already demonstrated loose linkage (27 map units) between the major gene for blister rust resistance in sugar pine and a 6-phosphogluconate dehydrogenase locus. For isozyme loci to be really useful for isolating genes with unknown products, such as the gene for blister rust resistance, the two must be very tightly linked. Linkage maps are being constructed for several species in the Pinaceae (e.g., Conkle 1981). Because of the apparently high degree of conservatism in evolution of the conifer karyotype, linkage maps in one conifer are likely to approximate those in others. The same linkages are repeated in the pines, firs, and spruces investigated so far (e.g., Conkle 1981, King and Dancik 1983, Neale and Adams 1981).

Restriction site mapping using enzymes that cut the DNA at specific base sequences, combined with isozyme mapping, would provide an extensive map in a short time. The development of isozyme technology in conifers provided a rapid means for chromosome mapping, but its utility is limited; only about 60 isozyme marker loci are available. While 60 is a considerable number, especially compared to virtually none 10 years ago, restriction fragment mapping could expand the number of markers to hundreds. Recombinant DNA techniques do not depend on expression of a gene; fragments can be assayed at any time. By contrast, genes coding for enzymes, such as alcohol dehydrogenase, may be expressed only during a restricted period of development or in certain tissues (Conkle 1971). Furthermore, fragments need not include functional genes in order to be valuable markers. Any fragment can be used that can be recognized by its banding pattern in molecular hybridization analysis.

Genes in heterozygous combination. Of special interest is the relation between heterozygosity and growth. In trees, growth and fitness are closely related, and they are correlated with heterozygosity. The notion that vigor and heterozygosity are related is not new; explanations for hybrid vigor, or heterosis, go back at least to the work of East and Shull over three-quarters of a century ago (Shull 1952) and was the subject of Lerner's (1954) classic book, "Genetic Homeostasis". However, the development of enzyme

[2] M.T. Conkle and B.B. Kinloch, Institute of Forest Genetics, U.S.D.A. Forest Service, Berkeley, California

electrophoresis revealed variation of such proportions that it was difficult to explain it all as a result of balancing selection; i.e., selection favoring heterozygotes (Lewontin 1974). Nevertheless, in a variety of organisms, including trees, growth and heterozygosity for isozyme loci are positively correlated in natural populations, a new finding (Ledig, Guries, and Bonefeld 1983). These results must be extended to additional species and to controlled environments, to determine their generality.

The newly found correlation between growth and heterozygosity in forest trees raises several questions regarding the conduct of tree improvement programs. In the initial stage of tree improvement programs, trees are selected one per stand and interplanted as grafted clones in seed orchards. Are realized gains from seed orchards the result of crossing among unrelated parents, which would favor heterozygosity? If so, will gains from a second generation of selection be much lower than expected? When trees are selected in natural populations based on growth, do heterozygotes have a greater probability of selection? Would a better scheme be to select and cross trees that differ at the maximum possible number of loci to produce highly heterozygous progeny?

Before the proper tree improvement strategy can be identified, research is needed to determine whether all the isozyme loci have an effect on growth or just specific genes. And, do all the loci involved have equal effects or are some more important than others? In fact, do the isozyme loci themselves control growth rate or are they simply linked to other, more important genes? Heterosis could actually be the result of inferiority of homozygotes at linked deleterious loci. Inbreeding depression is the converse of heterosis.

Transformation offers a way to investigate some of these questions. It would be relatively simply to isolate alternative alleles of isozyme loci and introduce them into a homozygous background to investigate the effect of heterozygosity at single loci. Torrey pines (Pinus torreyana Parry ex Carr.) are a prime target, because they seem to be completely homozygous (Ledig and Conkle 1983). Of course, regeneration of conifers from transformed cells is still a barrier to completing such a critical experiment.

Research needs. If genes are to be isolated from forest trees, forest biologists will need several types of knowledge: an understanding of the physiological and biochemical mechanisms of traits of interest, their mode of inheritance, and the gene products involved. Molecular geneticists will profit from better linkage maps of the conifer genome. Tight linkage with genes for which probes are available would facilitate isolation of the right fragment.

At first, genetic engineering in forestry will rely on genes from other organisms because of the paucity of economically important, single-gene traits identified in tree species. In fact, forestry will benefit from the much larger research effort in agriculture and medicine. Genes for insertion in conifers or hardwoods can come from any living system, bacterial, fungal, plant, or animal. Some candidates for transfer are herbicide resistance and salt tolerance (Chaleff and Ray 1984, Le Rudulier et al. 1984). Incorporation and expression must be investigated in tree species if forestry is to make use of genes from other organisms. At present we know little about the structure of the genome in tree species -- why do conifers have so much highly repeated DNA? Do conifer genes have introns? What are the promoters like? DNA content

may vary among populations and individuals: is DNA content itself adaptive, perhaps related to drought or cold hardiness? These questions are researchable and should be attacked early in any program of genetic engineering.

The Cloning-Vectors

Cloning conifer DNA. Few difficulties are anticipated in cloning conifer DNA. R.R. Sederoff and P.D. Hodgskiss have inserted two copies from a highly repeated fraction of the sugar pine genome into the bacterial virus M13 and multiplied them in the colon bacteria. They have sequenced segments of about 400 base pairs in length and will extend this in the near future. These DNA clones will be useful probes to determine where the sequence occurs in the sugar pine genome and its homology with the highly repeated fraction in other pines and more distantly related conifers.

The crown gall bacterium. Transformation is being approached from two directions: through the use of the crown gall bacterium (Agrobacterium tumefaciens) and by direct microinjection. Crown gall is the most widely-used system for transformation in higher plants (Barton and Chilton 1983). It carries a loop of DNA, the Ti plasmid. In an infected plant, part of the plasmid DNA takes up residence in a linear chromosome of the host. The plasmid genes are faithfully transcribed by the host, resulting in production of substances necessary for growth and reproduction of the bacterium. The plasmid DNA has been mapped, and can be modified to carry foreign genes, providing a means to transform selected host-plants. However, crown gall was not known to infect pines, although it had been reported on firs, incense cedar (Calocedrus decurrens [Torr.] Florin), and other conifers (de Cleene and de Lay 1976). Within the last year, R.R. Sederoff, A. Stomp, L. Moore, and W.S. Chilton have found a strain that will transfer and express genes from the crown gall bacterium in loblolly pine (Pinus taeda L.).

Microinjection. Microinjection is a direct way of introducing DNA into target cells, and has been used successfully in animal systems (Lo 1983). Very fine needles are guided into isolated, suspension-cultured cells, using micromanipulators. DNA is moved from the needle into the cell by altering the charge. Either vectors, such as the Ti plasmid, or "raw" DNA fragments can be "injected". There are still many technical difficulties in applying the procedure to conifer cells. Primary among these is the difficulty of penetrating the thick cell wall. It may be simpler to inject naked protoplasts (i.e., cells whose walls have been stripped by a cellulase enzyme). However, for most conifers it has not been possible to regenerate viable cell suspension cultures from protoplasts, although Teasdale and Rugini (1983) were successful with loblolly pine. And it is not at all certain that injected DNA will move into the nucleus, be incorporated in the conifer genome, or if incorporated, be expressed. D.E. Harry and M. Freeling of the University of California at Berkeley are working on these problems in cooperation with the Institute of Forest Genetics.

Selecting transformed cells. Transformation usually happens with low frequency, so transformed cells must be selected from among a larger population of untransformed cells. A common way to accomplish this is to engineer a vector that will permit easy identification of cells in which it has incorporated. An example is the use of the kanamycin-resistance gene (neomycin phosphotransferase, or NPT) from the colon bacterium, which has been spliced

into several plant vectors. When plant cells are plated onto agar with G418, an aminoglycoside antibiotic that can be inactivated by NPT, only those that have been transformed (i.e., those that have incorporated and expressed the gene) survive.

For some genes, like the major gene for resistance to white pine blister rust, direct selection may be possible. Fungal mycelia invade sugar pine cells in callus culture, and resistance is expressed on the cellular level by a hypersensitive reaction (Diner, Mott, and Amerson 1984). Following microinjection, cell lines could be multiplied, subdivided, and one replicate challenged by the fungus to identify transformed lines.

The Final Step: From Transformed Cells to Trees

The inability to regenerate whole plants from transformed cells is the greatest barrier to genetic engineering of conifers: there is no guarantee that research efforts will be rewarded in the near future. On the other hand, whole plantlets have been regenerated from cell and tissue culture in some hardwoods (Karnosky 1981). Several laboratories in the United States, Canada, and other countries are attempting to induce somatic embryogenesis in pines and Douglas-fir (*Pseudotsuga menziesii* [Mirb.] Franco), so far without success. Perhaps, the problem should be sidestepped rather than met head-on.

For example, the megagametophyte of conifers has some features that might be used to circumvent the problem of regenerating plantlets from cells *in vitro*. During an extended period of time, the megagametophyte is in a free nuclear state (i.e., the nuclei are not separated by cell walls). Mitotic divisions result in over a thousand nuclei before cell wall formation begins, and some of these nuclei differentiate into eggs (e.g. Allen and Owens 1972). If DNA fragments or vectors could be injected into the megagametophyte during the free-nuclear stage, they would be unimpeded by cell walls and, hopefully, incorporate in the conifer DNA at a high rate. The target is large, apparently up to 0.7 mm for an egg cell alone in sugar pine (Haupt 1941). Judging by the size of the free-nuclear megagametophyte in Douglas-fir (Allen and Owens 1972), the free-nuclear gametophyte in sugar pine may be several millimeters long. Injury from injection should not cause irreversible damage to the megagametophyte; e.g., seed bugs sometimes penetrate the megagametophyte without destroying it (Krugman and Koerber 1969). After differentiation of the egg and fertilization, the ovule could follow its normal course of development and mature an embryo. It may be better to use the system in this way rather than attempt to force conifer cells to do something they do not normally do (i.e., undergo somatic embryogenesis). However, research is needed to develop techniques for the direct injection of megagametophytes through the cone scales in such a way that the cone can continue its normal development.

Regeneration of plants from cell and callus cultures remains the most critical need in forest research. Other barriers to genetic engineering already show signs of cracking, but there have been no major breakthroughs in conifer regeneration. Without the capability of producing trees from cell culture, the full benefits of transformation will not be realized. The inability to regenerate trees from cells or callus is not only a block to the use of genetic engineering, it prevents forestry from making full use of the products of conventional selection and hybridization. There are several interspecific hybrids and some desirable intraspecific crosses that cannot be

economically multiplied, and mass cloning would be an especially valuable technique. One research approach would be the intensive study of embryogenesis to chart the path of normal development, providing a guide to the necessary steps *in vitro*.

CONCLUSION

Recombinant DNA technologies will make it possible to modify trees on a time scale comparable to that of annual crops. Furthermore, manipulations at the cellular level will result in greater gains than previously possible by eliminating the barrier posed by the large size of trees; as long as whole plants had to be evaluated in the field, selection intensity could never be as great for space-consuming trees as for relatively smaller agricultural plants.

Already there are indications that these technologies can be applied to conifers and hardwoods. Within the last year it has been possible to demonstrate the insertion of the Ti-plasmid from crown gall in pine and prove gene expression. DNA cloning and sequencing techniques have worked as well on conifers as on other plants. While there are still only a few valuable, single-gene traits known in forest trees, there are many markers, and linkage maps are being constructed for conifer genomes. The massive research effort in medical and agricultural sciences will provide valuable genes for the genetic engineering of trees just as it has provided the tools. However, forest biology cannot rely entirely on research in sister sciences.

Research should proceed on four parallel lines: 1) the genetic system of forest trees; 2) transfer systems; 3) the physiological and biochemical basis of valuable traits; and 4) the developmental path leading to regeneration from cell culture. Of these, work on the genetic system and transfer systems shows signs of progress.

With respect to research on physiological processes and gene products, it is time to stop treating tree growth, form, disease resistance, etc. as black boxes. While traditional breeding using the metrics of quantitative genetics has proved quite successful, more effort is needed to identify underlying mechanisms for important processes, their genetic control, and gene products if forest genetics is to realize its full potential.

Regeneration of trees from cell and tissue culture remains the major barrier to progress in forest genetics, and not because of lack of effort. Yet, there is no reason that this barrier too cannot be overcome. When it is, forestry will reap enormous benefits.

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NORTH CENTRAL FOREST EXPERIMENT STATION BIOTECHNOLOGY PROGRAM-APPLICATION TO TREE IMPROVEMENT

Neil D. Nelson[1/]

Abstract.--In 1983 the USDA Forest Service initiated a new research program on the genetic engineering of forest trees. One-half of this initiative is the Biotechnology Multiproject Research Program of the North Central Forest Experiment Station (NC). The NC Biotechnology Program is centered at Rhinelander, Wisconsin, and also has scientists at St. Paul, Minnesota, and Madison, Wisconsin. The Program has nationwide responsibilities and cooperators at another Forest Experiment Station, five universities, and three biotechnology companies. The overall Program purpose is genetic tree improvement, complementing conventional breeding technologies. Program structure, studies, and early results are described.

Additional keywords: Genetic engineering, somaclonal, protoplast, recombinant DNA, herbicide resistance, disease resistance, Populus.

In 1983 the USDA Forest Service initiated a research program on the genetic engineering of forest trees, probably the first such major program in the world. One-half of this initiative is the Biotechnology Multiproject Research Program of the North Central Forest Experiment Station (NC). The NC Biotechnology Program, formally organized in 1984, has a nationwide responsibility. Centered at the Forestry Sciences Laboratory in Rhinelander, Wisconsin, the Program has scientists at Rhinelander, and Madison, Wisconsin, and St. Paul, Minnesota, and research cooperators at another Forest Service Experiment Station, five universities, and three firms in the biotechnology industry.

Two previous papers have described the NC Biotechnology Program (Nelson and Haissig 1984, Nelson et al. 1984). My purposes in this paper are to describe and update Program:

- structure
- studies
- early results

1/ Program Leader, Biotechnology Program, North Central Forest Experiment Station, USDA Forest Service, Forestry Sciences Laboratory, Rhinelander, WI.

Strategic Plan

We recognized early that strong strategic planning was necessary for success in biotechnology. In the formative stages, we collaborated with an internationally known biotechnology consulting firm, L. William Teweles & Co., on a strategic plan for forest biotechnology research (Kidd 1984). A unique research program evolved from that collaboration as well as from extensive further analysis by Program managers and scientists.

The Program's strategic plan is based on the following postulates about conventional tree breeding:

.The new biotechnologies and conventional tree breeding are complementary, rather than competing, technologies. Both are essential components of successful tree improvement. For example, resistance to a specific stress imparted through biotechnological techniques is of little value when the trait resides in an otherwise maladapted genotype. Improved and elite genotypes and populations resulting from conventional breeding programs provide the most desirable starting material for further specific biotechnological improvement.

.One of the most important benefits of the new biotechnologies is the potential ability to reduce the long time periods required for tree improvement using conventional genetic technology alone. Three recent major studies of biotechnology (Burg et al. 1983, National Academy of Sciences 1983, Skelsey 1984) have identified woody perennial crops as prime targets for genetic engineering A major conclusion was that combining the new biotechnologies with conventional breeding may produce relatively greater payoffs in forestry than in any other agricultural area (Skelsey 1984), largely because of the time-saving potential of biotechnology.

.The new bioiotechnologies provide potential means for introducing rare or foreign critical traits into otherwise desirable forest tree germplasm. Introducing such traits into tree genomes may be impractical or impossible through conventional breeding alone.

.The new biotechnologies may aid in the development of practical means for capturing non-additive, as well as additive, genetic variation in improved tree populations. The established biotechnology of micropropagation provides the main vehicle for capturing such improvement. The new biotechnologies of somaclonal selection, somatic hybridization, and perhaps, microinjection and recombinant DNA may improve the frequency and reliability of whole plant regeneration in micropropagation systems.

The NC Biotechnology Program is modeled after a typical startup biotechnology company, a unique organizational plan for a public research effort. The Program is based on four essential synergistic factors (fig. 1), also present in new private biotechnology firms:

. entrepreneurship

. focus

. integration

. flexibility

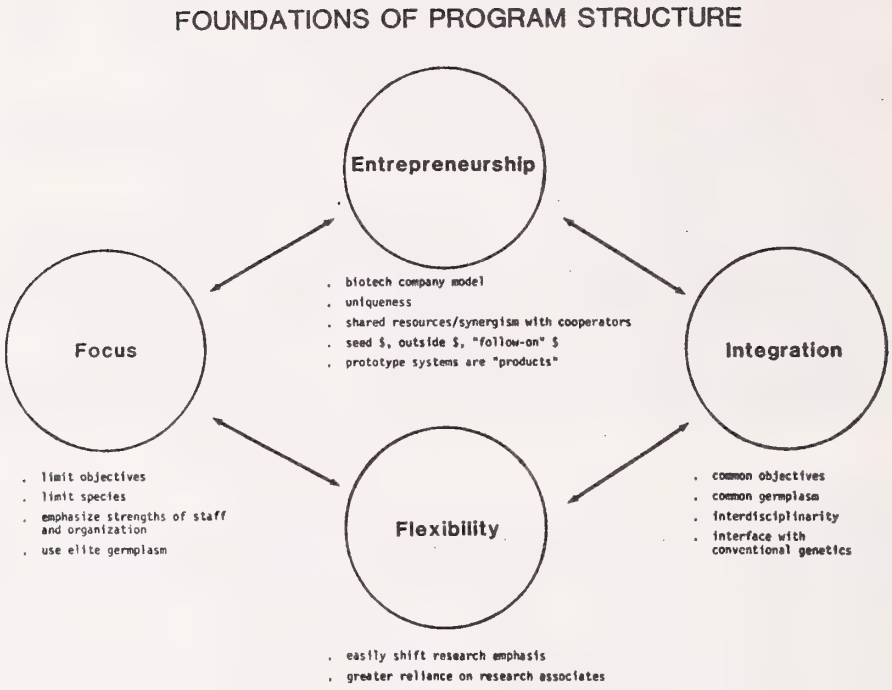

Figure 1. Principles of strategic planning and organization for the North Central Forest Experiment Station Biotechnology Program.

Entrepreneurship for the NC Program involves the forementioned emulation of biotechnology industry strategies. These strategic characteristics include ensuring uniqueness for the research program, sharing resources and developing a synergistic relationship with cooperators, and using "seed money" to help attract outside research contracts and grants and further "follow-on investment." In contrast to a private biotechnology firm, the NC Program produces prototype genetic transformation systems for forest trees, rather than commercial products.

Focus in the research program includes limiting the number of objectives and target species. It involves carefully selecting species based on both biological flexibility and commercial importance. The NC Biotechnology Program has optimized the use of our limited resources by emphasizing research on biologically amenable model species, while still maintaining research on some commercially important species at a lower but meaningful level. Focus in the NC Program also encompasses an emphasis on the strengths of the staff and parent organization in choosing research objectives. This analysis of endogenous strengths includes scientific, logistic, and financial considerations. An important part of research focus in the NC Program is the use of elite foundation stock from conventional genetics programs as starting material for biotechnological improvement, as mentioned above.

Integration within the NC Program means ensuring commonality across the Program, including all Forest Service and cooperating scientists. The commonality includes common objectives and common germplasm. Integration in the NC Program also includes interdisciplinary research planning and execution and a strong interface with conventional genetics and breeding efforts. The latter involves not only the selection of elite and well-defined germplasm as experimental material for the biotechnology research, but also the joint planning of how the biotechnologically improved genotypes will be delivered to users. In some cases the latter consideration may involve incorporating the improved trait in seed. Classical genetic analysis is an important part of analyzing and verifying the genetic effects of the new biotechnologies and constitutes another conventional genetics-biotechnology interface within the NC Program.

Flexibility in the NC Program includes building in the willingness and ability to shift research emphasis to follow up promising results. Research in biotechnology is "high risk" in that positive results often cannot be predicted. The history of research in this area also includes the common occurrence of unexpected results and important spinoff applications. A biotechnology research program must be flexible to capitalize on this situation. Another component of flexibility is a greater reliance on research associates and other employees on temporary appointments than has been common in Forest Service research.

Objectives

The NC Biotechnology Program is using established biotechnologies (conventional genetics and breeding, tissue culture) and new biotechnologies (somaclonal/gametoclonal selection, somatic hybridization, recombinant DNA) to accomplish three objectives:

- impart herbicide resistance to selected forest trees
- impart disease resistance to selected forest trees
- develop genetic guidelines for the regeneration of selected forest trees in tissue culture (regeneration genes)

The rationale for the choice of these objectives is fully explained in two previous papers (Nelson and Haissig 1984, Nelson *et al.* 1984). Current research is on the technologies of somaclonal selection, tissue culture, and breeding. We expect to gradually increase our commitment to somatic hybridization and recombinant DNA.

Species

The NC Program is working with some species selected primarily for biological reasons (models); some chosen primarily because they are commercially important, and one species selected for both biological (model) characteristics and potential commercial importance:

Model	Model/Commercial	Commercial
Populus spp.	*Pinus banksiana*	*Pinus taeda*
Larix spp.		*Pinus resinosa*

About 75 percent of current Program research is on Populus.

Poplars were selected as our primary experimental species because they are highly amenable to regeneration in a variety of tissue culture systems, can be easily vegetatively propagated, are the subject of ongoing breeding programs, are diploid, have a small genome size (Dhillon et al. 1984), are genetically variable, and have ample background information in genetics, physiology, and plantation silviculture. Because of these characteristics, the USDA Forest Service Genetic Engineering Workshop (F. T. Ledig, personal communication) recommended poplars as model species to include in forest tree biotechnology programs.

Staff

The NC Program currently includes three plant physiologists, a plant geneticist, a plant anatomist, a tissue culturist, and two plant pathologists (fig. 2). The Program Leader and Research Work Unit (RWU) NC-1403 are in

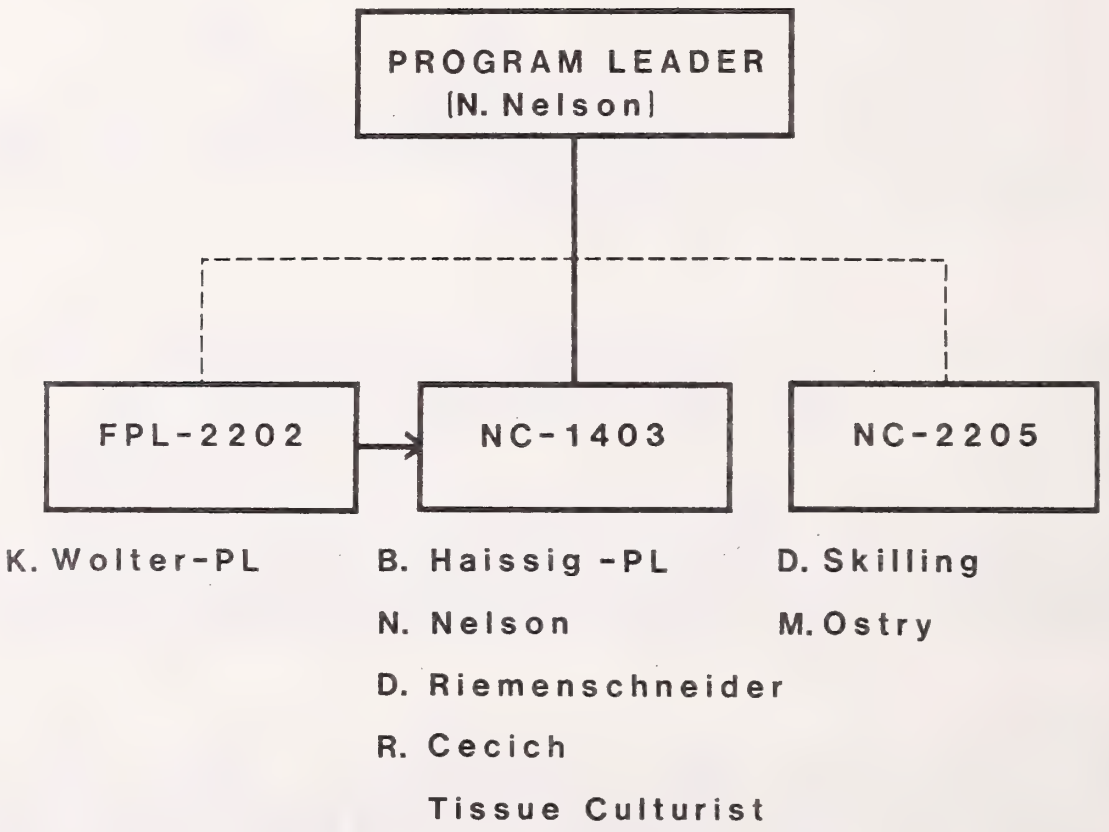

Figure 2. Organizational structure and in-house scientific staffing of North North Central Forest Experiment Station Biotechnology Program. Numbers are Research Work Units (RWU). NC-1403 is the Program core unit. PL is Project Leader of RWU. Arrow indicates research contribution to the core unit.

Rhinelander, Wisconsin; RWU FPL-2202 is in Madison, Wisconsin; and RWU NC-2205 is in St. Paul, Minnesota. NC-1403 is responsible for Program research on herbicide resistance and regeneration genes. FPL-2202 contributes tissue culture research to the work of NC-1403. NC-2205 is responsible for Program research on disease resistance.

Cooperators

Program cooperators, listed in table 1, work closely with the Forest Service scientists in the Program, using germplasm that is common across the Program. This planned research focus and coordination have created a synergistic organization.

STUDIES

The current studies of the NC Biotechnology Program are listed in table 2. Studies planned to begin soon are listed in table 3.

Table 1.--Research cooperators of the North Central Forest Experiment Station Biotechnology Program.

Government		University		Biotech. Industry	
Institution	Investigator	Institution	Investigator	Institution	Investigator
Southern Forest Exp. Station	O.Wells	Wisconsin	B.McCown	L.Williams Teweles & Co.	G.Kidd
		Minnesota	P.Read W.Hackett	DNAP	M.Sondahl
		N.C. State	S.Dhillon (J.Miksche)	Calgene	M.Moloney J.Fillatti
		Michigan Tech.	D.Karnosky A.Diner		
		New Hampshire	S.Minocha		

EARLY RESULTS

Most studies of the NC Biotechnology Program were initiated less than 1 year ago. Nevertheless, Program scientists have obtained a number of significant results. Some of the most noteworthy findings are listed below:

- A strategic plan for forest biotechnology was developed (Kidd 1984).

- A dozen Populus clones have been established in sterile shoot culture. Most have been established in a proliferative state (McCown 1984). Shoots of one clone were rooted and planted in a field test plot of 200 trees in northern Wisconsin (B. McCown and D. Riemenschneider, personal communication).

- Callus and leaf disc cultures have been established for several Populus clones. Nine clones have exhibited shoot and root formation from callus through organogenesis. High frequency regeneration of shoots from leaf discs can be readily obtained with two of these clones (T. Ettinger, B. McCown, N. Nelson, M. Ostry, P. Read, unpublished data).

Table 2.--Current studies of the North Central Forest Experiment Station Biotechnology Program.

Study	Principal investigators	Species	Program objectives[a]	Biotechnology emphasis
Stratigic plan in biotechnology for forest trees[b]	G.Kidd	all	all	general
Genetics of whole plant regeneration *in vitro*	B.Haissig,B.McCown, D.Riemenschneider, R.Cecich	*Populus*	rg	genetics/breeding, tissue culture
Anther culture for haploidy induction in *Populus*	K.Wolter	*Populus*	all	tissue culture
Tissue culture systems for red pine	M.Sondahl	*Pinus resinosa*	all	tissue culture
Genetics of hexazinone resistance in jack pine	D.Riemenschneider	*Pinus banksiana*	hr	genetics/breeding
Imparting glyphosate and sulfonylurea resistance to elite poplar germplasm	N.Nelson, B.Haissig	*Populus*	hr	somaclonal selection
Imparting *Septoria* resistance to elite poplar germplasm	M.Ostry,P.Read, W.Hackett	*Populus*	dr	somaclonal selection
Imparting scleroderris and needlecast resistance to selected *Larix*	D.Skilling, A.Diner, D.Karnosky	*Larix*	dr	somaclonal selection
Cotyledon and embryo culture of red pine to generate somaclonal variation	S.Minocha, N.Nelson, D.Riemenschneider	*Pinus resinosa*	all	somaclonal selection
Protoplast technology for *Populus*	B.McCown	*Populus*	all	somatic hybridization
Nuclear DNA changes in leaves of *Populus* and *Larix* during the growing season	S.Dhillon, J.Miksche, R.Cecich	*Populus*, *Larix*	all	recombinant DNA
Conferring glyphosate resistance on selected *Populus* through genetic transformation with *aro* A gene	M.Moloney,J.Filiatti, B.Haissig, B.McCown	*Populus*	hr	recombinant DNA

a/ All = all objectives, rg = regeneration genes, hr = herbicide resistance, dr = disease resistance.

b/ Completed.

Table 3.--Future studies of the North Central Forest Experiment Station Biotechnology Program, planned to begin in 1985.

Study	Principal investigators	Species	Program objectives[a]	Biotechnology emphasis
Genetic modulation of somaclonal variation in poplars	B.Haissig, N.Nelson D.Riemenschneider	*Populus*	hr	genetics/breeding, tissue culture, somaclonal selection
Genetics of regeneration *in vitro* for loblolly and jack pines	D.Riemenschneider, O.Wells, B.Haissig	*Pinus taeda*, *Pinus banksiana*	rg	genetics/breeding, tissue culture

a/ hr = herbicide resistance, rg = regeneration genes.

- An in vitro system has been developed for one Populus clone that allows continued viability of individual protoplasts and division and growth of these protoplasts to the large calli stage (> 1000 cells per callus) (B. McCown, unpublished data).

- An embryogenic cell suspension culture system has been developed for one Populus clone, apparently the first report of embryogenesis from cell suspension for the genus (B. McCown, unpublished data). This system is potentially useful for somaclonal selection for chemical and disease resistance as well as for genetic transformation through recombinant DNA.

- Populus shoot cultures were found to exhibit the highest sensitivity to cytokinin of any dicot deciduous tree species so far examined (Sellmer et al. 1985). This cytokinin sensitivity differs markedly by clone (Sellmer et al. 1985).

- Callus was obtained from the anthers of three male Populus clones in our research on haploidy induction and gametoclonal variation. Viable callus formed in 4 to 6 weeks, with spontaneous root formation after one or two subcultures. Sporadic shoot formation was also observed. No evidence of haploidy in any of these calli, roots, or shoots has yet been found (K. Wolter, unpublished data). Several experimental parameters are being modified to increase the probability of achieving haploidy.

- Populus cells were found to have very small chromosomes and only 1.5 pg of DNA per nucleus (Dhillon et al. 1984). At least 95 percent of the higher plant species so far examined have more nuclear DNA than this (J. Miksche, unpublished data). This surprisingly small genome size should facilitate genomic analysis through recombinant DNA approaches. Cytogenetic analysis, however, is made more difficult by the small chromosome size (R. Cecich, unpublished data).

- A leaf disc bioassay for Septoria susceptibility in Populus developed at NC Station has been refined so that there is a high correlation between bioassay results and Septoria resistance in field plantations (M. Ostry, unpublished data). This leaf disc bioassay is being used in our somaclonal selection system for Septoria resistance.

- Successful infection of Populus shoot and leaf disc cultures with Agrobacterium tumefaciens has been achieved, with gall formation in shoots in vitro (J. Fillatti and M. Moloney, unpublished data). Agrobacterium is the transformation vector in our North Central Station-Calgene-University of Wisconsin recombinant DNA herbicide resistance work (table 2).

- Large and highly significant differences were found between open pollinated families of Pinus banksiana in tolerance to the triazine herbicide, hexazinone (D. Riemenschneider, unpublished data). Work will soon begin on the mode of inheritance of this tolerance.

- Cotyledon culture systems of high regenerative capacity have been developed for Larix decidua (A. Diner, D. Karnosky, D. Skilling, unpublished data).

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BIOTECHNOLOGY AND FOREST GENETICS: AN INDUSTRY PERSPECTIVE

R. J. Dinus 1/

Abstract.--Biotechnology is not new to forestry or many other industries. Man has been using biotechnology, in its broadest sense, since he began domesticating crops. Recent advances, however, have made available new genetic and molecular techniques. The current excitement derives from their potential application across the spectrum of activities in forest industry--from producing wood through processing it to using wastes. Tree improvement, as an example, can be expedited and made less costly with techniques such as cell culture and gene transfer. Shortening the time required for selecting, breeding, and testing may allow forestry to benefit as much as or more than other industries dependent on plant material. In addition, forestry, for a change, may be on a par with other industries. New discoveries, or even genes, can be captured and used regardless of origin. Reaping dividends, however, requires that the scientific and industrial communities collaborate in selecting areas of work, choosing strategies, and planning research. The most promising areas must be identified; i.e., those with the most economic leverage. Coordinated strategies are likewise essential. Heavy spending on a narrow or applied front could be harmful. Biotechnology cannot replace other disciplines, rather it builds upon and provides tools for them. Balance must be maintained between fundamental and developmental work. Well-planned, far-sighted experimentation is more important than ever. Modifying or transferring genetic information provokes concern and questions. Precautions in executing research and deploying products are needed to avoid the perception that more problems are being created than solved. Without effective safeguards and education, the public may saddle the technology with unnecessary regulation. New knowledge, as accumulated, should be applied toward betterment of regulatory procedures.

Additional keywords Tree improvement, tissue culture, gene transfer, research management, *Agrobacterium tumefaciens*, *Pinus taeda*.

Biotechnology, in the broadest and oldest sense of the word, is not new to forestry or a variety of other industries. Early man applied and benefited from biotechnology when he began selecting desirable crop and animal variants, and took advantage of genetic variation to increase

1/ Forest & Biological Research Manager, Corporate Research Center, International Paper Company, Tuxedo Park, NY

productivity. In more recent times, the word has taken on a somewhat more restrictive meaning in that new genetic and molecular tools have become available. Such tools enable us to alter organisms of interest more dramatically and precisely than ever before, and further allow us to effect change much faster than by traditional means.

Such developments, not surprisingly, have provoked a wave of interest and excitement. Prospects for application of the new techniques to a host of industrial and commercial activities are manifold. In the forest products industry, potential applications span the spectrum from reducing the costs of producing raw material and increasing the efficiency of processing and manufacturing to converting wastes into harmless residues, salable products, or energy. Replacing even part of the energy and chemicals needed to make and bleach pulp has considerable economic leverage. Indeed, first applications in forestry may involve altered organisms or enzymes for bleaching pulp and/or decolorizing effluents.

Also exciting is the rate at which biotechnology has been advancing. Ten or so years transpired before restriction enzymes were understood or became usable as something other than mere research tools. Developing Agrobacterium tumefaciens into a workable vector for transferring genes among dicotyledonous plants took roughly seven years. And now, we read about new developments and products literally on a monthly basis.

Advances have occurred, and are now occurring quite rapidly, in forestry as well as in other disciplines and industries. Witness that in early 1983, an entire issue of Science was devoted to biotechnology and its implications for science, industry, and society. The sole article on forestry mentioned a number of techniques and applications, but did not discuss isolation, modification, and transfer of genetic information. As clearly demonstrated by other papers in these proceedings, involvement in such research has since increased and progress has been substantial. Within the last six or so months, evidence has been presented that Agrobacterium tumefaciens can infect and transform loblolly pine (Pinus taeda). The pace is thus rapid and undoubtedly will accelerate, as a variety of organizations and persons have worked to increase funding and support.

As a result, a number of opportunities and problems lie ahead, and we in research, education, and industry must prepare for them. My purpose then is to highlight a few accomplishments and explore some of the challenges.

CELL AND TISSUE CULTURE

The first area of concern is the art (and hopefully soon the science) of cell and tissue culture. Few coniferous species can be regenerated from protoplasts or single cells, and regeneration from organ culture has not proven an economical means for multiplying improved material. Even so, much knowledge has been garnered from efforts to accomplish such goals. What can now be gained from the experience? And, what direction should such research take in the future? What role should workers in the public and private sectors play?

Tree improvement has become an integral part of management in many forest regions, and generally is recognized as a worthy enterprise.

Improvement of southern pines essentially has become a business--a highly profitable one despite the considerable cost of entry and operation. Progress and profitability nevertheless remain limited by the lengthy reproductive cycle, the space and time required for testing, and the considerable expense associated with testing and selection.

Can tree improvement be expedited and made less costly by imaginative application of cell and tissue culture? The answer seems positive, provided we work together and focus talent on important issues. Doing so seems especially important in view of the economic situation facing us now and for the foreseeable future. Demand for raw material is no longer rising as rapidly as in earlier times, inflation has abated, and expectations have changed. The key to sustained profitability (and I might add, continued interest in research) may, therefore, rest on our ability to reduce costs of production. Indeed, lessening the time and expense of selecting, breeding, and testing may allow the forest industry to benefit from the new emphasis on biotechnology as much as or more than other industries dependent upon breeding and growing plants.

One example of such applications involves placing cell or callus cultures under stress, such as that provoked by low temperatures, restricted moisture availability, or toxins from pathogens. Testing and/or increasing selection intensity in culture can hasten identification and isolation of useful genetic variants. More entries can be evaluated in less time and space than in conventional tests. Approaches, such as protoplast fusion, can be used to increase variability. And, haploid material could be generated for use in research.

Realizing full benefit from such approaches, however, requires recovery of functional plants. Indeed, the utility of many new techniques will be limited until efficient, reliable means of organogenesis and embryogenesis are developed. Just how to effect that development most rapidly remains controversial. Some argue for allocating more funding and workers to the traditional empirical approach. Others hold that more emphasis should be placed on fundamental studies of differentiation. The problem of balance is serious, and need exists for work on both fronts.

What mechanisms control expression of the genes involved in differentiation? How do growth regulators, environmental conditions, and nutrients affect those mechanisms? What biochemical events occur in developing embryos and can we learn to provoke them in culture? Much remains to be learned, and answers to such questions will facilitate progress on cell and tissue culture, and perhaps hasten the day when we can generalize from an easily manipulated species to others of greater interest but difficult to culture. Knowledge about the processes and mechanisms of differentiations will also improve our understanding of growth in intact plants -- the components contributing to it, the underlying traits, and how they can be manipulated more easily. Adequate justification exists for continuing work on both approaches. We would be well advised, however, to provide somewhat greater support for work on processes and mechanisms than has been available in the past. Such a position, hopefully, would encourage continued movement of public sector scientists back to fundamental issues.

GENE ISOLATION AND TRANSFER

Another area of concern involves a set of techniques that has tremendous potential, that of isolating, cloning, modifying, and transferring genetic information. Work is progressing rapidly as indicated by another paper in these proceedings. Two specific strains of Agrobacterium tumefaciens, have been shown to infect loblolly pine and some bacterial genes were found to have been inserted into and expressed by the pine genome. Work on other gene transfer systems, such as micro-injection and liposomes, is also underway. Thus, we can expect within the near future to have available the technology to transfer single genes into the genomes of desirable trees. The ability to transfer the many genes presumed to control the most important traits, however, will not be within reach for some time.

Some other limitations should also be noted. Reaping dividends from such research, after all, requires that the gene be expressed at the correct time and place, that we can convert the transformed cell, callus, or organ to an intact, functional plant, and that we can multiply that plant, by sexual or asexual means, in a cost-effective manner.

Progress is also constrained by our understanding of only a very few forest tree genes well enough to attempt isolation and transfer. To some extent, this limitation can be overcome by borrowing genes for traits of interest from other organisms (for example, herbicide resistance from bacteria). This is one reason that biotechnology is so exciting for forestry. Within limits, new discoveries and even genes can be captured and utilized in forest research and development, regardless of origin. Thus, biotechnology may place forestry, for a change, on a par with other industries.

The larger problem nevertheless will persist for some time. Neither we nor other plant scientists know which genes are important or understand the activity of those that have been identified. Identifying genes and understanding gene action will not be easy, regardless of the plant or tree species. This aspect of biotechnology may well prove the most challenging. When considering plant genomes, one must contemplate which gene of perhaps one or more million is of interest. Several thousands or tens of thousands may be active in a particular organ or tissue at any given time. Which are active, what activates them, and how we capture the one of interest remain key questions. Such topics clearly deserve increased attention and seem best addressed by scientists in the public sector.

One might regard this situation, regrettably, as but one symptom of past neglect. The ebb and flow of research and education has been such that sufficient attention was seldom given to the basics of how trees grow. One danger inherent in the excitement about biotechnology, therefore, is that qualified workers will all rush to get on the "genetic engineering" bandwagon, reducing even further the magnitude of effort on fundamental issues.

Despite the many difficulties, work will continue and advances will occur. Before too long, useful genes will be moved into or among tree

species, and their expression will be confirmed. Ensuring continued interest and support, however, will be as difficult as doing the research. The economic climate of the present and foreseeable future is such that first results must be winning ones -- preferably seen as shortcuts to increased profitability. Careful selection of goals and areas of investigation is, therefore, necessary. Important traits must be identified and research strategies set such that early efforts will produce findings and/or material that can be moved quickly from research through development to commercialization. Continuing collaboration between the scientific and industrial communities in selecting areas of work, choosing strategies, and planning research is essential to ensure that investments in biotechnology are worthwhile.

CHALLENGES FOR THE FUTURE

Balancing on the High Wire

The foregoing sections were intended to provide a sense of the opportunities presented by forest biotechnology. They also should have surfaced some problems that must be resolved before the promises can be realized. Significant among the problems is the perennial tendency to regard new activities as panaceas or bandwagons. Though the associated dangers were mentioned earlier, the need to resist such tendencies must be reemphasized.

Bending biotechnology to yield real accomplishments in forestry requires a concerted effort of individuals from many disciplines and organizations. Molecular biology is exciting, but significant challenges also lie in the more traditional areas of tree breeding, physiology and biochemistry. Thus, there is still need and perhaps even greater need for increased research in such disciplines.

The new techniques are not a replacement for other disciplines, rather they are new tools for all to use. Indeed, their most significant near-term use may be in enhancing our understanding of tree growth and development--differentiation in cell and tissue culture being but one example. Molecular biology has as much to offer forest genetics as the latter discipline has for the former. Never before have we seen greater opportunities for collaboration among disciplines.

Coordinated and far-sighted strategies are thus essential to maintaining reasonable balance between disciplines and approaches. Heavy spending or plunging for headlines along narrow or applied fronts can do more harm than good. Without continued emphasis on the traditional disciplines and fundamental issues, progress will be as short-lived as it has been dramatic. Achieving a balanced research agenda is also essential if we are to attract the few brightest students to forestry, and train them to investigate, develop, and implement this attractive, but complex technology.

Maintaining an appropriate balance will not be easy. Economic conditions have made funding for research harder to obtain. On the positive side, attitudes about research have also changed, and so-called hard science has become more popular. The research community may therefore find it

easier to work on fundamental topics in both new and traditional areas than one might imagine at first glance.

The Sky May Fall

The emergence of forest biotechnology magnifies the traditional challenges about plantation monocultures and clonal forestry. Modifying and transferring genetic information naturally provokes concerns and questions from the public. Adding such concerns to the usual ones will generate more and harder questions.

Both our clients and the public will want to know more about what we investigate, what we produce, how we deploy it, and how it will affect the environment. Some actually may seek a role in determining what is done, why, and how. Not providing them information will create doubt and possibly fear. Inaccurate information or mere opinion will diminish credibility. Thus, well-planned and far-sighted research is more important than ever. Meaningful precautions must be taken in designing and executing research so as to avoid any perception that more problems are being provoked than are being resolved. We must also take the lead in educating our clients and the public. They must be assured of safety. Unless we accept and meet this challenge, all promises could be delayed or even forfeited. Without effective safeguards and education, the public may insist upon regulations that unnecessarily slow or complicate research, development, and commercialization. As responsible scientists, we must further provide accurate data to the agencies responsible for formulating and applying regulations. Our goal should be a responsive and responsible system of regulation that will satisfy public concerns and not inhibit sound research and development.

United We are Funded, Divided We Fall

As mentioned earlier, attitudes toward and the outlook for forest research have changed over the last decade. Funding, expressed in real dollars, has declined during most years, regardless of the organization--university, federal, or industrial. Some years have been better than others, but the average trend has been down or flat. Yet another trend has surfaced as well, that being the more careful choosing of research directions, the justification of expenditures, and the evaluation of payback. Just who does what research has also received more attention. Thus, public sector organizations are moving away from shorter-term, developmental activities to concentrate more on fundamental, longer-term issues. The Industry, on the other hand, is tending to concentrate less on hard science, and more on development and application. The outcomes have been several.

While cause and effect cannot be proved, one certainly can argue that such trends paved the way for increasing support of biotechnology. Most such research, regardless of the organism, was once conducted in a few universities, and largely supported by small federal grants. Now, many universities are establishing so-called Institutes of Biotechnology, aided by modest appropriations from their state legislatures. In addition, the U. S. Forest Service has initiated a modest program. Much impetus has also been lent by establishment of Competitive Grant Programs, first by the USDA, and

more recently, the Forest Service. Thus, increased activity on the biotechnology front can be expected in universities, the Forest Service, and eventually, industry.

Some other outcomes are also of interest. The several trends have led to establishment of strong cooperative (and sometimes contractual) relationships between universities, the Forest Service, and/or industry. Witness the formation of herbicide, nursery, and pest management cooperatives among others in addition to the long-standing tree improvement variety. Moreover, the industry has become more involved in research planning at the state, regional, and national levels. The National Forest Products Association, for example, now has a National Research Committee and five Regional Subcommittees. A special subcommittee monitors biotechnology research. Industry representatives are prevalent on advisory boards, and are frequent participants in formal and informal research reviews.

While such involvement is not new, the interactions are more intensive and considerably more harmonious than in earlier times, and generally of mutual benefit. That is, the several communities have learned much about their individual strengths and weaknesses, and are acting to help one another meet their respective needs. The research communities desire to perform more and better research and need support. Industry desires to promote the quality of research and to maintain the flow of research information germane to its goals. With time and effort, such interactions can be further strengthened, and used to secure a balanced research agenda and make the promise of biotechnology become reality.

TESTING AND DEPLOYMENT OF GENETICALLY ENGINEERED TREES

W. J. Libby[1/]

Chapter Outline for Bonga & Durzan, 2d Ed.

1. INTRODUCTION

1.1 What is meant by "genetically engineered", "testing" and "deployment"?
1.1 Some preliminary questions

2. TESTING

2.1 General considerations

- 2.1.1 Genetic variation within units
- 2.1.2 Environmental variation within and between test sites
- 2.1.3 The number of entries in a test
- 2.1.4 The location, preparation and care of test sites

3. CLONAL TESTING

3.1 Sensitivity to broad-sense heritability
3.2 Sensitivity to selection intensity
3.3 Large contiguous plots vs non-contiguous plots
3.4 Typical vs atypical sites
3.5 Possibility of testing for competitive compatibility

4. SPECIAL CONSIDERATIONS FOR GENETICALLY ENGINEERED TREES

4.1 The relationship of genetic novelty and test stringency

- 4.1.1 Two true stories

4.2 The heritability of genetically engineered characteristics

5. GENERAL DEPLOYMENT STRATEGIES

5.1 Provenances
5.2 Unusual sites

6. CLONAL DEPLOYMENT STRATEGIES

6.1 Maximum and minimum numbers of clones per locale
6.2 General-purpose vs. interactive clones
6.3 Wimps vs Moms

7. SPECIAL STRATEGIES FOR GENETICALLY ENGINEERED TREES

7.1 Minority mixes

1/University of California, Berkeley

BIOTECHNOLOGY II

MODERATED BY DR. MIKE GREENWOOD

University of Maine

DNA TRANSFER AND GENE EXPRESSION IN LOBLOLLY PINE

Ronald R. Sederoff[1], Anne-Marie Stomp[2], W. Scott Chilton[3], and Larry Moore[4].

We wish to report on a system for the transfer and expression of foreign genes in pines. The purpose of these experiments is to explore the use of the crown gall bacterium, Agrobacterium tumefaciens for the eventual goal of genetic engineering in important forest species. Previous work has described pines as resistant to infection by crown gall, however, we have found a strain of A. tumefaciens that will produce galls on loblolly pine. The frequency of gall formation is 3 percent. One of these galls has been removed and cultured on pine tissue culture medium. Cells from the resulting callus were extracted with ethanol and tested for the presence of opines by high voltage paper electrophoresis. The strain of crown gall that infects pine is known to synthesize agropine and mannopine in galls that have been induced in sunflower. Opines, particularly agropine, are found in abundance in callus derived from the pine gall, but are not detected in extracts of uninfected plants or uninfected loblolly pine callus. The presence of specific opines in infected pine cells provides strong evidence for the transfer and expression of foreign genes in pines. This system appears suitable for genetic engineering of commercially important conifers including loblolly pine.

1. Institute of Forest Genetics, Pacific Southwest Forest and Range Experiment Station, USDA Forest Service.
2. Department of Forestry, North Carolina State University.
3. Department of Botany, North Carolina State University.
4. Department of Botany and Plant Pathology, Oregon State University.

USE OF TISSUE CULTURE TECHNIQUES IN A HARDWOOD TREE IMPROVEMENT PROGRAM

D. W. Einspahr and S. R. Wann*

ABSTRACT

Tissue culture per se is not a method of genetic improvement but is instead a method of vegetatively propagating trees and plants. Tissue culture methods available to forestry are micropropagation, organogenesis, and somatic embryogenesis. A long-term industry-state cooperative conventional tree improvement program has used the techniques of selection, hybridization and polyploidy to produce rapid-growing Populus species hybrids. Aspen hybrid seedling populations have been produced that at 18 to 20 years grow approximately twice as fast, have 20 to 30 percent longer fiber length, and, 8 percent higher wood density than widely used native aspen. Only modest improvement, has been made, however, in producing hybrids that are resistant to the regions most serious forest management problem, hypoxylon canker (Hypoxylon mammatum Wahl., Miller).

Recent Ph.D. and related research has resulted in tissue culture procedures that allow us to readily screen seedling populations for seedlings that are highly resistant at the cellular level to the canker toxin, the reported determining factor in the disease. Also utilized are procedures for bioassaying field tested parent trees, hybrids, and young seedling populations for resistance. Planned is an expanded tree improvement program that will combine conventional tree improvement techniques and tissue culture procedures to produce hypoxylon resistant hybrid clones and seedling populations. Micropropagation and organogenesis methods are presently available for use in producing operational clonal plantings. At present there appears to be adequate natural resistance in existing seedling populations, so that the use of more sophisticated genetic engineering techniques (transformation, protoplast fusion, etc.) may not be required to solve this serious disease problem. Tissue culture techniques similar to those described could be expected to be useful in evaluating experimental crosses and screening and generating disease resistant parent trees in other hardwood tree improvement programs.

*The authors are, respectively, Senior Research Associate and Industrial Research Fellow, Forest Biology Section, The Institute of Paper Chemistry, Appleton, WI, 54912.

INTRODUCTION

Emphasis on the use of tissue and cell culture in the propagation of forest trees has increased dramatically in the last five to six years. The tissue culture methods available to forestry are micropropagation, organogenesis and somatic embryogenesis. Micropropagation is the *in vitro* propagation of plants using stem meristems, i.e., shoot tips and apical buds. Organogenesis is the *in vitro* propagation of plants from explants or callus where the organs (roots or shoots) are produced and then are manipulated to produce complete plants. Somatic embryogenesis is the *in vitro* propagation of plants, from single cells or small groups of vegetative cells, where the final stages of development produce embryolike structures that are capable of developing into intact plants. Although somatic embryogenesis appears to have the most promise for use with forest trees, each of the other methods also has its place in forest tree improvement work. The purpose of the discussion that follows is to illustrate how tissue culture techniques have and are being used in a hardwood tree improvement program in the Lake States. The hope is that in learning of these results, you will see ways you may be able to use similar approaches to solve problems associated with your tree improvement programs.

THE ASPEN GENETICS PROGRAM

The discovery of many rapid-growing, good-quality diploid and several triploid quaking aspen (*Populus tremuloides* Michx.) clones resulted in the establishment of an industry-sponsored aspen tree improvement program in 1955. The objectives of the program were to use the techniques of selection, hybridization and polyploidy to produce rapid-growing trees with improved wood properties. Many of the early ideas were those of Dr. Philip Joranson and were implemented by the first author and other researchers including Dr. Lawson Winton and Dr. J. P. van Buijtenen. During the 20 years that followed, more than 700 full-sib crosses were made, and the crosses included the hybridization of quaking aspen with *P. alba*, *P. grandidentata*, *P. tremula*, *P. davidiana*, and *P. canescens*. Crosses were made in the greenhouse using the cut branch technique of Wettstein[1]. The most often used procedure was to complete the crosses in February and March, produce 300-400 1-0 seedlings from each cross the following summer, and use these trees in one or two replicated field plantings. This crossing and field testing program resulted in the development of several types of crosses that appear to have potential for use by the paper industry. To date, the most useful crosses are those between *P. tremuloides* and *P. tremula*. Particularly promising have been a series of crosses using several *P. tremuloides* females and a tetraploid (4n) *P. tremula* male developed by H. Johnsson in Sweden[2]. As a result of this long-term research program, hybrid aspen seedling populations have been produced that at 20 years grow about twice as fast as native aspen, have 20-30% longer fiber length, and have about 8% higher wood density[3]. Associated with the described improved wood properties were improved paper properties (greater tearing and bursting strength and comparable tensile strength). Presently the forest management approach being suggested is to plant the triploid hybrid seedling populations at wide spacing (80-100 ft^2/tree) on medium to low quality northern hardwood sites, using conversion planting techniques. The prolific suckering ability of most aspen and aspen hybrids will then allow the management of the plantings using coppicing procedures for several rotations.

THE HYPOXYLON CANKER PROBLEM

Hypoxylon canker (Hypoxylon mammatum Wahl., Miller) is the most serious forest management problem influencing the use of aspen and aspen hybrids. Losses in the Lake States have been estimated to be 3% annually[4], an amount approximately equal to the annual harvest in 1983 of 280 million cubic feet[5].

One of the original objectives of the aspen genetics program was to select and breed for resistance to this serious canker disease. A modest amount of progress has been made by selecting and using parent trees that were free from hypoxylon. Progeny tests were evaluated at five-year intervals through age 25, and at present 20-year records exist for more than 60 full-sib aspen and hybrid aspen crosses. Typically, crosses with low resistance to hypoxylon will have infection levels of 46-68% at 20 years, whereas the best tremuloides x tremuloides crosses had 20-year infection rates of 0 to 18%*. Although progress has been reasonable, there is an urgent need to increase the number of highly resistant parent trees and to develop clones with a high degree of resistance for use in a planned clonal forestry program.

CHARACTERISTICS OF THE DISEASE

Hypoxylon canker is caused by the fungus Hypoxylon mammatum (Wahl., Miller). The wide geographic range of quaking aspen and the seriousness of the disease has resulted in the establishment of many research investigations into the nature of the disease and factors associated with spread of the disease. One of the most interesting was the discovery by Hubbes[6] of a diffusible substance produced by the fungus that elicited symptoms characteristic of the disease. The necrotic response to "mammatoxin" was later shown to be host-selective for species susceptible to hypoxylon canker and strongly suggests that the toxin is a determinant in the disease[7,8]. The necrotic response to mammatoxin resulted in the development of leaf bioassay by Bruck and Manion[9] in which the toxin was substituted for the pathogen in a procedure designed to identify disease-resistant clones.

Many important diseases of agronomic crops have been shown to have host-selective toxins as determinants in pathogenesis[10]. Employing toxin in a tissue culture system has resulted in the isolation of toxin-resistant cell lines. In those cases where plants were regenerated from these cultures, toxin resistance often persisted[11,12]. Further testing of the plants with the pathogen resulted in equating toxin resistance with disease resistance. Considering the above success in agriculture and that mammatoxin appears to be a determinant in hypoxylon canker, the use of mammatoxin in tissue culture systems to screen for toxin resistant seedlings was a logical approach to attempt to produce cellular-level resistance to hypoxylon canker.

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*% is the number of individuals lost or presently infected with hypoxylon canker divided by the total number of field planted trees exposed to the disease.

TISSUE CULTURE SELECTION OF TOXIN RESISTANT ASPEN

During the summer of 1983 a student research program was initiated that had the purpose of isolating and propagating mammatoxin-resistant quaking aspen. Part of this Ph.D. research program was to develop a procedure that allowed the regeneration of plantlets from cotyledon explants. The procedure that resulted (Wann and Einspahr[13]) involved induction of multiple adventitous buds on cotyledon and hypocotyl explants by culturing the explants on MS medium containing 0.1 mg/L NAA and 1.0 mg/L BA. Explants producing multiple buds were then transferred to 1/2 MS (macro- and microelements) containing 0.3 mg/L BA for elongation. Root formation was achieved by transfer to 1/3 MS containing 0.1 mg/L IBA. Normally about 90 percent of the elongated shoots rooted, and transfer to soil was accomplished with little difficulty by maintaining high humidity conditions for one month after transfer.

The procedure used to screen and propagate mammatoxin-resistant quaking aspen is illustrated in Fig. 1. Small hypocotyl and cotyledon explants were placed on the "bud proliferation" medium in which mammatoxin was substituted for part of the water. Following four weeks on this medium, organogenesis was evaluated, and surviving explants were rescued from the toxin-containing medium and transferred to the toxin-free elongation medium. The surviving elongating shoots were rooted and transferred to soil. The resulting plantlets were then grown for 18 weeks and bioassayed using the previously cited bioassay method developed by Bruck and Manion[9] and described by Griffin, et al.[14] and Stermer, et al.[15]. The bioassay method consisted of removing three leaves from the 18-week-old aspen plantlets and placing the petiole of leaves in a small vial of water. Small holes were made in the leaves with a minutin insect pin, and a 3 µL drop of the properly diluted toxin was placed over the hole. Usually, three holes were made in each half of the leaf blade. Following incubation in a humidified chamber at 28°C for 48 hours, the response to the toxin was measured as lesion diameter to the nearest 0.5 mm*.

An additional novel complementary study was run in which, for 120 seedlings, one cotyledon was removed and placed on the toxin containing screening medium. The original seedlings (minus one cotyledon) were grown for 18 weeks for use in leaf bioassay comparisons with those explants and resulting plants that survived the toxin screening and tissue culture propagation procedure. Figure 2 illustrates the procedure used. In this way, the bioassay response of a toxin-screened plantlet could be compared with the bioassay response of the donor plant.

The results of this toxin screening procedure and the leaf bioassay of the resulting plantlets turned out to be very interesting. When plants surviving the screening procedure were tested in the leaf puncture bioassay, this resistance was still maintained in the tissue-culture propagated ramets. For example, 5 clones comprising a total of 23 individuals were obtained from a full-sib cross, and all responded in a manner analogous to cottonwood, a species

*Additional details on producing the toxin and running the leaf bioassay are available in a paper by Wann and Einspahr[16].

that is resistant to hypoxylon canker and reacts negatively (lesion diameter < 1 mm) to the leaf bioassay. Equally interesting is that when the donor plants were compared with 22 clones of toxin-screened, cotyledon-derived plantlets (an ortet/ramets comparison), both the donor plants and corresponding toxin-derived plantlets tested resistant in the leaf bioassay. Figure 3 illustrates the results of *in vitro* toxin screening of two full-sib seedling populations.

As a result of these investigations it appears that mammatoxin can be used to rogue organ cultures of aspen seedlings for cellular level resistance to mammatoxin, and the plants propagated from these cultures retain this trait. The ability of an explant to survive and produce shoots on a toxin-containing medium is apparently inherent in the seedling from which it was derived, and not induced by the tissue culture system. Highly resistant individuals occurred to a significant extent in all crosses examined, indicating the resistance was of natural origin. This indicates that despite the current interest in genetic engineering (transformation, protoplast fusion, etc.), it appears the full genetic potential of many commercially important forest species is not now being fully utilized.

PLANNED EXPANSION OF THE ASPEN PROGRAM

Aspen is the most important pulpwood species in the Lake States Region. An attempt is being made to obtain an appropriate level of funding that will allow us to (1) determine the ability of the leaf puncture bioassay procedure to identify canker-resistant trees, (2) determine the heritability of mammatoxin resistance in quaking aspen, (3) verify the usefulness of tissue culture procedures in the production of a highly resistant parent tree for use in future breeding work, and (4) develop procedures for screening 15 to 25-year-old hybrid aspen populations for resistant individuals that can be used in a planned clonal forestry program.

The existence of appropriate parent trees and of 20-year field data on full-sib crosses will allow us to repeat crosses of interest and to compare the tissue culture seedling screening results and leaf bioassay data with the 20-year field results. The existence of parent trees and the ability to produce and evaluate progeny will also allow heritability estimates to be made. The existence of thousands of 15 to 25-year-old aspen hybrids in Northern Wisconsin plantings will serve as a source of rapid growing clones, a percentage of which may also turn out to be resistant to hypoxylon canker.

APPLICATION OF THE APPROACH TO OTHER HARDWOOD PROGRAMS

The tissue culture work by Wann described above represents the first instance where, for a commercially important forest tree species, a tissue culture system has been used to select resistant cultures and regenerate plants that maintain the resistance. This approach and similar procedures appear to be particularly appropriate for use with forest tree disease problems where the disease is of a toxin-determinate nature (*Fusaria* and *Alternaria* species, for example). This work also emphasizes the need for the development of several alternative tissue culture systems for the important U.S. tree species so that we can efficiently cope with future insect and disease problems.

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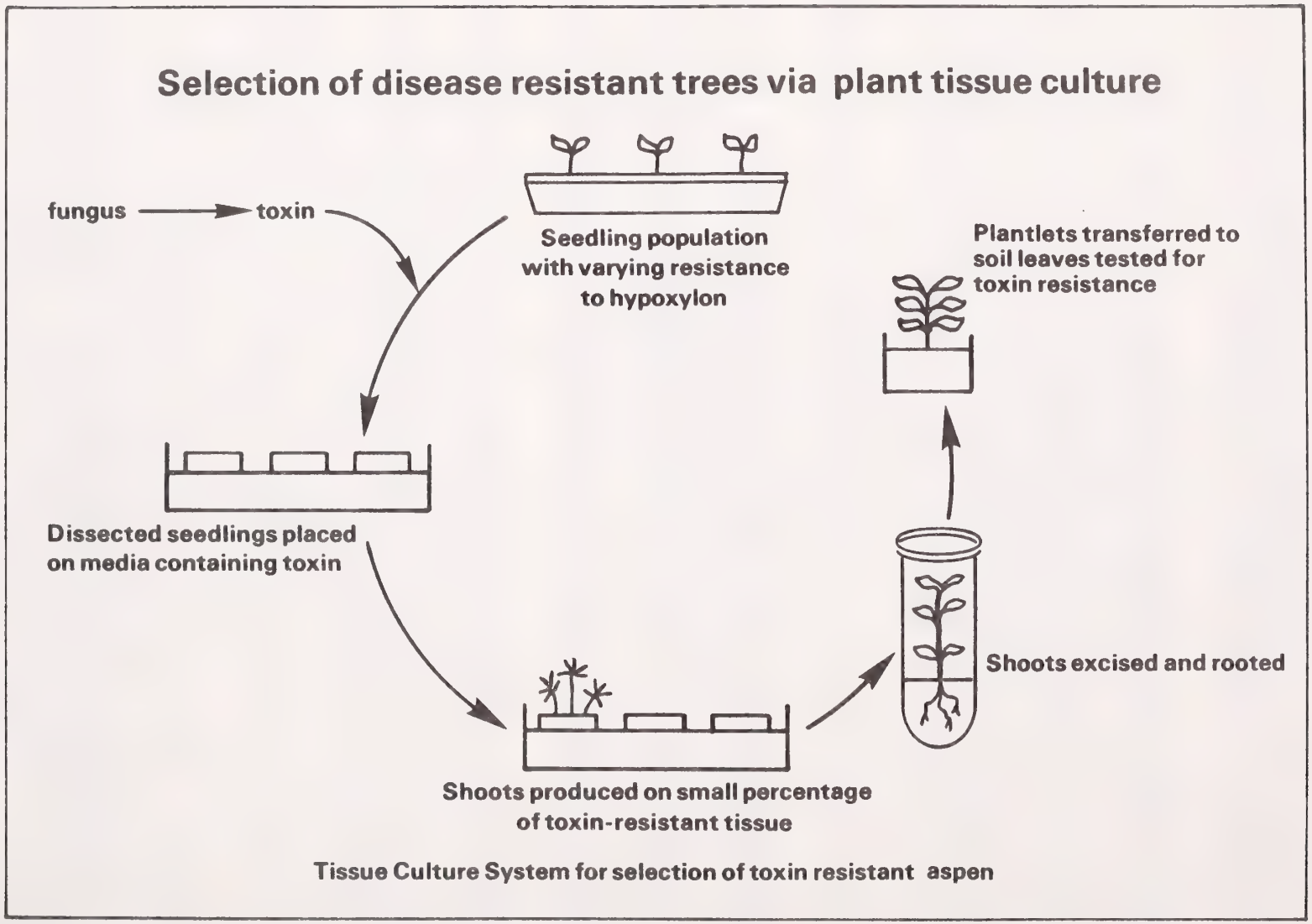

Figure 1. The method used for the selection of toxin resistant trees via plant tissue culture.

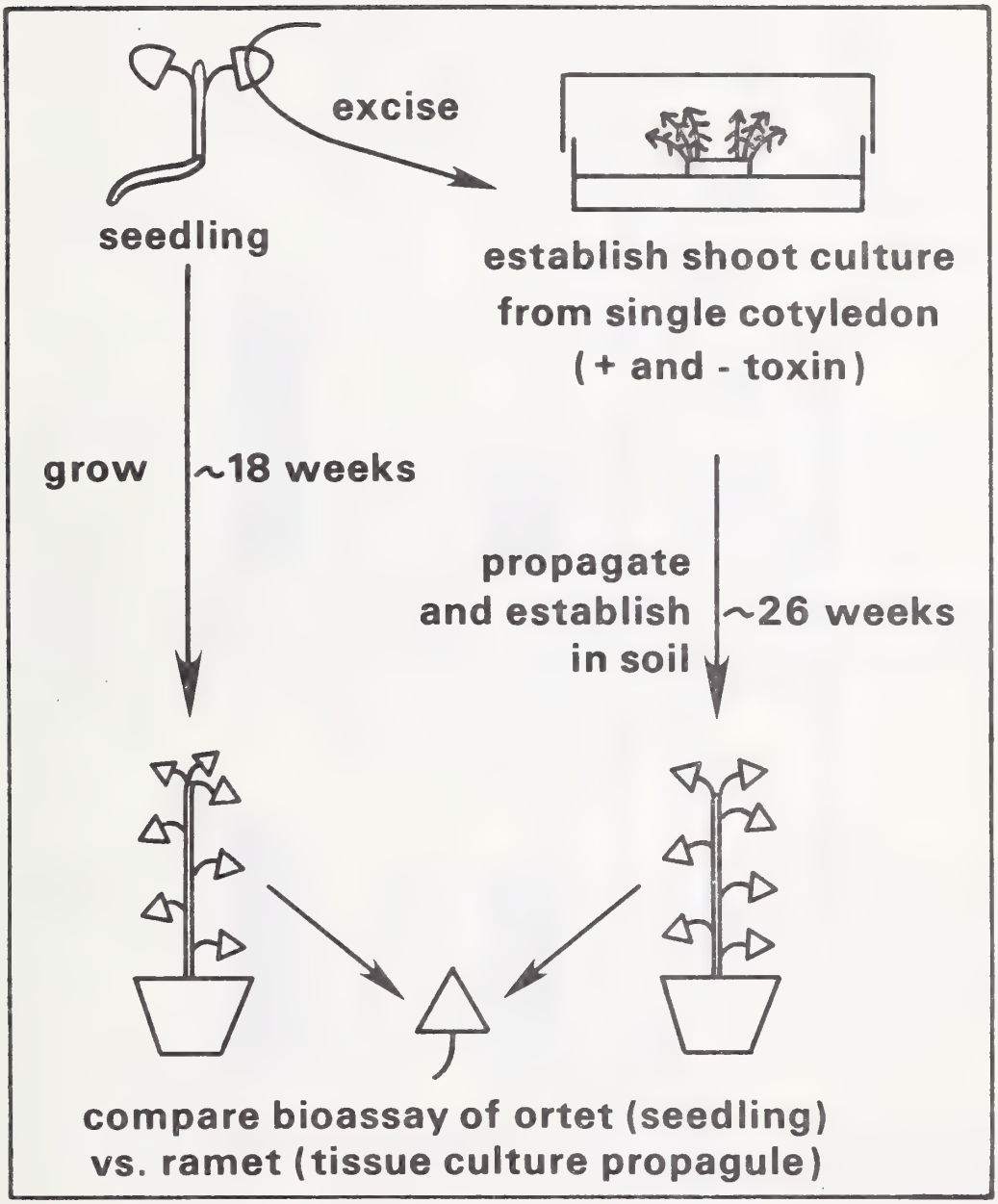

Figure 2. The methods used to propagate plantlets for the ortet vs. ramet bioassay comparison.

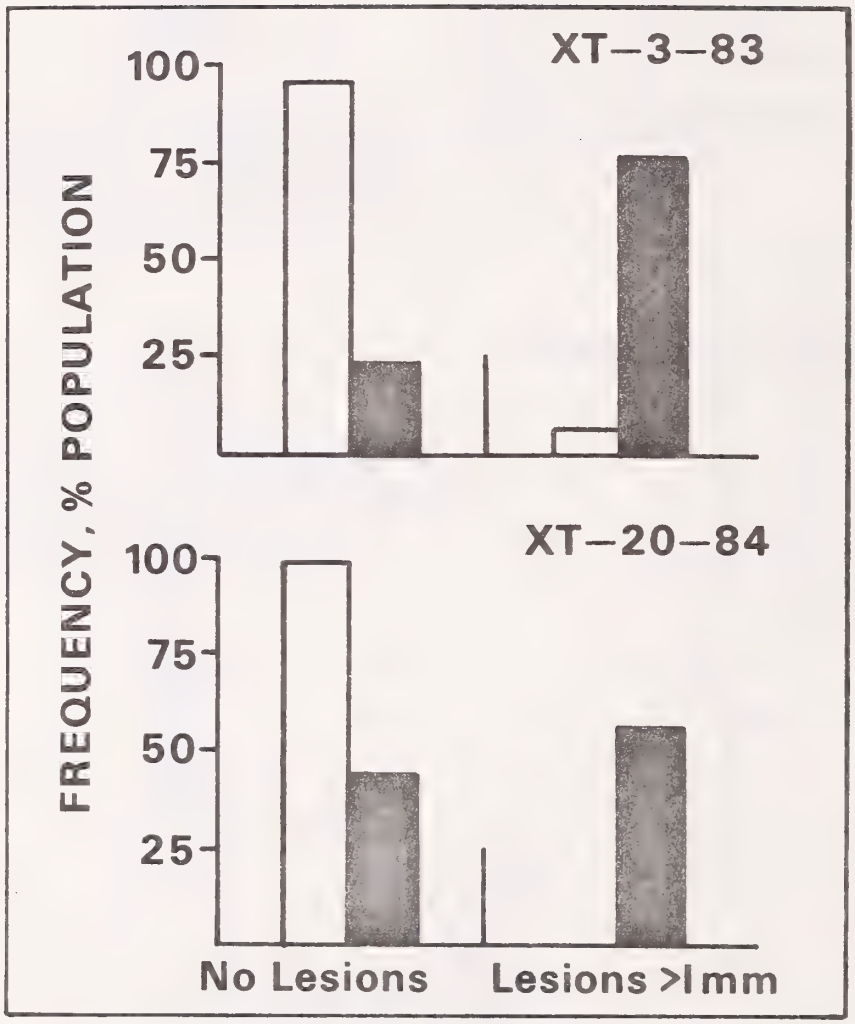

Figure 3. Illustrated by the open bars are percentages of toxin-resistant individuals (no lesions) vs. susceptible plants (lesions > 1 mm) from two aspen full-sib populations that were screened using the in vitro screening procedure. The closed bars indicate the inherent variation in toxin resistance in the original populations.

TISSUE CULTURE OF SWEETGUM (LIQUIDAMBAR STYRACIFLUA L.)

H. E. Sommer, H. Y. Wetzstein and N. Lee[1/]

Abstract.--An improved method for the tissue culture propagation of sweetgum (Liquidambar styraciflua L.) using a liquid culture stage is under development. This method produces more and larger shoots per culture than previous agar based methods. Plantlets from these shoots have been hardened off and grown in a nursery bed. The root collar diameters and heights of several clones after one season in the nursery are reported. Poor root form is the current problem limiting the use of these plantlets for field establishment. Photosynthesis, anatomy and alternate rooting methods have been studied for the evaluation of the efficiency and predictability of plantlets.

Liquidambar styraciflua is one of the major hardwood species in the Southeast United States. Once superior selections have been made, a method of propagation will be needed. Several alternatives include use of ½ sib families, conventional vegetative propagation and tissue culture. The latter two methods have the advantage of immediate genetic gain equal to the genotype of the selection through clonal replication. However, with Liquidambar, conventional vegetative propagation methods are inefficient. Thus alternative propagation methods using tissue culture are being investigated for this species. This report describes some of the refinements in tissue culture methods for Liquidambar.

MATERIALS AND METHODS

Agar Culture Methods

Seed was collected by the U.S. Forest Service from the Oconee National Forest and kept as half-sib lots. Seeds were surface sterilized and germinated under aseptic conditions on a modified Risser and White's basal medium (1,4,7). Hypocotyl sections were placed on a modified Risser and Whites medium with 1.0 ppm IAA and 5.0 ppm 2ip. After excision, the shoots were rooted on a modified Risser and White's rooting medium (4,7).

Liquid Culture Methods

Seed were prepared as described under agar culture methods. Shoots were initiated from hypocotyl sections on a modified Risser and White's medium with 0.1 ppm NAA and 0.5 ppm 2BA (4,7), multiplied

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[1/]Associate Professor, School of Forest Resources, Assistant Professor and Graduate Student, Department of Horticulture, University of Georgia, Athens, Ga.

on a modified Blaydes' medium with 0.01 ppm NAA and 0.5 ppm BA, and then placed on a modified Risser and White's basal medium for growth and rooting as previously described (6,7,8). Cultures were maintained at 25 ± 2°C with a 15 hr photoperiod, under cool white fluorescent lamps.

Nursery Bed Evaluations

Plantlets were removed from the agar rooting medium, and planted in Can Am pine tubes filled with a potting mix of vermiculite and sand (1:1 v/v). Plantlets were hardened off by gradually lowering the relative humidity (10,11), then maintained in a greenhouse or lathhouse prior to planting.

About 800 plantlets were planted on 4" centers in a cement block nursery bed between 30 May 1983 and 20 June 1983. Plantlets were lifted on 5 March 84. Stem length and root collar diameter were measured; root quality ratings were made.

Photosynthesis and Anatomy

Plantlets and seedlings were placed in a growth room maintained at 25 ± 2°C with a 16 hr photoperiod and placed under one of three quantum flux densities: $_{-2}^{50}{}_{-1}$ ± 5 (low light), 155 ± 10 (medium light), 315 ± 15 (high light) $\mu Em^{-2}s^{-1}$ (2). Net photosynthesis of seedlings and plantlets was determined using an infra-red CO_2 analyzer (9). Tissues were prepared for light and scanning electron microscopy as previously described (10,11).

RESULTS

Agar Culture

The results of the culture of sweetgum hypocotyl sections on agar have been reported (6). The yield of plantlets using the agar system is shown in Table 1. No net increase in plant number was obtained when considering initial seedling numbers. Even in terms of plants responding to culture, multiplication rates were low. Results were highly variable among seedlots. This method was the optimum of an investigation involving a 2x5x5 factoral experiment with 2400 cultures (7). It was thus felt that the limiting factor was not nutritional, but some other factor in the system. Agar in the medium may cause water stress, thus a liquid medium step was incorporated into the culture protocol.

Table 1.--Yield of plantlets from agar cultures

Seedlot	# of Seedlings	% Seedlings Giving Successful Cultures	Average # Shoots	% Shoots Yielding Plantlets
76-1B	80	28	2.9	36
76-5B	93	56	2.8	39
76-7B	50	80	3.9	28
76-10B	53	40	2.1	23
78-1B	23	26	2.2	23

Hypocotyl sections from seedlings were cultured on Risser and White's medium. After excision, shoots were rooted in medium without hormones.

Liquid Culture

For liquid culture experiments, buds were first initiated from hypocotyl sections on a revised Risser and White's medium, solidified with agar (4,7,8). Eight to 12 weeks following initiation, hypocotyls were transferred to a liquid Blayde's medium (8,12). After 8 weeks, some of the cultures had proliferated and started to produce shoots of varying sizes. The yield of shoots using liquid culture proliferation is shown in Table 2. Shoot number varied with clone. There exists the potential of harvesting large numbers of shoots per culture. In addition, several harvests and subcultures can be taken from each flask, which would greatly amplify the multiplication potential of this system. Table 3 shows the rooting of shoots obtained from liquid culture, when placed on basal modified Risser and White's medium. Most clones exhibited well over 50% rooting of shoots.

Table 2.--Yield of shoots from liquid culture

Clone	Seedlot Origin	# of Shoots
5529	80-5B	28
5587	81-8U	17
5595	81-8U	32
5635	81-2U	52
5637	81-2U	45
5646	81-2U	0
5659	81-2U	77
5688	81-14B	70
5718	81-4U	15
5727	81-4U	10
5757	81-4U	40
5780	81-76-8U	16

Hypocotyl sections were placed on modified Risser and White's medium 26 August 1983, removed 27 February 1984 and transferred to modified Blaydes liquid medium. The above data is from the harvest of 10 June 1984.

Growth of Plantlets in Nursery Beds

Growth of the six major clones in nursery beds is given in Table 4 and in Figures 1 and 2. From 74 to 86% of the plantlets were recovered; average height and root collar diameters varied among clones (Table 4). Variation within a clone was large (Figs. 1, 2). Most of the plantlets were less than 85 cm high (Fig. 1), which is considered the maximum height desired for planting. The root collar diameter of most of the plantlets exceeded 6.25 mm (minimum diameter for planting), and few exceeded 9.5 mm (preferred minimum for planting) (Fig. 2).

Table 3.--Rooting of shoots from liquid culture

Clone	Seedlot Origin	Total # of Shoots	% Rooted
5529	80-5B	27	63
5531	80-5B	4	50
5587	81-8U	12	67
5635	81-2U	38	55
5637	81-2U	37	73
5659	81-2U	78	73
5670	81-14B	12	92
5688	81-14B	77	90
5724	81-4U	20	60
5757	81-76-8U	40	73
5780	81-76-8U	16	44

Shoots excised from liquid multiplication culture 5-10 May 1984 and placed on rooting medium. Observations were made 2-21 August 84.

When the plantlets were lifted from the nursery beds in March 1984, it was surprising to find a high incidence of plantlets with girdling roots (Table 5). The girdling was traced back to the placement of the plantlets into the Can Am plugs. Manipulation during transplanting pushed the roots into a position promoting girdling root growth. This could similarly have occurred during transplant into the nursery beds. Plantlets were found with bent, "S"-shaped stems under the surface, proposed to result from transplant of the extremely flexible cultured shoots.

Table 4.-- Growth of sweetgum plantlets in cement block nursery beds

Clone #	Seed Lot Origin	Number Planted	% Recovered	Height (cm)	Root Collar Diameter (mm)
3070	79-6B	96	84	66.3	7.3
3083	79-6B	138	86	57.2	8.0
3090	79-6B	106	83	63.7	8.2
3201	78-1B	51	78	43.7	6.3
3346	77-10U	34	74	56.4	8.0

Plantlets were planted between 30 May 83 and 20 June 83, and lifted on the week of 5 March 84.

Table 5.--Plantlets with girdling roots in the nursery

Clone	Total # Plantlets	% Girdling
3070	81	68
3083	119	87
3090	88	93
3201	40	88
3346	25	80

Evaluation of roots of plantlets lifted the week of 5 March 1984 for girdling root systems.

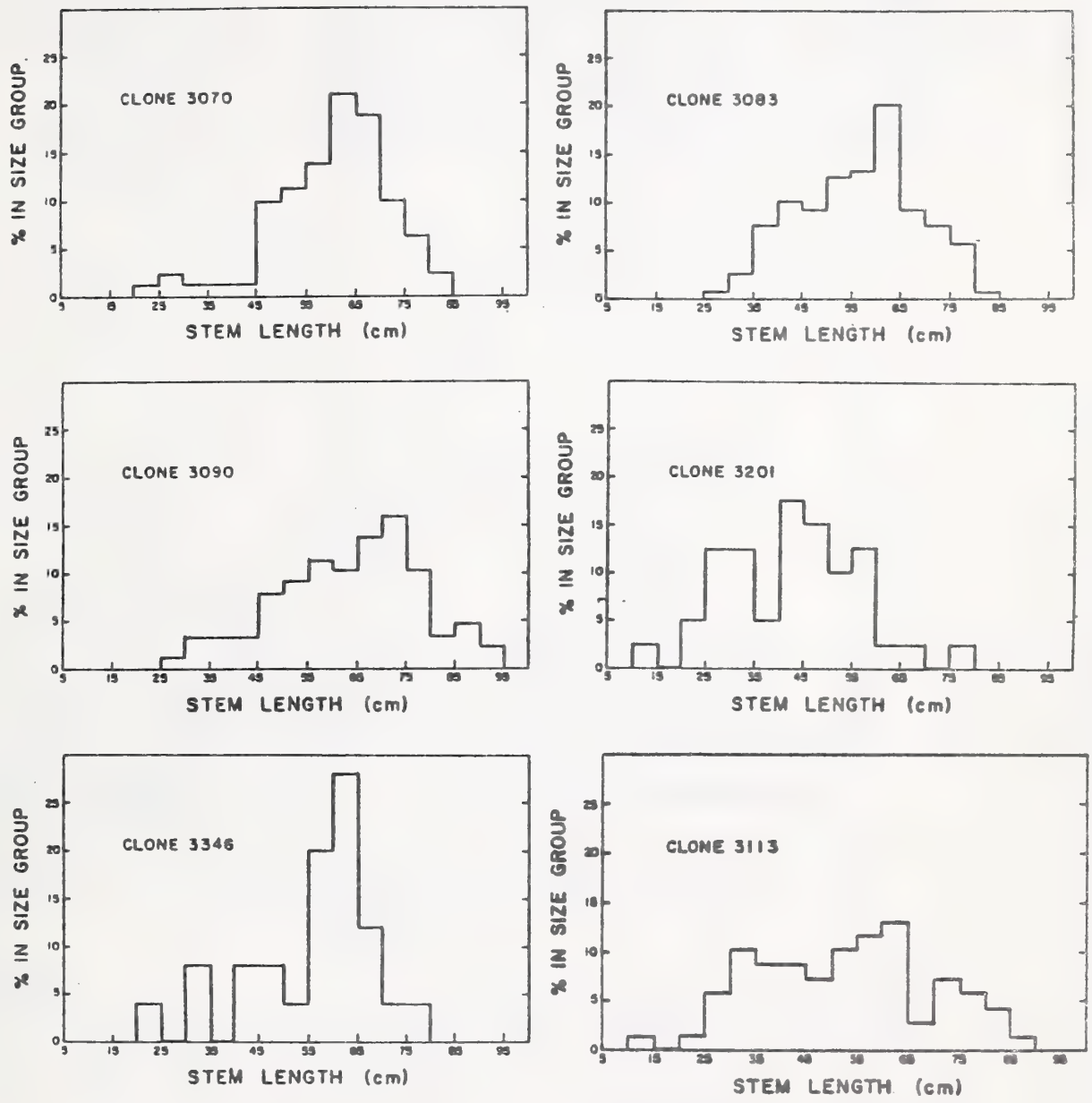

Figure 1. Stem length of 6 major clones grown in nursery beds.

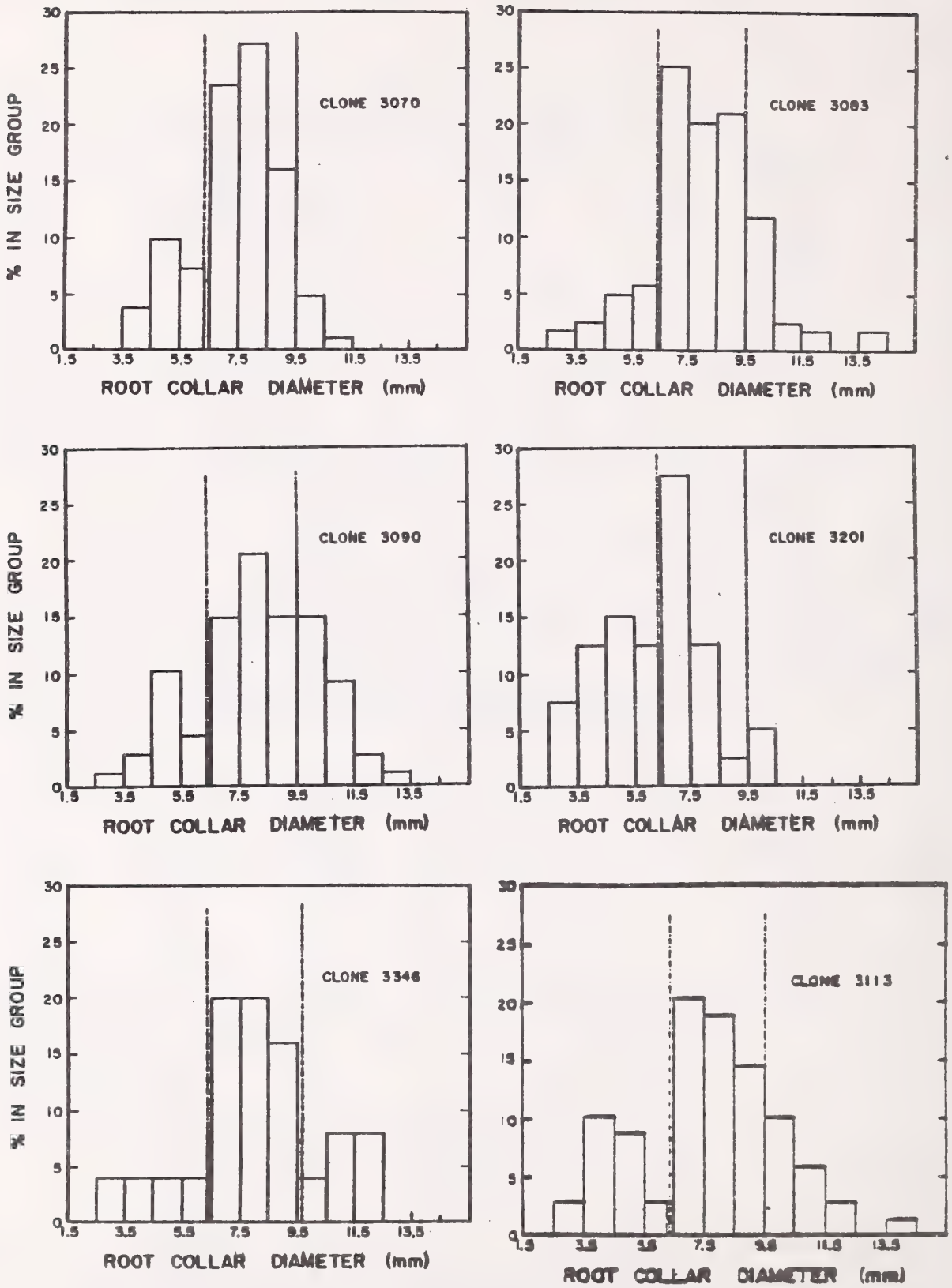

Figure 2. Root collar diameter of 6 major clones grown in nursery beds. Dotted lines indicate the minimum root collar diameter for planting and the preferred minimum root collar diameter for planting.

Photosynthesis and Anatomy

Net photosynthesis of seedlings and plantlets is shown in Figure 3. Seedlings showed typical light saturation curves when grown at the different light levels. High light seedlings had the highest maximum rate of photosynthesis. Photosynthesis of *in vitro* grown plantlets saturated at higher levels, with medium light plantlets attaining the highest photosynthetic rates. Plantlets exhibited much higher photosynthetic rates than seedling under all conditions.

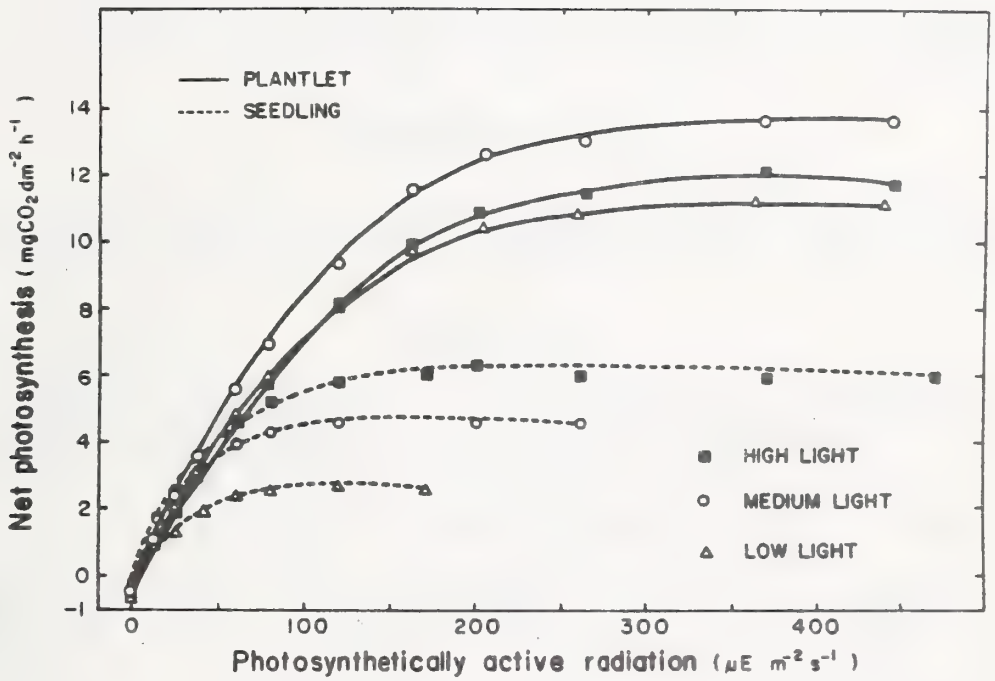

Figure 3. Net Photosynthesis of seedlings and plantlets grown under 3 quantum flux densities: 50 ± 5 (low), 155 ± 10 (medium) or 315 ± 15 (high) $\mu EM^{-2}5^{-1}$.

Anatomical observations of plantlet leaves showed that chlorplast structure, leaf thickness, and mesophyll development were affected by quantum flux differences in culture (2). The higher stomatal densities and sizes found in cultured leaves were not affected by light levels. Factors other than light are responsible for the atypical stomatal configurations which contribute to water loss and wilting of cultured plants.

DISCUSSION

The inclusion of the liquid medium step has greatly increased the number of shoots obtainable in culture. However, more research is needed to increase the percentage of cultures that respond in the liquid step.

The other obvious problem that needs solution is that of root girdling. McKeand and Wisniewski (3) reported a similar problem with pines, which was solved by using shorter roots at planting and a ridged tube instead of a pot. This may not be feasible for Liquidambar, however, in that cuticular development and stomatal functioning are less developed in cultured sweetgum than pine (8). A well developed root system is expected to be important in maintaining good water relations (10,11). Rooting in a stationary medium such as foam or peat plugs may be a possible solution.

We have determined that plantlets developed in vitro are capable of significant levels of photosynthesis. However, it is unknown if culture conditions (i.e. CO_2 and light levels) promote photosynthesis. The efficiency of our culture system could be improved if this photosynthetic capability were utilized. Further research is needed in this area as are anatomical observations for evaluating and predicting plantlet growth and culture efficiency.

ACKNOWLEDGEMENTS

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VEGETATIVE PROPAGATION OF SCOTS PINE (PINUS SYLVESTRIS L.) THROUGH TISSUE CULTURE

H.C. Sonia Tsai and F.H. Huang[1/]

Abstract.--A pulse treatment with a high-concentration NAA solution (125 mg/L) not only enhanced rooting up to 33% but also increased the number (up to 10 roots/propagule) and size of roots (2mm in diameter) in cultured Scots pine adventitious shoots. This induced multiple roots system should increase the vigor of regenerated plantlets, and hence, shorten the adaptation period while being transferred to soil.

Additional keywords: Adventitious buds, adventitious roots, pulse treatment, seedling, embryonic cotyledons.

Tissue culture methods have been evaluated for Scots pine by several research groups. Tranvan (8) studied the formation, localization of adventitious buds on seedlings, and the initiation of cotyledon adventitious buds. Bornman and Jansson (2) attempted to increase the rooting percentage of four types of explants by applying the growth-active compound, coumarin, alone or in combination with auxin. Shen and Arnold (7) completed the culture sequences to regenerate plantlets from cultured embryonic tissue with an overall regeneration rate of 10% over a 10-month period.

This experiment was directed to improve the survival rate at elongating stage of adventitious buds and then to promote the rooting percentage of those elongated adventitious shoots to provide mass, clonal propagules for improvement of a Christmas tree program.

METHODS

Scots pine seeds of Central Massif were chosen from germination tests as experimental material from among twelve varieties purchased from F.W. Schumacher Co.

Two types of explants were established from seeds by embryo culture. Seeds were pretreated with 1% H_2O_2 for 1 week to facilitate the removal of seed coats and to stimulate germination (6). After the removal of seed coats, seeds were surface-disinfected with 1/6-strength Clorox solution for 15 min and then rinsed with autoclaved, distilled water. Embryos were aseptically separated from endosperms and planted on culture media. Seedlings were produced by growing the embryos on 1/3-strength M.S. minimal organic medium (5), and cotyledonous adventitious buds were initiated on 1/3-strength M.S."B" medium with supplements of 1.0 mg/L kinetin and 30 g/L sucrose. The whorl of cotyledon with a stub of subtended hypocotyl was excised from 2- to 4-week-old seedlings and planted on the M.S."B"

[1/]Mrs. Tsai is a research assistant and Dr. F.H. Huang is an associate professor in the Department of Horticulture and Forestry. University of Arkansas, Fayetteville, AR 72701.

medium described above (Fig. 1A).

Four adventitious shoots, in addition to the apical shoot, were formed on the basal area of the cotyledon whorl (Fig. 1B). Those shoot bundles were separated from the mother explant at 2- to 3-month intervals and subcultured on M.S."B" medium for continuous multiplication. Cotyledonous adventitious buds were transferred to 1/6-strength M.S."B" medium to encourage the elongation of adventitious buds into shoots. Elongated shoots with a visible stem region were suitable to be rooted (4). Each of two chemical (adenine sulfate at 66 mg/L concentration and coumarin at 1.46 mg/L concentration) was incorporated into the basal medium (1/6-strength M.S."B" medium) separately to test for effect on rooting. Finally, a pulse treatment with a high concentration NAA solution (125 mg/L) was applied for 24 h before transferring the shoots to medium free of growth regulator (1, 3).

RESULTS AND DISCUSSION

Adventitious shoots were produced in two ways: 1) The cotyledon whorl excised from 2- to 4-week-old seedling readily produced four adventitious shoots on the basal area when it was planted on 1/3-strength M.S."B" medium with supplements of 1.0 mg/L kinetin and 30 g/L sucrose; 2) The adventitious buds initiated on embryonic cotyledons were transferred to 1/6-strength M.S."B" medium to encourage the elongation of buds into shoots. Those adventitious shoots developed on the basal area of the cotyledon whorl can be excised from mother explant at 2- to 3-month intervals and cultured on the same medium for continuous multiplication, or they are ready to be rooted.

The adventitious buds initiated on embryonic cotyledons were numerous, but the elongation of those adventitious buds was sporadic. Efforts to stimulate the growth of adventitious buds have been without much success so far. Increase in boron concentration to minimize the phenolic compound synthesis in order to prevent the stunting of the adventitious buds might be tried in further experiments.

Adenine sulfate and coumarin incorporation in culture medium did not show any significant effect over the control. But, those pretreated adventitious shoots responded quickly to pulse treatment with high-concentration NAA solution. In 3 months, ten adventitious shoots (33%) produced root primordium with four of them having actual root protrusion and root growth. The NAA solution pulse treatment not only increased the number of rooted propagules, but also increased the number and size of root formed in the individual propagule (Fig. 2A, 2B). A single root is typical in cultured pine tissue (6), but the above 8-month culture sequences resulted in an increase in number (up to 10 roots/propagule) and size (2 mm in diameter) of rooting which should increase the vigor of the regenerated plantlets, and hence, shorten the adaptation period when being transferred to soil. One regenerated plantlet from previous culture without the pulse treatment has been transferred to vermiculite for one year (Fig. 1D). A single rooting of 7 cm long was observed when being transferred to soil recently (Fig. 1C).

CONCLUSIONS

Before the survival rate at the elongation stage of cotyledonous adventitious buds can be increased or the embryogenesis method of Scots pine callus can be developed, the most practical way for vegetative propagation of Scots pine is through induction of adventitious shoots on the cotyledon whorl and rooting of those adventitious shoots with NAA solution pulse treatment.

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Fig. 1 (A) 4-week-old seedling from which apical slices were excised. Apical slices included the base of the cotyledonary whorl subtended by a stub of 2mm hypocotyl.

(B) Adventitious buds induced from apical slices after 4-week culture

(C) Plantlets regenerated in vitro after one month in the rooting medium.

(D) Plantlets potted in vermiculite.

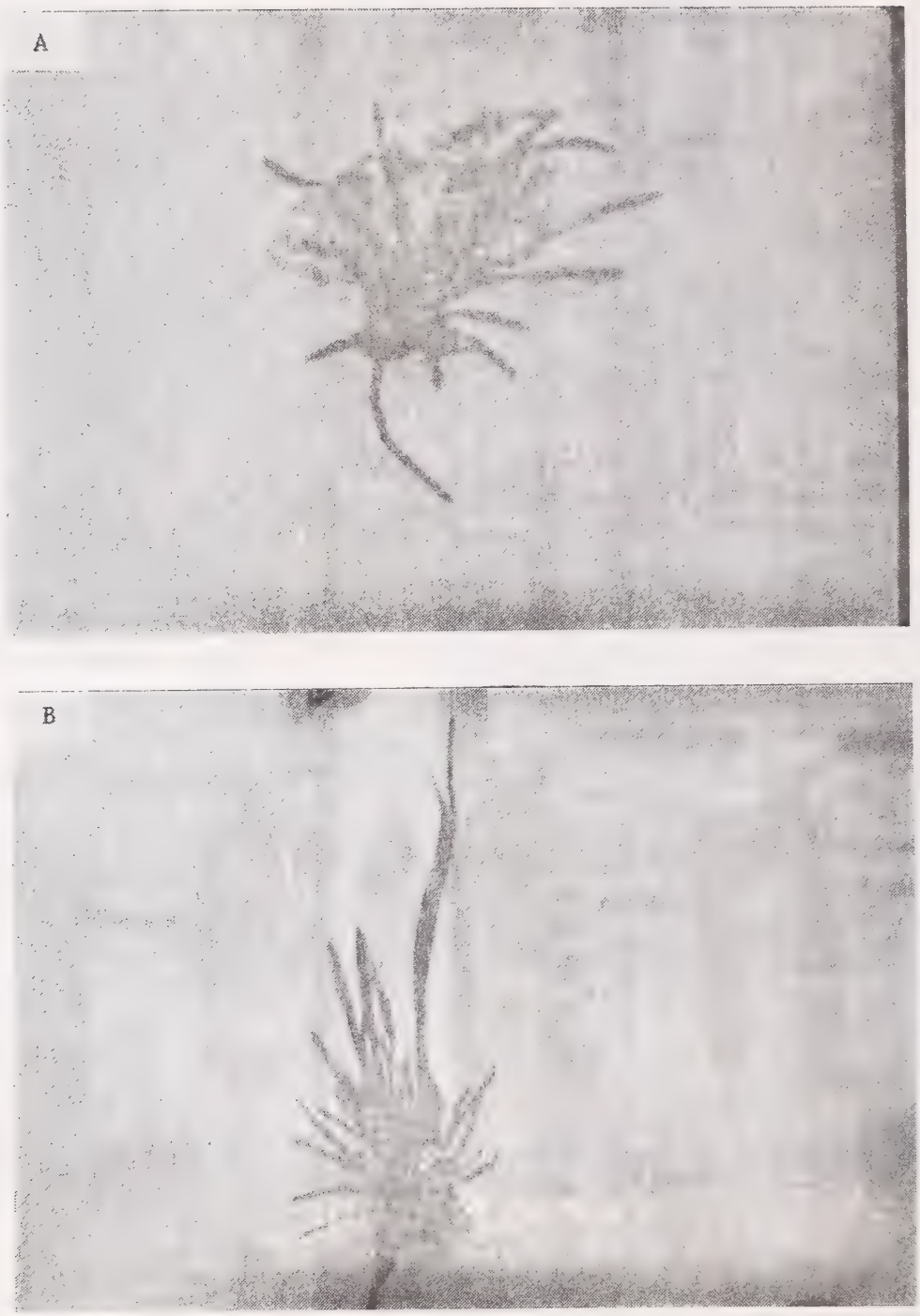

Fig. 2 (A) 10 roots per plantlet

(B) 2 mm in diameter root

MICROPROPAGATION OF *Eucalyptus viminalis*

M. W. Cunningham and R. L. Mott[1/]

Abstract.--Cooperators of the North Carolina State University Hardwood Research Program have selected over 50 *Eucalyptus viminalis* trees that demonstrated superior growth rates and tolerance to frost in test plantings in southern Georgia and northern Florida. Twenty-eight of these trees have been vegetatively propagated by grafting or rooting at North Carolina State University. These stock plants will be micropropagated as *in vitro* methods are developed, to establish a seed orchard on lands of Cartón de Colombia.

Results are presented for *in vitro* multiple shoot production, using single node explants. Genotype, node selection, and stock plant vigor influenced axillary shoot production of the original explants. The induction of multiple shoots on excised axillary shoots was not affected by the cytokinin concentrations or durations of treatments tested. When excised axillary shoots were cut into single nodes, there was a slight enhancement of multiple shoot production.

Additional Keywords: tissue culture, vegetative propagation

The need for a hardwood fiber source that would occupy upland sites and therefore be more readily available during wet seasons led members of the North Carolina State University Hardwood Research Program to begin screening seed sources of several species of *Eucalyptus* in 1971. The objective of this effort was to select species, seed sources, and trees within seed sources that would be fast-growing, of good form, and tolerant to both drought and frost. Over the years more than 100 species and 577 seed sources were tested. Initially, plantings ranged from the coastal plain of North Carolina to northern Alabama. As the program progressed, the plantings were restricted to southern Georgia and northern Florida because of harsh winter temperatures north of that area. The best suited species to this area were determined to be *E. viminalis*, *E. macarthurii*, *E. nova-anglica* and *E. camphora*.

The failure of any one seed source to consistently produce fast-growing trees that were also frost-tolerant emphasized the need to develop a land race of eucalyptus suitable for planting in the southeastern United States. The seedling seed orchard approach proposed by Purnell and Kellison (1983) for the genetic improvement of other hardwood species could not be used with the eucalypts because the species flowers during the winter months when freezing temperatures destroy the seed-bearing potential of the trees. The decision was thus made to establish a clonal seed orchard farther south, where there would be no danger of freezing temperatures. Container Corporation of America, Fernandina Beach, Florida, in conjunction with one of its South American subsidiaries, Cartón de Colombia, provided the funding for the orchard which is to be established near Popoyán, Colombia.

[1/]Graduate Research Assistant, Department of Forestry, and Professor of Botany, N. C. State University, Raleigh, N. C. (Funding for this project provided by Container Corporation of America, Fernandina Beach, Florida)

Eucalyptus viminalis, the species showing the most promise of the four mentioned previously, has proven difficult to propagate vegetatively. Attempts to root cuttings from induced epicormic shoots from mature trees, as is done with many other species of eucalypts, have met with minimal success. Grafting, while more successful than rooting cuttings, is also an unreliable method of vegetatively propagating the species. Micropropagation under controlled in vitro conditions may offer a viable alternative as a means to vegetatively propagate selected trees of E. viminalis.

Vegetative propagules have been produced by micropropagation for a number of species of eucalyptus (Hartney, 1983). Franclet and Boulay (1982), Boulay (1983), and Depommier (1981) described methods used by AFOCEL in France to successfully regenerate plantlets of E. gunnii and E. dalrympleana, using nodal explants derived from grafted or rooted cutting stock plants. Ages of the original ortets from which stock plants were derived ranged from 2 to 20 years. The general procedure followed in each of these papers included (1) induction of axillary shoot formation on single node explants, (2) multiple shoot formation from excised axillary shoots, (3) shoot elongation, (4) root initiation of excised shoots, and (5) root elongation and transfer to soil. Franclet and Boulay (1982) anticipated producing 25,000 plants per month, using this system.

The objectives of the Hardwood Research Program project are to employ and modify where necessary the techniques outlined by AFOCEL workers to vegetatively propagate 28 clones of E. viminalis. These clones will be shipped to Popoyán, Colombia, S. A., where they will be used to establish a seed orchard. The objectives of this paper are to report on the successes in inducing nodes to produce axillary shoots and the methods tested for multiple shoot formation from excised shoots.

ESTABLISHMENT AND MAINTENANCE OF CLONES

In 1983, cooperators of the Hardwood Research Program selected a total of 41 E. viminalis, E. macarthurii and E. nova-anglica trees that were phenotypically superior in growth rate and frost tolerance. Selections were made from seed source trials and operational test plantings three or more years old. In addition, 14 surviving E. viminalis clones from a previously established seed orchard near Ft. Green Springs, Florida were included, making a total of 55 selections. Twenty-eight of these selections have been vegetatively propagated by rooted cuttings or grafting and are being used as stock plants for the micropropagation work.

Cuttings were rooted following slightly modified techniques of Campinhos and Ikemori (1980). Basal epicormic shoots were induced on mature trees by girdling at a height of one meter. Shoots were cut into four-leaved, two-node cuttings, treated with a rooting powder consisting of 0.8% indolebutyric acid, and placed under intermittent mist. Rooting success was low for these basal sprouts, averaging less than 15% for all clones. Twelve of the 28 clones were propagated in this way. The remaining 16 clones were grafted, using scion material from the upper branches.

The stock plants were maintained in 4.5-l pots in the greenhouse with an extended photoperiod of 18 hours. They were fertilized weekly with a 0.5 g/l solution of 20-19-18 (N-P-K) fertilizer and sprayed twice weekly with Benlate, a systemic fungicide. The plants were maintained at a height of 30 - 60 cm by cutting them back at eight-week intervals.

INITIATION OF CULTURES

Initial explants were derived by cutting shoots into single-node sections and immediately sealing both ends in paraffin. The leaves were then trimmed to approximately four - mm squares. The explants were surface-sterilized for 10 minutes in a 10% solution of commercial bleach with two drops of surfactant added. The wax ends were then cut off, leaving an explant of 8 to 14 mm in length, with 3 to 4 mm above the node. The explants were then placed vertically into the medium, which was contained in sterilized petri plates. They were placed in the dark for one week and then moved to a continuous light environment supplied by two 40-watt, cool white fluorescent bulbs, approximately 15 cm above the top of the plates. The temperature in the culture room was 22° $\pm$ 2° C.

The basic medium used for all experiments consisted of half-strength salts and vitamins (Murashige and Skoog, 1962), 3.0% sucrose, and 0.7% agar. The pH of the media was adjusted to 5.6. For the initial nodal explant studies, 0.5 mg/l of benzylaminopurine (BAP) and 0.01 mg/l of napthaleneacetic acid. (NAA) were added.

By the end of the first week in culture, axillary shoots began to emerge from many of the explants. In most cases callusing occurred at the base of the explant and on the leaves where they were touching the media. Callusing occasionally occurred in the nodal region but this did not appear to influence the emergence of axillary shoots. By the third week on the medium, many of the axillary shoots had begun to elongate, some over one cm in length, and were ready to be excised.

As with most vegetative propagation methods, the genotype substantially influenced the response achieved. The responses of three clones that were used in a number of experiments are summarized in Table 1. Growth regulator concentrations were varied slightly from trial to trial but all clones were treated the same within a particular trial, and within a trial the explants for all clones came from stock plants at the same stage after pruning. Clones B16 and B6 consistently had response rates of greater than 50%, while explants from Clone 2594 never responded at rates above 50%.

Table 1.--Percentage of single-node explants producing axillary shoots in 3 - 4 weeks in four different trials

Clone	Trial 1	2	3	4
	Percent			
2594	33.3	8.6	8.3	-
B15	50.0	68.6	-	75.0
B6	84.6	60.0	50.0	66.7

For a given clone, the percentage of explants producing axillary shoots seemed to be influenced by stock plant vigor and node selection. In an experiment to determine which nodes were most useful, explants were grouped according to their distance from the growing point. The majority of stock plant shoots were no longer than 6 nodes in length, so explants were divided into three groups consisting of two nodes each (Nodes 1-2, 3-4, and 5-6, with Nodes 1-2 being closest to the growing point). After three weeks on the initiation medium, data were recorded for the number of axillary buds and shoots (shoots were distinguished from buds by the visible presence of at least a 1-mm internode) and the length of the longest shoot. The percentage of explants with buds or shoots was calculated. Percent and count data were transformed, using the arc-sine square root and the square root methods, respectively, for the analysis of variance.

The mean response of three clones after three weeks in culture showed that explants derived from Nodes 3 and 4 gave the highest response rate and the greatest number of shoots per plant (Table 2). While fewer explants from Nodes 1 and 2 responded than those from Nodes 5 and 6, those explants that did respond had more and longer shoots. This implies that explants from Nodes 5-6 are slower-growing and probably have many explants with buds that have yet to elongate. There were significant clone effects for all variables but the "clone by node" interaction was not significant, indicating that the influence of node number was consistent for all three clones tested.

Table 2.--Effect of node number on the response of single-node explants after four weeks in culture

Node Number	Percent with Buds or Shoots	Average Shoots per Explant	Average Length Longest Shoot(mm)
1-2	36.1 a[1/]	1.2 ab	3.3 a
3-4	53.3 b	1.5 a	3.4 a
5-6	47.2 ab	0.6 b	2.1 a

[1/] Values within a column followed by the same letter were not significantly different ($P \leq 0.05$) using Duncan's Multiple Range Test.

Stock plant vigor was also found to influence the response of nodal explants. In two separate trials, explants were collected from the same set of nodes and placed on the same medium. In one trial, explants were derived from stock plants that had been repeatedly pruned and fertilized weekly; while in the other experiment, stock plants were slower-growing, had not been fertilized on a weekly schedule, and had not been pruned for several months. For the two clones used in the trials, contamination and mortality rates were considerably higher and the number of explants producing axillary buds or shoots was much lower for the less vigorous stock plants (Table 3). These results emphasize the importance of maintaining pruned and vigorous stock plants as a source of explant material.

Table 3.--Effects of stock plant vigor on the response of nodal explants after 3 weeks in culture

Clone	Stock Vigor	Percent Contaminated	Percent Dead	Percent with Buds or Shoots
2604	Poor	50.0	25.0	25.0
	Good	4.2	4.2	66.7
B15	Poor	58.3	25.0	16.7
	Good	8.3	0	66.7

MULTIPLE SHOOT PRODUCTION

Buds and shoots continued to grow when left attached to the original node, about 3 mm by 3 weeks (Table 2) but reaching 7 to 20 mm by 6 weeks. However, the explants did not continue to make additional buds. Published methods require that shoots be excised both for additional bud production and for growth of shoots in preparation for the rooting stage.

At 3 to 4 weeks, some axillary shoots were sufficiently elongated to be excised and transferred to a new medium. In preliminary trials, excised shoots were placed on the same medium as the original explant (0.5 M.S. + 0.5 mg/l BAP + 0.01 mg/l NAA) under the same environmental conditions. After 2 to 3 weeks, explants began to turn red, older leaves senesced, and the explant died basipetally. Multiple buds were sometimes formed at the lowest node near the base of the explant. To determine if cytokinin concentrations influence this response, excised shoots from two clones were tested on the original medium, but with either 0.2, 0.5 or 1.0 mg/l BAP.

Neither clone nor BAP concentrations significantly ($P \leq 0.05$) influenced the number of buds, number of shoots, or shoot length (Table 4). Explants from all treatments responded the same as those from preliminary studies. Some of the explants produced clumps of buds at their base, but none of the buds elongated to more than 5 mm in length during the four weeks. Even at seven weeks, the average shoot length was 2.7 mm.

Table 4.--Effects of BAP concentration on the response of excised axillary shoots

BAP[1/] (mg/l)	Average Buds per Explant	Average Shoots per Explant	Average Length Longest Shoot (mm)
0.2	2.2	1.2	2.7
0.5	2.5	1.2	1.9
1.0	2.9	0.3	2.7

[1/] Analyses of variance showed no significant treatment effects ($P \leq 0.05$) for any of the variables measured.

In a second experiment, cytokinin pulses were tested to see if the length of time the explants were treated with BAP would influence their response. Shoots were excised from nodal explants of three clones and placed on the original medium with either 1.0 or 2.0 mg/l BAP for one, two or three weeks. The explants were then transferred to a medium (Franclet and Boulay, 1982) used for shoot elongation. This medium was the same as the original medium except for a reduced concentration of BAP (0.1 mg/l) and the addition of 1.5% activated charcoal.

Neither the duration nor the concentration of the BAP pulse significantly influenced the explant responses (Tables 5 and 6). The average number of buds and shoots was very similar to the results obtained from the BAP concentration study. Again, explants would respond with red foliage, callusing and senescing from the apex down and the formation of multiple bud clusters at the base of some of the explants. The use of charcoal appeared to make a slight improvement in the response. The average shoot lengths of explants in the pulse experiment (Table 5) were a little longer than those from the BAP concentration study (Table 4). It was noted in previous experiments that when multiple bud explants were transferred to a medium containing charcoal, the buds would begin to expand and elongate for two to three weeks. The explants would then begin to turn pale yellow and callus, probably as a result of the charcoal tying up needed growth regulators. Shoots seldom reached a length suitable for rooting.

Table 5.--Effects of length of BAP pulse on the response of excised axillary shoots

Pulse[1/] (wks)	Average Buds per Explant	Average Shoots per Explant	Average Length Longest Shoot (mm)
1	3.4	1.5	4.3
2	2.9	1.5	4.9
3	3.9	1.2	3.5

[1/] Analyses of variance showed no significant treatment effects ($P \leq 0.05$) for any of the variables measured.

Table 6.--Effects of BAP pulse concentration on the response of excised axillary shoots

BAP[1/] (mg/l)	Average Buds per Explant	Average Shoots per Explant	Average Length Longest Shoot (mm)
1.0	3.2	1.5	4.3
2.0	3.3	1.3	4.3

[1/] Analyses of variance showed no significant treatment effects ($P \leq 0.05$) for any of the variables measured.

The tendency of excised shoots to die back and produce multiple buds at the basal node led to a test of using excised shoots that had been further cut into single-node explants for multiple bud production. The explants were on a medium with 0.2 mg/l BAP and 0.01 mg/l NAA for three weeks. The group of single-node explants from one shoot tended to produce more buds and shoots than did comparable entire excised shoots (Table 7). This increase was the result of more explants producing bud clusters rather than an increase in the number of buds per cluster. The increased bud number and increased growth of the buds produced was probably a function of removing the apex from the explant, thus relieving the apical dominance; however, this hypothesis has yet to be tested.

Table 7.--Comparison of the response of single-node versus entire axillary shoots as an explant source for multiple shoot production

Explant Type	Average Buds per Explant	Average Shoots per Explant	Average Length Longest Shoot (mm)
Single Node	4.1	2.9	4.6
Entire Shoot	2.2	1.2	2.5

CONCLUSIONS

Nodal explants of E. viminalis could be induced to produce axillary shoots in vitro. The response varied from clone to clone and was improved by maintaining vigorously growing stock plants and by selecting the proper node. Proper greenhouse care to ensure rapidly growing, disease- and insect-free stock plants seems to be the most critical step for acceptable contamination rates and for the induction of buds and shoots. Axillary shoots, when left intact, continued to elongate through six weeks in culture. However, no additional shoots were initiated on the origianl explant after week four. Therefore, in order to multiply the number of plants for rooting, it is necessary to excise axillary shoots and induce multiple shoot formation.

Excised axillary shoots grew poorly, producing few buds, which failed to elongate. The response of the excised shoots was not influenced by cytokinin concentrations or the duration of these treatments. The addition of charcoal to the media was also ineffective in enhancing shoot elongation.

Boulay (1983) reported similar problems with several frost-tolerant eucalyptus species. He stated that for many clones, frequent subculturing was necessary before the explants would begin to grow vigorously and produce multiple shoots. We believe that this enhanced response after many subcultures probably resulted from better control of shoot vigor and/or node development stage of the in vitro stock plants used for serial subcultures. The influence of in vivo stock vigor and node number on the response of original explants found in our studies supports this hypothesis. From our preliminary data it appears that we can enhance multiple shoot production by selecting single nodes from in vitro-produced axillary shoots rather than using the entire shoot as an explant. However, the buds produced still did not elongate sufficiently for rooting purposes. We will be further investigating the effects of node selection of in vitro-produced axillary shoots to enhance multiple shoot production.

Multiple shoot production and shoot elongation cannot occur under the same in vitro conditions for E. viminalis. Future efforts will also be directed toward development of a separate medium for shoot elongation of multiple shoot explants and eventually the rooting of elongated shoots.

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GROWTH CHANGES IN LOBLOLLY PINE (*PINUS TAEDA* L.) CELL CULTURES IN RESPONSE TO DROUGHT STRESS

R. J. Newton, S. Sen[1], and J. P. van Buijtenen[2]

Abstract.-- Two *in vitro* systems; callus proliferation from (1) cotyledons and (2) pre-existing callus, were used to evaluate growth responses to low tissue water potential among two loblolly pine sources including 6 families. Both systems showed significant differences in growth response to low tissue water potential between sources from Louisiana and Texas. Both systems also showed significant differences between a fast-growing family from Louisiana (A-1-14) and a slow growing family from Texas (GR1-8). Louisiana sources sustained more growth under *in vitro* drought stress than did Texas sources. The minimum tissue water potential below which callus growth was halted was -1.0 MPa. These preliminary results suggest that *in vitro* drought stress techniques may have applicability for predicting growth potential in the field under stressed and nonstressed conditions and investigating drought tolerance mechanisms which appear to be distinct and separate from drought avoidance mechanisms. Additional research is in progress to confirm these results.

Additional keywords: Tissue culture, polyethylene glycol, callus, water potential, drought tolerance.

Several investigators have shown that there are differences in loblolly pine (*Pinus taeda* L.) source responses to drought. Most of these studies have been concerned with drought avoidance whereby plants tolerate drought by maintaining a high tissue water potential. Whole plant studies have shown that increased root growth (Youngman 1965, van Buijtenen et al. 1976, Bilan et al. 1978, Cannell et al. 1978), reduction in epidermal conductance (Thames 1963, Knauf and Bilan 1974, van Buijtenen et al. 1976, Bilan 1977) and reduction in evaporative surfaces (Wells and Wakeley 1966, Wright and Bull 1968, Woessner 1972a,b; Venator 1976) are mechanisms by which high tissue water potentials are maintained in loblolly pine resulting in drought avoidance. Only a few studies have investigated the mechanisms whereby drought is tolerated at low tissue water potential. These whole plant

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[1]Associate Professor and Research Associate, Department of Forest Science and the Texas Agricultural Experiment Station, Texas A&M University, College Station, TX 77843.
[2]Professor, Department of Forest Science and the Texas Forest Service, Texas A&M University, College Station, TX 77843.

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studies have shown that solutes accumulated (Hodges and Lorio 1969) and that growth potential was maintained through osmotic adjustment (Hennessey and Dougherty 1984) as low tissue water potentials were experienced. Other workers have shown differential tolerance to low tissue water potential among loblolly pine families (Kaloyereas 1958, van Buijtenen et al. 1976, Newton and van Buijtenen 1984) when whole plants were subjected to desiccation.

A recently developed technique for investigating drought tolerance at low tissue water potential is in vitro tissue culture. Tissue culture has several advantages over whole plants: (1) drought avoidance mechanisms such as increased rooting, decreased stomatal conductance and leaf drop can be dismissed, (2) cellular mechanisms of drought tolerance at low water potential can be investigated, and (3) it could be used to screen a large number of different sources of germplasm for drought tolerance in a short period of time and in a small space. Therefore, if the suitability of tissue culture as a selection tool for drought tolerance could be demonstrated, it could aid existing tree improvement programs, particularly those in the western edge of the loblolly pine region.

The first objective of this investigation was to evaluate two tissue culture systems for their suitability in differentiating between loblolly pine sources in their growth response to low tissue water potential. To accomplish this objective, seed sources from Louisiana and Texas were compared. Based on previous, percent-survival data in response to drought, the Louisiana sources have been designated as drought-susceptible and the Texas sources as drought-hardy (Newton and van Buijtenen 1984). Drought-hardy and drought-susceptible families have been selected for avoidance and maintenance of high tissue water potential rather than tolerance of low tissue water potential. We wanted to evaluate them for drought tolerance at low water potential by exploiting the unique system of cell culture.

The second objective was to determine the tolerance limit of callus growth in loblolly pine. That is, we wanted to determine minimum water potentials whereby growth could be sustained when callus was subjected to drought stress. This information would be important for comparison with other callus systems, particularly crop plants and other woody species. Furthermore, drought responses of cell cultures have not been previously reported for loblolly pine or for any other woody plant species. The data presented here show that in vitro cultures can be used for determining differences between loblolly pine source tolerance to low water potential and that cell growth is not sustained at water potentials lower than -1.0 MPa.

MATERIALS AND METHODS

Six half-sib loblolly pine families from known sources were used. Three of the families (BA3R13-41, BA3L11-1, GR1-8) were taken from the western edge of the loblolly pine range in Texas (TX) and three families

(A-1-4, A-1-7, A-1-14) were obtained from Crown Zellerbach Corporation and originated from Louisiana (LA). Explant and media procedures were modified from Mott and Amerson (1982). Seeds were placed in sterile flasks containing 1% H_2O_2 at room temperature (27°C) to stimulate germination (Ching and Parker 1958). After 4-5 days, seeds were further surface sterilized in 15% clorox solution for five minutes followed by three rinses of sterile water. Embryos were then aseptically removed and the cotyledons were excised as explants. In one in vitro system, the cotyledons were inoculated onto the experimental liquid media and allowed to produce callus (cot->cal) and in the second system, excised cotyledons were first placed on a complete agar media, allowed to proliferate callus for four weeks and then callus was inoculated onto the experimental media (cal->cal). Both the agar and the experimental liquid media were amended with napthalene acetic acid (NAA) and benzylamino purine (BAP) (see RESULTS). The cotyledons or callus were inoculated on Heller supports in test tubes containing Gresshoff-Doy (GD) (1972) liquid media with polyethylene glycol (PEG, Mol. Wt. 8000, Sigma) added to provide varying water potential (ψw). The media water potential was determined with a microvoltmeter (Wescor HR 33T) and thermocouple psychrometer chamber (Wescor C52). The standard media without PEG had a water potential of -0.4 MPa. Six sets of PEG media were prepared with a water potential of -0.6, -1.0, -1.3, -1.8, -2.3 and -2.7 MPa. In the cot->cal system the initial cotyledon explant weights were between 1.1 and 1.5 mg, and there were no significant differences between families in regard to their initial weights. In the cal->cal system, 80 to 90 mg of callus tissue were placed into each tube. The inoculated tissues were subjected to drought stress for eight weeks in a controlled environment chamber with a temperature of 21°C and continuous light (fluorescent and incandescent) with an intensity of $\mu Em^{-2}sec^{-1}$. Each treatment was replicated ten times. Test tubes were placed in a randomized block design and the position of each rack of tubes was randomly arranged every 24 hours to ensure equal treatment. After eight weeks the resulting callus was frozen in liquid nitrogen, lyophilized, and the dry weights recorded.

All data were analyzed with Analysis of Variance and Duncan's Multiple Range Test.

RESULTS

It was important first to determine which combination of napthalene acetic acid (NAA) and benzyl-amino-purine (BAP) would provide the most callus growth. One mg/lNAA and 3mg/lBAP resulted in maximum growth of callus after 8 weeks on a GD-agar medium. This modified medium was used thereafter as the standard medium for all subsequent experiments.

In vitro fresh weight growth of callus from cotyledons (cot->cal) and from pre-existing callus (cal->cal) over an eight week period in normal media with no PEG added are shown in Table 1. The water potential was -0.4 MPa. In both systems, family A-1-14 increased in fresh weight more than the other five families and family GR-1-8 had the least growth. Family rankings based on mean fresh weight were also similar in both systems with

the exception of BA3R13-41 and A-1- 4 which were reversed.

TABLE 1. In vitro fresh weight growth of 6 loblolly pine families at a water potential of -0.4 MPa.

		Mean Fresh Weight (mg)	
Family	Source	Cot->Cal[1]	Cal->Cal[1]
A-1-1	LA	58.0 a[2]	263.8 a[2]
A-1-7	LA	54.9 a	237.8 ab
A-1-4	LA	43.3 ab	173.9 ab
BA3R13-41	TX	42.8 ab	184.8 ab
BA3L11-1	TX	29.5 ab	128.4 ab
GR-1-8	TX	18.1 b	108.9 b

[1] 10 replicates per family
[2] means sharing a common letter are not significantly different at an alpha level of 0.05

Cultures from each family were next tested for their capacity to grow when PEG was added to the media. Cot->cal and cal->cal systems were both used with water potentials ranging from -0.6 to -2.7 MPa and -0.6 to -2.3 MPa for the two systems, respectively. The overall mean cot->cal fresh weight at 6 different water potentials was the largest for family A-1-14 and was smallest for family GR1-8 (Table 2). The overall mean cal->cal fresh weight at 5 different water potentials was also larger for A-1-14 and smaller for GR1-8 (Table 2). Growth of GR1-8 was 60 and 40% less than growth of A-1-14 in the cot->cal and cal->cal systems, respectively. Fresh weight growth under stressed and nonstressed conditions was significantly different between these two families (Table 1,2). Analysis of Variance showed significant differences at the 6% level between sources in response to drought stress (Table 2). Louisiana sources sustained more growth under drought stress than did Texas sources.

TABLE 2. *In vitro* fresh weight growth of 6 loblolly pine families averaged over all water potentials.

		Mean Fresh Weight (mg)	
Family	Source	Cot->Cal[1,4]	Cal->Cal[2,4]
A-1-14	LA	11.7 a[3]	93.7 a[3]
A-1-7	LA	10.9 a	84.3 ab
A-1-4	LA	9.4 ab	66.9 bc
BA3R13-41	TX	8.5 ab	67.3 bc
BA3L11-1	TX	8.2 ab	56.9 c
GR-1-8	TX	4.5 b	55.0 c

[1] 6 different water potentials with 10 replicates per family
[2] 5 different water potentials with 10 replicates per family
[3] means sharing a common letter are not significantly different at an alpha level of 0.05
[4] sources are significantly different at an alpha level of 0.06

It was most meaningful to determine the minimum tolerance levels for *in vitro* growth by loblolly pine since this has not been reported earlier. This was accomplished by pooling the family fresh weights for each water potential and comparing the overall means (Table 3). Mean fresh weight growth of the cot->cal system was 63% less than the control when subjected to drought stress at -0.6 MPa and 96% less than the control at -1.0 MPa. (Table 3). There was no growth at water potentials lower than -1.0 MPa. Fresh weight of the cal->cal system was decreased by 64% at -0.6 MPa and 74% at -1.0 MPa compared to the control. However, final fresh weight of the cal->cal tissue was smaller than the original inoculum fresh weight at all stress treatments; only the callus at -0.4 MPa was larger than the initial callus fresh weight (Table 3). At water potentials less than -1.0 MPa the fresh weight of both systems remained relatively constant. The minimum tolerance level for cot->cal growth was -1.0 MPa and was -0.6 MPa for cal->cal growth (Table 3).

TABLE 3. *In vitro* fresh weight growth of loblolly pine as influenced by decreasing water potential.

	Mean Fresh Weight (mg)	
ψw(MPa)	Cot->Cal[1]	Cal->Cal[1]
-0.4	41.6 a[2]	182.9 a[2]
-0.6	15.1 b	66.4 b
-1.0	1.6 c	47.9 bc
-1.3	1.3 c	45.9 bc
-1.8	1.0 c	43.1 c
-2.3	1.0 c	37.6 c
-2.7	0.8 c	

[1] n = 60 with 10 replicates per family
[2] means sharing a common letter are not significantly different at an alpha level of 0.05.

Dry weight decreased in the cot->cal system when subjected to drought stress, but cal->cal dry weight remained constant at all stress levels (Table 4). Dry weight of the cot->cal system was decreased by nearly 90% at -1.0 MPa compared to cultures at -0.4 MPa. Therefore, the decrease in fresh weight of cot->cal (Table 1) was due to both water loss and dry weight decrease (Table 3) whereas, the fresh weight decrease of cal->cal was primarily water loss (Table 1) with very little change in dry weight (Table 3).

DISCUSSION

Two *in vitro* culture systems were used to compare growth between loblolly pine families and sources. Both systems showed significant differences between fast-growing and slow-growing sources during drought stress. The family ranking of mean fresh weight growth under nonstressed conditions (Table 1) was the same as their ranking under stressed conditions (Table 2). Family A-1-14 from Louisiana consistently grew better under nonstressed and stressed conditions compared to the other 5 families and family G1-8 from Texas was a consistent, poor performer. These

TABLE 4. *In vitro* dry weight growth of loblolly pine as influenced by water potential.

	Dry Weight (mg)	
ψw(MPa)	Cot->Cal[1]	Cal->Cal[1]
-0.4	7.2 a[2]	20.2 a[2]
-0.6	4.4 b	22.3 a
-1.0	0.8 c	20.5 a
-1.3		21.8 a
-1.8		21.3 a
-2.3		20.7 a

[1] n = 60 with 10 replicates per family
[2] means sharing a common letter are not significantly different at an alpha level of 0.05

data indicated that fast growing families under nonstressed conditions also perform well under stressed conditions and that Louisiana sources grew significantly better than Texas sources. Although preliminary, these data also show that source tolerance responses to drought may be different from source avoidance responses.

Some interesting comparisons with field observations can be made here. Louisiana sources are known for their rapid growth under field conditions (Yeiser et al. 1981). Under drought stress, however they suffer excessive mortality (Zobel and Goddard 1955), although surviving trees may grow quite well for a period of time. Mortality of Louisiana source outplantings in drought prone areas decreases per acre yields compared to seed sources selected for drought resistance (van Buijtenen, unpublished). It will be most interesting to pursue the possibility that *in vitro* growth is indicative of growth rate observed under field conditions.

Callus proliferation from cotyledons appeared to be very limited if the water potential was less than -1.0 MPa. This is similar to the level of drought tolerance of other *in vitro* systems such as grain sorghum (Newton et al., submitted). At water potentials less than -1.0 MPa, the fresh weight of the cot->cal cultures (0.8 to 1.6 mg) were not significantly different from the weight (1 mg) of the initial, inoculated cotyledons

(Table 3). Control medium in these experiments was at a water potential of -0.4 MPa; higher water potentials were not tried. It would be most helpful to determine if growth is increased at these higher water potentials.

Even though callus fresh weight produced from callus was severely reduced by the slight stress of -0.6 MPa, the dry weight of the cal->cal system was not affected (Table 4), indicating that the tissue was becoming more dehydrated as it experienced more drought stress. For example, callus tissue at a water potential of -0.4 MPa contained 160 mg of water with a dry weight of 20.2 mg whereas tissue at -1.0 MPa had 27 mg of water with a dry wt of 20.5 mg (Table 3, 4).

In conclusion, Louisiana sources sustained more growth under in vitro drought stress than did Texas sources. The minimum tissue water potential below which callus growth ceased was -1.0 MPa. In vitro drought stress techniques may have applicability for predicting growth potential in the field under stressed and nonstressed conditions. Furthermore, the data indicate that this technique may be suitable for investigating drought tolerance mechanisms which appear to be distinct and separate from drought avoidance mechanisms. Additional research is in progress to confirm these preliminary results.

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CONIFER GENETICS I

LONGLEAF PINE

MODERATED BY MS. PAT LAYTON

University of Florida

TECHNIQUES FOR SUCCESSFUL ARTIFICIAL REGENERATION OF LONGLEAF PINE

MARC G. ROUNSAVILLE[1/]

Abstract.--Refinement of longleaf pine (Pinus palustris) artificial regeneration techniques over the past ten years on the Black Creek Ranger District has resulted in a five point program for success.

Longleaf pine has been reduced to less than 10% of its original range. This reduction is partly due to the problems and failures associated with artificial regeneration. The U.S.Forest Service aggressively attacked these problems in the early to mid-1970's. Five areas were identified as keys to improved survival.

METHODS

The five keys to successful artificial regeneration of longleaf are: 1. Well prepared sites; 2. Large, healthy, fresh seedlings; 3. Proper care and handling of planting stock; 4. Proper planting procedure; 5. Post planting care and management.

1. Well prepared sites. The site preparation method used on the Black Creek was usually shear, rake and disk. Other methods used are drum chopping, burning and sometimes disking. The method of choice is the one that will take control of the site and will allow access for the planting machines at the least cost; soils, topography, and the amount and kind of vegetation to be controlled also influence the choice of site prep method. Herbicides will replace disking for control of root competition in FY 1986.

2. Plant only large, healthy, fresh seedlings. Adequate survival cannot be expected with seedlings less than 0.4" root collar diameter. The length of storage also impacts survival. The seedlings grown at Ashe Nursery are graded to 0.4" RCD. White in his 1978 study on length of storage, RCD and their affect on survival showed that acceptable survival could not be expected with trees less than 0.4" RCD and those seedlings with RCD's in the 0.4" class would not survive if stored for three weeks or more. Brownspot needle blight control is enhanced by the use of Benlate at the nursery.

Seedling storage on the Black Creek is limited to less than one week by making seedling orders small and frequent. The nursery coordinates lifting with the districts and destroys seedlings after they have been in storage for ten days. Planting large seedlings will shorten the time seedlings remain in the grass stage since height will not start until the RCD is 0.7" to 1.0".

[1/]Silviculture Assistant, Black Creek Ranger District, National Forests in Mississippi, USDA Forest Service, Wiggins, Mississippi.

3. Proper care and handling of planting stock. Contracts on the Black Creek require the contractor to use an insulated storage box to transport the seedlings and store them in on the planting site. The box not only reduces exposure but also protects the seedlings from contamination by fuel, oil or other substance that may be in the bed of a truck. Seedlings are picked up daily from cold storage and any trees left at the end of the day are returned to cold storage.

Machine planting reduces the chances of exposure when compared to hand planting. Close administration of the contract ensures that the contractor meets the care and handling requirements of the contract.

4. Proper planting procedure. The planting contracts require that the seedlings be planted within 0.5" of the depth that they were grown in the nursery. Seedlings that are planted deeper than they were grown may survive for one to three years but will be much slower in intiating height growth than those planted at the correct depth. The seedlings planted too shallow will expose roots making them more susceptible to drought and fire.

The planting machine generally pushes up a small berm around the seedling as it is packed which will wash away from the seedling. The depth of planting should be based on the location of the root collar after the berm has settled away from the seedling.

Almost any planting machine in good working order will do a satisfactory job of planting longleaf. The machines used on the Black Creek are Reynolds double coulter machines.

The planting rated should be based on past survival and the number of trees per acre that achieve height growth in three to four years. One thousand trees per acre were planted until the 1984-1985 planting season. This has been reduced to 850 trees per acre for the 1985-1986 planting season.

5. Post planting care and management. Annual checks are made using 0.01 acre plots to determine survival, brownspot infestation, number of height growth seedlings and release needs. These plots are installed in grid fashion to yield a 1% inventory. The prescription for the stand is based on the information collected during this inventory. Brownspot control burns are made as needed in winter under conditions that will yield a "cool" burn. Normally this would mean burning with a head fire one to two days following frontal passage.

Cattle should be excluded from plantations until 300 trees per acre have achieved height growth. Fencing is the method normally used.

RESULTS

Prior to initiating these measures survival ranged from 57 to 67 percent on 3666 acres planted from 1973 to 1977 with the average for these years being 62 percent. This was not considered acceptable since the failure rate for these plantations ranged from 40 to 100 percent for this same period. These five "keys" were gradually phased in starting in 1973 as they were developed and refined (which continues today). The improvement in survival and plantation success rate is illustrated by the

results of the fiscal year 1984 planting season and the absence of plantation failures since fiscal year 1980. The survival for 793 acres planted in fiscal year 1984 was 97 percent.

Longleaf pine plantations can be established by paying close attention to these five keys and using good basic tree planting techniques.

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LONGLEAF PINE TREE IMPROVEMENT IN THE WESTERN GULF REGION

T. D. Byram and W. J. Lowe [1]

Abstract.--Longleaf pine collected from North Louisiana, Southeast Texas, South Louisiana and South Mississippi were outplanted in seven locations in the Western Gulf area. Family heritabilities across locations were 0.56 for second year survival, 0.54 for grass stage emergence and 0.72 for brown-spot needle blight resistance. Coefficients of genetic prediction indicated a positive relationship between survival and grass stage emergence but not between these traits and brown-spot resistance. Tentative conclusions based on one year's plantings indicate that North Louisiana, Southeast Texas, South Louisiana and South Mississippi can be considered one breeding zone for improving survival. Southeast Texas, South Louisiana and South Mississippi can be considered one breeding zone for improving emergence from the grass stage and brown-spot resistance.

Additional keywords: Pinus palustris, Scirrhia acicola, genotype by environment interaction, survival, grass stage emergence.

In recent years, increased interest has been shown in using longleaf pine (Pinus palustris Mill.) in artificial regeneration programs in the Western Gulf Region. This stems from 1) the increasing value of poles, pilings and other solid wood products, 2) the increasing losses to fusiform rust (Cronartium quercuum [Berk.] Miybe ex Shirai f. sp. fusiforme) on slash pine (Pinus elliottii Engelm. var elliottii), 3) an increasing emphasis on planting the proper species on appropriate sites, and 4) longleaf's suitability for planting in high fire hazard areas.

Many of the problems traditionally associated with the establishment and early growth of longleaf pine are manageable by improved nursery techniques, seedling care and silvicultural practices (Shipman 1960, Smith and Schmidtling 1970). Traits related to establishment are also under genetic control. Family heritabilities for early survival were estimated as 0.73 at one location (Rockwood and Kok 1977) and 0.35 across a wide range of environments (Goddard and Bryant 1981). Goddard and Bryant calculated that selecting the top one-half of the families in their study resulted in a 6.5 percent gain in survival. Family heritabilities for height initiation at two years of age across several environments ranged from 0.47 to 0.68 (Layton and Goddard 1982).

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[1]/Assistant Geneticist, Western Gulf Forest Tree Improvement Program, Texas Forest Service, College Station, Texas; and Associate Geneticist, Texas Forest Service, and Assistant Professor, Texas Agriculture Experiment Station, College Station, Texas. The authors wish to acknowledge the members of the Western Gulf Forest Tree Improvement Program working with longleaf pine for their efforts to establish, maintain and measure the test plantings reported in this study.

Snyder and others (1977) reported heritabilities for height initiation of 0.48 and 0.52 at three years of age. They also reported family heritabilities for brown-spot needle blight (Scirrhia acicola [Dearn.] Sigg.) resistance of 0.30 and 0.57.

Goddard and others (1973), and Snyder (1969) noted that selection of phenotypically superior plus trees was not particularly effective in improving juvenile traits and recommended a two step testing program. The Western Gulf Forest Tree Improvement Longleaf Pine Program is similar to the two step selection procedure developed by the Florida Cooperative Forest Genetics Program (Goddard and others 1973, Goddard and Rockwood 1978).

Western Gulf Longleaf Pine Program

Approximately 100 low intensity selections will be made from each of four provenances - Southeast Texas, South Louisiana, North Louisiana, and South Mississippi. The 400 selections will be included in short duration tests (three years) to evaluate juvenile traits. Families that exhibit acceptable performance will be established in long term, good growth and form progeny tests. Both stages of the testing program are designed to determine the effects of different provenances, the relative amount of family variation, and the presence of genotype by environment interactions.

Provisions have been made to meet seed needs with the establishment of seedling seed orchards concurrently with the establishment of the short term tests, in conjunction with the long term tests or by establishing clonal orchards based on the long term test results.

This paper presents the second year data from the first plantings of the short-term tests. The objectives are to estimate heritabilities and examine genotype by environment interactions for survival, grass stage emergence and brown-spot resistance.

Materials and Methods

In the spring of 1982, 100 families plus four bulk checklots were sown in three nurseries for outplanting at seven locations (Figure 1). Seedlings were sown at an initial density of nine per square foot and grown according to standard nursery procedures for longleaf pine. The two North Louisiana plantings were grown at one nursery, the two South Mississippi plantings were grown at a second nursery and the two Southeast Texas plantings and the South Louisiana planting were grown at a third. Sixty-four of the 100 families were common to all seven locations and ninety-three families were in at least five of the seven plantings. Field design consisted of eight replications at each location with four or five trees per row plot depending on the planting. Spacing was two by 10 feet at four locations, three by nine feet at two locations, and two by eight feet at a single location. Sites ranged from dry sand ridges to poorly drained flatwoods.

At the end of the second growing season survival, percent of living trees initiating height growth, and percent of foliage infected by brown-spot needle blight were scored. Brown-spot infection was scored on a 0 to five scale with 0 representing a brown-spot free individual. Higher scores represented the amount of foliage infected in 20 percent increments.

Figure 1. County/parish locations of the short-term longleaf pine plantings.

All percent data was transformed by the arc sine of the square root and each location was analyzed separately. The 64 families common to all seven locations were combined in one analysis across locations to examine the relative amount of genotype by environment interaction. Variance components were calculated for family within provenance and provenance effects to determine the relative importance of geographic variability in selection. Family heritabilities for survival, percent height initiation, and brown-spot severity were calculated at each of the locations with significant family effects as well as for the combined analysis according to the following formulas:

One location

$$h^2 = \frac{\sigma^2_F}{\sigma^2_F + \sigma^2_{E/r}}$$

Multiple locations

$$h^2 = \frac{\sigma^2_F}{\sigma^2_F + \sigma^2_{F(P)*L/l} + \sigma^2_{E/lr}}$$

Where

σ^2_F = variance among family means within provenances

$\sigma^2_{F(P)*L}$ = variance among families within provenance by planting location means
σ^2_E = error variance
l = number of locations
r = number of replications

The coefficients of genetic prediction (CGP) were used to examine the relationship between traits (Baradat 1976). They were also calculated for the same trait across locations to examine the amount and direction of genotype by environment interaction.

RESULTS AND DISCUSSIONS

Single Locations

Second year planting survival ranged from 26 to 95 percent (Table 1). There were differences between families within provenances in six of the seven locations. Family heritability for survival varied from 0.21 to 0.55. Low heritability estimates at the Tyler and Hardin County, Texas, tests are primarily caused by lack of variation due to uniformly good survival.

Table 1. Locations, averages, family heritability estimates, and standard errors for survival, grass stage emergence and brown-spot severity at seven plantings of two-year-old longleaf pine in the Western Gulf region.

Location County/Parish State	Trait								
	Survival			Grass Stage Emergence			Brown-spot Severity Code		
	%	$h^2 \pm$	SE	%	$h^2 \pm$	SE	$\bar{x}$	h^2	SE
Stone, MS	78	.47±	.16	58	.37±	.16	1.34	.70±	.15
Pearl River, MS	32	.55±	.17	45	.23±	.18	0.67	.49±	.18
Tyler, TX	95	.21±	.16	44	.40±	.16	2.88	.69±	.15
Hardin, TX	95	.29±	.16	93	---		.09	.23±	.16
Vernon, LA	72	---		27	.22±	.16	.65	.32±	.16
Bienville, LA	26	.34±	.16	64	---		.23	---	
LaSalle, LA	75	.28±	.16	82	---		.40	---	
Combined location		.56±	.18		.54±	.18		.72±	.18

The percent of living trees emerging from the grass stage ranged from 27 to 93 percent. The Hardin County, Texas, test showed the benefit of intensive competition control with both very high survival and a high percentage of living trees initiating height growth. There were differences between families within provenances for grass stage emergence in four of the seven locations. Family heritabilities ranged from 0.22 to 0.40.

Percent of trees infected with brown-spot needle blight varied from only 5 percent at the Hardin County, Texas, test to 96 percent at the Tyler County, Texas, test. In this planting, the average tree had almost 50 percent of its foliage infected. Brown-spot severity score was used for analysis because it was more heritable than the percent of trees infected. The average planting severity code ranged from a low of 0.09 to a high of 2.88. There were differences between families within provenances in five of the seven tests. Family heritabilities varied from 0.23 to 0.70. The low heritability estimate for the Hardin County test was primarily caused by the lack of infection. Brown-spot resistance was the most heritable of the three traits scored.

Combined Locations

When the 64 families common to all plantings were analyzed, there were differences between families within provenances for all three traits. There were also differences attributable to provenances for survival and grass stage emergence. The North Louisiana and Southeast Texas sources had slightly higher survival and grass stage emergence (Table 2). There was no provenance by planting location interaction for either trait. While there was no provenance effect for brown-spot infection, there was evidence of a provenance by planting location interaction (Figure 2). This interaction was statistically significant but not operationally meaningful. Although there were some changes in ranks at the Louisiana plantings, where the differences among provenances were small, the interaction resulted primarily from changes in magnitude. North Louisiana sources tended to be more susceptible while South Mississippi sources were affected least.

Table 2. Provenance means for survival and grass stage emergence for two-year-old longleaf pine planted in the Western Gulf region.

Provenance	Survival (Percent)	Growth Initiation (Percent)
North Louisiana	70.8	62.0
Southeast Texas	70.4	62.7
South Louisiana	68.1	59.6
South Mississippi	65.0	55.6

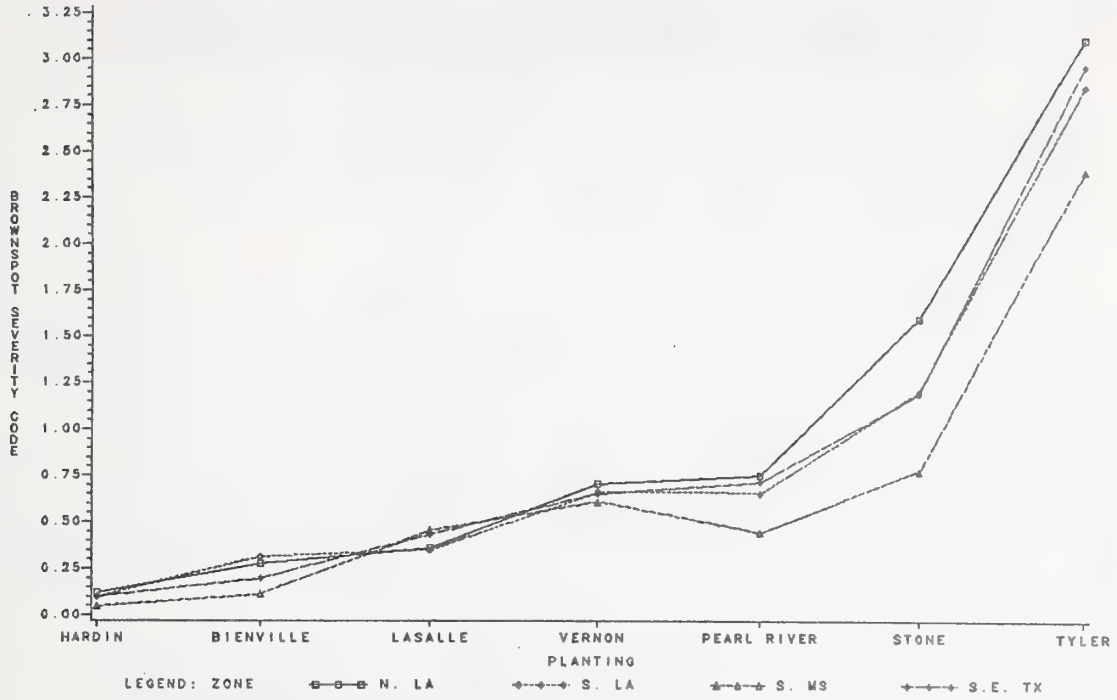

Figure 2. Provenance (zone) by planting averages for brown-spot severity in two-year-old longleaf pine.

There was a family within provenance by planting location interaction for all three traits. This interaction accounted for approximately 10 percent of the total phenotypic variation and was considered unimportant because of the large amount of family variation. Family heritability across all locations was 0.56 for survival, 0.54 for grass stage emergence and 0.72 for brown-spot resistance (Table 3). These moderate to strong heritabilities and the distribution of variation between the provenance effect and family within provenance effect (Table 4) indicate that emphasis should be placed on selecting the best individuals regardless of seed collection zone.

Table 3. Heritabilities and coefficients of genetic predition from the combined locations analysis for seven plantings of two-year-old longleaf pine in the Western Gulf region.

Trait	Survival	Grass Stage Emergence	Brown-Spot Severity Score
Survival	0.56	0.18	0.07
Grass Stage Emergence		0.54	0.05
Brown-Spot Severity Score			0.72

Table 4. Distribution of variation in percent between provenances and families within provenances for two-year-old longleaf pine planted in the Western Gulf region.

Type of Variation	Survival	Grass Stage Emergence	Brown-spot Severity Code
Provenance	28	35	10
Family (Provenance)	72	65	90

The coefficient of genetic prediction indicates that survival and the percent of trees initiating height growth are positively related (Table 3). Selection resulting in a one standard deviation increase in the phenotypic value for percent survival would be accompanied by an 0.18 standard deviation gain in breeding value for grass stage emergence. Brown-spot resistance at this age does not appear to be related to either survival or percent of trees initiating growth. This pathogen causes fatality by repeated defoliation and at least one of the mechanisms for escaping brown-spot needle blight is early height initiation. It may be that the relationship between these traits has not had sufficient time to develop in this study.

By considering the same variable across locations as different traits the coefficients of genetic prediction can be calculated across environments. This is a good device for examining genotype by environment interactions as suggested by Burdon (1977) for genetic correlations and demonstrated by Yeiser and others (1981). It is also useful in formulating seed movement recommendations and delineating breeding zones. The danger, when examining longleaf pine in this manner, is that most of the juvenile traits are strongly affected by nursery treatment. In this study, plantings in each zone were grown at the same nursery and can be expected to have similarities related to common nursery culture. Planting locations for which family variation was statistically insignificant, indicating no detectable additive genetic variance, were dropped from the CGP matrix.

CGP's for survival across locations are shown in Table 5. Because of the high overall survival at the Hardin and Tyler County, Texas, plantings, CGP's with these tests are very low. If these tests are ignored, it becomes apparent that survival at all of the other locations is positively correlated. For example, families selected for a one phenotypic standard deviation increase in survival at Stone County would have an increased breeding value of 0.46 standard deviations if planted at Pearl River County. In this example, it is impossible to separate the planting location effects from those contributed by a common nursery. Comparisons to the heritabilities along the diagonal indicate the relative efficiency of indirect selection.

Table 5. Coefficients of genetic prediction for survival in two-year-old longleaf pine across different test locations in the Western Gulf area.

Test	Stone MS	Pearl River MS	Tyler TX	Hardin TX	Bienville LA	LaSalle LA
Stone, MS	0.47	0.46	0.01	0.22	0.24	0.26
Pearl River, MS		0.55	-0.12	0.16	0.21	0.34
Tyler, TX			0.21	0.00	0.00	-0.05
Hardin, TX				0.29	-0.03	0.11
Bienville, LA					0.34	0.25
LaSalle, LA						0.28

Table 6 contains the CGP's for grass stage emergence. There appears to be a positive relationship between all test locations. This implies no special breeding zones are needed for east-west seed movement in the Western Gulf region. Seed movement recommendations for North Louisiana could not be made because the CGP's for these tests could not be calculated.

Table 6. Coefficients of genetic prediction for grass stage emergence in two-year-old longleaf pine across different test locations in the Western Gulf region.

Test	Stone MS	Pearl River MS	Tyler TX	Vernon LA
Stone, MS	0.37	0.51	0.48	0.34
Pearl River, MS		0.23	0.25	0.14
Tyler, TX			0.40	0.37
Vernon, LA				0.22

The coefficients for genetic prediction for brown-spot severity are listed in Table 7. CGP's for brown-spot are very similar to those for percent height initiation. There is a strong positive relationship between all test locations with possible exceptions of the North Louisiana tests for which CGP's could not be calculated. Again, positive gains in brown-spot resistance at any of the five southern tests would result in positive gains at any of the other southern tests.

Table 7. Coefficients of genetic prediction for brown-spot severity in two-year-old longelaf pine across different test locations in the Western Gulf region.

Test	Stone MS	Pearl River MS	Tyler TX	Hardin TX	Vernon LA
Stone, MS	0.70	0.41	0.68	0.35	0.45
Pearl River, MS		0.49	0.36	0.20	0.26
Tyler, TX			0.69	0.23	0.52
Hardin, TX				0.23	0.59
Vernon, LA					0.32

CONCLUSIONS

1. Good gains can be made in longleaf pine through selection for survival, grass stage emergence, and brown-spot resistance.

2. Family selection will result in twice as much gain as provenance selection.

3. Survival and grass stage emergence are positively related while brown-spot resistance is independent of either trait at this early age.

4. The same selection criteria will improve survival for all areas within the Western Gulf region.

5. Southeast Texas, South Louisiana and South Mississippi can also be considered one zone when selecting families for grass stage emergence and brown-spot resistance.

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POLYMORPHIC ISOENZYMES FROM MEGAGAMETHOPHYTES AND POLLEN OF LONGLEAF PINE: CHARACTERIZATION, INHERITANCE, AND USE IN ANALYSES OF GENETIC VARIATION AND GENOTYPE VERIFICATION

Stuart E. Dubal/

Abstract.--Segregation of isoenzyme variants of 13 enzyme systems, assayed in longleaf pine (Pinus palustris Mill.) indicated control by 19 separate loci in megagametophytes and embryos of control-crossed and open-pollinated seeds. Along with megagametophyte evaluations, pollen contributions to embryos proved to be suitable for genetic evaluation and determination of genotypes. A unique multi-locus genotype was determined for each parent involved in control-crosses. Exact parentage of hybrids was determined from female and male contributions to hybrid multi-locus genotypes. Unique genotypes were determined for 62 of 68 parents evaluated. Considerable genetic variation in isoenzymal characteristics was found among 24 natural populations from the Central Gulf Coast. Allele frequencies per locus differed significantly among populations although in most cases one allele was more frequent in all populations. Numbers of polymorphic loci per population ranged from 31.6 to 57.9 percent and were correlated with latitudes at which the populations occurred ($r = 0.63$, $P<0.002$).

Additional keywords: Pinus palustris Mill., electrophoresis, number of alleles, polymorphic loci, genetic distance.

Isoenzyme analyses, by means of electrophoretic separation, are no longer a novelty in forest genetics investigations, yet the number of species and populations that have been studied is limited. Loblolly (Pinus taeda L.) is the only southern pine that has been dealt with in much detail (Adams and Jolly 1980, Conkle and Adams 1977, and Florence and Rink 1979). Conkle and Adams (1977) included some longleaf pine (Pinus palustris Mill.) seeds in a survey of southern pine banding patterns and concluded that similar genes were probably present in all of them. However, inheritance of isoenzymes must be established before they can be used in genetic analyses. A very limited amount of isoenzymal inheritance data has been gathered for longleaf pine (Snyder and Hamaker 1978). To be of much utility in arriving at unique genotypes for a large number of parent trees, a large number of polymorphic loci must be available and their inheritance must be understood. Part I of this study was designed to elucidate the inheritance of 19 loci representing 13 enzyme systems in longleaf pine when evaluations were made of both the female gametophyte and the contribution due to pollen. Although isoenzymes may be expressed in both embryo and megagametophyte tissues, the pollen

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1/Research Associate, School of Forestry, Alabama Agricultural Experiment Station, Auburn University, AL 36849. Support of this research by the Georgia Forestry Commission is sincerely appreciated.

contribution to the embryo genotype is not always apparent because of confounding of the banding patterns. The reliability of determining genotypes from pollen contributions of control-crosses was determined. Both embryo and megagametophyte tissue were used to establish unique genotypes for parents and verify the parentage of crosses.

Genetic variation has been demonstrated among seed sources of longleaf pine for metric traits such as height growth (Wells and Wakeley 1970) and disease resistance (Synder and Derr 1972). An appreciable amount of genetic variation has been found for height growth which has allowed for the differentiation of geographic zones based on growth potential. The question arises whether similar variation is present for isoenzymal characteristics and if so, what is its extent and distribution. Also, if it is present can it be used to differentiate populations. Isoenzymal analyses are currently used to estimate heterozygosities, genic diversity, and the extent of population differentiation.

Isoenzymal variation has been demonstrated for single trees of loblolly pine (Adams and Jolly 1980) and for longleaf pine (Duba, 1983). Variation has also been demonstrated for particular isoenzyme loci of loblolly pine (Florence and Rink 1979), pitch pine (Pinus rigida Mill.) (Guries and Ledig 1982), Norway spruce (Picea abies K.) (Lundkvist 1979), and Douglas-fir (Pseudotsuga menziesii (Mirb.) Franco) (Yeh and O'Malley 1980) populations, although when averaged over several loci the variation has not always shown extensive population differentiation. Some clinical trends have been indicated (Guries and Ledig 1982, Yeh and O'Malley 1980), but more isoenzymal genetic variation has been found to exist within populations than between them. Native conifers differ in the kind and amount of isoenzymal genetic variation they contain (Conkle 1980) and, in general, possess high levels as would be expected when considering their life history characteristics (Hamrick et al. 1979).

Longleaf pine is a long-lived, wind pollinated species with a large natural range that does not span dramatic climatic differences. Still, patterns of variation in growth characteristics have been associated with climatic factors, particularly temperature and rainfall. In connection with a study of geographic variation in growth potential of longleaf pine, various sources were utilized in Part II of this study to evaluate variation in isoenzymal characteristics. The level of genetic diversity within the species was determined and patterns of variation among populations were evaluated by analyzing protein polymorphisms that were revealed by electrophoretic separations.

MATERIALS AND METHODS

Part I

Control-cross seeds were utilized for the majority of these analyses, but observations from open-pollinated seeds were also included. The control-cross seeds represented 10 crossing combinations from 5 parents that were included in the U.S. Forest Service's longleaf breeding program at Gulfport, Mississippi. Open-pollinated seeds represented 63 longleaf parents from sources located in Alabama and adjoining states.

For a detailed description of the electrophoretic run conditions, consult Duba (1983). Mobility of electrophoretic bands was used to identify differing zones of activity. Segregation of band patterns in each zone was evaluated in conjunction with mobility of the zones of activity to identify separate loci and alleles (variants) at each locus. Inheritance of the enzymes was postulated based on segregations observed in band patterns of both megagametophyte and embryo tissues. Whenever a locus was determined to be heterozygous, chi-square values were calculated to evaluate the goodness-of-fit to the expected 1:1 ratio of segregation. The segregation in pollen gametophytes was compared to that from megagametophytes to evaluate the suitability of using pollen contributions to detemine genotypes.

From analyses of megagametophytes, the multi-locus genotype of the 5 parents involved in the 10 crosses and of the 63 source parents was determined. Multi-locus genotypes and segregation ratios of progeny these crosses were evaluated. Verifications of the parents responsible for particular crosses were made.

Part II

The primary center of sampling was the central gulf coast region of the natural range of longleaf pine. The entire sample consisted of 22 populations distributed through Alabama, southeast Mississippi, southwest Georgia, and the panhandle of Florida, plus 2 distant sources, 1 in central Florida and 1 in North Carolina (Figure 1). Each source was evaluated as a separate population to determine the extent and distribution of isoenzymal genetic variation.

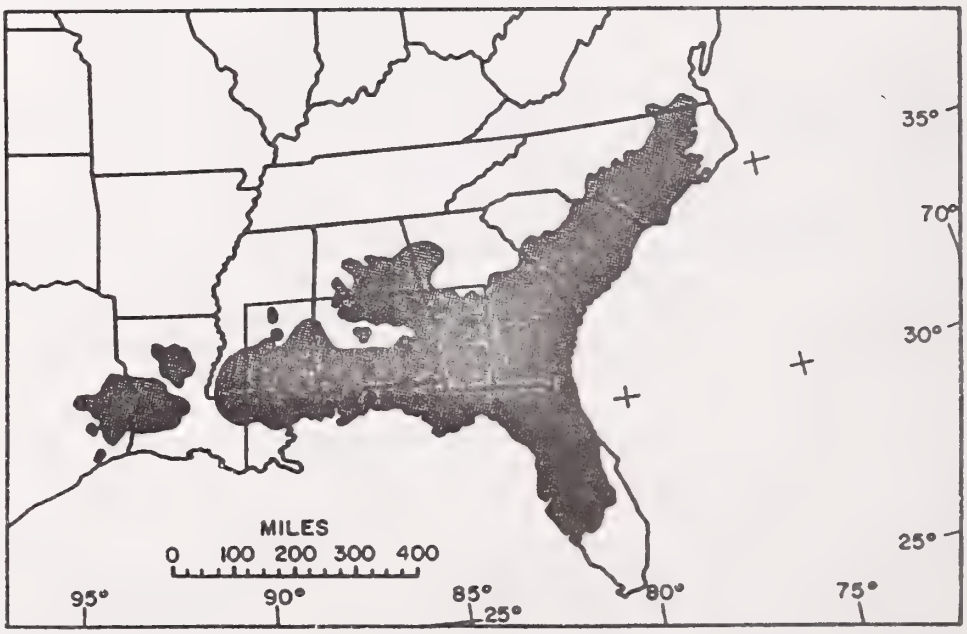

Figure 1.-- Species range of longleaf pine and relative locations of sampling points.

Direct count allele frequencies at each locus were obtained for each of the 24 populations. Within each population, genetic variation was quantified by determining the average number of alleles per locus, the proportion of polymorphic loci, and the Hardy-Weinberg expected proportion of heterozygous loci per individual. Heterogeneity chi-square values were calculated for allele frequencies among all populations to determine if frequencies were different from one population to another. Linear correlations were also computed between certain isoenzymal characteristics and latitude and longitude as well as growth potential.

RESULTS AND DISCUSSION

Part I

From analyses of electrophoretic banding patterns, 19 consistently staining zones of activity were observed in 13 enzyme systems. Evidence collected from segregation analyses of megagametophytes and pollen contributions to embryos of control-cross seeds (Table 1) demonstrated directly that 8 zones (ALAP, LAP-1, PGI-2, GOT-1, GOT-3, SKDH, MDH-2, AND PGD-1) were each controlled by a single locus. Evaluations from megagametophytes of open-pollinated seeds indicated control of 8 more zones (ADH, FLEST, LAP-2, PGI-1, PGM-1, PGM-2, GPD-3, and PGD-2) by a single locus each. Indirect evidence from embryos of open-pollinated seeds indicated control of the final 3 zones (GDH, GPD-1, and IDH) also by a single locus each.

Analysis of embryo bands in control-cross and open-pollinated seeds gave evidence that pollen contributions can be reliably ascertained (Table 1), although caution was required in evaluating one allelic combination at the MDH-2 locus. Pollen contributions can be utilized to verify male parents in hybrids and evaluate allele frequencies in population studies where the contributions to embryos can be consistently scored.

Table 1.--Allozyme segregation in megagametophytes and pollen produced by heterozygous parent trees

Enzyme locus	Estimated from[a]	Allelic combination[b] X	Y	Observed number X	Total	Deviation (1)	P
ALAP	G	1	2	18	39	0.23	>.50
	P	1	2	12	24	0.00	>.90
LAP-1	G	1	2	17	40	0.90	>.25
	P	1	2	11	24	0.17	>.50
PGI-2	G	1	2	20	44	0.36	>.50
	P	1	2	15	24	1.50	>.10
	G	1	3	56	115	0.08	>.75
	P	1	3	42	84	0.00	>.90
GOT-1	G	1	3	18	40	0.40	>.50
	P	1	3	14	24	0.67	>.25
GOT-3	G	1	2	22	40	0.40	>.50
	P	1	2	15	24	1.50	>.10
SKDH	G	1	2	51	89	1.90	>.10
	P	1	2	20	42	0.09	>.75
MDH-2	G	1	2	17	41	1.19	>.25
	P	1	2	7	18	0.89	>.25
	G	1	5	38	89	1.90	>.10
	P	1	5	15	31	0.03	>.75
	G	2	5	14	40	3.60	>.05
	P	2	5	43	65	6.78	<.01
PGD-1	G	1	4	16	41	1.97	>.10
	P	1	4	11	21	0.05	>.75
	G	1	5	80	120	13.33	<.01
	P	1	5	40	62	5.22	<.02

[a] Estimated from female gametophyte (G) or pollen (P) contributions.
[b] Allele 1 is the most frequent allele at each locus.

Segregation distortion was demonstarted in two combinations of PGD-1. Both combinations showed a deficiency for the same allele. Normal segregation was indicated for the other alleles at this locus. Therefore, caution must be exercised when using this locus for genetic evaluations. Part of this problem may have been due to band resolution between two alleles. The allele that was deficient migrated to a location between the two alleles that had excesses when in heterozygous combination. Although bands for this locus were very clear, the closeness in position of these bands may have been partly responsible for the excess numbers observed.

Every locus evaluated in longleaf pine had at least two electrophoretic variants (Figure 2) although the second variant (allele) at several loci was very rare. The reason for this observation may be that samples from a large portion of the longleaf pine natural range in Alabama, Florida, Georgia, and Mississippi were included in the open-pollinated seed collection, thus constituting a very broad sample.

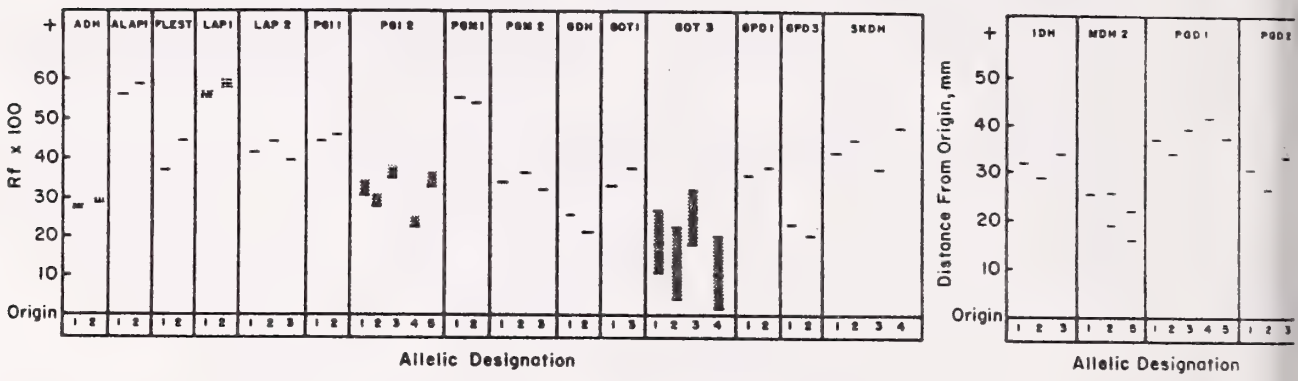

Figure 2.-- Band patterns and their allelic designations for 19 loci in longleaf pine.

An important utility of isoenzymal analyses to applied tree breeding has been the identification of parents (Adams and Jolly 1980). Offspring genotypes and frequencies have been predicted from genotypes of the parents involved in control-crosses. If each parent involved had a unique genotype, then the parents of any particular cross should be verifiable. The number of loci evaluated in this study was sufficient to allow for the determination of unique genotypes for each parent involved in control-crosses (Table 2). These multi-locus genotypes were suitable for evaluations of male and female contributions to hybrid embryos and the exact establishment of parentage (Table 3). The discovery that 62 of a total of 68 parents had unique genotypes was reassuring. If a large enough number of loci were included in evaluations, determinations of unique genotypes for applied breeding utilization is feasible.

Table 2.-- Unique genotypes of the five parents used in control crosses

Parent	Genotype (by locus) ADH	ALAP	FLEST	LAP1	LAP2	PGI1	PGI2	PGM1	PGM2	GDH	GOT1	GOT3	GPD1	GPD3	SKDH	IDH	MDH2	PGD1	PGD2	Number of alleles by which two parents differ
1	1	2	1	2	1	1	1,3	1	1	1	1	1	1	1	1,2	1	1,5	1,5	1	6, 6, 1, 7, 4, 6, 7, 3
2	1	1	1	1	1	1	1,2	1	1	1	1	1	1	1	1	1	1,2	1,4	1	
3	1	2	1	2	1	1	1,3	1	1	1	1	1	1	1	1,2	1	2,5	1,5	1	
4	1	1,2	1	1,2	1	1	1	1	1	1	1,3	1,2	1	1	1	1	1,5	1,5	1	
5	1	1	1	1	1	1	1,3	1	1	1	1	1	1	1	1	1	2,5	1	1	

Table 3.—Observed genotypes from ten control crosses used in hybrid verification

Cross		Genotype (by locus) ADH	ALAP	FEST	LAP1	LAP2	PGI1	PGI2	PGM1	PGM2	GDH	GOT1	GOT3	GPD1	GPD3	SKDH	IDH	MDH2	PGD1	PGD2
1 x 2	♀♂	1/1	2/1	1/1	2/1	1/1	1/1	1,3/1,2	1/1	1/1	1/1	1/1	1/1	1/1	1/1	1,2/1	1/1	1,5/1,2	1/1,4	1/1
1 x 3	♀♂	1/1	2/2	1/1	2/2	1/1	1/1	1,3/1,3	1/1	1/1	1/1	1/1	1/1	1/1	1/1	1,2/1,2	1/1	1,5/2,5	1,5/1,5	1/1
1 x 4	♀♂	1/1	2/1,2	1/1	2/1,2	1/1	1/1	1,3/1	1/1	1/1	1/1	1/1,3	1/1,2	1/1	1/1	1,2/1	1/1	1,5/1,5	1,5/1,5	1/1
1 x 5	♀♂	1/1	2/1	1/1	2/1	1/1	1/1	1,3/1,3	1/1	1/1	1/1	1/1	1/1	1/1	1/1	1,2/1	1/1	1,5/2,5	1/1	1/1
2 x 3	♀♂	1/1	1/2	1/1	1/2	1/1	1/1	1,2/1,3	1/1	1/1	1/1	1/1	1/1	1/1	1/1	1/1,2	1/1	1,2/2,5	1,4/1,5	1/1
2 x 5	♀♂	1/1	1/1	1/1	1/1	1/1	1/1	1,2/1,3	1/1	1/1	1/1	1/1	1/1	1/1	1/1	1/1	1/1	1,2/2,5	1,4/1	1/1
3 x 4	♀♂	1/1	2/1,2	1/1	2/1,2	1/1	1/1	1,3/1	1/1	1/1	1/1	1/1,3	1/1,2	1/1	1/1	1,2/1	1/1	2,5/1,5	5/5	1/1
3 x 5	♀♂	1/1	2/1	1/1	2/1	1/1	1/1	1,3/1,3	1/1	1/1	1/1	1/1	1/1	1/1	1/1	1,2/1	1/1	5/2,5	1,5/1	1/1
4 x 1	♀♂	1/1	1,2/2	1/1	1,2/2	1/1	1/1	1/1,3	1/1	1/1	1/1	1,3/1	1,2/1	1/1	1/1	1/1,2	1/1	1,5/1,5	1,5/1,5	1/1
4 x 2	♀♂	1/1	1,2/1	1/1	1,2/1	1/1	1/1	1/1,2	1/1	1/1	1/1	1,3/1	1,2/1	1/1	1/1	1/1	1/1	1,5/1,2	1,5/1,4	1/1

The utility of isoenzymes in genetic studies has been described previously (Adams 1979, Allard et al. 1975). Results on the inheritance of isoenzymes in longleaf pine allow for its addition to the list of species for which isoenzymal evaluations can enhance genetic analyses.

Part II

Allele frequencies were determined for each population as a first measure of genetic variation (Table 4). At least two loci were observed in each population sample, although several loci were essentially monomorphic. A single allele was more frequent in all populations for 16 of the 19 loci. Contingency chi-square analysis indicated significant differences for all loci except GPD-3 and PGI-1 (Table 5). Thus, in the allele frequency data, an appreciable amount of genetic variation was reflected among populations, but the distribution was not readily apparent.

Table 4.-- Allele frequencies at 19 loci in natural populations of longleaf pine

Locus	Allele	Populations																						
		3	5	7	9	11	13	16	19	23	25	29	30	31	32	36	37	38	41	42	43	45	46	50	51
ADH	1	1.00	1.00	1.00	0.97	0.94	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	0.98	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00
	2				0.03	0.06									0.02										
ALAP	1	0.80	0.95	0.94	1.0	0.86	0.80	0.64	0.85	0.91	1.00	0.95	0.89	0.95	0.97	0.92	0.96	0.89	0.91	0.97	0.92	0.86	0.80	0.88	0.62
	2	0.20	0.05	0.06		0.14	0.20	0.36	0.15	0.09		0.05	0.11	0.05	0.03	0.08	0.04	0.11	0.09	0.03	0.08	0.14	0.20	0.12	0.38
FLEST	1	0.97	0.93	0.97	0.87	0.95	0.94	0.89	0.90	0.88	0.89	0.97	0.83	0.86	0.95	1.00	0.91	1.00	1.00	0.82	0.95	0.98	0.97	0.93	0.90
	2	0.03	0.07	0.03	0.13	0.05	0.06	0.11	0.10	1.12	0.11	0.03	0.17	0.14	0.05		0.09			0.18	0.05	0.02	0.03	0.07	0.10
LAP-1	1	0.91	0.71	0.92	0.86	0.89	0.74	0.69	0.64	0.98	1.00	0.94	0.83	0.81	0.91	1.00	0.83	0.89	0.94	0.88	0.95	0.82	0.86	0.55	0.69
	2	0.09	0.29	0.08	0.14	0.11	0.26	0.31	0.36	0.02		0.06	0.17	0.19	0.09		0.17	0.11	0.06	0.12	0.05	0.18	0.14	0.45	0.31
LAP-2	1	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	0.88	0.98	1.00	0.94	1.00	1.00	1.00	1.00	1.00	1.00	1.00	0.98	0.95	0.98	1.00	1.00
	2									0.12	0.02		0.06										0.02		
	3																				0.02	0.05			
PGI-1	1	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	0.98	1.00	1.00	1.00	1.00	0.98	1.00	1.00	1.00	1.00	1.00
	2														0.02					0.02					
PGI-2	1	0.50	0.34	0.42	0.33	0.41	0.35	0.56	0.14	0.29	0.41	0.17	0.42	0.30	0.63	0.75	0.42	0.46	0.60	0.38	0.41	0.34	0.79	0.78	0.74
	2	0.42							0.07		0.03	0.04	0.04						0.09		0.04				0.02
	3	0.03	0.53	0.48	0.63	0.44	0.54	0.17	0.61	0.42	0.35	0.65	0.33	0.65	0.21	0.08	0.32	0.18	0.09	0.33	0.24	0.31	0.08	0.05	0.19
	4	0.05													0.03		0.03			0.03	0.04			0.02	0.02
	5		0.12	0.10	0.04	0.15	0.11	0.27	0.18	0.29	0.21	0.14	0.20	0.05	0.13	0.17	0.23	0.36	0.22	0.25	0.26	0.34	0.13	0.15	0.02
PGM-1	1	1.00	1.00	1.00	0.95	0.91	1.00	1.00	0.97	1.00	1.00	1.00	0.97	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00
	2				0.05	0.09			0.03				0.03												
PGM-2	1	1.00	1.00	1.00	0.88	0.94	0.86	0.99	1.00	0.92	0.97	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	0.96	0.96	1.00	1.00	1.00
	2				0.12	0.06	0.12	0.01		0.08	0.03											0.04			
	3						0.02														0.04				
GDH	1	1.00	1.00	1.00	1.00	1.00	1.00	1.00	0.94	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00
	2								0.06																
GOT-1	1	0.97	0.94	0.91	0.85	0.97	1.00	0.94	0.97	0.86	0.89	0.91	0.83	0.96	1.00	1.00	1.00	0.97	0.94	0.97	0.95	0.86	0.97	0.95	0.88
	3	0.03	0.06	0.09	0.15	0.03		0.06	0.03	0.14	0.11	0.09	0.17	0.14				0.03	0.06	0.03	0.05	0.14	0.03	0.05	0.12
GOT-3	1	0.91	0.97	0.95	0.91	0.94	0.77	0.97	0.97	0.97	0.91	0.91	0.97	0.79	0.97	1.00	0.97	1.00	0.88	1.00	0.94	0.89	0.94	0.95	0.98
	2	0.09		0.03	0.06	0.03	0.20	0.03	0.03	0.03	0.06	0.06	0.03	0.21	0.03		0.03		0.12		0.06	0.08	0.06	0.02	
	3		0.03	0.02	0.03	0.03	0.03				0.03	0.03										0.03		0.02	
	4																								0.02
GPD-1	1	1.00	1.00	1.00	1.00	1.00	1.00	1.00	0.97	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00
	2								0.03																
GPD-3	1	1.00	0.98	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	0.98	1.00	1.00	1.00	1.00	1.00	1.00
	2		0.02																0.02						
SKDH	1	0.69	0.46	0.71	0.58	0.67	0.73	0.81	0.48	0.45	0.70	0.53	0.80	0.88	0.67	0.92	0.73	0.68	0.56	0.79	0.74	0.64	0.61	0.86	0.83
	2	0.31	0.54	0.26	0.42	0.33	0.27	0.14	0.52	0.48	0.27	0.47	0.11	0.12	0.24	0.08	0.27	0.29	0.44	0.15	0.24	0.27	0.39	0.05	0.12
	3			0.03						0.03	0.03		0.09					0.03		0.06					0.02
	4							0.06		0.03					0.09						0.02	0.09		0.09	0.02
IDH	1	1.00	0.94	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	0.94	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00
	2		0.03									0.06													
	3		0.03																						
MDH	1	0.48	0.35	0.30	0.52	0.47	0.70	0.42	0.42	0.50	0.50	0.39	0.56	0.50	0.59	0.54	0.39	0.56	0.17	0.36	0.38	0.27	0.48	0.57	0.45
	2	0.32	0.08	0.34	0.33	0.41	0.18	0.26	0.15	0.26	0.36	0.20	0.35	0.33	0.27	0.33	0.27	0.41	0.38	0.44	0.29	0.33	0.52	0.17	0.07
	5	0.20	0.58	0.36	0.15	0.12	0.12	0.32	0.42	0.24	0.14	0.41	0.09	0.17	0.14	0.12	0.34	0.03	0.45	0.20	0.33	0.41		0.26	0.48
PGD-1	1	0.89	0.82	0.77	0.89	0.88	0.73	0.94	0.79	0.56	0.80	0.79	0.97	0.24	0.77	0.88	0.81	0.80	0.83	0.62	0.88	0.82	0.76	0.76	0.88
	2			0.04			0.04	0.04	0.03		0.03	0.03		0.10	0.06	0.04	0.16	0.14	0.03		0.09		0.03	0.07	
	3	0.06	0.15		0.08	0.09	0.09	0.01	0.14	0.17	0.02	0.14	0.03	0.07	0.09	0.08	0.03	0.06	0.08			0.17	0.06	0.02	0.07
	4	0.02	0.03						0.03	0.03									0.03			0.02			
	5	0.03		0.18	0.03	0.03	0.14		0.02	0.24	0.15	0.04		0.60	0.08				0.03	0.38	0.03		0.15	0.14	0.05
PGD-2	1	1.00	0.97	0.98	1.00	0.97	0.97	0.97	1.00	1.00	1.00	0.97	0.97	0.95	0.94	0.92	1.00	1.00	1.00	0.97	0.98	0.97	1.00	1.00	1.00
	2					0.03	0.03	0.03				0.03	0.03	0.05	0.03	0.08				0.03					
	3		0.03	0.02											0.03						0.02	0.03			

Table 5.--Contingency chi-square analysis for 19 loci pooled over populations

Locus	No. of alleles	Chi-square	D.F.	P
ADH	2	69.95	23	0.000
ALAP	2	161.89	23	0.000
FLEST	2	58.71	23	0.000
LAP-1	2	175.73	23	0.000
LAP-2	3	195.06	46	0.000
PGI-1	2	23.22	23	0.448
PGI-2	5	725.86	92	0.000
PGM-1	2	91.03	23	0.000
PGM-2	3	178.91	46	0.000
GDH	2	98.96	23	0.000
GOT-1	2	69.98	23	0.000
GOT-3	4	166.73	69	0.000
GPD-1	2	48.60	23	0.001
GPD-3	2	27.66	23	0.229
SKDH	4	320.74	69	0.000
IDH	3	136.56	46	0.000
MDH	3	272.28	46	0.000
PGD-1	5	517.87	92	0.000
PGD-2	3	79.20	46	0.002

As a second measure of isoenzymal genetic variation, the average number of alleles per locus, the percentage of polymorphic loci, and the average heterozygosity per individual were compared among populations (Table 6). Although 2 or more alleles were found for every locus over all populations, the range in number of alleles per locus for individual populations was only 1.5 to 1.9. There were no clear-cut differences between any two populations in number of alleles, but it was evident from the data that populations differed in the presence of specific alleles and in the frequencies of these alleles. The percentage of polymorphic loci per population ranged from 31.6 to 57.9 percent and was correlated with latitude of the populations ($r = -0.63$, $P < 0.002$). The more southern populations had a higher percentage of polymorphic loci.

Table 6.--Variation of isoenzyme characteristics in 24 natural populations of longleaf pine

Population		Average number of alleles per locus	Percentage of loci polymorphic[a)]	Percent heterozygosity per individual
3	Washington, FL	1.7 ± 0.2	36.8	13.8 ± 4.7
5	Okaloosa, FL	1.8 ± 0.2	47.4	15.8 ± 4.8
7	Santa Rosa, FL	1.8 ± 0.2	36.8	14.1 ± 4.9
9	Escambia, FL	1.8 ± 0.2	47.4	16.2 ± 4.5
11	Baldwin, AL	1.9 ± 0.2	57.9	16.2 ± 4.5
13	Mobile, AL	1.8 ± 0.2	47.4	17.9 ± 4.8
16	Stone, MS	1.8 ± 0.2	42.1	16.0 ± 5.1
19	Worth, GA	1.9 ± 0.2	42.1	17.6 ± 5.1
23	Geneva, AL	1.9 ± 0.2	47.4	18.6 ± 5.6
25	Pike, AL	1.8 ± 0.2	36.8	14.3 ± 5.0
29	Conecuh, AL	1.9 ± 0.2	42.1	14.8 ± 4.7
30	Washington, AL	1.8 ± 0.2	42.1	15.6 ± 4.6
31	Wayne, MS	1.7 ± 0.2	42.1	17.2 ± 4.9
32	Perry, MS	1.9 ± 0.2	31.6	13.5 ± 4.6
36	Lawrence, MS	1.5 ± 0.2	31.6	8.8 ± 3.8
37	Taylor, GA	1.6 ± 0.2	31.6	14.0 ± 5.2
38	Meriwether, GA	1.6 ± 0.2	31.6	12.6 ± 4.8
41	Autauga, AL	1.8 ± 0.3	42.1	14.0 ± 4.9
42	Bibb, AL	1.7 ± 0.2	31.6	15.2 ± 5.3
43	Hale, AL	1.9 ± 0.2	31.6	14.1 ± 5.0
45	Scott, MS	1.9 ± 0.2	42.1	18.0 ± 5.2
46	Tallapoosa, AL	1.7 ± 0.2	36.8	13.6 ± 4.2
50	Marion, FL	1.8 ± 0.2	36.8	13.9 ± 4.4
51	Richmond, NC	1.8 ± 0.3	42.1	15.0 ± 4.5

a) Considered polymorphic if the frequency of the most common allele does not exceed 0.95.

As a third measure of genetic variation, the genetic distance between pairs of populations was determined (Table 7). Genetic distance coefficients combined over all loci ranged from 0.0 for 2 populations (populations 37 and 43) from the same latitude but separated in longitude, to a high of 0.048 between the North Carolina population (population 51) and a Mississippi population (population 31). In general the genetic distance coefficients were small and of the same order as those for pitch pine (Guries and Ledig 1982), but were much larger than those for Douglas-fir (Yeh and O'Malley 1980). Distribution of the largest coefficients was essentially random although for some pairs, such as 31 and 51, they also were separated by a large geographic distance.

Genetic distance coefficients also were calculated for each locus separately to evaluate specific locus contributions to overall coefficients. There were six loci (ALAP, LAP-1, MDH, PGD-1, PGI-2, and SKDH) that seemed to contribute the most to the overall coefficients. Of these six, PDG-1 and PGI-2 both had coefficients ranging from 0.0 to approximately 0.98, and were the largest contributors to overall distances. The wide variation attributable to separate loci indicated the necessity for evaluating large numbers of loci in order to correctly evaluate population differentiation. Although these analyses identify substantial variation, the distribution among populations suggested a generally random distribution with only slight population differentiation.

Table 7.-- Genetic distance[1] coefficients between longleaf pine populations

Population	3	5	7	9	11	13	16	19	23	25	29	30	31	32	36	37	38	41	42	43	45	46	50
3	*****																						
5	0.027	*****																					
7	0.015	0.011	*****																				
9	0.022	0.011	0.007	*****																			
11	0.011	0.015	0.004	0.003	*****																		
13	0.020	0.019	0.012	0.008	0.007	*****																	
16	0.014	0.023	0.015	0.025	0.012	0.016	*****																
19	0.028	0.001	0.014	0.010	0.013	0.013	0.022	*****															
23	0.024	0.015	0.009	0.009	0.010	0.015	0.029	0.015	*****														
25	0.012	0.020	0.003	0.005	0.002	0.011	0.018	0.021	0.006	*****													
29	0.025	0.004	0.006	0.005	0.009	0.014	0.029	0.005	0.007	0.010	*****												
30	0.013	0.024	0.009	0.009	0.004	0.012	0.009	0.022	0.018	0.004	0.019	*****											
31	0.047	0.043	0.020	0.029	0.030	0.019	0.047	0.038	0.024	0.023	0.031	0.033	*****										
32	0.009	0.020	0.007	0.012	0.004	0.011	0.013	0.024	0.013	0.003	0.018	0.007	0.031	*****									
36	0.011	0.036	0.013	0.024	0.010	0.021	0.012	0.042	0.027	0.008	0.032	0.008	0.043	0.002	*****								
37	0.012	0.010	0.002	0.009	0.004	0.011	0.009	0.011	0.012	0.004	0.009	0.006	0.030	0.004	0.009	*****							
38	0.011	0.025	0.010	0.014	0.004	0.013	0.012	0.023	0.013	0.004	0.019	0.006	0.037	0.003	0.006	0.005	*****						
41	0.011	0.016	0.008	0.021	0.012	0.028	0.016	0.024	0.017	0.011	0.017	0.020	0.048	0.011	0.015	0.007	0.012	*****					
42	0.021	0.025	0.005	0.016	0.009	0.016	0.018	0.024	0.011	0.004	0.019	0.010	0.013	0.009	0.015	0.007	0.010	0.018	*****				
43	0.009	0.015	0.002	0.011	0.004	0.014	0.009	0.017	0.012	0.002	0.010	0.006	0.033	0.004	0.006	0.0	0.004	0.005	0.008	*****			
45	0.015	0.009	0.004	0.011	0.006	0.015	0.008	0.011	0.010	0.007	0.008	0.010	0.035	0.010	0.016	0.003	0.008	0.005	0.012	0.002	*****		
46	0.011	0.033	0.016	0.022	0.009	0.020	0.015	0.035	0.020	0.011	0.032	0.015	0.041	0.005	0.008	0.014	0.006	0.011	0.016	0.013	0.017	*****	
50	0.021	0.032	0.023	0.034	0.023	0.021	0.009	0.035	0.039	0.024	0.043	0.016	0.040	0.012	0.013	0.015	0.018	0.026	0.021	0.019	0.021	0.017	*****
51	0.018	0.025	0.020	0.032	0.022	0.022	0.003	0.029	0.037	0.027	0.035	0.017	0.049	0.018	0.017	0.016	0.024	0.022	0.028	0.017	0.018	0.022	0.008

[1] Unbiased genetic distance (D) according to Nei, 1978.

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CONIFER GENETICS II

MODERATED BY DR. TONY SQUILLACE

Universtiy of Florida

Genetic and Cultural Factors Affecting Growth Performance of Slash Pine

G. L. Reighard, D. L. Rockwood, and C. W. Comer[1/]

Abstract.--Five- to seven-year growth performances of genetically select slash pine progenies planted at five northern Florida sites were evaluated for differences due to family, provenance, plantation site, competition (pure and maximum), plot design (block and Nelder), spacing (472-43,100 trees/ha), and age. Significant family differences were found for growth. Family x site interactions were important on poor sites. Intergenotypic competition and plot design did not affect family performance. Spacing influenced diameter and volume, but a family x spacing interaction was not apparent. Growth trends detected at age five continued at age seven, but variation among families decreased.

Additional Keywords: Pinus elliottii var. elliottii, genetic tests, genotype x environment interaction, spacing.

INTRODUCTION

Numerous genetic tests of slash pine (Pinus elliottii var. elliottii Engelm.) have been established in the southeastern United States during the past 25 years. Data from these tests have been used to select fast-growing, rust-resistant genotypes for clonal seed orchards. Recently, Franklin (1979, 1983) and Stonecypher and McCullough (1981) have advocated shortening the evaluation period in progeny tests by planting at high, non-conventional densities to create competition at earlier ages. Furthermore, factors such as plot design, age of measurement, and environment interactions require additional study to determine if present genetic evaluations are appropriate for wide geographic plantings of selected slash pine families. The objectives of this paper were to evaluate the effects of family, provenance, site, intergenotypic competition, plot design, spacing, and age on growth performance of slash pine progenies at five northern Florida sites.

[1/]Assistant in Forest Biomass, Associate Professor, and Assistant Research Scientist, respectively, Department of Forestry, University of Florida, Gainesville, FL. Research reported here was supported by the Cooperative Forest Genetics Research Program, Oak Ridge National Laboratory under subcontract No. 19X-09050C, and a cooperative program between the Institute of Food and Agricultural Sciences of the University of Florida and the Gas Research Institute entitled "Methane from Biomass and Waste."

MATERIALS AND METHODS

Nine slash pine progeny tests located at five northern Florida sites were measured in 1984 (Table 1). Height, diameter at breast height (DBH), and survival were recorded. Individual tree volume was calculated using an equation developed by Goddard and Strickland (1968) for five-year-old slash pine. Six tests had block plot designs with tree densities ranging from 1,121 to 10,000 trees/ha. The three remaining tests were Nelder designs with eight spacings. One Nelder test had densities ranging from 472 to 3,089 trees/ha, whereas the densities of the other two ranged from 4,800 to 43,100 trees/ha. Detailed descriptions of the tests and test sites are given by Rockwood (1983).

Analyses of variance were performed on data from either randomized complete block or split-plot designs in each study. Coefficients of variation were determined for height and DBH data from five tests. Spearman's rank correlation coefficients were calculated for volume data between common families of comparison tests. Rank correlations were also calculated between the volume data from each of the nine tests and the volume clonal evaluations assessed by the Cooperative Forest Genetics Research Program at the University of Florida.

RESULTS AND DISCUSSION

Family and Site Factors

Growth varied by progeny test (Table 1) and was influenced by site quality. Good growth occurred in tests 8-78-1, 8-78-2, and 0-58, which were planted on similar lower coastal plain sites. Tests 0-59 and 0-60 were planted on less fertile, flatwood sites and had only average growth. Growth differences due to site were also evident between the tests at Gainesville, FL (10-N, 10-P) and Trenton, FL (11-N, 11-P). Early survival, however, was not affected by site quality.

Growth differences due to family were statistically significant in every test except 10-P and 11-P. In tests that contained a commercial checklot (i.e., 0-58, 0-59, 0-60), selected slash pine families generally grew and survived better than the check; although an average of 19% of the selected families had less plot volume than the check for a given site. Since some families occasionally performed poorly on particular sites, family mixes would be preferred over family blocks for certain sites if no families have demonstrated superiority.

Family ranks for individual tree volume in each of the tests were not statistically correlated with the standardized clonal evaluations (Table 2), which are based on row-plot progeny tests planted at operational spacings. The low correlations for sites other than Cantonment suggested that genotype x environment interactions were present. Earlier comparisons of other families across various test sites also suggested such interactions (Goddard *et al.* 1976, Goddard *et al.* 1982) whenever site differences were large. Most highly-rated families were consistent, however, in these tests. Since fifth-year performance in

Table 1. Mean growth performance for all slash pine families in each progeny test.

Progeny Test No.	Florida Location	Age (yrs)	Ht. (m)	DBH (cm)	Vol. (m^3)	Surv. (%)
8-78-1	Cantonment	6	7.7	10.4	.0299	86
8-78-2	Cantonment	6	7.3	10.7	.0297	90
0-58	Cantonment	7	7.5	10.6	.0296	87
0-59	Perry	7	6.0	8.8	.0189	89
0-60	Yulee	7	5.9	8.6	.0182	89
10-N	Gainesville	5	3.1	3.3	.0064	96
10-P	Gainesville	5	3.6	3.9	.0074	95
11-N	Trenton	5	5.3	5.5	.0111	96
11-P	Trenton	5	5.8	5.8	.0121	94

Table 2. Spearman's rank correlation coefficients between progeny test performance and composite clonal evaluations.

Progeny Test No.	Florida Location	No. Common Families	Volume
			--r---
8-78-1	Cantonment	7	.32
8-78-2	Cantonment	7	.61
0-58	Cantonment	22	.40
0-59	Perry	22	.04
0-60	Yulee	19	.25
10-N	Gainesville	32	.08
10-P	Gainesville	21	-.08
11-N	Trenton	25	.19
11-P	Trenton	17	.06

conventionally spaced tests is not as well correlated with subsequent measurements as are 10th-year data, the higher correlations for the Cantonment sites may be due to the excellent growth which was equivalent to that of older trees.

Genotype x environment interactions were less apparent in the comparisons between common families in the nine tests (Table 3). Excluding comparisons involving test 10-P, rank correlations were positive (r=.16 - .73). Therefore, family rankings for growth performance within these tests did not change significantly. The failure of the standardized clonal evaluations to demonstrate genotypic stability for family performance across sites limits the usefulness of the evaluations for selecting superior families for planting. Further study of the methodology used in family evaluation is needed, since comparisons between the progeny tests (Table 3) indicated genotypic stability did occur at a modest level.

Test comparisons (Table 3) emphasizing site differences rather than plot designs generally had positive rank correlations (r=.33 - .57). Since plot designs and spacings were identical, the largely non-significant correlations from these paired tests suggested family x environment interactions were occurring. These interactions affected growth of some families on the poorest site, test 10-P. Correlations from the three paired tests involving 10-P were negative, and two were significant. Possibly the slash pine families that grew well on moderate to good sites may have been more sensitive to site quality and therefore, were physiologically predisposed to site and spacing interactions on poor sites.

Family performance was also linked to geographic origin in tests having commercial spacings (i.e., 0-58, 0-59, 0-60). Families from southeastern Georgia and northeastern Florida consistently grew the best. Volume growth of these families averaged 3% more than southern Alabama and Mississippi families and 9% more than north-central Florida families. However, these comparisons may be influenced by the unequal and limited sample sizes from the geographic regions. Slightly different geographic patterns were observed in the fifth-year data (Goddard et al. 1982).

In addition to family performance across sites, intergenotypic competition was investigated in test 8-78-1. Contrary to findings by Williams et al. (1983) for loblolly pine, no differences in growth were found between pure plots (one family) and mixed plots (nine families) which agreed with results reported by Franklin (1983) for studies on loblolly family competition. Survival, however, was significantly higher (+9%) in the mixed plots.

Plot Design and Spacing Factors

Comparisons of tests from the same site but having different plot designs, from different sites with the same plot design, and from different sites with different designs showed that, with the exception of the 10 tests, plot design affected family volume rankings less than did site differences (Table 3). Families in Cantonment tests 8-78-1

Table 3. Spearman's rank correlation coefficients for volume data of common families across different tests and plot designs.

Test Comparison	Plot Design Comparison	No. Common Families	Volume (m^3) --r---
8-78-1/8-78-2	Block/Nelder	9	.73*
10-P/10-N	Block/Nelder	20	-.46*
11-P/11-N	Block/Nelder	13	.57*
0-58/0-59	Block/Block	14	.57*
0-58/0-60	Block/Block	14	.47
0-59/0-60	Block/Block	14	.36
10-P/11-P	Block/Block	17	-.05
10-N/11-N	Nelder/Nelder	25	.33
10-P/11-N	Block/Nelder	18	-.48*
10-N/11-P	Nelder/Block	15	.16

*Significant at the 5% level.

and 8-78-2 were significantly correlated (r= .73) despite the differences in plot design and spacings between the two tests. The 11-P and 11-N test comparison gave similar results. The similarity of family volume production across two plot designs with different spacings suggested family x spacing interactions were not influencing family rankings. This was in contrast to Stonecypher and McCullough's (1981) observations from a eight-year-old Nelder test of Douglas-fir. They found family x density interactions at spacings (735 to 26,300 trees/ha) greater than those tested in the 10-N and 11-N tests. There were, however, significant spacing x family interactions for height in tests 8-78-2 and 10-N. Therefore, some slash pine families were affected differentially by spacing at ages five and six, although most family ranks changed little.

Spacing significantly affected family height and/or diameter (DBH) growth in all spacing tests except 8-78-1. DBH was influenced by spacing in tests 8-78-2, 0-58, 0-59, 0-60, 10-N, and 11-N. Height was affected in tests 10-N and 11-N. In the Nelder tests, spacing influenced height, DBH, and volume in a manner similar to that reported by Stonecypher and McCullough (1981) for Douglas-fir. DBH and volume increased with each subsequent decrease in density. Height also increased with decreasing density up to the two lowest densities where it decreased slightly. Therefore, growth differences between the progeny tests reflected spacing as well as site differences.

Age Factors

Spearman correlations between family height, DBH, and volume data for years three versus five, four versus six, and five versus seven were significant (r=.75 - .93) in all tests. Family performance rankings changed little over two years, irregardless of the different spacing treatments.

The coefficient of variation (CV) for growth data recorded in 1982 and 1984 from four tests planted at two operational spacings decreased over time (Table 4). The CV for height of trees planted at wide spacings varied from 0.9% less than to 1.5% more than the CV of narrow spacing trees. The CV for DBH was 1.5-1.7% larger at the narrow spacings in three of four tests. Similarly, the CV for DBH at five spacings (4800, 8400, 14,600, 25,100, and 43,300 trees/ha) in Nelder plots from test 11-N decreased from the densest to the widest spacing at both ages three (34% to 25%) and five (27% to 21%).

Table 4. Coefficients of variation (CV) for height and DBH of all families tested at two spacings and measured in 1982 and 1984.

Progeny Test (Planting Yr.)		CV for Height Wide/Narrow Spacings[a] 1982	1984	CV for DBH Wide/Narrow Spacings 1982	1984
		(%)			
8-78-1	(1978)	13.2/14.4	11.5/11.8	18.4/20.7	15.9/17.5
0-58	(1977)	13.6/13.2	11.9/11.9	18.1/18.3	14.8/16.5
0-59	(1977)	21.9/18.5	16.6/15.1	29.3/26.2	22.1/21.3
0-60	(1977)	21.2/22.5	16.5/17.4	29.3/31.5	20.5/22.0

[a] Spacings were 1,223 and 2,446 trees/ha, respectively.

Even though high densities appeared to encourage expression of intrafamily variation at three, five, and seven years of age, no family x spacing interactions for DBH were found in the Nelder tests (8-78-2, 10-N, 11-N) where high densities were tested. Furthermore, the Nelder data and the decreasing CV with time suggested that significant family x spacing interactions may not occur in these tests in the future. Thus the merits of using narrow spacings and alternate plot designs to evaluate the growth potential of slash pine families at young ages have yet to be demonstrated.

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TWO-STAGE EARLY SELECTION: A METHOD FOR PRIORITIZATION AND WEIGHTING OF TRAITS

Cheryl B. Talbert[1/]

Abstract--A simulation approach was used to evaluate the impact of a two-stage early-screening-plus-field-test selection program on predicted total selection-progress in a 'mature-stand' selection-goal. Early family-screening , when followed by field testing for a specified time period, can lead to more, less or the same expected gain than a standard 'field-test-only' program, depending upon the correlations among the early-screening, field and mature-stand traits chosen. If the early-screening and field-test criteria chosen are either strongly positively or strongly negatively correlated, both total gain and gain per unit time may be less than if the early screening had never been carried out. This effect can be reduced by appropriate allocation of selection intensity between the early-screening and field steps. Economic analysis will be necessary to evaluate whether the benefits of early screening (in quality of the early-test environment(s), reduced field-test size, and/or larger family-size and greater selection efficiency for a given field-test size) will outweigh its negative impacts under the conditions faced by a particular organization.

INTRODUCTION

Selection considerably prior to harvest-age is an operational reality in loblolly pine tree improvement, as a result of persistently high alternative rates of return and the crushing resource costs of carrying large field tests over long periods of time. The excellent research of the past decade into methods of greenhouse, laboratory and nursery selection for improvement of later field performance (reviewed by Lambeth, 1983 and by Talbert and Lambeth, 1984) has not yet produced results conclusive enough to cause operational programs to move away from conventional field tests of 4 to 8 years duration. However, a number of organizations are seriously planning one- or two-year greenhouse, lab or nursery trials to 'screen out' their poorest families for growth, quality and/or adaptability, in order to reduce the size of their field-tests.

A likely scenario for such a program is selection in two or more stages, where some proportion of a population of half-sib and/or full-sib families would be discarded at some early age, or at several early ages, on the basis of seedling traits. After this initial truncation, field tests would be planted with reserve seed from the remaining families, and the final set of orchard parents or selections for the subsequent generation would then be chosen from those tests based upon survival, pest-response and height or volume. This discussion will use the term early screening to define truncation-selection based upon seedling traits.

Considerable theoretical work in the agronomy literature has shown that, when different selection criteria are correlated, culling of the population for one criterion can drastically impact gain-potential from other criteria, and the total progress that can be achieved is strongly

1/ Scientist, Forestry Research Division, Weyerhaeuser Company WTC-2H2, Tacoma, Washington 98477.

affected by the relative correlations of each criterion with the desired mature-stand traits, and by the relative economic importance of those mature-stand traits (Jain and Amble, 1962; Namkoong, 1970; Cunningham, 1975). Multi-stage culling can actually *reduce* total 'value gain' if the ordering of traits and the selection pressure applied to each trait is not properly matched to the genetic and economic characteristics of the population. At the same time, early family-screening can provide a number of advantages over conventional 'field-only' testing, which could offset these disadvantages (Talbert and Lambeth, 1984) - advantages in quality of the test environment(s) (for example, the ability to evaluate rust resistance in a high-inoculum environment), in reduced field-test size, and/or in larger family-size (and greater selection efficiency) for a given field-test size.

This report will explore the impact of early truncations on total gain from a fairly simple two-stage 'early-screening + field-test' selection program, and will explore alternatives for maximizing gain from such a program in populations with a variety of characteristics.

The Quantitative Basis for Two-Stage Selection

Selection is usually justified by the expectation that some desired change will occur as a result, in one or more correlated traits (the simplest example would be genetic value for the original trait). By the same token, any early truncation of a population will influence the mean and variance of later-stage, correlated traits, thereby affecting the progress achievable from selection on those later-stage traits. The impact of sequential truncations on predicted gain can be quantified based upon the characteristics of the multivariate-normal distribution (Eisen, 1983). The general theory is adapted to the current example below.

Because early selection is most commonly carried out to improve average genetic value for one or more harvest-age traits, gain in a harvest-age trait M resulting from early selection for the same or different trait J is appropriately described using an equation for indirect selection:

$$\Delta G_{M,J} = i_J Cov(M,J) / \sigma_{p_J} \quad , \qquad (1)$$

where i_J = the selection intensity practiced on J,

Cov(M,J) = the covariance between genetic value for the harvest-age trait and phenotypic value for the early trait, and

σ_{p_J} = the phenotypic standard deviation for J.

For individual selection this formula reduces to the familiar form:

$$\Delta G_{M,J} = i_J h_J h_M r_{G_{MJ}} \sigma_{p_M} \quad , \qquad (2)$$

where h = square root of the heritability, and

rGMJ = genetic correlation between the mature trait M and the early trait J.

Now, instead of one early-selection trait J, consider the case of two early-selection criteria J and F. In the current context, J would be the greenhouse/lab/nursery trait and F would be a field-test trait; therefore, J must be assessed prior to F. If a proportion of families p(J) is selected based upon J, retaining a reduced population having a standardized selection differential i_J, the variance of the field trait F is reduced whenever J and F are correlated:

$$V_F* = V_F - \frac{[Cov(J,F)^2 \times i_J(i_J - c_J)}{V_J}, \qquad (3)$$

where * = designation of adjustment for 1st-stage selection,

V_F = the variance of F prior to selection on J,

Cov(J,F) = the covariance between J and F prior to selection on J,

and c_J = the standardized truncation point for J.

In addition, the selection intensity which can be applied to F after selection on J, i_F*, is reduced, regardless of the correlation between J and F, due to the fact that the proportion left for field selection out of a fixed total proportion P to be selected, is reduced.

The covariance between F and M are also affected by prior selection whenever either F or M are correlated with J:

$$Cov(F,M)* = Cov(F,M) - \frac{[Cov(J,F) \times Cov(J,M) \times i_J(i_J - c_J)]}{V_J} \qquad (4)$$

The correlated gain in M resulting from the selection on J can be predicted by equation (1). However, the gain which can be obtained from subsequent selection on F will be altered by the first-stage truncation, because of the impact of the truncation on i_F, on the variance of F, and on the correlation of F with M. Therefore, gain in M resulting from two-stage truncation selection on J and F will be:

$$\Delta G_{M,J+F} = \Delta G_{M,J} + i_F Cov(F,M)* \; / \; \sigma_{p_F}* \; .$$

The Simulation Analysis

To illustrate the impacts of first-stage selection on total progress from a two-stage selection program, several simulated populations were carried through equations 1-5. A number of combinations of genetic correlations between J, F and M were used, chosen to represent a range of combinations rather than to model any specific biological scenario (Table 1). For purposes of the simulation, individual-tree narrow sense heritabilities are assumed to be 0.2 for J, F and M, and the phenotypic variances for J, F and M are held at 1, 10 and 100, respectively, for all of the simulations. Finally, it is assumed that each population consisted of 100 half-sib families, with a constant family-size of 200 for the early screening and 60 in the field. Half-sib family selection is used in the early-screening stage, and a combined half-sib-family and within-family index is used in the field stage. It is important to note that, since reserve seed

is used to plant the field-test in our example, none of the same trees would be measured for both J and F, although half-sibs would be.

Table 1. Ranges of selection intensity and inter-trait genetic correlations used in the simulation analysis.

Parameter	Definition	Range
i_J	selection intensity for J	0.35 - 1.73
p_J	proportion of families selected for J	80% - 10%
rG_{JF}	genetic correlation between J and F	-0.4, 0.1, 0.4, 0.8
rGJM	genetic correlation between J and M	-0.4, -0.2, 0.2, 0.4
rG_{FM}	genetic correlation between F and M	-0.2, -0.4, 0.4, 0.2

RESULTS

Figures 1a and 1b illustrate the impact of increasing intensity of early half-sib family screening for a trait J on the phenotypic variance of a field-trait index F, and on the correlation between genetic value for M and the phenotypic index value for F, when the genetic correlation between J and M and between F and M prior to the early screening are equal in absolute value at rG = +0.4 or -0.4.

Regardless of the genetic correlations between J and M and between F and M, when the genetic correlation between J and F is strongly positive, the variance of the field-trait index F decreases to less than two-thirds of its original (rG(J,F)=0) value as the proportion of families selected for J reaches 20 percent (or 20 of 100 families selected). If the genetic correlations between J and M, F and M and J and F prior to the early-screening are of the same sign, selection for J results in a decrease in the absolute value of the correlation between F and M[2/]. On the other hand, if the genetic correlations between J and M and between F and M are of the same sign and the correlation between J and F is of the opposite sign, then early screening for J will decrease the absolute genetic correlation between F and M if rG(JM) is positive, and will increase that absolute correlation if rG(Jf) is negative. Finally, if rG(JM) and rG(FM) are of opposite sign, then early screening for J will increase the absolute value of rG(FM) if the genetic correlation between J and F is positive, and will decrease the absolute value of rG(FM) if rG(JM.F) is negative.

2/ The importance of changes in rG(FM) result from the direct proportionality of rG(FM) with expected second-stage gain. If expected gain from selection on a given variable turns out to be negative, then selection on the opposite end of the scale for that variable will make the gain positive, and of the same magnitude. Therefore, absolute correlations and expected-gain values are the quantities of interest.

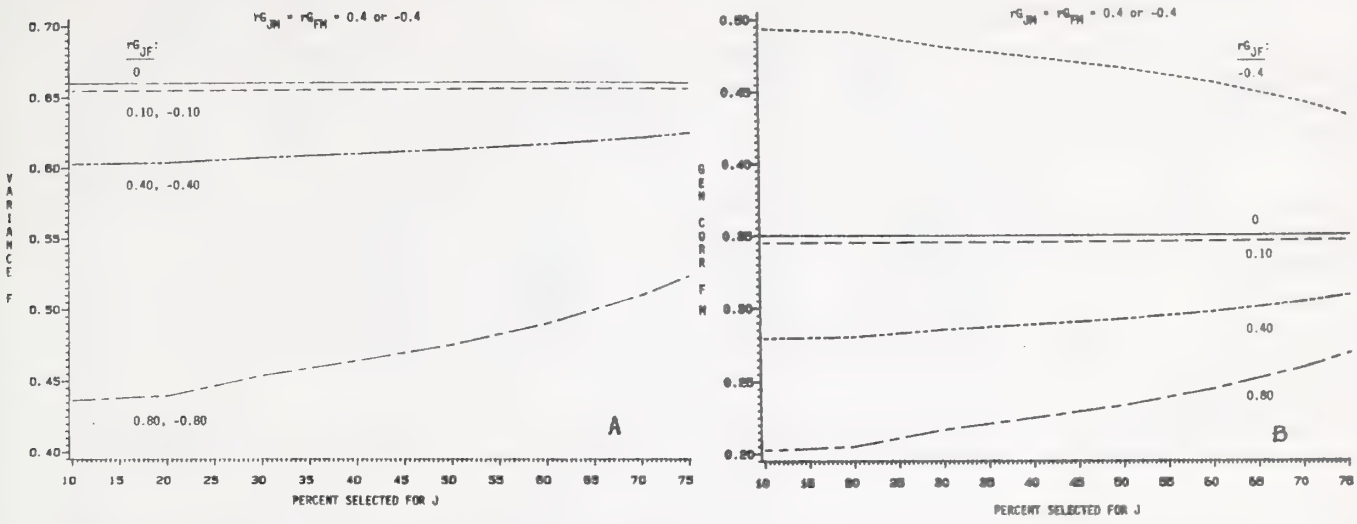

FIGURE 1. The impact of early screening for a trait J on (A) the variance of a correlated field-trait F, and (B) the correlation of F with a selection goal M. (Assumptions and definitions in text and in Table 1)

How can selection for one variable actually increase the correlation between two other variables? This can happen because, under certain conditions, removal of entries on the low end of the scale for J means removal of entries which are simultaneously low for M (due to a positive correlation of J and M) and high for F (due to a negative correlation of J with F). An opposite but equivalent example exists if J and M are negatively correlated and J and F are positively correlated. The outcome is an upward pressure on the absolute value of the correlation between F and M.

The combined impact of these effects is illustrated in Figures 2a-2c, in terms of the predicted progress in the mature trait M which would be expected from the two-stage selection program relative to that which would be expected from selection for the field-trait index alone, when the genetic correlations of J with M and of F with M are again equal in absolute value at +0.4 or -0.4.

Regardless of the proportion selected in the early-screening step, when the genetic correlation between J and F is less than 0.4 or negative and the correlations of J and F with the selection goal are both either +0.4 or -0.4, selection for both J and F yields more expected gain (in M) than does field selection alone. In fact, under these conditions, if the genetic correlation between J and F is negative the expected gain in M from the second-stage, field-selection step *alone* is greater than the total expected gain would have been from a single-stage field selection on F. On the other hand, if J and F are strongly positively correlated (>0.4), and both rG(JM) and rG(FM) are either +0.4 or -0.4, two-stage selection yields *less* expected gain in M than does one-stage selection for F alone.

The opposite behavior occurs when the genetic correlations between J and M and F and M are opposite in sign and equal in magnitude at |0.4|. In

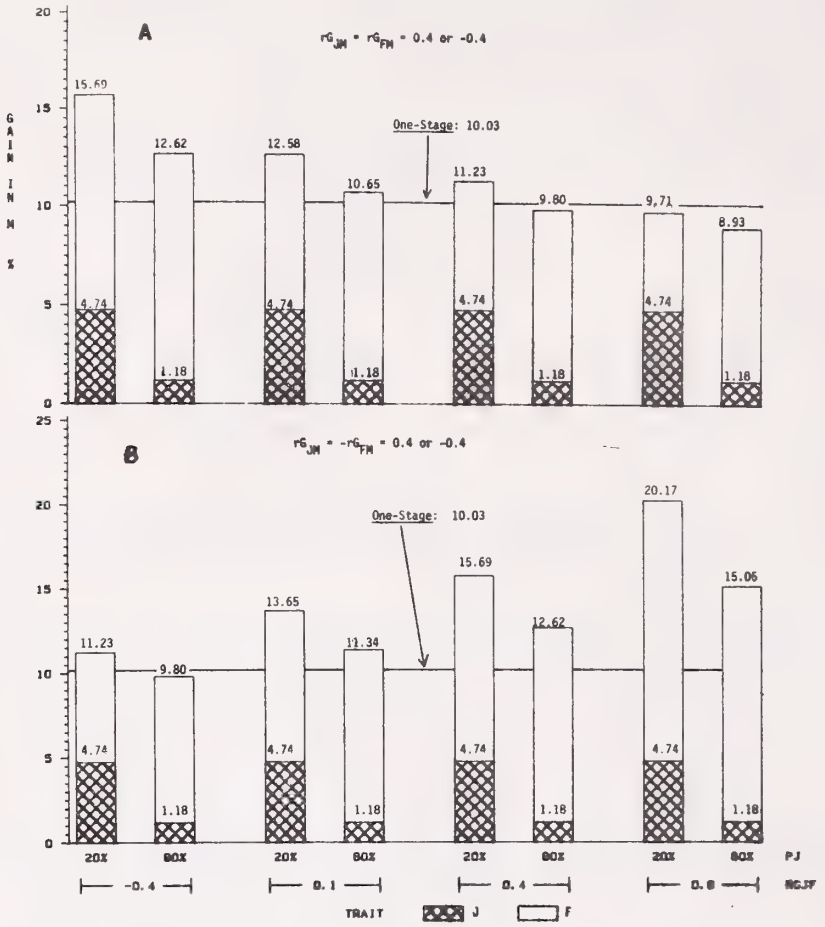

FIGURE 2. Predicted gain in a mature-stand trait M resulting from two-stage 'early-screening-plus-field' selection for a seedling trait J and a field trait F, compared to field-selection only, for two early-screening intensities: (a) when rG(JM) and rG(FM) are both 0.4 or both -0.4; and (b) when rG(JM) and rG(FM) are opposite in sign but equal in magnitude at |0.4|. (Assumptions and definitions are contained in Table 1)

such a scenario, unless J and F are strongly negatively correlated, two-stage selection yields greater expected gain than one-stage selection.

As expected, gain from two-stage selection varies in magnitude with the degree of selection-pressure placed on the early-screening trait J, while expected gain from the one-stage 'field-only' method remains unaffected. For every population scenario tested, rigorous early screening yields greater gain overall than does low-intensity early screening. This occurs despite the fact that greater early-screening gain sometimes results in reduced gain from field-test selection; the increase in gain resulting from the increased early-selection intensity even in these scenarios is greater than the decrease in gain from the field-selection step. Therefore, it appears that it is possible to allocate selection intensity between early-screening and field-test steps in a two-stage selection program so as to make two-stage selection at least as effective, and often much more effective, in overall expected gain than a one-stage field-test-only program.

Needless to say, it is not realistic to presume that the genetic

correlation between a chosen early-screening trait J and the 'mature-stand' selection goal M will always be the same as the genetic correlation between the chosen field-test trait (F) and M. Figures 3a-3d illustrate the effects of differences between J and F in the strength of their genetic relationships with M, ranging from a case where the early-screening trait J is less-strongly correlated with M than is the field-trait F (at rG = 0.2 and 0.4 respectively), to a case where J is twice as strongly correlated with M than is F (rG = 0.4 and 0.2 respectively). (Again, the attempt here is to utilize a range of theoretically-possible scenarios, rather than to pick a single empirical example, so that readers will have maximum latitude in finding their own 'most useful' case.) Of course, when rG(FM) is |0.2|, the absolute gain from field selection in both the one-stage and the two-stage examples is less than it is when rG(FM) is |0.4|. Relative to the 'conventional' one-stage approach, however, two-stage gain is affected in a predictable way. In situations where the early screening has a detrimental impact on gain from field-selection, a lower genetic correlation between J and M reduces expected gain from early-selection, and therefore decreases the detrimental impact; the two-stage approach 'looks better' relative to one-stage selection. In situations where early screening actually increases expected gain from field-selection, a stronger genetic correlation between J and M increases the magnitude of the positive effect, and two-stage selection becomes proportionally better relative to one-stage selection. Even under these altered scenarios the greatest two-stage gain is produced when rigorous selection is applied in the early-screening step.

It is important to note that the relationships and results discussed here are dependent only upon underlying biological relationships, and are independent of scale. If the scale of J, F or M is reversed, all of the relationships of that variable with other variables in equations 3-5 will be affected, and the combined effects will cancel one another. It is assumed in this analysis, however, that selection will be carried out appropriately relative to scale: in other, words, that when rG(JM) or rG(FM) is negative, the 'lowest value' entries for J or F will be selected, so that expected progress in M will always be positive.

DISCUSSION, RECOMMENDATIONS

Quantitative methods are most appropriately used not in *making* decisions, but rather as tools to augment the knowledge and experience of the decisionmaker. In the case of two-stage screening, the quantitative tools used in this analysis provide four guidelines:

. Early family screening for a seedling trait J, when followed by selection for a trait F in a conventional field-test, may actually lead to reduced total expected gain in a 'mature-stand' selection goal (M) from the two stages combined, compared to what would be expected from field selection alone. This will occur if the genetic correlations between J and M and F and M are of the same sign, and the early-screening trait is strongly positively correlated with the field-test trait ($rG > 0.4$), or alternatively, if rG(JM) and rG(FM) are opposite in sign and rG(JF) is strongly negative ($rG < -0.4$). In all other scenarios tested, two-stage selection provided at least as much and generally more total expected gain than one-stage 'field-only' selection.

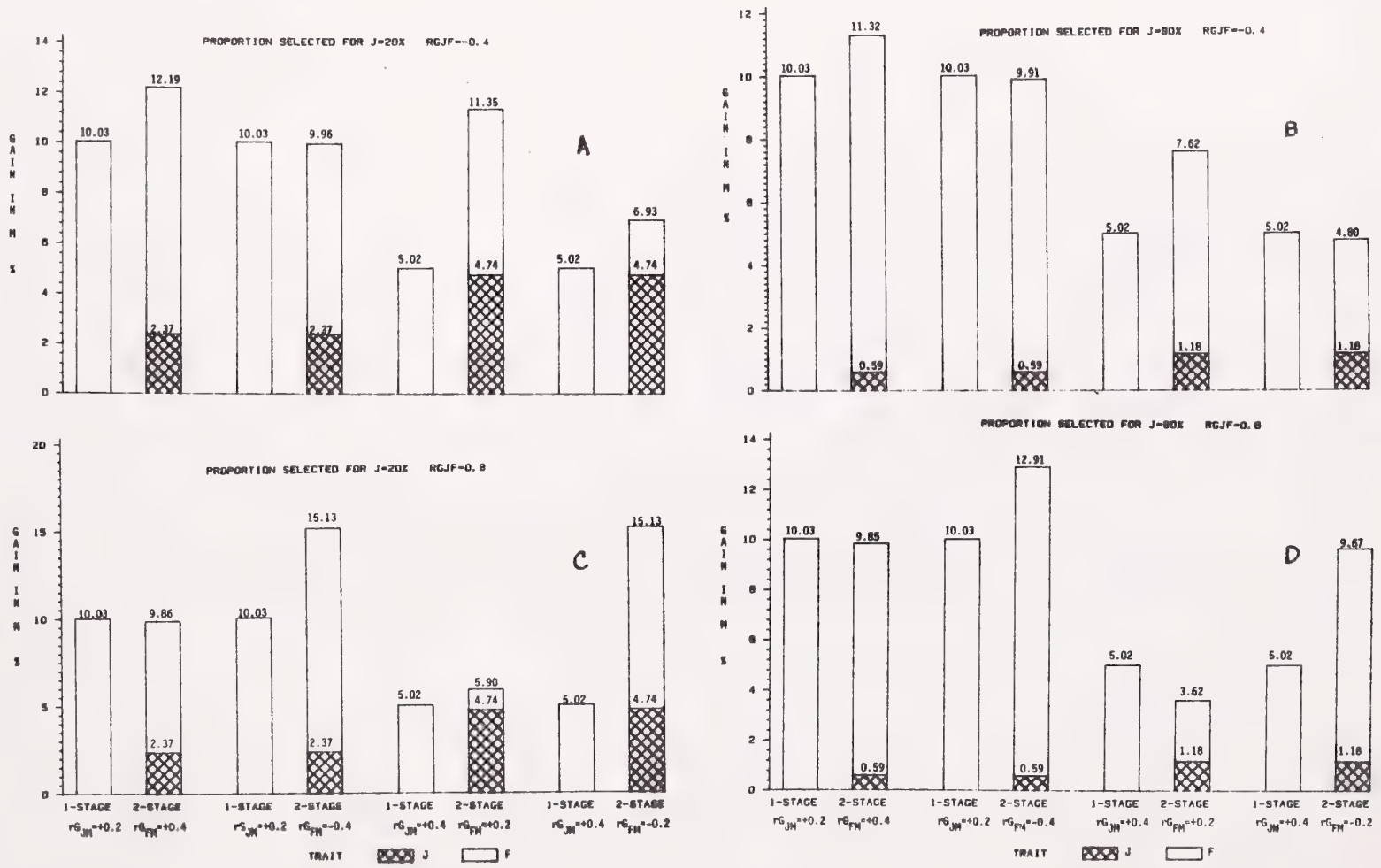

FIGURE 3. Predicted gain in a selection goal M resulting from two-stage selection, for an early-screening trait J followed by a field-test trait F: (a) when rG(JF) is negative and rigorous early selection is applied; (b) when rG(JF) is negative and weak early selection is applied; (c) when rG(JF) is strongly positive and rigorous early selection is applied; and (d) when rG(JF) is strongly negative and weak early selection is applied. (Assumptions and definitions are given in text and in Table 1.)

. If a two-stage selection program is assumed to require 1-2 more years to complete than a one-stage program, most two-stage scenarios tested yield less expected gain per year than one-stage selection, for the same rG(FM).

. The stronger the genetic correlation between J and M, the greater the gain from first-stage truncation, and the greater the resulting impact on second-stage gain. Under conditions where early screening is detrimental to field-test-selection progress, the detrimental impact is more severe when rG(JM) is increased, and less severe when rG(JM) is decreased in absolute value. On the other hand, when early screening increases expected field-test-selection progress, an increase in rG(JM) increases gain from both the first and the second-stage.

. Regardless of the scenario, if a two-stage selection approach is chosen, the greatest gain will result when strong selection pressure is placed on the early-screening traits - the resulting increase in gain from the early screening offsets the corresponding reduction in predicted gain from the later field-selection step.

Most tree-improvement decisionmakers operate with some 'feeling' for the general magnitude of interrelationships among selection-criteria, and between selection-criteria and selection-goals; in many cases, there are actual published relationships. Although experience-based parameter-estimates will not always be unbiased, they are likely to be sufficiently accurate to allow for decisionmaking based upon the above results. To ignore these guidelines is to implicitly assume that the correlations among early-screening and field traits are zero.

Because of today's grueling economic conditions, early-screening may appear very attractive as a means of reducing long-term field-testing costs. These results show, however, that two-stage selection may not always yield greater gain for the additional effort. The analytical methods discussed here allow the tree improver to choose the most appropriate relative magnitude of selection-emphasis to apply to different selection criteria throughout a given testing-cycle, to determine the maximum possible two-stage expected gain under the specific conditions suspected to be operating in the population of interest. It is critical that a careful economic analysis then be carried out to determine whether two-stage selection is a desirable course of action.

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DESIGN EFFICIENCIES WITH PLANNED AND UNPLANNED UNBALANCE FOR ESTIMATING HERITABILITY IN FORESTRY

Barbara G. McCutchan[1/]

Abstract.--Both balanced and unbalanced data can be analyzed for variance component estimation with Modified Maximum Likelihood estimates in a unified approach. Design efficiencies are evaluated for the estimation of heritability using this methodology, assuming knowledge of the variance components. Rules for obtaining efficient randomized block designs are established. The effect of number of blocks, plot size, number of families, variance on family size and total number of observations on design efficiency is examined across the range of heritability and under 100%, 90%, 80% and 60% survival.

Additional keywords: Modified Maximum Likelihood, Design allocation rules.

INTRODUCTION

One of the problems that the experimenter faces in forestry designs for the estimation of means and also variance components is the use of large blocks in balanced experimental designs. Large blocks necessitate employment of either a restricted set of environments where small plot variances can be found or the inclusion of block-type variation among plots within blocks. In this latter situation, the error in estimating family means is increased, effectively decreasing heritability.

Anderson (1975, 1981) and others suggest and evaluate intentionally unbalanced designs for the estimation of variance components. These planned unbalanced designs allow for the redistribution of the degrees of freedom to the variance components of interest. Various unbalanced two-way designs, useful for forest genetics trials of half-sib families with small randomized blocks, are also possible to design (McCutchan 1985).

A complicating factor in most forestry experiments, and one which makes design evaluation difficult, is that some level of unplanned loss occurs in a genetic trial subsequent to its establishment. Roughly 10% loss occurs in loblolly pine (Pinus taeda L.) genetic trials after one year of field growth, with up to 30% loss occurring by age 10, depending upon the incidence of fusiform rust (Cronartium quercuum f. sp. fusiforme) (R. J. Weir, pers. comm.). The efficiency of a design for the estimation of particular variance components or functions thereof, under states of loss, is of consequential interest.

1/ Quantitative Geneticist, Westvāco Forest Research, Summerville, SC. This research was conducted while the author was a Graduate Research Assistant at North Carolina State University, Raleigh, NC. The author graciously acknowledges Drs. Gene Namkoong and Francis Giesbrecht for their guidance with this research, the Statistics Department at North Carolina State University, Raleigh, for providing the necessary computing funds, Ronnie Hise for his drawing of the graphics, and the North Carolina State Hardwood Research Cooperative, Raleigh, NC, for releasing the 1981 sycamore data.

The analysis of unbalanced data--either planned or unplanned unbalance--is therefore important for two reasons: (1) after an experiment is completed, an efficient estimator is needed and (2) before an experiment is conducted, designs need to be analyzed for the possible efficiency with which parameter estimates will ultimately be made (Namkoong 1981). Unfortunately, the most commonly used analytical procedure, namely Henderson's Method 3 (1953), is ambiguous as to which sum of squares is most appropriate; such estimators retain only their unbiased property with unbalanced data. The maximum likelihood type estimators have been known theoretically, but have not been available practically. Giesbrecht (1983) has written an efficient algorithm by which Modified Maximum Likelihood (MML), Maximum Likelihood (ML) and MInimum Norm Quadratic Unbiased Estimates (MINQUE) can be computed. The MML approach is used for the remainder of this paper. The MML estimates, for which normality is assumed, are chosen because of their desirable properties regardless of the state of balance in the data. The estimates maximize the likelihood, use the same information as the full ML estimates do and account, in some sense, for the estimation of fixed effects; with balanced data, MML estimates are also those obtained through the analysis of variance (AOV), which is not true for ML estimates. The MML estimates are obtained by iterating the MINQUE. The MML method is a unified approach to the estimation of variance components and/or for comparing design efficiency.

The objective of this paper is to compare design efficiencies of planned balanced and unbalanced designs for the estimation of heritability (h^2). The unbalanced designs allow for the inclusion of a large number of families in relatively small blocks. The variance of the estimate of h^2 ($var(\hat{h}^2)$) from each design is compared to other designs across the range of h^2 and with 10%, 20% and 40% random loss of individuals. Design efficiency is examined over the range of h^2 to indicate the quality of the design at any level of realized h^2 or for multiple traits which may have different h^2 in the same experiment. The design structure studied is a randomized block design on one location; the treatments are unrelated half-sib families using either single-tree or two-tree contiguous plots. The variance components are assumed known which enables calculation of the variances of the variance components. An overview of the results from McCutchan et al. (submitted) and McCutchan (1985) is presented.

METHODOLOGY

The notation and the computational methodology for Modified Maximum Likelihood follow Giesbrecht's (1983). His procedure for variance component estimation is written as a temporary Statistical Analysis System (SAS™) program entitled Procedure MIXMOD.

The statistical model for each design considered is:

$$\underset{nx1}{Y} = \mu \underset{nx1}{1} + \underset{nxb}{U_B}\,\underset{bx1}{e_B} + \underset{nxf}{U_F}\,\underset{fx1}{e_F} + \underset{nxs}{U_P}\,\underset{sx1}{e_P} + \underset{nx1}{e_W}$$

where Y is the column vector of n observations; μ is the overall mean; U_B, U_F and U_P are design matrices pertaining to block, family and plot effects, respectively, with all elements equal to zero or one (where there are b blocks, f families and s filled combinations of the families and blocks); for

single-tree plots, U_P is the identity matrix of size n; e_B, e_F, e_P and e_W are independent column vectors of independent random variables, each with zero mean and variance-covariance matrix $I_b\sigma_B^2$, $I_f\sigma_F^2$, $I_s\sigma_P^2$ and $I_n\sigma_W^2$, respectively.

The variance-covariance matrix for the vector of observations (Y) is:

$$V(Y) = U_B U_B' \sigma_B^2 + U_F U_F' \sigma_F^2 + U_P U_P' \sigma_P^2 + I_n \sigma_W^2 ,$$

where σ_B^2, σ_F^2, σ_P^2 and σ_W^2 are the variance components due to the block, family, plot and within-plot effects, respectively. Letting $V_i = U_i U_i'$ and for convenience $V_W = I_n$, V(Y), based on the parameters, can be rewritten as:

$$V_{\sigma^2} = V(Y) = V_B\sigma_B^2 + V_F\sigma_F^2 + V_P\sigma_P^2 + V_W\sigma_W^2 . \qquad (1)$$

It is assumed that the f unrelated families chosen are a random sample of the reference population, that those trees planted are all to be assessed save for those lost and that blocks of different sizes are placed on different parcels of land such that for a given set of variance components $(\sigma_B^2\ \sigma_F^2\ \sigma_P^2\ \sigma_W^2)'$ different sizes of blocks and plots can be compared.

If the variance components (σ_i^2) were known, then the variance-covariance matrix for the resulting MML estimates of the components $(\sigma_B^2\ \sigma_F^2\ \sigma_P^2\ \sigma_W^2)'$, which would then be MInimum Variance Quadratic Unbiased Estimates, would be:

$$2\{tr(Q_{\sigma^2} V_i Q_{\sigma^2} V_j)\}^{-1} \qquad i,j = B,F,P,W \qquad (2)$$

where $Q_{\sigma^2} = V_{\sigma^2}^{-1} - V_{\sigma^2}^{-1} 1 (1' V_{\sigma^2}^{-1} 1)^{-1} 1' V_{\sigma^2}^{-1}$, and V_{σ^2} is defined in Eq. 1.

The dispersion matrix (Eq. 2) of the variance components is a function of the variance components themselves plus the design matrices (U_i). It is therefore possible to calculate this dispersion matrix for a given set of true variance components and a design. The observational values (Y) are not needed to calculate the dispersion matrix for the variance components. Heritability is calculated based on the experimental structure as:

$$h^2 = 4\sigma_F^2/(\sigma_F^2 + \sigma_P^2 + \sigma_W^2) = X/Y. \qquad (3)$$

Since variance component estimation is based on the experimental design in only one environment, any environment by additive genetic interaction variance is confounded with the estimates of the additive variance σ_A^2. Such an estimate is appropriate for inferences only on this site type. The variance of

the estimate of h^2 can be approximated by the variance of a ratio using a Taylor's series expansion:

$$\text{var}(\hat{h}^2) \approx (1/Y)^2\text{var}(\hat{X}) - 2(1/Y)(X/Y^2)\text{cov}(\hat{X},\hat{Y}) + (X/Y^2)^2\text{var}(\hat{Y}). \quad (4)$$

The computation of $\text{var}(\hat{h}^2)$ is based on the calculation of $\text{var}(\hat{X})$, $\text{cov}(\hat{X},\hat{Y})$ and $\text{var}(\hat{Y})$ from the dispersion matrix (Eq. 2). The values of X and Y are calculated from the set of variance component parameters. The approximation of $\text{var}(\hat{h}^2)$ relies on large sample theory.

The $\text{var}(\hat{h}^2)$ is calculated for each of the designs in Figure 1 for two types of variance component sets. Only the type $\sigma^2_F = \sigma^2_B$ is reported here, where $\sigma^2_B = 2$, $\sigma^2_W = 1$ and $\sigma^2_F = \sigma^2_P$ take on values of .5, .1, .05 and .005 for h^2 of 1.0, .33, .18 and .02, respectively. The actual assignment of the families to the blocks is not given, as it was found to be inconsequential in terms of design efficiency (McCutchan et al. submitted). In each design the effect of the random loss of 10%, 20% and 40% of the individuals on design efficiency is examined. The $\text{var}(\hat{h}^2)$ reported for cases of loss is actually an average of two independent samplings of individual loss.

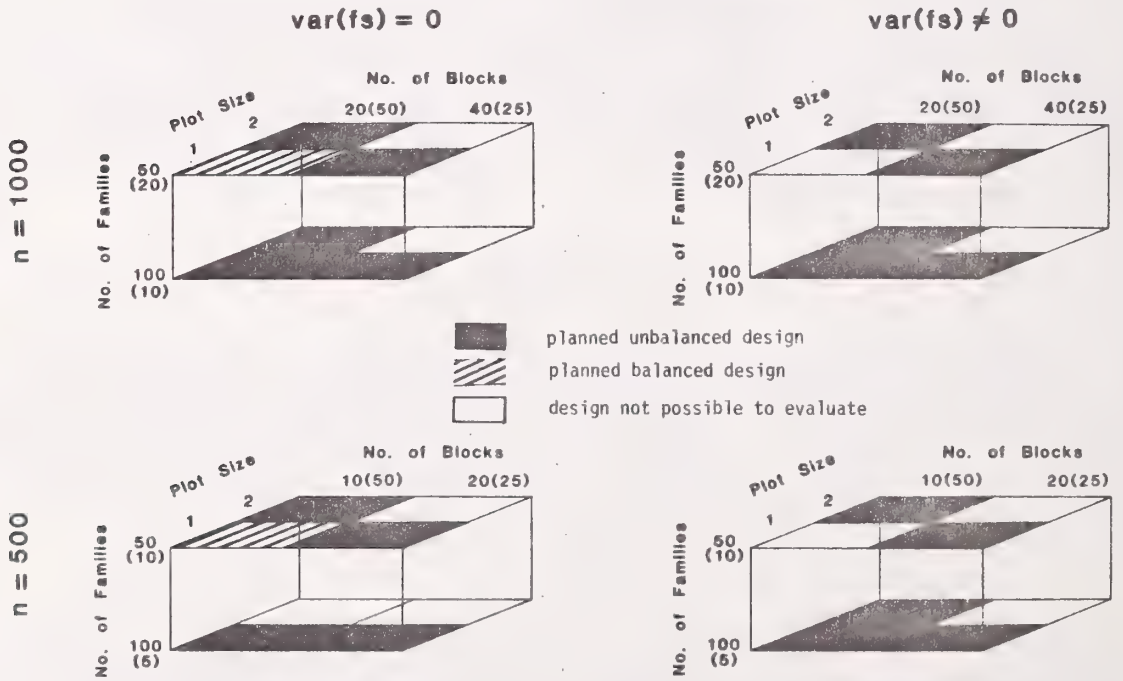

Figure 1. Schematic diagram of designs studied for 1000 and 500 observations (n), and for equal (var(fs)=0) and variable (var(fs)≠0) family sizes. The var(fs) is proportional to the mean family size and to n. The number in parentheses indicates the size of the effect; in the case of variable family size, the number is the mean family size.

The effects of block size, plot size, family size, variance of family size and total experimental size on design efficiency are each assessed at four levels of survival across the range of h^2. To examine any one effect, the $var(\hat{h}^2)$ from each of two designs, which differ only in their levels of this effect, are compared in a ratio. If the ratio is one, then one level of the effect is just as efficient as the other level. If the ratio is greater than one, then the design whose $var(\hat{h}^2)$ is in the numerator of the efficiency ratio is less efficient. In assessing the efficiency ratios for each effect, the buffering to loss is discussed as is the comparison of $var(\hat{h}^2)$ to a particular criterion (Namkoong and Roberds 1974). The criterion is established on a CV = 50% = $(\sqrt{var(\hat{h}^2)} / h^2) \times 100\%$ for $h^2 \geq .2$ and on $std(\hat{h}^2) = .10 = \sqrt{var(\hat{h}^2)}$ for $h^2 \leq .2$. Ideally, a design is sought whose $var(\hat{h}^2)$ profile falls beneath that of the standard across the range of h^2.

RESULTS AND DISCUSSION

All design efficiency ratios are computed based on a given set of variance components $(\sigma_B^2\ \sigma_F^2\ \sigma_P^2\ \sigma_W^2)$, regardless of block, plot or family size.

The effect of block size on design efficiency is illustrated in Table 1 for the 100-family single-tree plot (STP) design with 1000 observations. The ratio of the $var(\hat{h}^2)$ from the 20-block block design is less than that from the 40-block design across the range of h^2. The larger block design is uniformly more efficient than a design with more smaller blocks, given the same set of variance components.

The effect of random loss on such a comparison is shown in Table 2 for $h^2 = .33$. The ratio decreases with loss indicating that the larger block design is better buffered to loss. This is true also across the range of h^2. The specifics for other comparisons of the block effect are given by McCutchan (1985).

Table 1.--Design efficiency ratios as affected by number of blocks for 100% survival, 100 families, STP and n=1000

h^2	Number of Blocks	
	40	20
	$var(\hat{h}^2)$	$E_{b=20}$ [1/]
1.00	.0219	.982
.33	.0096	.977
.18	.0068	.975
.02	.0041	.973

Table 2.--Design efficiency ratios as affected by number of blocks for $h^2 = .33$, 100 families, STP and n=1000

Survival (%)	Number of Blocks	
	40	20
	$var(\hat{h}^2)$	$E_{b=20}$
100	.0096	.977
90	.0109	.974
80	.0128	.970
60	.0195	.958

[1/] $E_{b=20}$ is the ratio of the $var(\hat{h}^2)$ from the 20-block design divided by that from the 40-block design.

The initial motivation for examining the usefulness of smaller blocks was the observation that smaller homogeneous sites are more frequent than larger homogeneous sites. In these comparisons designs have been examined

for the same set of variance components over the range of h^2 and a range of random loss, regardless of block and plot size. The designs with larger blocks are 2% to 3% more efficient than those with 25-tree plots, and are better buffered to loss, given the same set of variance components. The practical application of these results includes consideration of the frequency at which these larger sites can be found. For a fixed n, fewer larger sites would be required than small sites; whether b large blocks could be found for a given site type, of course, depends upon the site. Considering the use of 20 blocks, the results show that by using designs with blocks half the size, a 2% to 3% loss in efficiency is incurred. (The loss in efficiency indicates the increase in $var(\hat{h}^2)$ in having used 40 blocks versus 20 blocks). This cost in efficiency has to be balanced against the cost of obtaining and maintaining half as many blocks, each of twice the size. This latter cost may include not only difficulties in locating such blocks, but also bias in representing planting sites.

The effect of plot size on design efficiency is illustrated in Table 3 with the comparison of a single-tree plot (STP) design to a two-tree plot (TTP) design given 100 families, 20 blocks and n=1000. The STP design is more efficient than that with TTP across the range of h^2 (Table 3), with the advantage in efficiency at 100% survival decreasing with h^2. The STP design remains more efficient with the imposition of random 10%, 20% and 40% loss (Table 4).

Table 3.--Design efficiency ratios as affected by plot size for 100% survival, 100 families, 20 blocks and n=1000

h^2	Plot Size	
	STP	TTP
	$var(\hat{h}^2)$	E_{TTP} 1/
1.00	.0215	1.2351
.33	.0094	1.1815
.18	.0066	1.1676
.02	.0039	1.1570

Table 4.--Design efficiency ratios as affected by plot size for h^2 = .33, 100 families, 20 blocks and n=1000

Survival (%)	Plot Size	
	STP	TTP
	$var(\hat{h}^2)$	E_{TTP}
100	.0094	1.1815
90	.0107	1.1768
80	.0124	1.1685
60	.0187	1.1675

1/ E_{TPP} is the ratio of the $var(\hat{h}^2)$ from the TTP design divided by that from the STP design.

The premise of using a TTP design is to protect the data set against loss of plots. An AOV can be used for balanced data on a plot mean basis. Loss of plots is not a computational or interpretative obstacle with the MML methodology. There is a statistical cost to using TTP, as observed here, which even at that fails to insure plot survival.

The large number of small family design is more efficient for 100% survival and high heritabilities than the small number of large family design (Table 5, $E_{f=50}$). This result is reversed for low heritabilities, where the larger family design is more efficient. The effect of random loss on the design efficiency is given in Table 6 by $E_{f=50}$ based on 10%, 20% and 40% random loss. At each h^2 given, the $var(\hat{h}^2)$ from the 50-family design increases

less than that from the 100-family design. The buffering capacity of the design to loss is greatly influenced with these size differences in families, there being roughly twice as much buffering capacity at $h^2 = 1.0$ for the 50-family design compared to the 100-family design, with this difference decreasing with h^2. This greater buffering capacity with 50-family designs is reflected in a decreasing $E_{f=50}$ with loss. The 50-family design, with this large difference in buffering capacity, becomes more efficient with 10% loss at $h^2 = .33$ in contrast to the block or plot effects. In neither of these designs are families lost through random loss of individuals.

Table 5.--Design efficiency ratios as affected by number of families for 100% survival, 40 blocks, STP and n=1000

h^2	Number of Families	
	100	50
	$var(\hat{h}^2)$	$E_{f=50}$ [1/]
1.00	.0219	1.476
.33	.0096	1.026
.18	.0068	.824
.02	.0041	.517

Table 6.--Design efficiency ratios as affected by number of families for $h^2 = .33$, 40 blocks, STP and n=1000

Survival (%)	Number of Families	
	100	50
	$var(\hat{h}^2)$	$E_{f=50}$
100	.0096	1.026
90	.0109	.982
80	.0128	.935
60	.0195	.830

1/ $E_{f=50}$ is the ratio of the $var(\hat{h}^2)$ from the 50-family design divided by that from the 100-family design.

The implications for design recommendations are that STP and large blocks provide low $var(\hat{h}^2)$ across the range of h^2, but that the family size that should be employed depends on the heritabilities of interest. The 100-family design is more efficient across a large portion of the range of h^2. If design allocations included only balanced designs, this efficient 100-family, 20-block design would not be a viable alternative. If all the traits of interest have low heritabilities, for example, less than .2, then a 100-family design would not be the most efficient design to use. The 50-family design would be more efficient in this range and have greater buffering to loss.

The effect of variable family size in contrast to equal family size on design efficiency is illustrated in Table 7 for 100 families of average size 10. The equal family size design is more efficient at $h^2 > .33$ than the variable family size design at 100% survival. The variable family size design is more efficient below this level of h^2, increasingly so as h^2 decreases. These results confirm the suggestion (McCutchan et al. submitted) that increased variance on family size might result in increased efficiencies for low h^2. They found that for 100 families of average size 10, the design with var(fs) = 7 based on a binomial distribution with mean 10 was 2% less efficient at $h^2 = 1.0$ than the equal family size design. The variable family size design became more efficient at $.25 > h^2 > .21$ than the equal family size design, having a $var(\hat{h}^2)$ 6% less than that of the equal family size design at $h^2 = .02$. Variance of family size equal to 60 is examined here. The variable family size design is less efficient at $h^2 = 1.0$, 21% higher $var(\hat{h}^2)$, and more efficient at $h^2 = .02$, 35% lower $var(\hat{h}^2)$, than the equal family size design.

In addition to the 100% survival case studied by McCutchan et al. (submitted), the effects of 10%, 20% and 40% random loss on this comparison are given (Table 8). The variable family size design is better buffered to loss at heritabilities other than 1.0. The buffering is such that with 40% loss at $h^2 = .33$, the variable family size design becomes more efficient.

Table 7.--Design efficiency ratios as affected by variance on the family size for 100% survival, 40 blocks, STP, 100 families and n=1000

h^2	Variance of Family Size 0 $\text{var}(\hat{h}^2)$	Variance of Family Size 60 $E_{V=60}$ [1/]
1.00	.0219	1.2067
.33	.0096	1.1052
.18	.0068	.9596
.02	.0041	.6534

Table 8.--Design efficiency ratios as affected by variance on the family size for $h^2 = .33$, 40 blocks, STP, 100 families and n=1000

Survival (%)	Variance of Family Size 0 $\text{var}(\hat{h}^2)$	Variance of Family Size 60 $E_{V=60}$
100	.0096	1.1052
90	.0109	1.0749
80	.0128	1.0432
60	.0195	.9633

[1/] $E_{V=60}$ is the ratio of the $\text{var}(\hat{h}^2)$ from the variable family size design (with variance equal to 60) divided by that from the equal family size design.

The variable family size effect on design efficiency is an extended version of the family size effect. Use of variation on the family size effectively increases the average family size through an asymmetric effect of the larger families. The deliberate use of variable family size can be viewed, consequently, in a similar light as family size, in that its use depends upon the portion of the range of h^2 in which interest in estimation lies. If family sizes are unequal due to differential fecundity or survival, then for an average family size $\bar{n}$, a variable family size design will actually be more efficient at the lower range of h^2 than an equal family size design.

A general comment can be made concerning n=1000 and n=500 designs in relationship to the criterion. Only at the 60% survival level for either $h^2 = .18$ and/or $h^2 = .02$ are $\text{var}(\hat{h}^2)$ values from the 1000-observation designs greater than the criterion. However, values from the 500-observation designs are generally greater than the criterion for all levels of survival at $h^2 = .33$, .18 and .02.

As an example of evaluating a given design for the estimation of h^2, h^2 and $\text{var}(\hat{h}^2)$ are estimated from a North Carolina Forest Service installed American sycamore (Platanus occidentalis L.) mother tree trial. The experiment, located in McDowell County, N.C., has 7 blocks, 30 half-sib families in 10-tree row plots and a total of 1866 surviving trees (89% survival). Variance components were estimated on eight-year-old height data (ft.), converging in one iteration: $\hat{h}^2 = .25$ and $\hat{\text{var}}(\hat{h}^2) = .01$. The estimated variance on h^2 is less than that suggested by the standard, namely .016. The results of the research show that for a design established primarily for the estimation of h^2, an equivalent level of $\text{var}(\hat{h}^2)$ can be obtained with half as many total observations as in this trial.

CONCLUSIONS

Utility of efficient Modified Maximum Likelihood estimators is afforded by recent computational methodology. Both balanced and unbalanced data can be analyzed for variance component estimation in a unified approach. Design efficiencies are evaluated for the estimation of heritability using this methodology and assuming knowledge of the variance components. Rules for randomized block design allocation are established based on using the same set of variance components regardless of block, plot or family size. Single-tree plots in large blocks are recommended if the plots within blocks have small homogeneous variances--smaller blocks if the above is not possible. Recommendation of a particular family size depends on the portion of h^2 range in which estimation interest lies. Five hundred observations are insufficient to achieve the set standard on estimating h^2. One thousand observations will achieve this standard if survival is at least 80%. The rules indicate that there is not one design allocation which will uniformly provide a low var($\hat{h}^2$) across the range of h^2.

The research has based design efficiency on the estimation of h^2. Heritability is but one function of the variance components; the methodology is laid out for the examination of other functions of variance components. This sort of a priori examination of design efficiency offers the experimenter a strong tool in achieving experimental design objectives.

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WITHIN-TREE VARIATION IN CORTICAL MONOTERPENES OF SLASH PINE

Susan V. Kossuth and H. David Muse

Abstract.--Cortical monoterpene composition, bud diameter, and length of the current flush from all buds (545) on a 10- to 12-year-old grafted, high gum-yielding Pinus elliottii Engelm. (slash pine), were analysed to determine within-tree variability. Approximately equal numbers of high-, medium- and low-vigor buds were sampled from the upper, middle, and lower crown position from the north and south aspects in the spring, summer, fall, and winter. No north or south differences were found. Beta-pinene content was significantly greater in the spring than in the other seasons and the inverse was true for α-pinene and β-phellandrene. Alpha-pinene content was significantly greater in buds from the lower part of the crown. Beta-pinene and β-phellandrene did not vary with crown position. Alpha-pinene content decreased from low- to high-vigor buds and the opposite was true for β-phellandrene. Beta-pinene content was highest in low-vigor buds. Bud diameters and lengths of the flushes decreased progressively in size from the upper to lower crown, and from high- to low-vigor buds. Bud diameters were similar among seasons, and lengths of the current flushes were slightly longer in the spring than summer.

Additional keywords: terpene, oleoresin, gum, clone, ramet, seed orchard, α-pinene, β-pinene, β-phellandrene, limonene, myrcene, α-phellandrene, Pinus elliottii.

1/ Supervisory Research Geneticist and Station Statistician, respectively, USDA Forest Service, Southeastern Forest Experiment Station, 1143 Fifield Hall, University of Florida, Gainesville, FL 32611, and Forestry Sciences Laboratory, Carlton Street, Athens, GA 30602.

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INTRODUCTION

Analyses of the composition of monoterpenes in conifers has been a useful tool for identifying the geographic source of seed in plantations, identifying hybrids and inbreeding, and for identifying ramets in seed orchards (Squillace 1976; Kossuth and McCall 1984). Damage to trees from grazing animals and resistance to insects and disease have also been linked to the monoterpene composition of trees (Squillace 1976). In an early study it was suggested that before large-scale studies of monoterpene composition are undertaken, the best place to sample on a tree should be determined so that repeated sampling would give consistent results (Hanover 1966) for individual tree phenotypes. The objectives of this study were to determine the effects of season, aspect, position in crown, and bud vigor on monoterpene composition of one slash pine (Pinus elliottii Engelm.) ramet.

METHODS

One 10- to 12-year-old grafted ramet of high gum yielding slash pine clone number 335 in a seed orchard at the USDA Forest Service, Southeastern Forest Experiment Station, Olustee, Florida, was used for the study. It was 12.9 m tall, and had a live crown height of 9.8 m.

The ramet was divided into north and south aspects, and these were subdivided into three equal lengths of crown for upper, middle, and lower position. In the spring, all the buds within each of the six sections on the ramet were classified as high, medium, or low vigor. A count was made of the number of buds in each vigor class by section and ramet. One-fourth of the buds from each section on the tree were sampled for cortical monoterpenes in the spring (5/5/82), summer (7/12/82), fall (10/6/82) and winter (1/12/83). All 545 buds were sampled.

Samples were taken by removing approximately the terminal centimeter of the bud, collecting the cortical oleoresin that flowed out, and immediately placing it in vials containing pentane. If the quantity of oleoresin was low, the bud--with bud scales removed--was extracted in the pentane. Gas-liquid chromatographic analysis was conducted according to the method of Kossuth and Munson (1981) by using a 5840-A Hewlett-Packard gas chromatograph with an automatic sample injector and programmable integrator. The amount of each monoterpene is presented as a percentage of the total monoterpenes since this method has been shown to have the least variation (Powell and Adams 1973). At the time of sampling, each bud diameter was measured. The length of the current flush for the bud sampled was measured in the spring and summer only.

The percentage data was subjected to an arcsin square root transformation and analysed by analysis of variance (ANOVA) and Duncan's multiple range test. If significant interactions were present, then additional ANOVA was performed on each level of each factor involved to further investigate main effects. Higher order interaction mean squares were used to approximate associated error terms for each analysis.

RESULTS

The average monoterpene content for the tree was 30.8, 43.0, and 21.6 percent α-pinene, β-pinene, and β-phellandrene, respectively. Of the other constituents in the monoterpene fraction, camphene, myrcene, α-phellandrene and limonene each contributed less than 4 percent and were not analysed statistically. These low levels are probably of little physiological significance. Alpha-pinene, β-pinene and β-phellandrene contents ranged from 24.5-47.7, 18.2-54.5, 11.1-31.9 percent, respectively.

Overall, the content of the three major monoterpenes showed no significant differences for the north and south aspects (Table 1).

Table 1. Significance levels for monoterpene composition, bud diameter, and length of flush determined by analysis of variance, by source of variance.

Source of variance	α-pinene	β-pinene	β-phellandrene	Bud diam.	Flush length
Aspect					
Position	*			***	**
Season	**	**	**		
Aspect x season					
Position x season					
Vigor	****	***	***	****	****
Vigor x aspect					
Vigor x position				***	****
Vigor x season					

*, **, ***, **** indicate significance differences at the 0.05, 0.01, 0.001, and 0.0001 levels, respectively.

Alpha-pinene content increased progressively with decreasing bud vigor and the opposite was true for β-phellandrene (Table 2). Beta-pinene content was significantly lower in the high- and medium-vigor buds than in the low vigor buds. Alpha-pinene and β-phellandrene were significantly lower in the spring than in the other seasons, and the inverse was true for β-pinene for high- medium- and low-vigor buds (Table 3).

Alpha-pinene content was higher in the lower part of the crown than in the middle and upper crown. Beta-pinene and β-phellandrene content did not vary with crown position (Table 2).

Bud diameter and current flush length of the buds sampled decreased from high to medium to low-vigor buds, and from the upper to the lower crown (Table 2, 4,). Bud diameter and flush length varied significantly with position in the crown and bud vigor (Table 1). There was a significant interaction of bud vigor and crown position for both bud diameter and flush

TABLE 2. Cortical monoterpene composition, bud diameter, and current length of flush for buds collected from grafted high gum-yielding slash pine ramet number 335, by aspect, bud vigor, season, and position in crown.

Source	No.	α-pinene	β-pinene	β-phellandrene	Bud Dia.	Flush lgth.	No.
		Percent			mm	cm	
Overall	545	30.8± 2.4	43.0± 3.8	21.6± 3.7	6.8± 1.6	13.5± 8.8	259
Range		24.5-47.7	18.2-54.5	11.1-31.9	3.0-13.0	1.7-63.7	
Aspect							
North	280	31.0± 2.3a	43.4± 3.5a	21.2±3.7a	6.6± 1.6a	13.0± 9.1a	144
Range		25.2-40.2	34.2-54.0	11.2-31.9	3.0-13.0	1.7-63.7	
South	265	30.6± 2.5a	42.6± 4.0a	22.1± 3.8a	7.1± 1.7a	14.3± 8.5a	115
Range		24.5-47.7	18.2-54.5	11.1-30.5	4.0-13.0	3.1-52.2	
Vigor							
High	62	29.9± 2.2c	42.1± 3.7b	23.2± 3.5a	9.2± 1.6a	27.5±14.1a	24
Range		25.2-34.9	34.2-54.5	14.6-31.9	5.0-13.0	11.8-63.7	
Medium	335	30.6± 2.2b	42.6± 3.6b	22.3± 3.4a	7.2± 1.0b	14.5± 5.6b	94
Range		24.5-37.6	32.1-54.5	12.8-30.5	4.0-11.0	1.7-38.5	
Low	148	31.7± 2.8a	44.3± 4.0a	19.5± 3.8b	5.0± 0.9	6.5± 3.5c	75
Range		25.1-47.7	18.2-54.0	11.1-29.5	3.0- 8.0	2.7-26.3	
Season							
Spring	120	28.9± 2.4b	47.1± 2.7a	18.2± 2.1b	6.8± 1.4a	14.5± 7.4a	120
Range		24.5-36.3	40.2-54.5	11.1-24.8	4.0-10.0	3.1-47.6	
Summer	138	31.0± 2.7a	41.1± 3.1b	23.1± 4.4a	6.7± 1.8a	12.9± 9.8a	138
Range		26.1-40.2	34.2-51.2	11.9-31.9	3.0-13.0	1.7-63.7	
Fall	137	31.0± 1.5a	42.3± 3.2b	22.6± 3.2a	7.0± 1.9a	--	
Range		27.8-36.9	35.7-52.5	12.9-29.5	3.0-13.0	--	
Winter	150	31.9± 2.1a	42.0± 3.3b	22.1± 3.0a	6.7± 1.3a	--	
Range		29.1-47.7	18.2-50.2	11.8-28.4	4.0-12.0	--	
Position							
Top	171	30.5± 3.0b	42.7± 4.1a	22.2± 3.6a	7.8± 1.8a	20.5±12.0a	74
Range		24.5-47.7	18.2-54.5	12.9-30.5	4.0-13.0	1.7-63.7	
Middle	227	30.7± 2.0b	43.5± 3.6a	21.1± 3.8a	6.6± 1.4b	10.9± 5.1	113
Range		25.9-37.8	34.2-54.0	11.2-31.9	4.0-11.0	3.0-25.3	
Lower	147	31.2± 2.3a	42.6± 3.7a	21.7± 3.9a	6.0±1.3c	10.7±4.7c	72
Range		25.9-40.2	34.8-50.4	11.1-29.5	3.0-9.0	2.7-26.3	

Mean ± standard deviation. Column values within groups followed by different letters are significantly different at the 0.05 level.

TABLE 3. Cortical monoterpene composition, bud diameter, and length of flush for buds collected from one slash pine ramet, by bud vigor and season.

Bud vigor and season	No.	α-pinene	β-pinene	β-phellandrene	Bud Dia.	Flush lgth.
		. . . . . Percent			mm	cm
High						
Spring	12	27.2± 1.5	47.3± 2.8	18.7± 1.9	9.1± 0.9	27.2± 9.6
Range		25.2-29.3	43.1-54.5	14.6-21.5	8.0-10.0	15.7-47.6
Summer	15	29.5± 2.1	39.2± 2.3	26.4± 2.4	9.5± 1.9	27.8±17.2
Range		26.1-33.9	34.2-42.0	23.1-31.9	7.0-13.0	11.8-63.7
Fall	16	30.0± 0.8	41.5± 3.3	24.2± 2.7	9.6± 2.1	--
Range		28.6-32.1	37.8-49.2	18.4-27.5	5.0-13.0	--
Winter	19	31.9± 1.5	41.7± 1.8	22.5± 2.1	8.6± 1.2	--
Range		29.8-34.9	39.4-45.7	19.3-26.3	7.0-12.0	--
Medium						
Spring	73	28.8± 2.2	46.8± 2.8	18.8± 1.7	7.1± 0.9	15.4± 4.7
Range		24.5-34.7	40.2-54.5	15.3-24.8	5.0-10.0	5.3-32.1
Summer	84	30.5± 2.4	40.8± 3.0	24.0± 3.9	7.1± 1.0	13.8± 6.2
Range		26.4-37.6	36.2-48.7	12.8-30.5	5.0-11.0	1.7-38.5
Fall	79	30.8± 1.3	42.0± 3.1	23.1± 2.8	7.6± 1.2	--
Range		27.8-30.0	35.7-52.5	14.9-28.5	5.0-10.0	--
Winter	99	31.7± 1.7	41.5± 2.2	22.8± 2.5	7.0± 0.8	--
Range		29.3-37.4	32.1-46.6	15.9-28.1	4.0-10.0	--
Low						
Spring	35	29.7± 2.6	47.6± 2.4	17.0± 2.3	5.4± 1.2	8.3± 4.1
Range		25.0-36.3	43.3-54.0	11.1-21.3	4.0- 8.0	3.1-26.3
Summer	39	32.7± 2.6	42.6± 3.1	20.1± 4.4	4.7± 0.8	5.1± 1.4
Range		27.9-40.2	35.5-51.2	11.9-27.7	3.0- 7.0	2.7- 8.5
Fall	42	31.7± 1.8	43.4± 3.1	20.9± 3.5	5.0± 0.8	--
Range		28.0-36.9	35.8-51.0	13.0-30.0	3.0- 7.0	--
Winter	32	32.4± 3.1	44.0± 5.4	19.7± 3.7	5.0± 0.6	--
Range		29.1-47.7	18.2-50.2	11.8-28.4	4.0- 6.0	--

Mean ± standard deviation.

Table 4. Diameter and flush length of buds by bud-vigor class and position in crown.

Source of variance	Bud diam. (mm)	Flush length (cm)
VIGOR		
High x		
Upper	10.3a	39.1a
Middle	8.8b	20.7b
Lower	7.6b	16.1b
Medium x		
Upper	7.9a	19.8a
Middle	7.1b	13.2b
Lower	6.5b	11.3b
Low x		
Upper	5.8a	9.41a
Middle	4.9b	5.7a
Lower	4.5b	5.7a
POSITION		
Upper x		
High	10.3a	39.1a
Medium	7.9b	19.8b
Low	5.8c	9.4c
Middle x		
High	8.9a	20.7a
Medium	7.1b	13.2ab
Low	4.9c	5.7b
Lower x		
High	7.6a	16.1a
Medium	6.5b	11.4b
Low	4.5c	5.7c

Column values in each group followed by different letters are significantly different at the 0.05 level.

length (Table 1). Further analysis by bud vigor to determine position effects showed that any conclusions about these effects depended on bud vigor (Table 5). However, analyses by position to determine bud vigor

Table 5. Significance levels of bud diameter and flush length determined by analysis of variance of effects of bud-vigor class on position in crown, and of position effects on vigor.

Variable	Bud diam.	Flush length
Vigor effects on position		
Upper	****	*
Middle	****	*
Lower	****	**
Position effects on vigor		
High	**	**
Medium	*	*
Low	**	--

*, **, ***, **** indicates significantly different at the 0.05, 0.01, 0.001, and 0.0001 levels.

effects showed that conclusions about position effects can be made without regard to bud-vigor considerations. High-, medium- and low-vigor buds tended to be larger in the upper crown and decrease toward the lower crown (Table 4). Flush lengths followed the same general pattern.

DISCUSSION

The seasonal differences in monoterpene composition found in this study are consistent with those found in P. taeda L. (Rockwood 1973) and in Picea glauca (Moench) Voss, P. pungens Engelm., P. mariana (Mill.) B.S.P., and Abies balsamea (L.) Mill., when several samples from each tree were combined for each determination (vonRudloff 1972, 1975a, 1975b; vonRudloff and Granat 1982). Sampling in the spring seems to result in the most variation and should be avoided. Based on the standard deviations and ranges for the monoterpenes, β-pinene and β-phellandrene vary the most, and α-pinene is the most stable.

Because of the significant differences in monoterpene composition associated with bud vigor and the lack of any interaction with season, trees

should be sampled by using buds of the same vigor. Because low- vigor buds are small and difficult to sample there is a greater chance of getting some xylem oleoresin, which tends to be lower in β-phellandrene (Squillace and Fisher 1966) in the sample. Sampling is easiest from the lower crown where more low-vigor buds are found. Although α-pinene was somewhat higher in content here, the differences were not large. Kossuth and Muse (1985)[2/] determined that combining five buds is adequate to ensure an error of, at most, 5 percent in phenotypic determinations of the three monoterpene concentrations with a 95 percent confidence limit. Based on the data in this study, it is recommended that sampling for determining individual tree phenotypes be done in the summer, fall, or winter from the lower crown from buds of similar vigor or size, preferably large buds.

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FIELD PERFORMANCE OF LOBLOLLY PINE TISSUE CULTURE PLANTLETS

L. John Frampton, Jr., Ralph L. Mott and Henry V. Amerson[1/]

Abstract.--Loblolly pine tissue culture plantlets of cotyledon origin were compared to seedlings from the same half-sib families after three growing seasons in the field. Early growth in the field was slower for the plantlets than the seedlings although the plantlets appeared more resistant to fusiform rust. Morphologically, the plantlets appear more mature than the seedlings. Further studies to understand and manipulate these differences are underway.

Additional Keywords: Pinus taeda, vegetative propagation, tree improvement.

INTRODUCTION

A major potential benefit of tissue culture to forestry operations is its use as a method of vegetative propagation of elite genotypes from tree improvement programs. Seed for planting stock produced from currently applied seed orchard technology captures only additive genetic effects; however, commercial propagation of planting stock via tissue culture techniques could utilize all (additive and nonadditive) genetic effects. In loblolly pine (Pinus taeda L.), such technology could conservatively increase genetic gains by one-third to one-half (McKeand 1981). Based on published estimates of genetic variance components (McKeand et al. 1985), genetic gains for some traits such as volume growth and disease resistance may double by employing tissue culture technology.

In addition to greater genetic gains, a large decrease is expected in the length of time between selection of improved individuals and production of planting stock from tissue culture as opposed to seed orchard propagation. Loblolly pine seed orchards require a minimum of eight to ten years from grafting until large-scale seed production begins. Hopefully, tissue cultured propagules from select trees could be mass-produced one or two years following selection. Therefore, tissue culture technology offers not only the opportunity to capture greater genetic gains, but also to utilize this genetic gain earlier than with conventional seed orchard technology (McKeand and Frampton 1984, Amerson et al. 1985).

These and other (Durzan and Campbell 1974, Mott 1981, Sommer and Brown 1979) benefits of tissue culture have great potential, but the technology

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[1/]Assistant Professor, Dept. of Forestry, Professor, Dept. of Botany and Assistant Professor, Dept. of Forestry and Botany, respectively, North Carolina State University, Raleigh, N. C. The laboratory production of plant material by the technical support staff of Dr. Henry V. Amerson is gratefully appreciated.

necessary for operational propagation of loblolly pine via tissue culture is not yet available. Ultimately, embryogenesis or organogenesis from callus or cell suspensions are desired to facilitiate mechanization of propagation for mass-production as well as integration with molecular genetic biotechnology. Currently, organogenic propagation of loblolly pine from needle fascicles (Mehra-Palta et al. 1977), cytokinin-treated winter dormant buds (Abo El-Nil 1982) and cotyledon explants (Mott and Amerson 1981) is possible. While the technology to propagate from other sources is less reliable, clonal propagation of loblolly pine from cotyledon explants on a research scale is routine. The use of cotyledons as starting material offers less genetic advantage than propagation from older trees of proven genetic value. However, until more reliable methods of propagation from older trees are available, studies of the field performance of tissue culture plantlets from cotyledon origin will be useful in identifying and understanding general problems associated with tissue culture propagation and facilitate amelioration of these problems when other propagation systems are employed.

For this reason, the Project on Tissue Culture of the Southern Forest Research Center at North Carolina State University has established a series of field plantings containing tissue culture propagules of cotyledon origin. This paper compares the growth and development of these plantlets with seedlings after three growing seasons in the field.

METHODS

Laboratory

The propagation system used to establish these studies was reported in 1981 (Mott and Amerson) and involves the timely application and removal (i.e., pulsing) of growth regulators to progress from shoot initiation through rooting. Although this sequence has been and continues to be improved, a summary of the process follows. Basal media used in this study was BLG (Brown and Lawrence 1968) with glutamine (10 mM) substituted for NH_4 and NO_3 and 10 mM Kcl added, or GD (Gresshoff and Doy) based media diluted by one-half (Mott and Amerson 1981).

Seeds scarified at the micropylar end were partially germinated in hydrogen peroxide (typically three days in 1% aqueous H_2O_2 followed by one to two days in 0.03% H_2O_2 at 28-30°C). Subsequent to seed coat removal and surface sterilization, the embryos were aseptically excised from the female gametophyte. Next the cotyledons were surgically removed and planted horizontally on a shoot initiation medium (BLG) which was cytokinin-rich [typically 44μM benzylaminopurine (BAP)]. Cotyledons were maintained on this medium for 14 to 28 days. On this medium, cell divisions occurred in the peripheral areas of the cotyledons producing a warty, meristematic surface. Cotyledons were removed from this medium prior to the actual observance of shoots and placed on a hormone-free ($GD_1 \cdot 1/2$) medium containing charcoal to further aid cytokinin removal. On this medium, shoot apices became recognizable on the cotyledons and the shoots began to elongate. Shoot growth continued during further monthly subcultures on hormone-free (BLG) medium. The multiple shoots crowded on the cotyledons were individually excised and separated from the cotyledon for further growth.

Following growth, shoots about 1-2 cm in length were transferred to auxin-rich ($GD_1 \cdot 1/2$) medium [typically, α-Naphthaleneacetic acid (NAA) at

2.5μM]. Shoots were freshly cut at the base, implanted upright and pulsed for six to nine days on the auxin medium. Pre-root cell divisions formed near the cambial region at the stem base, resulting in a swollen, callusy region. To facilitate organization and rapid root growth, the shoots were transferred to hormone-free ($GD_1 \cdot 1/2$) medium. Plantlets typically were ready for transfer to greenhouse soil three to five weeks after the root initiation treatment.

Greenhouse

Plantlets were transferred from the agar medium to the greenhouse when their total shoot lengths (including needles) exceeded 1-2 cm and their individual root lengths exceeded 3-4 mm. Plantlets meeting these requirements were carefully removed from the agar medium and planted in 164 cc RL Super Cells containing a fine textured mix of peat, vermiculite, and perlite (2:2:1). The plantlets were grown in a mist bench the first three to six weeks in the greenhouse. After the first week, they were fertilized three to five times weekly with Peters 15-30-15 mixed at 30 ppm N. Plantlets in the mist bench were sprayed weekly with a fungicide, Captan, to reduce damping off and other disease problems. When necessary, the photoperiod of the plantlets was extended to 16 hours using incandescent lights (approximately 4 Wm^{-2}) to prevent dormancy.

Although the initial growth of the plantlets in the greenhouse was very slow, after about six weeks, new vigorous growth appeared. At this time, plantlets were removed from the mist bench and fertilization was changed to Peters 20-19-18 mixed at 40 ppm N applied three to five times weekly. After removal from the mist bench, plantlets were watered as needed with pH 5.5 water. Generally, plantlets reached a suitable size for field planting (about 20-30 cm in height and 3-5 mm in caliper) after six months in the greenhouse.

Using similar procedures, seedlings were also grown in the greenhouse to use in field tests for comparison purposes. Seedlings generally required only four months to attain plantable size so that it was often necessary to manipulate the watering and fertilization regime of the plantlets and seedlings in order to coordinate their growth.

Before field planting, both the plantlets and seedlings were gradually adapted to conditions outside the greenhouse. The succulent growth was hardened-off by first stopping fertilization and reducing watering. Next, the trees were transported outside for two to four weeks in order to adapt to direct sunlight, natural photoperiod and outdoor temperatures.

Field

The field tests were carefully site-prepared, hand-planted and intensively managed. At the time of establishment, a soil analysis was conducted and any nutrient deficiencies were corrected. Additionally, approximately 50 g N usually in the form of ammonium nitrate was applied to every tree during the spring to enhance growth. Weeds in the plantings were controlled either by periodic mowing or with herbicides. Nantucket pine tipmoth (Rhyacionia frustrana Comst.) which often kills young loblolly pine shoot tips was controlled with Furadan applications. Spacing was typically 3.05 x 3.05 m.

The North Carolina State University Project on Tissue Culture has established 16 field plantings across the Southeast (Figure 1). Over 3000 trees each of plantlets and seedlings have been planted representing over 25 half-sib families of loblolly pine. Results discussed in this paper will be limited to the first series of eight plantings that were established in 1981 with brief mention of results from two of the plantings established in 1982.

All of the 1981 field plantings contain paired row-plots of plantlets and seedlings from several half-sib families. The plantlets in a plot represent one clone produced from the cotyledons of a single embryo. The trees of the seedling plots were grown from seed of the same half-sib family from which the plantlets were derived. The 1981 field plantings contained from 16 to 49 plots. Plot size varied from two to 46 trees depending on the number of plantlets produced in a clone. Planting size varied from 158 to 324 trees. Several of the field plantings established after 1981 in addition to row-plots contain clonal block plots of 16 or 25 plantlets compared to block plots of seedlings from the same half-sib family.

Total height and the incidence of fusiform rust (caused by Cronartium quercuum (Berk.) Miyabe ex Shirai f. sp. fusiforme (Cumm.) Burds. et Snow) were recorded annually in all field tests. Additional measurements of morphological characteristics were also made in some of the plantings.

Figure 1. Location of the North Carolina State University Tissue Culture Project's loblolly pine field plantings.

Data Analysis

Analyses of variance were utilized to determine differences between plantlets and seedlings at each location. Although the field design necessitated a different model for some plantings, height measurements at most locations were analyzed using the following sources of variation: 'plant type' (plantlets verus seedlings), 'family', ' plant type x family', 'plot(plant type x family)' and 'tree(plot(plant type x family))'. Incidence of fusiform rust was analyzed on plot means employing a similar model. Plant type differences in the analysis of variance were tested using the 'plant type x family' interaction as the error term. Plant type differences across all locations were tested by a paired t-test where the difference between plant type means at each location was weighted by its number of observations.

RESULTS AND DISCUSSION

Height Growth

Average second year survival exceeded 94 percent for both the plantlets and seedlings in the eight 1981 field studies. Third year height averaged 2.72 and 3.38 m, respectively, for the plantlets and seedlings (Table 1). The plantlet height averaged 74 to 84 percent of the seedling height and except for one location was statistically ($P \leq 0.05$) shorter than that of the seedlings.

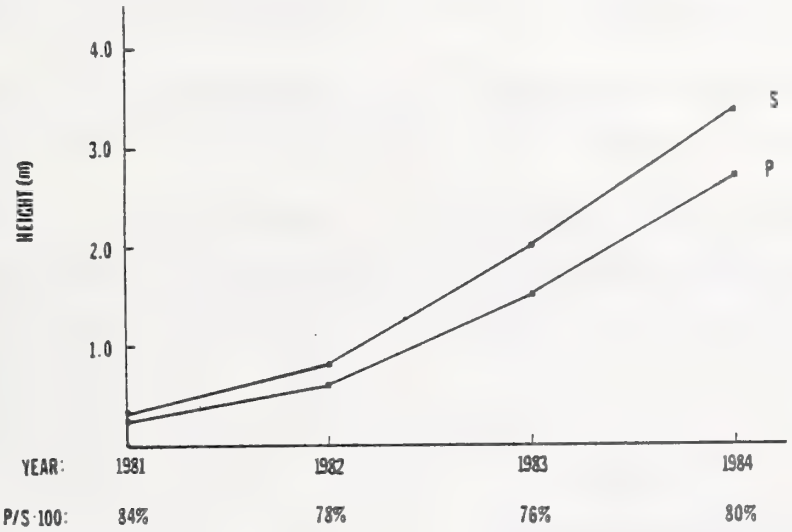
Table 1.--Mean third year heights of the eight field plantings established in 1981 by the North Carolina State University Tissue Culture Project.

Organization	Location	Total Height(m) Year 3		
		P	S	P/S*100
Federal Paper Board Co., Inc.	Lumberton, NC	2.96 *	3.95	75
Brunswick Pulp Land Co.	Jesup, GA	4.21 *	5.14	82
Westvaco Corp.	Summerville, SC	3.45	3.98	87
Scott Paper Co.	Monroeville, AL	3.31 *	3.99	83
N. C. State University	Raleigh, NC	1.38 *	1.80	77
ITT Rayonier, Inc.	Yulee, FL	2.99 *	3.72	80
Champion International Corp.	Newberry, SC	1.60 *	2.16	74
Crown Zellerbach Corp.	Bogalusa, LA	3.49 *	4.40	79
Weighted Mean		2.72 *	3.38	80

[1/] P = plantlet, S = seedling, * = significantly different at $p \leq .05$ level.

Figure 2 compares the height growth curves of the plantlets and seedlings averaged over the eight 1981 field plantings. Within each year, the seedling height significantly exceeded the plantlet height (McKeand and Frampton 1984, Amerson et al. 1985). Although every effort was made to establish the plantlets and seedlings at similar heights and stages of development, the plantlets averaged only 84 percent of the seedling height at the time of field planting. The plantlet height fell further behind the seedling height during the first and second growing seasons. However, after the third growing season, plantlets had gained to only a net loss of four percent from the time of establishment.

Figure 2.--Mean height growth curves of tissue culture plantlets and seedlings in the eight field plantings established in 1981 by the North Carolina State University Tissue Culture Project.



1/ P = Plantlet, S = Seedling

Thus, the difference between the third year height of the plantlets and seedlings is largely related to differences in initial planting size and a first year lag in plantlet height growth in the field. In these studies, the seedlings will most likely remain taller than the plantlets in absolute height through rotation age. Since plantlet growth rates are similar to that of seedlings during the third year in the field, cultural treatments which overcome the plantlets' initial slow growth should yield plantlets of comparable size as seedlings in future studies. Measurements will continue in these tests to monitor long-term growth trends.

Fusiform Rust Resistance

After three growing seasons in the field, the plantlets had less fusiform rust incidence than the seedlings in all eight of the 1981 field plantings (Table 2). Overall, the plantlets averaged 27.7 percent infection while the seedlings averaged 47.6 percent, a statistically significant difference ($P \leq 0.05$). In some high hazard regions of the Southeast, such reductions in fusiform rust incidence would be of great economic benefit and could offset initial slower growth. The nature of the plantlets' relative resistance is not yet understood.

Table 2.--Mean third year incidence of fusiform rust in the eight field plantings established in 1981 by the North Carolina State University Tissue Culture Project.

Organization	Location	Fusiform Rust Infection (%) Year 3 1/	
		P	S
Federal Paper Board Co., Inc.	Lumberton, NC	1.8	8.4
Brunswick Pulp Land Co.	Jesup, GA	56.0	71.0
Westvaco Corp.	Summerville, SC	54.4 *	83.5
Scott Paper Co.	Monroeville, AL	29.8 *	73.5
N. C. State University	Raleigh, NC	1.7	7.3
ITT Rayonier, Inc.	Yulee, FL	34.1 *	57.8
Champion International Corp.	Newberry, SC	4.1	18.0
Crown Zellerbach Corp.	Bogalusa, LA	39.4	61.7
Weighted Mean		27.7 *	47.6

1/P = plantlet, S = seedling, * = significantly different at $p \leq .05$ level.

Morphological Characteristics

Many differences between plantlet and seedling shoot morphology have been observed in the field plantings. Although the plantlets originated from embryonic material, their morphology appears more mature-like than seedlings. Older loblolly pine generally has larger needles, fewer branches per unit of height, fewer growth cycles per season and slower growth rates than younger material (Greenwood 1984). A detailed measurement of these and other characteristics (after two growing seasons) at the 1981 field planting in Jesup, Georgia, has verified quantitatively that the plantlets are expressing more mature-like morphology than the seedlings (McKeand 1985). Examples from that study are presented in Table 3.

Additionally, early production of female stobili has been observed on many plantlet clones. One clone which is represented by 75 plantlets at each of two of the 1982 field plantings, located at Rincon, Georgia, and Cantonment, Florida, represents the extreme of this phenomenon. After one full growing season in the field, 85 percent of the plantlets of this clone had produced female strobili. Only five percent of the seedling plots from the same half-sib family had produced female strobili at the Cantonment, Florida, study while no trees in the seedling plots had produced strobili at the Rincon, Georgia, study (McKeand 1985).

Table 3.--Second year measurements of the loblolly pine tissue culture planting established in 1981 at Jesup, Georgia (McKeand 1985).

Trait	Plantlets		Seedlings
Terminal Bud Length (cm)	3.4	NS[1/]	3.1
Terminal Bud Diameter (mm)	6.3	*	5.4
Needle Length (cm)	19.1	NS	18.6
Needle Dry Weight (g)	0.16	*	0.12
Percent Fusiform Rust Infection	47.4	NS	68.8
Number of Cycles	4.6	*	5.4

1/ * = significantly different at $P \leq 0.05$ level.

The cause of this apparent early maturation of loblolly pine tissue culture plantlets relative to seedlings is unknown.

CONCLUSION

Early results from field trials of loblolly pine tissue culture plantlets have identified differences in growth, fusiform rust resistance and morphology between plantlets and seedlings. In an attempt to better understand the nature of these differences, new studies have been initiated. These include: (1) exploring alternative treatments for producing shoots and roots *in vitro*, (2) investigating the effect of cultural practices such as root pruning on subsequent plantlet development, (3) establishment of field plantings to compare seedlings having tissue culture-produced root systems, plantlet shoots grafted onto seedling roots and seedling shoots grafted onto plantlet roots and (4) excavation of both plantlet and seedling root systems after several growing seasons in the field. Forthcoming results from these and other studies will not only provide knowledge necessary to improve tissue culture propagation but also provide some insight into the control processes of development in loblolly pine.

The use of trade names throughout this paper does not imply endorsement of these products, nor criticism of products not named.

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PARENT VS. OFFSPRING SELECTION: A CASE STUDY

Gary R. Hodge[1]/

Abstract.--A comparison was made between genetic gains and program benefits to be expected from parent selection after progeny testing, and offspring selection of the best trees in the best families, given the constraints of the N. C. State Industry Tree Improvement Cooperative disconnected half diallel mating scheme. At moderate heritability levels ($h^2 = 0.15$), offspring selection yielded higher expected genetic gains. However orchards established with juvenile scion material from genetic tests may not reach commercial production levels as rapidly as orchards established with scion material from older parent trees. Economic analysis shows that a delay of only one year in reaching full production levels can make offspring selection less profitable than parent selection, i.e., the cost of delay exceeds the increased return of higher genetic gains.

INTRODUCTION

Parent selection is a selection strategy where individuals are selected on the basis of the performance of their progenies. The best individuals (the parents of the best progeny families) form the commercial production population. Offspring selection is a strategy where the selected individuals are the best members of the progeny families. The main advantage of offspring selection is that it offers breeders the opportunity to do within family selection at high selection intensities. This generally allows the achievement of higher expected genetic gains, and thus is quite attractive to breeders. The primary objective of breeding programs, however, is not to achieve maximum genetic gains, rather it is to generate maximum dollar value or economic return. In making a decision about which selection strategy to use, it is important to consider factors other than those which maximize genetic gain. A case study of the situation involving the N. C. State Industry Tree Improvement Cooperative illustrates this point.

At this time, the N. C. State Cooperative plans to use offspring selection to select its third generation population of loblolly pine (*Pinus taeda* L.) (Anon., 1983). There has been some concern, however, that seed orchards established after offspring selection may not develop strobili as quickly as orchards established after parent selection. This possibility arises because the two selection strategies will yield scion material of quite different ages and in different states of sexual maturity. Loblolly pine grown in the field does not begin to flower consistently until it is 10 to 15 years-old (Dorman and Zobel, 1973). Since the N. C. State Cooperative plans on making selections in its genetic tests at age eight (McKeand and Weir, 1983), scion material from offspring selection will be sexually immature. Scion material from parent selection would be sexually mature, and the two

1/Research Assistant, N. C. State University, Raleigh, North Carolina.

types of orchards could conceivably show different patterns of strobili production over time. The objective of this case study is to compare genetic gains expected from parent and offspring selection, and to examine the effect of production delays on expected program benefits.

ESTIMATION OF GENETIC GAINS

The following assumptions were made in calculating genetic gains:

a. The seed orchard will be composed of 24 unrelated individuals.

b. The foundation population consists of 480 unrelated individuals.

c. The foundation population will be mated using six parent disconnected half-diallels formed at random with respect to general combining ability.

d. Non additive genetic variance equals additive genetic variance.

e. Field trials of the diallels will be conducted according to the N. C. State Cooperative Genetic Testing Manual (Talbert et al., 1981).

The specific calculations for genetic gains for both parent and offspring selection are presented in the Appendix. Calculations for genetic gains from offspring selection were made using separate formulae for family and within family selection, following a technique outlined by Squillace (1973). Estimates of genetic gains obtained from this technique are conservative, as combined selection would give greater genetic gains (Falconer, 1981). However, in calculating genetic gains from combined selection a priori, one cannot account for the requirement to maintain unrelatedness.

Expected genetic gains (Table 1) are presented in terms of phenotypic standard deviations of individuals (σ_p), and this was assumed to be equal for both parent and offspring populations.

Results of Genetic Gain Calculations

As expected, at most heritabilities offspring selection yielded higher expected genetic gains than parent selection. At very low heritabilities (individual, narrow sense), parent selection was more efficient, although a change occurred between $h^2 = 0.10$ and $h^2 = 0.15$. One should note that this was primarily due to the effect of within family selection. As heritability increased from 0.05 to 0.50, gains from half-sib, full-sib, and parent selection increased approximately three-fold. Over the same range, gains from within family selection increased ten-fold.

If the decision between the two selection strategies were simply a question of maximizing genetic gains, one need only determine the heritability of the trait of interest to make the correct decision. As the program objective is to maximize economic return, however, other factors must be considered.

Table 1.--Expected genetic gains[a] from offspring and parent selection.

h^2	Half sib	Full sib	Within Family	Selection: Offspring[b]	Selection: Parent
.05	.2197	.0935	.0707	.3839	.4379
.10	.3167	.1377	.1442	.5986	.6354
.15	.3903	.1711	.2209	.7823	.7581
.20	.4522	.1991	.3009	.9522	.9107
.25	.5065	.2236	.3847	1.1148	1.0209
.30	.5556	.2456	.4727	1.2739	1.1204
.40	.6426	.2847	.6632	1.5905	1.2967
.50	.7192	.3191	.8773	1.9156	1.4517

[a]Values expressed in units of phenotypic standard deviation of individuals.

[b]Gain from offspring selection equals the sum of gains from half-sib, full-sib, and within family selection.

SEED ORCHARD DEVELOPMENT

Grafted seed orchards (using sexually mature scion material) have generally reached commercial production levels 10 to 15 years after orchard establishment (Anon., 1979), and a rule of thumb developed in the N. C. State Cooperative is that 8 to 12 years usually elapse before meaningful production occurs (Talbert et al., 1983). Good choice of seed orchard sites, along with intensive irrigation and fertilization can promote the development of young seed orchards (Jett, 1983). The possibility that seed orchards established with sexually mature material may come into production sooner than orchards established with juvenile material forces one to consider the economic costs of a delay in production. To do this, one must know when the returns from the increased genetic gains will be available, i.e. when the improved trees will be harvested.

TIME LINES

The following assumptions were used in developing time lines scheduling the harvest of improved trees:

a. Two years to establish orchard after selection.
b. Breeding and testing for the subsequent generation begins immediately and will be completed in 14 years. A new orchard will then be established.
c. Two years after first commercial cone harvest until actual planting of the improved seedlings.
d. 25 years rotation.
e. Seed orchards go from zero to full strobili production in a single year.

Assumption e. is unrealistic and was made only to simplify analysis. Using these assumptions, one can generate a timeline for an orchard established after parent selection, assuming eight years to reach full production (P_8):

Event	Year
Year to finish orchard establishment	2
Year to first commercial production	10
Year to first planting	12
Earliest that next generation orchard could be established	16
Time when next generation orchard would produce	24
Last year planting	25
First year next generation material planted	26
Years of harvest	37 to 50

A similar time line can be developed for offspring orchards. Comparisons were made between the P_8 situation above and offspring orchards taking 9, 10, and 11 years to reach full production (O_9, O_{10}, and O_{11}, respectively).

One should note that in the P_8 situation, harvests are made from year 37 to year 50. For all offspring orchard situations, the next generation orchard is assumed to take eight years to develop. This would be the case if offspring selection was utilized, and then followed by parent selection for the next cycle of improvement. Thus for the O_9 situation, the years of harvest are years 38 to 50, and it is the harvests over these years that are compared to the P_8 situation.

DISCOUNTING PROCEDURES

In order to compare the economic values of the two selection schemes, one needs to convert the genetic gains calculated earlier into dollar values. The information needed is the mean of trait p, the standard deviation of trait p, and the relationship of trait p to dollar value. At the time that the selections would be made, this information would be in hand. For the sake of comparison in this general analysis, however, one can make the assumption that trait p is linearly related to dollar value. This is a reasonable assumption for volume growth with a product objective of pulpwood. It then becomes possible to make relative comparisons between the two selection schemes, simply treating genetic gain as if it were dollar value. Another assumption is that the amount of land planted and harvested is equal each year and from year to year. For example, the organization may be planting and harvesting 10,000 acres every year. The organization would then receive an annual annuity over the years of harvest outlined above.

One can calculate the present value of a terminating annual annuity with the formula:

$$V_{O(n)} = a \frac{(1+i)^n - 1}{i\,(1+i)^n}$$ (Lundgren, 1971)

where: a = value of annuity
i = interest rate
n = year the annuity terminates

The present value of an annual annuity to be received between the year n and year x is:

$$V_{O(n \text{ to } x)} = V_{O(x)} - V_{O(n-1)}$$

including heritability, interest rate, economic value function, and generation interval. The effects of heritability and interest rate have already been discussed. Economic value function can also be important. In this study, an assumption was made that the trait of interest is linearly related to dollar value. In fact, the economic value function could be some type of stepwise function where an increase of the trait beyond a certain point yields a very large increase in dollar value. If the trait has this sort of relationship to dollar value, the discounted genetic gain estimates for offspring selection may be low relative to parent selection. The more the population mean is increased, the greater the probability the population will reach the next step in dollar value.

Generation interval is also important. In this study, it was assumed that six years would be necessary to complete the matings, and eight years to complete the field testing, for a generation interval of 14 years. If selections were made at six years instead of eight, the generation interval would be 12 years. Under this circumstance, the relative cost of missing the first year of production is greater than with a 14 year generation interval. Therefore, if a breeder expected orchards established with offspring scion material to be slower in reaching full production than orchards of parent scion material, a 12 year generation would tend to push him even more in the direction of parent selection.

For this specific case study, the primary question becomes "Will there be a difference in the development of seed orchards established with sexually mature and immature scion material?" Although there is very little in the literature on this subject, I suspect that there would be little differnce. Consider that if selection occurs at eight years, it takes two years to establish the orchard, and a minimum of six to eight years are necessary for the trees to have enough vegetative growth to support full production levels, offspring scion material would be 16 to 18 years-old, and would probably be sexually mature. More concrete evidence is presented by Talbert et al. (1982). In a study of four seed orchards, although sexually immature grafts tended to produce more pollen catkins and less female strobili than mature grafts, the differences were not statistically significant.

Other Time Factors

A difference in the rate of seed orchard development is not the only way that a time difference could have an impact on the decision between parent and offspring selection. Another source of difference might be the time required for orchard establishment. It may take more time to establish offspring seed orchards from single eight year-old trees than parent orchards from numerous ramets kept in a greenhouse or clone bank.

Breeders should also consider that it is generally possible to identify the best families in field tests earlier than it is possible to identify the best individuals in those families. In the case of the N. C. State Cooperative, it is likely that one could be nearly as effective in parent selection at age four or six as at age eight. To identify the best individuals in offspring selection, however, is very difficult at younger ages. One could then argue that a breeder is imposing a two year delay on his program in order to gain the additional benefit of within family selection. The results of this study would suggest that this is not worthwhile.

One can then discount the genetic gains presented in Table 1 by multiplying by the appropriate value for $V_{0(n \text{ to } x)}$. This allows a comparison of the two selection schemes taking the delay in reaching full production into account.

Discounted Genetic Gains

Genetic gains were discounted at interest rates of 6% and 9% (Tables 2, 3). Discounted genetic gains were calculated at $h^2 = 0.15$. Solely on the basis of genetic gains, offspring selection was more efficient at all heritabilities in Tables 2 and 3. But comparing the P_8 and O_9 situations at 6% interest, the cost of a one year delay in reaching full production levels was enough to make parent selection more valuable than offspring selection at heritabilities up to 0.25. Not unless h^2 was as high as 0.30, did the increased genetic gains from offspring selection offset the cost of missing the first year of production.

Longer delays had higher costs. Comparing the P_8 and the O_{10} situations in Table 2, a two year delay at 6% interest, only at a heritability of 0.50 was offspring selection more valuable than parent selection. For a three year delay, P_8 and O_{11}, parent selection was always more valuable.

The effect of higher interest rates was to place a higher premium on reaching production earlier. At 9% interest with a one year delay, offspring selection became more valuable than parent selection at $h^2 = 0.40$, as opposed to a $h^2 = 0.30$ with 6% interest.

Table 2.--Discounted genetic gains at an interest rate of 0.06.

h^2	P_8	O_9	O_{10}	O_{11}
.15	.8649	.8019	.7165	.6349
.20	1.0390	.9761	.8721	.7739
.25	1.1647	1.1428	1.0210	.9061
.30	1.2783	1.3059	1.1668	1.0354
.40	1.4794	1.6304	1.4567	1.2928
.50	1.6562	1.9637	1.7545	1.5570

Table 3.--Discounted genetic gains at an interest rate of 0.09.

h^2	P_8	O_9	O_{10}	O_{11}
.15	.2340	.2119	.1848	.1598
.20	.2811	.2580	.2249	.1945
.25	.3152	.3020	.2633	.2278
.30	.3459	.3451	.3009	.2603
.40	.4003	.4309	.3757	.3249
.50	.4481	.5189	.4525	.3914

DISCUSSION

The most striking result of this study was that only a one year delay had a significant impact on the choice between parent and offspring selection. In making this decision, tree breeders must consider a number of factors

CONCLUSIONS

In making a decision between parent and offspring selection, tree breeders should consider when genetic gains will be available, in addition to the size of those genetic gains. Even a delay of as little as one year can change which selection scheme yields the highest overall benefit to the program.

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Appendix.--Genetic Gain Calculations.

A. Conditions

480 P_1 selections, divided into 80 groups of 6 each.
Selections mated to prduce 5 full-sib families within each half-sib family.

Field test design involves (4 locations) (6 reps/loc.)
(6 trees/family/rep) yielding 144 trees/cross.
n_f = 144 = number of full-sib family members
n_h = 720 = number of half-sib family members

B. Offspring Selection Scheme

1. All half-sib and full-sib families are ranked.
2. The highest ranking half-sib family is identified (Family A).
3. The best of the five full-sib families involving Family A is identified (Family AxB).
4. The best individual tree in the full-sib family AxB is identified to be grafted into the seed orchard.
5. Half-sib families and full-sib families involving A or B are eliminated from the list of candidate families.
6. Return to Step 2.

C. Genetic Gain From Parent Selection

$$R_p = i\sigma_p h^2 \sqrt{\frac{n}{4 + (n-1)h^2}} = 2.063\ \sigma_p h^2 \sqrt{\frac{720}{4 + (720-1)h^2}}$$

where:

R_p = expected response from progeny testing (parent selection)

i = selection intensity expressed in standard measure

σ_p = phenotypic standard deviation of individuals

h^2 = individual tree heritability

n = number of progeny = 720

Selection of parents of the top 24 half-sib families = 24/480 yielding $i = 2.063$.

D. Genetic gain from offspring selection - adapted from Squillace (1973).

$$R_O = R_{HS} + R_{FS} + R_{WF}$$

where:

R_O = expected response from offspring selection
R_{HS} = expected response from half-sib family selection
R_{FS} = expected response from full-sib family selection
R_{WF} = expected response from within family selection

1. Half-sib selection

$$R_{HS} = i\sigma_p h^2 \frac{1 + (n_h-1)r_{eh}}{\sqrt{n_h[1 + (n_h-1)\hat{t}_h]}} = 2.013\ \sigma_p h^2 \frac{1 + (720-1)(.2987)}{\sqrt{720[1 + (720-1)(.3495h^2)]}}$$

where:

variables as before

n_h = number in half-sib family

r_{eh} = effective coefficient of relationship between members of half-sib families (Lush, 1943)

$$r_{eh} = \frac{r_{wh} - r_{bh}}{1 - r_{bh}} = \frac{.2999 - .0017}{1-.0017} = .2987$$

r_{wh} = average coefficient of relationship within half-sib families

$$= \frac{n_h(f_h + 1) - 2}{4(f_h n_h - 1)} = \frac{720\ (5 + 1) - 2}{4[(720)(5) - 1]} = .2999$$

f_h = number of full-sib families per half-sib family

r_{bh} = average coefficient of relationship between half-sib families

$$= \frac{3p - 2}{4(N-1)(p-1)} \quad \frac{(3)(6) - 2}{4(480-1)(6-1)} = .0017$$

where:

N = total number of parents = 480

p = number of parents per diallel group = 6

$\hat{t}_h$ = phenotypic correlation between trees in half-sib families = .3495 h^2

If all genetic variance was additive, t could be calculated for a half-sib family by multiplying the h^2 by the average coefficient of relationship: $t = h^2 r$. In this case, however, non-additive variance equals additive variance, and may contribute to phenotypic correlation as the half-sib families are not truly half-sibs, but have full-sibs in them. Full-sib families have a covariance of $1/2V_a + 1/4V_d$. If all non-additive variance is dominance, one can calculate t for full-sibs as $t_f = (1/2V_a + 1/4V_a)h^2$. Thus $t_f = 0.75\ h^2$. One can then determine $\hat{t}_h$ for half-sib families by weighting t_h and t_f by the probability of selecting two half-sibs and two full-sibs, respectively, when randomly choosing two members of a half-sib family.

$$\hat{t}_h = Pr(FS) + t_f + PR(HS)t_h$$
$$\hat{t}_h = 143/719\ (.75h^2) + [(719-143)/719](.25h^2) = .3495h^2$$

Selection Intensity: We are selecting 24 half-sib families, but our selection scheme eliminates two half-sib families each time through the cycle. We may not be able to actually select the top 24 half-sib families. It is possible to determine, however, the probability that a diallel contains zero of the top 27 half-sib families:

$$\Pr(\text{diallel has zero of top 27}) = {}_{(480-27)}C_{6} \,/\, {}_{480}C_{6}$$

$$= (453 \times 452 \times 451 \times 450 \times 449 \times 448)/(480 \times 479 \times 478 \times 477 \times 476 \times 475)$$
$$= 0.7052$$

0.7052 x 80 diallels = 56 diallels that contain none of the top 27 families; therefore 24 different diallels contain the top 27. We can then be confident of selecting the top 27 half-sib families from 480 yielding i = 2.013.

2. Full-sib selection

$$R_{FS} = i\sigma_p h^2 \frac{1 + (n_f-1)r_{ef}}{\sqrt{n_h\,[1 + (n_h-1)t_f]}} = 1.163\sigma_p h^2 \frac{1 + (144-1).333}{\sqrt{144[1 + (144-1)(.75h^2)]}}$$

where:
variables as before
r_{ef} = effective coefficient of relationship between members of full-sib families

$$= \frac{r_{wf} - r_{bf}}{1-r_{bf}} = \frac{0.5 - 0.25}{1 - 0.25} = 0.333$$

r_{wf} = coefficient of relationship within full-sib families = 0.5
r_{bf} = coefficient of relationship between full-sib families = 0.25
t_f = phenotypic correlation between members of full-sib families = $0.75h^2$

Selection intensity is 1 of 5 yielding i = 1.163.

3. Within family selection

$$R_{WF} = i\sigma_p h^2\,(1-0.5)\sqrt{\frac{n_f - 1}{n_f\,(1-t_f)}} = 2.784\,\sigma_p h^2(0.5)\sqrt{\frac{144-1}{144(1 - .75h^2)}}$$

where:
variables as before

Selection intensity is 1 of 144 yielding i = 2.784.

GENETIC VARIATION IN LOBLOLLY PINE ROOT GROWTH POTENTIAL

L.E. DeWald, P.P. Feret, and R.E. Kreh[1]

Abstract.--Half-sib families of 1-0 loblolly pine seedlings were evaluated for genetic variation in their ability to regenerate new roots (root growth potential (RGP)) when outplanted. Half-sib family variation was significant with heritabilities ranging 0.34-0.37 for two independent samples of seedlots lifted in March 1983 and 1984. Variation patterns of root growth potential were different for half-sib families lifted at different times during the nursery growing season, and RGP of March lifted half-sib families was related to both first- and second-year field performance.

Additional keywords: RGP, field performance, height growth, nursery management, lifting season, Pinus taeda.

INTRODUCTION

Loblolly pine (Pinus taeda L.) is typically regenerated in the southern U.S. by planting bare-root 1-0 seedlings. Although nearly one billion loblolly pine seedlings are planted annually, (Johnson et al. 1983), highly variable transplanting success has resulted in decreased survival rates since 1960 (Weaver et al. 1981).

Analysis of the ability of bare-root seedlings to regenerate new roots (root growth potential) has improved the understanding of plantation establishment failures of western North American conifers (Ritchie and Dunlap 1980, Jenkinson and Nelson 1978). A direct relationship between root growth potential (RGP), and field survival and height growth has been established for many western conifer species. Among the factors found to affect western conifer RGP are genetic origin, nursery practices, and handling procedures (Ritchie and Dunlap 1980). Jenkinson (1980) determined that one of the keys to successful plantation establishment of western yellow pines is knowing when to lift seedlings from the nursery. Similar results have been obtained for Douglas-fir (Pseudotsuga menziesii (Mirb.) Franco) (Jenkinson 1984, Jenkinson and Nelson 1983) and for other western species (Stone and Norberg 1979). Subsequently, optimum lifting periods or "windows" have been established for different species which are based on genetic background and climatic data.

Stimulated by the success of RGP research in the west, and the studies in the southeast which indicate a relation between loblolly pine RGP and field performance (Feret and Kreh 1985, Feret et. al 1985) a project was undertaken to examine the role of genetic variation in RGP among half-sib families of loblolly pine seedlings. The specific objectives were to determine if root growth potential is affected by genetic orgin; to describe the relationship between root growth potential, genetic origin, and field performance; and to

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[1] Graduate Research Assistant, Associate Professor, and Research Associate, respectively, Dept. of Forestry, VPI & SU, Blacksburg, VA 24061.

determine if there is a lift date by genetic origin interaction.

MATERIALS AND METHODS

Two separate studies were established to examine genetic variation in loblolly pine RGP. The first study, conducted in 1982-1983, measured the genetic variation in RGP among 15 half-sib families and determined the relationship between RGP, genetic origin, and field performance. The second study, which measured genetic variation in RGP over the nursery lifting season, was conducted in 1983-1984 using 14 half-sib families completely independent of those used in the 1982-1983 study.

The 1982-1983 study used seedlings grown from seed donated by the N.C. State Cooperative Tree Improvement Program, and a Virginia Division of Forestry (VDF) nursery mix. The seedlings were grown in four replicate nursery beds at the VDF nursery in Providence Forge, Va. using standard nursery practices, except they were not undercut or top pruned.

On March 15, 1983, 90 seedlings per half-sib family (seedlot) were hand-lifted; 30 seedlings per seedlot were evaluated for RGP and 60 outplanted on the Virginia Piedmont Plateau, in Patrick County, Va. The outplanted seedlings were randomly assigned to 4 five-tree plots per seedlot within each of three blocks using a 0.5 by 1.0 m spacing. The outplanting site was a southwest facing slope originally containing a mixed pine-hardwood stand that was clearcut, followed by a chop and burn site preparation. The soil was a Hayesville fine sandy loam (SCS 1973). Seedling height and survival were measured at the start of the growing season and again in December of 1983 and 1984 when growth had ceased.

Root growth potential was measured by root pruning the seedlings to 12 cm below their root collars and then planting 15 seedlings per seedlot into two acrylic trays (46 x 10 x 40 cm) containing Promix Bx®, a commercial growth media containing peatmoss, pearlite and nutrient additives. The trays were watered to field capacity, sealed and placed in a waterbath at 20 C for 24 days under a 16 hour photoperiod in a greenhouse maintained above 15 C at night and below 24 C during the day.

After 24 days, seedlings were excavated from the trays and the number of new roots greater 0.5 cm (easily distinguished from old roots by their white color) were removed and counted. Root growth potential was expressed as the number of new roots produced by each seedling. Each seedling was also characterized at harvest by measuring root collar diameter, and shoot and root dry weights (dried at 70 C to constant weight).

The 1983-1984 study used 14 half-sib families donated by the VDF plus a VDF nursery mix. The seedlings were grown operationally from seed at the same VDF nursery as the previous study in 8 replicate nursery blocks. They were top pruned but not undercut. Severe 1983 spring storms caused poor survival in the nursery and, consequently, only four lift dates were possible. On October 25, November 22, 1983 and on February 2 and March 13, 1984 two randomly selected nursery field replicates were handlifted, common seedlots from both replicates were combined, and the RGP of 28 seedlings per seedlot measured.

A simpler less expensive RGP testing system, which gives similar relative results to the soil system used in the 1982-1983 study (DeWald et al. 1985), was used in the 1983-1984 study. In this system, 4-seedling plots per seedlot were grown hydroponically in a greenhouse under a 16-hour photoperiod in 7 replicate 37.8 liter fish aquariums for 15 days. The seedlings were suspended in aerated tap-water by inserting them at their root collars in floating sytrofoam blocks. The water temperature was maintained at ambient air temperature (minimum of 16 C nights and up to 27 C days) and 0.5 g of 20-20-20 (nitrogen-phosphorus-potassium) was added to the water (approximately 13 ppm final concentration). After the 15 days of hydroponic growth the RGP of the seedlings was quantified in the same manner as the 1982-1983 study.

Root growth potential and field performance (expressed as total height, height increment and survival) were analyzed using analyses of variance. Seedlot means were separated with Duncan's multiple range tests. Heritability of root growth potential was calculated (Falconer 1983) using data from the half-sib families (excluding the VDF nursery mix). Regression analysis was used to elucidate the relationship between RGP and field performance of the different seedlots. Since the 1982-1983 seedlings were not top pruned regression analyses were conducted using annual height increments (obtained by subtraction).

RESULTS

1982-1983 Study

The number of new roots averaged over all seedlots ranged from 14.6 to 23.5 with a mean of 17.9 (standard deviation = 3.4), and seedlot variation was significant (α=0.01). The heritability estimate for RGP was 0.34 ($\pm$0.12). First-year height increment averaged over seedlots ranged from 14.9 to 22.2 cm with an overall mean of 17.1 cm (standard deviation = 3.38). Second-year height increment averaged over seedlots ranged from 43.4 to 60.7 cm with a mean of 50.4 (standard deviation = 5.1). Survival after the first growing season was 95.9 percent and remained unchanged after two years. The regression of field performance on number of new roots was significant for both first- and second-year height increment (α=0.01), with R^2 values of 0.59 (standard error = 1.86 cm) and 0.28 (standard error = 4.46 cm), respectively. The relationships of RGP and height increment are illustrated in Figures 1 and 2.

Regression analyses of mean seedlot shoot dry weight, root dry weight, root-shoot ratio (based on dry weights), and root collar diamter versus mean number of new roots revealed that only root-shoot ratio was significantly (α=0.05), although weakly, related (r=0.25).

1983-1984 Study

Root growth potential varied significantly (α=0.01) among seedlots and among lift dates. In general, RGP and heritability were lowest for October and highest for the February lift. There was little RGP variation among seedlots for these two lifts, with only one seedlot differing significantly (α=0.05) from the rest. RGP variation among seedlots for the November and March lift dates was similar and greater than in October and February. The heritability estimates for the March and November lift dates were also

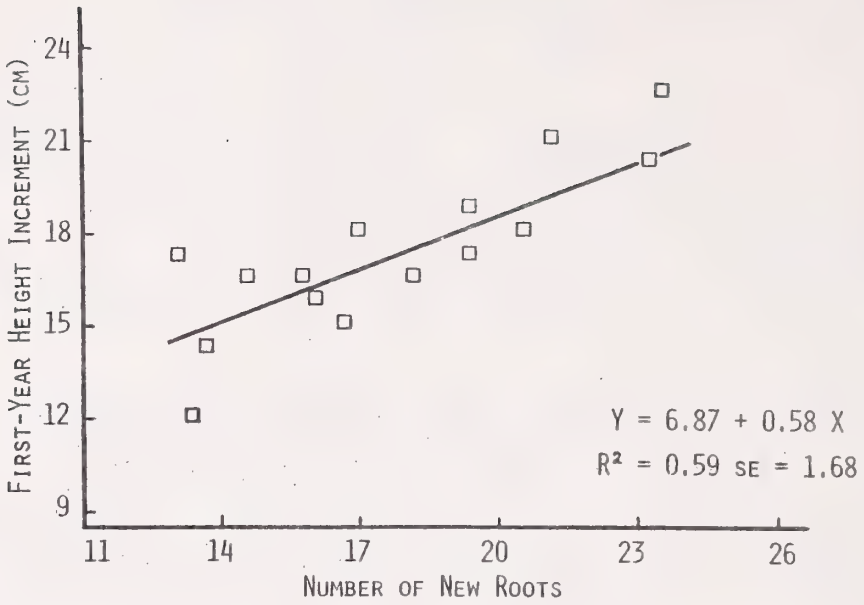

Figure 1. Relationship of first-year field performance and root growth potential of loblolly pine half-sib families lifted and outplanted on the Virginia Piedmont Plateau, Patrick County, Va., in March 1983.

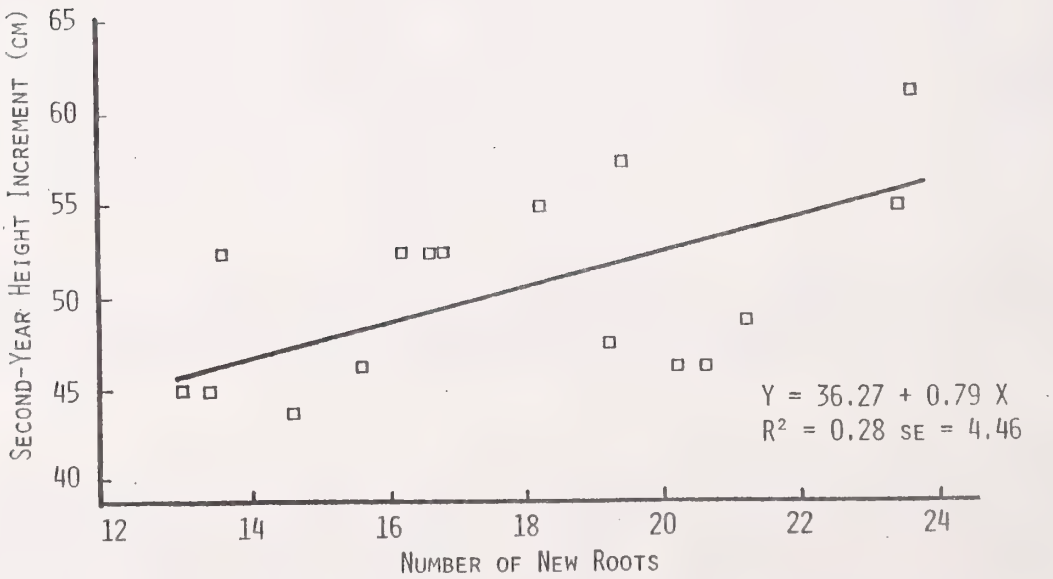

Figure 2. Relationship of second-year field performance and root growth potential of loblolly pine half-sib families lifted and outplanted on the Virginia Piedmont Plateau, Patrick County, Va., in March 1983.

similar. The RGP results and heritabilities for each lift are summarized in Table 1.

The seedlot by lift date interaction was also significant (α=0.01). This interaction was both a relational (rate) as well as a complete reversal type of interaction. In general, the October RGP was always the lowest and either the February or March lift dates had the highest RGP. The specific direction of change in RGP from date to date varied depending on the seedlot. This relationship is illustrated in Figure 3.

Table 1. Summary of half-sib loblolly pine seedling root growth potential performance over the 1983-1984 nursery lifting season.

Time of Lift	Seedlot Variation Significance	Number of New Roots (Seedlot Means)		Heritability h^2	SE
		Mean	Range		
October	α = 0.05	0.44	0.14-1.14	0.15	±0.03
November	α = 0.01	6.17	2.71-9.29	0.30	±0.04
February	α = 0.01	8.97	6.54-18.36	0.50	±0.05
March	α = 0.01	8.32	4.39-13.42	0.37	±0.05

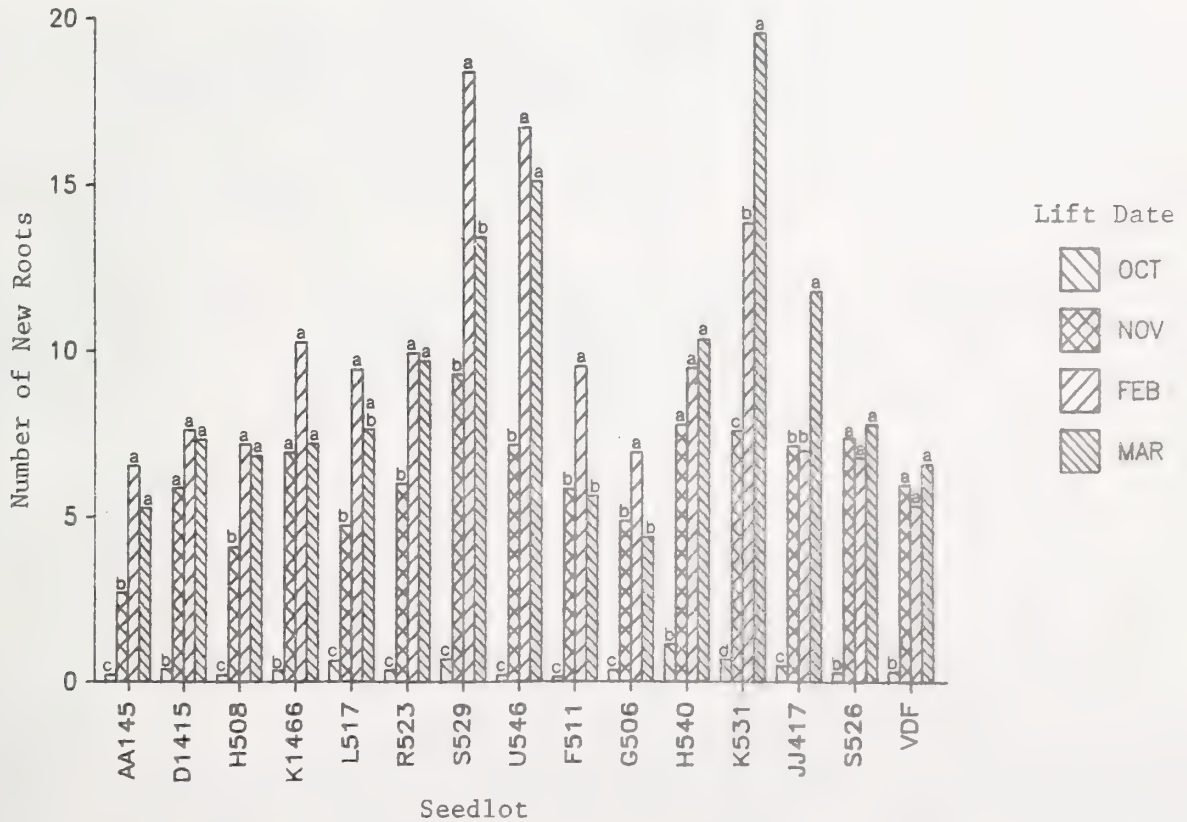

Figure 3. Loblolly pine seedlot root growth potential performance over the 1983-1984 lifting season. Means followed by the same letter within each seedlot do not differ significantly (α=0.05).

DISCUSSION

Results from both years indicate strong genetic control for loblolly pine RGP and selection would probably be successful for improving this trait. The heritabilities for March lifted seedlings are similar for both years even though two completely independent groups of half-sib families were used. The different heritabilities for the four lift dates indicate that different degrees of environmental control exist over the growing season, but this difference is in part due to the lift date by seedlot interaction.

The interaction between time of lift and genetic origin suggests that different optimum lifting and planting dates exist for different loblolly pine seedlots. It appears that lifting "windows", such as those described for western species (Jenkinson (1980, 1984), also exist for loblolly pine.

Field performance, genetic origin, and RGP appear to be closely linked and the results indicate that seedlots with superior RGP will have greater height increment the first growing season, with this effect lasting at least two years. The relationship between field performance and RGP is consistent with other loblolly pine studies where treatments with low RGP had poor field performance (Feret and Kreh 1985, Feret et al. 1985). When interpreting the field performance data of individual seedlots, it must be kept in mind that some of the RGP and subsequent height differences may have been due to March not being an optimum time for lifting some seedlots.

The differences in RGP responses over the lifting season suggest that family-block plantings in the nursery have an advantage over composite seedlot plantings since time of lift could be scheduled to maximize RGP for groups of families that respond similarly. In the western U.S. nurseries that utilize family-block plantings can use lifting windows ensuring appropriate lifting schedules for each seedlot, thereby increasing the probability of plantation success (Jenkinson 1984).

In addition to lifting schedules, seedlot blocks in the nursery also allow cultural practices to be tailored to maximize RGP. For example, certain cultural treatments such as fertilization might result in increased RGP of some seedlots at a time when their RGP would otherwise be low. These seedlots could then be lifted at what would have been a non-optimal time.

The genetic control of RGP permits screening of seedlots for an indication of potential relative field performance. This can be particularly useful for the selection of seedlots for difficult sites or difficult years. In addition, the lift date by seedlot RGP interaction may be a partial cause of obscure genotype by environment interaction in progeny tests.

The differential RGP response among seedlots over the lifting season should be considered in the establishment of progeny tests. Since all seedlots are not at their optimal RGP for lifting simultaneously, progeny tests should perhaps be established over a period of weeks to minimize seedlot differences caused by non-optimal lifting dates. This procedure would allow more precise estimates of the actual field performance rankings of improved families by minimizing error variance due to non-optimal lifting from the nursery.

CONCLUSIONS

Results of this research show RGP is a genetically controlled trait, and suggests that RGP should be considered in improvement programs as well as in nursery management of loblolly pine seedlings. Genetic selection for improved RGP should be successful and yield seedlings better able to withstand transplanting. If the relationship between RGP and height growth continues beyond the first two years, RGP may be a screening tool for the early selection of superior genetic stock.

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RESPONSE OF FOUR SOURCES OF LOBLOLLY PINE TO SOIL ACIDITY EXTREMES

Michael C. Harbin[1/]

Abstract--Four sources of loblolly pine from Florida and adjacent regions were grown on two soil regimes, one very acidic and the other nearly neutral, in a greenhouse. All sources had greater height, root collar caliper, and dry weight on the acidic soil. There was no significant interaction of sources or families within sources with acidity levels. The Gulf Coast source was significantly shorter in height than the other three sources. While no important differences were found between the two Florida sources, some Gulf Hammock families performed well on the soil with acidity level of pH 6 to 7.

Additional keywords: Marion County, edapic ecotypes, G X E interaction, Pinus taeda.

INTRODUCTION

Much interest has been expressed in Florida loblolly pine (Pinus taeda L.) because of its status as the southern-most source and its reputation for fast growth (Ladrach, 1980 and Draper, 1975). It is a logical choice for planting as an exotic in more tropical regions of the world.

Over the years, foresters have come to refer to Florida loblolly pine as either the Marion County or Gulf Hammock source. The Marion County material is typically found on deep sandy sites with soil acidity levels of pH 4 to 5. In contrast, Gulf Hammock refers to Levy and Dixie Counties on the Gulf Coast and is typified by wet marl soils with soil acidity of pH 6 to 7. Since soil acidity is such a dominant factor in soil chemistry and the Florida sources are under intense selection pressure by virtue of the fact they are on the edge of species' natural range, an interesting question is whether the two sources have evolved differently into edaphic races.

Another concern involving Florida Loblolly pine is the restricted breeding population and dim prospects for locating more select phenotypes. Presently, about 140 selections have been located by the organizations working with Florida loblolly pine. This is far below the 200 to 400 genotypes generally suggested as the minimum for a base breeding population (Krug, 1979; Burdon, et al., 1977; and McKeand and Beineke, 1980). The dearth of natural stands precludes more mass selection to bolster the breeding population.

This paper reports on a greenhouse study to determine if the two Florida sources are distinct genetically and whether they could be combined with adjacent sources of loblolly pine in an expanded breeding population.

1/ Tree Improvement Project Leader, Chesapeake Corporation, West Point, Virginia.

MATERIALS AND METHODS

Fifteen half-sib families were chosen from seed orchard seed lots to represent ortets originally selected in each of the following geographical seed sources: Gulf Hammock, Florida (GH); Marion County, Florida (MC); the Gulf Coastal Plain of Mississippi - Alabama (GCP); and the Atlantic Coastal Plain of Georgia - South Carolina (ACP). The GH seed was collected from Georgia - Pacific's Gulf Hammock orchard and the MC seed came from clonal collections at Brunswick Pulp Land Company's Florida Loblolly Seed Orchard. The other seed lots were selected at random from seed in storage at North Carolina State University. Relative quality was ignored in choosing seed lots.

A split-plot design was used with three replications. Two soils with acidities of pH 4.5 and pH 6.5 were the main plots, seed sources were sub-plots, and the half-sib families were nested within sources. Families in the main plots were represented by 10 seedlings. A base soil media of pH 4.5 was developed using equal parts of river sand, peat moss, and coarse vermiculite. Calcium carbonate was used to adjust the soil acidity to pH 6.5. The seedlings grew for 16 weeks in cylindrical containers of 164 cc volume. The temperature settings in the greenhouse were 26^{o}C days and 22^{o}C nights.

Poor germination caused the deletion of 3 families of the Atlantic Coastal Plain and 1 family of the Gulf Coast source. Seedlings were given optimum conditions for growth, other than soil acidity, which proved to be fairly easy to regulate. Approximately two whole pH units were maintained between the main plot treatments over the course of the study, although they varied from the target pH levels of 4.5 and 6.5.

At the end of the growth phase, the growth variables looked at were seedling height, root collar caliper, and stem dry weight. An analysis of variance was done on plot means for each trait.

RESULTS

Seedling Height

Seed weight accounted for only a small part of the variability associated with seedling height ($r = -0.19$) and was not significant. The analysis showed a significant acidity effect. Seedlings on the low pH soil averaged 27.77 cm, 13% taller than seedlings on the high pH soil (Table 1). There were marked differences in appearance as well. Seedlings looked healthy and vigorous at the lower pH level while those growing under the acidity level of pH 6.5 looked distressed and were less bushy with needles that were a paler shade of green.

Source rankings were exactly the same under both acidity regimes, with no significant interaction of acidity and sources (Table 1). This suggests that none of the sources tested are especially adapted genetically to either high or low soil acidity. The fact that Marion County was tallest and Gulf Hammock was second is in agreement with previous seed source studies (Draper, 1975 and Labrach, 1980) and is encouraging evidence that the Florida sources respond similarly to soil acidity.

Table 1.--Seedling height for all four seed sources, by pH level and combined over pH levels.

Seed Source	Seedling Height		
	pH 4.5	pH 6.5	overall
	- - - - - - Centimeters - - - - - -		
Marion County	28.76	25.44	27.10 b [a/]
Gulf Hammock	28.26	24.93	26.60 b
Atlantic Coastal Plain	27.97	24.87	26.42 b
Gulf Coastal Plain	26.08	22.95	24.51 c
Means	27.77	24.55	26.16

[a/] Means within a soil pH level no sharing the same superscript are significantly different at the 1% level.

The source effect was highly significant in the analysis. A multiple comparison procedure using Waller-Duncan's Bayesian K-ratio t-test revealed that the Gulf Coast source was significantly different at the K=100 level (approximately equal to the 0.05 level). This result was surprising since the GCP source had the heaviest seed and was ranked first in height at age ten weeks. This apparent genetic difference in height growth would be a considerable handicap if it persisted well into the rotation.

There was a large and highly significant family within source effect. The large amount of variation within sources indicates that mass selection would be effective in a breeding program.

While there was no significant family X acidity interaction, a closer look at the two Florida sources revealed interesting family rank changes. Using Spearman's Coefficient of Rank Correlation (Steel and Torrie, 1980), families of the MC source exhibited a high degree of stability over acidity regimes (r_s = 0.85). The rank of GH families over acidity regimes was very poorly correlated (r_s = 0.08), indicating a lack of stability to soil acidity extremes. These results are supported by linear correlation r values of 0.90 for MC and 0.36 for GH.

Some GH clones apparently are genetically adapted to either low or high soil pH values. For instance, clone 23-34[1/] ranked second under pH 4.5 but dropped to last in pH 6.5, while clones 22-33, 22-32, 22-23, and 22-4 performed well on the high pH soil.

Root Collar Caliper

Root collar caliper is more sensitive than seedling height to stocking density (McGilvary and Barnett, 1981). It has limited value as a measure of growth because of the high seedling sensity in the study (527 seedlings per square meter) and the small amount of variation detectable with the measuring

[1/] Clone number assigned by the North Carolina State University - Industry Cooperative Tree Improvement Program.

instruments on small stems. Also, stem dry weight accounted for 76% of the variability associated with root collar caliper.

Diameter growth was significantly better on the low pH value soil where the mean caliper was 8.5% greater. The analysis found no interaction between soil acidity and sources or families within sources, although there was some rank change amoung the sources between pH levels (Table 2). The Gulf Coastal Plain source had the largest caliper on both pH levels, with no clear second place. The fact that seed weight accounted for 39% of the variation in caliper ($r = 0.63$) and that the Gulf Coastal Plain had the heaviest seed may explain the good showing of this source material.

Table 2.--Root collar caliper for all four sources by pH levels and combined over pH levels.

Seed Source	Root Collar Caliper		
	pH 4.5	pH 6.5	overall
	- - - - - Millimeters - - - - -		
Gulf Coastal Plain	2.85	2.65	2.75 b [a/]
Marion County	2.82	2.58	2.70 b
Atlantic Coastal Plain	2.84	2.56	2.70 b
Gulf Hammock	2.78	2.60	2.69 b
Means	2.82	2.60	2.71

[a/] Means within a soil pH level sharing the same superscript are not significantly different at the 0.05 level.

For this trait, sources were basically equal, with no significant differences. However, once again there were important family within source differences, indicating enough potential variation to make mass selection worthwhile. Variability among families ranged from a high of 3.00 mm for family 24-1 (GCP source) to 2.45 mm for family 22-22 (MC source), a difference of 22% in root collar caliper and 150% in cross sectional area.

As the data in Table 2 shows, the GH source had slightly larger caliper (2.60 mm vs. 2.58 mm) than MC on the pH 6.5 soil. When the GCP and ACP sources were dropped from the analysis, the Florida sources exhibit a significant interaction with acidity levels at the 0.05 level. The Gulf Hammock source may be better adapted to high pH levels; some families did show the ability to grow well on the adverse soil of pH 6.5. Families 22-32 and 22-33 had larger root collar caliper on the high pH value soil than on the low pH value soil, and family 22-23 was nearly the same on both soil acidities. These three Gulf Hammock clones were also top performers for seedling height. This suggests that these clones are tolerant of soil acidity in the range of pH 6.5. At the same time, they are adaptable enough to show good growth on soil with acidity more typical of loblolly pine sites.

Each Florida source had good correlation of family ranks between acidity extremes when using Spearman's Coefficient of Rank Correlation (r_s values were

0.53 and 0.86 for GH and MC). A linear correlation check revealed a lower correlation of r = .46 for GH versus r = .84 for MC. Only one Marion County clone grew reasonably well on the soil with pH value of 6.5.

Stem Dry Weight

Seed weight or common environment effect contributed 40% of the variation in stem dry weight at age 16 weeks (r = 0.63). Similar results have been reported at age 24 weeks when the correlation coefficient was 0.57 (Waxler and van Buijtenen, 1981).

The analysis showed that stem dry weight was strongly affected by soil acidity levels. Overall, seedlings grown on the low pH soil were 33% heavier (Table 3). Fluctuations in the target acidity levels indicated that growth was severely restricted when the soil acidity rose to approximately pH 7; the ability of the seedlings to absorb nutrients, even if abundantly supplied, and function well physiologically was impared at pH 6.5 and above.

Table 3:--Stem dry weight for all from sources by pH levels and combined over pH levels.

Seed Source	Stem Dry Weight		
	pH 4.5	pH 6.5	overall
	- - - - - - - - grams- - - - - - - -		
Gulf Coastal Plain	1.454	1.139	1.297 b [a/]
Marion County	1.467	1.062	1.265 b
Atlantic Coastal	1.422	1.028	1.225 b
Gulf Hammock	1.299	1.009	1.154 b
Means	1.411	1.060	1.235

[a/] Means within a soil pH level sharing the same superscript are not significantly different at the 0.05 level.

As in the other two traits measured, there was a significant family response. Dry weights for families, averaged over pH levels, ranged from 0.925 grams to 1.569 grams, a difference of 70%. There obviously is sufficient genetic variation to make good gains with selection in a breeding program.

Sources were not found to be significantly different in their dry weights, at least at the 0.05 level. The Gulf Coastal Plain and Marion County sources were nearly equal, with a slight edge to the GCP material (Table 3). The Gulf Hammock source was consistently last in dry weight, despite the fact with GH material exhibited good height growth and average diameter growth. Seedlings of this source apparently contained more water in the above ground biomass. The reason for this is not known.

Neither sources nor families within sources were found to vary in performance with respect to soil acidity; no statistically significant evidence of ecotypic adaptation was found. Both of the Florida sources gave high rank

correlations when Spearman's procedure was used to compare family ranks on the high and low pH value soils, (r_s was 0.64 for GH and 0.68 for MC). Again, clones 22-32, 22-33, and 22-23 of GH source grew very well on the less acidic soil. These clones appear to be well adapted to a wide range of soil acidity levels.

CONCLUSIONS

The most reliable growth trait examined, seedling height, gave strong evidence that the two Florida seed sources, Gulf Hammock and Marion County, are genetically similar in their response to soil acidity. Combining the Florida sources into one breeding program seems to be justified. Additionally, there is evidence for including selections from adjacent geographical sources in an expanded breeding population for Florida loblolly pine, particularly selections from the Atlantic Coastal Plain of Georgia and possibly South Carolina. Use of selections from the Gulf Coastal Plain should be avoided until more reliable data is available. These results must be considered tentative until field trails either confirm or dispute the findings.

Fortunately, field trials using the same open-pollinated seed lots for each source have been established in a join project between the N. C. State University - Industry Cooperative Tree Improvement Program and University of Florida Cooperative Forest Genetics Program. The tests are located at nine sites chosen to sample the environment within the provenances and provide information on the extent of genotype X environmental interaction.

While no sources exhibited edaphic adaptation to either high or low soil acidity, there was evidence of intra-source G X E within the Gulf Hammock material. Several GH families consistently grew very well on the pH 6.5 soil and could represent physiological ecotypes. As proposed by Bridgwater and Stonecypher (1978), the real opportunity here is for realized genetic gains, made possible by assigning half-sib families to sites to which they are specifically adapted.

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Winner of the First TONY SQUILLACE AWARD

DYNAMICS OF IMPROVED LOBLOLLY PINE PLANTATIONS AND THE IMPLICATIONS FOR MODELING GROWTH OF IMPROVED STANDS

Marilyn A. Buford and Harold E. Burkhart[1]

Abstract.--A study was initiated to examine the dynamics of genetically improved loblolly pine plantations and to develop guidelines for incorporating the effects of genetic improvement into various types of growth and yield models for loblolly pine plantations. The limited data base from stands of improved stock dictated that any modeling effort concentrate on the synthesis of fragmented information from many types of studies rather than the usual data fitting procedures.

A series of hypotheses concerning stand dynamics and growth patterns in stands of improved stock relative to stands of unimproved stock were developed and tested. Results of these tests indicate: 1) at the seed source and family levels, the shape of the height-age curve is dictated by the site, but the level of the height-age curve is dictated by the seed source or family; 2) at the seed source and family levels, the shape of the height-diameter relationship at a given age is determined by site and initial density while the level of the height-diameter relationship is determined by the seed source or family and is directly related to the dominant height of the seed source or family at that age; and 3) given that silvicultural treatments are the same and are equally intense and successful, variances of height and diameter in stands originating from selected genotypes are not different from those in genetically unimproved stands.

Implications for modeling growth of stands originating from selected genotypes are: 1) genetic improvement affects the rate at which stands develop, but does not fundamentally alter the pattern of stand development from that of unimproved stands; 2) changes in genetic material on a given site will likely affect the level, but not the shape, of such basic relationships as the height-age and height-diameter relationships; and 3) correctly characterizing the height-age profile will be very important.

Additional keywords: Pinus taeda L., height-age relationship, height-diameter relationship, stand-level variance.

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1. Champion International Postdoctoral Fellow and Thomas M. Brooks Professor of Forest Biometrics, Virginia Polytechnic Institute and State University, Blacksburg, Virginia. The authors acknowledge Champion International Corporation, the Loblolly Pine Growth and Yield Research Cooperative, North Carolina State University-Industry Tree Improvement Cooperative, Crown Zellerbach Corporation, and the Southern Forest Tree Improvement Committee for support and data release.

In modeling the growth and yield of forest stands, we seek to mathematically interpret the biological relationships that underlie and drive stand development. The logical way to approach the problem of modeling the growth and yield of genetically improved stands of loblolly pine (Pinus taeda L.) is by studying the dynamics of such stands. An understanding of the growth and dynamics of genetically improved stands relative to unimproved stands is important for decisions regarding selection and breeding as well as forest management.

Certain basic relationships are key components of stand dynamics. They are: 1) the development of dominant height through time or the height-age relationship; 2) the development of the height and diameter distributions through time; 3) the relationship of the mean height by diameter class across the range of diameters at a given age, or the height-diameter relationship; and 4) the mortality-time relationship. Hypotheses concerning the similarities and differences between stands of improved and unimproved stock were developed and tested for the first three relationships listed above. This paper details the hypotheses tested, the test results and the implications of the results for modeling growth of stands of genetically improved stock.

DATA

The data available to this study were diverse types and not, by study design or plot structure, the kind usually used for growth and yield analysis. This dictated that the hypotheses be tested and the implications be proposed by the synthesis of fragmented information rather than the usual modeling techniques.

The data base used is comprised of three components: 1) the Loblolly Phase of the Southwide Pine Seed Source Study up to age 25 (Wells and Wakeley 1966); 2) a 15 year-old block-plot half-sib progeny test planted near Bogalusa, Louisiana, belonging to Crown Zellerbach Corporation; and 3) three 16 year-old, ten-tree row-plot progeny tests located in eastern Virginia, eastern South Carolina and north-central Alabama released to this project by the North Carolina State University-Industry Tree Improvement Cooperative. The row-plot progeny test data were grouped into half-sib families on the male parent thereby combining rows from different locations within a rep in an attempt to overcome the environmental artificiality of the row-plot design. The row-plot tests were then analyzed at the half-sib level.

The block-plot progeny test is replicated 4 times at the same location with each rep containing twelve 121 tree plots (11 x 11 trees) with the inner 49 trees as measurement trees. Spacing is 8 x 8 feet. Each rep contains 11 plots of selected genotypes and 1 plot of local woodsrun origin as a control.

A complete description of the Southwide Pine Seed Source Study design is given in Wells and Wakeley (1966).

Analysis of the Seed Source Study allowed hypotheses to be tested regarding similarity or difference of growth pattern of different seed

sources at the same location and at different locations. Analysis of the half-sib block-plot progeny test allowed hypotheses to be tested regarding growth patterns of half-sib families at the same location. The row-plot progeny test data were used to further test hypotheses accepted using the Seed Source Study and the half-sib block-plot progeny test data.

METHODS AND RESULTS

Height-age relationship

When modeling stand development, the most important relationship to understand and correctly characterize is the height-age relationship. The analysis of the height-age profiles in the data available was therefore of primary interest.

Nance and Wells (1981) used the Southwide Pine Seed Source Study and a fairly inflexible model for height growth to study differences in site index among different seed sources. One result of their work is that at any given location of the Seed Source Study, the shape of the height-age curve is the same for all seed sources, but the level of the curve is affected by seed source and block. To determine whether or not these results were an artifact of the model used by Nance and Wells (1981) the analysis was repeated using the very flexible Richards' function:

$$H = A(1-\exp(-b*age))^{**}c \qquad (1)$$

where: H = height at any given age
A = asymptotic or maximum height
b = rate parameter
c = shape parameter.

Equation (1) was fitted to the tallest seven trees at each age (roughly analogous to the tallest 100 trees per acre) for each seed source x block x location combination. Analysis of variance for A, b, and c and graphical analysis of the resulting curves for each location showed significant seed source and block effects on A, the asymptotic height, but generally no seed source or block effects on b and c, the rate and shape parameters, respectively.[2] Differences did occur among all the parameters from location to location.[2] These results support the findings of Nance and Wells (1981) and also indicate that an extremely flexible function is not necessary for this analysis.

Extending the conclusions about height-age patterns in the Seed Source Study to a general hypothesis, patterns of the height-age profiles in the block-plot progeny test were examined. The equation

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2. In the interest of space, exhaustive tables of test and fit statistics are not given in this paper. Such tables can be obtained from the senior author on request. All tests of significance were carried out with $\alpha = 0.05$.

$$\log (H) = a + b(1/age), \qquad (2)$$

where: H = height at any given age
a = level or intercept parameter
b = slope or shape parameter
log = logarithm base e,

was fitted to the tallest seven trees at each age for each plot (family x block combination). An analysis of variance for the estimates of the slope parameter, b, was done following the form of Table 1. Results of this analysis showed that there are no family and no rep effects on the shape parameter. That is, the height-age curves are the same shape for all the families across all the reps. Equation (2) was fitted to the data from each plot again while maintaining a common slope parameter, b, for all plots. An analysis of variance (Table 1) was performed on the estimates of the intercept and the results showed significant family effects on the intercept, but no rep effects on the intercept.

Table 1.--Form of analysis of variance for block (rep) and seed source (family) effects on the parameters of the height-age and height-diameter relationships.

Source of Variation	Degrees of Freedom	Mean Square	F-ratio
Block (Rep)	b-1	MSB	F_B=MSB/MSE
Seed Source (Family)	s-1	MSS	F_S=MSS/MSE
Error	(b-1)(s-1)	MSE	

Extending this analysis to the row-plot progeny tests, equation (2) was fit to the tallest 15% of the trees at each age for each plot (half-sib family x rep combination) for the three progeny tests. An analysis of variance on the estimated slopes was done following Table 1. Results of this analysis showed that there are no family and no rep effects on the slope or shape parameter, b. Equation (2) was fitted to the data from each plot again while maintaining a common slope parameter, b, for all plots within a test. An analysis of variance (Table 1) was performed on the estimated intercepts and the results showed significant family and rep effects on the intercept term in all three tests.

The conclusion of this phase of the study is that at a given location, the shape of the height-age profile is the same among seed sources or families, but the level of the height-age curve differs by seed source or family. This result is consistent from the Southwide Pine Seed Source Study to the block-plot half-sib progeny test to the row-plot progeny tests.

Height-diameter relationship

Height-diameter relationships are used in many growth and yield models to predict the mean height for a given diameter or diameter class.

The diameter and predicted height values are then used in stand volume and value calculations. The wide use of this relationship dictated its investigation. Only data from unthinned studies or unthinned parts of studies were suitable for analysis of the height-diameter relationship as thinning destroys stand structure and, with it, the base line development of the height-diameter relationship. Nine locations of the Seed Source Study unthinned by age 15 were used (see Table 2). The block-plot progeny test was thinned after age 8. Given that the dominant heights ranged from 38 to 50 feet at age 8 in these plots, the height-diameter relationship was well-developed at age 8 and these data were appropriate for the analysis. The three row-plot progeny tests were unthinned and the age 16 data from these studies were used in the analysis.

Table 2.--Location of the nine Southwide Pine Seed Source Study plantations used in the analysis of the height-diameter relationship.

Location	County or Parish	State	Series
C AL (2)	Coosa	Alabama	2
E MD (1)	Worcester	Maryland	1
N MS (2)	Winston	Mississippi	2
S MS (1)	Pearl River	Mississippi	1
S MS (2)	Pearl River	Mississippi	2
SE LA (1)	Washington	Louisiana	1
SE LA (2)	Washington	Louisiana	2
SW GA (1)	Dooly	Georgia	1
W SC (2)	Newberry	South Carolina	2

The function used to model the height-diameter relationship in this analysis was:

$$\log(H) = a + b(1/D) \qquad (3)$$

where: H = mean height for a given diameter, D
a = level or intercept parameter
b = slope or shape parameter
log = logarithm base e.

Equation (3) has been found to work well for loblolly pine and examination of residual plots showed that it fit the data from all the studies well.

Equation (3) was fitted to all the height-diameter pairs for each seed source x block combination (plot) for each of the nine locations of the Seed Source Study used. An analysis of variance (Table 1) was done on the estimated slope parameters, b, at each location. At 8 of the 9 locations, seed source did not affect the shape of the height-diameter curve. At 8 of the 9 locations, blocks did not affect the shape of the height-diameter curve. Given these results, equation (3) was refitted to each plot while maintaining a common slope within each location. The analysis of variance indicated in Table 1 was performed on the estimates of the intercept, a,

within each location. At 2 of the 9 locations, blocks significantly affected the intercept. At 3 of the 9 locations, seed source significantly affected the intercept, and at 3 of the 9 locations, block and seed source significantly affected the intercept. Recall that there were significant block and seed source effects on the intercept of the height-age relationship. The estimated slopes were significantly different across locations. Simple linear regressions were calculated for the estimated intercepts on dominant height at age 15 of the appropriate plot. Eight of the 9 regressions had r^2 values greater than 0.79. Using r^2 simply as a measure of association, the intercept, or level, parameter of the height-diameter relationship at age 15 is strongly related to the dominant height at age 15 of that source on that site. The conclusion from this portion of the analysis is that the height-diameter relationships are the same shape across seed sources at any given location at age 15 and the level of the relationship is directly and strongly related to the dominant height of the source at age 15.

Extending this conclusion to a general hypothesis, the same type of analysis was performed on the block-plot progeny test data at age 8. Equation (3) was fitted to the height-diameter pairs for each plot (family x block combination). The analysis of variance indicated in Table 1 was performed on the estimates of the slope, b. The results of the analysis of variance showed no block or family effects on the slope parameter. Using a common slope, b, equation (3) was refitted to the data from each plot. An analysis of variance of the form in Table 1 was carried out on the estimated intercepts. There were no rep effects, but there were significant family effects on the intercept parameter. Recall that there were no rep effects but there were significant family effects on the intercept of the height-age equation for these plots. The simple linear regression of the estimated intercepts on the dominant height of the plots had an r^2 of 0.93. The conclusion from this portion of the analysis is that the height-diameter relationships are the same shape across families at age 8 and the level of the relationship is directly and strongly related to the dominant height of the family at age 8.

Performing the same type of analysis with the row-plot progeny tests, equation (3) was fitted to the height-diameter pairs for each plot (half-sib family x rep) for the three separate tests. For each of the three tests, an analysis of variance (Table 1) was conducted on the estimates of the slope, b. There were no significant rep or family effects on the slope parameter, b, in any of the three row-plot progeny tests. Maintaining a common slope for each test, equation (3) was refitted to the height-diameter pairs for the three tests. An analysis of variance (Table 1) was performed on the resulting estimates of the intercept, a. There were significant family and rep effects on the intercept in all three row-plot progeny tests. Recall the significant rep and family effects on the estimates of the intercept for the height-age relationship for these data. A simple linear regression of the estimated intercepts of the height-diameter relationship on the plot dominant height was calculated for each of the three progeny tests. All had r^2 values greater than 0.88. The slope parameters were different in the three tests. From this analysis of the three row-plot progeny tests, the conclusion is that the height-diameter relationships are the same shape across families within a test at age 16 and the level of the relationship is directly and strongly related

to the dominant height of the family at age 16.

The conclusion of this phase of the study is that at a given location, the shape of the height-diameter relationship at any age is the same among seed sources or families, but the level of the height-diameter curve at that age differs by seed source or family. In addition, the level of the height-diameter curve is directly and strongly related to the dominant height of that seed source or family at that age. These results are consistent for the Southwide Pine Seed Source Study, the block-plot half-sib progeny test, and the row-plot progeny tests.

Stand-level variance

The shape or spread of the diameter and height distributions, as well as their relative location, determine the ultimate value of a stand. Accordingly, the variances of the height and diameter distributions are of interest in modeling the growth and yield of loblolly pine stands. It has been conjectured that while increasing the mean tree size, genetic selection could reduce the stand value by reduction of the stand-level variance and possible production of fewer large trees (Nance and Bey 1979, Thurmes 1980).

To test this proposal, data from unthinned family block plots were needed. Because the block-planted half-sib progeny test had been thinned after age 8, the data at age 8 were used, since given the initial spacing of 8 x 8 feet and the dominant heights ranging from 38 to 50 feet, the distributions of height and diameter were well developed.

Simple variances of height and diameter were calculated for each plot (family x rep combination) of the block-plot half-sib progeny test. Bartlett's test for homogeneity of variance was performed for both diameter and height in each rep; that is, the variances of the 12 families within a rep were compared. The hypothesis of homogeneity of variance was accepted for the variance of diameter in each of the four reps. The hypothesis of homogeneity of variance was accepted for the variance of height in three of the four reps. These results indicate that the variances of height and diameter generally did not differ among the families within a rep.

An observational analysis was done to determine the relative sizes and ranks of the variances of height and diameter of the twelve families within each rep. For each rep, the variances of height and diameter were ordered from largest to smallest. There were no consistent family rankings across the reps for diameter or height. The variance of diameter for the woodsrun control family ranked largest in one rep, third largest in one rep, and seventh largest in two reps. The variance of height for the woodsrun control family ranked fourth largest in one rep, sixth largest in one rep, seventh largest in one rep, and tenth largest in one rep. It is clear that the height and diameter variances of the woodsrun control family are not consistently larger than those of the eleven selected genotypes.

The general conclusion to be drawn is that given that silvicultural treatments are equally intense and successful, the variances of height and diameter in stands originating from selected genotypes are not different or consistently smaller than those in stands originating from genetically

unimproved stock.

CONCLUSIONS AND IMPLICATIONS

The major conclusions to be drawn from the work presented here are: 1) given that silvicultural treatments are the same and are equally intense and successful, variances of diameter and height in stands originating from selected genotypes are not different or consistently smaller than those in stands originating from genetically unimproved stock; 2) at the seed source and family levels, the shape of the height-diameter relationship at a given age is determined by the site and initial density while the level of the height-diameter relationship is determined by the seed source or family and is directly related to the dominant height of the seed source or family at that age; and 3) at the seed source and family levels the shape of the height-age curve is dictated by the site, but the level of the height-age curve is dictated by the seed source or family. A very important result of this work is the consistency of the results concerning the height-diameter relationship and height-age relationship from the Southwide Pine Seed Source Study to the block-planted half-sib progeny test to the row-plot progeny test. This consistency suggests implications for growth and yield modeling.

Implications for modeling growth of stands originating from selected genotypes are: 1) genetic improvement affects the rate at which stands develop, but does not fundamentally alter the pattern of stand development from that of stands of unimproved stock; 2) changes in genetic material on a given site will likely affect the level, but not the shape, of such basic relationships as the height-age and height-diameter relationships; 3) from the analysis of the height-diameter relationship, it is likely that the development of the height and diameter distributions will follow from the development of dominant height; and 4) correctly characterizing the height-age profile for a given site will be of primary importance.

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SEED ORCHARD MANAGEMENT

MODERATED BY DR. RAY GODDARD

University of Florida

ESTIMATING POLLEN CONTAMINATION IN LOBLOLLY PINE SEED ORCHARDS BY POLLEN TRAPPING

Michael Greenwood[1/] and Terry Rucker[2/]

Abstract.--The construction of an inexpensive, easy-to-build pollen trap is described, which rotates to keep the trap oriented into the wind at all times. These traps have been deployed for several years, both within and surrounding four loblolly pine (Pinus taeda L.) seed orchards ranging in age from 7 to 25y. A comparison of pollen caught on both the orchard and background traps permits an estimate of how much pollen the orchard is producing relative to background. The resulting estimates of background contamination are very similar to those obtained with other methods.

Keywords: pollen contamination, pollination, seed orchards, Pinus taeda.

Loblolly pine seed orchards of the North Carolina State and Western Gulf Cooperative Tree Improvement Programs produced over 60 tons of improved loblolly pine seed in 1982, enough to grow about 1 billion seedlings (Talbert et al., 1985; Byram et al., 1985). After genetic roguing of the orchards, the predicted volume gain at rotation from these improved seedlings is about 12%. However, these gains assume that there is no pollen contamination from outside the seed orchards. Squillace and Long (1981), citing several types of estimates of background contamination, suggest that contamination may range from 30 to over 80%, even in mature orchards surrounded by isolation zones. Friedman and Adams (1981), estimate that outside contamination in a 16-year old loblolly pine seed orchard was 28%, based on detection of several allozyme gene markers via electrophoresis. Pollen contamination of 30 or 80% will reduce a projected 12% gain to 10.2 or 8.2% respectively. Given the amount of seed now being produced by seed orchards, minimizing the impact of background pollen should be a major concern. Therefore, measuring orchard pollen production and the contribution of background pollen should be a routine part of quality control.

The most accurate way of assessing background contamination is by using genetic markers for such traits as allozymes or monoterpenes. But these methods, although potentially precise, require relatively sophisticated equipment and methods. A simple and possibly quite accurate method of determining background contamination is to compare pollen production within the orchard to background pollen trapped nearby. Koski (1970) used a similar method for estimating background contribution to total pollination in Scotch pine plantations in Finland. Here we describe a simple method of measuring the relative amount of pollen contributed by both the orchard and background.

[1/] Professor, College of Forest Resources, University of Maine, Orono, ME.
[2/] Lead Technician, Southern Forestry Research Dept., Weyerhaeuser Co., Hot Springs, AR.

Contamination is then estimated as a simple ratio of background production to total orchard production. Estimates of background contamination by pollen trapping are being verified by comparison with estimates made by analysis of allozymes.

METHODS

Pollen flight in four Weyerhaeuser Co. loblolly seed orchards was assessed by means of pollen traps changed at 24-hour intervals throughout the period of pollen flight. The daily peak in pollen flight was detected by changing traps at hourly intervals on 4/11/80 (see Fig. 1). Each trap consists of a glass microscope slide bearing a piece of double coated tape (about $1cm^2$), held at a 45° angle by a clothespin mounted on a vane which keeps the trap oriented into the wind at all times (see Fig. 1, inset).

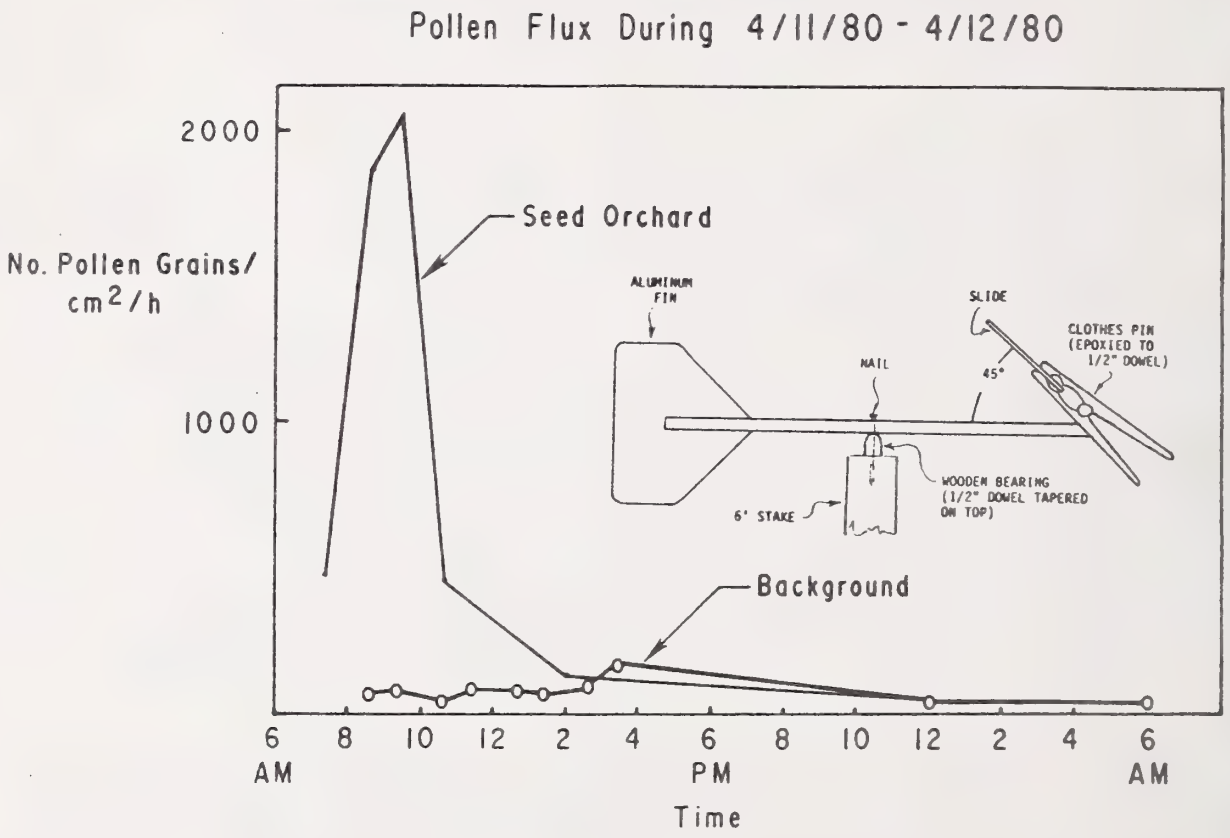

Figure 1.--Diurnal pattern of seed orchard and background pollen flight, diagram of pollen trap.

Materials required to construct the vane-type pollen trap are: 1) 0.5 in. diameter wooden dowels; 2) wooden clothespins; 3) aluminum nails (2.5 in. long x .12 in. dia.); 4) aluminum sheets (very thin aluminum plates used for the printing of newspapers, when cleaned, work very well and are cheap); 5)

2.5x7.6 cm (1x3 in.) glass microscope slides with frosted end; 6) Scotch brand #666 double coated tape with liner (0.5 in. width). The 0.5 in. wooden dowels are cut into 1-ft. lengths. One end of the dowel is then cut at a 45° angle to serve as the attachment point for the wooden clothespin. A thin slit, approximately 2 inches long, is cut in the other end of the dowel for attachment of the aluminum vane. The clothespin is glued to the dowel using contact cement or some other waterproof glue. The clothespin is oriented so that the glass slide, when held by the clothespin, is angled toward the vane end of the dowel. At this point, the dowel with clothespin attached can be painted. The aluminum vane, when cut to a desired size and shape, is inserted into the slit and attached to the dowel with aluminum nails. To avoid splitting the dowels, the heads should be cut from the nails to allow them to be mounted into a drill for insertion. Once the aluminum vane is attached, the excess nail should be cut off and filed flush with the surface of the dowel. To determine where to drill the hole in the dowel for attachment to a wooden stake, a slide is mounted on the clothespin and the balance point for the pollen trap is located. A hole large enough to allow rotation of the trap about the aluminum nail is drilled in the dowel at the balance point. A 1.25-inch segment of dowel with one end rounded serves as a bearing for pollen trap rotation (see Fig. 1).

Keeping the traps oriented into the wind resulted in up to a 7-fold increase in pollen catch over a trap fixed in a SW direction. Whenever possible, the traps were changed before rainfall, since rain will wash off significant quantities of pollen (0.6 cm of rain washed off over 60% of pollen trapped previously). After drying, however, the adhesive properties of the tape were restored.

In order to monitor pollen flight within the seed orchard, the traps were placed in open areas where trees had been removed, to minimize the impact of individual trees. Although Koski (1970) reports maximum pollen catch at crown level in Scotch pine, mounting traps in tree crowns is very inconvenient. During heavy pollen flight in the J. P. Weyerhaeuser seed orchard, we compared pollen catch on traps within the crowns of 5 different trees at a height of about 12M, with a trap located near each tree approximately 1M from the ground. The mean catch over a 24-hour period for the crown traps was about 3100 grains/cm compared with 2,800 for the ground traps. Since the difference was small, we adopted the easier option of deploying traps on 1.8M tall stakes so they could be easily reached.

Background pollen was trapped in open areas at least 300M from the edge of the orchard or any non-orchard sources of loblolly pine pollen. At one seed orchard, wind direction and velocity were recorded with a vane anemometer, while at the other three, the wind direction during orchard pollen flight (which usually occurs in the morning) was noted visually and recorded. At least four background traps were located approximately north, south, east, and west of the orchard. Traps located downwind during heavy production of orchard pollen could then be excluded from background averages. Ten traps were located throughout the seed orchard in open areas where trees had been removed.

Several transects, consisting of 4 traps 30M apart, were also placed downwind from the edge of the orchard to assess how pollen catch changes with

distance from the orchard. At Lyons, background traps were also located in the center of a 300-acre field about a mile from the orchard site (the Cato traps - see Fig. 2).

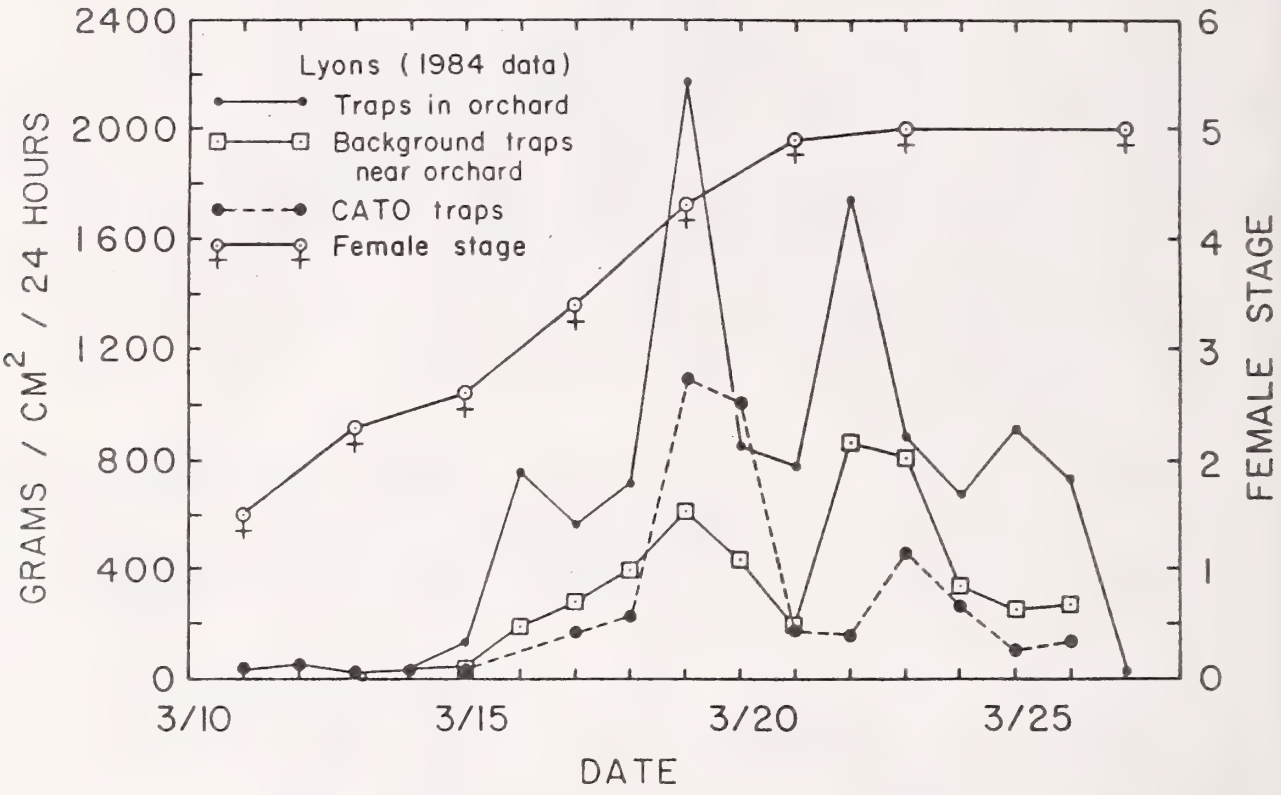

Figure 2.--Seasonal pattern of pollen flight for orchard located in Lyons, GA. Background trapped near the orchard and in a 300 acre field about 1 mile from the orchard (Cato) is also shown.

The traps were collected at the same time every day (either early morning or late afternoon, depending on the location), and the tape was covered with a plastic cover slip to prevent contamination. Number of pollen grains trapped per cm^2 super cestimated by counting 10 separate fields of view at 100x or 430x using a compound microscope. The area of the field of view was calculated after measuring its diameter with a stage micrometer. The results are presented as number of pollen grains/cm^2/24h.

Stage of female development was estimated for 10 clones, representing early, late, and average occurrence of female receptivity. Fifty or more strobili were observed from a lift truck on each of 3 ramets/clone every one or two days, and stage of development (Bramlett and O'Gwynn, 1980) was recorded for each.

RESULTS and DISCUSSION

After several years of observation, the orchard pollen almost always flies in mid to late morning after the catkins have dried out and when the wind is sufficiently strong to move the branch (see Fig. 1). A similar diurnal pattern is observed with other wind pollinated species (Ogden et al., 1969). Occasional afternoon pollen flights have been seen if morning rain was followed by a period of clearing and drying. Virtually no pollen is shed on rainy days. A comparison of hourly pollen catch for both an orchard and nearby background trap (upwind from the orchard) during heavy orchard pollen flight is shown in Figure 1. Note the great bulk of orchard pollen flew between 9 and 11 a.m., while background catch was fairly uniform, showing a slight peak between 3 and 4 p.m. Little pollen was trapped between 3 p.m. and 12 midnight, or 12 midnight to 6 a.m. the next day.

Since the background catch shown in Fig. 1 did not peak at the same time of day as the orchard, we can be reasonably certain that the background trap, located upwind from the orchard, was not receiving residual pollen from the seed orchard. Nonetheless, on a daily basis, background peaks, whether adjacent to the orchard or a mile away, do occur at roughly the same time as orchard peaks (see Figure 2). This suggests that the background pollen trapped was probably shed the same day as the orchard pollen. If we assume 1) that background pollen was shed at the same time as the orchard pollen; 2) that the wind velocity averaged from 10-15 mph on 04/11/80; and 3) that background catch peaks at 3 p.m. (about 6 hours after peak shed in the orchard see Fig. 1), then some of the pollen trapped late in the day could have traveled 60-90 miles.

To assess the possible impact of orchard pollen on background traps located downwind from the orchard, transects were located in open areas normally downwind from the orchards. Table 1 shows the catch averaged over several days versus distance from the edge of the orchard (Table 1).

Table 1.--Impact of orchard pollen on background estimates - pollen catch vs. distance downwind from orchard.

Location	Orchard	30M	60M	90M	120M	Background
J.P. Weyerhaeuser[a/]	4723	3159	2372	1979	2037	1112
Comfort[b/]	2464	2440	1846	1947	1428	909
Lyons[c/]	908	647	593	614	402	351

a/ Results from 2 transects over 8 days.
b/ Results from 2 transects over 4 days.
c/ Results from 4 transects over 12 days.

At 120M downwind from the orchard, the pollen catch diminished considerably from that in the orchard but was still 13 to 45% higher than the mean of background traps not located downwind. Wang et al. (1960) showed that while pollen catch from a single tree declines logarithmically with distance,

the decline from a stand is much less steep and appears somewhat linear. Background traps that are downwind from the orchard on a given date should be excluded from background estimates. We recommend that background traps be located at least 300M from the periphery of the orchard or any local source of pollen.

A comparison of pollen trapped throughout the pollination season both inside and outside four seed orchards, over two successive years, is shown in Table 2. Note that the older seed orchards produce the most pollen, but there is considerable variation by year. Also, total background catch is not correlated well with orchard production (total orchard minus background). Variation in orchard production only explains 15% of the variation in background catch across all seed orchards and years (r^2=0.15), so the magnitude of background catches shown here are not significantly related to seed orchard pollen production.

The patterns of pollen flight and female receptivity shown in Fig. 2 are representative of all orchards studied here. Both background and orchard pollen flies during the receptive period of most female strobili within the orchard.

Pollination in loblolly pine is a two-step process, the first being accumulation of pollen on the micropylar horns, the second being transfer of the grains to the nucellus by rain or the pollen drop (Brown, 1984; Greenwood, 1985). Any pollen grain reaching the micropylar horns, whether it arrives early or late, has an equal chance of reaching the nucellus and presumably germinating (Greenwood, 1985). An estimate of the contribution of background pollen to total pollination (in the orchard) should, therefore, be equal to the ratio of total background to total orchard (the latter includes both background and orchard). Estimates of contamination expressed as % total orchard pollination contributed by background are shown in the last column of Table 2.

Table 2.--Background pollen catch as percent total catch in 4 seed orchards, for two yeas each. Both background and orchard pollen were trapped throughout pollination period.

Orchard	Year	TOTAL TRAPPED Background	TOTAL TRAPPED Total Orchard	Background as % Total Orchard
Washington, NC.	1983	10,868	34,362	32%
Est. 1959	1984	15,935	50,968	31%
Comfort, NC.	1983	16,342	27,086	60%
Est. 1974	1984	7,579	19,299	39%
Lyons, GA.	1983	8,372	11,209	75%
Est. 1978	1984	4,782	11,330	42%
Magnolia, AR.	1982	11,345	12,838	88%
Est. 1972	1983	16,130	23,783	68%

Estimates of contamination range from 31 to 88%, similar to the range presented by Squillace and Long (1981). As expected, the oldest orchard showed the least contamination. However, the Magnolia orchard has sustained very high contamination because background there was very high both years. On the other hand, background was consistently low for both years at Lyons. Clearly, orchard location can significantly affect background load, especially when the orchard is young.

We are currently verifying the estimation of background contamination at the Washington, N.C., seed orchard with allozyme markers, and our first results show close similarity to those presented here (N. C. Wheeler and M. S. Greenwood, unpublished data). As mentioned earlier, other workers, also using biochemical genetic markers, have obtained a comparable range of estimates for southern pine seed orchards.

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SUPPLEMENTAL MASS POLLINATION OF SINGLE CLONE ORCHARDS FOR THE PRODUCTION OF SOUTHERN PINE HYBRIDS

Donald R. Knezick, John E. Kuser1/, and Peter W. Garrett2/

Abstract.--Supplemental mass pollination, "mistblowing", of a multiclone pitch pine orchard with loblolly pine pollen produced an average of 11% hybrid seed. Results of a controlled pollination study indicate that mistblowing isolated single clone pitch pine orchards would produce much higher percentages of pitch x loblolly hybrids. This technique could also be used to mass produce other commercially valuable southern pine hybrids.

Additional keywords: Pinus rigida, P. taeda, inbreeding depression, seed orchard, supplemental mass pollination.

The northern range of loblolly pine, Pinus taeda L., extends into Maryland, Delaware, and even southern New Jersey (Little, 1971). Unfortunately when loblolly is planted in colder areas to the north and west, it is susceptible to snow and ice damage and winter desiccation. Pitch pine, P. rigida Mill., although cold hardy, is noted for slow growth and poor form. Having observed fast growing hybrids between the two species growing around loblolly plantations in Maryland and New Jersey, Dr. Silas Little of the U.S. Forest Service saw the potential of using the hybrid for reforestation in the northeastern United States.

In the early 1960's, the U.S. Forest Service and Westvaco signed a cooperative agreement to breed and field test pitch x loblolly pine hybrids. Under the direction of Dr. Little and Fred Trew of Westvaco, the cooperative intensively selected 33 loblolly pines in Maryland and Delaware, and 32 pitch pines from Virginia, West Virginia, Maryland, New Jersey, Pennsylvania, New York, Massachusetts, New Hampshire, and Maine. In 1964, a clonal breeding orchard was established at the Northeastern Forest Experiment Station field office in New Lisbon, NJ. By 1968, enough female strobili were present to initiate controlled pollinations. The first test plantations were established in 1971 (Little and Trew, 1979). To date, there are over 50 hybrid plantations in several northeastern and midwestern states.

Although there currently is a strong demand for hybrid seedlings, the controlled pollination technique used to produce the hybrid seed is far too costly for mass production. Reforestation with hybrids on a large scale is dependent upon development of economical mass production techniques. Supplemental mass pollination is one of several techniques under consideration.

1/ Instructor and Assistant Professor, Rutgers University, Department of Horticulture and Forestry, New Brunswick, NJ 08903.
2/ Geneticist, Northeastern Forest Experiment Station, USDA Forest Service, Durham, NH 03824. Funding for this study was provided, in part, by a fellowship grant from the Jessie Smith Noyes Foundation, through Rutgers University's Center for Coastal and Environmental Studies, Division of Pinelands Research. Special thanks are extended to Silas Little, Fred Trew, Francis Roesch, and Douglas Eberhardt.

Mistblowing experiments have been conducted at the multiclone New Lisbon orchard for several years. Allozyme analysis of seed collected from the 1976 mistblowing indicate that an average of 11% of the seed was hybrid (Joly and Adams, 1983). The remaining seed was either self pollinated or was outcrossed to other clones of pitch pine in the orchard or wild pitch pines from the surrounding vicinity. Much higher percentages of hybrids must be produced in order for mistblowing to become operational.

A single clone pitch pine orchard, isolated from all outside sources of compatible pollen, mistblown with loblolly pollen can yield only two types of seed: self pollinated and hybrid. If the pitch clone selected exhibits a significant degree of inbreeding depression, most of the seed will be hybrid.

MATERIALS AND METHODS

All breeding work was conducted at the U.S. Forest Service field office in New Lisbon, NJ. Controlled crosses were made in loblolly pine and pitch pine seed orchards established in 1963-64 by the Northeastern Forest Experiment Station and Westvaco. Each clonal orchard consists of phenotypically superior selections planted in rows of 8-16 ramets to facilitate controlled pollination.

Four clones of pitch pine were selected as female parents for the study. The clones were chosen on the basis of precocious flowering and their demonstrated ability to produce good pitch x loblolly hybrids. Two clones each of loblolly pine and pitch pine were chosen as pollen parents. All pollen used was fresh and tested for viability. Ortet data on the female and male clones used are listed below.

clone #	county	state	height	age
		Pitch Pine Females		
62	Tompkins	New York	95′	160
71	Plymouth	Massachusetts	85′	114
76	Carroll	New Hampshire	68′	63
79	Oxford	Maine	75′	110
		Pitch Pine Males		
15-54	Rabun	Georgia	90′	51
16-269	Burke	North Carolina	64′	40
		Loblolly Pine Males		
4-32	Worcester	Virginia	92′	42
7-56	Williamsburg	South Carolina	90′	36

Pollination bags were mounted on twenty branch tips per ramet, each with a minimum of two female strobili (from hereon referred to as conelets). Four branch tips per ramet were marked as open pollinated controls. Only one ramet per female clone was used in order to avoid possible variation between ramets. Six pollen treatments were applied to each clone. Each treatment was replicated in four pollination bags. The pollen treatments were:

1) No Pollen: unpollinated to test for complete conelet isolation.

2) Self: pollen from the same clone to test for self compatibility versus inbreeding depression.
3) Self + Loblolly: pollen from the same clone plus loblolly pollen in a 1:1 mix to simulate the conditions in a mistblown single clone orchard.
4) Loblolly: a mixture of two loblolly pollens to test the ability of the pitch clone to hybridize with loblolly.
5) Outcross Pitch: a mixture of two pitch pollens to test the effectiveness of the controlled pollination technique with presumably highly compatible pollen.
6) Open Pollinated: unbagged wind-pollinated control.

All crosses were made using standard control pollination techniques. Beginning in early May 1982, sausage casing style pollination bags were mounted over branch tips with the aid of aluminum rings for added support. Conelets were bagged while in stages I and II (Bramlett and O'Gwynn, 1980). When they reached stage V, 0.50cc - 0.75cc of fresh pollen was injected by hypodermic syringe into each bag. The conelets were treated twice at two day intervals to bracket the period of maximum receptivity. The bags were removed when the conelets reached stage VI.

In September 1983, the cones were harvested. They were kept separate according to treatment and bag number. Conelet abortion was determined by subtracting the number of cones harvested from the number of conelets pollinated. Cones were placed in used paper pollination bags with clear plastic on the upper side in an unheated greenhouse for drying. Cones from clones 71, 76, and 79 opened by January 3, 1984. Cones from clone 62 were serotinous and were opened by heating in an oven at 40° - 45° C. Seeds were extracted from each cone by hand and tallied for each bag separately. The number of seeds per cone was determined by dividing the number of seeds per bag by the number of cones per bag.

As fresh pitch pine seed does not require stratification (USDA, 1974), the seed was ready for germination. All seed was surface sterilized with 0.7 molar NaOCl for 15 seconds and placed directly on moist blotter paper in germination trays. Two trays of approximately 60 seeds each were prepared for the four replicates of each treatment. Seed was germinated under eight hours of light at 30° C and 16 hours of darkness at 20° C for 14 days.

At the end of the germination period, the numbers of normal and abnormal germinants were counted. The remaining seeds were opened to determine whether or not they were filled. The percentage of filled seed was determined by dividing the total number of germinants plus the number of ungerminated filled seed by the initial number of seeds placed in each germination tray. Numbers of filled seeds per cone were determined by multiplying the number of seeds per cone by the percentage of filled seed. Numbers of germinated seedlings per cone were determined by multiplying the number of seeds per cone by the percent germination of all seed (filled and empty).

In February of 1984, 28 self pollinated, 28 loblolly pollinated, and 28 self + loblolly pollinated seedlings from clone 76 were planted in Ray Leach super cells. (Only clone 76 was used because there were not enough seedlings from the other clones). The seedlings were grown in a heated greenhouse under supplemental lighting. In September of 1984 the seedlings were transplanted into one gallon containers.

RESULTS

Clones 71, 76, and 79 did not produce any cones when unpollinated (table 1), but clone 62 developed eight cones from the initial 13 conelets bagged. The cones were significantly smaller than those resulting from other pollen treatments. Although normal wings developed, the seeds were small and rudimentary, and are not counted as seeds in table 1. Abortion of self pollinated conelets varied by clone. While all self pollinated conelets of clones 71 and 76 developed into mature cones, in clone 79 only one of fifteen developed. In clone 62, self pollinated conelets aborted less often than those from the outcross pitch treatment.

The total number of seeds per cone was not affected by inbreeding depression. In clones 62 and 71, the number of seeds per cone in the self pollination treatment was actually greater than the number of seeds per cone in outcross pitch pollination. (Data from clone 79 are difficult to assess due to low sample size in the outcross pitch treatment because of damage to three of the four pollination bags during the breeding season).

The number of filled seeds per cone resulting from outcross pitch and loblolly pollination of clones 62, 71, and 76 was far greater than those in the self pollination treatment. Clones 71 and 76 produced more filled seed from outcross pitch pollination than from loblolly pollination, but clone 62 produced more with loblolly pollination. In clones 62, 71, and 76 the self + loblolly mix treatment is intermediate between self pollination and loblolly pollination.

Inbreeding depression effect on germination was determined by comparing percent germination of filled seed. In all three clones, percent germination follows the same pattern: outcross pitch > loblolly > self + loblolly > self. In all cases, self pollination yielded fewer seedlings per cone than the other pollen treatments. The self + loblolly pollen treatment always produced more seedlings per cone than self pollination but fewer than loblolly pollination.

As of June 1985, seedling survival is 93% for the hybrids, 54% for the selfs and 61% for the self + loblolly pollinated seedlings. The hybrid seedlings are an average of three times taller than the selfs. There is no overlap between the two groups as the shortest hybrid is still taller than the tallest self. The self + loblolly pollinated seedlings have segregated into two populations, fast growing and slow growing. The average height of the fast growing seedlings is equivalent to that of the hybrids and the average height of the slower growing seedlings is equivalent to the of the selfs.

DISCUSSION

Use of inbreeding depression to facilitate production of hybrids is certainly not a new idea. It is widely used in crop breeding and its application to forest genetics was discussed by Wright in 1976. The effects of inbreeding depression can be used to increase the percentage of pitch x loblolly hybrids in various ways. In the four clones of pitch pine tested, self pollinated cones always had fewer filled seeds that loblolly pollinated cones. Although southern pine pollen is capable of self pollination and fertilization (Bramlett, 1981), embryo collapse is more likely to occur when a pitch pine ovule is self pollinated than when loblolly pollinated. Thus there

Table 1.--Summary of controlled pollinations

Clone #	Pollen Treatment	Conelets Pollinated	Cones Matured	Seeds per Cone	Filled Seeds per Cone	% Germination of Filled Seed	Germinants per Cone
62	No Pollen	13	8	0.0	-	-	-
	Self	16	11	94.8	3.4	82.4	2.8
	Self + Loblolly	13	6	110.3	53.7	84.0	45.1
	Loblolly	12	4	120.0	60.4	87.8	53.0
	Outcross Pitch	12	5	89.2	30.6	92.4	28.3
	Open Pollinated	18	17	116.0	45.5	82.3	37.7
71	No Pollen	15	0	-	-	-	-
	Self	14	14	92.5	13.6	69.1	9.4
	Self + Loblolly	20	20	78.7	17.6	82.4	14.5
	Loblolly	16	16	87.5	27.5	94.9	26.1
	Outcross Pitch	17	13	83.2	67.1	96.9	65.0
	Open Pollinated	17	15	82.7	46.9	95.1	44.6
76	No Pollen	18	0	-	-	-	-
	Self	16	18	58.3	25.7	72.0	18.5
	Self + Loblolly	15	14	62.1	27.0	82.6	22.3
	Loblolly	12	12	78.2	31.7	87.8	27.8
	Outcross Pitch	12	12	71.3	59.9	95.3	57.1
	Open Pollinated	12	12	81.3	73.0	97.0	70.8
79	No Pollen	14	0	-	-	-	-
	Self	15	1	100.0	11.5	55.7	6.4
	Self + Loblolly	12	0	-	-	-	-
	Loblolly	11	7	118.0	57.3	97.0	55.6
	Outcross Pitch	4	1	90.1	79.2	96.5	76.4
	Open Pollinated	17	16	125.3	102.1	97.5	99.5

should be more hybrids than selfs in mistblown seedlots (assuming equal pollen volumes).

All four clones of pitch tested had lower seed germination upon selfing than with loblolly pollination. Reduced germination of selfed seeds in the nursery seedbed will further increase the percentage of hybrids.

Inbreeding depression in survival and growth of selfed seedlings, as seen in clone 76, indicates yet another means of increasing the percentage of hybrids. As the seedlings grow in the seedbed, there is competition for light, nutrients, water, and space. Many of the smaller self pollinated seedlings will suffer and die as a result. In addition, many of the surviving selfs can be rogued out of harvested mistblown seedlings during standard nursery grading operations. In our greenhouse experiment, it appears that all of the selfs could be rogued out of the self + loblolly seedling group.

It is impossible to accurately estimate percentages of hybrids which could be produced by mistblowing single clone orchards based solely on data from this experiment. The actual percentage of hybrids produced will depend upon the degree of inbreeding depression expressed by the orchard clone, compatibility of the orchard clone to the pollens used, the amount of pollen mistblown, and the timing of the application. Even so, it seems safe to predict that in clone 62, where 53.0 seedlings per cone developed when loblolly pollinated compared to only 2.8 per cone when self pollinated, the percentage of hybrids in mistblown seedlots would be very high.

CONCLUSION

Results of this experiment indicate that mistblowing isolated single-clone pitch pine seed orchards with loblolly pine pollen may be an effective technique for mass producing pitch x loblolly hybrids.

Establishing several single clone orchards and mistblowing each with a variety of compatible loblolly pollens would reduce problems associated with low genetic variability. Ease of access must be weighed against adequate pollen isolation when deciding upon the number of orchards to be located in any particular area.

In order for this technique to become accepted, it must be cost effective. Currently, mistblowing is being carried out operationally in several southern pine seed orchards. Mistblowing in seed orchards may become commonplace as a means of alleviating pollen shortages and to circumvent inbreeding, with increases in seed yield and variability justifying the additional expense (Kellison, 1971) (Bridgewater and Trew, 1981). Mistblowing single clone orchards should be equally cost effective.

While this study was designed to test the feasibility of single clone orchard production of pitch x loblolly hybrids, the technique appears to be readily adaptable for the production of other commercially valuable southern pine hybrids.

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THEORETICAL IMPACT OF POLLEN VIABILITY AND DISTRIBUTION ON THE NUMBER OF STROBILI TO USE FOR CONTROLLED POLLINATIONS IN LOBLOLLY PINE

D. L. Bramlett, F. E. Bridgwater, J. B. Jett, and F. R. Matthews[1/]

Abstract.--The number of female strobili to pollinate per controlled cross in loblolly pine can be estimated from three factors: (1) *in vitro* pollen viability, (2) pollen distribution frequency within the ovules, and (3) the expected cone survival of pollinated flowers. In this study empirical data on the number of pollen grains per ovule were obtained from sampling loblolly pine conelets 2 weeks after pollination. The frequency distribution for the number of pollen grains per ovule was then used to develop a nonlinear model to estimate pollination effectiveness. Adjustments for less than the maximum number of seeds per cone and for empty seed losses resulted in estimates of filled seed per cone for varying levels of pollen viability. From these data, the numbers of flowers required to produce 300 filled seed per cross are presented for four levels of cone survival. These guidelines can improve breeding effectiveness and efficiency for loblolly pine.

Additional Keywords: *Pinus taeda*, tree breeding, tree improvement

INTRODUCTION

Controlled pollinations are a vital component of the recurrent selection and breeding program of southern pines. Production of adequate seeds for genetic testing requires both an effective and efficient controlled pollination procedure. An effective program would have a high success rate for completion of the attempted prescribed crosses. For example, it is important to have all the cells of the mating design completed before outplanting. However, an effective program could have a high completion rate but would not necessarily be efficient in terms of the required resources such as the number of pollination bags installed, the amount of pollen required, or the labor employed.

[1/] The authors are respectively: Plant Physiologist, Southeastern Forest Experiment Station, Macon, GA; Plant Geneticist, Southeastern Forest Experiment Station, Raleigh, NC; Associate Director, Cooperative Tree Improvement Program, School of Forest Resources, N.C. State University, Raleigh, N.C.; and Plant Pathologist, Southeastern Forest Experiment Station, Athens, GA.

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An efficient program could provide a high completion rate for attempted crosses yet should not utilize more than the necessary amount of resources. Some safety margin is prudent, but consistent overproduction of cones or seeds is certainly not cost effective. It would be better to refine the breeding program so that more crosses were completed in a given year rather than to overproduce seeds from a smaller number of crosses.

In research studies to evaluate the factors affecting the success of controlled pollinations, pollen viability appears to be the single most important factor relating to high seed yield per cone (Matthews and Bramlett 1985). Correct timing of female receptivity and the delivery of the pollen to the ovules are important factors but if the pollen is not of high quality, the seed yield and seed quality may be seriously reduced.

Large-scale breeding programs must routinely deal with the subject of pollen viability because of the impact this variable has on how aggressively breeding efforts can be pursued. The North Carolina State Cooperative Tree Improvement Program maintains a large, centralized pollen storage bank to expedite the Cooperative's second-generation breeding program. The Cooperative's program involves the sharing of the control-pollination workload and the exchange of pollens. In order to meet the biological time constraints imposed by the pollination season, it is necessary to use stored pollen.

The Cooperative annually receives and processes for storage some 600 to 800 pollen lots. In the course of processing, several decisions are made that take into account pollen viability. Moisture content and percentage of germination <u>in vitro</u> are determined upon receipt of the pollen. When moisture content exceeds 15 percent, the pollen is dried in an extractory as described by Sprague and Snyder (1981) to a moisture content of 8 to 10 percent. This initial drying is done to stabilize pollen quality until final preparations for long-term storage can be completed. Subsequent drying to approximately 4 percent moisture is done by freeze-drying and the pollen is vacuum sealed in 10 ml vials for long-term storage. Germination tests (0.5 percent agar) are only done before pollen storage. Both research and experience indicate that properly stored pollen suffers little loss in viability for at least several years (Goddard and Matthews 1981).

Pollen lots with viabilities as low as 10 percent are stored when no other pollen of higher viabilitiy is available. This approach assumes that even at viabilities as low as 10 percent, some seed set will be achieved when appropriate amounts of pollen and proper timing are utilized in making controlled pollinations. The low-viability pollen will be employed where required rather than delay breeding for a year. When sufficient pollen is available with viability greater than 50 percent, 80 ml of each pollen lot are put into storage (eight vials of 10 ml each). This amount is considered sufficient to complete the anticipated crosses involving any given pollen even when pollen viabilities approach 10 percent. When the viability for a pollen lot is less than 50 percent, additional pollen is requested the following spring to upgrade the inventory. As long as a cross has not been completed, the plan is to replace or upgrade pollen inventories when the prestorage viability is less than 50 percent.

The complexities of managing a large pollen bank with constantly shifting inventories are eased with a computer-based record system. Matching pollen availability to the availability of female strobili also requires good records to avoid inefficiencies in breeding efforts and lost time.

This paper presents the impact of pollen viability on the effectiveness and efficiency of controlled pollinations in loblolly pine. Empirical data were used to construct a generalized model to generate the pollination effectiveness at varying viability levels. The pollination effectiveness was then transformed to the expected seed yields per cone and the number of strobili estimated to produce 300 filled seed for progeny testing.

EFFECTIVE CONTROLLED POLLINATIONS

The pine reproductive system consists of the megasporangiate strobilus (female flower), and the microsporangiate strobilus (male catkin). The female flowers are in an upright position on the tips of the new vegetative growth and are phenologically synchronized in development to be at maximum receptivity during the peak of pollen release from the male catkins. As the wind transports pollen to the female flower, individual pollen grains adhere to micropyle arms and are transported to the pollen chamber via a pollination droplet. In controlled pollination, breeders try to simulate the natural wind pollination process. Wind pollen is excluded with an isolation bag and the selected pollen is injected into the bag by the breeder. Key elements of successful breeding are (1) correct timing of pollen application, (2) providing adequate quantities and distribution of the pollen to the flowers, and (3) maintaining a high viability and vigor of the pollen.

Up to 10 million pollen grains may be injected into a single pollination bag, and the female flower may have a large amount of pollen between the cone scales, yet only a very small number of pollen grains are found in the pollen chamber. Because only those pollen grains in the pollen chamber are capable of producing seed, the effectiveness of the controlled pollination can be evaluated by examining the pollen chambers and recording the number of pollen grains present. Matthews and Blalock (1981) have described the procedures for making the pollen chamber count and this method has been a valuable tool to quantify the effectiveness of both controlled- and wind-pollinated pines. By using the pollen count technique, the timing of pollen application has been found to be more flexible than once thought. It appears that 2 days before or after maximum receptivity (Stage 5) are nearly as effective as pollination at stage 5 (Bramlett and Matthews 1983).

The pollinator used to supply the pollen can be an important part of the seed yield and many types of pollinators have been successfully used (Bramlett and O'Gwynn 1981). Any device that effectively delivers the pollen to the ovules can be used and may include a camel's-hair brush, wash bottle, syringe, or cyclone pollinator. The quantity of available pollen may influence the choice of pollinator. For very limited amounts of pollen, the camel's-hair brush is effective. When pollen is abundant, the cyclone pollinator uses high volumes of air and pollen to completely distribute pollen to all flowers within the bag.

Best results have been achieved by using the cyclone pollination with 1 cc of pollen applied per bag. Normally, one application at the correct stage of flower development is adequate, but up to three pollen applications per bag may be used when flower development within the bag is widely divergent.

FREQUENCY DISTRIBUTION OF POLLEN WITHIN THE OVULES

In several controlled pollination experiments we have quantified the pollen catch per ovule. From these studies, the pollen distribution approaches but does not equal the average number of pollen grains per ovule for wind-pollinated flowers. In the data presented in Figure 1, four quantities of pollen 0.25, 0.50, 1.00, and 2.00 cc were applied to three separate female clones in the Georgia Forestry Commission's Arrowhead Seed Orchard. Conelets were collected 10 to 14 days after pollination and approximately 1,500 ovules were observed for pollen counts within the pollen chamber. An analysis of variance of the data set indicated no statistical differences for the mean pollen count among the 0.25, 0.50, and 1.00 quantities of pollen. These three quantities were combined in the frequency distributions shown in Figure 1A and had a mean value of 2.22 pollen grains per ovule. In this distribution 12 percent of the ovules had zero pollen grains. If an ovule has no pollen grains or has pollen grains that are not viable, the ovule aborts soon after pollination and no seeds are formed. If the ovule has at least one viable pollen grain, however, the ovule continues development and forms a mature seed coat unless the normal development is disrupted by destructive agents. In Figure 1B, the pollen counts for ovules pollinated with 2.0 cc of pollen had a mean value of 2.78 pollen grains per ovule and lower frequencies of ovules with 0, 1, or 2 pollen grains. Thus, the percentage of ovules with at least one viable pollen grain would be expected to increase with increasing mean values and consequently a low-frequency distribution of ovules with 0, 1, or 2 pollen grains per ovule. For example, in Figure 1C, the frequency distribution for wind-pollinated ovules illustrates a mean value of 3.97 with small numbers of ovules with 0, 1, or 2 pollen grains per ovule.

Regardless of the distribution frequency of the pollen grains in the ovules, the probability that some ovules will have no viable pollen grains increases as the viability decreases. Thus, for each distribution function, the empirical pollination effectiveness can be calculated by summing the probabilities of ovules with at least one viable pollen grain for each pollen count class.

DEVELOPMENT OF A PREDICTIVE MODEL

If the pollen viability and distribution are known, a probability model can be developed for varying quantities of pollen. For example, with 50 percent viable pollen and the pollen distribution as shown in Figure 1A, the combined probabilities would give 60 percent of the ovules with at least one viable pollen grain. This ratio of ovules with one or more viable pollen grains to the total number of ovules per cone (seed potential) has been termed the pollen effectiveness (PE) (Bramlett 1981). Therefore, the maximum predicted seed yield for pollen lots with 50 percent viability and 2.2 pollen grains per ovule would be PE x SEED POTENTIAL or 0.60 x 160 = 96 developed seeds per cone. Obviously the actual yield of developed and filled seeds would be lower than the maximum as will be discussed later in the paper.

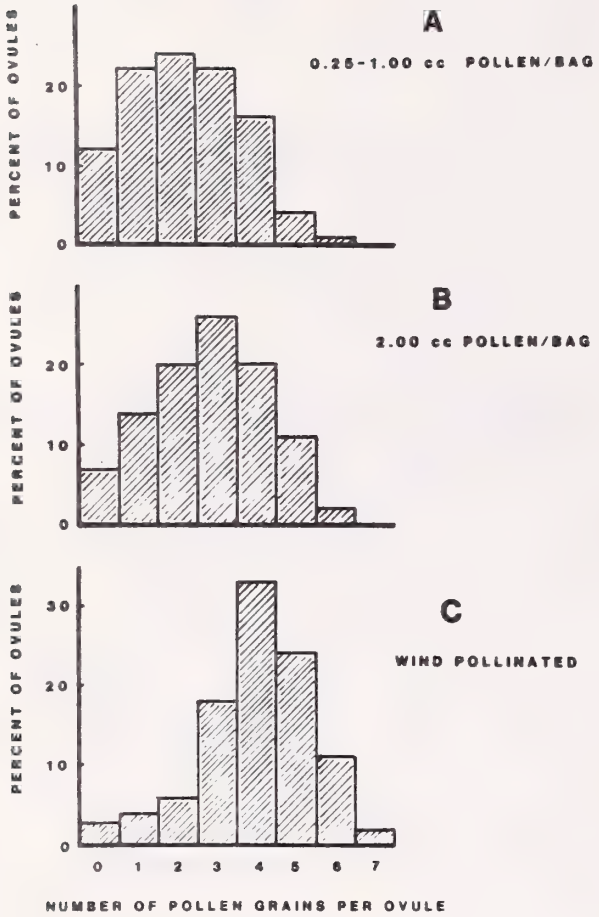

Figure 1.--Number of pollen grains per ovule for loblolly pines following controlled pollinations and wind pollinations (A) Controlled pollinations with 0.50 to 1.0 cc of pollen per bag (B) Controlled pollination with 2.0 cc of pollen per bag (C) Wind pollinations.

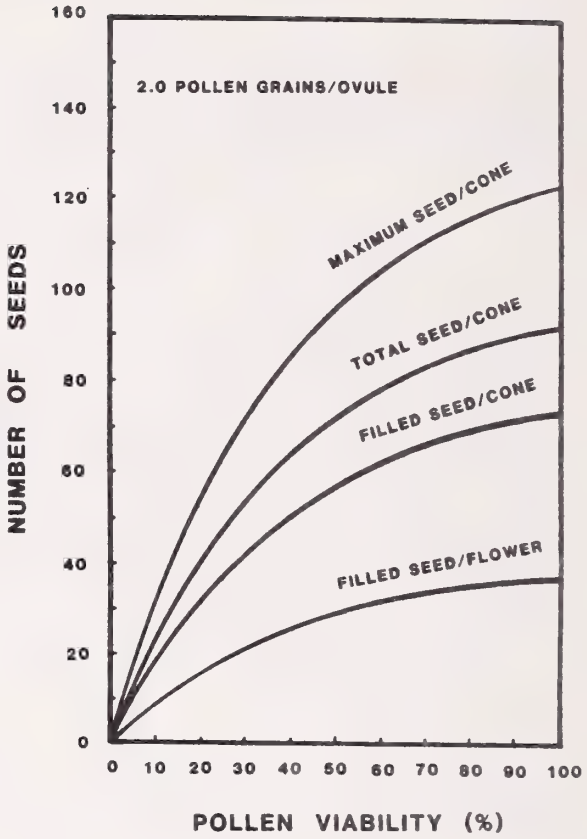

Figure 2.--Predicted maximum seed yield per cone, total developed seed per cone, filled seed per cone, and filled seed per flower for controlled pollinations in loblolly pine. The predicted values are generated from a model with 2.0 pollen grains per ovule and a seed potential of 160 ovules per cone and are shown for varying pollen viabilities.

The next development of the general predictive model was derived from pollen count data collected from a pollination timing study in 1982. In this study, pollen was applied to loblolly pine (Pinus taeda L.) flowers at varying female flower development stages (Bramlett and Matthews 1983). Pollen application before or after the date of maximum receptivitiy reduced the mean number of pollen grains per ovule. Using this data set a nonlinear predictive model was derived by fitting the observed data with a function described by Richards (1959):

$$\text{Pollination effectiveness} = a \times (1 - e^{(-b \times VIAB)})$$

Where: $a = 8.0402 + (53.4169 \times MPC) - (7.7432 \times (MPC^2))$

$b = 0.00737 + (0.0087 \times MPC)$

with VIAB = Viability of applied pollen

and MPC = mean pollen count per ovule

From this predictive equation, values of pollination effectiveness were generated for the values of the mean number of pollen grains per ovule (MPC) ranging from 0.50 to 4.0 (Table 1). The range of MPC are realistic values that have been observed in controlled pollination studies. Expected values in controlled pollinations average between 2.0 to 3.0 pollen grains per ovules. Counts approaching 4.0 have been observed with heavy applications of pollen but would generally be expected to be the upper limit of average number of pollen grains per ovule. Counts below 1.0 occur when low quantities of pollen are applied or when the pollen is not applied at the correct stage of flower development.

Table 1.--Predicted pollination effectiveness and apparent pollination effectiveness for loblolly pine at varying pollen viabilities.

Mean Pollen Grains/Ovule	Pollen Viability (%)				
	10	25	50	75	90
	--------Pollination effectiveness--------				
0.5	0.036	0.083	0.146	0.192	0.214
1.0	0.080	0.178	0.297	0.376	0.411
2.0	0.184	0.388	0.596	0.708	0.749
3.0	0.281	0.559	0.801	0.906	0.938
4.0	0.337	0.638	0.860	0.937	0.956
	----Apparent pollination effectiveness----				
0.5	0.027	0.062	0.110	0.144	0.160
1.0	0.060	0.133	0.223	0.282	0.308
2.0	0.138	0.292	0.447	0.531	0.562
3.0	0.211	0.419	0.601	0.680	0.704
4.0	0.253	0.478	0.645	0.703	0.717

PREDICTED SEED YIELD FROM CONTROLLED POLLINATIONS

The PE value can be transformed to seed yields by multiplying PE value by seed potential. In Figure 2, the predicted number of developed seed per cone for a mean pollen count of 2.0 and varying pollen viabilities is illustrated. The highest values are the maximum seed per cone that could be expected with the given parameters (Maximum Seed/Cone, Fig. 2). However, not all of the ovules with one or more viable pollen grains per ovule will continue development and form a seed coat. Numerous causes may exist but one primary problem is insect damage to the ovule resulting in ovule abortion before seed coat formation.

If the pollen grains have not been counted in the pollen chambers, the apparent pollination effectiveness (APE) can be measured as the ratio of the total developed seed to the seed potential (Total Seed/Cone, Fig. 2). APE approximates PE when insect losses are minimal. From seed yield data when varying quantities of pollen were applied to three loblolly pine clones, APE was 75 percent of the pollination effectiveness as determined by the pollen count per ovule data. Thus the APE for a given tree could be expected to average 0.75 x PE (Table 1).

A second factor in the reduction of seed yield is that not all of the developed seeds are filled. Again, several causes of empty seed are known but insect damage and embryonic abortion are principal reasons that only a percentage of the total seeds are filled and thus capable of germination.

The values in Table 1 for APE can be converted to predicted filled seed yields per cone by expanding to the seed potential for loblolly pine and then adjusting for the percentage of total seeds that are filled. Although the percentage of filled seeds may vary from year to year depending on the level of insect protection and other factors, an average of 80 percent filled seed is a reasonable number to use for seed orchard controlled pollinations. Thus the predicted filled seeds per cone would equal:

Filled seed (FS) = APE x SP x PFS
APE = Apparent pollination effectiveness
SP = Average seed potential for loblolly pine (160)
PFS = Percent filled seed (0.80)

Values of predicted filled seeds per cone for varying viabilities and pollen distribution functions are shown in Table 2. As expected, the seed yields in Table 2 indicate that low seed values are associated with low mean pollen counts per ovule. These low mean values could result from inadequate distribution of pollen to the ovule or if flowers were not pollinated at the proper time. More important, low values of filled seed would also be expected when the viability is reduced. For example, with a mean value of 2.0 pollen grains per ovule, predicted filled seed yields with 90 percent viable pollen are four times the yields when using 10 percent viable pollen. In Figure 2, yield of filled seed per cone can be compared with the maximum seed and total developed seed per cone.

Table 2.--Predicted number of filled seeds per surviving cone for control-pollinated loblolly pine at varying levels of pollen viability.

Mean Pollen Grains/Ovule	Pollen Viability %				
	10	25	50	75	90
	filled seed				
0.5	3	7	14	18	20
1.0	7	17	28	36	39
2.0	17	37	57	67	71
3.0	27	53	76	87	90
4.0	32	61	82	89	91

NUMBER OF FLOWERS TO POLLINATE

The final factor for controlled pollination guidelines to be both effective and efficient is to know the number of flowers to pollinate based on the viability of the pollen source. As stored pollen viability can be determined *in vitro* before installation of pollination bags, guidelines are needed to adjust the number of bags to use per cross based on the expected seed yield. Currently 144 seedlings are required for outplanting each cross in the N.C. State Tree Improvement program (Talbert *et al.* 1981). To ensure adequate seedlings, approximately 300 filled seeds per cross are needed.

The number of flowers required to provide 300 filled seed per cross are estimated in Table 3. The table gives four levels of cone survival. Rarely would 100 percent of the flowers pollinated reach cone maturity. Based on experience, more reasonable survival rates are 50 to 75 percent of the flowers pollinated. In Figure 2, the number of filled seeds per pollinated flower are presented for 2.0 pollen grains per ovule at varying pollen viabilities. Survival rates of 25 percent indicate serious problems involving insects or other factors including poor pollination techniques. With pollen viability of 75 to 90 percent and two or more pollen grains per ovule, less than 10 flowers would be required to produce the 300 seeds for progeny testing. This number may be lower than is currently being used for operational breeding programs. Obviously, it is important to be sure that adequate seed are available, and the numbers of flowers given in Table 3 should be considered as minimum requirements. Also Table 3 illustrates the guidelines to follow for loblolly pine. Other species could be adjusted based on pollen counts per ovule and differences in the seed potential.

Table 3.--Minimum number of loblolly pine flowers to pollinate to produce 300 filled seed per cross, at varying levels of pollen viability.

Cone Survival	Mean Pollen Grains/Ovule	Pollen Viability %				
		10	25	50	75	90
		no. of flowers				
25 percent	0.5	400	172	86	67	60
	1.0	172	71	43	34	31
	2.0	71	33	22	17	17
	3.0	45	23	16	17	14
	4.0	38	20	15	14	14
50 percent	0.5	200	86	43	34	30
	1.0	86	36	22	17	16
	2.0	36	17	11	9	9
	3.0	23	11	8	9	7
	4.0	19	10	8	7	7
75 percent	0.5	134	57	29	22	20
	1.0	57	24	15	12	11
	2.0	24	11	8	6	6
	3.0	15	8	5	5	5
	4.0	13	7	5	5	5
100 percent	0.5	100	43	22	17	15
	1.0	43	18	11	9	8
	2.0	18	9	6	5	5
	3.0	12	6	4	5	4
	4.0	10	5	4	4	4

ADDITIONAL REMARKS

The theoretical model for the effectiveness of controlled pollinations with varying levels of pollen viability indicates that tree breeders sacrifice a large amount of efficiency when low pollen viabilities are used. However, our model indicates that only a very few female flowers per cross are required to produce adequate seeds when correct timing, adequate distribution of pollen within the bag, and highly viable pollen are used.

One of the untested assumptions of the model is that the ratio of developed seeds to the maximum percentage of ovules with at least one viable pollen grain would be the same regardless of the pollen viability. This assumption may not be valid. Some preliminary work indicates that when pollen viability is low, that pollen vigor may also be reduced. Pollen vigor is a loosely defined term that currently is not measured *in vitro*. The effects of low pollen vigor are reduced percentages of filled seed per cone. This apparently is a result of pollen that germinates on the nucellus but does complete fertilization. Thus ovules pollinated with low-vigor pollen could have had relatively high *in vitro* viability but rather low filled seed yields per cone.

The number of flowers to pollinate per cross does not indicate how many bags should be used. Frequently large numbers of flowers can be enclosed within one bag. The danger is that a broken branch would be disastrous. Therefore, a minimum number of bags should be five or six. Also, when large numbers of flowers per bag are used, the flower development may extend over several days, requiring more than one pollination per bag.

Finally, the number of flowers required to complete a cross can be used in two ways. The first way is increase the effectiveness and efficiency of the breeding program. The second way is to evaluate current breeding results. If large numbers of flowers are required to complete each cross, this would appear to be a warning signal that one or more of the several components of pollination are not favorable. Pollen viability would be the most likely item but pollen distribution and flower mortality could also be part of the low efficiency. Taking corrective action should mean that seed yields increase and the breeding program becomes both more effective and efficient.

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A SEVEN-YEAR-OLD OCALA SAND PINE SEEDLING SEED ORCHARD

Ralph A. Lewis, Timothy LaFarge and James L. McConnell 1/

Abstract.--A 20 acre Ocala sand pine seedling seed orchard was established in 1978 near Ocala, Florida. First year survival was 67%. An apparent adaptive mechanism that allows some trees to survive unfavorable environmental conditions was noted. After 7 years and 2 thinnings, the tallest trees were over 26 ft. and the tallest family averaged over 19 ft. Although a few cones were observed after 3 years, the orchard is just starting to produce a significant amount of seed.

Additional keywords: Orchard management, seed orchard design, single-tree plot, Pinus clausa.

The theory for design and management of seedling seed orchards is well known but few production orchards of this type have been established in the South. In January of 1978, a production seedling orchard of the Ocala race of sand pine (Pinus clausa var. clausa Ward) was planted on the Ocala National Forest in central Florida.

METHODS AND MATERIALS

Orchard Design and Layout

Design.--The orchard is designed for 30,000 seedlings, consisting of 120 families with each family represented by a single tree plot in each of 250 blocks (replications). A block consists of 10 rows of 12 trees planted on a 5 ft. by 5 ft. spacing. Actual orchard area is slightly less than 17 acres with another 3 acres devoted to roadways and border strips. Border rows were planted around the exterior and along both sides of all interior roadways.

Layout.--The size and complexity of the planting required that the layout be simple but precise. All blocks and rows within blocks were tagged. Every planting point was marked with a wire stake flag. In order to help guide the planters, each block was staked with a single color with all adjacent blocks staked with different colors. This was accomplished by alternating red and white blocks next to alternating blue and yellow blocks. Another color (orange) was used exclusively for border rows.

1/ Forester, Eastern Zone Geneticist, and Regional Geneticist respectively, USDA Forest Service, Region 8, Atlanta, Georgia.

Materials

Site.--The planting site is located in central Florida on the Lake George Ranger District, Ocala National Forest. Soils are excessively drained, stongly acidic deep sands of the Astatula series. The area is part of a "longleaf island" and it has a slightly higher clay content than the more typical sand pine sites. The topography ranges from flat to slightly rolling. The site was prepared by removing all woody vegetation followed by raking and double disking.

Seed.--Wind-pollinated seeds from 131 select trees (all growing in wild stands on the Ocala N. F.) were collected in 1976. Sufficient seed from each collection to produce at least 250 healthy seedlings were planted in April of 1977 in the Chipola Experimental Forest nursery near Marianna, Florida.

Seedlings.--The early care and culture of the seedlings were routine but in late summer, both moisture and fertilization were gradually reduced in order to induce hardening-off. Although this procedure tended to produce slightly smaller seedlings, it prepared the seedlings to better cope with planting shock. Lifting began during the first week in January,1978. Trees were individually tagged with family identification, sorted into "block" bundles (one tree each of 120 families), and packed in kraft bags. After transportation to the vicinity of the planting site, the bags were stored under refrigeration until planted.

Planting and Mapping

Planting.--Each block was planted by a single two man crew using a standard dibble. Randomization of families in a block was obtained by planting the seedlings in the sequence they were removed from the bag (trees were throughly mixed during packing) and by starting the planting of each block on a row picked at random.

Mapping.--Mapping of each block was done as quickly as possible after planting. The family identity of each seedling was recorded from an attached paper label along with the planting location (row and point within row). These data were double checked in the field and edited by computer for duplicate family numbers within block.

RESULTS

Survival

First year.--Two survival counts were made during the first year. The first count was done in May on about 26% of the orchard. Overall survival was estimated to be 66.5%. Both block and family survival

appeared to vary greatly. In October, a complete inventory counted 20,047 live trees for a survival rate of 66.8%. Individual families ranged from 27% to 86%. Of the 120 families, 24 had survival rates of 75% or more while 8 had rates less than 50%. Block survival ranged from 3% to 89%. The pattern of survival (by block) indicated that some mortality was not random. Of the 20 blocks in the orchard with survival below 40%, 16 were located in the southwest corner of the orchard. An exact cause for the poor survival in this area could not be determined.

Third year.--Additional mortality between the first and third year was very small. Survival was 64.2% for a net loss of 794 trees.

Growth

Third year.--Height growth for individual trees varied from less than 1 foot up to 14 feet. Mean height for the entire plantation was 7 feet with family means varying from 4.6 to 9.4 feet.

Seventh year.--Height growth continued at a rapid rate for most remaining trees. Mean height was 19.5 feet with some trees exceeding 26 feet tall. Individual family means ranged from 17.9 to 20.9 feet. Mean diameter (d.b.h.) was 2.9 inches.

Apparent Survival Mechanism

Description.--During the analysis of the first-year survival checks, inconsistencies were found in the data that indicated live trees were growing in spots previously tallied as dead. At first these were thought to be simple recording errors but a field check indicated the data was correct. Close examination of the seedlings revealed that they probably appeared to be dead during the early survival check because all foliage had turned brown and dropped off, leaving only the naked stem. At some time later, a new sprout appeared near the top of the old stem and took over as the terminal shoot. By the time of the second survival check, many of these "dead" trees appeared almost normal.

Frequency of Trait.--Since the first survival check only examined 26% of the plantation, it is not possible to determine the full extent of this trait. Comparison of data between the two first-year checks and the third-year check indicated that about 1.5% of the trees were involved. All families except one contained at least one tree that had the trait. The maximum frequency of occurence within family was 4%.

Growth.--Generally, height growth of the trees exhibiting this trait was inferior to other trees of the same family. Also, these trees were usually shorter than their neighbors in the block. There were a few exceptions where trees displayed outstanding growth and these exceptions seem to be concentrated in a few families.

Cultural Practices

In the first three years after planting, cultural practices were limited to spot control of competing vegetation and other activities to maintain the health and safety of the orchard. After the third-year measurements were analyzed, the first thinning was done. All stunted, deformed or badly overtopped trees were removed. Trees were also removed in order to improve spacing but care was taken to retain a good representation of all families. A subsequent thinning in 1984 removed more trees for spacing and rogued the four worst (in terms of height growth) families. This reduced the tree count to 7,400. Another thinning is currently underway to rogue several more of the poorest families based on combined orchard and supplemental test data.

Seed Production

Sand pine is well known as an early and prolific seed producer. By the third year after planting, several cones were observed on scattered trees. A majority of the trees were producing a few cones at the fifth year. The 1984 cone crop was estimated at 10 bushels per acre. Any serious effort to collect these cones has been precluded by the relatively dense and irregular spacing of the trees.

DISCUSSION

Successful establishment and maintenance of a large seedling seed orchard involve complex and demanding tasks that must be done at the proper time. Orchard development will be very rapid for a species such as sand pine. Frequent inventories and measurements are necessary in order to plan for thinnings and roguing. These data can also provide additional insight into the species and the population being grown.

For example, Ocala sand pine is difficult to transplant. Nevertheless, our data indicate considerable variation in survival by family. This information should be of value for both future testing and regeneration.

The apparent survival mechanism displayed by a small number of trees is interesting but it will probably have little influence in this orchard. Poor height growth by most of these trees has resulted in their removal during the first or second thinning. On the other hand, knowledge of the existence of such traits may be useful when selecting for tolerance to unfavorable site conditions.

A seedling seed orchard will never be as neat and tidy as its clonal counterpart. The close spacing during early development makes any vegetation management practices very difficult. The manager should be prepared to do much of the early thinning by hand. Mortality and removals due to thinnings are almost always randomly spaced so mowing and cone collection may be hampered for several years.

MONITORING CONEWORMS WITH PHEROMONE TRAPS: A VALUABLE PEST DETECTION PROCEDURE FOR USE IN SOUTHERN PINE SEED ORCHARDS

J. C. Weatherby, G. L. DeBarr, and L. R. Barber

Abstract.--Sticky traps baited with synthetic pheromone were used to detect the presence of the webbing coneworm, *Dioryctria disclusa* Heinrich, during 1981-1984 in southern pine seed orchards. The blister coneworm, *D. clarioralis* (Walker), and the loblolly pine coneworm, *D. merkeli* Mutuura and Munroe, were also trapped during 1982-1984. More than 80 orchards were surveyed in 1984. Trapping data indicate that outbreak populations of *D. disclusa* present during 1981 in eastern North Carolina and eastern South Carolina declined each year, while populations remained relatively stable or increased in orchards in central Georgia, Alabama and Mississippi during this same period. Trap catches of *D. merkeli* remained high (25-49 males/trap/year) or very high (>50 males/trap/year) during 1984 in 57% of the orchards which detected high or very high catches in 1983. Trap catches of *D. clarioralis* remained relatively stable during the 3 year trapping period. Regional seasonal activities of the insects, and pest management decisions for individual orchards are discussed.

Keywords: *D. disclusa, D. merkeli, D. clarioralis*, IPM, *Dioryctria*.

Several species of coneworms, *Dioryctria* spp., are considered key pests damaging cone and conelet crops in southern pine seed orchards (Ebel et al. 1980). Prior to 1981 blacklight traps were occasionally used by orchard managers to detect and monitor coneworm populations (Yates and Ebel 1975). This procedure was time consuming and accurate identifications of the various coneworm species were difficult.

The discovery (DeBarr and Berisford 1981) and the identification of the sex pheromone produced by female *D. disclusa* moths for the purpose of attracting males (Meyer et al. 1982) led to the development of a highly specific bait which could be used to monitor *D. disclusa* populations. In 1981 traps baited with synthetic sex pheromone of the webbing coneworm, *D. disclusa* were installed in 63 pine seed orchards (DeBarr et al. 1982). This survey demonstrated "the value of pheromone-baited traps as part of an integrated pest management approach to coneworm control in southern pine seed orchards."

Results of the 1981 survey and research aimed at identifying the pheromone produced by *D. clarioralis* females (Meyer et al. 1984) showed that both *D. clarioralis* and *D. merkeli* males were frequently attracted to bait with the single chemical component of the *D. disclusa* pheromone. Hanula et al. (1984a) demonstrated that traps baited with *D. disclusa* pheromone could be used to detect the presence of 3 coneworm species — *D. disclusa, D. clarioralis* and *D. merkeli*. Because of the success of the 1981 survey and the discovery of cross attractancy, the 1982 survey was expanded to monitor populations of all three of these insect pests.

Survey cooperators in industrial, state and Federal forestry organizations are compiling historical data files for their orchards which are also being used to detect regional changes in yearly trap catches. Comparisons of trap catches from individual orchards are used to locate potential "hot spots" of activity. This information provides the orchard manager with an early warning system to indicate years and locations where coneworms are likely to cause substantial damage. Interpretations of trap catch data are also helping to define the regional flight periods and the population phenologies of the major coneworm species. This information is expected to increase the effectiveness of sprays timed to suppress adults or newly hatched larvae.

METHODS AND PROCEDURES

The coneworm survey is a cooperative effort between the USDA-Forest Service and cooperating orchards. Entomologists with Region 8, Forest Pest Management (FPM) and the Southeastern Forest Experiment Station supply cooperators with instructions, data sheets, and pheromone baits. Cooperators are responsible for obtaining, installing and checking traps. Orchard managers are also requested to submit their data to FPM for use in summaries and regional interpretations of trap catches.

Baits and Traps

Rubber septum dispensers impregnated with 100 µg of 98% pure (Z)-9-tetradecenyl acetate (Z9-14:AC) dispensed in 5 ul carbon disulfide are used as baits. A seasonal supply of baits is mailed to each cooperator. Baits are stored at below-freezing temperatures prior to use in the field. Either of two commercially available traps, the Pherocon 1C (Zoecon Corp., Palo Alto, CA) and Sentry wing trap (Albany International Corp., Needham, MA) are effective. In 1981, six traps were used in each orchard (DeBarr et al. 1982). Since then, the recommended procedure is to install 10 traps in a selected seed source at each orchard site. The traps are assembled and 1 bait is placed in the center of each trap bottom.

Trapping Procedures

Trapping locations are randomly selected on a grid drawn to overlay a map of the entire trapping area. The tallest tree in the general vicinity of each trapping location is selected (Hanula et al. 1984c). Selected trees are to be at least 90 feet apart.

Traps are placed in the top 10 feet of the tree crown (Hanula et al. 1984b). Traps are hung by running a nylon cord from the ground, through a wire loop which is attached at the designated height to a branch, and back to the ground (DeBarr et al. 1982). Both ends of the nylon cord are attached to the trap so that the trap can be raised and lowered from the ground.

The traps are checked once or twice a week. The numbers of moths of each species caught in each trap are recorded, and the moths are removed. Baits are changed the first of each month. Trap bottoms are changed as needed.

Traps are deployed in late March or early April and trapping continues through mid-November. Trapping is discontinued in late October or November when no moths are caught for 2 weeks at any trapping location. Data are submitted to FPM monthly.

Reporting and Summary Procedures

The Southern Region was divided into 3 areas based upon average daily January temperatures (Fig. 1). All cooperating orchards located below the 50° F isotherm are placed in Area I. Orchards located between the 45-50° F isotherm are in Area II. The remaining orchards are in Area III.

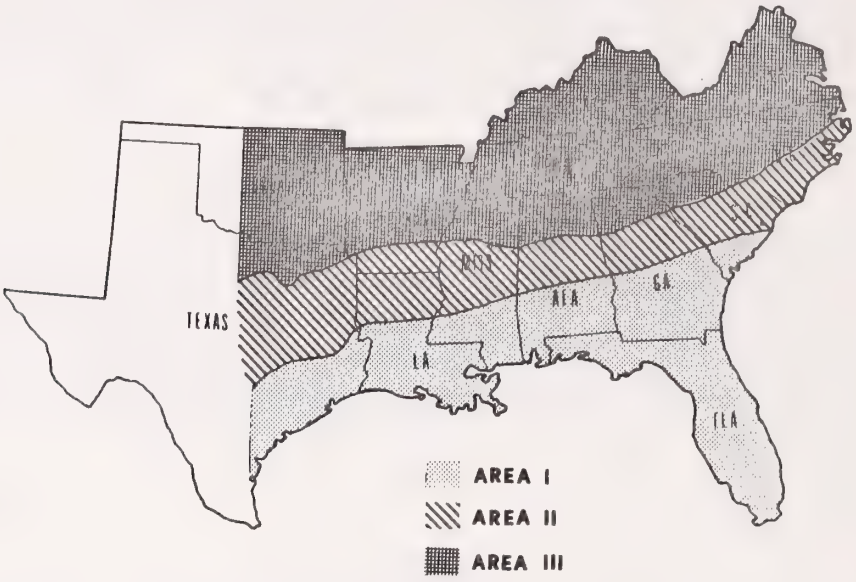

Figure 1.--Pheromone survey areas within the Southern Region.

Each cooperating orchard receives a monthly summary which ranks the total trap catch of each species for all orchards within each area. Also included in the monthly summary are 2 sets of 3 graphs. One set compares the orchard trap catch for the current year with the area trap catch for the previous year on a weekly basis. The other set of graphs compares the weekly trap catches for the orchard and the area during the current year.

RESULTS AND DISCUSSION

Interpretations of Monthly Reports

During the last week of every month each orchard manager receives a survey summary for the previous month. A case study of the trap catches at Orchard #101 illustrates how a typical monthly report might be used by a seed orchard manager in developing a pest management strategy.

Orchard #101 is located in central Alabama above the 50° F isotherm. A monthly report for Orchard #101 summarizes the trapping data from the orchard with data from other orchards within Area II. Table 1 lists the orchard rankings by total moth catch for orchards located in Area II. During the 1984 trapping season, Orchard #101 captured 105 *D. clarioralis*, 17 *D. disclusa* and 149 *D. merkeli*. While the absolute relationship between total trap catch and coneworm impact is unknown, orchard managers are encouraged to compare their data with data from other similarly managed orchards. Based on these relative comparisons, a sizeable *D. clarioralis* population was present in 1984 at

Orchard #101 and the potential for significant losses was anticipated. Trap catches of *D. disclusa* detected the presence of a minimal population in 1984 and suggested that damage during 1985 caused by *D. disclusa* should be low. Approximately 40% of the orchards located in Area II caught more *D. merkeli* males in 1984 than did Orchard #101. However, the relatively large *D. merkeli* trap catch (149 total) indicated that populations were present and this species should probably be considered in the pest management program.

Table 1.--Ranking by total moth catch for Orchard 101 compared to other orchards in Area II (March - November 1984)

Rank	Orchard	No. of DC	Orchard	No. of DD	Orchard	No. of DM
1	111	182	130	249	61	402
2	101	105	13	227	63	380
3	109	75	115	187	103	375
4	128	63	55	149	123	267
5	13	62	103	97	14	236
6	105	58	116	93	111	201
7	54	57	14	78	115	198
8	131	48	114	66	114	191
9	55	43	105	58	116	163
10	122	40	61	56	138	155
11	130	38	109	44	101	149
12	138	31	63	35	55	141
13	115	16	17	33	128	137
14	14	14	124	29	13	101
15	116	11	28	27	109	94
16	123	8	111	22	122	71
17	103	4	131	22	28	40
18	15	1	101	17	126	5

In addition to the ranking tables a typical monthly report contains 2 sets of graphs. One set of graphs (Fig. 2) plots the total weekly trap catches of *D. clarioralis* from all orchards located in Area II for the 1983 season. Superimposed upon the area graph is a plot of the total weekly trap catches of *D. clarioralis* from Orchard #101 for the 1984 season. This graph summarizes the seasonal flight patterns for the previous year, and by studying it the orchard manager can anticipate when current flights might begin, peak, and end. Similar graphs plotting trap catch data for *D. disclusa* and *D. merkeli* would be included in a typical monthly report.

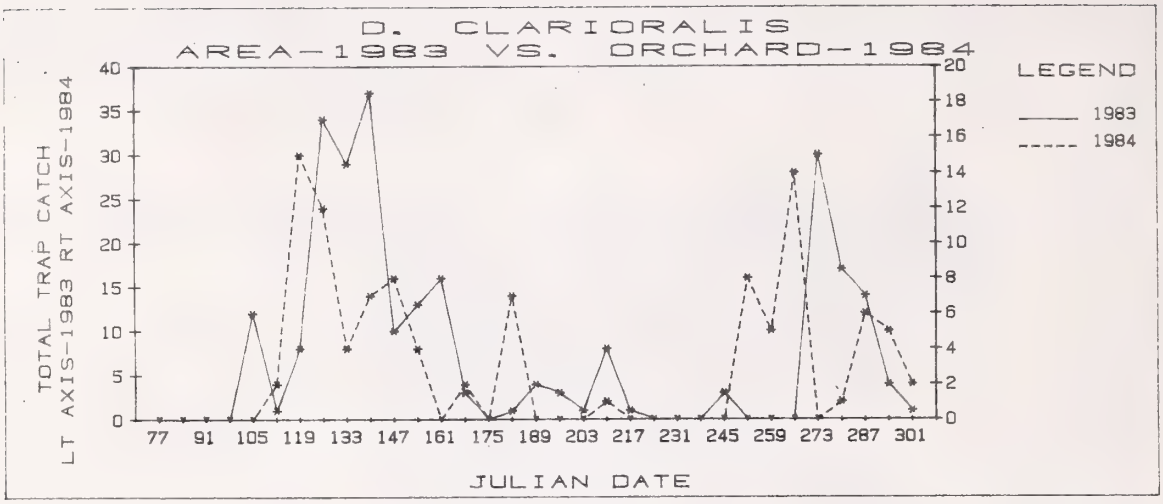

Figure 2.--Total trap catch for 1984 from Orchard #101 superimposed upon the total trap catch for all orchards in Area II which submitted data in 1983.

The other set of graphs (Fig. 3) displays the total weekly catches of *D. clarioralis* from Orchard #101 and the total weekly catches for all the orchards in Area II for the 1984 trapping season. Orchard managers can determine if the flights detected in their orchard correspond to the area flights. Similar graphs plotting the trap catch data for *D. disclusa* and *D. merkeli* would also be included in a typical monthly report.

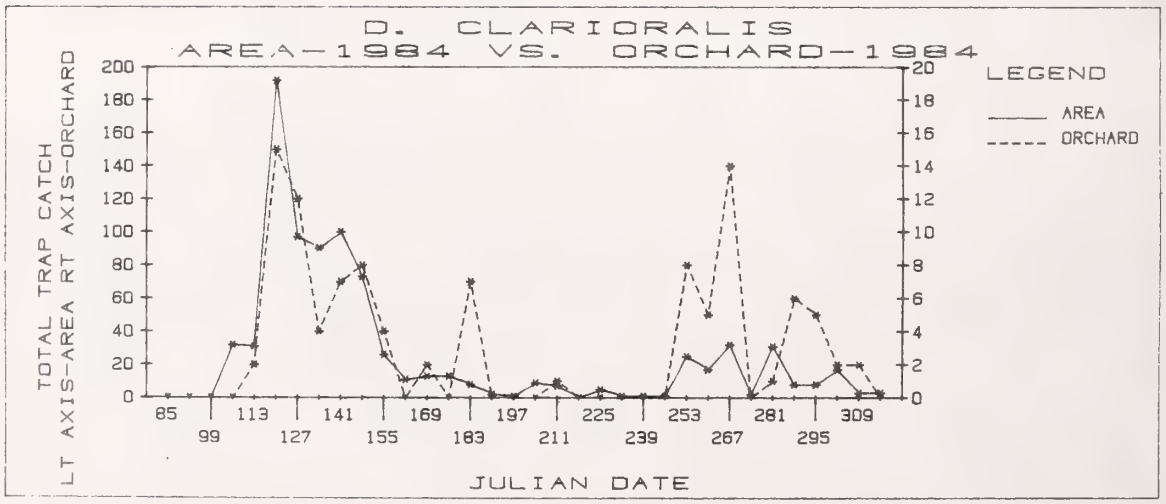

Figure 3.--Total trap catch for 1984 from Orchard #101 superimposed upon the total trap catch for 1984 from all orchards in Area II.

Pest Management Decisions Based Upon Trap Catches

Early Warning System

Seed orchard managers are using trap catches to detect the presence of *D. clarioralis*, *D. disclusa*, and *D. merkeli*. The relative importance of each member of the coneworm pest complex varies by orchard and season. Although, the exact relationship between trap catch and potential damage is unknown, population trends are also evident. Table 2 lists trap catches from 2 orchards with different coneworm complexes. Trapping data from Orchard A indicate that *D. disclusa* and *D. merkeli* are the most important pests. At Orchard B, the major coneworm is *D. clarioralis* and pest management efforts should be aimed at suppressing this species. Based upon our trapping experience and observations the following arbitrary scale of seasonal trap catches has been developed in order to rank the relative potential for cone attacks by each species: very high, >50 moths/trap; high, 25-49 moths/trap; moderate, 10-24 moths/trap; low, 1-9 moths/trap; very low, <1 moth/trap. Each season, seed orchard managers use this arbitrary scale to determine which species pose the greatest threat to next years cone crop. Management decisions are modified in order to target control actions for major pest species at each location.

Table 2.--Total trap catches for 1982-1984 at Orchard A (Perry Co., MS) and Orchard B (Geneva Co, AL)

Orchard	Year	*D. clarioralis*	*D. disclusa*	*D. merkeli*
A	1982	27	323	230
	1983	3	415	1
	1984	52	76	1
B	1982	120	4	155
	1983	227	3	21
	1983	125	0	18

In addition to identifying the coneworm complex found within each orchard, trapping data indicate population trends over time. Figure 4 illustrates two different population trends which have been detected with pheromone baited traps. Orchard C, located in Beaufort Co., NC had a *D. disclusa* outbreak which peaked in 1981. Since 1981 the trap catch has steadily declined. Using the previously described population scale, the 1984 population level can be classified as low (1-9 moths/trap). Therefore the predicted potential cone loss for 1985 should be minimal. Pest management actions designed to control *D. disclusa* in 1985 are probably not necessary. Orchard D, located in Putnam Co., GA has also had a population outbreak of *D. disclusa*; however trap catches have remained high (25-49 moths/trap) or very high (>50 moths/trap) during the last 5 years. Trap catches indicate a steady increase from 1982-1984. Therefore, pest management actions targeted for *D. disclusa* are advisable at Orchard D.

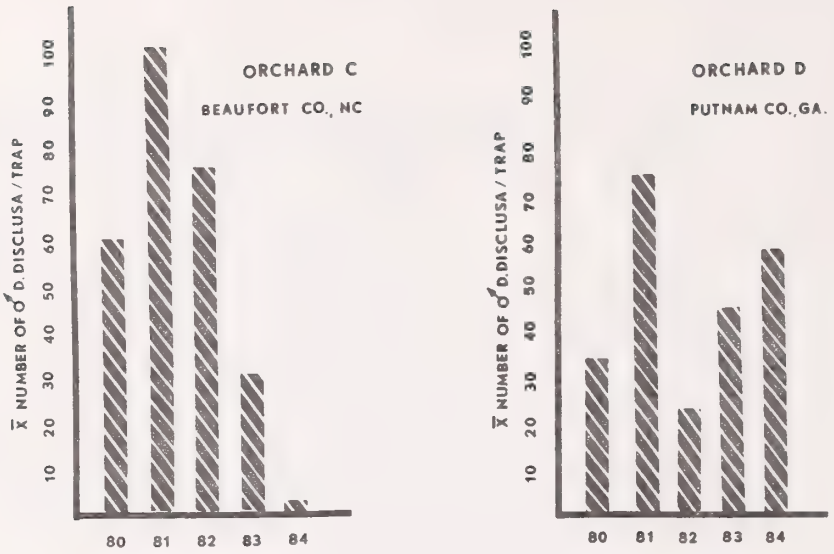

Figure 4.--Mean numbers of male *D. disclusa* per trap captured during 1980-84 in two loblolly pine seed orchards.

Seasonal Activity

Coneworm development and population phenologies are controlled predominantly by temperature accumulations (Hanula 1984b). Temperature accumulations vary at each orchard and, as a result, moth flights often begin, peak and end on different dates at different trapping locations. Peak trap catches for *D. disclusa* in the more northern orchards are 3 to 4 weeks later than the most southern orchards (Fig. 5). Figure 6 is a graph of weekly total trap catches of *D. disclusa* at 3 different orchards. Orchard E is located in Washington Parish, LA (Area I); Orchard F is located in Webster Parish, LA (Area II); and Orchard G is located in Murray Co., GA (Area III). In 1984, flights of *D. disclusa* males began on Julian dates 141, 162, and 169 at Orchard E (Area I), Orchard F (Area II), and Orchard G (Area III), respectively.

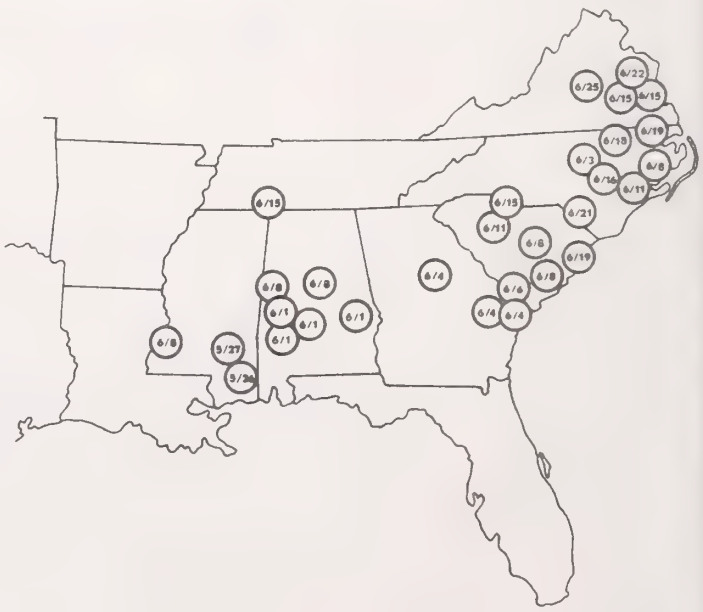

Figure 5.--Peak catch dates (month-day) for *Dioryctria disclusa* males during 1981 (From DeBarr et al. 1982).

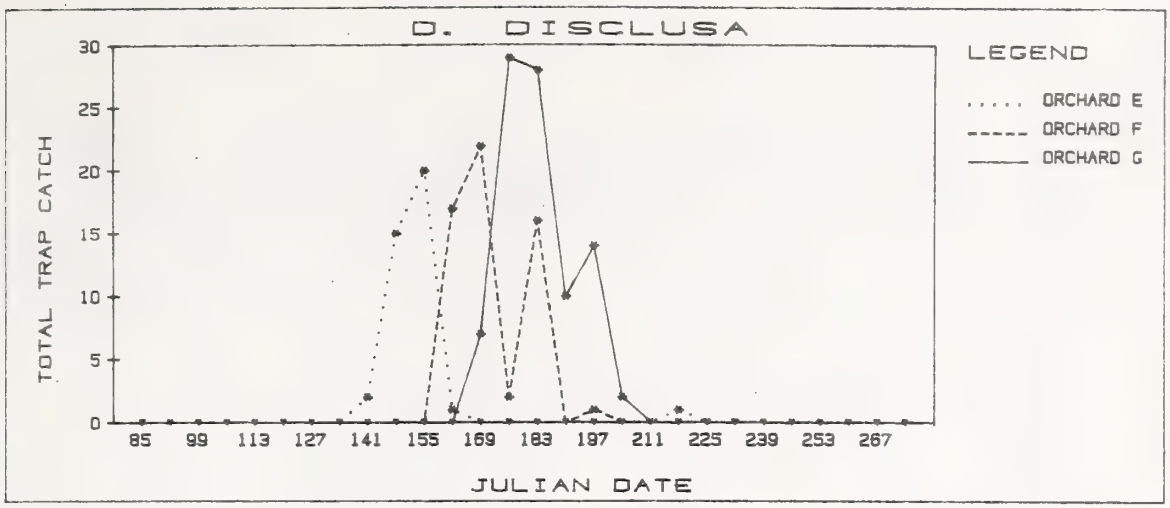

Figure 6.--Total weekly trap catchs of *D. disclusa* for 1984 at Orchards E (Area I), F (Area II), and G (Area III).

The population phenology of *D. merkeli* is also affected by temperature accumulations. Unlike *D. disclusa* which flies in late spring, *D. merkeli* flies in late summer and fall. With the onset of cooler temperatures and shorter day lengths typical of late August, September and October, adult flights of *D. merkeli* in more northern orchards occur earlier than flights in southern orchards (Fig. 7). The earlier flight is a survival mechanism which insures that adult emergence, mating and egg development occur before low temperatures prevent normal behavioral activities and development. Figure 8 is a graph of the weekly total trap catches of *D. merkeli* at 3 orchards. Orchard H. is located in Monroe Co., AL (Area I); Orchard I is located in Greene Co., AL (Area II); and Orchard J is located in King William, Co., VA (Area III). In 1984 the adult flights of *D. merkeli* began on Julian dates 267, 246, and 225 at Orchard H (Area I), Orchard I (Area II) and Orchard J (Area III), respectively.

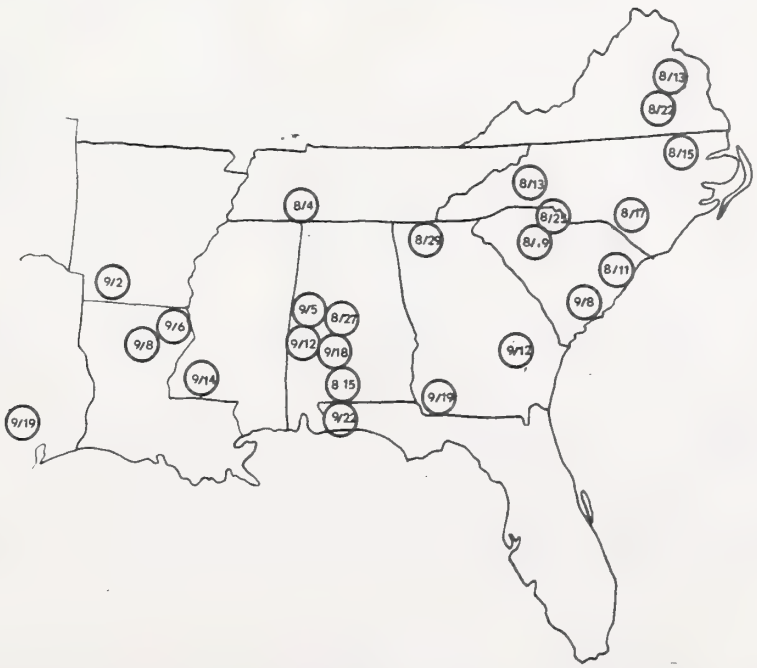

Figure 7.--First catch dates (month-day) for *Dioryctria merkeli* males during 1984 (Only orchards which caught 50 or more males/season and trapped through Julian day 270 are included).

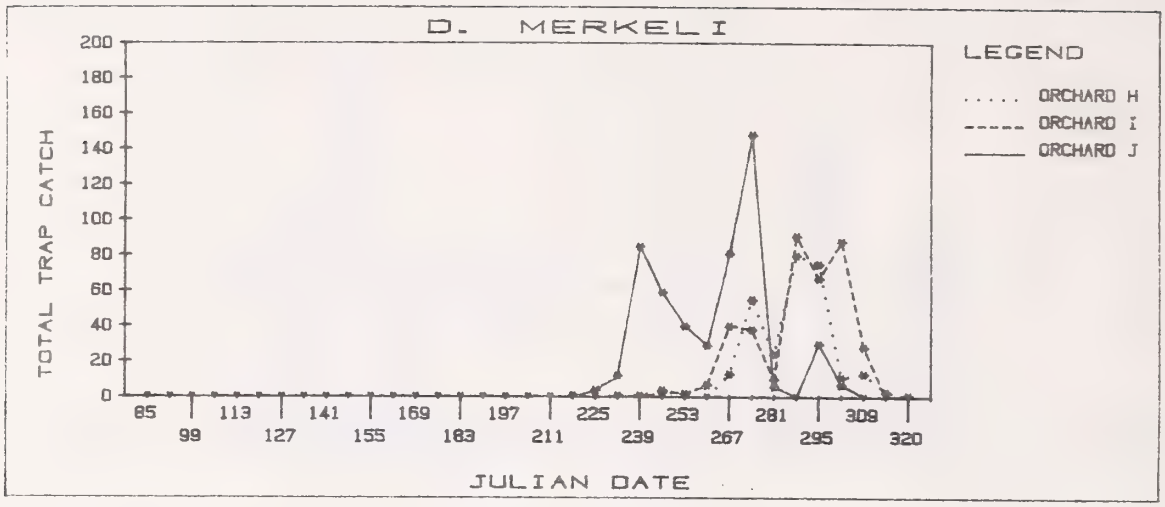

Figure 8.--Total weekly trap catches of *D. merkeli* for 1984 at Orchards H (Area I), I (Area II), and J (Area III).

For the present, pheromone traps provide the orchard manager with local seasonal activity patterns for the major coneworm species. Research is underway to develop degree-day prediction models for use with pheromone traps. In the future, temperature accumulations will be set to coincide with the beginning of moth flight and insecticide applications will be timed for maximum effectiveness against susceptible lifestages.

Regional Interpretations

The regional population levels for the 1981-1984 *D. disclusa* survey are shown in Figure 9. Each dot on the map represents the total seasonal catch per trap for each cooperating orchard. Dot size indicates the relative population classified according to the previously described population scale. In 1981 outbreak populations were detected at several orchards along the east coast in North Carolina, South Carolina, and Georgia. By 1984, populations in this area had declined. However, "hot spots" were detected at several orchards scattered throughout the south. Figure 10 shows regional trends for orchards which increased or decreased one or more population classes. Comparisons between catches in 1982 and 1983 indicate increasing population trends in Mississippi and Alabama. A similar trend was apparent in comparisons between catches in 1983 and 1984. The majority of the orchards which trapped moderate or high populations in 1984 detected increasing population trends from levels detected in 1983. Orchard managers are encouraged to consider both the relative population size and trend when developing pest management strategies.

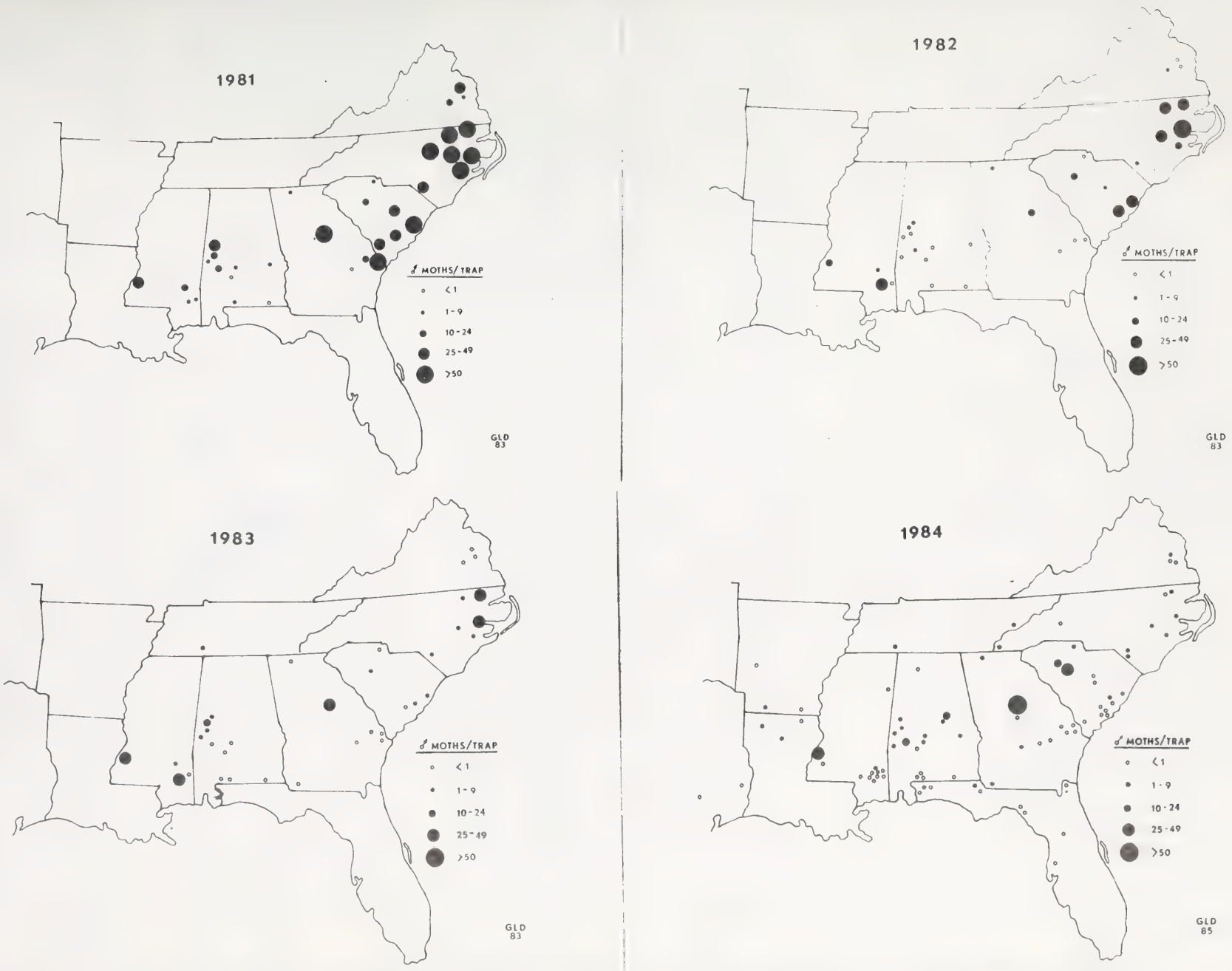

Figure 9.—Average numbers of male *D. disclusa* caught per trap for Pherocon 1C traps (N=10) baited with synthetic sex pheromone at each orchard location during 1981-1984.

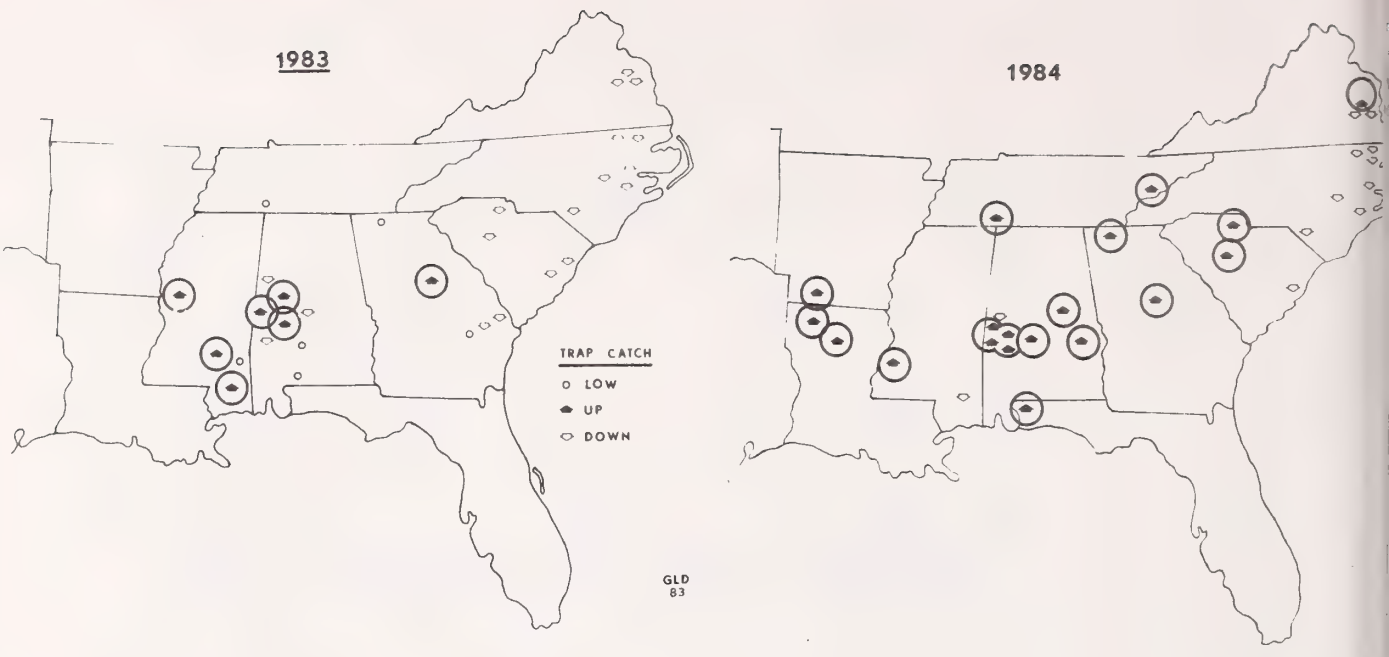

Figure 10.--Regional population trends for pheromone trap catches of *D. disculsa* from 1982 to 1983 and 1983 to 1984. (Up and down arrows reflect a change of one or more population classes).

Similar regional population maps have also been drawn for *D. merkeli* and *D. clarioralis*. Catches of *D. merkeli* for orchards which trapped through Julian date 270 indicated high to very high populations in southwestern Alabama, eastern North and South Carolina, and Virginia during 1984 (Fig. 11). Scattered "hot spots" also occurred at several other locations. In contrast, only two locations had high trap catches of *D. clarioralis* during 1984 (Fig. 12).

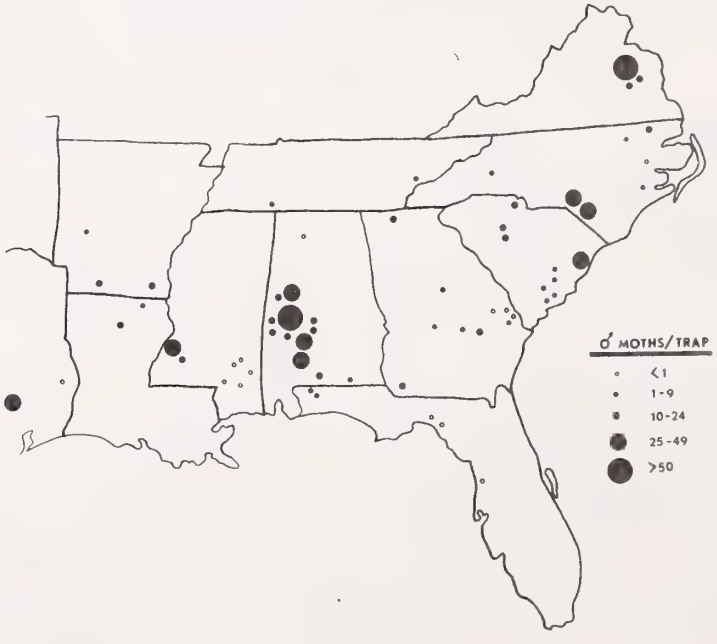

Figure 11.--Average numbers of male *D. merkeli* caught per trap for Pherocon 1C traps (N=10) baited with synthetic sex pheromone at each orchard location during 1984.

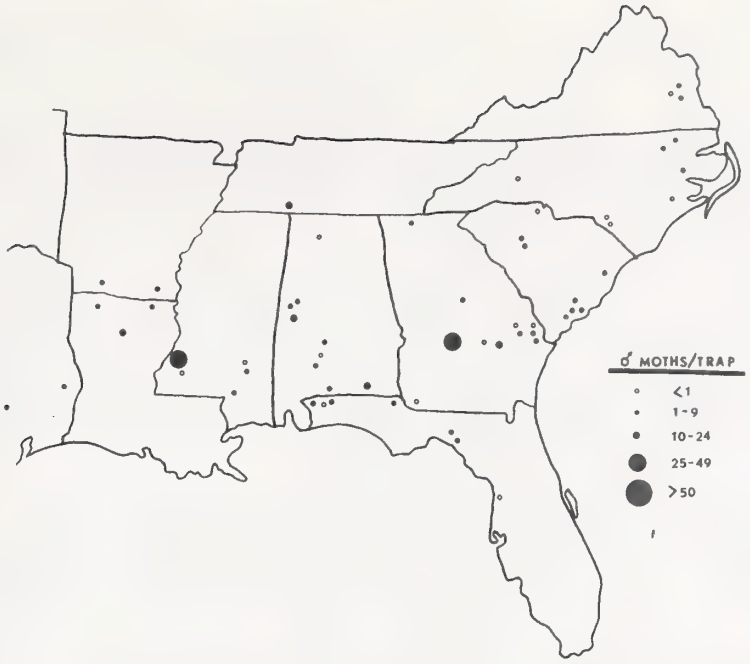

Figure. 12.--Average numbers of male *D. clarioralis* caught per trap for Pherocon 1C traps (N=10) baited with synthetic sex pheromone at each orchard location during 1984.

CONCLUSIONS

Data from the southwide coneworm survey are providing seed orchard managers, pest management specialists, and researchers with invaluable information. Orchard managers are using historical data files to make informed decisions concerning pest management strategies targeted for specific key pests. In addition, survey data are helping to define regional population trends and pest phenologies.

Efforts are being made to improve the quality of the survey data. Species identification causes confusion and occasionally errors have been detected. Incomplete data sets are fairly common particularly for fall flights of *D. clarioralis* and *D. merkeli*. Orchard managers are encouraged to continue trapping throughout the fall emergence period. Research concerning trapping procedures (Hanula et al. 1984c) indicates that minor deviations from suggested procedures, particularly trap height, can cause considerable variations in trapping data. Data interpretation for individual orchards continues to be a major problem. Efforts are being made to decrease the turn-around time so that orchard managers can use current data to adjust pest management strategies.

Despite a few minor problems, the southwide coneworm survey has been an extremely successful and valuable regional effort. In the future the survey will continue to function as an early warning system, alerting seed orchard managers to potential problem species. Field tests with a synthetic pheromone for the Southern pine coneworm, *D. amatella* (Hulst.), have been completed (Meyer et al. 1985) and baits for this species were deployed at 20 locations throughout the South. Research efforts are being directed toward gaining a better understanding of the relationship between relative trap catch and damage potential, as well as developing timing systems for insecticide applications using trapping data and temperature accumulations.

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SELECTION POTENTIAL FOR CONEWORM AND SEED BUG RESISTANCE IN LOBLOLLY PINE SEED ORCHARDS

George R. Askew, Roy L. Hedden, and Gary DeBarr[1/]

Abstract.--Current seed orchard practices rely heavily on pesticide control of cone and seed insects. Advanced generation orchards will have a greater need for control as seed becomes more valuable. However, as environmental concerns about pesticide use change political pressure may force changes in management practices. Increasing costs of pesticides and the need for new formulations may make the use of inherent resistance breeding seem more plausible. Intensive breeding programs utilizing decreased generation lengths can be adapted to allow for the inclusion of several resistant parents. Levels of resistance within the orchards can be increased slowly, a little each generation, and coupled with selective spraying regimens without great disruption of current management schemes. Advanced generation selections made without regard to infestation potential, perhaps because pesticide use has virtually eliminated the problem in the 1st generation orchard, may result in a 2nd or 3rd generation orchard with an infestation potential that has increased beyond the control capabilities of available pesticides.

Additional keywords: Pesticide, Dioryctria, breeding

Coneworms (Dioryctria spp.) and seed bugs have been recognized as serious threats to seed orchard production (Ebel et al. 1981) with damage estimates as high as 90% in some untreated southern pine orchards. Dioryctria amatella, D. disclusa, D. merkeli, Leptoglossus corculus and Tetyra bipunctata are the main problem pests for southern pines. Coneworms present a major problem for seed orchard managers because the entire cone is destroyed. Seed bugs destroy individual seeds and hence even though damage may be extensive some seed may be salvageable from infested cones. Together they constitute a management problem that is currently dealt with by spray applications of insecticides such as fenvalerate. The commercial value of seed orchard seed, particularly those from advanced generation orchards, warrants every effort to minimize insect predation.

Even in orchards with great amounts of damage, the pattern of infestation is not consistent among all trees. Some trees are virtually eliminated as seed producers while others receive no appreciable damage. Differentiation of heavily infested and lightly infested trees has been found to be associated with clonal affiliation in some orchards, Askew et. al (1985). They found that among 22 loblolly pine (Pinus taeda) seed orchards ranging in age from 9 to 13 years, 13 had significant clonal variation in coneworm damage. We have found 8 of these same 22 orchards with significant

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1/ Associate Professor of Forestry, Belle W. Baruch Forest Science Institute, P. O. Box 596, Georgetown, SC 29442, Professor of Forestry, Clemson University, Clemson, SC 29631 and Research Entomologist, U.S.D.A. Forest Service, Athens, GA.

levels of clonal variation in seed bug damage. If lower infestation levels are due to a resistance mechanism as suggested by Merkel et al. (1966) then the variation among clones suggests that breeding for resistance may enhance current chemical control measures. This paper will discuss some alternatives to current pesticide application techniques and their implications for the breeding program.

SELECTION

Selection for coneworm and seed bug resistance may be effective if the controlling mechanism is genetic in nature. However, selection for this type of resistance is much different than selection for other types of pest resistance that are associated with product degradation in the outplanted progeny. Such selection places emphasis on a non-commercial product in the sense of fiber, lumber, etc. Improvement in seed production will not necessarily improve the potential genetic gain obtainable in the orchard, but will provide a greater resource for planting improved trees. Consideration of the increase in base population size necessary for incorporation of an additional independent criterion is of primary importance. The additional criterion will not be burdensome if the resistance level is positively correlated with the major commercial traits being evaluated. However, the number of trees to be screened may be increased by several fold, perhaps to an impractical level if a strong negative correlation is found. Askew et al. (1985) found little or no correlation of seed bug or coneworm infestation levels with height growth or diameter growth performance values. They found that the probability of a tree selected on its commercial value having less than 5% coneworm infestation was approximately 0.35. Hence, if no conscious selection was practiced for resistance, 35 out of every 100 select trees would be expected to be 95% resistant. Only 40% of the clones examined had less than 5% of their seed damaged by seed bugs. However, if coneworm damage and seed bug damage are viewed as independent events. Then only 14% of the trees selected for commercial traits could be expected to suffer less than 5% damage from both coneworms and seedbugs without effective pesticide applications.

In general, it seems to be an undesirable proposition to include resistance as a selection criterion for every first generation selection. Commercial value of the wood product far outweighs the potential problem of seed and cone predation. Even if the resistance criterion was mandatory it would be difficult to evaluate the potential for infestation of trees growing in the wild. The opportunity for infestation may not exist at the time of evaluation or may not be a problem in that particular geographic region. Low levels of cone production in forest stands may be prohibitive to the formation of large insect populations and hence infestation levels may be low even on highly susceptible trees. Indirect selection is also a problem because at present there is no morphological trait that is known to be highly correlated with the trees' infestation potentials. A breeder would be limited to selecting trees on the basis of commercial value and then estimating the number of resistant trees that will be included by chance. Thus, it would be difficult to assess the potential for cone and seed insect problems until the orchard was established and their trees began to produce substantial cone crops.

In light of the problems of developing methods of selecting base trees for resistance it seems feasible to use selection for resistance when the orchard receives its first roguing. If cone and seed insects are a major problem despite spraying, then a substantial gain in seed production and perhaps a reduction in insecticide costs can be obtained by tailoring the roguing criteria to include insect problems as well as commercial genetic value. There are several implications of selecting against insect damage at the time of roguing. First, you must determine what level of infestation is tolerated for your orchard. You may have to rogue 65% of your trees if a level of 5% infestation is the maximum you wish to allow. As you reduce the stringency of your criterion the number of trees that will meet your needs will increase. In any case it will be necessary to begin with a larger base orchard in order to assure an orchard of sufficient size after roguing.

Rather than placing a strict resistance criterion on every selection it may be possible to develop selection indices that incorporate both commercial traits and insect resistance with each being weighted relative to its importance. Another possibility would be to use several "completely" resistant trees as parents for second generation orchard trees. This would allow resistance to be bred into the commercially superior base population. As the breeding program advance, resistance would be accumulated as a companion trait. Introduction of resistance in this manner would allow for basic selection on commercial value for the majority of the trees but would rely on a highly heritable resistance trait in the few trees to be used as donors.

SELECTIVE SPRAYING

If breeding for resistance is undesirable, a well structured spraying program may be a viable alternative. Current orchard spraying regimens usually involve a series of whole-orchard sprayings. Heavily infested trees receive the same treatment as lightly infested or noninfested trees. DeBarr et al. (1972) suggested spraying heavily infested clones on an individualized basis. Noninfested and lightly infested trees could be ignored or receive an abridged treatment. This idea bears some merit but several assumptions must be met if it is to be successful. First, the breeder must be able to accurately identify the heavily infested trees at a sufficiently early stage in order to prevent damage. Secondly, the insect population must be choosing the trees that they heavily infest because they are more susceptible than the others. If the selection is by chance, or because they are attracted to these trees rather than being repelled from others the insect population may merely shift its host base and the problem will still exist. If these criteria are met, the selective spraying technique may provide the breeder with a more economical and more effective treatment program.

Combining the selective spraying ideas with inbred resistance would be the next logical step. During the early years of the breeding program resistance levels would be low and spraying would be necessary to minimize the losses. As the level of resistance is increased with advancing generations, the spraying requirements should decline and may eventually be unnecessary.

Long generation lengths may make this combined approach technically viable but practically inoperative. However, advanced generation breeding

techniques which reduce generation length may soon put forest tree improvement efforts on a par with agronomic crop improvement programs. The main point is: tree improvement programs are already in place for many corporations and public agencies and a minimal effort would be required to introduce some highly specific genetic material to attain a gain in pest resistance. Reliance on pesticides causes a continued need for new pesticides as they lose their effectiveness. Utilizing a natural resistance mechanism may provide the orchard manager with an opportunity to produce more seed for less money.

FUTURE PROBLEMS

A potential problem needs to be considered if cone and seed insect resistance is strongly heritable. Breeding and production programs that are currently experiencing complete control of cone and seed pests by pesticide application may not see the justification for increasing the selection criteria burden. However, infestation potentials may increase with advancing generations as genes from highly susceptible clones are unknowingly combined to produce a new parent generation. Potential infestation levels could accidentally increase to the point being uncontrollable by pesticides alone. Corrective measures during 4th or 5th generations would be extremely costly in money and time.

It is important to remember that tree improvement programs in the South are in their infancy. One or two generations of breeding and selection are not satisfactory for determining future successes and patterns. Information that is currently superfluous may be crucial in several generations. Lessons learned by crop breeders need to be remembered by tree breeders. Our failure to prepare for future problems cannot be readily corrected. Our problems may develop slower than those in agriculture but, in all liklihood, they will develop.

NEEDS

Much of this proposed breeding strategy is based on a small data base and personal observations. Many factors still need to be examined in detail. Heritability of insect resistance needs to be examined. If the trait is highly heritable and is an additive effect then the prospects for its incorporation into the commercial orchards gene pool are good. If dominance factors are the controlling mechanisms the prospects are poor. Linkage may also be a factor that needs to be carefully evaluated. If the resistance mechanism is tightly linked to undesirable characteristics, a more exhaustive search of the base population may be necessary to find qualified trees. The material necessary for evaluating the heritability of resistance already exists in progeny test plantings and second generation orchards. Several years of cone collections using statistically rigorous sampling schemes should provide breeders with the necessary information for evaluating the potential of this technique.

Spraying studies need to be conducted in existing orchards to evaluate the population dynamics of the undesirable insect species. Correct timing of pesticide applications will be crucial to the success of a "custom made" breeding and spraying program. Annual changes in infestation levels will need to be studied before generalized spraying recommendations can be

prepared. Confirmation that uninfested trees remain uninfested when the insect population is excluded from their prime hosts will require careful observation and cone analyses.

Finally, a method of evaluating the potential for infestation of trees in previously unaffected regions or orchards will need to be established. Identification of defense mechanisms and the identification of morphological markers or biochemical markers that can be easily and positively identified in the field or laboratory are of prime importance.

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PREDICTING LOBLOLLY PINE SEEDLING PERFORMANCE FROM SEED PROPERTIES

T H Shear and T O Perry [1]

Abstract.- Seed properties affect seedling performance, and may confound evaluations of progeny tests and determinations of the best seed handling practices. The total seed weight, coat weight, gam/emb weight, lipid content, calories per gam/emb, days to seedling establishment, and the weights of the seedlings were determined for the seeds of 19 clones of loblolly pine. There were large variations among clones in all seed and seedling properties measured. Various combinations of seed properties accounted for as much as three fourths of the variation in seedling weight.

INTRODUCTION

Performance of the same progenies in different field tests is often not consistent. One source of variation could arise from differences in seed properties and behavior among clones. This paper reports on clonal variations in seed properties of loblolly pine (Pinus taeda L.) and their effects on the initial performance of progenies.

In an investigation of loblolly pine seeds, Perry and Hafley (1981) examined seed weight, embryo condition, seed coat thickness, stratification requirements, and the number of days required to shed the seed coat. At best, these factors only accounted for about 20% of the variability in seedling size. To explain more of this variation, it is proposed here that differences in loblolly pine seedlings are associated with differences in energy content of the gametophyte and embryo of the seeds, as well as other seed properties.

The objectives of this study were to:

1. Identify genetic differences in the following seed properties of loblolly pine: seed coat, gametophyte and embryo, and total seed weight; total lipid content; energy content (calories per seed); and number of days to seedling establishment,

2. Measure the performance of the seedlings produced from the seeds, and

3. Correlate seedling performance with seed properties.

MATERIALS AND METHODS

Seeds were obtained from open pollinated clones of loblolly pine in the Weyerhaeuser Corporation orchard in Washington, North Carolina. Samples from the seedlots of 10 clones from the 1981 collection and 9 clones from the 1982 collection were obtained.

Fifty seeds were chosen randomly from each seedlot. The seed coat was removed. Each seed coat and each combined gametophyte and embryo (gam/emb) were weighed and saved for subsequent measurements.

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[1] Unemployed Ph.D. Forest Scientist, and Professor, Dept. of Forestry, North Carolina State University, Raleigh, NC

Fifty seeds were chosen randomly from each seedlot. The seed coat was removed. Each seed coat and each combined gametophyte and embryo (gam/emb) were weighed and saved for subsequent measurements.

The total lipid of each seed was extracted by a modification of the procedure outlined by Christie (1982). Each gam/emb was crushed in 2 ml of chloroform:methanol (2:1) and treated with a sonic disintegrator for about 30 seconds to insure complete homogenization and dissolution of all lipid. The resulting mixtures were filtered into weighed tubes. Distilled water, equal to one-quarter of the total volume of the filtrate, was added to each filtrate (approximately 0.38 ml water to 1.52 ml filtrate). The solvents partitioned into an aqueous upper phase and an organic lower phase. The lower phases, containing the extracted lipid, were dried in a vacuum dessicator connected to a dry-ice/acetone cold trap and a vacuum pump. The amount of lipid in each dried extract was determined gravimetrically. A correction factor which accounted for the amount of lipid lost from a gam/emb during the extraction procedure was determined by performing the entire extraction process on 25 seed lipid aliquots of known weight, as if each were a single seed.

Seven clones representing the entire range of lipid concentration (gm lipid per gm gam/emb) were chosen for measurement of total energy content using a bomb calorimeter. One-hundred seeds of each clone were decoated and divided into two groups of 50 gam/embs each and weighed. Each group was weighed, placed in a gelatin capsule, and burned. The mean calories per gram of gam/emb determined for each of these clones were regressed against the lipid concentrations. This regression equation was then used to estimate the calories per gram of gam/emb of the seeds of all clones.

Forty-nine stratified seeds and 49 unstratified seeds of each clone were planted at the same time in "Cone-tainers" (1 seed in each). Seeds were stratified by placing them in a pipette washer with cold running water for 48 hours and then storing them for 40 days at 7° C. They were arranged in random groups of 7 in racks in a greenhouse (only stratified or unstratified seeds in each group), watered when necessary, and fertilized approximately every 10 days. The number of days to seedling establishment (DTE) was recorded for each seed and the mean DTE was calculated for each seedlot. Seedling establishment is defined here as the number of days from planting to the day of hypocotyl straightening.

An establishment value (EV) was calculated for each seedlot by substituting seedling establishment for germination into the formula for the germination value proposed by Czabator (1962). The establishment value is a measure of both the speed and the completeness of seedling establishment. Calculations were made at 30 days for unstratified seedlots and 20 days for stratified seedlots.

After 75 days from planting, the seedlings from stratified seeds were harvested (both roots and shoots) because their roots had almost filled the containers and there was no further seedling establishment. The seedlings from unstratified seeds were harvested 97 days after planting because there was still some seedling establishment after 75 days. All seedlings were oven-dried at 80°C for 24 hours and then weighed.

Seed and Seedling Properties

There were significant differences among clones in all seed properties measured (Table 1).

The loss of lipid in the extraction procedure was determined to be 10% of the actual lipid weight and the weight of lipid from each gam/emb was corrected accordingly. The gam/embs varied in lipid concentration from 36% to 70% (mean = 47%).

The regression used to estimate the calories per gram of gam/emb from lipid concentration had an R^2 of 0.88.

Significant differences were evident among mean DTEs of both stratified and unstratified seeds of many clones (Table 2). Stratification reduced the mean DTE and its variance by 59% and 70%, respectively. As a result, there were fewer differences among clones in DTE for stratified seeds.

Prediction Equations

Unstratified seeds.- Every combination of mean DTE and means of seed properties was examined by regression analysis for prediction of seedling weight (Table 3). DTE alone accounted for 63% of the variation in seedling size. When the calories per gam/emb were added to the relationship, 76% of the variation could be explained.

The number of calories per gam/emb was closely related to the gam/emb weight ($R = 0.99$), probably because the calculation of total calories per individual gam/emb was very sensitive to even small changes in the gam/emb weight. When the lipid concentration was halved, only a 20% increase in the gam/emb weight was necessary to maintain the same total number of calories. Because of this large dependence, the gam/emb weight could be substituted for the calories per gam/emb in the regression without affecting the ability to predict seedling size ($R^2 = 0.76$). These were the only models which accounted for a large amount of the variation in the size of seedlings from unstratified seeds.

Model 2 supports the hypothesis that seedling weight is affected by the energy content of the seed. However, Model 3 is more useful for predictive purposes. It is easier to weigh the gam/embs than to burn them.

Combinations of variables for regression models were reexamined, substituting EV for DTE. There were no improvements in the regression coefficients for the previous models and no new models were found.

Stratified seeds.- There were still significant differences in DTE and seedling weights among clones when the seeds were stratified. However, there were no significant correlations between DTE and any other measure of the stratified seeds.

Table 1. Comparisons among clones of means of seed properties. All mean weights are expressed in grams. Means followed by the same letter were not determined to be significantly different by Tukey's studentized range test (alpha = 0.05).

CLONE	TOTAL SEED WEIGHT	SEED COAT WEIGHT	GAM/EMB WEIGHT	PERCENT OF SEED AS SEEDCOAT	PERCENT OF SEED AS GAM/EMB	LIPID WEIGHT	PERCENT GAM/EMB AS LIPID	CALORIES PER GRAM GAM/EMB	CALORIES PER GAM/EMB
H	0.0390 a	0.0266 a	0.0124 abc	68.0 a	32.0 h	0.0055 c	40.0 ij	6613 ghi	90.7 ab
J	0.0376 a	0.0247 a	0.0129 ab	65.7 abc	34.3 fgh	0.0049 cde	39.6 ij	6605 ghi	82.1 bcd
S	0.0374 a	0.0255 a	0.0119 bcd	68.1 a	31.9 h	0.0052 cd	56.8 c	6942 bc	65.5 fg
N	0.0317 b	0.0180 bcde	0.0137 a	56.8 g	43.2 b	0.0050 cde	46.9 edf	6750 defg	73.7 def
I	0.0303 bc	0.0185 bcd	0.0119 bcd	60.5 def	39.5 cde	0.0041 fg	34.5 k	6505 i	77.6 de
E	0.0301 bc	0.0191 b	0.0109 de	63.4 bcd	36.6 efg	0.0042 efg	47.4 def	6759 defg	60.8 gh
D	0.0301 bc	0.0185 bcd	0.0116 bcd	61.3 def	38.7 cde	0.0045 def	57.9 c	6964 bc	55.4 h
G	0.0298 c	0.0179 bcde	0.0119 bcd	59.7 efg	40.3 bcd	0.0045 def	40.9 hi	6632 efghi	72.8 ef
K	0.0298 c	0.0185 bcd	0.0113 cd	61.9 def	38.1 cde	0.0051 cd	43.8 fghi	6687 defgh	79.1 de
F	0.0297 c	0.0160 ef	0.0137 a	53.5 h	46.5 a	0.0046 cde	40.3 ij	6619 fghi	77.0 de
L	0.0297 c	0.0188 bc	0.0110 de	63.3 bcd	36.7 efg	0.0065 b	50.8 d	6825 cd	87.9 abc
Q	0.0267 d	0.0153 fg	0.0114 cd	57.3 g	42.7 b	0.0047 cde	48.1 def	6772 def	66.2 fg
O	0.0266 d	0.0168 cdef	0.0098 ef	63.2 bcd	36.8 efg	0.0035 gh	44.9 efgh	6710 defg	52.9 h
P	0.0255 de	0.0160 ef	0.0095 fg	62.8 bcd	37.2 def	0.0066 b	48.4 de	6779 de	92.6 a
B	0.0242 ef	0.0163 def	0.0080 h	67.3 a	32.7 h	0.0028 h	36.1 jk	6537 hi	53.0 h
M	0.0242 ef	0.0162 def	0.0079 h	67.5 a	32.5 h	0.0078 a	70.2 a	7205 a	81.2 cde
A	0.0239 ef	0.0157 ef	0.0081 gh	66.1 ab	33.9 gh	0.0052 cd	45.7 efg	6726 defg	76.7 de
R	0.0236 fg	0.0149 fg	0.0087 fg	63.1 bcd	36.9 efg	0.0054 c	63.3 b	7070 ab	61.5 gh
C	0.0221 g	0.0131 g	0.0090 fg	59.0 fg	41.0 bc	0.0050 cde	41.7 ghi	6647 efghi	79.2 cde
MEANS	0.0291	0.0182	0.0108	62.5	37.5	0.0050	47.2	6755	72.9

Table 2. Mean numbers of days to seedling establishment (DTE), establishment values (EV), mean seedling weights, and comparisons among clones. Standard deviations (SD) of the mean DTEs show how the variance was reduced by stratification. Seedlings from unstratified seeds and stratified seeds were grown for 97 and 75 days, respectively. Means followed by the same letter were not determined to be significantly different by Tukey's studentized range test (alpha = 0.05).

	UNSTRATIFIED SEEDS			STRATIFIED SEEDS			SEEDLING WEIGHT (grams)	
CLONE	MEAN DTE	SD	EV	MEAN DTE	SD	EV	UNSTRATIFIED SEEDS	STRATIFIED SEEDS
Q	55.4 a	23.5	0.23	18.9 a	12.3	9.94	0.1998 de	0.3432 abc
K	49.4 ab	23.5	0.56	12.4 cd	3.7	0.56	0.3161 cde	0.2424 bc
N	47.1 ab	20.7	0.40	15.3 abc	8.6	17.41	0.4157 abcde	0.4387 a
S	45.9 ab	20.1	0.50	18.2 ab	4.8	15.16	0.2265 de	0.3288 abc
C	41.9 abc	17.2	0.39	13.5 bcd	6.8	12.49	0.1439 e	0.2087 bc
G	38.6 bcd	23.6	1.17	13.6 bcd	8.6	23.87	0.4952 abcd	0.3984 ab
H	36.1 bcde	21.8	0.78	12.6 cd	3.3	22.38	0.3747 bcde	0.3712 abc
J	35.9 bcde	14.2	0.56	10.6 cd	3.1	26.85	0.3254 cde	0.3868 abc
R	34.3 bcdef	21.2	0.67	13.0 cd	3.9	14.72	0.4674 abcde	0.3675 abc
F	33.7 bcdef	24.9	2.63	12.4 cd	2.7	21.86	0.5216 abcd	0.3996 ab
I	29.0 cdefg	16.7	2.17	10.7 cd	2.0	24.98	0.4370 abcde	0.3266 abc
A	27.3 cdefg	11.7	2.11	11.1 cd	1.6	36.10	0.3800 abcde	0.3757 abc
O	22.9 defg	15.9	3.67	11.3 cd	2.2	28.88	0.6145 abc	0.4253 a
D	21.9 efg	15.4	7.19	10.7 cd	2.9	26.85	0.6472 abc	0.4526 a
B	21.5 efg	8.2	1.41	12.7 cd	6.0	6.42	0.3821 abcde	0.2891 abc
L	18.6 fg	13.0	5.50	10.4 cd	2.0	33.43	0.6482 abc	0.4313 a
P	17.6 g	6.5	6.78	12.1 cd	2.7	24.48	0.7042 ab	0.4186 ab
M	16.6 g	16.6	2.28	11.9 cd	2.5	24.47	0.5052 abcd	0.3947 ab
E	14.9 g	3.9	14.01	10.8 cd	3.9	23.00	0.7254 a	0.3963 ab
MEANS	31.1	20.9	2.80	12.6	6.3	21.03	0.4484	0.3691

Table 3. Summary of important regression models for the dependent variable seedling weight. Unless indicated, all parameter estimates for independent variables were significant at alpha = 0.05.

MODEL	INDEPENDENT VARIABLES	R-SQUARE
For Unstratified Seeds		
1	DTE	0.63
2	DTE, calories per gam/emb	0.76
3	DTE, gam/emb weight	0.76
For Stratified Seeds		
4	coat weight, gam/emb weight, total weight	0.37
5	coat weight, gam/emb weight, total weight, calories per gam/emb[1]	0.50
6	EV	0.46
7	EV, coat weight, gam/emb weight, total weight	0.72

[1] parameter estimate significant at alpha = 0.10

Although other models had higher R^2 values, Model 4 (R^2 = 0.37) would be the easiest to use because it requires only weights which are easily obtained. Calories per gam/emb accounted for an additional 13% of the variation in seedling size (Model 5). However, the parameter estimate for calories was significant only at the 90% level. These were the only models in which DTE and seed properties accounted for a large amount of the variation in the weight of seedlings from stratified seeds.

All models were retested, substituting EV for DTE. Model 6, with EV as the only independent variable, accounted for 46% of the variation. The weights of the seed parts and total seed weight to the model accounted for another 26%.

DISCUSSION

The total seed weight, often proposed as being related to seedling size, was of no predictive value in this study. The seed coat represented the major portion of the seed (approximately 63% by weight). However, seed coat weight was not closely correlated with the gam/emb weight (R = 0.51), but was highly correlated with the total seed weight (R = 0.95). These correlations demonstrate that seed coat weight may change among some clones without

relative changes or even absolute changes in gam/emb weight. Some seeds were heavier only because they had heavier seed coats.

It might be expected that larger seed coats would offer greater mechanical restriction, have more inhibitory chemicals, etc., and result in longer germination and establishment times (Barnett 1972). However, there was no correlation between seed coat weight and DTE, which does not support this view. Gam/emb weight appears to be more important than seed coat weight in determining seedling size. Total seed weight, primarily determined by the seed coat weight, is a poor predictor of seedling weight.

The EV accounted for variation in the weights of seedlings from stratified seeds that the DTE could not explain. The establishment (germination) value varies directly and proportionally with both speed of establishment and total establishment and is relatively sensitive to minor differences in either. The EV is an absolute value while the DTE is a mean. Since there were few differences between DTEs or seedling weights for stratified seeds, it was not likely that a relationship between the two could be developed. In contrast, the EVs spanned a large range and the differences among clones in them were quite large. For unstratified seed, there were many differences in DTE and it did not matter if DTE or EV was used to develop relationships.

CONCLUSIONS AND IMPLICATIONS

As expected, there were large differences among clones in all of the seed and seedling properties examined. There were no simple relationships between total seed weight and seedling performance. Seed size should be closely correlated with seed weight. It does not appear that sorting seeds by size is a useful nursery practice for controlling the size of seedlings. Rather, seeds should be planted separately by clone, a standard practice in some nurseries.

Many attempts to reduce the time required to test the performance of tree progenies have been unsuccessful. Seed size has often been thought to be related to early progeny performance, and has been proposed as a possible quick indicator of progeny performance. But the correlation between seed size and progeny performance is usually poor.

In attempts to shorten progeny testing, age:age correlations (i.e., juvenile:mature correlations) are used to determine if differences among clones in progeny characteristics (i.e., height) are consistent throughout the lives of the plants. The correlations between seedling size and size at older ages decrease with time, but do not disappear. Despite some arguments to the contrary, trees that start out big seem to remain big (Bengston 1963, Grigsby 1975, Hatchell et al. 1972, Sluder 1979, Wakely 1969, Zarger 1965). Indeed, exponential growth models demonstrate that the rate of growth is a function of the initial size of the organism (Lotka 1956). The effect of initial size can be amplified in the nursery where large seedlings often suppress smaller ones.

If half to three-quarters of the variation in seedling size is attributable to nongenetic and nonheritable properties of the seeds, and if seedling size is correlated to tree size, then estimates of genetic gain may be overstated. When genetic gain is calculated from progeny tests, the effect of initial size is not considered. Thus, it becomes included in the estimates of genetic gain. Along with heritable factors, there are many nonhereditary

factors and cultural practices that affect seed properties. Energy content and the number of days to seedling establishment (DTE) may also be strongly influenced by these factors.

The small embryo represents less than 10% of the total seed and is the only part that contains chromosomes from both parents. Maternal influences on the seed may have many effects on the resulting seedling (Perry 1976). First generation progeny tests may partly select the best seeds rather than genetically superior progeny. While there is certainly genetic gain as a result of progeny testing, it is likely that it is not being accurately estimated. To accurately predict future growth on the basis of early progeny performance, other genetic and nongenetic factors that regulate germination and growth must be taken into account.

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AN OCALA SAND PINE PROGENY TEST COMPARED WITH A SEEDLING SEED ORCHARD

Timothy LaFarge, Ralph Lewis, and James L. McConnell 1/

Abstract.--Sixty-three half-sib families of Ocala sand pine were grown in a seedling seed orchard and a progeny test in Central Florida. At age 7, differences among families were significant for height, dbh, and survival in the seed orchard, for survival in the progeny test, and for dbh and survival in the combined analysis of variance of both locations. In the combined analysis the location X family interaction was only significant for survival. However, inspection of family means of each trait showed that some of the best and worst families changed rank dramatically between tests.

Additional keywords: Half-sib family, genotype X environment interaction, Pinus clausa, seedling seed orchard, progeny test.

Geneticists of the Region 8 Tree Improvement Program are assessing the performance of 120 half-sib families of sand pine (Pinus clausa (Chapm.) Vasey) in Central Florida on the Lake George Ranger District north of Silver Springs. In addition to the seed orchard, 106 of these families, plus three check lots, were also planted the same year (1978) in a progeny test on State Highway 19 about 20 miles from the orchard. Both the seedling seed orchard and the progeny test also serve as progeny tests for most of the ortets represented in a clonal seed orchard growing contiguous to the seedling seed orchard.

The purpose of the family assessments is to provide data on which to base thinnings and roguings in the seedling seed orchard. If family performance is uniform on the two sites, then genetic gain can be maximized by roguing a large number of the worst half-sib families from the seedling orchard. However, if there are strong genotype X environment interactions, then family selection must be decreased and heavy thinnings accomplished primarily by means of removal of the worst trees within each family.

METHODS

Both the seedling seed orchard and the progeny test were established in January and February 1978. In the seedling orchard 120 families were planted in 250 blocks as non-contiguous single-tree plots at a spacing of 5 feet. In the progeny test 106 families plus 3 check lots were established in 10-tree row plots in 7 replications in a randomized complete-block design.

The seed orchard has been thinned twice, once in 1981 and once in 1983. Of the 30,000 trees planted, 7,466 remain. Two more planned thinnings will reduce this number to less than 2,000. The progeny test has only been thinned by natural mortality, and survival is currently 45 percent. The progeny test was included in a RARE II Wilderness area soon after establishment, so that no competition control or cultural activity of any kind has been possible.

1/ Eastern Zone Geneticist, Forester, and Regional Geneticist respectively, USDA Forest Service, Region 8, Atlanta, Georgia.

Table 1. Degrees of freedom, mean squares and significances of differences for three traits among 63 sand pine half-sib families in a seedling seed orchard, a progeny test, and a combined analysis of variance of both tests.

SOURCE OF VARIATION	DEGREES of FREEDOM	MEAN SQUARES for: PERCENT SURVIVAL	HEIGHT	DBH
SEEDLING SEED ORCHARD				
Replicate (R)	3	0.146	6.274	1.433
Family (F)	62	0.023 **	1.469 **	0.121 **
RF	186	0.005	0.458	0.030
PROGENY TEST				
Replicate (R)	3	0.257	8.389	0.170
Family (F)	62	0.088 **	5.513 ns	0.177 ns
RF	186	0.045	6.189	0.186
COMBINED ANALYSIS OF VARIANCE				
Location (L)	1	4.715 **	8,296.046 **	250.190 **
F	62	0.060 **	3.513 ns	0.169 **
R(L)	6	0.204	7.331	0.796
LF	62	0.051 **	3.469 ns	0.125 ns
RF(L)	372	0.025	3.324	0.108

* Difference is statistically significant at the .05 level.
** Difference is statistically significant at the .01 level.
ns Difference is not statistically significant.

Both the orchard and the test were measured in 1981 at age 3. The orchard was also measured in 1983 at age 5 and in 1984 at age 6. The progeny test was measured a second time in March, 1985 at age 7. Both height and dbh were included in the last measurements at each test, and it is this set of measurements which we will investigate.

We performed a combined analysis of variance for survival, height, and dbh from both the orchard and the progeny test. To adapt the seed orchard and the progeny test to compatible field designs, we had to modify the field design of each. A road system divides the seed orchard into three large blocks of roughly equal size. Each family plot in a block is represented by a number of non-contiguous single-tree plots. Hence, for the purpose of data analysis we treated each of these large blocks as a replication. In the progeny test we used data from only the first four of the seven replications. These were, in fact, the most complete replicates anyway.

A subset of 63 families was then chosen because those families contained no missing plots in the four replications at each location. Because the orchard contained no check lots, none were included in the analysis. In summary the combined analysis of variance comprised two locations, each containing four replications and 63 half-sib families.

There are some important differences between the seed orchard and the progeny test. First, the seed orchard is mostly on a longleaf (Pinus palustris Mill.) island, but the progeny test is on a deep sand, a typical sand pine site. In the orchard each family plot is a number of noncontiguous single-tree plots randomly scattered throughout each replication, whereas in the progeny test each family plot is a single 10-tree row plot in each replicate.

The anlyses of variance were performed by means of program P8V of the Biomedical Data Processing Statistical Software system (Jennrich and Sampson 1983). Also, Duncan's multiple range tests were performed by hand.

RESULTS

In table 1 there were significant differences among families in the orchard for survival, height, and dbh. But survival was the only trait showing significant family differences in the progeny test. In the combined analysis of variance, survival and dbh showed significant differences.

The analyses considered here were based on measurements taken at ages 6 and 7 on the seed orchard and progeny test respectively. However, height at age 3 in the progeny test, with essentially the same subset of families considered, showed significant differences among families. The family ranking for height at age 6 differ little from those at age 3. Lack of competition control probably has allowed environmental noise (replicate X family interactions and variation among trees within plots) to mask family height growth differences. In table 1 only survival shows significant location X family interactions. However, we believe that an examination of the data will show some strong location X family interactions for height and diameter as well.

Table 2. Rank comparisons of the best and worst sand pine half-sib families for three traits in a seedling seed orchard and a progeny test.

FAMILY I.D.	SEEDLING SEED ORCHARD			PROGENY TEST		
			SURVIVAL			
	Percent	Rank	p = .05	Percent	Rank	p = .05
8	25.7	22	de-ij a/	77.5	1	a
7	43.5	1	a	32.5	47	de-gh
101	41.5	3	a	75.0	2	ab
116	11.5	63	l	15.0	63	h
			HEIGHT			
	Feet	Rank	p = .05	Feet	Rank	p = .05
12	20.7	2	ab	14.0	1	a
67	20.8	1	a	11.4	33	abc
97	17.6	63	f	11.9	21	abc
118	19.7	18	ab-ef	8.9	63	c
120	18.5	58	ef	8.9	63	c
			D B H			
	Inches	Rank	p = .05	Inches	Rank	p = .05
67	3.4	1	a	1.6	14	ab
39	2.9	25	bcde	2.0	1	a
40	3.0	9	bcd	2.0	1	a
97	2.5	63	f	1.6	14	ab
118	3.0	9	bcd	1.1	63	b
11	2.7	59	def	1.1	63	b

a/ Means not followed by the same letter are significantly different at the .05 level

In table 2 we have listed some of the rank changes of certain families for best and worst performance in all three traits. Some families maintain their rank as best or worst very well in both the orchard and the progeny test. Two examples are: (1) family 12 ranked 2 and 1 for height in the seed ochard and progeny test respectively; (2) family 120 ranked 58 and 63 for height in the orchard and the progeny test respectively. However, other families made dramatic shifts. Two examples are: (1) family 97 ranked 63 for height in the seed orchard but 21 for height in the progeny test; (2) family 118 ranked 9 for dbh in the orchard, but 63 for dbh in the progeny test.

Family 97 typifies a short term roguing problem. If the seed orchard were our only source of information, we would rogue this family. But in the progeny test on a site representative of the kind of site on which it will probably be planted, it grows well. Family 118 represents a long term problem. Without the progeny test we would select its best trees for further breeding, based on its rank of 9 in the seed orchard, but its rank of 63 in the progeny test should persuade us to exclude it from most sites on which we are likely to be planting sand pine.

CONCLUSIONS

The results in the combined analysis seem to bear a warning. Since the progeny test site is representative of the sites on which we intend to re-establish sand pine, we should probably pay heed to the progeny test results in our selection and roguing of families. If we should plan to put any operational plantings on longleaf islands, then we have some families, which seem to be better adapted to those sites also. Other families may be planted on either site without risk.

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HARDWOOD GENETICS

MODERATED BY DR. BRUCE BONGARTEN

University of Georgia

GEOGRAPHIC PATTERNS OF VARIATION IN GROWTH OF SWEETGUM IN EAST TEXAS

A. F. Stauder, III, and W. J. Lowe[1]/

Abstract.--Two groups of open-pollinated sweetgum progeny tests from 202 selections were planted in east Texas. Study A consisted of three tests and was established in northeast Texas and southeast Texas. This study contained seedlings from 100 families representing 12 counties in east Texas. Significant provenance differences were observed for height and diameter but not for survival and volume. The planting location by family within provenance interaction was significant for survival, diameter and volume. Two other tests (Study B) were established in east-central Texas and southeast Texas and contained seedlings from 102 families representing east Texas, south Arkansas and west Louisiana. The provenance effect was significant for survival and volume, and the family within provenance term was highly significant for all traits. There were no important genotype by environment interactions. The data indicate that sweetgum seed should be collected from Polk, Tyler, Newton and Jasper Counties for use in east Texas.

Additional keywords: Liquidambar styraciflua, progeny tests, heritability.

Sweetgum (Liquidambar styraciflua L.) is a southern bottomland species and occurs mostly on rich, moist, alluvial soils (Harlow and Harrar 1969). The range of sweetgum extends from Connecticut southward throughout the eastern United States to central Florida and east Texas, as well as scattered locations in Central America (Fowells 1965). Besides being an important commercial hardwood species in the United States, sweetgum is a desirable ornamental because of its attractive shape and brilliant autumn leaf coloration. The North Carolina State University-Industry Hardwood Research Cooperative, the Western Gulf Forest Tree Improvement Program-Hardwood and the Texas Forest Service Urban Tree Improvement Program have active tree improvement programs underway with sweetgum, and selected trees have been accepted for use in breeding arboretums and seed orchards (North Carolina State University 1984, Byram et al. 1984). Sweetgum is an intolerant species and large, pure even-aged natural stands are not uncommon. It appears

1/Silviculturist, Texas Forest Service, Alto, Texas; and Associate Geneticist, Texas Forest Service and Assistant Professor, Texas Agricultural Experiment Station, College Station, Texas. The authors express appreciation to Bosch Development Company, Champion International and Temple-Eastex Forests for providing land to establish these tests.

that sweetgum is relatively slow to establish itself; however, once established growth is relatively fast on many bottomland sites.

The objectives of this study were to examine the amount of genetic variation present in stands of sweetgum in east Texas and develop seed movement guidelines. Estimated genetic gains from family selection are also described.

METHODS

Study A

Open-pollinated seed from 110 sweetgum selections representing 12 counties and six provenances throughout east Texas were collected in 1969 and 1970. The seedlings from these selections were grown in 1970 and 1971 in three replications at Indian Mound Nursery located near Alto, Texas. One progeny test containing 100 families was established in early spring, 1971 in Jasper County, Texas (Figure 1). Two other progeny tests containing 110 families were planted in 1972 at Harrison County and Montgomery County. All seedlings were root pruned to eight inches before planting.

Data from the 100 common families among the three plantings were used in the analysis. The field design for all tests was a six-replication, randomized complete block with four-tree family row plots. Spacing was 10 by 10 feet. A single border row was used at each location to offset edge effects; however, the border row was partially destroyed at the Harrison County location.

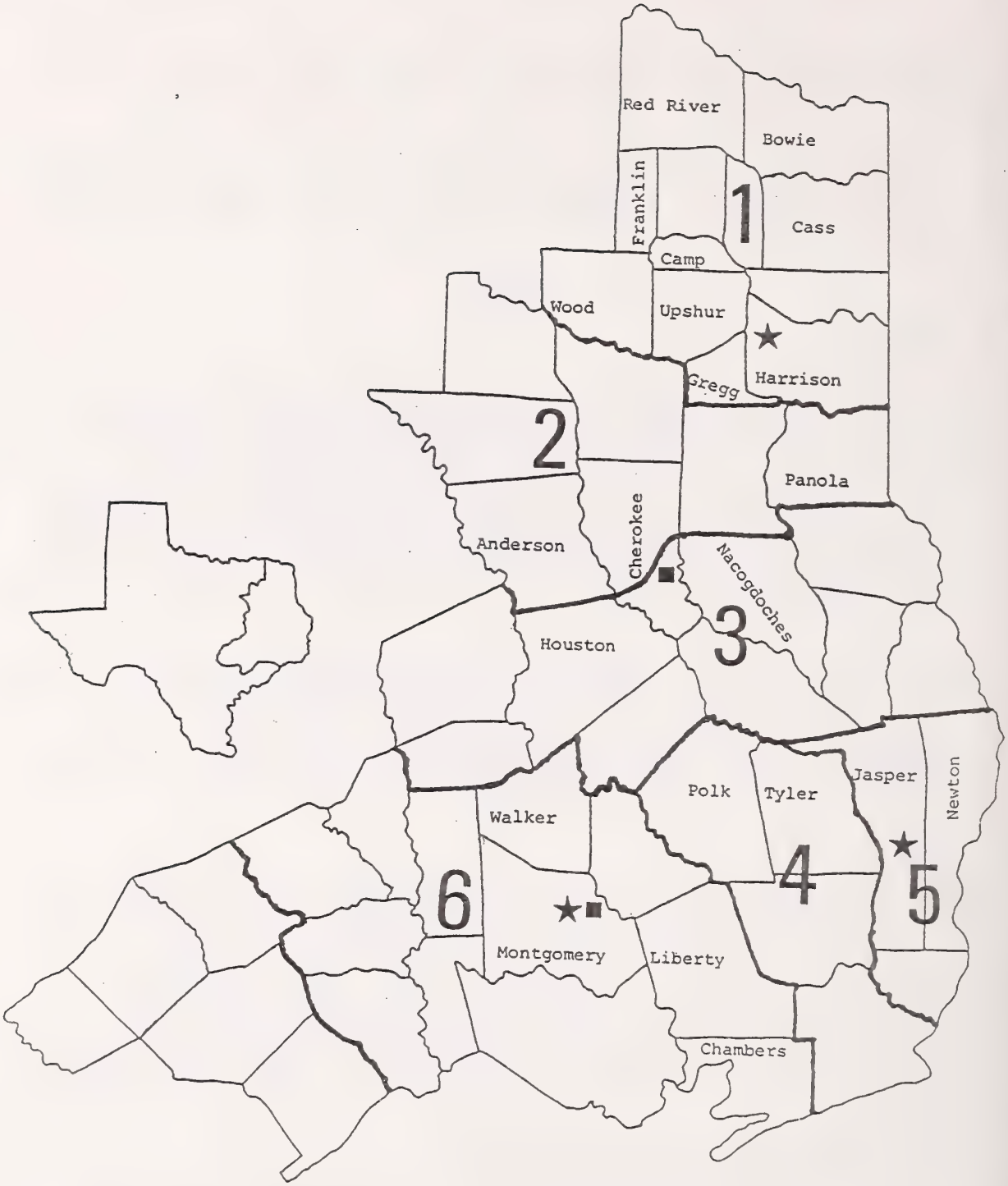
Study B

Open-pollinated seed were collected in 1972 from 106 sweetgum selections representing 16 counties and six geographic areas of seed collection from east Texas as well as two counties each from south Arkansas and west Louisiana. As in Study A, these seedlings were grown at Indian Mound Nursery. Two progeny tests containing 104 families each were established in 1974 in Cherokee County and Montgomery County (Figure 1). Data from 102 families in common between the two locations were used for the analysis. The field design was the same as that used for Study A.

Site Description and Cultural Treatments

The Harrison County planting was cleared, previously forested land of silty loam soil. The Cherokee County planting site was sandy loam soil and was also cleared, previously forested land. Both sites in Montgomery County were cut over areas on clay loam soils that were previously planted in pine and abandoned one year after planting. The test in Jasper County was an old clearcut on a sandy clay loam soil.

Weeds and sprout competition were controlled in all tests by disking during the first three years and by mowing thereafter. The



★ = plantation locations for Study A

■ = plantation locations for Study B

Figure 1.--Seed collection and plantation locations in east Texas for the sweetgum progeny tests.

test at Harrison County was fertilized in 1973 and the plantations in Cherokee and Montgomery Counties were fertilized in 1974.

Analysis

Provenance and family within provenance variation were examined by a least squares regression approach using the General Linear Model (GLM) procedure of the Statistical Analysis Systems (SAS Institute, Inc. 1982). Plot means were used in each of the combined analyses. The provenance effect was considered as fixed, while locations, replications and families within provenance were considered random effects. Dead trees were assigned a zero volume to account for survival differences. A Satterthwaite-F (pseudo-F) test was used in the absence of valid tests (Hicks 1973). Family heritability and gain estimates were calculated where appropriate for survival, height, diameter and volume.

RESULTS AND DISCUSSION

Study A

Survival ranged from 58 to 91 percent and averaged 77 percent for the three plantings at age 10 (Table 1). Height, diameter and volume averaged 5.9 m, 7.2 cm and 7.7 dm^3, respectfully. The test in Jasper County showed the best growth while the one at Montgomery County had the slowest.

Table 1.--Plantation means for five ten-year old sweetgum tests in east Texas.

Plantation	Survival (%)	Height (m)	Diameter (cm)	Volume (dm^3)
Study A				
Harrison County	83	5.7	6.9	6.9
Montgomery County	58	5.1	7.0	4.9
Jasper County	91	6.8	7.8	11.2
Study B				
Cherokee County	82	7.6	10.5	21.0
Montgomery County	86	7.7	10.9	25.2

The analysis of variance indicated significant provenance effects for height and diameter (Table 2). Trees from provenances four and five in east Texas were the largest (Table 3). These provenances were represented by families collected from Polk, Tyler, Jasper and Newton Counties. There was no detectable planting location by provenance interaction for the observed traits.

Table 2.--Combined analysis of variance for survival, height, diameter and volume of three sweetgum progeny tests (Study A).

Source of Variation	df	Mean squares for Sur.	Ht.	Dia.	Vol.
Location	2	75505.83**	272.03**	76.90	3316.45**
Replication(Loc.)	15	2269.04**	22.36**	56.40**	369.28**
Provenance	5	1672.49	8.78**	20.45*	84.41
Family(Provenance)	94	697.31	1.44**	3.00	27.76
Loc. x Provenance	10	1045.40	0.75	4.38	46.79
Loc. x Family(Prov.)	188	840.82**	0.93	2.58*	26.94**
Rep.(Loc.) x Prov.	75	421.17	0.92	2.87*	21.45
Error	1332	374.19	0.79	2.08	19.03

*Significant at 0.05 level of significance
**Significant at 0.01 level of significance

Table 3.--Provenance means for the combined analysis for the ten-year-old sweetgum progeny tests in east Texas.

Provenance	Height (m)	Diameter (cm)	Volume (dm^3)
Study A			
1	5.8	7.0	7.5
2	5.8	7.0	7.5
3	6.0	7.4	8.3
4	6.2	7.5	8.7
5	6.1	7.6	8.7
6	5.7	7.0	7.3
Study B			
1	7.3	10.2	20.8
2	7.7	10.9	23.1
3	7.2	9.9	17.9
4	8.1	11.7	29.6
5	8.0	11.1	26.7
6	7.7	10.8	21.1
15 (La.)	7.9	10.9	24.3
18 (Ark.)	7.8	10.9	24.0

Family within provenance differences could only be detected for height. Family heritability and gain estimates for height was 0.35 (SE=0.16) and 0.16 m (3%), respectively. The planting location by family within provenance interaction was significant for survival, diameter and volume. This indicates that family rankings for these traits differed among the plantations. The results from Study A indicate that sweetgum seed can be collected from any area within these boundaries and planted in east Texas without a loss in survival and volume growth. Height and diameter growth can be significantly increased by collecting seed from trees in provenances four and five. They also indicate that any family differences in survival, diameter and volume growth are masked by the significant interactions.

Study B

Survival was good (84 percent) for the average of the two 102-family progeny tests at Cherokee County and Montgomery County. Average volume was 23.1 dm^3, which was larger than that for any of the three tests in Study A (Table 1).

Results from an analysis of variance for these two plantings revealed no differences between the planting locations (Table 4). Survival and volume varied significantly among provenances. As shown in Table 3, trees from provenances four and five were clearly the largest. These same geographic areas produced the tallest trees in Study A.

Table 4.--Combined analysis for survival, height, diameter and volume at age ten of two sweetgum progeny tests (Study B).

Source of Variation	df	Mean squares for Sur.	Ht.	Dia.	Vol.
Location	1	267.28	2.30	5.58	1232.73
Replication(Loc.)	10	2079.18**	51.96**	124.03**	3050.05**
Provenance	7	1593.10**	15.45	36.15	1585.87*
Family(Provenance)	94	728.72**	3.72**	8.44**	321.11**
Loc. x Provenance	7	74.33	3.74	9.45	274.48
Loc. x Family(Prov.)	94	364.52	1.62	3.96	125.41
Rep.(Loc.) x Prov.	70	386.15	2.58	5.05	190.15
Error	894	371.24	2.04	3.96	164.83

* Significant at 0.05 level of significance
** Significant at 0.01 level of significance

In this study, the family within provenance term was highly significant for survival, height, diameter and volume. There were no significant genotype x environment interactions for these plantings, indicating consistent family rankings between the tests. Selected families as well as provenances performed well at both locations. This study indicates that a single breeding population can be used in central and southeast Texas.

Family heritability and gain estimates were calculated for all four traits (Table 5). Estimates ranged from h^2=0.50 for survival to h^2=0.54 for volume. Genetic gain by selecting the best 15 families out of 102 for survival, height, diameter and volume were 6.15 percent (7%), 0.47 m (6%), 0.71 cm (7%), and 4.72 dm^3 (20%), respectively. These gains appear to be sufficient for use in an operational tree improvement program.

Table 5.--Family heritabilities (h^2) and estimated genetic gains for the combined analysis of two sweetgum tests (Study B).

Variable	Family $h^2 \pm$ SE	Gain
Survival	0.50±0.16	6.15%
Height	0.51±0.14	0.47 m
Diameter	0.53±0.16	0.71 cm
Volume	0.54±0.14	4.72 dm^3

CONCLUSIONS

The results from Study A indicated that the geographic area of seed collection within east Texas affected height and diameter growth but not survival or volume. Individual family differences could only be detected for height (h^2=0.35). There was also a significant location by family interaction for survival, diameter and volume. The results from Study B revealed a significant effect of geographic area of seed collection on survival and volume as well as significant family differences for all traits. Growth traits appeared to be moderately inherited in sweetgum (volume h^2=0.54). By selecting the 15 best families, expected genetic gains were six percent for height, seven percent for survival and diameter, and 20 percent for volume growth.

Based on these studies, it appears that sweetgum seed should be collected from Jasper, Newton, Polk and Tyler Counties for use in east Texas.

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JUVENILE GROWTH PERFORMANCE IN A PROVENANCE TEST OF SWEETGUM

by Kim C. Steiner, Bruce Bongarten, and Randall J. Rousseau[1]

Abstract.--In a provenance test of sweetgum planted at four locations, the tallest trees after four growing seasons in the field were generally of non-local origin. Family-within-provenance variation was significant at two locations, and in every plantation was 23 to 44 percent as large as the provenance component. Sweetgum improvement programs should incorporate both provenance selection and progeny testing of wild parents.

Additional keywords: Liquidambar styraciflua, height, progeny test, natural variation.

Prior genetic evaluations of sweetgum (Liquidambar sytraciflua L.) have focused on that part of the species' natural range in which it is planted most frequently, the Piedmont and Coastal Plain (Mohn and Schmitt 1973, Sprague and Weir 1973, Texas Forest Service 1975, Wells et al. 1979). However, sweetgum is also planted commercially in bottomlands of the central interior states, and it is a common street and ornamental tree as far as 200 km north of the natural range.

This study was created to fill the need for a provenance test appropriate to the northern portion of the sweetgum commercial region. Its purpose was primarily to evaluate in northern environments the performance of populations native to the northern two-thirds of the species' range, although two plantations in more southern locations provide a useful opportunity to compare performance. All or portions of the collection have been established in experimental plantations in Georgia, Illinois, Iowa, Michigan, New York, Pennsylvania, South Carolina, Vermont, and West Virginia. Growth performance after one year in three West Virginia plantations was reported by Prowant et al. (1983). We are reporting performance after four growing seasons in plantations in Georgia, Illinois, Pennsylvania, and South Carolina.

METHODS

Seed collections were made fall 1975 from 1 to 4 open-pollinated trees in each of 47 populations of sweetgum distributed broadly, but mostly north of the Coastal Plain (Figure 1). A "population" was arbitrarily defined as occupying an area no larger than 25 km^2, and parent trees were essentially unselected as to phenotype. The trees in most populations were presumed to

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[1]Respectively, Associate Professor, School of Forest Resources, Pennsylvania State University, University Park, PA; Assistant Professor, School of Forest Resources, University of Georgia, Athens, GA; and Research Geneticist, Timberlands Division, Westvaco, Wickliffe, KY. This research was supported in part by the U.S. Department of Agriculture, Cooperative Regional Research Projects NE-27 and S-23, and in part by Grant No. 23-773 from the U.S. Forest Service, Consortium for Environmental Forestry Studies. Thanks are due to O. O. Wells for his comments on an earlier version.

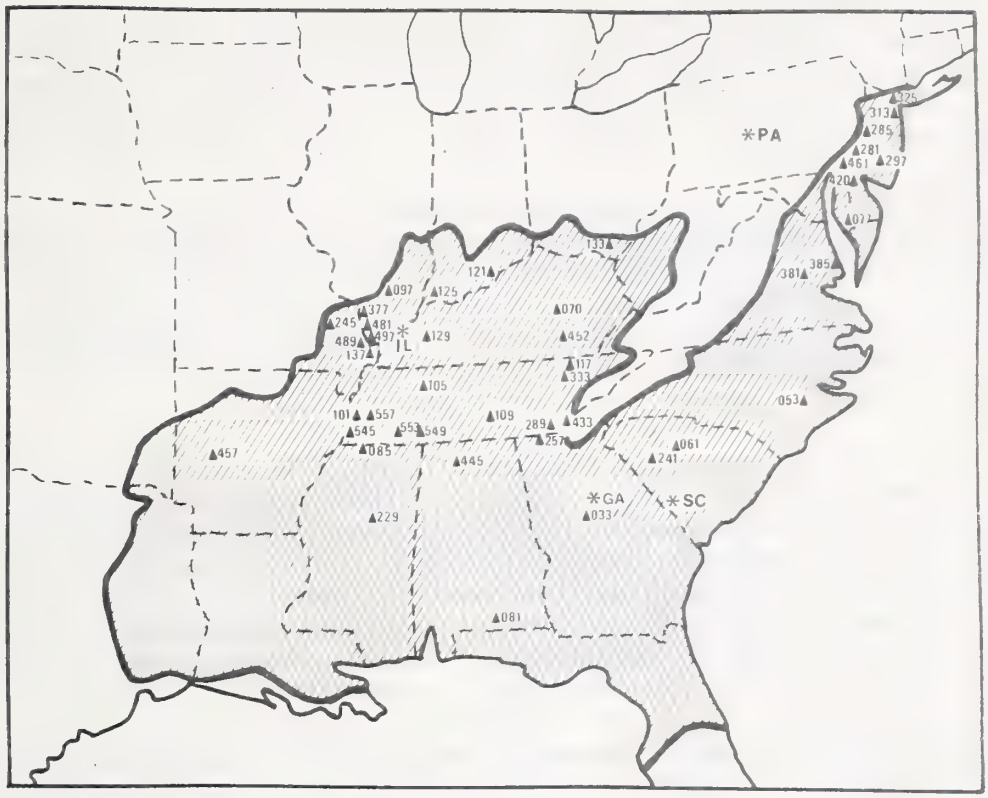

Figure 1.--Provenance locations of sweetgum evaluated for growth rate. Plantation locations are also indicated. Not shown are several collections of undetermined origin and four provenances in Georgia and Mississippi sampled specifically for the GA and SC plantations.

be native in origin. Identities of seeds and progenies were maintained according to female parent.

The seeds were distributed to each cooperator, who grew his own seedlings for outplanting. Plantations SC and GA (see below) came from a common set of nursery stock. The description of each plantation is as follows:

Centre County, Pennsylvania (PA). Planted April 1981 with 1-1 stock in 8 randomized blocks of 33 provenances in 4-tree row plots. Each provenance plot consists of four 1-tree family plots (compact family design). Spacing is 2.4 x 2.4 meters between trees. Soil is a Hagerstown silty clay loam (upland); site previously in crops, plowed and disked prior to planting and cultivated for three years afterward.

Aiken County, South Carolina (SC). Planted January 1980 with 1-0 stock in 6 randomized blocks of 26 provenances in 4-tree row plots as in PA plantation. Spacing is 1.2 meters between trees in rows and 2.4 meters between rows. Soil is a Fuquay sandy loam (upland); site previously clearcut of pine and the slash windrowed and burned, cultivated for two years after planting and fertilized in second year with 560 kg/ha of 10-10-10.

Putnam County, Georgia (GA). Planted May 1980 with 1-0 stock in 4 randomized blocks of 26 provenances in 4-tree row plots as in PA plantation. Spacing is 2.4 x 2.4 meters between trees. Soil is a Vance sandy loam (upland); site previously clearcut of mixed pine and hardwood and root-raked prior to planting, mowed for three years afterward and fertilized in second year with 560 kg/ha of 10-10-10.

Massac County, Illinois (IL). Planted April 1979 with 1-0 stock in 4 randomized blocks of 83 families in 8-tree row plots, the families representing 31 provenances. Family plots were aggregated by physiographic region, a grouping ignored for the present analysis. Spacing is 3.4 x 3.4 meters between trees. Soil is a Sciotoville silt loam (bottomland terrace); site clearcut in 1977 and the slash burned (ash deposits not planted), disked prior to planting and cultivated for three years afterward.

Each plantation was evaluated for height at the end of its fourth growing season in the field. Height data from the PA, SC, and GA plantations were subjected to analysis of variance for provenance effects. Because of the compact family designs in those plantations, family effects were examined by separate analysis of variance for each provenance. Sums of squares and degrees of freedom for family and error terms were then pooled across provenances to get an overall estimate of the significance of family-within-provenance effects.

For the IL plantation, a separate analysis of variance for provenance and family-within-provenance effects was performed for each physiographic region by which field plots were grouped, and the sums of squares and degrees of freedom were pooled across regions. Sums of squares and degrees of freedom for regional effects, from an analysis of all the data, were combined with those for provenance effects.

Block, provenance, and family were treated as random effects in all analyses. Variance components were calculated for each effect as follows, using mean squares from the pooled analyses:

Provenance	$VAR_{BxF/P} + (b)VAR_{F/P} + (f)VAR_{BxP} + (bf)VAR_{P}$
Family/Provenance	$VAR_{BxF/P} + (b)VAR_{F/P}$
Block x Provenance	$VAR_{BxF/P} + (f)VAR_{BxP}$
Block x Fam./Prov.	$VAR_{BxF/P}$

Because provenances in the IL plantation were represented by variable numbers of families, component coefficients for that plantation were generated using the VARCOMP procedure of SAS (SAS 1982).

No analysis of variance across all plantations was performed because of the differences in experimental design. Instead, provenance means in each plantation were standardized (by subtracting plantation mean and dividing by standard deviation of provenance means) and provenance contributions to provenance x plantation interaction sum of squares were calculated as follows for each of the six pairs of plantations:

$$SS_i = \sum_{j=1}^{2} (x_{ij} - x_{i.})^2,$$

where x_{ij} = mean of provenance "i" in plantation "j" and $x_{i.}$ = mean of provenance "i" across both plantations. This is Wricke's "ecovalence" formula (Shelbourne 1972), but simplified because plantation means using standardized data are 1.0. The use of standardized provenance means eliminates contributions to the interaction that are purely a function of scale as a result of provenances being more variable in some plantations than others. It also enables the comparison of contributions for a given provenance across plantation-plantation combinations.

RESULTS AND DISCUSSION

Mortality after four growing seasons was low in all plantations (SC - 2.6%, GA - 5.3%, PA - 7.8%, and IL - 14.6%). Differences among provenances were generally minor, but there were two apparent trends. Provenances native within 300 km of the IL plantation site had slightly lower mortality ($\bar{x}$ = 11.1%) at that location than those from more distant locations ($\bar{x}$ = 17.2%). It is obviously not a strong difference, but one which will be worth watching as the plantations develop. In the PA plantation, trees of southern origin have been repeatedly winter-injured, and this is beginning to show up in mortality. Provenances native south of latitude 35° had higher mortality ($\bar{x}$ = 15.3%) than those of more northern origin ($\bar{x}$ = 4.9%), and the difference will probably increase with time.

Plantation mean heights varied from 1.4 m in PA to 3.1 m in IL (Table 1). Provenance was a significant source of variation in all plantations (Table 2), and the best provenance in each grew 10 to 20 percent faster than the plantation mean. In general, the best provenances at each plantation were native to locations distant to the plantation site. The tallest 10 percent of the provenances at PA were native to Illinois and Indiana; and at IL the tallest trees were native to North Carolina, Illinois, and Georgia (Table 1). For these two plantations, there was a definite growth advantage in provenances of somewhat more southern origin than the plantation site. In the case of the PA plantation at least, this advantage was associated with no sacrifice in hardiness, since southern Indiana and Illinois trees appear at this time to be as hardy as those from coastal New Jersey and Pennsylvania.

The tallest 10 percent of the provenances at SC were native to Mississippi and Alabama; and at GA, to Mississippi and North Carolina (Table 1). Two of the three provenances involved in each case came from milder, more coastal locations than the respective plantation sites. Mississippi and Alabama sources were consistently superior at GA and SC and showed a 7 or 15 percent average height advantage over Georgia and South Carolina provenances.

Except at GA and SC, there was little positive correspondence between provenance means at different plantations (Table 3). IL means showed only a very weak positive correspondence with those at all three other locations. PA means were significantly and negatively correlated with those at the two southernmost plantations, probably as a result of the interrelationships

TABLE 1.--Relative heights (percentage of plantation mean) of 51 provenances after four growing seasons in four plantations.

Provenance		Plantation			
Number	State	PA	SC	GA	IL
001	GA	--	107.9	106.8	--
006	GA	--	105.8	99.2	--
011	GA	--	109.4	102.7	--
016	MS	--	113.6	120.1	--
033	GA	87.5	101.3	98.8	--
053	NC	91.2	107.4	115.0	--
061	SC	85.3	--	--	--
070	KY	--	--	--	93.2
077	MD	95.6	--	--	--
081	AL	80.2	116.5	109.8	--
085	MS	90.5	102.0	117.7	--
097	IL	114.0	93.7	99.4	--
101	TN	100.8	109.7	97.8	--
105	TN	97.8	--	--	--
109	TN	100.8	--	--	--
117	TN	90.5	97.5	96.2	104.0
121	IN	119.1	92.5	92.0	103.7
125	IN	116.9	--	--	103.4
129	KY	102.2	96.7	101.4	--
133	OH	--	96.3	83.1	100.2
137	MO	--	--	--	94.4
229	MS	89.7	111.6	112.4	--
241	SC	86.0	95.6	95.2	--
245	MO	98.6	--	--	--
257	GA_1	96.3	104.0	95.7	108.2
261	cv	--	--	--	96.7
281	PA	--	98.2	100.4	--
284	NJ	--	--	--	107.8
285	PA	115.5	77.7	74.3	--
289	TN	--	--	--	106.6
297	NJ	109.6	--	--	93.8
313	NJ	106.6	85.3	95.2	99.5
325	NJ	--	--	--	86.8
329	TN	--	--	--	101.8
333	TN	--	--	--	95.1
377	IL	119.9	--	--	108.2
381	VA	90.5	103.6	85.8	98.3
385	VA	90.5	100.5	107.7	103.7
420	DE	--	--	--	90.6
433	NC	--	--	--	110.4
445	AL	100.8	--	--	96.3
452	KY	--	--	--	100.2
457	AR	86.8	--	--	89.0
461	PA_1	111.8	--	--	94.8
473	cv	91.2	81.5	83.7	102.1
481	IL	116.9	--	--	100.2
489	MO	114.0	--	--	103.4
545	TN	102.2	91.8	99.4	102.1
549	TN	100.0	103.6	104.6	104.3
553	TN	100.8	102.0	98.3	104.3
557	TN	--	--	--	97.3
Plantation mean:		1.36 m	2.63 m	1.91 m	3.13 m

[1]Cultivated origin.

TABLE 2.--Variance components for age 4 height at four plantations.

Source	Component (% of total less "block") for: PA	SC	GA	IL
Provenance	11.1***	10.4***	8.2*	12.3**
Family/Provenance	4.0**	3.5*	1.9	5.4
Error[1]	84.9	86.0	89.8	82.3

[1]Pooled over "block x provenance" and "block x family-within-provenance".

*, **, *** Effect statistically significant at $P < 0.05$, $P < 0.01$, and $P < 0.001$, respectively.

between growth potential, cold tolerance, and latitude of origin. For age 1 performance of the same material in plantations in West Virginia and Maryland, Prowant et al. (1983) documented a negative relationship between latitude of origin and annual growth increment, but a positive relationship between latitude and height as a result of winter dieback on southern trees in the nursery.

Provenance x plantation interactions for the six plantation-plantation combinations are shown more clearly in Table 4. Provenances that contributed most to interactions involving PA and the two most southern plantations were of either extreme northern or extreme southern origin, an obvious consequence of the slow growth of the former in GA and SC and winter injury to the latter in PA. To a degree, the same situation occurred in the PA and IL comparison.

TABLE 3.--Coefficients of correlation between provenance mean heights at four plantations.

	SC	GA	IL
PA	-0.57**	-0.44*	+0.20
SC	--	+0.68**	+0.38
GA	--	--	+0.50

*, **Statistically significant at $P < 0.05$ and $P < 0.01$, respectively.

TABLE 4.--Provenance contributions to provenance x plantation interaction sum-of-squares in six plantation/plantation comparisons.

Provenance		Contributions to sum-of-squares[1] for plantation combinations:					
No.	State	PA + SC	PA + GA	PA + IL	SC + GA	SC + IL	GA + IL
033	GA	(-)0.709	(-)0.416	--	(+)0.029	--	--
053	NC	(-)1.067	(-)2.184	--	(-)0.221	--	--
081	AL	(-)5.930	(-)3.508	--	(+)0.295	--	--
085	MS	(-)0.488	(-)2.896	--	(-)1.063	--	--
097	IL	(+)2.225	(+)1.222	--	(-)0.173	--	--
101	TN	(-)0.306	(+)0.101	--	(+)0.721	--	--
117	TN	(-)0.137	(-)0.074	(-)1.549	(+)0.005	(-)0.043	(-)0.035
121	IN	(+)3.690	(+)3.766	(+)0.379	0.000	(-)0.336	(-)0.224
125	IN	--	--	(+)0.272	--	--	--
129	KY	(+)0.229	(+)0.030	--	(-)0.112	--	--
133	OH	--	--	--	(+)0.740	(+)0.454	(-)0.123
229	MS	(-)2.056	(-)1.967	--	0.000	--	--
241	SC	(-)0.281	(-)0.250	--	0.000	--	--
257	GA	(-)0.193	(+)0.025	(-)2.121	(+)0.330	(-)0.461	(-)1.693
281	PA	--	--	--	(-)0.024	--	--
285	PA	(+)7.522	(+)8.284	--	(+)0.010	--	--
297	NJ	--	--	(+)1.972	--	--	--
313	NJ	(+)2.557	(+)0.789	(+)0.167	(-)0.555	(-)0.031	(+)0.758
377	IL	--	--	(+)0.001	--	--	--
381	VA	(-)0.653	(+)0.181	(-)0.189	(+)1.465	(+)3.355	(+)0.138
385	VA	(-)0.347	(-)1.080	(-)1.439	(-)0.228	(+)0.024	(+)0.820
445	AL	--	--	(+)0.236	--	--	--
457	AR	--	--	(+)0.404	--	--	--
461	PA	--	--	(+)1.986	--	--	--
473	cv	(+)0.692	(+)0.377	(-)0.862	(-)0.064	(-)1.402	(-)0.636
481	IL	--	--	(+)0.942	--	--	--
489	MO	--	--	(+)0.112	--	--	--
545	TN	(+)0.698	(+)0.097	(-)0.053	(-)0.307	(-)0.057	(+)0.338
549	TN	(-)0.027	(-)0.034	(-)0.468	(-)0.002	(+)0.074	(+)0.217
553	TN	0.000	(+)0.080	(-)0.407	(+)0.068	(+)0.017	(-)0.007

[1]A "+" indicates provenances that grew relatively better in the first plantation listed, a "-" provenances that grew better in the second.

However, interactions involving the IL plantation were also distinguished by the almost consistently superior performance in IL of provenances from the Cumberland Plateau and associated highlands near southeastern Tennessee, and one collection (473) of cultivated origin. Interactions between SC and GA were small.

Family-within-provenance was a significant source of variation in two of the four plantations (Table 2). Depending upon plantation, family variance components were 23 to 44 percent as large as provenance components, with an

average of 34 percent. This compares closely with an analogous figure of 27 percent that can be calculated from Sprague and Weir's (1973) ANOVAs for age four height in ten plantations containing overlapping sets of 10 to 12 stand collections each represented by five open-pollinated families. Wells et al. (1979) also found significant within-stand variation in growth rate of progenies from 138 stands predominantly in Mississippi.

To determine whether some provenances were consistently more variable than others, we performed separate ANOVAs for family effects in each provenance at each plantation (Table 5). No provenance exhibited significant family variation at more than one location, and in fact there was hardly any consistency across plantations in the relative size of the family mean squares for each provenance. In other words, the expression of within-population variation was too inconsistent from site to site to permit generalization, and family x plantation interactions would probably have been large if we had analyzed for them. Of course, this has little practical import because provenance selection would preclude most opportunities for the selection of identical families for two or more of these plantation locations, except perhaps GA and SC.

CONCLUSIONS

After four years in field plantings, best growth was generally obtained on provenances native fairly large distances from the plantation site. For the respective plantations, the fastest growing trees originated as follows:

PA -- southern Illinois and Indiana

IL -- Cumberland Plateau and associated highlands in Georgia, North Carolina, and Tennessee, and one provenance each in New Jersey and Illinois.

SC -- Mississippi, Alabama, and Tennessee

GA -- Mississippi, Alabama, and North Carolina

Whether these patterns will persist, and whether such provenance transfers would entail some risk in adaptation, will require further study to determine. The results must be regarded with caution because vigorous height growth is just beginning to occur in the plantations.

There was little consistency in provenance performance except between SC and GA. The only interpretable interactions were those attributable to winter injury to southern provenances in contrasts between PA and the two most southern plantations.

Although family-within-provenance was a significant source of variation in only two plantations, it consistently accounted for at least 23 percent as much height variation as provenance. This is especially remarkable considering the fact that provenance representation was nearly range-wide. Cooper (cited in Wells 1979) has shown no advantage to plus-tree selection in this species. Consequently, sweetgum improvement programs should incorporate both provenance selection and progeny testing of wild parents.

TABLE 5.--Mean squares for family-within-provenance effects, by provenance and plantation.

Provenance		Plantation			
Number	State	PA	SC	GA	IL
33	GA	0.3980	0.0175	0.0563	--
53	NC	0.7975	0.8886**	0.1383	--
61	SC	0.6144*	--	--	--
77	MD	0.2510	--	--	--
81	AL	0.4161	0.3164	0.1441	--
85	MS	0.2412	0.3220	0.3886	--
97	IL	0.2739	0.2099	0.2597	--
101	TN	0.4043	0.0853	0.0675	--
105	TN	0.7411	--	--	--
109	TN	0.5197	--	--	--
117	TN	0.7160*	0.1641	0.0020	0.1185
121	IN	0.2859	0.0531	0.0653	0.0508
125	IN	0.2505	--	--	0.0692
129	KY	1.0624**	0.1262	0.1285	--
133	OH	--	0.1278	0.1557*	0.0000
229	MS	0.1052	0.2764*	0.1978	--
241	SC	0.3670	0.4867	0.1193	--
245	MO	0.2634	--	--	--
257	GA	1.0633*	0.5233	0.0290	--
261	cv	--	--	--	0.0005
281	PA	--	0.0195	0.0903	--
285	PA	0.1680	0.0313	0.0518	--
297	NJ	0.3408	--	--	0.1329
313	NJ	0.0279	0.3697	0.1623	0.0048
333	TN	--	--	--	0.0140
377	IL	0.2336	--	--	0.1625
381	VA	0.5248	0.2140	0.1449	0.1051
385	VA	0.4521	0.2938	0.2827	0.0840
445	AL	0.8113	--	--	0.4608
457	AR	0.7143	--	--	0.0009
461	PA	0.3220	--	--	0.1826***
473	cv	0.4605	0.0247	0.1872*	0.2174
481	IL	0.5267	--	--	0.1097
489	MO	0.2564	--	--	0.2270
545	TN	0.1074	0.2765	0.0639	0.0918
549	TN	0.0469	0.2175	0.0475	0.1537
553	TN	0.0587	0.0561	0.1338	0.1617

*, **, *** Statistically significant at $P < 0.05$, $P < 0.01$, and $P < 0.001$, respectively.

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GENETIC PARAMETERS FOR TWO EASTERN COTTONWOOD POPULATIONS IN THE LOWER MISSISSIPPI VALLEY

G. Sam Foster[1]/

Abstract.--Genetic variances and heritabilities were compared between samples of two populations of eastern cottonwood tested on adjacent sites. Data for fourth-year growth and second-year survival yielded little difference between families in either population with most of the genetic variation associated with clones-within-families. Resultant estimates of additive genetic variance were low with much higher estimates of dominance variance. Consequently, narrow-sense heritabilities ranged from 0.00 to 0.27, and broad-sense heritabilities ranged from 0.01 to 0.45. A more efficient future test design includes smaller blocks and noncontiguous family and clonal plots.

Reforestation of eastern cottonwood (Populus deltoides Bartr.) has generally utilized clonal planting stock. Consequently, tree improvement efforts have been focused primarily on testing and selecting clones with little emphasis on recurrent selection programs. Without genetic recombination, a clonal selection program will eventually reach a plateau beyond which no further genetic gain can be obtained.

A recurrent selection program provides an opportunity for continuing advancement in gain over time; but to be efficient, breeders need estimates of genetic parameters for use in program planning. Estimates of additive genetic variance (Farmer and Wilcox 1966, Farmer 1970, Cooper and Randall 1973, and Ying and Bagley 1976), narrow-sense heritability (Farmer and Wilcox 1966), and dominance variance (Cooper and Randall 1973) have been published. However with the exception of one seven-year-old study (Ying and Bagley 1976), the reports have all described one and two-year-old data.

In this report, genetic parameter and heritability estimates are presented using second-year survival and fourth-year growth data from two populations of eastern cottonwood. Recommendations on future cottonwood test design are also made.

MATERIALS AND METHODS

Populations

Population 1.--Female parent trees were chosen in stands growing near Lake Albemarle, just north of Vicksburg, Mississippi. Criteria for

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1/ Manager-Forestry Research/Research Forester-Tree Improvement, Crown Zellerbach Corp., Bogalusa, LA 70427. The author would like to acknowledge the work of Dr. Tom Cooper, recently of the USFS, and the South. For. Exp. Stn. for installing both studies.

selection included straightness and general appearance compared to neighboring trees. Open-pollinated seeds were collected and sown in a replicated nursery in July, 1971. The seedlings were cut back each following winter. In the spring of 1977, the best of the surviving trees were cloned and planted in another nursery. After being cut back in 1978, the clones with good survival and growth were again cloned and planted in a new nursery.

On February 15, 1980, clones were established in a field trial on Crown Zellerbach Corporation land near Fitler, Mississippi. The trial included 15 families with an average of 9.8 cloned individuals per family (range of 7 to 15; 147 clones total).

Population 2.--Female parent trees were chosen from stands in western Tennessee, 150 miles north of Stoneville, Mississippi. Using the same criteria as in Population 1. Open-pollinated seeds were collected, and seedlings were grown in a nursery in 1978 and cloned into a nursery in 1979.

The field study was planted on February 15, 1980 on a site adjacent to the test for Population 1. The trial included 17 families with an average of 6.2 cloned individuals per family (range of 1 to 17; 105 clones total).

Experimental Design

The experimental design consisted of five replications of two-tree plots planted at a 12x12 ft. spacing. The design employed a compact family block configuration in which families were arranged as randomized complete blocks with cloned individuals randomized within their respective families.

Two unrooted, 20 inch cuttings were planted at each planting spot. During the second growing season, survival was recorded; and if more than one tree survived per spot, then the second one was cut.

The test received standard cultural maintenance with several diskings during the first growing season (personal communication, Pat Weber, Fitler Managed Forest, Crown Zellerbach Corp.).

Analyses

Three traits were measured in each test including: second-year survival of the two cuttings planted at each planting spot, total height (ft) at age four, and d.b.h. (inch) at age four. Merchantable tree volume (to a three inch top) was calculated using equation 4 from Mohn and Krinard (1971).

The analysis of variance (Table 1) utilized plot mean data and employed a least-squares procedure due to imbalance of clones-in-families. Variance components were calculated by equating mean squares with expected mean squares, and coefficients of variance components were adjusted for the data imbalance (Searle 1971).

The calculation of narrow-sense and broad-sense heritabilities

utilized standard formulas (Sorensen and Campbell 1980, Foster et al. 1984). Estimates of additive and dominance variance were derived by equating variance components to their genetic expectations (Bohren et al. 1965). Nonadditive variance was assumed to be due solely to dominance variance. The ratio of dominance variance to phenotypic variance provided a measure of its relative importance.

Table 1.--Form of the analysis of variance for growth and survival traits for two populations of eastern cottonwood

Source	D.F.	Expected mean squares[a/]
Families(F)	(f-1)	$\sigma^2 + r\sigma^2_{C/F} + c\sigma^2_{FR} + rc\sigma^2_{F}$ [b/]
Replications(R)	(r-1)	$\sigma^2 + c\sigma^2_{FR} + cf\sigma^2_{R}$
FxR	(f-1)(r-1)	$\sigma^2 + c\sigma^2_{FR}$
Clones(C)/F	(c-1)f	$\sigma^2 + r\sigma^2_{C/F}$
Error	remainder	σ^2
Total	frc - 1	

[a/] Population 1: r = 2.9; c = 9.3; f = 14.0
Population 2: r = 3.9; c = 5.7, (5.9 for survival); f = 9.0
[b/] Synthetic F test (Cochran 1951)

Uniformity of family-mean performance between replications was estimated by calculating appropriate correlation coefficients.

RESULTS

Population 1

Tree growth in this study was considered good for the test site conditions for the first three replications, but replications four and five were inadvertently located on very wet areas and suffered from low survival. For this reason, further analyses refer only to the first three replications. Total height, d.b.h., survival and volume averaged 41.3 ft., 5.4 inch, 78.1 percent, and 2.0 cu. ft., respectively.

Analysis of variance results were similar among the four traits. No significant variation occurred among families for any of the traits (Table 2), with family variation accounting for a very small proportion (0.0 to 1.0 percent) of the total variation (Table 3). Replication effects were significant for all traits (p = 0.10) (Table 2) and accounted for 2.5 to 12.9 percent of the total variation (Table 3). The replication x family interaction was surprisingly large and significant for all traits (Table 2), representing an average of seven percent of the total variation (Table 3). For survival, d.b.h., and volume, this interaction equaled or exceeded the replication effect in importance. Considering height, d.b.h., and volume, the most important effect, except error, in the analysis arose from clones-in-families (Table 2)

representing 27 percent of the total variation (Table 3). No differences occurred among clones-in-families for survival.

Table 2.--Mean squares and F tests for the analysis of variance for Population 1 of eastern cottonwood

Source	D.F.	Mean Squares			
		Height	D.B.H.	Survival	Volume
Families(F)	14	40.98^{NS}	1.74^{NS}	899.27^{NS}	1.35^{NS}
Replications(R)	2	331.55**	3.49^{1}	4789.03**	4.57**
FxR	28	24.78**	1.11**	836.63*	0.76*
Clones(C)/F	132	21.61**	1.12**	501.89^{NS}	1.14**
Error	251	10.37	0.48	489.85	0.45

^{1} Significant at p = 0.10
* Significant at p = 0.05
** Significant at p = 0.01
^{NS} Nonsignificant at p = 0.10

The large replication x family interaction manifested itself in the family-mean correlations between replications. While the correlation between replications one and two was significant (p = 0.05) and positive (0.65), the correlations between replications one and three (-0.32) and two and three (-0.25) were nonsignificant.

Given nonsignificant family effects and highly significant clones-in-family effects (except for survival), estimates of additive variance were small and nonsignificant (Table 3); estimates of dominance variance were significant and apparently accounted for all the genetic variance (Table 3).

Heritability estimates reflect the importance of genetic variance to phenotypic variance; therefore the trends for heritabilities followed previous results for variance components. Narrow-sense heritability for height was 0.05, for survival was 0.01, and 0.00 for the other two traits (Table 3). Broad-sense heritability ranged from 0.25 to 0.33 for height, d.b.h., and volume and equaled 0.01 for survival (Table 3). Broad-sense heritability based on clone-means ranged from 0.51 to 0.60 for height, d.b.h., and volume (Table 3).

Dominance variance as a proportion of phenotypic variance equaled broad-sense heritability for d.b.h. and volume and was only slightly lower in the case of height (Table 3).

Population 2

Although tree growth in Population 2 did not equal that of Population 1, it was still acceptable for the site. Average fourth-year height, d.b.h., survival, and volume reached 38.1 ft., 5.1 inch, 77.2 percent, and 1.5 cu. ft., respectively. Correlations of family-means

Table 3.--Variance components and genetic parameter estimates for Population 1 of eastern cottonwood

Parameter	Estimate							
	Height		D.B.H.		Survival		Volume	
σ^2_F	0.18	(1.0)[d]	0.00	(0.0)	1.88	(0.3)	0.00	(0.0)
σ^2_R	2.36	(12.9)	0.02	(2.5)	30.36	(5.4)	0.03	(4.0)
σ^2_{FR}	1.55	(8.5)	0.07	(8.9)	37.29	(6.6)	0.03	(4.0)
$\sigma^2_{C/F}$	3.88	(21.2)	0.22	(27.9)	4.15	(0.7)	0.24	(32.0)
σ^2	10.37	(56.4)	0.48	(60.7)	489.85	(87.0)	0.45	(60.0)
V_A[a]	0.72		0.00		7.52		0.00	
V_D[b]	3.34		0.22		0.00		0.24	
h^2	0.05		0.00		0.01		0.00	
H^2	0.25		0.29		0.01		0.33	
H^2_x[c]	0.51		0.55		0.03		0.60	
V_D	0.21		0.29		0.00		0.33	
phen. variance								

[a] V_A = additive genetic variance = 4 (σ^2F)
[b] V_D = dominance genetic variance = $\sigma^2C/F-(3)(\sigma^2F)$

[c] H^2_x = broad-sense heritability of clone means

[d] Variance components as a percent of total variation

between replications (although nonsignificant in most cases) were positive except with replication two (Table 4). The importance of this result will be discussed fully later, but it was used as rationale to delete replication two from further analyses.

Table 4.--Correlation coefficients between replications based on family means of eastern cottonwood

Replications	Replications			
	2	3	4	5
1	-0.03^{NS}	0.35^{NS}	0.15^{NS}	0.67^{**}
2		-0.40^{NS}	-0.13^{NS}	-0.17^{NS}
3			0.32^{NS}	0.41^{NS}
4				0.17^{NS}

NS Nonsignificant at p = 0.10
** Significant at p = 0.01

The analyses of variance for Population 2 followed a somewhat different pattern than for Population 1. Differences among families achieved significance (p = 0.10) for height (Table 5) and accounted for

Table 5.--Mean squares and F tests for the analysis of variance for Population 2 of eastern cottonwood

Source	D.F.	Mean Squares			
		Height	D.B.H.	Survival	Volume
Families(F)	16	49.50[1]	1.68[NS]	1397.18[NS]	1.73[NS]
Replications(R)	3	63.90**	0.40[NS]	3006.03*	0.99[1]
FxR	48	11.30*	0.42[1]	903.57**	0.41*
Clones(C)/F	88	24.40**	1.33**	963.94**	1.17**
Error	251[a/]	7.20	0.31	488.07	0.29

[1] Significant at p = 0.10
* Significant at p = 0.05
** Significant at p = 0.01
[NS] Nonsignificant at p = 0.10
[a/] D.F. for survival = 264

six percent of the total variation (Table 6). The family source of variation for the other three traits was nonsignificant (Table 5) and accounted for little of the total variation (0.1 to 3.5 percent of the variation) (Table 6). Replication effects were significant for height and survival (p = 0.05) as well as volume (p = 0.10) but not for d.b.h. (Table 5). Variation represented by replication effects ranged from 0.0 to 7.0 percent (Table 6). Family x replication interaction was again significant for all traits (Table 5) and accounted for an average of 5.4 percent of the total variation (Table 6), a smaller percentage than in Population 1. Clones-within-families clearly exceeded all other sources of variation in importance. It was highly significant (Table 5) and contributed an average of 32.8 percent of the variation (Table 6).

The family x replication interaction appears to be largely due to the unusual family rankings in replication two (Table 4). Replications one, three, four, and five are positively correlated (though only replications one and five were significantly correlated), while replication two was clearly an outlier. With replication two in the analyses, the family x replication interaction represented an average of 9.7 percent of the variation.

Though still nonsignificant for three of the four traits, additive genetic variance estimates were all positive (Table 6). Dominance variance clearly represented the major proportion of the total genetic variation for d.b.h., survival, and volume; while additive variance for height was double the dominance variance.

With one exception, narrow-sense heritabilities were still quite small, and broad-sense heritabilities considerably exceeded the

Table 6.--Variance components and genetic parameter estimates for Population 2 of eastern cottonwood

Parameter[a/]	Estimate							
	Height		D.B.H.		Survival		Volume	
σ^2_F	0.90	(6.0)	0.01	(1.7)	0.75	(0.1)	0.02	(3.5)
σ^2_R	1.00	(7.0)	0.00	(0.0)	39.59	(5.5)	0.01	(1.8)
σ^2_{FR}	0.70	(5.0)	0.02	(3.3)	70.42	(9.8)	0.02	(3.5)
$\sigma^2_{C/F}$	4.40	(31.0)	0.26	(43.3)	118.97	(16.6)	0.23	(40.3)
σ^2	7.20	(51.0)	0.31	(51.7)	488.07	(68.0)	0.29	(50.9)
V_A	3.60		0.04		3.00		0.08	
V_D	1.70		0.23		116.72		0.17	
h^2	0.27		0.07		0.004		0.14	
H^2	0.40		0.45		0.18		0.45	
H^2_x	0.73		0.77		0.45		0.76	
$\frac{V_D}{\text{phen. variance}}$	0.13		0.43		0.17		0.30	

[a/] Parameter symbols explained in Tables 1 and 4.

narrow-sense. Narrow-sense heritability for height equaled 0.27; it ranged from 0.004 to 0.14 (Table 6) for the other three traits. Broad-sense heritabilities averaged 0.37 with a range of 0.18 to 0.45 (Table 6). Clone-mean heritabilities ranged from 0.45 to 0.77 (Table 6).

The ratio of dominance variance to phenotypic variance exceeded the ratio of additive genetic variance to phenotypic variance (narrow-sense heritability) for all traits but height (Table 6).

DISCUSSION

Family differences achieved significance only for height in Population 2, with nonsignificant variation for the other traits in both populations. The low amount of family variability led to either no additive genetic variation or a small amount, at best, for the traits. These results were unexpected based on the significant findings of Farmer and Wilcox (1966), Farmer (1970), and Ying and Bagley (1976).

Clones-within-families comprised the major portion of the genetic variation in these two studies. Ying and Bagley's (1976) results also concurred that, for growth traits, clones-within-families comprised a larger proportion of the total variation than families. In the present study, the clonal variation derived mainly from dominance genetic effects rather than additive genetic effects for all traits except survival in Population 1 and height in Population 2. Cooper and Randall (1973) found that additive genetic variance accounted for three times the level of dominance variance for first-year height and one-fifth the level of dominance variance for first-year survival.

Three possibilities exist for the results of this study compared to earlier studies. The findings may be real but unique (compared to earlier studies) for the two sampled populations. Intra-locus genetic interactions (dominance) may actually be the major cause of genetic variation in these populations. Selection pressure in these populations may favor survival of heterozygous individuals with very similar genotypes. The earlier studies cited above sampled many more populations and therefore had a greater chance of sampling ones with significantly different gene frequencies.

The second possibility is that these results are an artifact of analyzing data only for fourth-year growth traits and second year survival. Only one of the cited studies in the literature examined data for a range of ages (Ying and Bagley 1976) while the others examined data only for first and second-year traits. Ying and Bagley's fourth-year analysis agreed that a larger proportion of variation was due to clones-within-families compared to families; but families were still a significant source of genetic variability.

The last explanation relates to the large microsite variability in this flood-prone test site and the experimental design. The area is situated behind a levee and is not subjected to major river flooding but still receives regular backwater flooding from the Mississippi River. Undoubtably though, the soil profile originally resulted from alluvial deposits from the river and is characterized by ribbons of fairly different soil types (Wynn et al. 1961). The topography is slightly undulating and water pools up in the low spots following rains or flooding. The interaction of these site factors yields a large amount of microsite variability. Block sizes of 0.7 to 1.0 acre were probably too large and included too much within-block variability. Incomplete block designs (i.e., as described by Schutz (1966) and Libby and Cockerham (1980) hold promise for reducing block size thereby increasing efficiency of test results. In addition as Lambeth et al. (1983) demonstrated, contiguous family plot configurations (as compared to noncontiguous plots) cause larger block-by-family interactions and larger coefficients of variation for family means. A compact family plot design was used in this study as well as (contiguous) row plots for clones-within-families which probably contributed to the high family x replication interaction and nonsignificant differences among families. The efficiency of future tests of this type could be increased by using smaller blocks and a noncontiguous configuration of both clones-within-families and ramets-within-clones.

Results from this study as well as others (Ying and Bagley 1976) demonstrate the larger importance of clone-within-family variability compared to family variability. A tree improvement program should be designed which, while taking advantage of additive genetic variation through family selection, lends major emphasis to clone-within-family selection thereby tapping the large amount of dominance variance. One alternative includes a main line program emphasizing family and within-family selection for gains from additive genetic variation while in each generation utilizing a production population derived mainly from pure clonal selection.

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GENETIC VARIATION AMONG OPEN-POLLINATED FAMILIES OF BALDCYPRESS SEEDLINGS PLANTED ON TWO DIFFERENT SITES

Patricia Faulkner, Furcy Zeringue, and John Toliver[1/]

Abstract.--After two years of growth on two different sites in south Louisiana, baldcypress seedlings averaged 126.3 cm in height and 2.05 cm in diameter. Available soil moisture significantly influenced seedling growth between the two sites, with the wetter site producing the largest seedlings. Geographic variation was not found. However, family-within-source variation was significant for both height and diameter.

Additional keywords: crawfish, geographic variation, Procambarus clarkii, Taxodium distichum.

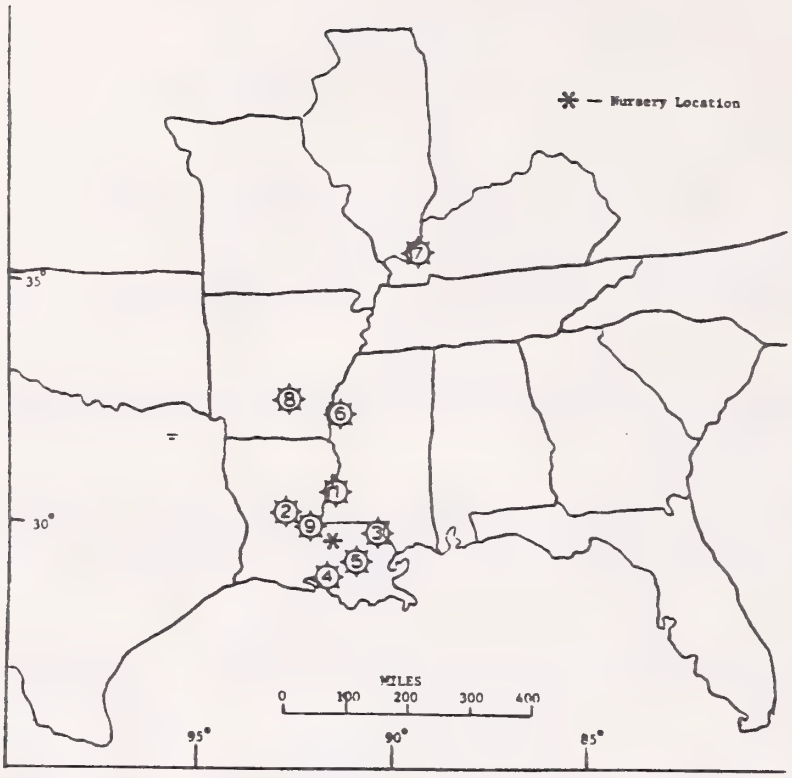
Baldcypress [Taxodium distichum (L.) Rich.] is an important commercial species in the swamps and bottomlands of the southern and southeastern United States. Throughout the South, there is an estimated 5.5 billion ft^3 (155.7 million m^3) of baldcypress growing stock on 3 to 5 million acres of commercial timberlands (Williston et al. 1981). Much of this timber will reach merchantable size within the next 30 years. Baldcypress is adapted to permanently or periodically flooded sites that are difficult to restock by natural regeneration. Before existing stands of cypress are harvested, an alternative method of regeneration must be investigated to improve cypress resources for future demands. Planting of baldcypress seedlings is a viable alternative, and numerous successful plantings have been reported (Bull 1949, Foil and Merrifield 1966). Researchers at Louisiana State University are studying the genetic variation of baldcypress in an effort to enhance knowledge of regeneration and management techniques.

METHODS

Seedlings from 26 half-sib baldcypress families representing 9 geographic seed sources (fig. 1) were planted at two locations in southern Louisiana in early 1983. The 1-0 seedlings had been grown at the Louisiana State University, School of Forestry, Wildlife, and Fisheries nursery at Baton Rouge, LA. Field design was a ten-replicate, randomized block design with five-tree-row family plots. Seedlings were planted on a 3-m x 3-m spacing, and a single row of border trees was planted around each plantation. All seedlings were root-pruned to eight inches to facilitate planting and graded by height and diameter. The largest seedlings of each family were planted in block 1, and successively smaller seedlings were planted in later blocks.

The first out-planting is located on the Thistlethwaite Wildlife Management Area in St. Landry Parish, Louisiana. This site had been under cultivation for approximately 25 years prior to which it was a bottomland hardwood site. The soils at the Thistlethwaite plantation consist of approximately 80 percent Baldwin silty clay loam and 20 percent Dundee silty clay loam (USDA Soil Conservation Service 1976). The second plantation is on a

1/ Graduate Research Assistant, Research Associate, and Associate Professor, respectively, Louisiana State University, School of Forestry, Wildlife, and Fisheries, Louisiana Agriculture Experiment Station, LSU Agriculture Center, Baton Rouge, La. 70803



Source locations
1 - Natchez, MS
2 - Alexandria, LA
3 - Bogalusa, LA
4 - Gibson, LA
5 - La Place, LA
6 - Stoneville, MS
7 - Illinois/Kentucky
8 - Monticello, AR
9 - Bunkie, LA

Figure 1. Relative location of selected geographic seed sources of baldcypress (Adapted from Faulkner and Toliver 1983).

bottomland hardwood/swamp site owned by the St. Martin Land Company in St. Martin Parish, Louisiana. This site was cleared of timber and diked for management as a crawfish (Procambarus clarkii) pond. The soil here is a Sharkey clay (Murphy et al. 1977). The St. Martin plantation was flooded with water to a depth of 20 cm for 2-3 weeks in May 1983 to stock the site with crawfish and then was inundated again from October 1983 through May 1984 for crawfish production. Both plantation sites were disked prior to planting, and weed competition after planting was controlled by a combination of disking, mowing, and herbicide applications. Soil moisture and climatic factors were monitored and recorded on a bi-weekly basis at each plantation from April through September of 1983.

An analysis of variance on the height and diameter of the seedlings taken at the time of planting indicated several significant differences attributable to the seedling grading procedure. Therefore, in order to remove the effect of this initial variation, geographic source and family components of the 3-year-old seedlings were examined by analysis of covariance using the General Linear Model (GLM) procedure of the Statistical Analysis System (SAS) (SAS Institute 1982). Further adjustment of the statistical model was necessary because of animal damage to seedlings that occurred at both plantations. In the early summer of 1983, white-tailed deer (Odocoileus virginianus) browsed 47.2 percent of the seedlings at the Thistlethwaite plantation. In the spring of 1984, crawfish either partially or completely girdled 77.8 percent of the

seedlings at the St. Martin site. In both cases the damage rate was negatively correlated to the height and diameter of 1-0 seedlings. In order to remove the effect of seedling damage on the genetic and site components of the statistical model, a damage factor was added for analysis of covariance of the 3-year-old seedling data.

RESULTS AND DISCUSSION

Site Variation

After two growing seasons in the field, the combined survival rate of the two plantations was 96.4 percent. The Thistlethwaite seedlings had a survival rate of 98.3 percent, while survival at St. Martin was 94.5 percent. Combined mean height was 126.3 cm and mean diameter was 2.05 cm. Analysis of covariance indicated a highly significant difference in height and diameter of seedlings between the two plantations. Mean height and diameter at Thistlethwaite were 125.8 cm and 1.72 cm, respectively, as compared with a mean height of 127.2 cm and a mean diameter of 2.40 cm for the seedlings at the St. Martin plantation. Mean growth at Thistlethwaite was 15.2 cm in height and 0.60 cm in diameter as compared to a growth of 28.1 cm in height and 1.48 cm in diameter at the St. Martin site. The superior growth rate of the St. Martin seedlings is attributed to the more favorable soil moisture conditions at this site brought about by the periodic inundation of the plantation area for crawfish production. Since baldcypress is naturally adapted to bottomlands subjected to seasonal flooding, the spring and early summer flooding of the St. Martin site did not hamper seedling growth. Instead it appeared to enhance growth by delaying the development of weeds and summer moisture stress conditions, therefore promoting early height and diameter growth. Precipitation and temperature were not significantly different between the two sites.

Genetic Variation

Geographic variation was not significant for either height or diameter of the 3-year-old seedlings. Faulkner and Toliver (1983) found a similar lack of geographic variation among 1-0 baldcypress seedlings. It is possible that the scope of both studies was not large enough to detect geographic variation. Only localities along the Mississippi River floodplain were sampled, while testing of provenances from a wider range might have provided more geographic diversity. This could also be a result of seed dispersal down the Mississippi River during flood conditions. Flood waters could easily have carried seed from northern sources southward, resulting in less genetic diversity among provenances along the floodplain.

Family-within-source variation was significant for height ($p<.05$) and highly significant for diameter ($p<.01$) of the 3-year-old seedlings (table 1). Thus it appears that there is greater genetic diversity among individual trees within natural baldcypress stands than among provenances. The wide range of variability among families points to a potential for genetic gain in the growth of baldcypress through family selection. If the best three families (top 10 percent) were selected for mean height (table 2), one each would come from the Stoneville, MS; Gibson, LA; and La Place, LA sources resulting in a realized gain of 13.3 percent (16.8 cm). A gain of 17.6 percent (0.36 cm) in diameter can be obtained by selecting the best three families, two families from the Stoneville source and one from the Gibson source (table 2). These

Table 1.--Analysis of covariance of baldcypress seedling heights and diameters after two growing seasons on two different sites in Louisiana.

Source of variation	Degrees of freedom	Mean squares	
		Heights	Diameters
Damage = D	3	81739.89	41.07
Plantation = P	1	86166.83[a/]	128.94[a/]
Block-within-plantation = B(P)	18	2155.90[a/]	1.33[a/]
D x B(P)	27	566.35	0.46
Source = S	8	1086.49	0.42
D x S	24	693.04	0.28
P x S	8	783.17	0.64[b/]
B(P) x S	144	485.32	0.26
Family-within-source = F(S)	17	906.57[b/]	0.52[a/]
P x F(S)	17	285.94	0.16
Error	597	407.36	0.22

a/ Significantly different at the .01 level of probability.
b/ Significantly different at the .05 level of probability.

gains could be extremely important to the successful establishment of baldcypress plantations. Planting of larger seedlings could overcome the problems of periodic high water levels, weed competition, and animal damage, and thus result in higher survival rates. It should be remembered, however, that rapid early growth in either height or diameter of a particular family does not necessarily indicate that gains will continue through an entire rotation. Further testing is essential to determine if the magnitude of these family gains and rankings will remain consistent.

Genotype x Environment Interaction

Plantation-by-source interaction was significant ($p<.05$) for seedling diameter (table 1). This genotype x environment interaction indicates that some geographic sources of baldcypress may be site specific. Certain sources performed very well on one site and poorly at the other in comparison to the other sources. In particular, the Stoneville source ranked first at the St. Martin site with a mean diameter of 2.91 cm, but dropped to seventh place at the Thistlethwaite site with a mean diameter of 1.67 cm. The Natchez, MS source was second at the Thistlethwaite plantation (mean diameter = 1.79 cm) and ranked ninth at St. Martin (mean diameter = 2.07 cm). If this interaction

continues to exist after further testing, then future plantings should be made by matching provenances to the proper site to obtain maximum tree growth.

Table 2.--Ranking of families by height and diameter across both plantations.

Seed Source	Family Code	Mean height	Mean diameter
		----------(cm)----------	
	a/		
Stoneville, MS	6-4	147.75	2.51
Gibson, LA	4-2	140.84	2.32
La Place, LA	5-2	140.68	2.26
Stoneville, MS	6-2	140.47	2.39
Alexandria, LA	2-3	139.88	2.26
Gibson, LA	4-1	135.46	2.19
Stoneville, MS	6-3	132.54	2.30
Alexandria, LA	2-1	131.66	2.21
La Place, LA	5-1	131.10	2.09
Stoneville, MS	6-1	131.06	2.19
La Place, LA	5-5	127.31	1.86
Bogalusa, LA	3-5	125.94	2.02
Bunkie, LA	9-2	125.14	2.08
Bunkie, LA	9-3	125.07	1.89
Bunkie, LA	9-4	124.58	1.95
Monticello, AR	8-2	124.03	2.19
Monticello, AR	8-6	123.09	2.12
La Place, LA	5-3	122.52	1.93
La Place, LA	5-4	121.68	1.84
Bunkie, LA	9-5	116.26	1.80
Natchez, MS	1-2	116.19	1.79
Illinois/Kentucky	7-2	115.86	1.78
Illinois/Kentucky	7-3	115.57	1.82
Monticello, AR	8-1	115.55	2.01
Illinois/Kentucky	7-1	107.45	1.66
Illinois/Kentucky	7-4	105.58	1.86

a/ The first number refers to geographic seed source (see fig. 1), and the second number refers to family-within-source.

CONCLUSIONS

Site conditions are important to height and diameter growth of baldcypress seedlings. Adequate available soil moisture is of particular importance in this respect and should be considered in site selection. Significant family variation for both height and diameter and potential early growth gains through family selection warrant the further testing of baldcypress families. Also, the existence of a genotype x environment interaction indicates the need for progeny testing of baldcypress over a wider range of sites to increase the potential for gains through consideration of site characteristics. Finally, the planting of larger seedlings (taller than 1.0 m in height and larger than 1.25 cm in diameter) should reduce the incidence of crawfish damage and deer browse and improve early survival and growth.

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BIOMASS CHARACTERISTICS OF SYCAMORE COPPICE INFLUENCED BY PARENTAGE AND TYPE OF PLANTING STOCK

S. B. Land, Jr. and E. B. Schultz[1/]

Abstract.--Three years after clearcutting a six-year-old sycamore progeny test in northeast Mississippi, stem dry weight of coppice averaged 2.27 Mg/ha and represented 63% of the above-stump dry biomass. Stumps of trees established from unrooted cuttings produced fewer coppice sprouts, smaller sprouts, and 40% less coppice stem dry weight than stumps of trees established from seedlings. There were no differences between stumps originating from top-pruned and unpruned seedlings. Progeny families differed in survival, dry weight yield of five-year-old trees before the clearcut, and number and maximum diameter of coppice sprouts per stump. Small, positive correlations between stump diameter before clearcutting and the resulting coppice characteristics were found, and these relationships may differ among families.

Additional keywords: Coppice growth, genetic differences, Platanus occidentalis.

Growing American sycamore (Platanus occidentalis L.) in plantations under short coppice rotations for fiber has received much publicity as the "silage sycamore concept" (Georgia Forest Research Council 1973). The concept was originally advocated for pulp and paper production, but now has applicability to energy plantations. Age of tree, spacing, and season of cutting can influence coppicing ability and coppice yields (Kennedy 1975 and 1980; Kormanik et al. 1973). Objectives of the present study are to elucidate effects of parentage and type of planting stock on coppicing ability in a young sycamore progeny test and to compare coppice yields three years after clearcutting with stand yields prior to the clearcut.

MATERIALS AND METHODS

A nine-year-old open-pollinated sycamore progeny test in Oktibbeha County, Mississippi (33°18' North, 88°55' West) that was clearcut at age six to produce coppice was used for the study. Three types of planting stock were utilized to establish each of ten families in the test in June, 1974: (i) unrooted top cuttings from 1-0 seedlings, (ii) top-pruned 1-0 barerooted seedlings, and (iii) whole (unpruned) 1-0 barerooted seedlings. The first two types were obtained by clipping a seedling at 2.5 cm above the root collar to provide a complete top cutting and a detopped (top-pruned) root system. The ten families came from eight geographic seed sources in the Gulf South (Figure 1), with two families per source being used from sources "A" and "F" and one family per source from the other six. The test was arranged as a split-plot

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1/ Professor, Department of Forestry, and Research Associate, School of Forest Resources, Mississippi Agricultural and Forestry Experiment Station (MAFES), Mississippi State, MS 39762. MAFES contribution No. 6134.

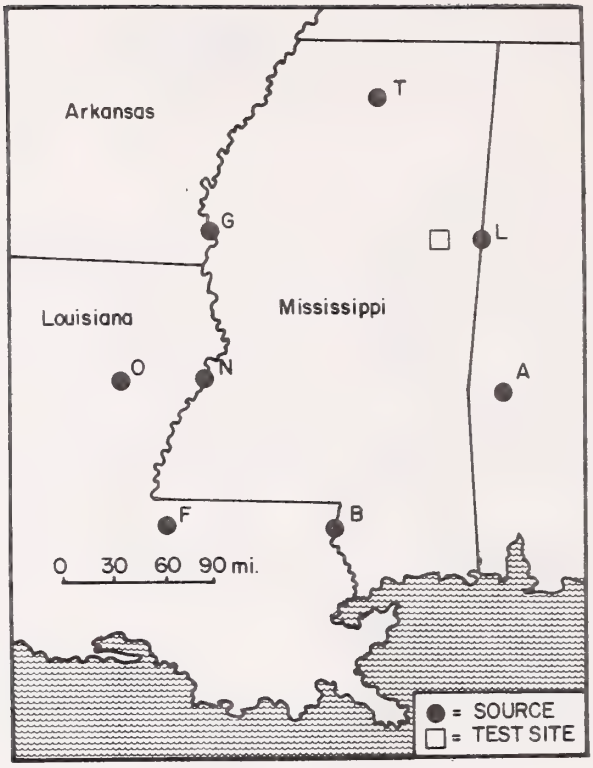

Figure 1.--Geographic locations of seed sources and test site for a sycamore progeny test used to study effects of parentage and type of planting stock on biomass characteristics of three-year-old coppice.

design with families as whole units and with four replications. Spacing was 3 x 3 meters, and each rep-by-family-by-stock-type plot contained five trees.

Stump diameters at 15 cm above ground, stem diameters at breast height (DBH), and tree heights were measured at plantation ages three and five. During the sixth growing season the test was clearcut, and at stump age nine the three-year-old coppice was measured for number of sprouts per stump, sprout diameter at 7.5 cm above stump, and sprout height above stump. Results of the fifth-year measurements before the clearcut have been reported by Land et al. (1981), but are expressed in metric terms in this paper for comparison with the coppice results.

A sample of 257 coppice sprouts from 36 stumps was measured for diameter and height during the fourth growing season of the coppice, and these sprouts were then destructively sampled for green and dry weights of stems, limbs, and leaves separately. Ratios of dry weight to green weight were used to obtain total dry weight of each component of each sprout, and the 257 records were utilized in developing dry-weight prediction equations for individual sprouts from regression on sprout diameter and height.

The above equations were applied to the three-year-old-coppice data to predict the dry weight of each sprout. Totals for all sprouts on a stump (kg/stump) were obtained and used as the basic records in analyses of variance for effects of families and types of planting stock. Survival times number of

trees planted per hectare times dry weight per stump provided plot values for coppice yields in kg of dry weight per hectare.

RESULTS AND DISCUSSION

The 257 destructively-sampled coppice sprouts averaged 3.1 cm for diameter at 7.5 cm above stump and 3.5 m for height above stump. Minimum-maximum values were 1.3 cm to 8.9 cm and 1.2 m to 7.6 m, respectively. Dry weight/green weight ratios were 0.374 for leaves, 0.455 for limbs, and 0.451 for stems. The resulting means and ranges in dry weight per sprout for each component were 0.222 kg for leaves (0.004 to 1.874 kg), 0.229 kg for limbs (0.000 to 3.030 kg), and 0.833 kg for the stem (0.037 to 6.325 kg). The prediction equations derived from the samples (Table 1) should only be used on coppice within these size ranges.

Diameter squared times height (D^2H) was a better predictor of sprout dry weight than diameter squared (D^2), as indicated by smaller standard errors of estimate ($S_{y \cdot x}$) and larger coefficients of determination (R^2) for D^2H than for D^2 in Table 1. The no-intercept models with D^2H were chosen for predicting dry weights in the three-year-old coppice, since these models did not greatly increase the $S_{y \cdot x}$ values over those of the corresponding intercept models and since the no-intercept estimates for stem, limb, and leaf components can be added together to equal predicted total values.

The additive feature of the no-intercept predictions allows the calculation of predicted weights for other components if the predicted weight for one component is given. This feature also means that identical analysis-of-variance results and rankings of treatment means will be obtained for the predicted weights of the different components. Therefore, only the stem dry weights per tree (or per stump for coppice) and per hectare will be presented, and the other components' green and dry weights can be calculated from the conversion equations in Table 2 if desired.

Stem dry weight yield of the three-year-old coppice on nine-year-old stumps averaged 2.27 Mg/ha (1.0 tons/ac) and represented 63 percent of the above-stump dry biomass (Tables 2 and 3). These yield and percentage values are lower than those reported for other sycamore coppice studies (Kennedy 1975 and 1980; Kormanik *et al.* 1973), because of the wider spacing used here than in those studies. However, the three years of coppice growth produced approximately as much biomass as the first four years of tree growth in the progeny test, as indicated by a stem dry weight yield of 0.66 Mg/ha for the three-year-old progeny test and 5.75 Mg/ha for the five-year-old test. The average stump at nine years of age contained 3.9 coppice sprouts that were greater than 1.8-cm diameter at 7.5 cm above the top of the stump, and the largest sprout per stump averaged 4.42-cm diameter and 4.35 m height. No mortality of stumps occurred following clearcutting, so that the 80.5-percent survival of the nine-year-old stumps was the same as the survival of the three-year and five-year-old trees before clearcutting.

This investigation of effects of parentage and type of planting stock on coppice dry weight yields used the initial working hypothesis that stump survival, number of sprouts per stump, and size of the largest sprout per stump were the primary determinants of yield. It was also hypothesized that diameter of the stump when the trees were clearcut would influence these

Table 1. Equations for prediction of dry weights of stems, limbs, and leaves for five-year-old trees and three-year-old coppice in a sycamore progeny test in northeast Mississippi

Dependent Variable (= Y)	Prediction Equation[a/]	$S_{y.x}$	R^2
Five-Yr.-Old Trees			
Oven Dry Wt. (kg/tree)			
Stem	$Y = 0.1579(DBH)^2$	0.9480	
Limbs	$Y = 0.0808(DBH)^2$	1.0206	
Leaves	$Y = 0.0459(DBH)^2$	0.8800	
Total	$Y = 0.2846(DBH)^2$	2.1410	
Three-Yr. Coppice on 9-Yr. Stump			
Oven Dry Wt. (kg/sprout)			
Sprout Stem	$Y = -0.1345 + 0.00736(D^2)$	0.2680	0.957
	$Y = 0.0810 + 0.0108(D^2H)$	0.1939	0.977
	$Y = 0.0698(D^2)$	0.2880	
	$Y = 0.0111(D^2H)$	0.2058	
Sprout Limbs	$Y = -0.1094 + 0.0258(D^2)$	0.1908	0.843
	$Y = -0.0343 + 0.0038(D^2H)$	0.1778	0.864
	$Y = 0.0227(D^2)$	0.2093	
	$Y = 0.0037(D^2H)$	0.1799	
Sprout Leaves	$Y = -0.0348 + 0.0195(D^2)$	0.0977	0.922
	$Y = 0.0239 + 0.0028(D^2H)$	0.0945	0.927
	$Y = 0.0185(D^2)$	0.1014	
	$Y = 0.0029(D^2H)$	0.0965	
Total Sprout	$Y = -0.2787 + 0.1189(D^2)$	0.4920	0.945
	$Y = 0.0705 + 0.0174(D^2H)$	0.4012	0.963
	$Y = 0.1111(D^2)$	0.5387	
	$Y = 0.0177(D^2H)$	0.4050	

[a/] DBH = diameter at breast height in cm; D = diameter of sprout in cm at 7.5 cm above stump; H = height of sprout in m above stump.

determinants of yield, and this diameter would be subject to effects of parentage and type of planting stock.

Trees originating from unrooted cuttings produced significantly fewer coppice sprouts per stump, smaller diameters and heights for the largest sprout per stump, and less coppice stem dry weight per stump than did trees coming from seedlings, but there were no differences between pruned and unpruned seedlings (Table 3). The trees from cuttings also averaged smaller stump diameters at age five prior to the clearcut. Since stump diameter of the young trees may be an index of the size of the root system and thus of the absorption and storage capacity of the plant, analysis of covariance was used to adjust coppice means to equivalent stump diameters for each of the types of planting stock. The smaller size of the largest sprout per stump for cuttings than for seedlings was related to the effect of smaller stumps from cuttings, since covariance adjustment removed the significant differences in sprout

Table 2. Distribution of green and dry weights among stems, limbs, and leaves of young sycamore trees and coppice sprouts, and conversion equations for calculating these components from stem dry weight

Trait	Percent Distribution in Tree or Sprout	Conversion Equations (Wts. in kg/tree, or Mg/ha)
Plantation Trees (3 & 5 Years Old)		
Stem Dry Wt. (= StDW)	56	StDW = given in Tables 3&5
Limb Dry Wt. (= LmDW)	28	LmDW = 0.5117(StDW)
Leaf Dry Wt. (= LvDW)	16	LvDW = 0.2907(StDW)
Total Dry Wt. (= TotDW)	100	TotDW = 1.8024(StDW)
Coppice Sprouts (3 Yrs. on 9-Yr. Stump)		
Stem Dry Wt. (= CStDW)	63	CStDW = given in Tables 3&5
Limb Dry Wt. (= CLmDW)	21	CLmDW = 0.3295(CStDW)
Leaf Dry Wt. (= CLvDW)	16	CLvDW = 0.2639(CStDW)
Total Dry Wt. (= CTotDW)	100	CTotDW = 1.5934(CStDW)
Stem Green Wt. (= CStGW)	61	CStGW = 2.2188(CStDW)
Limb Green Wt. (= CLmGWW)	20	CLmGW = 0.7214(CStDW)
Leaf Green Wt. (= CLvGW)	19	CLvGW = 0.7034(CStDW)
Total Green Wt. (= CTotGW)	100	CTotGW = 3.6436(CStDW)

sizes. Additional factors--such as stump vigor, root system arrangement, or response to clearcutting--that are not highly related to stump diameter may be more influential in determining number of sprouts per stump. Covariance analysis did not remove the significant difference between cuttings and seedlings for number of sprouts per stump.

Survival was lower for trees established from cuttings than for trees coming from seedlings (Table 3). This effect of planting stock occurred prior to plantation age three, and there was no stump mortality after clearcutting. Since coppice dry weight per stump was also lower for cuttings than seedlings, stumps from cuttings produced 40 percent less coppice stem dry weight per hectare (1.2 Mg/ha) than did stumps from seedlings (2.85 Mg/ha). In the five-year-old trees prior to clearcutting there was also a significant difference in stem dry weight yields between pruned and unpruned seedlings, but the difference was not present for three-year-old trees or three-year-old coppice.

The primary effect of parentage (family) on coppice characteristics was in number of sprouts per stump, although the maximum and minimum families for diameter of largest sprout per stump were also significantly different (Table 4). Stump diameter at age five differed significantly among some families, and when used as a covariate it removed the one significant family difference in diameter of largest sprout. Adjustment for stump diameter did not remove family differences in numbers of sprouts per stump, however. There were no significant family differences in coppice stem dry weight per stump or per

Table 3. Means and tests of significance for effects of type of planting stock on traits of 3-year-old trees, 5-year-old trees, and 3-year-old coppice in a 9-year-old sycamore progeny test

Trait	Overall Study Mean	Type of Planting Stock[a]			F-test Prob.[b]
		Cuttings	Seedlings		
			Top Pruned	Whole	
Stump Diam. (cm) at Age 5	8.51	7.3	8.7	8.9	.0001**
No. Sprouts/Stump:					
Unadjusted[c]	3.9	3.3	4.0	4.2	.0001**
Adjusted	3.9	3.4	3.9	4.2	.008**
Diam. (cm) of Max. Sprout/Stump:					
Unadjusted	4.42	4.1	4.5	4.5	.001**
Adjusted	4.42	4.2	4.5	4.4	.243
Ht. (m) of Max. Sprout/Stump:					
Unadjusted	4.35	4.1	4.4	4.4	.001**
Adjusted	4.35	4.2	4.3	4.3	.759
Survival (%) at Stump Age 9	80.5	56	90	96	.0001**
Coppice Stem Dry Weight:					
kg/Stump	2.62	2.0	2.7	2.8	.0001**
Mg/ha	2.27	1.2	2.8	2.9	.0001**
Tree Stem Dry Weight:					
Age 3 (Mg/ha)	0.66	0.3	0.9	0.9	.0001**
Age 5 (Mg/ha)	5.75	3.0	6.6	7.7	.0001**

[a] Means underlined by same line are not significantly different at the .05 probability level.

[b] F-test probability level for "Types of Planting Stock" in analysis of variance.

[c] Means unadjusted and adjusted for differences in age 5 stump diameter.

hectare, even though family differences in survival and dry weight yield of the five-year-old trees were detected prior to clearcutting (Table 5).

Relationships were examined between stump diameter and dry weight yields, number of sprouts per stump, and diameter of largest sprout per stump to elucidate the nature (or lack) of family effects on coppice production (Figure 2). Coppice stem dry weight, number of sprouts, and diameter of the largest sprout per stump all increased as stump diameter increased, but the percent of variation explained by regression (R^2 x 100%) was low. Other factors, such as families, types of planting stock, and many non-measured variables (stump height, date cut, etc.), all contributed to the unexplained variation. The effects of families are illustrated by the plotted family means. Although family differences were not significant for stem dry weight at coppice age three, several interesting trends can be observed by comparing graphs. Family 'A2' is consistently a poor performer from tree age five through coppice age

Table 4. Means and tests of significance for effects of family on 3-year-old coppice characteristics in a 9-year-old sycamore progeny test

Trait	Family Rank[a] #1	#2	#3	#4	#5	#6	#7	#8	#9	#10	F-test Prob.[b]
Stump Diam. (Age 5):											
Family	G1	F1	T1	L1	F2	A1	B1	N1	O1	A2	.145
Diam. (cm)	9.0	8.9	8.9	8.8	8.6	8.5	8.0	8.0	7.6	7.2	
No. Sprouts/Stump:											
Family	T1	B1	F2	G1	L1	A1	O1	F1	N1	A2	.003**
Number (unadj.)[c]	4.9	4.2	4.0	4.0	3.9	3.8	3.7	3.5	3.4	3.3	
Family	T1	B1	F2	G1	L1	O1	A1	F1	A2	N1	.002**
Number (adj.)	4.7	4.2	4.0	3.9	3.8	3.8	3.7	3.4	3.4	3.3	
Diam. Max. Sprout/Stump:											
Family	L1	O1	G1	F1	A1	T1	F2	N1	B1	A2	.493
Diam. (cm) (unadj.)	5.0	4.7	4.5	4.5	4.4	4.3	4.2	4.1	4.0	3.8	
Family	L1	O1	G1	F1	A1	F2	N1	B1	T1	A2	.504
Diam. (cm) (adj.)	4.9	4.8	4.4	4.4	4.4	4.1	4.1	4.1	4.1	4.0	
Ht. Max. Sprout/Stump:											
Family	A1	L1	T1	G1	O1	F2	F1	B1	A2	N1	.381
Ht. (m) (unadj.)	4.8	4.7	4.7	4.5	4.4	4.2	4.1	4.0	4.0	3.9	
Family	A1	L1	O1	T1	G1	A2	F2	B1	F1	N1	.464
Ht. (m) (adj.)	4.7	4.6	4.5	4.5	4.4	4.2	4.2	4.1	4.0	3.9	

[a] Means underlined by same line are not significantly different at the .05 probability level.

[b] F-test probability level for 'Families' in analysis of variance.

[c] Means unadjusted and adjusted for differences in age 5 stump diameter.

Table 5. Means and tests of significance for effects of family on survival and stem dry weights of 3-year and 5-year-old trees and 3-year-old coppice on 9-year-old stumps in a sycamore progeny test

Trait	Family Rank[a] #1	#2	#3	#4	#5	#6	#7	#8	#9	#10	F-test Prob.[b]
Survival (Stump Age 9):											
Family	F2	G1	F1	L1	O1	A1	A2	N1	B1	T1	.152
%	98	93	83	80	78	78	78	75	70	70	
Coppice Stem Dry Wt.:											
Family	L1	T1	G1	A1	O1	F2	B1	F1	N1	A2	.512
kg/stump	3.5	3.4	3.2	3.2	2.4	2.1	2.1	2.1	1.8	1.7	
Family	G1	L1	A1	T1	F2	O1	F1	B1	N1	A2	.470
Mg/ha	3.3	3.0	2.9	2.6	2.3	2.1	1.9	1.8	1.5	1.4	
Tree Stem Dry Wt. (Age 3):											
Family	A1	N1	F2	G1	L1	F1	T1	O1	B1	A2	.668
Mg/ha	0.8	0.8	0.8	0.8	0.7	0.7	0.6	0.6	0.5	0.5	
Tree Stem Dry Wt. (Age 5):											
Family	F2	G1	F1	L1	A1	O1	N1	B1	T1	A2	.064
Mg/ha	7.5	7.3	6.6	6.5	6.2	5.0	5.0	4.7	4.6	4.2	

[a] Means underlined by same line are not significantly different at the .05 probability level.

[b] F-test probability level for 'Families' in analysis of variance.

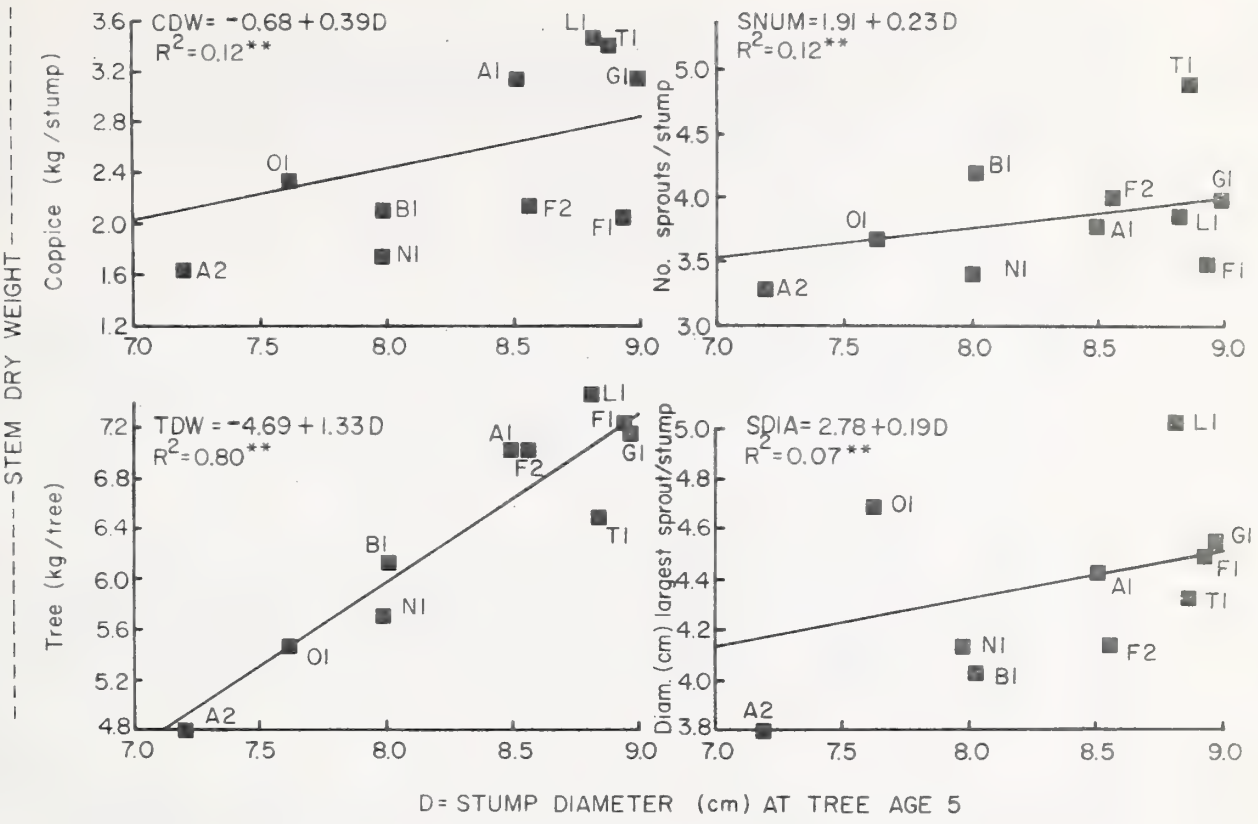

Figure 2.--Regressions and plotted family means for relationships between stump diameter at age five and (i) stem dry weight of tree at age five and (ii) characteristics of three-year-old coppice on nine-year-old stumps.

three. Families 'L1', 'T1', 'F1', and 'F2' illustrate contrasts in coppice dry weight production. All had large stump diameters at age five, and all but 'T1' had high stem dry weights at that age. Four years later the stem dry weight yields of the three-year-old coppice for 'F1' and 'F2' were well below their predicted values based on stump diameter, whereas 'L1' and 'T1' were well above the predicted values. The reasons differ between the two sets. 'L1' is high because of large diameter sprouts and average number of sprouts per stump, whereas 'T1' is high because of large numbers of sprouts and average sprout diameter. 'F1' is low because of low numbers of sprouts and average sprout diameter, whereas 'F2' is low because of small diameter sprouts and average number per stump. If these trends continue for older coppice, where family differences in coppice dry weight may be significant, the desirable families to breed for coppice production would be typified by 'L1': high biomass on a few, large sprouts per stump.

SUMMARY AND CONCLUSIONS

Dry and green weights of sycamore coppice sprouts ranging in size from one to nine cm in basal diameter and one to eight meters in height were reliably predicted from no-intercept linear regression equations presented in this paper with basal diameter squared times height as the independent variable. Using these equations, stem dry weight yield of three-year-old coppice on nine-year-old stumps in a sycamore progeny test planted at 3 x 3 m

spacing in northeast Mississippi averaged 2.27 Mg/ha. This yield fell between the yields for the three-year-old and five-year-old trees in that test prior to clearcutting. Sixty-three percent of the above-stump dry coppice biomass was located in the stems. There was no mortality of stumps during the three years following clearcutting, probably because of the wide spacing used.

Trees established from unrooted cuttings of seedlings' tops had poorer early survival and grew slower than trees established from pruned or unpruned bareroot seedlings. When the trees were clearcut, those from cuttings had smaller stump diameters and subsequently produced fewer and smaller coppice sprouts than those from seedlings. The end result was 40 percent less coppice stem dry weight produced on stumps of trees from cuttings than on stumps of trees from seedlings. Top pruning of bare-root seedlings before planting had no effect on coppice characteristics when compared with unpruned seedlings.

Families significantly affected survival and growth of trees during the first five years after planting. When the stand was cut at age six and allowed to coppice for three years, family differences were detected for number of sprouts per stump, but not for coppice stem dry weight per stump or per hectare. Small, positive relationships between stump diameter and coppice characteristics were observed, however, and family means varied around this relationship. Selection for families with the fastest growth rates during the first five years after planting, and thus the largest stump diameters when clearcut to produce coppice, may not always be the families that produce the most coppice biomass or the most biomass in the most acceptable form (few stems).

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Predicted Genetic Gains Adjusted for Inbreeding for an Eucalyptus grandis Seed Orchard

K. V. Reddy, D. L. Rockwood, C. W. Comer and G. F. Meskimen[1/]

Abstract.--One-half of a genetic base population (GPOP77) of Eucalyptus grandis Hill ex Maiden containing 529 families representing four generations of selection was harvested in August 1978. Family and individual tree heritabilities for 64-month coppice height, diameter at breast height (DBH) and volume were 0.65, 0.65 and 0.59, and 0.31, 0.32 and 0.27, respectively. Predicted gains were adjusted for inbreeding due to mating of related individuals as well as for natural selfing. The predicted genetic gains through different selection strategies ranged from 41% to 90%. Impressive gains were also predicted for clonal propagation of selected individuals.

Additional keywords: heritability, coppice, inbreeding, selfing.

In southern Florida Eucalyptus grandis has been the focus of intensive genetic improvement (Franklin 1978). It is recommended on the palmetto prairie (low fertility) and acid flatwoods (Geary et al. 1983). Trees planted in southwest Florida consitute a landrace developed through three generations of selection and progeny testing. Each generation of selection enhances the landrace's adaptation to local conditions (Meskimen 1983).

In south Florida, substantial genetic gain for juvenile height and volume growth at 2.5 years of age was found in 33 E. grandis progenies tested (Rockwood and Meskimen 1981).

[1/]Graduate Research Assistant, Associate Professor, Assistant Research Scientist and Adjunct Assistant Professor, respectively, Department of Forestry, University of Florida, Gainesville, FL. Research reported here is supported by Oak Ridge National Laboratory under subcontract no. 19X-09050C and a cooperative program between the Institute of Food and Agricultural Sciences of the University of Florida and the Gas Research Institute, entitled "Methane from Biomass and Waste". The USDA For. Serv., Southeastern Forest Experimental Station, installed and maintained the experimental materials through September, 1984.

In Florida the current E. grandis base population consists of 529 families (Geary et al. 1983). These families were all derived from 228 original introductions, mainly from Australia and South Africa, that went through up to four generations of selection. Consequently, some of the 529 families in the current base population were related due to common ancestry leading to a certain degree of inbreeding.

In a tree improvement program, care should be taken to avoid the deleterious effects of inbreeding. These effects may be of greater importance in eucalypts than has previously been considered. The most significant consequence of inbreeding depression is the reduction of the mean phenotypic value manifested by characters connected with reproductive capacity, physiological efficiency and general vigor of the offspring. Selfing, the most severe form of inbreeding has been shown to adversely affect most characteristics in E. grandis (Van Wyk 1981). Estimation of inbreeding consequences is of practical importance in continuing development of E. grandis seed orchards in southern Florida. This paper reports the effect of inbreeding due to mating of relatives as well as to natural selfing on predicted gains from a 50-family seed orchard.

MATERIALS AND METHODS

The E. grandis genetic base population (GPOP77), located at LaBelle, Florida, was planted on July 1977 and includes 529 open-pollinated families representing four generations of selection (Table 1). Each family was represented 60 times in a completely randomized single-tree plot design on 17.3 hectares. Spacing was 1.8 m between trees on paired beds spaced 2.3 m within pairs and 3.5 m between pairs, for a density of 1,916 trees per hectare. The southern half of the plantation was harvested in August, 1978. Coppice height and DBH at 64 months were used to calculate the volume according to the formula D^2H, where H and D are height and DBH, respectively. Genetic gains were predicted for different selection strategies for both individual and combined selection, using selection intensities obtained from tables provided by Namkoong and Snyder (1969).

The effect of inbreeding on predicted gains was studied for a 50-family seed orchard with one tree per family. The study assumed that the original introductions themselves were not inbred. The inbreeding coefficients for the progeny of all possible matings among the 50 families were calculated by tracing the pedigree of each mating to the common ancestor and computing the probabilities at each level (Li 1976) as shown in Figure 1.

Table 1. Proportion of families per generation in GPOP77.

Generation	% Families
1	27
2	40
3	24
4	9

RESULTS AND DISCUSSION

High genetic variation was observed for height, DBH and volume with the best trees producing 2-3 times more than others. Estimates of individual and family heritabilities of five traits (seven-month seedling height, three- and 64-month coppice growth, height and DBH) were not significantly different (Reddy *et al*. 1985). At 64 months after harvest, the family and individual heritabilities for coppice height, DBH and volume were 0.65, 0.65 and 0.59, and 0.31, 0.32 and 0.27, respectively.

The inbreeding coefficients (F) for possible crosses ranged from 0 to 0.25 (Table 2). The mean F value for all the matings was low (0.5%) because only 13% of all possible crosses were related. Additionally, in related individuals two or three generations have passed since direct relationship by common ancestry.

Hodgson (1976a) reported a reduction in seed yield of 53% to 98% following self-pollination. In deliberately selfed progenies of *E. grandis*, a loss of 8% to 49% in height growth is reported (Eldridge 1978, Hodgson 1976b). Moreover, selfed progeny were found to be more

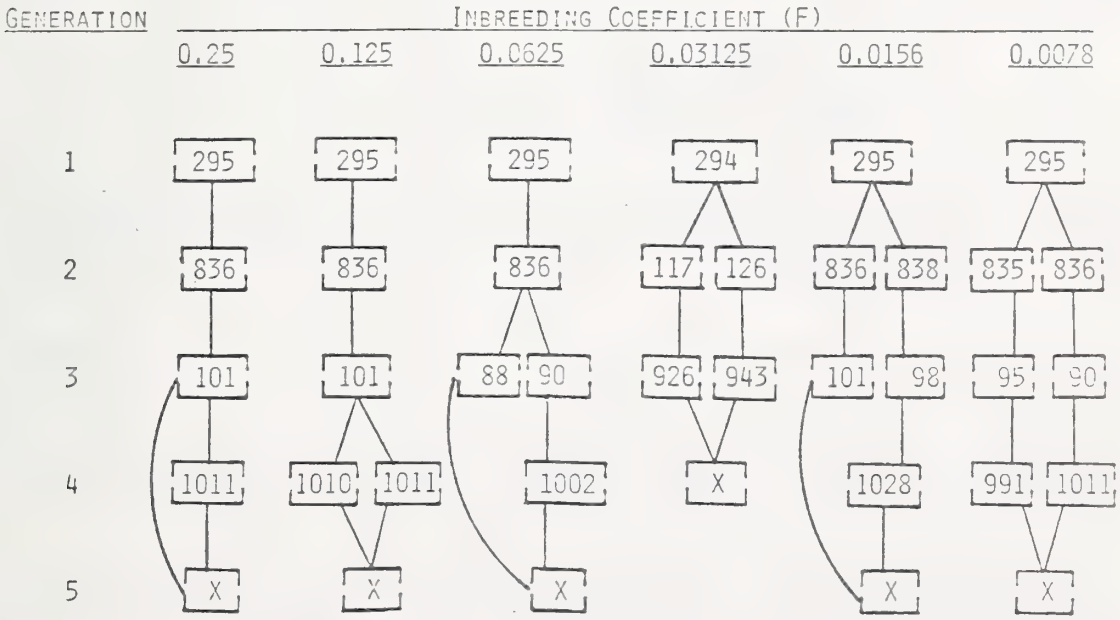

Figure 1. Representative pedigrees and inbreeding coefficients of different degrees of relationships among families in GPOP77.

Table 2. Inbreeding coefficients (F) and number of all matings in a 50-family seed orchard.

F		Number of Matings
0		1064
0.25		2
0.125		13
0.063		6
0.031		93
0.016		19
0.008		28
Mean 0.005	Total	1225

crooked and have 30% abnormal seedlings. The amount of natural selfing that occurs in eucalypts is higher than reported for pines. An average of 7% selfing is reported in many pines (Wright 1976). Published estimates for the degree of natural selfing in eucalypts vary with species: 24% in E. obliqua (Brown et al. 1975), 37% in E. pauciflora (Phillips and Brown 1977), 23% in E. delegatensis (Moran and Brown 1980) and 18% in E. stoatei (Hopper and Moran 1981). Eldridge (1978) reported 20% to 40% selfing occurring in E. grandis and Van Wyk (1981) estimated an average of 30%. Using a conservative approach for the range of inbreeding depression reported for E. grandis, a 50% loss in growth for selfed progenies (F=0.5) amounts to 10% loss for every 10% of inbreeding coefficient.

Using Eldridge's (1978) estimate of 30% natural selfing in seed orchards of E. grandis, the mean F value for the offspring of all possible matings will increase due to selfing. Thirty percent of the offspring will have an F of 0.5 and 70% will have an F of 0.005. Thus the estimated inbreeding coefficient of the offspring will be 15.4% (F=0.154). Therefore, the predicted genetic gains from the seed orchard should be decreased by 15.4% to account for the loss due to selfing and mating of relatives.

Based on the results from the 50-family seed orchard, similar adjustments for inbreeding were made for the predicted genetic gains from different selection strategies. This assumed that the mean inbreeding coefficient for the progenies of the families in the seed orchard is the same (F=0.005) for any selection strategy that has a variable number of families and number of trees per family.

The predicted genetic gains that could be achieved through different selection strategies are given in Table 3. Higher genetic gains through family and combined selection than mass selection agrees with the results reported in E. robusta (Dvorak et al. 1981). The greatest gain in volume (90%) over the whole population can be achieved when combined selection is performed, selecting the top 100 families with three trees per family. Similar gains can be achieved by selecting one tree from the top 300 families (96%). A gain of 80% can be realized through mass selection of the top 200 trees. Genetic gain through

Table 3. Predicted genetic gains in 64-month coppice volume for alternative improvement strategies with Eucalyptus grandis.

Selection Strategy	Genetic Gain (%)
200 best trees	80
300 best trees	69
10 top families (30 trees per family)	41
30 top families (10 trees per family)	61
100 top families (3 trees per family)	90
300 top families (1 tree per family)	86
Clonal propagation of 200 best trees	441

clonal propagation of the best trees shows considerable promise. A gain of 441% is predicted through the clonal propagation of the top 200 trees. This estimate, however, is biased upward by the fact that it assumes 100% survival, which is unrealistic. Nevertheless, it does indicate the potential that exists in clonal propagation.

CONCLUSIONS

Natural selfing is an important factor to be considered in predicting genetic gains. The estimates should be adjusted to account for the inbreeding depression that occurs due to selfing as well as due to mating of related families which share a common ancestor. In this study the mean relationship among families was low (0.5). However, the predicted gains were decreased by 15.4% to account for natural selfing which is reported to be 30% in E. grandis.

High genetic variation for growth was observed in E. grandis with the best trees producing 2-3 times more than the others. This suggested the obvious potential for improvement through clonal propagation of the best individuals to capture full genetic superiority. Impressive gains are also predicted through combined selection of various selection intensities. A gain of 90% could be achieved by selecting three trees from the top 100 families.

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CAN LATERAL ROOT CHARACTERISTICS BE A MAJOR FACTOR IN ASSESSING SEEDLING QUALITY

Paul P. Kormanik[1]

Abstract.--A grading standard for tree seedlings should be an easily observed or measured characteristic that is strongly indicative of performance after outplanting. Lateral root morphology may be such a characteristic. Recent work with sweetgum indicates that at least four strong lateral roots may be needed to make a seedling competitive for artificial regeneration. Tests with scores of half-sib seedlots indicate that at least 40 percent of the nursery seedlings produced may not have this number of lateral roots developed when they are lifted for planting.

Additional keywords: Root grading, root morphology, sweetgum.

Seedling Quality: What is it? Although foresters readily agree on the need for quality seedlings for outplanting, few would have enough confidence to write a prescription for judging seedling quality in the nursery. The need for production of quality seedlings is certainly well known and equally well documented (SIFRC 1984, Duryea and Landis 1984, Wakeley 1954). There are, unfortunately, no reliable criteria for assessing seedling quality for any species of forest tree. This lack of agreement on what constitutes seedling quality has both land and nursery managers in a serious dilemma. Development of the technology to assess seedling quality is among the highest priorities throughout the United States and it is particularly important in the South because of the large acreages of trees being artificially regenerated (SIFRC 1984).

In the early 1920's and 30's morphological grading of southern pines seemed most promising, and it seemed to be working (Wakeley 1954). As the grading procedure became well accepted and more universally applied during the 1930's, erratic performance of graded seedlings began to appear in many field locations. It soon became apparent that nursery locations, different soil and management practices were altering seedling development enough that morphological grades from different nurseries were no longer comparable and uniform (Wakeley 1954).

By the early 1960's as the costs of nursery stock began to increase and when seed from genetically improved stock became more widely used, erratic plantation performance became a major concern of land managers. Improved techniques in nursery management did little to improve field performance of planted seedlings, even those grown from improved seedlots. Over a period of years, different criteria for judging seedling quality were offered but these proved to be of limited value and now seedlings are sold predominantly by weight.

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[1] Silviculturist, Institute for Mycorrhizal Research and Development, Southeastern Forest Experiment Station, USDA Forest Service, Athens, GA.

No attempt will be made here to thoroughly cover the seedling grading standards that have been proposed, but they generally can be classified as assessments of either morphological traits or physiological attributes. These seedling characteristics may carry the classification of Material Performance Attributes (Ritchie 1984), Stock Type, or Physiological Condition (Duryea 1984). Morphological criteria--something that is readily visible to the naked eye or easily measured or assessed--for grading all planting stock is more desirable than expensively obtained physiological parameters from a few seedlings. Wakeley (1954), however, pointed out that any nursery practice which improves the physiological status of nursery stock will also materially alter the morphological grade of the seedlings and he felt the best future for grading seedlings would have to rely heavily on some physiological criteria.

Land managers generally use morphological grades and many feel that root collar diameter (RCD) is the best measure of seedling quality. However, Webb (1969) cautioned against using RCD as a grading criteria for sweetgum (Liquidambar styraciflua L.) seedlings because seedlings grown at different nurseries or even from the same nursery but at different seedbed densities varied considerably in RCD.

The questionable reliability of seedling physical measurements as a grading criteria may be responsible for Burdon and Sweet's (1976) comment that land managers aren't really interested in nursery performance of seedlings but desire some measurable attribute on seedlings from the nursery that is well correlated with later field performance of planted seedlings. They concluded that possibly genotypic differences might be found within populations of seedlings that could be used to improve field performance. Any morphological trait that varies by nursery location or is readily altered with fertility practices would be of questionable value.

During the past 7 years working with sweetgum at the Institute of Mycorrhizal Research and Development (IMRD), Athens, Georgia, we feel that a relatively stable morphological root relationship has been found that may be of value in assessing seedling quality and which may be suitable for use in grading sweetgum nursery stock. It can be of value in realistically assessing the percentage of plantable seedlings one could expect from a given nursery and might be helpful in judging mother trees before they are established in orchards. Preliminary data suggests that comparable lateral root assessments may be equally common and readily recognized on seedlings from most forest tree species.

Early Nursery and Field Experiments

Beginning in 1973 and through 1978, numerous nursery experiments were run on sweetgum and other hardwoods at the U.S. Forest Service's experimental nursery maintained at the University of Georgia's Whitehall Experimental Forest. The purpose of these experiments was to determine the effects of different vesicular-arbuscular mycorrhizal (VAM) fungi and fertility regimes on seedling development. In general, these experiments showed that hardwood seedlings did not develop normally in the absence of VAM fungi when concentrations of available soil phosphorus were low (12 to 25 ppm, Bray II) (Kormanik and others 1977, 1982, Schultz and others 1981). When available soil P exceeded a

level near 75 ppm, however, seedling growth was not adversely affected by the absence of mycorrhizae. If available soil P was in the range of 50 to 75 ppm, about 95 percent of the mycorrhizal sweetgum seedlings exceeded the minimally acceptable RCD limits of from 0.64 cm for outplanting. A comparable percentage of plantable nonmycorrhizal sweetgum seedlings was produced when available soil P was in excess of 100 ppm.

On the average early performance of mycorrhizal and high P nonmycorrhizal seedlings were about the same--rather poor. To find out why, additional sweetgum plantations were established on the Savannah River Forest Station, Aiken, South Carolina, in 1977 and 1978. Fifty to sixty percent of the planting locations in these plantations were occupied by two seedlings planted about 30 cm apart. The plan was to excavate one of each pair without excessive damage to the roots of the other. The original purpose of these excavations was to follow vesicular-arbuscular mycorrhizal development in seedlings after outplanting and to correlate stem growth with degree of mycorrhizal development observed. Within 6 to 8 weeks after plantation establishment, we observed that all seedling roots, regardless of original nursery treatments, had comparable mycorrhizal development. This comparability in mycorrhizal development was not accompanied by uniform growth or survival of seedlings from within the different nursery treatments. We found that a seedling's development appeared to be correlated with the number of lateral roots on the excavated seedlings. Unfortunately, when these early plantations were established our primary concern was the presence or absence of mycorrhizae on the seedlings when they were lifted from the nursery. It was later that I thought of assessing lateral root development.

From 1978 through 1981, 8,000 to 10,000 seedlings a year from four to six different half-sib sweetgum seedlots were grown and lifted separately at the IMRD Experimental Nursery at the Whitehall Experimental Forest. All seedlings were grown in the same 8 to 12 nursery beds each year at a seedbed density of $62/m^2$ (ca $6/ft^2$). Fertility and mycorrhizal variables differed somewhat among years.

The purpose of these early experiments was to develop a preliminary prescription for grading sweetgum seedlings based primarily on number of permanent lateral roots. Over the years, we had come to recognize at least three distinct types of lateral roots occurring along the taproot of sweetgum. The first are small, thin feeder roots seldom exceeding 2.5 cm in length which are uniformly distributed along the entire taproot. A second type has a similar spacial distribution, but lacks rigidity, are threadlike and can attain lengths of up to 12 cm. Some of these roots have diameters exceeding 1 mm and have attached many small feeder roots of varying lengths up to about 1.0 cm. The third type, which we consider to be a part of the permanent lateral root system, develops primarily within 20 cm of the root collar. These roots are rigid and have diameters from 1 to 5 mm, lengths exceeding 35 cm, and higher orders of branching upon which abundant terminal feeder roots develop. Only the permanent lateral roots generally withstand the rigors of lifting and packaging in the nursery.

Based on data collected from field excavations, we developed a preliminary prescription for grading sweetgum nursery stock based primarily on the number of recognizable permanent lateral roots present on a given seedling. The poorest (grade 3) seedlings were those with three or fewer permanent lateral roots. The intermediate (grade 2) seedlings had from four to six permanent lateral roots while the best (grade 1) seedlings have seven or more permanent lateral roots. In these early nursery studies where percentage of seedlings in each grade was being evaluated, seedlings were destructively sampled and no plantations were established. We simply wanted to determine how nursery fertility and different mycorrhizal symbionts affected lateral root development for half-sib seedlots. At this time we suspected that nursery management practices would alter root morphology as clearly as it did stem morphology.

Results from Early Nursery Experiments

In 4 years of nursery testing of 18 different half-sib seedlots, the number of grade 3 seedlings ranged from about 35 to 60 percent for different seedlots. The average number of grade 3 seedlings annually approached about 50 percent. Specific half-sib seedlots were tested annually in up to 10 different nursery treatment combinations. We found that the distribution of seedlings by root grade was comparable across all treatments for a given half-sib seedlot, even when VAM and fertility treatments resulted in seedlings with ranges of mean heights of 0.75 m to 1.0 m and mean RCD of 0.25 to 1.1 cm.

Nursery practices significantly increased seedling size, but the increases obtained in RCDs were not normally accompanied by the development of more permanent lateral roots. More important, however, even the best mother trees produced a high percentage of "carrot-rooted" progeny--those with fewer than three strong lateral roots. This information may be of considerable importance for it suggests that regardless of improvement in nursery seedling stem characteristics, a significant percentage of seedlings may not be genetically capable of being competitive in nature because of limitations in root development.

During this early testing, seedlings from a mixed seedlot in a state nursery and one industrial nursery were also evaluated. In both nurseries, approximately 45 percent of the seedlings graded had three or fewer permanent lateral roots--figures comparable to those observed in our nursery testing.

Current Testing

In each of the 1982 and 1983 growing seasons, seedlings from four half-sib seedlots were grown in nursery beds receiving eight different treatment combinations. Seventy-eight seedlings were randomly selected from each seedlot/treatment combination in both years to provide data on root grade distribution. Four thousand seedlings from the 1982 nursery test were outplanted in a field study with a split plot design in which the effects of both nursery treatments, seedlots and root grades, could be evaluated. From the four 1982 seedlots, the grade 3 seedlings represented 53, 58, 48, and 50 percent of the seedlings produced. There were no biologically significant differences among treatments. Table 1 contains nursery seedling information for each half-sib seedlot for all nursery treatment combinations as well as first year growth and survival data for seedlings from all three grades.

Table 1.--Height and root collar diameter (RCD) of lifted seedlings, and height, RCD, and survival at the end of the first growng season in the field, by half-sib seedlot and root grade

Treatment and root grade	Initial size		End of growing season		
	Height	RCD	Height	RCD	Survival
	m	cm	m	cm	percent
80-5B					
Grade 1	1.04a	1.31a	0.87a	1.30a	84a
Grade 2	1.02a	1.10b	0.65b	0.96b	68a
Grade 3	1.00b	0.80c	0.40c	0.58c	52c
81-12B					
Grade 1	1.04a	1.34a	0.73a	1.18a	74a
Grade 2	1.04a	1.16b	0.58b	0.89b	64b
Grade 3	1.02a	0.85c	0.29c	0.52c	49c
81-3U					
Grade 1	1.04a	1.35a	0.82a	1.26a	80a
Grade 2	1.05a	1.12b	0.53b	0.81b	72b
Grade 3	1.02b	0.80c	0.33c	0.55c	52c
81-5U					
Grade 1	1.10a	1.41a	0.82a	1.32a	78a
Grade 2	1.11a	1.17b	0.57b	0.90b	66b
Grade 3	1.09a	0.82c	0.32c	0.57c	50c

Within columns and treatments, values followed by the same letter do not differ significantly ($P = 0.05$) according to Duncan's New Multiple Range Test.

Even though grade 3 seedlings would qualify as plantable stock by current standards for sweetgum, their survival percentage was not acceptable by any standard. Drought was severe through most of the spring and summer of 1983 in the Piedmont of South Carolina and Georgia. Survival in this plantation was poor as it had been in all plantations in those areas. The drought impact, however, was far more severe on seedlings with few lateral roots. Second-year data have not been statistically analyzed, but it appears that the relatively poor performance of root grade 3 trees is unchanged from the previous year. Seedlings from the two better root grades are growing well and, with some half-sibs, it is difficult to distinguish between them. Some half-sib grade 2 seedlings show greater variation in stem development than is apparent in grade 1 half-sib seedlings. This variation within grades may be reduced when new data are available for determining how many lateral roots are required for a seedling to be competitive.

The results from the 1983 nursery trials were similar to those obtained in the 1982 tests. Heights averaged ca 0.91 m with no biologically significant difference among grades. The RCDs for grade 3 seedlings in 1983 averaged 0.90 cm, which was significantly smaller than the 1.17 cm obtained from the root grade 1 seedlings. However a 0.90 cm diameter would be acceptable under present sweetgum grading standards. Half-sib seedlot 81-12B was used in both the 1982 and 1983 nursery trials. Among the eight VAM-fertilizer treatments in 1982, an average of 58 percent of the seedlings from this seedlot were placed in root grade 3. Comparable treatments used in 1983 resulted in 52 percent of the seedlings from this seedlot being placed in this group. The percentage of grade 3 seedlings from the other three 1983 half-sib seedlots were 36, 41, and 47.

Where Are We and Where Do We Go From Here

Our research to date indicates that the percentage of grade 3 seedlings in a seedlot is quite stable and predictable. It appears that the number of lateral roots that develop on individual seedlings may not be significantly altered by fertility practices. At low fertility levels, although the seedlings are smaller and the diameters of their permanent lateral roots are smaller, we still were able to separate seedlings into different morphological root grades. Under higher soil fertility regimes the seedlings were larger and the permanent lateral roots were also larger but we were still able to separate them from other emphermal lateral roots. Based on examination of seedlings sent to the Forestry Sciences Laboratory, Athens, Georgia, from different nurseries, soil texture appears to have limited affect on lateral root numbers and distribution. Both soil fertility and soil texture, of course, can affect the feeder root development but this has not affected the number of permanent lateral roots produced in our tests.

We now need to determine how extremes in seedbed density affect the development of permanent lateral roots and our ability to distinguish root grades. This is the objective of the 1985 nursery testing program. We do know, however, that diameters of lateral roots of seedlings in the interior of the beds are significantly smaller than those seedlings lifted from the exterior border rows. The data do not indicate, however, that the number of lateral roots from the border rows represent a different distribution than occurs on the interior seedlings. Within a given half-sib seedlot, I do not believe it is very important how big the permanent lateral roots are initially, within reasonable limits, but rather how many are present just as long as we can identify them as permanent or emphermal.

There is little doubt that there exists a distinct distribution of lateral root grades on sweetgum seedlings lifted from the nursery. This distribution appears to be stable from selected half-sib progeny and a comparable distribution has been found with seedling populations obtained from mixed seedlots of unknown parentage. Extensive nursery trials show that 40 to 50 percent of all seed germinated produce seedlings with fewer than four permanent lateral roots, which may be a minimum for satisfactory early plantation performance. This assumption must be tested in the field over time but two sweetgum plantations established in 1982 and 1983 appear to support it.

The ease with which aboveground characteristics can be changed without altering lateral root characteristics may in part explain why improved nursery practices, even with seed from selected trees, have not resulted in improved performance of nursery stock in the field. If lateral root distribution is positively correlated with plantation performance, a biological grading scheme may be fairly easy to develop. Standards, of course, will probably vary by species. Our early data indicate that lateral root characteristics are quite different for the seven different commercially important forest species we have examined. Six lateral roots may be sufficient for sweetgum seedlings to be competitive, but oaks (Quercus spp.) and black walnut (Juglans nigra) may need twice this number.

Recent surveys indicate that productivity of plantations is not as good as predictions indicated. The cause may be production of seedlings with large tops but without accompanying large root systems. If we are correct in our assumption that number of lateral roots occurring on young seedlings is a good estimator of potential productivity of that seedling and that this trait may be predictable within a given species, the economic gains in yield per hectare would warrant extensive trials of biological grading. Unfortunately, at this time, we may not be able to alter root morphological characteristics as readily as we can change aboveground characteristics. Thus, we must determine how important lateral root development of seedlings really is and be prepared to cull many seedlings if marginal plantation performance is shown to be correlated with lateral root development.

What is a quality seedling? Could it be one with at least a specific number of lateral roots?

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CONIFER GENETICS III

MODERATED BY DR. HANS VAN BUIJTENEN

Texas A&M University

MONOTERPENE PHENOTYPES IN LOBLOLLY PINE POPULATIONS: NATURAL SELECTION TRENDS AND IMPLICATIONS

A.E. Squillace, Harry R. Powers, Jr., and S. V. Kossuth[1]

Abstract.-- The degree of discrepancy between observed proportions of various monoterpene phenotypes involving two or more loci and those expected under random association between loci was studied in 111 populations scattered throughout the range of loblolly pine. Results suggest that some phenotypes are being favored by natural selection while others are being disfavored. Natural selection varies among regions and appears related to variation in resistance to fusiform rust.

Additional keywords: Linkage disequilibrium, fusiform rust.

Previous work has shown that contents of four of the major monoterpenes in cortical oleoresin of loblolly pine (Pinus taeda L.) are largely controlled by single genes, with high content being dominant over low in all cases (Squillace et al. 1980). The four loci involved have also been shown to be rather closely linked (Squillace and Swindel, in press). The objectives of the present study are to: (1) examine deviations between observed proportions of phenotypes involving two or more monoterpene loci and those expected under random association between alleles at different loci (linkage disequilibrium) in loblolly pine populations, (2) interpret the results from the standpoint of natural selection, and (3) seek relationships with resistance to fusiform rust (Cronartium quercuum f. sp. fusiforme).

MATERIALS AND METHODS

In this study we utilized data previously reported by Squillace and Wells (1981) and McRae and Thor (1982). In those reports, cortical monoterpenes were sampled in trees from a total of 111 loblolly pine populations scattered throughout the species range (Table 1). Most of the trees were in seed source study plantations containing trees of known geographic origin. Details on sampling and analytical techniques are available in the two publications cited. In some of the plantations sampled by Squillace and Wells (1981), data on occurrence of fusiform rust infection were also available and were utilized in interpreting results of the disequilibrium analyses.

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[1] Squillace is Adjunct Professor, University of Florida, and Volunteer, USDA Forest Service, Gainesville, FL. Powers and Kossuth are Project Leaders, Southeastern Forest Experiment Station, USDA Forest Service, Athens, GA and Gainesville, FL, respectively. The study was also sponsored by the Georgia Forestry Commission. Journal Series Paper 6469 of the Florida Agricultural Experiment Station.

Table 1.--Numbers of loblolly pine populations and trees sampled for cortical monoterpene composition by regions of the southern U.S.

Region[a/]	Populations	Trees
Western	21	565
Central	85	2141
Northeastern	5	143
Totals	111	2849

[a/] Western = populations west of the Mississippi River.
Central = populations between western and northeastern regions.
Northeastern = populations in Virginia, Maryland, and Delaware.

In order to study linkage disequilibrium between pairs of loci, we first determined the frequencies of trees in each phenotypic class. Since dominance occurs at each locus, there are four possible phenotypes for each pair of monoterpenes. For example, designating two monoterpenes as A and B, with capital letters representing high amounts and lower case letters as low amounts, the four possible phenotypes are:

1. AB, which includes genotypes AABB, AABb, AaBB and AaBb
2. Ab, which includes genotypes AAbb and Aabb
3. aB, which includes genotypes aaBB and aaBb
4. ab, which includes only aabb genotypes

An estimate of linkage disequilibrium, $\hat{D}$, is given by the following (Cavalli-Sforza and Bodmer 1971, Hill 1974)

$$\hat{D} = \sqrt{\frac{x_4}{n}} - \frac{\sqrt{(x_2 + x_4)(x_3 + x_4)}}{n}$$

in which x_2, x_3, and x_4 are numbers of phenotypes Ab, aB, and ab in the population, respectively, and n = total number.

If the product of the AB and ab phenotypes (coupling types) exceeds the product of Ab and aB phenotypes (repulsion types), D will be positive. If the reverse is true, D will be negative. A positive D indicates that the proportion of coupling types observed is greater than that expected from random association. It can mean, for example, that natural selection is favoring coupling over repulsion types. A negative D would suggest the reverse.

To test the significance of $\hat{D}$, we used the likelihood criterion (K) given in Hill (1974):

$$K = \frac{4\, n\, \hat{D}^2}{\hat{p}\,(2-\hat{p})\,\hat{q}\,(2-\hat{q})}$$, which is a χ^2 distribution, with 1 d.f.,

where $\hat{p}$ = estimated frequency of the A allele = $1-\sqrt{\frac{x_3 + x_4}{n}}$,

and $\hat{q}$ = estimated frequency of the B allele = $1-\sqrt{\frac{x_2 + x_4}{n}}$.

As an example, $\hat{D}$ and K will be computed for β-pinene vs. myrcene in population #2 (Marion Co., FL). The numbers of phenotypes BM, Bm, bM, and bm were 14, 2, 6, and 1, respectively (summed from the second column of table 2). Substituting these values into the above equations, we obtain: $\hat{D}$ = .009, $\hat{p}$ = .448; $\hat{q}$ = .639, and k = .01.

Since our data involved four loci, we were also interested in determining evidence of disequilibrium that may occur among four-locus phenotypes. With four loci showing dominance, there are 16 possible phenotypes as shown in Table 2. We could find no procedure in the literature for estimating and testing linkage disequilibrium in such cases and hence used the following procedure to get indications of natural selection favoring or disfavoring each phenotype. Expected frequencies (proportions) of each phenotype were computed on the basis of frequencies of single-locus phenotypes and these were subtracted from observed phenotypic frequencies. We shall designate the differences by D'.

Table 2.--Computation of observed-expected proportions of four-gene phenotypes (D'), for Population #2, Marion Co., FL

Phenotype [a]	Observed #	Observed prop.	Expected prop. [b]	D'
BMLP	2	.087	.101	-.014
BMLp	0	.000	.005	-.005
BMℓP	11	.478	.478	.000
BMℓp	1	.043	.022	.022
BmLP	1	.043	.015	.028
BmLp	0	.000	.001	-.001
Bmℓ P	1	.043	.072	-.028
Bmℓp	0	.000	.003	-.003
bMLP	0	.000	.044	-.044
bMLp	0	.000	.002	-.002
bMℓP	6	.261	.209	.052
bMℓp	0	.000	.010	-.010
bmLP	1	.043	.007	.037
bmLp	0	.000	.000	.000
bmℓP	0	.000	.031	-.031
bmℓp	0	.000	.001	-.001
Totals	23	.998	1.001	.000

[a] B, M, L, and P represent high amounts of β-pinene, myrcene, limonene, and β-phellandrene, respectively, while lower case letters represent low amounts.

[b] Computed from observed proportions of one-gene phenotypes, summed from column three, above: B = .696, b = .304, M = .870, m = .130, L = .174, ℓ = .826, P = .956, and p = .044. Thus, for example, the expected proportion of BMLP trees is (.696)(.870)(.174)(.956) = .101. See text.

Thus, an estimate of D' for phenotype BMLP was computed as:

$$D'_{BMLP} = f_{BMLP} - f_B f_M f_L f_P,$$

in which f_{BMLP} is the observed frequency of the BMLP phenotype in the population, and f_B, f_M, f_L and f_P are the observed frequencies of B, M, L, and P phenotypes in the same population, respectively. D' values were likewise obtained for the other 15 phenotypes:

$$D'_{BMLp} = f_{BMLp} - f_B f_M f_L f_p,$$
$$D'_{BM\ell P} = f_{BM\ell P} - f_B f_M f_\ell f_P,$$
$$\vdots$$
$$D'_{bm\ell p} = f_{bmlp} - f_b f_m f_\ell f_p.$$

An example is given in Table 2. The above procedure was also used for getting estimates of disequilibrium for groups of populations (regions). It should be noted that expected four-locus phenotypic frequencies can also be computed from observed frequencies of individual alleles--rather than using observed frequencies of individual phenotypes--with the same result, but the latter procedure is simpler.

The numbers of trees in phenotypes within individual populations were too few to make reliable tests of significance because in most populations many phenotypes were represented by four or less trees, preventing reliable use of chi-square tests. However, we tested significance of average differences for each phenotype across all populations, using a t-test with the null hypothesis that the observed-expected values = zero. When pooling populations within regions, we tested for significance by computing

$$\frac{(\text{observed number} - \text{expected number})^2}{\text{expected number}}$$

for each phenotypic class in which expected numbers were five or more and summing these to obtain χ^2. Degrees of freedom here were presumed to be total number of four-locus phenotypes minus number of single-locus phenotypes used in computing expected values.

RESULTS

Two-locus Phenotypes

Significant pair-wise linkage disequilibrium was found rather frequently (Table 3). Myrcene vs. limonene was especially notable--84 of the 98

populations permitting this test showed negative $\hat{D}$ values, with 31 of them being significant. None of the positive values were significant. Curiously, the populations showing positive values were clustered in three areas:

Table 3.--Results of linkage disequilibrium ($\hat{D}$) analyses in 111 loblolly pine populations

Loci compared	Range of $\hat{D}$ values	Positive $\hat{D}$ values		Negative $\hat{D}$ values	
		Total no. [a]	No. significant [b]	Total no. [a]	No.significant [b]
β-pinene & myrcene (B,M)	0.08 to -0.27	17	0	50	16
β-pinene & limonene (B,L)	.13 to - .27	43	0	17	8
β-pinene & β-phellandrene (B,P)	.22 to - .23	8	1	43	2
Myrcene & limonene (M,L)	.07 to - .37	14	0	84	31
Myrcene & β-phellandrene (M,P)	.15 to - .27	31	1	46	8
Limonene & β-phellandrene (L,P)	.17 to - .27	48	1	22	8

[a] The total number of positive and negative values in each comparison are less than 111 because in many populations both the observed and expected proportions of phenotypes were zero, negating a test.

[b] Significant at 0.05.

southwestern Alabama, southeast Georgia-northeast Florida, and the Carolinas. The results suggest that Mℓ and mL phenotypes are being favored by natural selection, while ML and mℓ types are being disfavored in most portions of the species range. Note also that $\hat{D}$ values forβ -pinene vs. myrcene and for myrcene vs. β-phellandrene also tended to be negative, judging from both numbers of negative vs. positive values and significance (Table 3). These findings suggest that BmLP trees are being favored by natural selection, which will be examined further.

Four-locus Phenotypes

Analyses of four-locus phenotypes in individual populations showed that observed frequencies of some phenotypes exceeded expected values while for others the reverse was true (Table 4). Phenotype BmLP had both the greatest positive average deviation and the greatest proportion of populations showing positive deviations. Values obtained for this phenotype are plotted in Figure 1. Note that positive values prevail in all areas except southwest Alabama, southeast Georgia-northeast Florida, and the Carolinas. Thus, as suggested earlier, BmLP phenotypes seem to be favored by natural selection over most portions of the species range. Phenotype BMℓP also showed a significant trend toward positive deviations. Finally, the relatively rare phenotype bMℓP also showed a significant but small positive average deviation. These results may be partly a reflection of the very strong negative disequilibrium between myrcene and limonene (Mℓ and mL types being favored), noted earlier.

Table 4.--Results of analyses of observed vs. expected proportions of each monoterpene phenotype, within populations

Phenotype [a/]	No. of populations in test [b/]	Percent of populations showing + deviations	Average deviation over all populations	t value [c/]
BMLP	102	18	-.039	9.38**
BMLp	71	19	-.005	2.44*
BMℓP	109	68	.030	5.73**
BMℓp	79	55	.004	1.12
BmLP	100	82	.043	9.79**
BmLp	70	22	.002	.88
BmℓP	107	25	-.034	7.33**
Bmℓp	78	31	-.001	.24
bMLP	61	21	-.001	.52
bMLp	45	6	.001	.72
bMℓP	67	66	.012	3.64**
bMℓp	52	21	-.001	.67
bmLP	60	14	-.001	.57
bmLp	45	4	.001	.56
bmℓP	66	12	-.010	4.92**
bmℓp	52	2	-.001	1.68

[a/] B, M, L, and P represent high amounts of β-pinene, myrcene, limonene, and β - phellandrene, respectively, while lower case letters represent low amounts.

[b/] These values are less than the total (111 populations) because in many cases both the observed and expected proportions were zero, in which case no test was possible.

[c/] Test of the hypothesis that the population average is zero, with N-1 d.f. An * = significant at the 0.05 level; ** = significant at the 0.01 level.

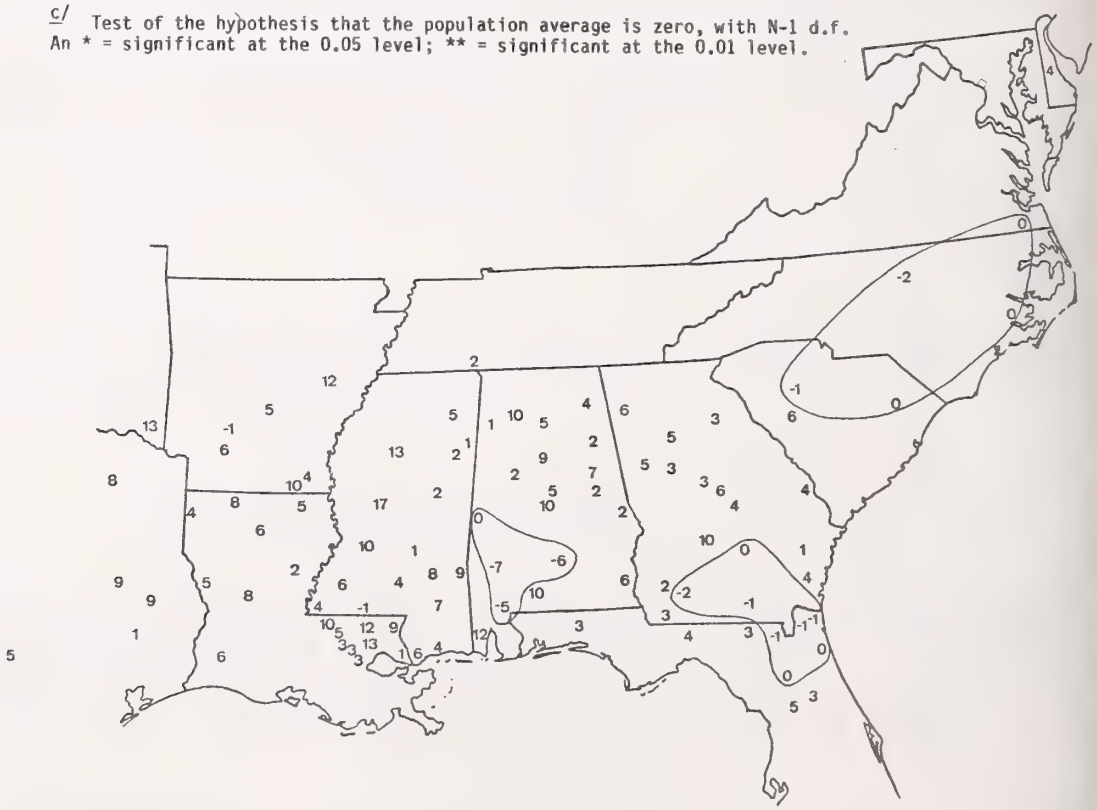

Figure 1.--Observed-expected percent of BmLP phenotypes. Clusters of zero and negative values are outlined.

Analyses in which populations were pooled by regions gave similar results (Table 5). However, the deviations of observed vs. expected values were generally largest in the central region, smaller in the West, and very small in the northeast.

Table 5.-- Observed (O) and expected (E) numbers of trees in each of 16 monoterpene phenotypes within regions[a/] and tests of significance

Phenotype [b/]	Western populations			Central populations			Northeastern populations		
	O	E	$\frac{(O-E)^2}{E}$	O	E	$\frac{(O-E)^2}{E}$	O	E	$\frac{(O-E)^2}{E}$
BMLP	140	177	7.7	249	348	28.2	3	3	--
BMLp	0	1	--	21	35	5.6	0	0	--
BMℓP	78	44	26.3	1039	953	7.8	89	91	.0
BMℓp	0	0	--	107	95	1.5	8	6	.7
BmLP	307	268	5.7	260	143	95.7	2	1	--
BmLp	1	1	--	20	14	2.6	0	0	--
BmℓP	30	66	19.6	290	392	26.5	21	21	.0
Bmℓp	1	0	--	34	39	.6	1	2	--
bMLP	4	2	--	11	21	4.8	0	1	--
bMLp	0	0	--	2	2	--	0	0	--
bMℓP	3	1	--	80	57	9.3	16	14	.3
bMℓp	0	0	--	8	6	.7	0	1	--
bmLP	1	4	--	9	9	.0	0	0	--
bmLp	0	0	--	1	1	--	0	0	--
bmℓP	0	1	--	9	24	9.4	3	3	--
bmℓp	0	0	--	1	2	--	0	0	--
Totals [c/]	365	565	59.3**	2141	2141	192.7**	143	143	1.0

[a/] Western = populations west of the Mississippi River.
Central = populations between western and northeastern regions.
Northeastern = populations in Virginia, Maryland, and Delaware.

[b/] B, M, L, and P represent high amounts of β-pinene, myrcene, limonene, and β-phellandrene, respectively, while lowercase letters represent low amounts.

[c/] The totals of $(O-E)^2/E$ are chi-squares, with 12 d.f. Classes having fewer than five expected values were omitted in computing χ^2.

** = significant at the 0.01 level.

IMPLICATIONS

The results strongly suggest that natural selection in most portions of the range of loblolly pine is favoring certain monoterpene phenotypes and disfavoring others. Here we examine possible reasons.

Note first that the phenotype which seems to be most strongly favored by selection (BmLP) is very prevalent in the western region, $^{307}/_{565} = 54$ percent being of this type (Table 5). It is much less prevalent in the central region (12 percent) and almost absent in the northeast. The geographic pattern is shown more clearly in Figure 2. Note further that in many respects it conforms to regional patterns of resistance to fusiform rust (Grigsby 1973, Squillace and Wells 1981). Western trees have large

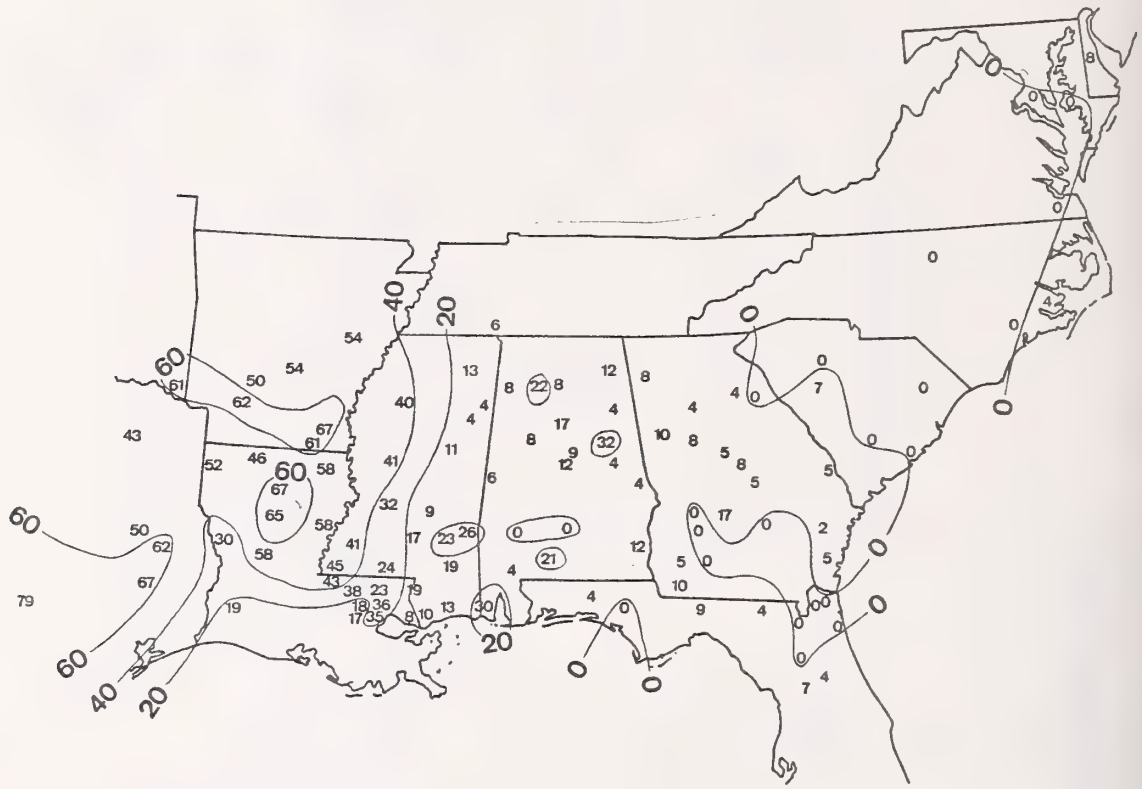

Figure 2.--Percent of BmLP phenotypes.

proportions of BmLP trees and are relatively resistant, and this relationship tends to extend into western Mississippi, although to a lesser extent. Populations in the panhandle of Florida and in southeast Georgia-northeast Florida have few BmLP trees and are very susceptible to rust. The pattern fails in the northeast where BmLP trees are scarce and resistance is high. We shall return to this point later.

With these observations in mind, we hypothesize that natural selection is favoring BmLP trees because they tend to be more resistant to fusiform rust than other types. Data from progeny tests reported by Squillace et al. (1984) also suggest that BmLP trees tend to be more resistant than other types. Perhaps natural selection has been favoring such trees in the West over a longer period than in the central region, explaining its greater prevalence in the West.

A somewhat similar situation seems to exist for phenotype bM&P, which also tended to be favored by selection. The proportion of such trees was 11.2 percent in the northeast, 3.7 percent in the central region, and 0.5 percent in the West (Table 5). As is well known, northeastern populations tend to be relatively resistant [see, for example Grigsby (1973)]. Thus, natural selection for this phenotype may also be a reflection of resistance to rust.

CONCLUSIONS

Natural selection is definitely favoring BmLP trees in both the west and central regions of loblolly pine. This phenotype presently comprises a large proportion of Western populations, which are relatively resistant to fusiform rust. Although it is now rather infrequent in central populations, it is presumably increasing with each generation. We hypothesize that BmLP trees tend to be more resistant to fusiform rust and that this is why they are favored by natural selection. A similar situation seems to occur for bM&P trees, although this is less certain. The latter phenotype is relatively most prevalent in the northeast, where resistance to rust also occurs and it is presumably being favored by natural selection, especially in central populations.

The nature and degree of the relationship between monoterpenes and fusiform rust resistance is still unclear. Although significant relationships were found on a regional basis (Squillace and Wells 1981), we have not yet completed studies comparing trees of different phenotypes within families and populations. We do not believe monoterpene composition actually affects resistance--more likely it may be an indicator of the presence of some types of resistance. It is possible, for example, that the monoterpene phenotypes being favored by natural selection are results of hybridization or introgression with other species, such as shortleaf pine (P. echinata Mill.)(Hare and Switzer 1969) and pond pine (P. serotina Michx.)(Saylor and Kang 1973). Studies of cortical monoterpenes in these species would be desirable. Also, it would be desirable to artificially inoculate trees having different monoterpene phenotypes and observe their reactions to the rust.

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ONE-QUARTER CENTURY OF TREE IMPROVEMENT ON NATIONAL FORESTS IN THE SOUTHERN REGION

Robert N. Kitchens 1/

Abstract.--In 1983, the Southern Region of USDA Forest Service Harvested over 15 tons of first generation orchard seed from six seed orchards. This represented a milestone for a tree improvement program that began in 1959. That was the year researchers and foresters made plans for breeding improved trees for restocking National Forest lands. The program is large and complex, encompassing some 13 species and 52 geographic sources, and serves a land base of about 10 million acres. Some tough seed orchard management problems were solved along the way - examples, Net Retrieval system for seed harvest and aerial application of pesticides. Payoffs are impressive by any measure. Early progeny test results indicate that large gains in volume and other traits can be expected through genetic tree improvement. Over 400 acres of progeny tests have been planted mainly to provide a source of selections for another generation of breeding. The Second Generation Plan has been developed and is being implemented.

Additional keywords: Seed production, progeny testing, hardwood tree improvement, net retrieval system.

The Southern Region's Tree Improvement Program is one of the really great success stories of the USDA Forest Service. In the last 2 years, 47,000 pounds of first generation orchard seeds were harvested. Through the 1985 planting season, approximately 325,000 acres of improved trees have been planted on National Forests in the South. This acreage is increasing at the rate of about 50,000 acres per year.

Payoffs are great by any measure. These trees will produce 10 to 20 percent more volume than average wild trees. They are also bred to be straighter, more disease-resistant, and to have better wood qualities than their wild cousins.

It took 25 years of hard work by many people to get to this point, but the most important phase lies just ahead--that of second generation superior trees. These trees are expected to grow a whopping 35 to 45 percent faster than the wild population!

First Generation Program

In 1959, under the guidance of Thomas F. Swofford, the first Regional Geneticist, planning for the program was started. He consulted many people and organizations in developing the program. The following is a list of some who contributed and deserve recognition: John Kraus, Bruce Zobel, Hans Van

1/Regional Silviculturist, Timber Staff Unit, Southern Region, Atlanta, Georgia

Buijtenen and Ray Goddard, Southern, Southeastern and Southwestern Forest Experiment Stations, and Tree Improvement Cooperatives at North Carolina State University, University of Florida, and Texas A & M University. Programs already underway in Portugal, Sweden and England were also examined. Swofford retired in 1975 and was replaced by Jim McConnell, the present Regional Geneticist.

The pine program was started first. Actual selections begun in 1961 and were essentially completed by 1967. Thirty-eight species-geographic source combinations were recognized in the original selection program; consisting of shortleaf (Pinus echinata Mill.), loblolly (P. taeda L.), longleaf (P. palustris Mill.), slash (P. elliottii Englem.), eastern white (P. strobus L.), Virginia (P. virginiana Mill.), and Ocala sand pines (P. clausa var. clausa D.B. Ward) [McConnell 1978]. All original orchard sites and species were successful except for sand pine. It was first included as part of the Erambert Seed Orchard in south Mississippi. Survival and growth was poor so the sand pine orchard was moved to central Florida, which is in the native range of the species.

Approximately 6 million acres of National Forest pinelands were intensively searched for the very best trees as candidate parent trees for first generation orchards. After a candidate was found, it had to pass several screens before it was finally accepted. Faster growth, pruning ability, straightness, disease resistance, and specific gravity were the traits sought after in the superior tree selections. About 50 selections for each species-geographic source were approved. Then the selections were grafted into clonal orchards. The Ocala sand pine orchard has both a clonal orchard and a 120-family seedling seed orchard. The Region now has 2,177 pine selections in 1,256 acres of pine seed orchards at 6 orchard sites.

The first collectible crop of seed was harvested in 1970; through 1984, collections have totalled 81,000 pounds. Most pine sources in the program are now producing enough seeds for total planting requirements.

The hardwood program started in 1968. Six species are in the program--black (Quercus velutina Lam.), white (Q. alba L.), northern red (Q. rubra L.), and chestnut oaks (P. prinus L.), cherry (Prunus serotina Ehrh.) and yellow-poplar (Liriodendron tulipifera L.). To-date, 382 selections have been made for clonal orchards and 29 acres of clonal orchards have been established. A 220-family, 16 acre, northern red oak seedling seed orchard was established, which was originally a Tennessee Valley Authority progeny test on National Forest land.

Of the hardwood clonal orchards, only yellow-poplar and black cherry are producing enough seed for operational plantings. Sure-fire techniques for successful oak seeding or planting are still not developed; however, within a few years, crops will be harvested from the orchard and attempts at using them for reforestation will be made.

Managing first generation orchards presented some unique problems that had to be solved. Foremost was how to harvest all the cones or seeds without harming the trees. During regular woods cone collection, trees were usually

cut down and the cones picked; however, since cutting trees was not possible in a seed orchard, various methods were used to place people in the trees, including ladders and bucket trucks. Besides being expensive and slow, these methods were somewhat hazardous.

With the cooperation of the Georgia Forestry Commission, a new system was developed, called the Net Retrieval System. Netting was placed on the ground where the seeds fell, and a combine-type machine was used to roll the net and separate the seeds. The Net Retrieval System is now in operation on all or part of 4 Forest Service orchards (Edwards and McConnell 1983, McConnell and Edwards 1985), and other organizations are considering using this system.

Because a seed orchard has many trees of the same age, it is an attractive home for insects--especially those that like to eat cones and seeds. Safe and effective ways had to be found to control these seed-destroying insects. Entomologists worked closely with orchard managers on pesticide formulation, application, and timing for effective control. With the help of several organizations, technology for the aerial application of insecticide was developed. Now an orchard can be treated in hours instead of weeks that were required for ground application methods. In addition, aerial applications place the insecticide in the top portion of the crown, where the cones are. This means less insecticide is necessary to do an effective job.

Progeny Testing

In 1974, controlled crosses among orchard trees began according to a plan that employed disconnected half-diallels for all species except sand pine. Individual matings were made to match desirable characteristics as indicated by the original scoring sheets, fusiform rust disease resistance screening tests, and progeny performance (McConnell 1983). Over 9,500 individual crosses will have been made when the plan is completed.

Progeny testing was done to; (1) measure gains, (2) test worth of parents, and most importantly, (3) as a source of selections for second generation orchards. A few open-pollinated tests were installed, mainly for demonstration purposes. To date, over 250 tests have been planted representing about 6,500 families. About 20 percent of the tests are 5 years old or older. Early results have been quite surprising. Of course, early results must be used with a great degree of caution. Nevertheless, they indicate that large genetic gains can be made.

A white pine open-pollinated test at the Cradle of Foresty, on the Pisgah National Forest in North Carolina, showed orchard trees to have a 25 percent superiority in diameter growth (dbh) and a 15 percent superiority in height growth over general forest area stock at age 5. The 10-year results for the same test showed an accelerating difference--28 percent in height and 36 percent in diameter. The 10-year mean for orchard stock was 24.75 feet tall and diamater of 4.97 inches; for general forest area stock the respective means were 19.25 feet and 3.65 inches.

Another important result, and one expected by geneticists, was that the range of trait variation for height and diameter was the same for seed orchard material as general forest area stock. Only the mean of the two populations was different. This is evidence that orchard populations will continue to have large amounts of variation for some traits.

One of the largest actual heights and diameters occurred in a loblolly pine test in Southern Mississippi. At age 5, the average of all orchard families was 16.6 feet, the tallest family was 18.2 feet, and the tallest individual tree was 29.1 feet.

Other early results are quite impressive. However, the number of tests analyzed is small relative to the total number planned. During the next few years many more tests will be analyzed so that greater confidence can be placed in the gain percentages.

Plans are to use 8- to 10-year test results to begin making selections for second generation orchards. That time is almost here.

Second Generation Breeding

The second generation plan for pines has been developed. Actually, it goes beyond the second generation because selection of new genetic material to infuse into second and successive generation breeding is also planned.

As stated earlier, since second generation gains are expected to double first generation gains, full speed ahead is in order.

Orchard site selection has already begun. In general, second generation sites will be near first generation sites in order to efficiently utilize present personnel, facilities, and equipment.

The 38 pine geographic source-species combinations used in the first generation were streamlined into 20 breeding populations for the second generation. The breeding populations are based on seed movement and planting zones as defined by research results in most cases, but a few were designed using a combination of intuition and/or administrative necessity (Wells and McConnell 1983). Reducing the breeding population to 20 will increase program efficiency. It also gives a broader genetic base for second generation selections since some first generation populations were combined.

The 20 breeding populations for the second generation have been prioritized based on the species importance in National Forest reforestation and on progress of first generation progeny tests. The highest priorities will be developed first and others will be done as timing and budgets allow. Other flexibilities have also been built into the plan so that developing technology can be incorporated along the way.

Guiding Principles

Several guiding principles have been used which contributed greatly to the success of the program.

Knowledge and experience of a large part of the tree improvement community have been drawn upon in formulating strategic plans and critiquing the program. The Regional Geneticist and a small staff are responsible for strategic planning, but they consult frequently with many others. John Kraus and Ozzie Wells, Southeastern and Southern Forest Experiment Stations respectively, are constant advisors. The interchange of tree improvement information under the umbrella of the Southern Forest Tree Improvement Committee is used. One almost has to be a part of tree improvement in the South to fully realize the spirit and degree of cooperation within this community.

Maximum involvement of Forest and District personnel is fostered. There is no tree improvement organization as such below the Regional level. Forest Supervisors are responsible for the program on their respective forests. Orchard Managers and workers who do actual test plantings and measurements are on the District Ranger's staff. By having this type of involvement, ownership of the program is vested in all levels of the organization and each level takes pride in program accomplishments. Work is accomplished in a timely fashion and the quality of work is high.

The Tree Improvement Program is continually evaluated with respect to current silvicultural practices used on National Forest lands. Greater productivity is the goal of tree improvement. Greater productivity cannot take place without the proper integration of genetics and silviculture. In fact, on the National Forests, tree improvement is viewed as an integral part of silviculture.

Closing

A quarter-century has brought the Southern Region's program a long way, but the challenge to breed another generation of trees to reach higher production goals is great and exciting. The next 25 years will no doubt bring accomplishments unimaginable today.

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THIRD-YEAR COMPARISONS OF LOBLOLLY AND SLASH PINE SEED SOURCES FOR FUSIFORM RUST RESISTANCE AND GROWTH POTENTIAL IN NORTH CENTRAL FLORIDA

John A. Pait, III, Lee Draper, Jr., and Robert A. Schmidt [1/]

ABSTRACT

Four loblolly and three slash pine seed sources were evaluated for rust resistance and growth potential in a provenance test established in 1981 in a high rust incidence area in northcentral FL. The site was moderately well-drained and the soil was a sandy loam overlying clay at 24 inch depth; site index was 75 ft at 25 years. Loblolly pine seed sources were East Texas, Livingston Parish, Marion County, FL and FL seed orchard. Slash pine seed sources were FL and GA seed orchards and a rust-rogued seed production area in North FL. Survival, diameter, height and rust incidence (% trees with one or more galls) were measured after the third growing season. Seed sources within species only were compared statistically. Within loblolly sources, survival, diameter and height were greatest in Marion County and least in East Texas; Livingston Parish and FL seed orchard were intermediate. All loblolly sources were significantly different for rust incidence. East Texas was least rust-infected (9.6%), Livingston Parish was intermediate (21.0%), Marion County was highly infected (50.6%), and the Florida seed orchard source was the most infected (68.7%). Within slash pine sources survival, diameter and height were greater in the seed orchard sources than in the rust-rogued seed production area, but the latter had significantly less rust (27.4%) compared with 59.0% and 61.7% for the FL and GA orchards, respectively. For loblolly sites in this area recommendations are to plant East Texas sources on the highest rust incidence sites, Livingston Parish sources on the intermediate rust incidence sites and Marion County sources in eastern area where rust incidence is low. For slash pine sites where rust incidence is high, seed from the rust-rogued seed production area is recommended.

Additional Keywords: *Pinus elliottii* var *elliottii*, *P. taeda*, *Cronartium quercuum* f. sp. *fusiforme*, disease resistance, provenance tests.

[1/] Research Foresters, Container Corporation of America, Timber Research and Development, Callahan, Florida, 32011, and Professor of Forest Pathology, Department of Forestry, University of Florida, Gainesville, 32611, respectively.

INTRODUCTION

In areas where the incidence of fusiform rust, caused by *Cronartium quercuum* f. sp. *fusiforme*, is great forest managers must consider alternatives to reduce the impact of this disease (Schmidt et al. 1977,

Schmidt and Klapproth 1982, Anderson et al. 1984). Currently, the primary means of mitigating the epidemic in high rust incidence areas is planting rust-resistant seed sources (Schmidt et al. 1985). Several sources of resistance are available. These include Livingston Parish and East Texas provenances of loblolly pine and rust-rogued seed production areas for slash pine (Goddard and Wells 1977, Schmidt et al. 1981). Recommendations for a specific area must consider both growth and rust response. Decisions are best made with data obtained from tests established in the immediate area, since both growth and disease resistance (perhaps pathogenic variability) vary geographically (Draper 1975, Powers and Matthews 1980, Pait and Draper 1983). The objectives of this study were to compare the growth and rust resistance of several loblolly and slash pine seed sources in a high rust incidence area in North Central FL for the purpose of providing management recommendations.

METHODS AND MATERIALS

Location and Site Characterization. The seed source test is located in North Central FL in Marion County, approximately ten miles northwest of Ocala. The soil is a moderately well-drained, fine sandy loam overlying clay at a 24 inch depth. Site index is 75 ft at 25 years for loblolly and slash. Fusiform rust is considered a serious problem in this location, e.g., rust incidence on a seven-year-old loblolly plantation in this area exceeded 75% of the trees infected.

Seed Sources. Four sources of loblolly and three sources of slash pine seed were planted. These sources represented the best regeneration alternatives at the time and all were bulk seed collections. The loblolly pine sources were from 1) East Texas 2) Livingston Parish, and 3) Marion County, FL provenances, and 4) Container Corporation of America's (CCA) loblolly seed orchard selections. The slash pine sources came from CCA's 1) GA slash pine seed orchard, 2) FL slash pine orchard, and 3) a rust-rogued seed production area in Madison County, FL. Seed orchard sources of both species were generally unimproved for rust resistance. The East Texas and Livingston Parish provenances were known to contain appreciable rust resistance (Wells and Wakely 1966, Wells and Switzer 1975) and the Marion County provenance has shown good growth and, on occasion, some rust resistance (Draper, 1975). The rust-rogued slash pine seed production area was located in a high rust incidence area in Madison County, FL and was expected to have considerable rust resistance (Goddard et al. 1975), but was not previously tested in an appropriate trial.

Site Preparation and Planting: Sites were prepared by pushing debris from the area of the plots, followed by single drum chopping and burning. In January 1981 seedlings were hand-lifted from the nursery at Archer, FL and dibble-planted at a spacing of 5.5' x 12' (660 trees/acre).

Study Design. The study design was a randomized complete block. Within species each seed source was randomly planted in each of nine replications (plots). Each plot consisted of 130 seedlings (10 rows of 13 seedlings each.) Species were analyzed separately since they were not mixed, and were separated by a fire break.

Maintenance and Data Collection. In May of 1982 hardwood sprouts, which threatened survival and growth, were killed with cut-surface application of Banvel CST. The strips between the rows were mowed in the summer of 1982 and 83.

After the third growing season (October 1983) survival, DBH, total tree height and rust incidence were measured. Rust incidence was recorded on the total number of living trees and included trees with 1) stem galls only, 2) branch galls only, and 3) both branch and stem galls. Rust associated mortality averaged < 1% and was excluded from the analyses. Within species data were analyzed with the general linear models procedure for analyses of variance (Statistical Analysis System) and seed source means were compared with Duncan's Multiple Range test ($p \leq 0.05$).

RESULTS

Survival, Height and DBH (Table 1).

Loblolly. The four loblolly pine seed sources averaged 77.3% survival, 6.6 ft in height and 0.78 inches DBH. There were significant differences among seed sources for each of these parameters. The Marion County source performed best and the East Texas source performed the poorest; the Livingston Parish and FL seed orchard sources were intermediate.

Slash. The three slash pine seed sources averaged 77.6% survival, 5.9 ft in height and 0.92 inches DBH. There were significant differences among seed sources for each of these traits. Generally, the FL and GA seed orchards sources performed the best and the seed production area source performed the poorest for these growth traits.

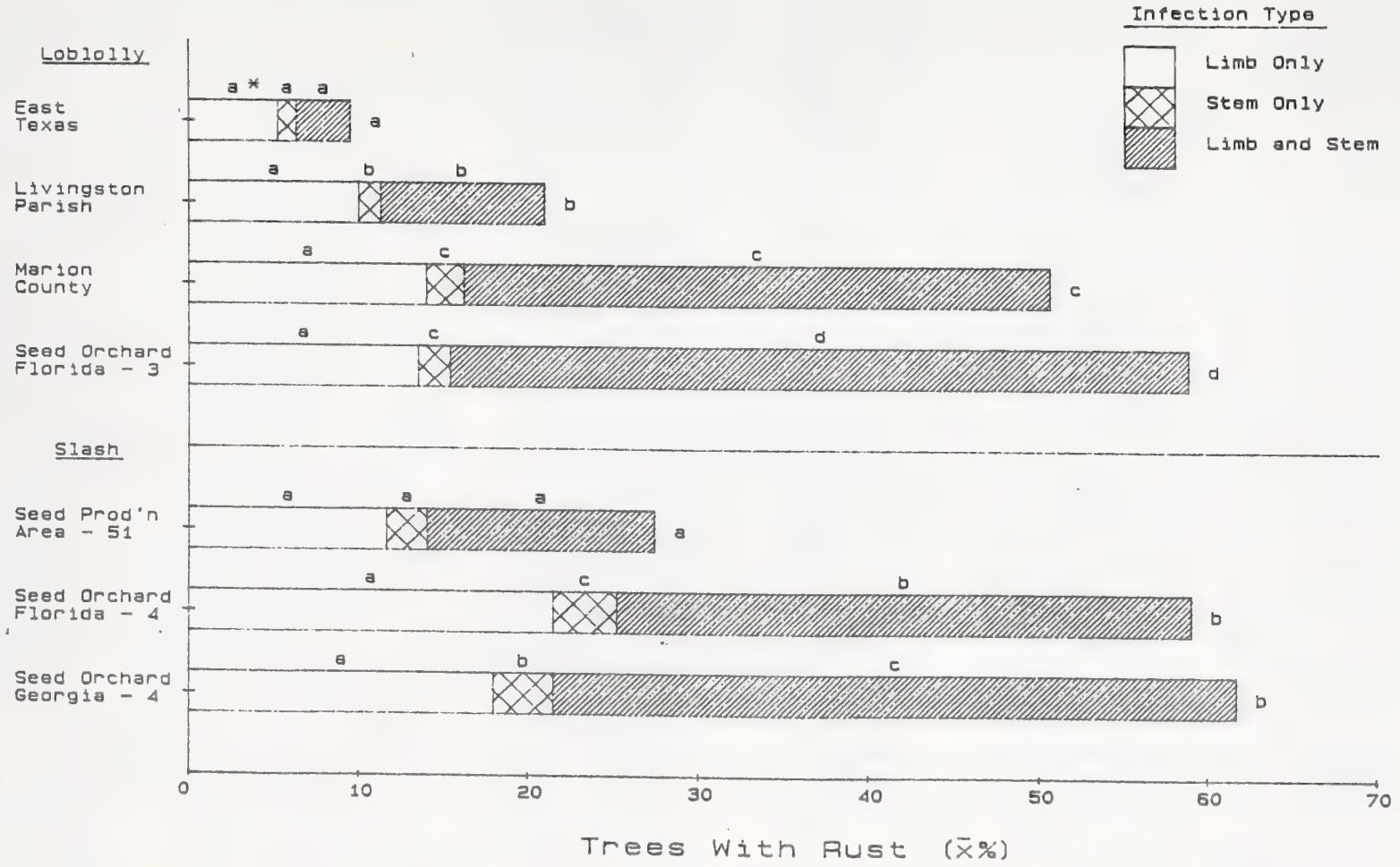
Table 1. Comparison of mean survival, DBH and height at age 3 years for loblolly and slash pine seed sources planted in Marion Co., Florida.

Species	Source	Survival (%)	DBH (inch)	Height (ft)
Loblolly	Marion County, FL	83.4 a2/	0.87 a	7.2 a
	FL Seed orchard	73.7 c	0.78 b	6.8 b
	Livingston Parish	79.2 b	0.78 b	6.5 c
	East Texas	72.9 c	0.69 c	5.8 d
Slash	FL Seed orchard	74.6 b	0.97 a	6.2 a
	GA Seed orchard	80.9 a	0.93 a	6.1 a
	Seed production area 1/	74.6 b	0.85 b	5.5 b

1/ Rust-rogued and located in a high rust incidence area in Madison County, FL

2/ Statistical comparisons are within a parameter (column) among seed sources within species; means followed by different letters are significantly different (Duncan's Multiple Range Test, ($p \leq 0.05$)

Figure 1: Mean percentage of live trees with rust galls at age three for pine seed sources planted in Marion County Florida



*Total rust infection (bar lengths) and infection types among seed sources and within species are not significantly different if accompanied by similar letters according to Duncan's Multiple Range Test ($p \leq .05$).

Fusiform Rust (Figure 1).

Loblolly. The four loblolly pine seed sources averaged 1.6% stem galls only, 10.8% limb galls only, 25.1% both limb and stem galls, and 37.5% total rust infected live trees. Average rust associated mortality among these sources was ≦ 1%. The mean total rust incidence on live trees was 9.6, 21.0, 50.6 and 68.7% in the East Texas, Livingston Parish, Marion County and FL seed orchard sources, respectively. Rust incidence was significantly different for each loblolly source.

Slash. The three slash pine seed sources averaged 3.2% stem galls only, 17.1% limb gall only, 29.1% both limb and stem galls and 49.4% total live trees with rust. The mean total rust incidence on living trees was 27.4, 59.0 and 61.7% in the seed production area and FL and GA seed orchards sources, respectively. The seed production area source exhibited significantly less rust than the seed orchard sources.

DISCUSSION

Average survival (loblolly, 72.4-83.4%; slash, 74.6-80.4%) and average rust incidence (loblolly, 9.5-68.7%; slash 27.4-61.7%) were sufficient for a reliable test of these seed sources. Growth rankings among sources may change with time and these data must be considered preliminary. Rust incidence will increase with time, but it is unlikely that relative rankings among seed sources will change.

As suggested in earlier tests (Draper 1975, Pait and Draper 1983) the Marion County source has superior growth in this area, as well as in some northern areas. The East Texas and Livingston Parish sources grew more slowly corroborating age five results published previously (Pait and Draper 1983). The slash pine seed orchard sources grew significantly better than the seed production area source. Among the seed sources, survival, height and DBH variation was greater in loblolly than in slash pine.

Rust incidence on the East Texas source was significantly less than all other loblolly sources. Similar results were reported by Pait and Draper (1983) for this and other areas in FL and GA. Although a statistical comparison was not appropriate - due to the experimental design - the East Texas source was less infected than all slash pine sources. Livingston Parish exhibited good rust resistance in this area, but other data (Pait and Draper 1983) suggests this source is very susceptible when planted in Madison County, FL, 100 miles northwest of Marion County, FL. The reason for the poor performance of Livingston Parish in the earlier study is not known.

As was reported by Goddard et al. (1975) the seed from heavily infected rust-rogued stands possess substantial rust resistance. This was substantiated here as the seed production area source was significantly less infected than the other slash pine sources. In fact, the rust-rogued seed production area source performed nearly as well for rust resistance as the Livingston Parish source. In the absence of rust improved orchard seed and resistant provenance sources, seed from rust-rogued slash pine seed

production areas provides a good alternative for planting in areas of high or intermediate rust incidence.

Despite a report (Schmidt et al. 1985) that rust incidence is higher on loblolly than on slash pine in this geographic area, susceptible loblolly (68.7%) was only slightly more infected than was susceptible slash pine (60.4%).

CONCLUSIONS

Among the loblolly sources East Texas had significantly less fusiform rust, but also had the poorest survival and growth. The Livingston Parish source was intermediate in rust incidence and growth. The Marion County source grew best, but had significantly more rust than either the East Texas or the Livingston Parish source. The seed orchard source had significantly more rust than all other sources and was intermediate in survival and growth.

Among the slash pine sources the rust-rogued seed production area had significantly less rust than the seed orchard sources. In fact the resistance of the rust-rogued seed production area source compared favorably with the Livingston Parish source, although a statistical comparison was not appropriate because of the experimental design.

Indications from these early observations, combined with information from previous tests suggest the following seed source allocation.On loblolly sites East Texas and Livingston Parish sources should be utilized on the high and intermediate rust incidence areas, respectively. The Marion County source should be restricted to the eastern portion of this area where rust incidence has been low. On slash pine sites the seed production area source should be planted in the high and intermediate rust incidence areas and the seed orchard sources elsewhere on the low rust incidence areas only.

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COMPARATIVE PHYSIOLOGY OF LOBLOLLY PINE SEEDLINGS FROM SEVEN GEOGRAPHIC SOURCES AS RELATED TO GROWTH RATE

Bruce C. Bongarten, Robert O. Teskey and Brett A. Boltz[1]

Abstract.--Growth, photosynthesis and water relations characteristics were examined in loblolly pine seedlings from seven diverse geographic sources. At the end of the first year, Florida trees were largest in height and dry weight, while Texas and Arkansas trees were smallest. Seed source size rankings were established by the fifteenth week of growth and were correlated with seed weights and both earliness and completeness of bud-set. They were also correlated with net photosynthesis at each of eleven dates during the growing season. This appears to result primarily from differences in leaf area accretion. When photosynthesis was measured on a unit leaf area basis, differences among the provenances were absent, except late in the year when Florida trees were most active.

Few differences in water relations characteristics were shown among the provenances. No differences in osmotic potential at saturation or turgor loss were detected. The degree of osmotic adjustment appeared to be equal, as well. Continental seedlings (Texas, Arkansas and Georgia Piedmont) exhibited greater stomatal conductance than seedlings from coastal origins when drought stress was never imposed, however, in trees pretreated with drought, no provenance differences were observed.

Differences in first year growth rate appear to be due, in part, to differences in seed weight, leaf production, and late season growth and photosynthesis. The measured water relations traits do not appear to be important although other water relations traits may be.

Additional keywords: Genetic differentiation, provenance testing, photosynthesis, water relations, pressure-volume curves, Pinus taeda.

INTRODUCTION

Thirty years of loblolly pine provenance testing have clearly demonstrated the presence of geographic differentiation for growth rate. Principally, trees from south coastal areas, with mild winters and heavy summer rainfall, are faster growing than trees from interior or north coastal regions

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[1]Assistant Professor, Assistant Professor and Graduate Research Assistant, respectively, School of Forest Resources, University of Georgia, Athens, GA 30602.

(Wells, 1983; Wells and Wakeley, 1966). Differences are sufficiently great that trees from non-local provenances are often planted to improve productivity. This is particularly true in Arkansas where loblolly pines from coastal North Carolina are now widely planted with projected volume increases of 20-30% over the local stock (Lambeth, et al., 1984).

In this work, we examined some of the possible physiological causes for provenance differentiation in growth rate in the seedling phase. The growth superiority of trees from regions with short, mild winters and wet summers suggests that provenance differences may be due to differences in the duration of growth activities, including photosynthesis, and/or differences in water relations traits. We have, therefore, emphasized these in our initial work. The information obtained may be useful in predicting responses of trees to different environments, thus providing a means for assessing the risks incurred upon planting trees of foreign provenance. Additionally, knowledge of the physiological basis for growth rate differences may be used to design inter-provenance breeding plans which maximize growth by combining complementary components. As our understanding of the physiological basis for growth increases, it may also be possible to select more effectively for rotation-age volume in the juvenile stage.

MATERIALS AND METHODS

Seedlots

Seeds were obtained from seven first generation seed orchards representing different portions of the loblolly pine range (Figure 1). The North Carolina, South Carolina, Florida and Louisiana sources are considered "Coastal", while the Georgia, Arkansas/Oklahoma and Texas sources are considered "interior" or "continental". The Georgia Piedmont and Coastal Louisiana seed orchards had been rogued, while the others had not. The Texas seed orchard was composed of ramets from ortets selected in nursery beds for drought resistance by the Texas Forest Service; most were originally from Bastrop and Lee Counties, Texas. Ortets for the other orchards were selected primarily for phenotypic superiority in volume, and crown and form factors.

Growth and Photosynthesis

In May, 1984, seeds from six seedlots (all except Georgia Piedmont) were sown in DEEPOT containers ($646cm^3$) in a greenhouse using a randomized complete block design with four replicates. Each replicate contained one plot of 20 trees per seedlot. Seedlings were kept well-watered and fertilized throughout the study period.

Beginning 12 weeks after sowing, and recurring every 10 to 14 days through mid-December, seedling height, root collar diameter and net photosynthesis were measured on the interior six trees from each plot. The number of seedlings with buds was also recorded on each date. In all, measurements were made on eleven dates. Seed weights, based on 500 seeds, were obtained before sowing.

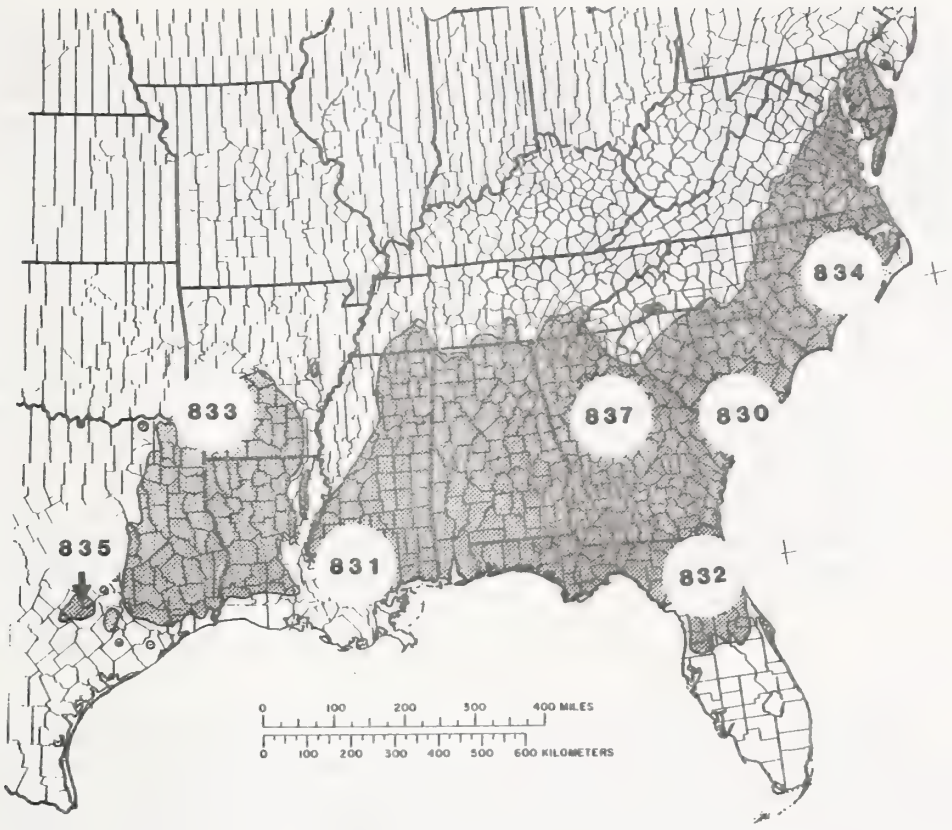

Figure 1.--Locations of the seedlots used in this study. Shaded area represents the natural range of loblolly pine.

Net photosynthetic rates were obtained under steady-state conditions. Trees were placed in a cuvette in which the temperature was maintained at 30°C, relative humidity at 50%, light at 1500 µmol m^{-2} s^{-1} (middle of the cuvette; equivalent to full sun light) and CO_2 concentration at 340 ppm. Carbon dioxide (1%) was pumped continuously from a compressed gas cylinder into the chamber to maintain the CO_2 level at 340 ppm. Steady-state equilibrium was achieved when a constant flow of CO_2 maintained the prescribed cuvette CO_2 concentration. Because the volume of CO_2 pumped into the chamber displaced an equal volume of air having the ambient CO_2 concentration, the rate of CO_2 fixation was calculated as the product of the CO_2 flow rate and the difference in CO_2 concentration between the pumped and displaced gases (Griffiths and Jarvis, 1981). Approximately one hour was required to achieve steady-state equilibrium for each sample of six trees from a seedlot-replicate combination. For each measurement period 24 observations (six seedlots x four replications) were made over two days.

The cuvette, constructed of clear Lexan, measured 47 x 21 x 41 cm in length, width and height, respectively. Only the shoot portion of the tree

was enclosed within the chamber. Light was provided from above by a sodium vapor lamp, and from the sides by two incandescent lamps. Temperature was controlled with two copper, radiator type heat exchange units controlled by Neslab thermostats (Portsmouth, NH). Relative humidity was regulated by a Honeywell dehumidifier control (H46C 1000) which directed chamber air through a desiccator column as required.

Estimates of leaf areas on each of the sample dates were obtained from regressions of height on leaf area developed at the end of the study. In this way photosynthesis per unit area could be estimated without destructive sampling. Such regressions are subject to errors because of leaf and internode growth subsequent to each measurement. However, these errors may be roughly compensating and of minor importance, particularly near the end of the study, when growth was terminating.

Analysis of variance was used to test for seedlot differences on each measurement date. Differences between dates within seedlots were analyzed by paired t-tests.

Water Relations

Water relations measurements were also taken on trees grown in DEEPOTS in a greenhouse. Eight blocks, each containing one plot of 20 trees from each of six seedlots (coastal South Carolina excluded), were used. Trees in four of the blocks were kept near field moisture capacity by watering on alternate days (high moisture regime). Trees in the other four blocks were subjected to recurring drought cycles; rewatering occurred only when flaccid shoot tips were observed in early morning (low moisture regime). Before the first measurements were taken, trees in the low regime had experienced eight drying cycles and were approximately one-third the size of the trees in the high moisture regime.

Stomatal conductances were determined for each seedlot, in both high and low moisture regimes, under three different humidity conditions. Steady-state methodology was employed. A cuvette measuring 15 x 20 x 15 cm was constructed of glass, Lexan and propafilm-c. Individual seedlings were placed in the cuvette, and dry air was introduced, displacing the moist chamber air. When a constant flow of dry air maintained the chamber air at a prescribed relative humidity, steady-state equilibrium was achieved. Transpiration and stomatal conductance were calculated from the water lost in the displaced air.

The prevailing environmental conditions included a temperature of 30°C, and light at 1500 umol m^{-2} s^{-1}. Measurements were made at relative humidities of 77%, 54% and 31%, which corresponded to absolute humidity deficits of 7, 14 and 21 g/cm^3, respectively. For each seedlot seven to nine seedlings were sampled, individually, in each the high and low moisture regimes. Differences between seedlots, moisture regimes and humidity treatments were assessed by analysis of variance.

Pressure-volume curves were also developed to examine differences in turgor loss points, osmotic potentials at saturation, and osmotic adjustment

(Tyree and Hammel, 1972). Seedlings were cut near the root-collar and placed in water in a dark closet overnight to insure full saturation. Xylem water potentials, measured with a Scholander pressure bomb, and relative water contents (the proportion of total shoot water content) were then periodically recorded as the seedlings were allowed to dry. The curves were plotted as the inverse of xylem water potential versus relative water deficit (RWD), the proportion of water lost (Figure 2). Turgor loss points were estimated visually for each seedling. Osmotic potentials at saturation were estimated

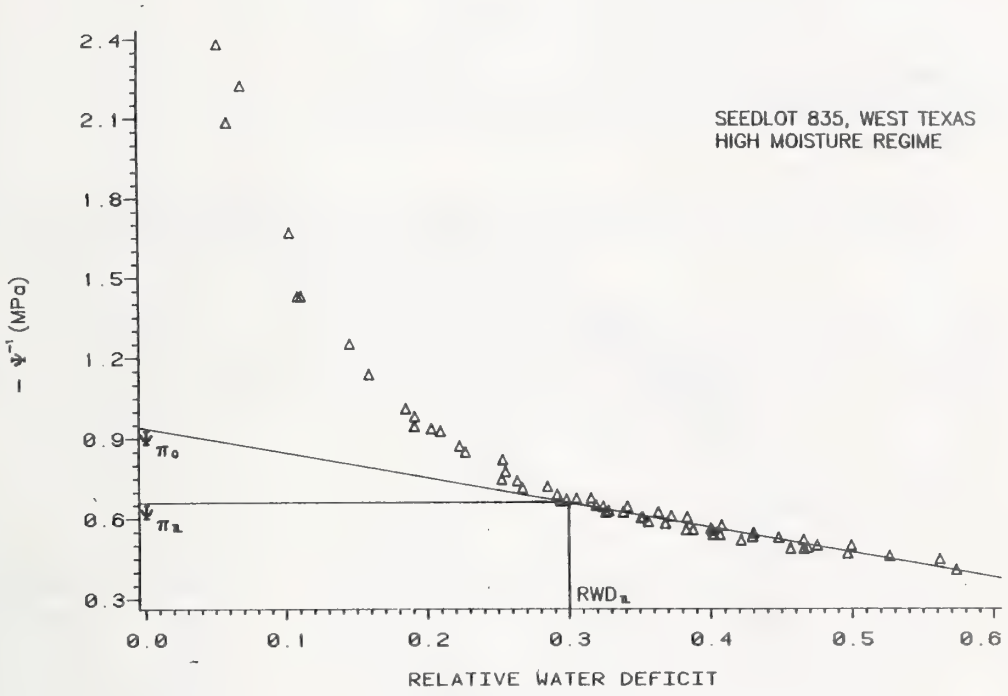

Figure 2.--A sample pressure-volume curve indicating the osmotic potential at saturation (ψ_o), and at turgor loss (ψ_{tl}), and the relative water deficit at turgor loss (RWD_{tl}). This plot was constructed from the combined data of six sampled trees.

for each seedlot-moisture regime combination as the intercepts of linear regression lines based on all points beyond turgor loss (Figure 2). Osmotic adjustment was determined as the difference in osmotic potentials between trees in the high and low moisture regimes. Differences between seedlots were tested with analyses of variance.

RESULTS

Growth

Of the seedlings monitored for photosynthesis, those from each of the Coastal Plain origins grew faster than those from Arkansas or the Lost Pines region of Texas. Seedlings from Florida grew fastest, achieving 50% greater height and dry weight than seedlings from Arkansas, which, on average, were smallest (Figure 3).

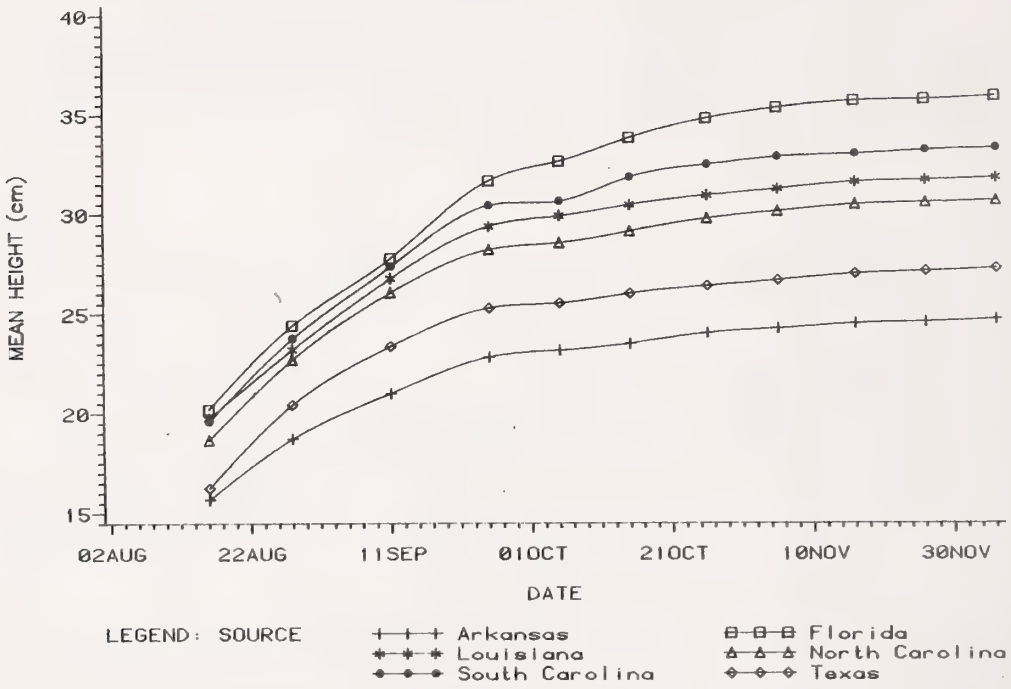

Figure 3.--Height growth in the first year for loblolly pines from six provenances.

Provenance differentiation in height was apparent by the first measurement, 12 weeks from sowing. At that time, seedlings from Texas and Arkansas were already significantly shorter than seedlings from Coastal sources. This early size difference may be related to seed weight as seeds from Texas and Arkansas averaged 2.7 mg, compared to 3.4 mg for seed from Coastal sources. Significant differences in height at 12 weeks were also detected among the

Coastal provenances (Louisiana and Florida seedlings being taller than North Carolina seedlings), but they were not related to seed weight. The early difference between Coastal and Continental seedlings in height growth was accentuated by differences in bud-set later in the study. Compared with Coastal trees, those from Texas and Arkansas began bud-set earlier, and more of them had terminal buds at the end of the study (Table 1). Among the Coastal seedlings, those from Florida showed significantly less bud-set than the others. Overall, provenance differences in bud-set closely parallelled final tree heights and dry weights, although provenance differences in seedling height were readily apparent well before the first seedlings set buds.

At the end of the study, provenance differences in height more or less mirrored differences in root collar diameter, total dry weight and dry weights of leaves, stems and roots (Table 1). By contrast, Florida and Texas seedlings, which represented the extremes in size, had the greatest shoot-root ratios. However, shoot-root ratios vary with seedling size (Ledig and Perry,

Table 1.--Growth measurements at the end of the first growing season for loblolly pines from six provenances.[a]

Source	Height	Dry weight	Shoot-root	Bud-set
	cm	g		% of seedlings
SC	33.3^{ab}	3.7^{ab}	0.52^{a}	45.8
LA	31.8^{b}	3.5^{abc}	0.49^{b}	50.0
FL	35.9^{a}	4.2^{a}	0.64^{a}	12.5
AR	24.7^{d}	2.8^{c}	0.52^{b}	87.5
NC	30.6^{bc}	3.6^{ab}	0.46^{b}	41.7
TX	27.2^{cd}	3.1^{bc}	0.46^{b}	58.3

[a]Means not having a superscript in common differ at the 5% level of significance.

1965) and time of bud-set (Cannell and Willett, 1976). Therefore, the shoot-root ratios calculated here may not be indicative of the real differences in the relative growth of shoots and roots. This essential information may only be obtained by sampling shoot and root weights throughout the study period.

Photosynthesis

The seasonal pattern of seedling net photosynthesis was similar for each of the six examined seedlots. From August 15 photosynthesis rose slowly until October 1, then rapidly to a maximum in late October. Thenceforth, net photosynthesis fell until levelling off in November (Figure 4). The rise in

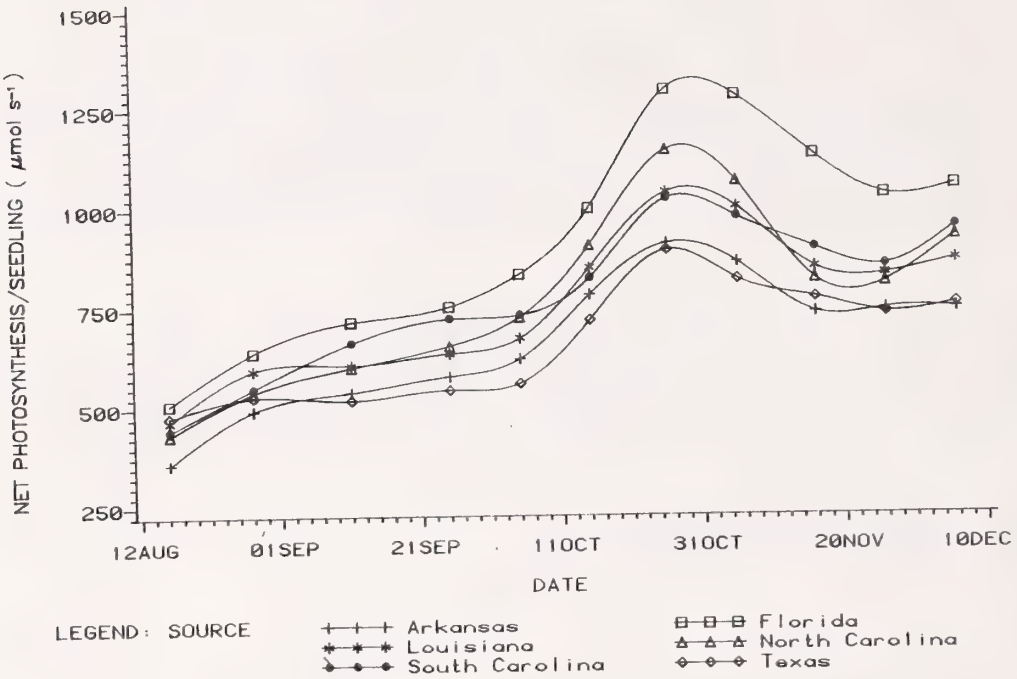

Figure 4.--Seasonal course of net photosynthesis per seedling. Each point represents a mean of 24 trees.

net photosynthesis through October resulted primarily from accretion of leaf area. The increases were modest through early October as respiration from the production of new needles largely offset the increased photosynthetic capacity. However, when the rate of new leaf production declined, net photosynthesis rose dramatically. The decline in net photosynthesis after October was apparently related to internal physiological changes, and is common in temperature zone trees entering the winter season (Ledig, 1976).

When leaf area effects were removed by considering net photosynthesis on a unit leaf area basis, maxima were observed in August and late October, while minima occurred in early October and after mid-November (Figure 5). The decline in net photosynthesis per leaf unit area from August to early October probably resulted from increased respiration due to leaf production. The increase in photosynthesis thereafter until late October reflected a reduction in leaf production. Finally, the decline after late October indicated a transition in physiological activity associated with the onset of winter, as mentioned previously.

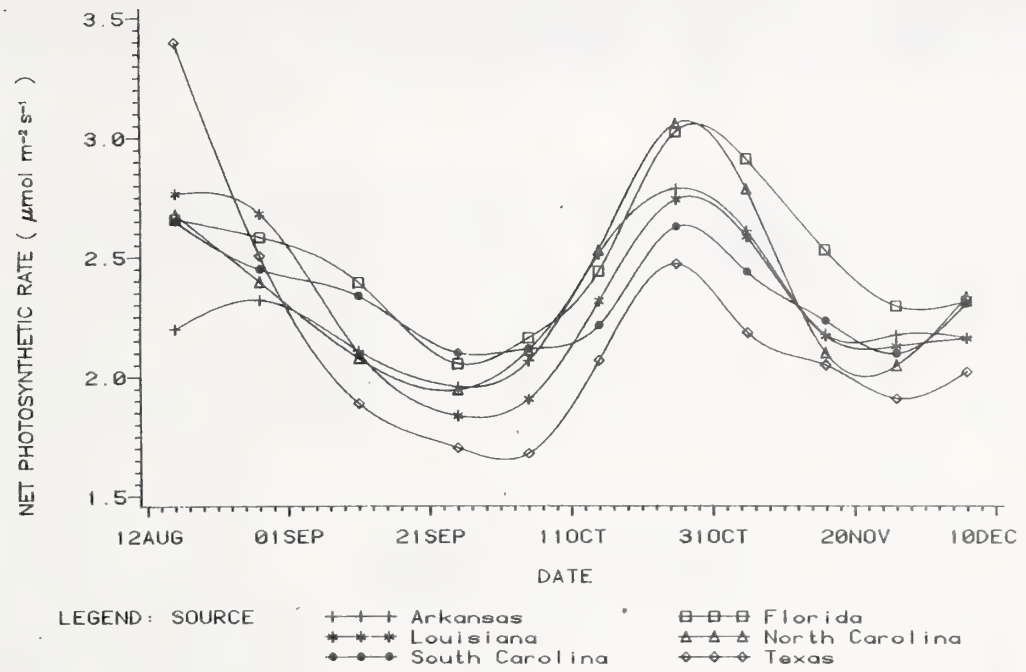

Figure 5.--Seasonal course of net photosynthesis per unit leaf area. Each point represents a mean of 24 seedlings.

Significant provenance differences (0.1 level) in net photosynthesis per seedling were found on only five of the eleven measurement dates. However, provenance rankings were consistent throughout the study leading to large differences in total CO_2 assimilation (the areas under the curves in Figure 4). The rankings of the seedlots in total net assimilation closely corresponded to their rankings in mean seedling size, seedlings from Florida having the largest values and those from Texas and Arkansas the smallest. This correspondence is expected because of the dominating influence of leaf area on total net photosynthesis.

When calculated on a unit leaf area basis, differences among the provenances in net photosynthesis were detected only on one date near the end of the study, and the seedlot rankings, in general, did not correspond well with those for mean seedling size. The late-season net photosynthetic rates were an exception. Florida seedlings, which had the greatest late season growth, also showed a significantly slower decline in net photosynthetic rate. Thus,

it appears that differences in leaf area accretion are primarily responsible for differences in net assimilation and growth among the seedlots. Differences in photosynthetic rate appear to be relatively unimportant except that the longer period of high photosynthetic rate found in Florida seedlings may account for their greater late-season growth.

Stomatal Conductance

Stomatal conductances at three absolute humidity deficits are shown in Figure 6 for droughted and non-droughted seedlings. Trees which had never

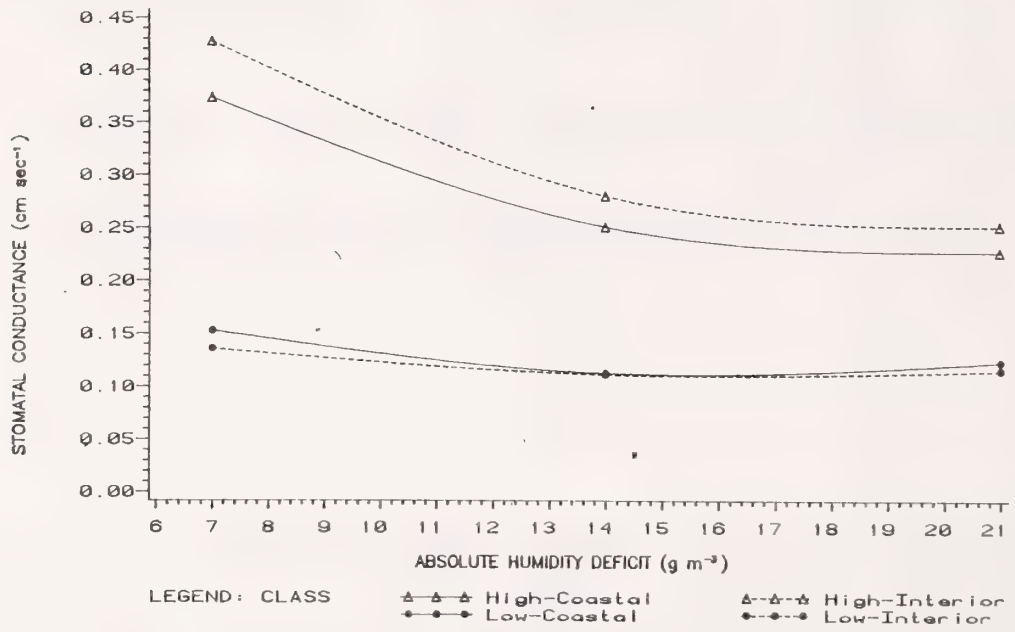

Figure 6.--Stomatal conductances, at three absolute humidity deficits, for Coastal and Interior loblolly pines seedlings grown under moist and droughty regimes.

been subjected to drought had conductances which were more than twice as great as those which had. In the trees which had never been subjected to drought, stomatal conductance declined markedly as the absolute humidity deficit increased from 7 to 14 g/m^3 (equivalent to a change from 77% to 54% relative humidity at 30°C), while the droughted trees showed little stomatal response

to changing absolute humidity deficit. Curiously, among the trees which had never been droughted, those from the three interior origins (Texas, Arkansas, and Georgia) had consistently greater stomatal conductance. After drought pretreatment, though, no differences in stomatal conductance were observed among the seedlots.

Pressure-volume Curves

Four water relations parameters were estimated from pressure-volume curves: osmotic potential at saturation (ψ_0), osmotic potential at turgor loss (ψ_{tl}), relative water deficit at turgor loss (RWD_{tl}), and the change in osmotic potential with declining water content (slope). These parameters are shown in Table 2. No seedlot differences were detected for any of the

Table 2.--Pressure-volume curve parameters for seedlots and moisture regimes.

Water Regime	Source	Water potential (MPa)		RWD_{tl}	Slope
		Saturation	Turgor loss		
HIGH	LA	-1.03	-1.47	0.29	-0.099
	FL	-1.03	-1.41	0.26	-0.102
	AR	-1.02	-1.48	0.29	-0.098
	NC	-1.23	-1.53	0.29	-0.081
	TX	-1.00	-1.42	0.28	-0.107
	GA	-1.01	-1.44	0.28	-0.106
	MEAN	-1.05	-1.46	0.28	-0.099
LOW	LA	-1.00	-1.58	0.34	-0.108
	FL	-0.98	-1.57	0.34	-0.114
	AR	-0.96	-1.53	0.32	-0.123
	NC	-0.96	-1.61	0.35	-0.114
	TX	-0.99	-1.55	0.37	-0.116
	GA	-0.88	-1.59	0.36	-0.142
	MEAN	-0.96	-1.57	0.35	-0.120

parameters. However, trees in the high moisture regime differed significantly from those in the low moisture regime in each of the four parameters. Droughted trees had greater initial osmotic potentials, but lower osmotic potentials at turgor loss than non-droughted trees. Furthermore, the droughted trees reached turgor loss at lower water contents than the non-droughted trees. Such differences between droughted and non-droughted trees are common and are termed "osmotic adjustment." The seedlots could not be shown to differ in their degree of osmotic adjustment, either.

DISCUSSION

When undamaged by winter cold or ice or biotic agents, loblolly pines of Coastal origin grow faster than those of Continental origin over a wide range of sites. Similarly, southern loblolly pines outgrow northern ones (Wells and Wakely, 1966; Wells, 1983). In our work we have sought to determine the causes for these well documented trends in order to better understand the risks incumbent with seed transfer and to assess opportunities for improving growth rate with inter-provenance hybrids.

In the investigations considered here, the seedlings conformed to the expected geographic differences in growth rate. The differences among seedlots in total growth were well correlated with differences in total net photosynthesis; however, the correlation resulted primarily from differences in leaf area and is of little value in selection or breeding. Differences between Coastal and Interior seedlings in early growth rate may, however, be due to the much smaller seed weights and earlier bud-set of the latter. Furthermore, the superiority of the trees from Florida may result, in part, from their longer period of high photosynthetic activity. Although differences in photosynthesis per unit leaf area or partitioning of photosynthate are often suggested as causes for differences in growth rate, we could not show significant differences among seedlots in either respect.

The growth measures presented here are based on high levels of moisture and nutrients and cannot be considered to simulate field conditions. Differences among the seedlots in growth rate may also be due to factors affecting growth during water or nutrient stress (Cannell et al., 1978). In our investigations we have also considered some of the factors that might affect growth during periods of drought stress, including (1) changes in stomatal conductance with increasing evaporative demand, (2) differences in osmotic potential, and (3) differences in osmotic adjustment (the latter two may result in differential stomatal closure with increasing water loss). Although measured with great precision, no differences among seedlots for these characteristics was detected. Other drought resistance characteristics which might affect growth rate, such as rapid stomatal closure in response to water stress and longer and deeper roots, have been shown to differ among seedlots from the Western Gulf region (van Buijtenen et al., 1976), but these were not examined in this work.

It is obvious that at this point in time our knowledge of the physiological basis for growth rate differences is incomplete, and we are not in a position to make recommendations applicable to field conditions. However, this is a rather new area of research endeavor, and, if considered holistically, promises to offer faster, more effective methods of selection and improved breeding strategies.

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RESISTANCE TO THE DEVELOPMENT OF PITCH CANKER IN OPEN-POLLINATED SLASH PINE FAMILIES

G.A. Lowerts, M.H. Zoerb, Jr., and J. Pait [1/]

Abstract.--Open-pollinated slash pine (Pinus elliottii Engelm. var. elliottii) families displayed a significant amount of family variation in resistance to the development of pitch canker (Fusarium moniliforme Sheld. var. subglutinans Wollenw. and Reink). Fertilized slash pine families possessed a significantly greater level of infection than nonfertilized families. Percent infection ranged from 13 to 69 among fertilized and from 6 to 39 percent in nonfertilized families. Slash pine families originating from selections indigenous to Florida were significantly more resistant than families originating from Georgia.

INTRODUCTION

Pitch canker infection of slash pine plantations became a serious forest management problem on Union Camp Corporation land in late 1975 and early 1976 (Broerman, 1976). A survey of pitch canker incidence on company land revealed that 40 to 90 percent of all trees were infected within slash pine plantations in the Florida counties of Clay, Putnam, Flagler, and Volusia. In these highly affected areas, the entire crown of a tree would be infected in contrast to infection of the terminal and perhaps a single branch when pitch canker was present at an endemic level. Losses due to mortality and decreased growth were estimated to be in excess of 1.5 million dollars (Broerman, 1976). In response to the high level of pitch canker infection and the resultant growth loss, the company decided to: (1) document the distribution of the disease and assess the intensity and rate of disease development, (2) develop a management strategy to implement salvage cuttings when necessary, (3) support basic research on the pathogen and means of transmission and (4) screen for potential resistance among open-pollinated slash pine families in the company's first (1.0) generation seed orchards. This paper contains the results of a genetic test to determine the extent of resistance to pitch canker infection among open-pollinated slash pine families.

MATERIALS AND METHODS

An adequate level of inoculum must be present to screen slash pine families for pitch canker resistance. Therefore, a test site was located

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[1/] Geneticist and Senior Project Leader, respectively, Union Camp Woodlands Research, Savannah, Georgia, and Research Forester, Container Corporation of America, Callahan, Florida.

within a high disease incidence area on Union Camp land [1/] in Volusia County, Florida. The slash pine plantation present prior to test establishment was harvested prematurely due to extensive pitch canker induced damage and loss. The slash pine plantations surrounding the test site were also heavily infected. Ninety-two families were available for pitch canker resistance screening from the Union Camp Corporation's first generation seed orchards.

In January 1977, the 92 families were planted in two blocks. Due to space restrictions, Block I and Block II did not receive the same number of families. Block I and Block II were randomly assigned 56 and 36 families, respectively. Four commercial checks were the only "families" common to each block. Each block contains 20 replications with each family planted in five tree row plots. At the time of planting, Block II received 250 lbs./acre of an 18-40-0 fertilizer applied to the planting beds and each tree in both blocks received 7 grams of Furadan 10G.

In summer 1984, height, diameter and pitch canker incidence were measured on each tree. Height was measured to the nearest foot and diameter to the nearest tenth inch. Trees were scored as either being infected with pitch canker or not infected. The magnitude of infection in each tree was not assessed.

Since the commercial checks were the only entities common to each block, a paired t-test was used to compare block means for height, diameter and percent infected trees. Replication, family and family by replication effects were analyzed separately for each block using analysis of variance procedures. The family by replication interaction was not significant in the nonfertilized block, but was significant in the fertilized block. This interaction involved a minor family rank order change of no biological significance. Within each block, the family by replication effect was then pooled with experimental error. Based on family means, height, diameter and percent infected trees were analyzed with families and replications as the sources of variation.

The families tested in this study originated from selections in the Atlantic Flatwoods and Upper Coastal Plain provinces of Georgia and from Florida. Based on the county of origin of the select parent tree, each family in both the fertilized and nonfertilized blocks was clustered into one of four groups: (1) Upper Coastal Plain of Georgia; (2) Northern Georgia Atlantic Flatwoods; (3) Southern Georgia Atlantic Flatwoods and (4) Florida. Duncan's new multiple range test was used to compare mean pitch canker infection, height and diameter among the four groups.

RESULTS AND DISCUSSION

Slash pine families in the fertilized block possessed a significantly higher incidence of pitch canker infection and a significantly larger mean height and diameter than those in the nonfertilized block (table 1).

[1/] The test site was subsequently purchased by Container Corporation of America.

Table 1.--Mean tree height and diameter and the proportion of trees infected with pitch canker in the fertilized and nonfertilized blocks. 1/

Block	Height (ft.)	Diameter (in.)	Proportion of trees infected with Pitch Canker
Fertilized	26.3	4.8	40.5
Nonfertilized	24.3	4.0	20.6
	$t=10.40^{ns}$	$t=2.93^{*}$	$t=8.87^{**}$

1/ where ns = not significant
* = significant at five percent level
** = significant at one percent level

Twenty-three percent of the trees in the fertilized block were infected with pitch canker while only twelve percent of the trees were infected in the nonfertilized block. A significantly greater incidence of infection also occurred among the commercial checks in the fertilized block than in the nonfertilized block (table 2). The commercial checks in the fertilized block possessed a significantly larger mean height and diameter. Since the commercial checks were the same in both blocks, the increased rate of infection in the fertilized block was apparently due, in part, to fertilization and is probably not a result of the random assignment of families to each block. A prolonged growing season and an increase in the amount of succulent tissue as a result of fertilization may have predisposed the slash pine to

Table 2.--Degrees of freedom, mean and F-value of height, diameter and proportion of trees infected with pitch canker among the commercial checks between the fertilized and nonfertilized blocks. 1/

Source of Variation	DF	Block	Height(ft.) $\bar{X}$	Height(ft.) F	Diameter(in.) $\bar{X}$	Diameter(in.) F	% Pitch Canker $\bar{X}$	% Pitch Canker F
Block	1	Fert.	24.6	**	4.5	**	45.6	**
		Nonfert.	23.6	6.48	4.5	35.80	20.1	7.39
C.C.	3			2.07^{ns}		0.68^{ns}		1.39^{ns}
C.C.* block	3			3.15^{*}		1.27^{ns}		0.41^{ns}

1/ where ns = not significant
* = significant at five percent level
** = significant at one percent level
C.C. = commercial check

pitch canker (Dwinell, et al., 1981). In a general way this possibility is corroborated by other work indicating that an imbalance in plant nutrition because of an over abundance or shortage of nutrients can lead to greater levels of infection by a pathogen (Agrios, 1978). The incidence of pitch canker infection has been shown to be associated with increased levels of fertilization (Wilkinson, et al., 1977). Fertilization, applications of pesticides and mechanical wounding may also be associated with the occurrence of pitch canker in loblolly (Pinus taeda L.) and slash pine seed orchards (Dwinell, et al., 1981).

In both the fertilized and nonfertilized block, a weak inverse correlation existed between family mean height and diameter with family percent pitch canker infection (table 3). Although family mean height was

Table 3.--Correlation coefficient (r) of family mean height and diameter with the proportion of trees infected with pitch canker in the fertilized and nonfertilized blocks. 1/

Block	N	Ht	Diameter	
Fertilized	40	-0.45^{**}	-0.26^{ns}	With proportion of trees infected with pitch canker
Nonfertilized	60	-0.47^{**}	-0.246^{ns}	With proportion of trees infected with pitch canker

1/ where ns = not significant
** = significant at the one percent level

significantly correlated with percent pitch canker infection, the correlation accounted for only 20 and 22 percent of the variation between height and percent pitch canker infection in the fertilized and nonfertilized blocks, respectively. The weak height and diameter correlation with percent pitch canker infection suggests that the growth rate of the slash pine families in this study probably did not directly influence the host-pathogen disease complex to any great extent. Growth rate was not correlated with pitch canker resistance in a slash pine screening study conducted by McRae, et al. (1985). Arvanitis, et al. (1984) also found that diameter was not related to pitch canker infection in nonfertilized slash pine plantations.

The proportion of trees infected with pitch canker varied significantly among the slash pine families in both the fertilized and nonfertilized blocks (table 4). In the fertilized block, pitch canker infection among slash pine families ranged from 13.0 to 69.0 percent and from 6.0 to 39.0 percent in the nonfertilized block. Family variation in the fertilized and nonfertilized blocks accounted for 35 and 32 percent, respectively, of the total variation present in each block. Resistance to pitch canker infection

Table 4.--Degrees of freedom and F-value for height, diameter and proportion of trees infected with pitch canker within the fertilized and nonfertilized blocks. 1/

Source of Variation	----- Fertilized Block ---- DF	Ht	Dia.	% Infect.	--- Nonfertilized Block ---- DF	Ht	Dia.	% Infect.
Replication	19	10.66**	8.5**	4.3**	19	14.53**	10.5**	6.13**
Family	39	2.34**	1.68**	2.94**	59	1.79**	1.10ns	3.49**

1/ where ns = not significant
** = significant at one percent level

also varies among clones in both slash and loblolly pine seed orchards (Phelps and Chellman, 1976; Dwinell, et al., 1977; Dwinell and Barrows-Broaddus, 1981; Kuhlman, et al., 1982). The results of this study suggest that the slash pine families in both the fertilized and nonfertilized blocks contain varying levels of resistance to pitch canker.

In both the fertilized and nonfertilized block, slash pine families which were indigenous to Florida displayed a significantly lower level of pitch canker infection than those families which were indigenous to Georgia (table 5). There was no significant difference in the level of pitch canker

Table 5.--Comparison of mean height, diameter and proportion of trees infected with pitch canker among slash pine clones originating from the Upper Coastal Plain, northern and southern Atlantic Flatwoods region of Georgia and from Florida. 1/

Region 2/	- Nonfertilized Block - Ht. (ft.)	Dia. (in.)	% Infect.	-- Fertilized Block --- Ht. (ft.)	Dia. (in.)	% Infect.
U.C.P. Ga.	24.6 ab	4.0 ab	19.7 a	26.0 a	4.7 a	42.2 a
A. Flat. N. Ga.	24.1 b	4.0 ab	23.0 a	26.1 a	4.8 a	45.6 a
A. Flat. S. Ga.	24.2 b	3.9 b	20.8 a	26.6 a	4.7 a	37.5 ab
Florida	24.9 a	4.1 a	13.7 b	26.4 a	4.7 a	30.9 b

1/ Means within a trait and block not sharing the same superscript are significantly different at the five percent level.

2/ U.C.P. Ga. = Upper Coastal Plain of Georgia
A. Flat. N. Ga. = Atlantic Flatwoods Northern Georgia
A. Flat. S. Ga. = Atlantic Flatwoods Southern Georgia

infection among Georgia slash pine families. However, slash pine families which possess good height and diameter growth with pitch canker resistance could be found in selections from Georgia and Florida. In Florida, natural stands of slash pine possess significantly lower levels of pitch canker infection than slash pine plantations; especially those plantations which originated from selections in southern Georgia (Blakeslee and Rockwood, 1978; Dwinell, et al., 1981). This study supports the views of several researchers that slash pine trees indigenous to Florida are, in general, more resistant to pitch canker.

The results of this study suggest that fertilization, directly or indirectly, increases the susceptibility of slash pine families to pitch canker infection. The role of fertilization and perhaps other environmental factors (eg. drought) in the host-pathogen interaction is still unknown. Elucidation of the effect of fertilization and environmental factors on the predisposition of slash pine to pitch canker infection is necessary since pitch canker remains a potential disease of epidemic proportions. The large amount of variation displayed among the open pollinated slash pine families suggests a tree improvement program to enhance pitch canker resistance may be possible. Additional testing is required in order to confirm the repeatability of resistance since the slash pine families employed in this study were only tested in one location. Even though the resistance of the slash pine families was determined in one location, the most resistant families should be preferentially planted in regions of high pitch canker infection.

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GROWTH MODEL EVALUATION: SOUTH-WIDE LOBLOLLY PINE SEED SOURCE STUDY

Fan H. Kung[1/]

ABSTRACT.--A growth model useful to the forest geneticists should have the following five P's: (1) perfection of fit to the data presented, (2) predictability of growth potentials, (3) possibility of inference based on parameter estimates, (4) power of discrimination among seed sources, and (5) persistency of regression coefficients over time.

To illustrate, the function of ln(HT) = B0 + B1/AGE + B2 ln(1 + 1/AGE) was fitted to the height growth of 15 seedlots of loblolly pine in a south-wide study. The degree of determination was 0.999 at least. The five-year and the ten-year projections were low by 3% and 5% respectively. The regression coefficients B0, B1 and B2 were highly significant among seedlots. When the three growth periods were compared, the coefficient of variation for the regression coefficients was less than 5%.

Additional Key Words: Growth projection, Model verification, Growth curve discrimination

INTRODUCTION

The mathematical characterization of growth is among the oldest scientific pursuits. Indispensable long-term planning in forestry requires reliable information about the growth of forest stands. A wide array of growth and yield models ranging from whole stand models to individual tree models has been developed for southern species.

Genetic field tests are subjected to many uncontrolled disturbances. However, height of dominant-codominant trees is much less dependent on density and therefore is a better measure of inherent growth differences. Growth and yield models can be used to translate differences in dominant-codominant height into stand differences expected in the absence of uncontrolled disturbances (Nance and Wells 1981).

Foresters have long used the mean height of dominant-codominant trees for site index, as a universal measure of the potential

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1/ Professor, Department of Forestry, Southern Illinois University, Carbondale, IL 62901. The author is grateful to Dr. Osborn O. Wells and the Southern Forest Experiment Station for providing the data for this study, and to Dr. Marilyn A. Buford for fruitful discussions.

productivity of forest land. Thus, a further improvement in precision for measuring genetic differences in growth rate would be removing the site effect and provenance by site interaction by using the regional mean from many plantations.

Suppose that we have determined to use the range-wide mean height to assess genetic differences, the question still remains with us as to which growth model should we use as a base for comparison.

Before growth models can be evaluated, one must outline the criteria for model selection. We believe a useful growth model should have the following characteristics:

1. perfection of fit to the data presented,
2. predictability on growth potentials,
3. possibility of inference based on parameter estimates,
4. power of discrimination among seed sources, and
5. persistency of regression coefficients over times.

In this paper, we use data from the loblolly pine south-wide study to illustrate such desirable properties of a growth function.

SOUTH-WIDE LOBLOLLY PINE SEED SOURCE STUDY

Complete details of the Loblolly pine experiment are given by Wells and Wakeley (1966). Fifteen seed sources are represented and 16 plantings survived after 25 years in the field.

Measurements of total height were made at various ages from 1 to 27 years. However instead of using only the dominant and codominant trees, we used all available trees measured in 3, 5, 10, 15, 20, and 25 years to calculate the mean height growth for each seed source. The reason for this selection is based on statistical and not on silvicultural ground. The statistical property of dominant and codominant trees is close to the extreme number distribution while the average height is close to the normal distribution. The latter is much easier for data analysis.

The south-wide regional means of the 15 seed sources are listed in Table 1. The mean standard deviation and the coefficient of variation are also presented.

GROWTH FUNCTION

Nonlinear growth functions have been proposed for total height. The monomolecular function is useful for site index curves for age 20 and older which show no point of inflection (Lundgren and Dolid 1970). Richards' curve gives a good fit for both height and volume growth of Douglas-fir provenances (Namkoong, Usanis, and Silen 1972). The Weibull function can describe the growth of trees and stands (Yang, Kozak and Smith 1978). However, we are in favor of linear models over nonlinear models because linear models give rise to unbiased, normally distributed, minimum variance estimators, whereas nonlinear

Table 1.--South-wide regional means of height growth among loblolly pine seed sources.

Seed Source	Regional Mean at Age					
	3	5	10	15	20	25
	- - - - - - - - - - - - 0.01 ft. - - - - - - - - - - - -
301	451	1089	2872	4174	5270	6092
303	455	1092	2907	4283	5388	6339
305	479	1137	3021	4440	5582	6410
307	391	948	2566	3907	5078	5907
309	481	1120	2866	4166	5331	6305
311	388	940	2599	3955	5146	5964
315	432	1043	2716	4085	5133	6007
319	457	1057	2790	4113	5243	6069
321	377	903	2545	3898	4976	6028
323	433	1079	2897	4244	5317	6162
325	438	1048	2793	4020	5006	5849
327	412	992	2654	3782	4843	5681
329	393	934	2608	3779	4831	5643
331	346	905	2585	3915	4912	5949
Mean	421	1014	2729	4039	5132	6018
St. Dev.	40	82	159	197	220	222
C.V. %	9.5	8.1	5.8	4.9	4.3	3.7

regression models tend generally to do so only as the sample size becomes very large (Ratkowsky 1983). With only a total of six data points we are not very comfortable with the results of a nonlinear model. Therefore, to illustrate that the five desirable properties are obtainable, we use the following curve with an intrinsically linear combination of parameters:

$$\ln (HT) = B0 + B1(1/AGE) + B2 \ln (1 + 1/AGE),$$

where ln is the natural logrithm transformation and B0, B1, and B2 are coefficients to be determined by regression analysis.

The function provides precise description of observed data points and provides trustworthy predictions. The function is differentiable and is applicable to many growth and yield characters in life sciences (Kung 1984).

PERFECTION OF FIT

Growth is a continual process but it may be subdivided into stages. Tree physiologists indicated that growth consists of division elongation, differentiation and maturation of cells (Kozlowski 1971). Forest geneticists divided stand development into juvenile-genotypic, mature-genotypic and codominance-suppression phase (Franklin 1979). Forest biometricians believe that a growth curve begins at the value of zero, climbs slowly at first and then more steeply. After a turning point, the increment diminishes and then asymptotically moves towards some final value

(Prodan 1968). Thus a growth function should act like an adjustable ship curve used in drafting that fits all data points through various stages equally well and not just for a single stage. For example, a polynomial, as well as the simple exponential function may fit a part of a growth series better than the more complex nonlinear models, but may have a poor fit elsewhere.

In the paper, three periods were used for comparison: (1) age 3 to 15, (2) 3 to 20, and (3) 3 to 25. The F value, the root mean square of error and the coefficient of determination are used to judge the fit of the model.

The F values ranged from a minimum of 3040 to a maximum of 999999 (Table 2). All models were significant at the 0.0001 probability of error.

Table 2. Fit between data and model among seed sources in three time periods at age 3 to 15, 3 to 20 and 3 to 25 years.

Seed Source	Model Statistics					
	F value			Root mean square		
	3 - 15	3 - 20	3 - 25	3 - 15	3 - 20	3 - 25
301	18,909	13,895	12,333	.009	.012	.015
303	58,791	32,671	9,387	.005	.008	.016
305	32,812	32,243	38,561	.007	.008	.008
307	29,514	4,855	6,166	.007	.022	.022
309	123,317	5,170	3,040	.003	.019	.029
311	999,999	7,173	10,099	.001	.018	.017
315	10,901	11,113	7,760	.011	.014	.012
317	70,568	7,852	4,596	.005	.017	.025
319	45,775	11,704	12,122	.006	.013	.014
321	483,648	72,226	5,834	.002	.006	.022
323	29,266	35,172	20,694	.007	.008	.011
325	4,926	13,345	10,270	.017	.012	.016
327	2,844	4,473	4,068	.023	.022	.029
329	2,104	5,331	7,232	.027	.020	.020
331	115,717	318,691	6,114	.004	.003	.022
Mean				.009	.013	.019
St. Dev.				.008	.006	.006
C.V. %				85	45	32

The root mean square of fitting errors ranged from a minimum of .001 to a maximum of .029. On the average the fitting error was .009 for the 3 to 15 year period and increased to .019 for the 3 to 25 year period. The average for the three periods was .014. For small values of e, we have approximation of Exp(e) = 1 + e. Therefore, the small size of the error term represents the relative error. In other words, the percent of error for the 3 - 15 year period would by only .9 percent, corresponding to the average RMSE of .009. The average of the relative error for the study is 1.4 percent.

The coefficient of determination ranged from 0.9997 to 1.000 among 45 regression models. It may lead one to wonder whether a perfect fit has been achieved.

PREDICTABILITY OF GROWTH POTENTIALS

One of the most rigorous tests of a fitted equation is cross verification with a second sample taken at another time (Daniel and Wood 1980). Because this is impossible, we have used a longitudinal verification for the growth curve. First, we developed regressions based on 3 to 15 and 3 to 20 year periods for each seed source. The second step was to project the height at age 25 from each regression. The final step was to compare the projected and the observed height. The results are presented in Table 3.

Table 3.--Comparison between projected and observed height at 25 years of age.

Seed Source	Observation at age 25	Projected from ages 3-15	Projected from ages 3-20	Error rate 3-15	Error rate 3-20
	0.01 ft.			%	
301	6,092	5,858	5,944	-3.9	-2.4
303	6,339	6,051	6,114	-4.5	-3.5
305	6,410	6,278	6,337	-2.1	-1.1
307	5,907	5,571	5,741	-5.7	-2.8
309	6,305	5,800	5,963	-8.1	-5.4
311	5,964	5,699	5,847	-4.4	-2.0
315	6,007	5,715	5,805	-4.8	-3.4
317	5,865	5,456	5,588	-7.0	-4.7
319	6,069	5,825	5,932	-4.0	-2.2
321	6,028	5,681	5,726	-5.8	-5.0
323	6,162	5,977	6,027	-3.0	-2.2
325	5,849	5,671	5,686	-3.0	-2.8
327	5,681	5,333	5,444	-6.1	-4.2
329	5,643	5,461	5,510	-3.2	-2.4
331	5,949	5,646	5,641	-5.1	-5.1
Mean	6,018	5,735	5,821	-4.7	-3.3
St. Dev.	222	228	239	1.6	1.3

All projections were lower than the observed heights. The 10 year projection was low by 2.8 feet or 4.7 percent; while the 5 year projection was low by 2.0 feet or 3.3 percent. However the standard deviation of the three groups were the same. The projections were as precise as the observations even though the accuracy may be off a little. The standard deviation for the relative error rate were 1.6 and 1.3 percent respectively for the 10-year and 5-year projections.

The bias in projection is not the fault of using transformation. The transform of the expected value does not equal the

expected value of the transform, although they are crude approximations of each other (Kruskal 1978). The percent of bias calculated according to the formula given by Wiant and Harner (1979) was less than 0.04 percent. Because of the small error variance in the regressions, the adjustment of the prediction (Baskerville, 1972) offered little reduction in the bias. This large and consistent bias needs to be rectified by adding 3 percent to the 5-year forecasting and 5 percent to the 10-year forecasting.

POSSIBILITY OF INFERENCE

Growth potential and growth rate are of interest to the forest manager. The function $\ln(HT) = B0 + B1(1/AGE) + B2 \ln(1 + 1/AGE)$ indicates that the asymptotic height should be near the value of Exp(B0). For example, the maximum value (9.334) for B0 among 15 seed sources was found in provenance 305, therefore the asymptotic height would be Exp(9.334) x .01 ft. = 113 ft. On the other hand, a minimum of B0 in seed source 327 indicated that the average asymptotic height could be 92 ft. Notice that the average height at age 25 for the complete study is 60 feet, the average site index for the complete experiment could be estimated as 90 ft. at age 50. Which is the average site for loblloly pine. Trees at that site grow slowly after 50 years of age, hence our estimate of asymptotic height seems to be reasonable.

The function can be differentiated. The instantaneous growth rate of log (Ht) at age x is:

$$d(\ln HT)/dx = -B1/x^2 - B2/(x^2 + x)$$
$$= (-x(B1 + B2) - B1)/(x^3 + x^2)$$

Using the coefficients developed from the 3 to 25 year as an example we found in Table 4 that seed source 321 would have the greatest growth rate of log (Ht) at age x as $((17.15x - 33.90)/(x^3 + x^2)$, while seed source 325 would have the smallest growth rate as $((14.81x - 24.58)/(x^3 + x^2)$ at the age of x years. Because of the extremely high correlation between B1 and B2 (r = 0.997), selection of growth rate can be simplified as selection for B1.

POWER OF DISCRIMINATION

If simple functional differences among genotypes were existent or nonexistent, a growth function should have the power of discrimination to prove or to disprove the differences in growth form. By fitting the mean height from age 5 to 55 years old of 13 populations of Douglas-fir in Wind River, Oregon, Namkoong, Usanis and Silen (1972) found that the parameters A, C, and m of the Richards' function were nonsignificant among populations. Using the Weibull function to quantify sweetgum germination, it was found that the coefficients b and c were significant among families within the stand, but all of the three coefficients (a, b, c) were not significant among stands (Rink et al. 1979). The significant difference in a given parameter indicated that selection in that characteristics of the growth curve may be possible. On the other hand, no growth rate can be selected if all growth curves are the same.

Table 4.--Summary of regression coefficients in growth models for 15 loblolly pine seed sources during three time period.

Seedlot	Coefficient B0				B1				B2			
	3-15	3-20	3-25	C.V.	3-15	3-20	3-25	C.V.	3-15	3-20	3-25	C.V.
301	9.222	9.248	9.267	.24	22.58	23.89	25.02	5.17	-36.98	-38.58	-39.96	3.87
303	9.272	9.289	9.318	.25	24.66	25.56	27.22	5.03	-39.53	-40.62	-42.65	3.87
305	9.309	9.324	9.384	.13	25.04	25.85	26.38	2.62	-39.93	-40.91	-41.56	2.01
307	9.217	9.267	9.290	.40	27.36	29.96	31.28	6.75	-42.98	-46.16	-47.77	5.34
309	9.206	9.253	9.298	.50	23.41	25.82	28.40	9.64	-37.65	-40.51	-43.74	7.50
311	9.252	9.296	9.311	.33	28.58	30.80	31.72	5.32	-44.55	-47.27	-48.38	4.22
315	9.212	9.239	9.266	.29	24.46	25.82	27.40	5.68	-39.26	-40.93	-42.85	4.38
317	9.196	9.236	9.275	.43	27.13	29.20	31.44	7.37	-42.75	-45.28	-47.99	5.78
319	9.240	9.270	9.289	.27	26.65	28.22	29.28	4.72	-41.71	-43.63	-44.92	3.72
321	9.268	9.281	9.322	.30	30.84	31.53	33.90	5.00	-47.33	-48.17	-51.05	3.99
323	9.249	9.264	9.281	.17	22.09	22.83	23.85	3.86	-36.65	-37.55	-38.79	2.85
325	9.193	9.197	9.220	.16	23.04	23.26	24.58	3.53	-37.51	-37.79	-39.39	2.65
327	9.125	9.160	9.194	.38	22.15	23.83	25.80	7.64	-36.35	-38.52	-40.92	5.92
329	9.189	9.204	9.223	.19	26.65	27.42	28.53	3.43	-42.06	-43.01	-44.35	2.67
331	9.235	9.236	9.277	.26	24.70	24.72	27.13	5.48	-40.41	-40.43	-43.35	4.10
Mean	9.226	9.250	9.278	.29	25.29	26.58	28.13	5.41	40.37	41.95	43.84	4.19
St. Dev.	.043	.042	.040	.10	2.54	2.80	2.93	1.83	3.18	3.46	3.62	1.45
C.V. %	.47	.45	.43		10.06	10.55	10.41		7.89	8.25	8.26	

The functions presented in this paper have great power of discrimination among seed sources of loblolly pine. All three coefficients B0, B1 and B2 are significant beyond the 0.001 probability of error (Table 5). The repeatability or provenance heritability calculated from the F value (Kung and Bey 1978) was 0.98 for each coefficient.

Table 5.--Analysis of variance of the regression coefficients.

Source	d.f.	Coefficient B0 MS	B0 F	B1 MS	B1 F	B2 MS	B2 F
Seed source	14	.0050	40[a]	22.20	63[a]	34.19	65[a]
Age period	2	.0101	82[a]	30.31	86[a]	45.22	86[a]
Error	28	.0001		.35		.52	
Total	44						

[a]Significant at the 0.0001 level.

PERSISTENCY OF COEFFICIENT

A good growth curve should be relatively independent of the range of data base. If the growth curve developed from the juvenile growth period were the same as that developed from the complete life span, we would be more successful in making early selection.

The persistency of coefficient in the three periods (3 to 15,

3 to 20, and 3 to 25 years of age) is evident in Table 4. The average coefficient of variation among three periods was only .29 % for B0, 5.4 % for B1 and 4.2 % for B2.

As the range of ages becomes wider, the absolute values of the B0, B1 and B2 also increases. The differences are significant beyond the 0.001 probability of error (Table 5). Although one would like to have a constant regression coefficient throughout the years if possible, the second best would be that for any given parameter, it may differ from one period to another, but it should vary in a predictable manner.

The persistency of coefficient can be shown also by the correlation coefficient for B0, B1 and B2 among three periods (Table 6). All correlations are significant at the 0.001 level. From the period, of 3 to 15 years to the period of 3 to 20 years, the B1 increases by 6 % and the B2 increases by 4 %; while from the period of 3 to 20 years to the period of 3 to 25 years both B1 and B2 increases only 2 %.

Table 6.--Correlations among three periods for parameters B0, B1 and B2.

Correlation between periods		Correlation coefficients for parameters		
		B0	B1	B2
3 - 20	3 - 25	.97	.98	.98
3 - 15	3 - 20	.94	.96	.96
3 - 15	3 - 25	.89	.94	.94

DISCUSSION

Many growth and yield models are available to tree improvement workers. From a practical point of view we recommend the 5-P criteria for model selection. However, we have not assigned any weight to each criterion which may differ from one program to another.

We use the range means of the seed source and not the individual tree in hope that if all the environmental errors could be averaged out we would have a more accurate evaluation of the performance of the growth model as well as the genetic difference among seed sources.

The function used for illustration is almost ideal in the 5-P criteria. However, we are still searching for a perfect curve which is totally independent of the age range. Would it be possible to have a model which regression coefficients are changeless between any period range from 1 to 100 years? or is it an impossible dream?

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Evaluation of Slash Pine for Resistance to Pitch Canker

C. H. McRae, D. L. Rockwood and G. M. Blakeslee[1/]

Abstract.--Two- to three-year-old orchard open-pollinated seedlings from 46 slash pine (Pinus elliottii Engelm. var. elliottii) clones were evaluated for resistance to Fusarium moniliforme var. subglutinans (FMS). These families, representing fast growing and/or fusiform rust resistant genotypes, were planted near Gainesville, Florida. Terminal and lateral shoots of 21 to 24 seedlings per family were wounded and inoculated with a polymix of four isolates of FMS. Family mean symptom expression ranged from 16.6 to 91.7%; shoot mortality ranged from 4.2 to 91.7%. Strong individual and family heritabilities suggested that genetic resistance may be useful in management of pitch canker. Estimated gains from four improvement options are presented. There were no significant correlations between pitch canker resistance and either fast-growth or fusiform rust resistance.

Additional Keywords: Pinus elliottii var. elliottii, Fusarium moniliforme var. subglutinans, heritability, genetic variation, genetic gain, fusiform rust, Cronartium quercuum f. sp. fusiforme.

INTRODUCTION

FMS infects many southern pines. This fungus has been especially damaging in slash pine, inciting resin-soaked cankers on the branches and main stem resulting in shoot dieback, stem deformity, reduced growth, and mortality (Blakeslee et al. 1980, Blakeslee and Oak 1979, Dwinell et al. 1985, Phelps and Chellman 1976). Prospects for long-term control of pitch canker are strengthened by studies showing genetic variation within pine species for resistance to pitch canker (Barnett and Thor 1978, Blakeslee and Rockwood 1978, Dwinell and Barrows-Broaddus 1979, 1981 and 1983, Dwinell et al. 1977). This paper reports further evidence of genetic variation in slash pine and estimates genetic gains that could be obtained from using resistant genotypes.

[1/]Graduate Research Assistant, and Associate Professors, respectively, Department of Forestry, University of Florida, Gainesville, FL. The assistance of the Cooperative Forest Genetics Research Program at the University of Florida is gratefully acknowledged.

MATERIALS AND METHODS

Open-pollinated progeny of 46 fast-growing and/or rust-resistant clones were planted in a randomized complete block design on a fertile, well-drained site in north central Florida in 1981 and 1982. The trees were planted at a 10' x 2' spacing in 10-tree row plots that were replicated three times.

From September 24 through October 18, 1983, the terminal and one lateral shoot of each tree were inoculated with an aqueous suspension of conidia of FMS. At the time of inoculation, the trees averaged 7.25 feet in height and were vigorous and healthy. The eight most vigorous of the ten trees planted per row plot were selected for inoculation. Precipitation and ambient temperature were monitored on the site during the inoculation period.

The inoculum consisted of four proven-pathogenic isolates of FMS obtained from slash pines in Volusia, Franklin and Gilchrist counties in Florida. The isolates were single spored and grown on carnation leaf-water agar for about 10 days prior to use. An aqueous suspension of conidia from each source (1-1.5 x 10^5 conidia/ml) was prepared daily, and equal aliquots of each source were combined just prior to use. Post-inoculation germination checks were made daily, and viability consistently exceeded 96%.

Prior to inoculation, shoots were surface sterilized by spraying with 95% ethanol and allowed to dry. Each shoot received two wounds at the same level, located on opposite sides of the third flush of 1983 growth. The wounds were made with an 18-gauge needle, and two drops of inoculum were placed in each wound. Control branches, selected at random within the family, were treated in the same manner except that sterile water was used in place of the inoculum.

At about 18-day intervals for the next eight months, each shoot was examined for symptom development. At each observation, the condition of the shoot was rated as 1 (no symptoms), 2 (foliage discolored) or 3 (foliage brown and shoot dead). So that maximum disease expression could be obtained for all trees, the shoots were harvested according to disease severity, with dead shoots being harvested and processed in the laboratory before living shoots were harvested. In July 1984 when new symptom expression had essentially ceased, all remaining shoots were collected and brought to the laboratory for detailed examination and isolation.

Two responses on terminal shoots were analyzed; percent of trees with pitch canker symptoms (conditions 2 and 3) and percent of trees with pitch canker induced mortality (condition 3 only). To determine significance of blocks and families, analysis of variance was conducted suing plot means. For genetic analyses, these two binomial responses were handled in the same manner as percent rust-infected data (Rockwood and Goddard 1973). Individual tree and family mean heritabilities were calculated on the assumption of half-sibs. Genetic gains employed techniques presented by Shelbourne (1969). Selection intensities assumed were: 10% in seed production areas and for tested clonal

orchards, 2% for untested clonal orchards and 40% in existing clonal orchards.

Correlations between traits were based on family means. Clonal evaluations for growth and rust resistance included in the correlations were weighted, standardized comparisons from numerous progeny tests involving open-pollinated progenies and an unimproved check lot.

RESULTS AND DISCUSSION

Symptom development followed a typical disease progress curve (Figure 1). Observed symptoms included those regularly associated with pitch canker on slash pine, via. discoloration and death of needles around the inoculation sites, exudation of pitch from infected tissues surrounding the inoculations, discoloration and death of needle and shoot tissues distal the the cankers, and, for those trees where advanced symptom development did not occur until the following spring, a second flush of symptoms coinciding with the spring flush of growth.

The pitch canker pathogen was reisolated from about 90% of the inoculated shoots and 2% of the control shoots, thus indicating that the observed symptoms were due to the pathogenic action of the introduced

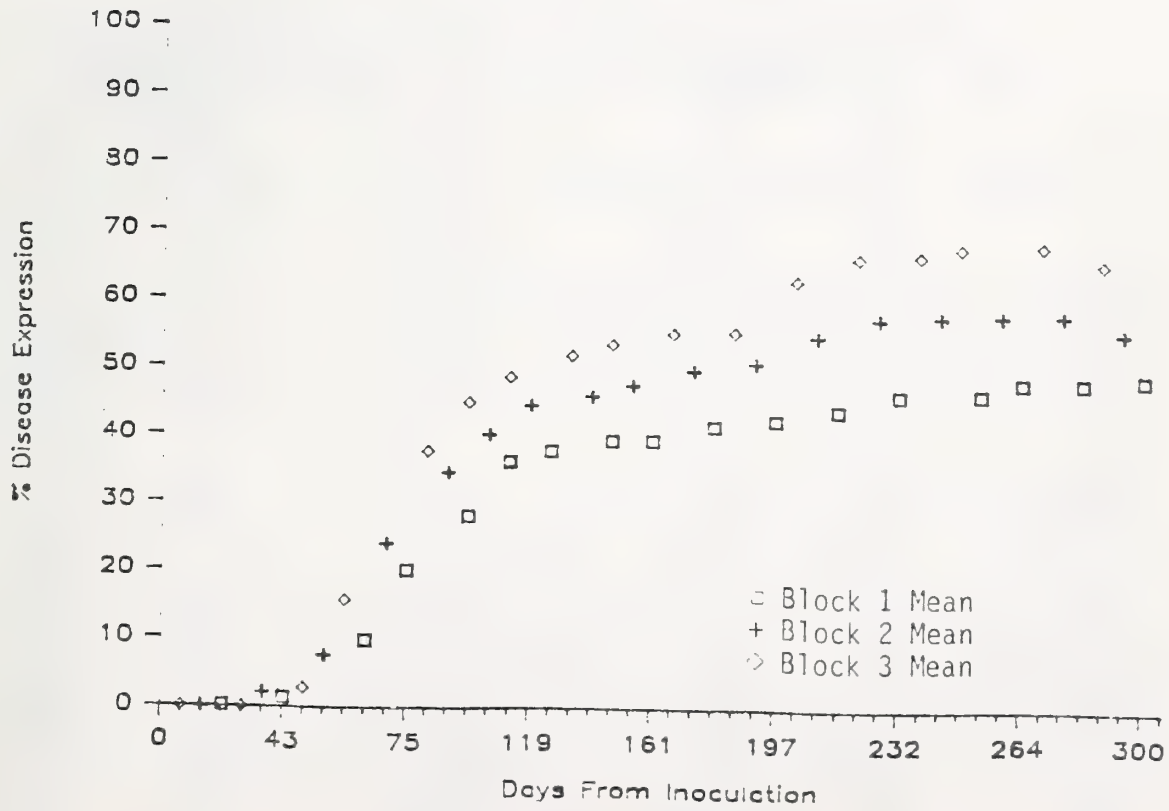

Figure 1. Pitch canker symptom expression disease progress curves based on block means.

fungus. Failure to obtain higher yields from the inoculated shoot reisolation may be due to incomplete isolation from the canker, or loss of viability of the fungus due to active defense of host, thus limiting tissue available for colonization. Reisolation of the pathogen from a small percentage of the control shoots can be readily attributed to the presence of idigenous FMS inoculum that can infect susceptible tissue when wounded.

Family mean responses to the pathogen were normally distributed, ranging from highly susceptible to highly resistant (Table 1). A similarily wide range of incidence has been observed in loblolly pine clones (Dwinell et al. 1977). However, there were certain trees that became infected but progressive tissue colonization did not occur as the canker was stabilized and the infection delimited by the production of hypertrophic callus around the canker. Those trees, based on mean symptom expression, would appear to be poor performers, but when evaluated with respect to shoot mortality, appear to have functional resistance. Overall, there was a strong correlation between symptom expression and mortality response.

Significant differences existed among families and among blocks (Table 2). The differences between blocks may be related to differences in edaphic conditions, topography, variations in rainfall during the inoculation period or the slightly higher percentage of younger (two-year-old) trees included in the third block.

Table 1. Slash pine family means for percent pitch canker symptom expression (Symp) and mortality (Mort) of terminal shoots.

	Pitch Canker			Pitch Canker	
Family	Symp	Mort	Family	Symp	Mort
1-60	16.6	8.3	56-56	62.5	33.3
23-59	20.8	16.7	327-56	64.9	26.2
M-835	29.2	8.3	102-57	66.7	29.2
57-56	33.3	12.5	261-56	66.7	29.2
239-56	37.5	25.0	285-55	66.7	41.2
M-204	40.3	13.9	69-56	66.7	29.2
205-55	45.8	12.5	163-57	68.3	38.3
24-60	45.8	16.7	173-57	70.8	25.0
13-59	47.0	30.6	33-58	70.8	41.2
89-57	48.2	17.6	48-59	70.8	37.5
M-308	50.0	8.3	60-56	70.8	29.2
16-59	50.0	25.0	B-106	75.0	20.8
163-58	50.0	12.5	65-56	75.0	45.8
31-60	50.0	29.2	91-58	75.0	50.0
66-73	50.0	20.8	M-109	76.4	45.8
27-58	50.8	14.3	13-56	79.2	66.7
M-817	54.2	4.2	293-55	79.2	54.2
64-56	54.2	37.5	106-56	83.3	58.5
357-56	55.4	26.8	265-55	83.3	66.7
57-61	58.3	25.0	76-58	83.3	45.8
100-56	62.5	29.2	130-60	87.5	30.9
330-56	62.5	41.7	347-56	87.5	75.0
342-56	62.5	50.0	70-56	91.7	91.7

Table 2. Analyses of variance, heritabilities and genetic gains for percent pitch canker symptom expression (Symp) and mortality (Mort) in slash pine.

		Pitch Canker			
		Symp		Mort	
		Analysis of Variance			
Source	DF	MS	F	MS	F
Block	2	3857.6	10.40*	1574.8	5.58*
Families	45	927.8	2.50*	1070.2	3.79*
Error	90	371.0		282.5	
		Heritabilities			
Individual		.253		.383	
Family Mean		.600		.736	
		Genetic Gains			
		----------(% of Mean)----------			
Short-term Options -					
Seed Production Area		39.4		107.7	
Tested Clones in Existing Seed Orchard		16.8		41.4	
Long-term Options -					
Orchard of Untested Select Trees		54.5		148.9	
Orchard of Tested Clones		60.7		149.3	

*Significant at the 1% level.

Family differences were somewhat greater for percent mortality than for percent symptoms, and this relationship was further evidenced in heritabilities and genetic gains (Table 2). Individual tree heritabilities were strong and family heritabilities also suggested potential for genetic gain.

A variety of short- and long-term options are available for realizing the genetic potential for reducing pitch canker incidence. Short-term alternatives include seed production areas in heavily-infected plantations or natural stands in epidemic areas, a very successful strategy for developing fusiform rust resistance (Goddard et al. 1975), and collection of seed from tested clones in established seed orchards. The more productive alternative appears to be seed production areas (Table 2). Assuming that 20 clones of a typical 50-clone orchard will be resistant and contribute seed, existing orchards provide a relatively small, but still meaningful, gain.

The long-term options involving new clonal orchards offer greater improvement. An orchard of proven pitch canker resistant clones would provide slightly more gain than an orchard of untested clones derived from pitch canker epidemic areas.

Simultaneous implementation of short- and long-term options, if possible, is desirable for developing progressively resistant planting stock. Conversion of stands to seed production areas is a relatively low cost alternative applicable to some forestry organizations. Initiation of new orchards is immediately possible due to ongoing screening efforts which have identified more than 25 resistant trees.

Correlations between pitch canker resistance, rust resistance, fast growth and tree height were insignificant (Table 3). Lateral shoot response showed a strong relationship to the response of terminal shoots (Table 3) notwithstanding the differences in shoot size and phenology . These results suggest that fast growth and rust resistance need not be compromised in selecting for pitch canker resistance. Several of these 46 clones were superior in all three characteristics.

Additional testing involving different environments and additional clones will be conducted in 1985-86. Data obtained will permit expanded examination of the results reported in this paper.

CONCLUSIONS

These results from inoculations of vigorous, healthy, field-grown trees suggest that genetic resistance to pitch canker is present in slash pine and that significant genetic gains may be realized.

Pitch canker resistance appears to be unrelated to either rust resistance or fast growth, indicating that fast-growing, rust-resistant and pitch canker resistant trees may be selected.

Table 3. Correlation coefficients among slash pine family means for terminal (Term) and lateral (Lat) shoots pitch canker symptom expression (Symp) and mortality (Mort), tree height and clonal rust and growth evaluations.

	Shoots				Clonal Evaluation	
	Term.	Lat.				
Trait	Mort	Symp	Mort	Tree Height	Rust	Growth
Term - Symp	.78*	.79*	.72*	.10	-.01	.05
Mort		.73*	.77*	.09	-.01	-.05
Lat - Symp			.87*	-.14	.03	-.04
Mort				-.11	-.01	-.01
Tree Height					.02	-.08
Clonal Rust						-.25

*Significant at the 1% level.

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COLD TOLERANCE VARIATION IN LOBLOLLY PINE NEEDLES FROM DIFFERENT BRANCH TYPES, FAMILIES, AND ENVIRONMENTS

T. E. Kolb and K. C. Steiner[1/]

Abstract.--The cold tolerances of loblolly pine needles from different open-pollinated families, branch types, field blocks, and test locations were measured by the electrical conductivity method. Significant differences in tolerance were found between families, "upper" and "lower" growth internodes, blocks, and locations. Family differences in tolerance were more pronounced among 1-year-old seedlings in a nursery environment than among 12- and 13-year-old trees in plantation environments. Results indicate that non-genetic sources of variation and genotype x environment interaction may bias assessments of cold tolerance genetic variation in loblolly pine.

Keywords: Pinus taeda, cold tolerance, genetic variation.

INTRODUCTION

Loblolly pine (Pinus taeda L.) is the preferred pulpwood species for many areas located immediately north of its natural range (Allen 1953, Aughanbaugh 1957, Lambeth et al. 1984). However, winter injury to loblolly pine in these areas may reduce growth rates or cause mortality, thus reducing productivity below the potential for the site (Boggess and McMillan 1954, Minckler 1952, Thor 1967, Wells and Rink 1984). Consequently, loblolly pine genetic improvement programs should stress the development of varieties possessing both cold tolerance and rapid growth rates.

Reliable techniques of screening loblolly pines for genetic differences in cold tolerance are needed to expedite the production of hardy varieties. Assessments of cold tolerance differences among progenies in field tests are desirable since the trees acclimate under natural environmental conditions. However, field assessments depend on the fortuitous occurrence of adequate test winters and may require observations over many years to detect anything but large differences in tolerance. A modification of the electrical conductivity method (Dexter et al. 1930, 1932) was used by Kolb et al. (1985) to accurately measure differences in cold tolerance among open-pollinated families of loblolly pine growing in a western Kentucky field test. This paper reports the results of two studies designed to identify non-genetic sources of variability which may bias assessments of cold tolerance genetic variation in loblolly pine. Study One emphasized within-tree and field block sources of variability, while Study Two emphasized ontogenetic and plantation sources of variability.

[1/]The authors are, respectively, Graduate Research Assistant, and Associate Professor of Forest Genetics, Forest Resources Laboratory, The Pennsylvania State University, University Park, Pennsylvania 16802. Journal Article No. of the Pennsylvania Agricultural Experiment Station. The authors wish to acknowledge the financial assistance provided by Westvaco Corp. in performing the research and the valuable contributions of Henry F. Barbour in executing the study.

STUDY ONE

Methods and Materials

On December 15, 1982, needle samples from three open-pollinated families (1-31, 6-20, 18-94) were collected from each of four field blocks in the Westvaco Corporation's 1976 progeny test in Calloway County, Kentucky. These families represent a wide range of hardiness based on assessments of winter injuries that occurred in the progeny test in 1977 (Kolb et al. 1985). Family 1-31 was the most hardy of these families, 6-20 was intermediate, and family 18-94 was the least hardy.

In each field block, collections consisted of two lower and two upper growth internodes from branches which flushed two or three times in 1982. The "upper" growth internodes on three-flush branches were defined as the middle internode, and "lower" internodes were consistently those in the lowermost position on both two-and three-flush branches. Two- and three-flush branches were collected from one tree each in a ten-tree row plot for each family in a block. The collection of samples was limited to branches formed from the main stem in 1981 on the north side of the tree. This sampling scheme produced 48 treatment combinations in factorial arrangement: 3 families, 4 field blocks, 2 branch types (two- and three-flush), and 2 internodal positions, each combination represented by samples from two branches on the same tree. Samples were promptly packed into coolers and mailed to University Park, Pennsylvania, where they arrived the morning following collection.

Samples were prepared for laboratory exposure to low temperatures by bulking an approximately equal number of needles from the two branches representing each treatment combination, cutting these into 5 cm segments from the fasicle end, and randomly choosing approximately 25 segments for each desired temperature exposure. Needle samples of each treatment combination were commonly exposed in a freezing chamber to the following temperatures: 5°C (unfrozen control), -10°C, -15°C, -20°C, -25°C, -30°C, -35°C, -40°C, -45°C. The temperature in the chamber was lowered at a rate of 4°C per hour, and each desired temperature exposure was maintained for 30 minutes. Needle samples were removed from the chamber following each desired exposure, and slowly thawed to an ambient temperature of 5°C. Electrolytes from each sample diffused into 10 ml of deionized water for 24 hours after thawing. The electrical conductivity of each diffusate solution was measured both before and after an autoclaving treatment at 245°C for 30 minutes.

As initially described by Dexter et al. (1930, 1932), the electrical conductivity of diffusate from plant tissues injured by low temperatures is higher than that of diffusate from uninjured tissues. To obtain a measure of injury due to low temperature exposure, a "relative electrical conductivity" was calculated for the diffusate solution from each sample by dividing the electrical conductivity before autoclaving by the electrical conductivity after autoclaving. This index eliminates spurious effects caused by the tendency of some samples to have a higher electrical conductivity due to differences in needle sample size or nutrient status (Wilner 1959).

Analysis of variance on observations of relative electrical conductivity were used to determine if cold tolerance varied between needles from different sources. In these analyses, the temperature x needle source interaction was of primary interest since the significance of this term indicated whether needles from different growth internodes, branch types, families, or blocks varied in their injury response to temperature and consequently cold tolerance.

Results

The first hypothesis of interest was that the cold tolerance of loblolly pine needles does not differ between lower and upper growth internodes. This was tested by an analysis of variance on observations of relative electrical conductivity for upper and lower growth internodes averaged over two- and three-flush branches and families. Needles from lower growth internodes were significantly ($p < 0.01$) less cold tolerant then those from upper growth internodes. This difference is illustrated in Figure 1-- needles from lower internodes were injured more rapidly in response to decreasing temperature than needles from upper growth internodes.

The second hypothesis of interest was that tolerance does not differ between needles from two- and three-flush branches. This was tested by analysis of variance which compared the tolerances of needles from the two branch types averaged over families for lower and upper internodes, respectively. Needles from comparable internodes (lower or upper) did not differ significantly in tolerance when obtained from either two- or three-flush branches. As shown in Figure 2, needles from lower internodes on two- and three-flush branches were injured approximately the same. However, Figure 3 shows a possible difference in response between needles from upper internodes of two- and three-flush branches. Thus, needles from upper internodes may differ slightly in cold tolerance between branch types (although not significant in this experiment), while needles from lower internodes appear to be relatively stable in tolerance with respect to branch type.

The final hypotheses of interest were that the cold tolerance of needles does not differ among the three families and four field blocks sampled in this experiment. To test these hypotheses, data for the three families were averaged over two- and three-flush branches using only lower internodes, as suggested by the results of the previous analysis. Families did not differ significantly in tolerance. However, the pattern of response of families to temperature shown in Figure 4 is identical to that suggested by winter injury to families under field conditions in 1977: family 18-94 was injured the most rapidly, 6-20 was intermediate, and 1-31 was injured the least rapidly. This comparison suggests that cold tolerance varied among families, but that these differences were not statistically detectable at the level of precision present in this experiment.

It was also apparent in this analysis that needles from the four field blocks differed significantly ($p < 0.005$) in tolerance. As shown in Figure 5, needles from block one were injured much more rapidly than needles from other blocks. These block influences on cold tolerance are presumably related to variations in microsite within the plantation and perhaps related to the fact that block one is located in a somewhat moister area than the others.

STUDY TWO

Methods and Materials

On February 8, 1984, needle samples were collected from each of four open-pollinated families of loblolly pine (3-4, 3-41, 6-8, 6-22) at Westvaco's 1972 test in Livingston County, Kentucky (13-year-old trees), Westvaco's 1973 test in Hickman County, Kentucky (12-year-old trees), and in Westvaco's progeny test seedlings at the J. P. Rhody (Kentucky State) Nursery, Marshall County, Kentucky (1-year-old trees). For each family, progenies at all three test locations originated from the same seed orchard, but not necessarily the same seedlot. Progenies 3-4 and 3-41 are from the Champion International seed orchard in Newberry, South Carolina, and progenies 6-8 and 6-22 are from the Champion International seed orchard in Tillary, North Carolina.

For the 1972 and 1973 tests, a lower internodal segment of 1983 twig growth from a lateral branch formed on the main stem in 1982 was collected from seven trees per family in each of three field blocks. For each family in the nursery, twenty whole seedlings were collected from each of two blocks. All collections were packed into coolers and mailed to University Park, Pennsylvania, where they arrived the next morning.

The preparation of samples, the freezing process, and the measurement of injury was identical to that described for Study One with the following exceptions: 1) needles collected from blocks in the field were divided into four replications for laboratory analysis, and 2) temperature treatments of -10°C, -40°C, and -45°C were excluded. Relative conductivity data from each of the test locations were subjected to analysis of variance to determine if genetic differences in tolerance among families were detectable in each environment. A combined analysis of variance was used to determine whether test locations influenced overall levels of tolerance, as well as relative differences among families.

To make specific comparisons of cold tolerance, injury response curves were formulated by regressing mean relative conductivity on temperature treatment using the following model:

$$Y_{ij} = b_{oij} + b_{1ij}X + b_{2ij}X^2 + E_{ij}, \text{ where}$$

Y_{ij} = mean relative conductivity for family "i" at test location "j" for each temperature treatment

X = temperature treatment

E_{ij} = residual for family "i" at test location "j"

Similar regressions were performed on overall means at each test location to compare injury responses among environments. The model fit the data adequately, as indicated by R^2 values which ranged from 0.91 to 0.99.

Results

Overall levels of tolerance differed significantly ($p < 0.005$) among the three test locations as illustrated by injury response curves shown in Figure 6. One-year-old seedlings from the nursery were generally the least tolerant, trees from the 1973 test were intermediate, and trees from the 1972 test were the most tolerant. Test location also influenced differences in tolerance among families (family x temperature x location interaction significant, $p < 0.005$). Injury response curves shown in Figures 7 and 8 indicate no significant differences in tolerance among families in the 1973 and 1972 test. In contrast, family 3-41 was significantly ($p < 0.005$) less tolerant than the other families in the nursery (Figure 9).

DISCUSSION

These studies indicate that differences in the cold tolerance of loblolly pine needles may arise from both genetic and non-genetic sources of variation. Consequently, confounding the sampling of tissues from families or clones with branch positions, field blocks, or test locations may seriously bias genetic assessments of cold tolerance. Consistent sampling is especially important when measuring cold tolerances by the electrical conductivity method because of its sensitivity in detecting differences in injury. Exploratory studies to identify potential sources of variation are a necessary precaution in using indirect measures such as the electrical conductivity method for genetic assessments of cold tolerance in any species.

The differential injury response of families from the three test locations suggests that genetic variation in tolerance may be more pronounced among seedlings in the nursery than among older trees in the field. The reasons causing this interaction cannot be determined in this study since environmental and ontogenetic effects were confounded. It is conceivable that expression of genetic variation was greatest in the nursery environment because of a correlation between tolerance and some aspect of seedling physiology such as response to fertilizers or other cultural treatments used in the nursery. Useful assessments of cold tolerance in loblolly pine breeding programs will be complicated if such genotype x environment or genotype x age interactions are common.

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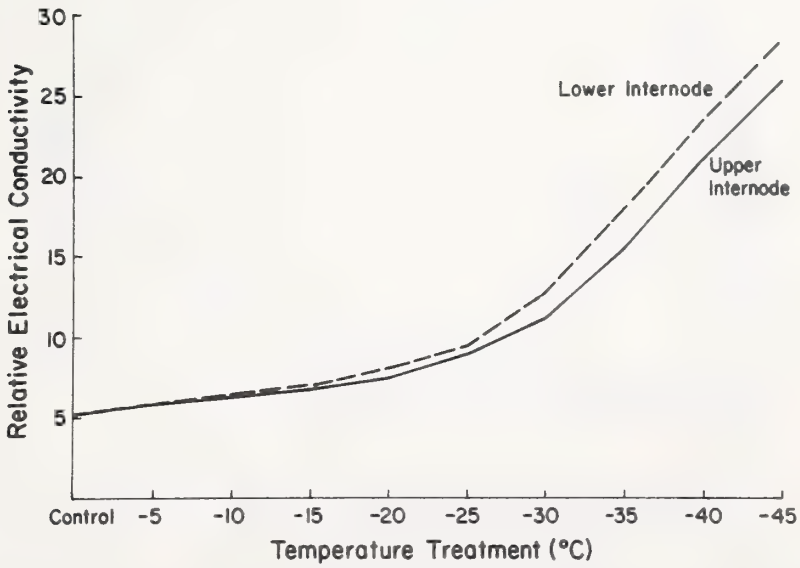

Figure 1.--December 1982 injury (Relative Electrical Conductivity) to needle tissues versus temperature treatment for "lower" and "upper" growth internodes. Graphs adjusted to a common y-intercept.

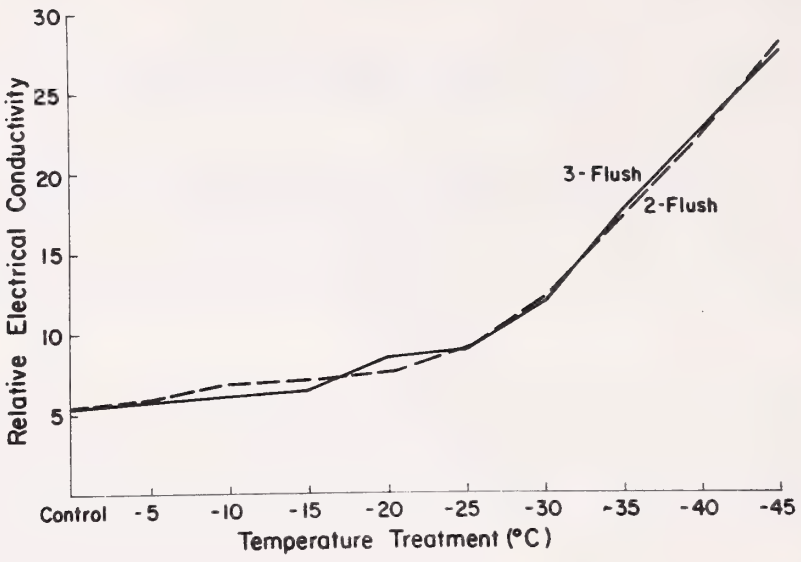

Figure 2.--December 1982 injury (Relative Electrical Conductivity) to needle tissues versus temperature treatment for lower internodes of "two-flush" and "three-flush" branches. Graphs adjusted to a common y-intercept.

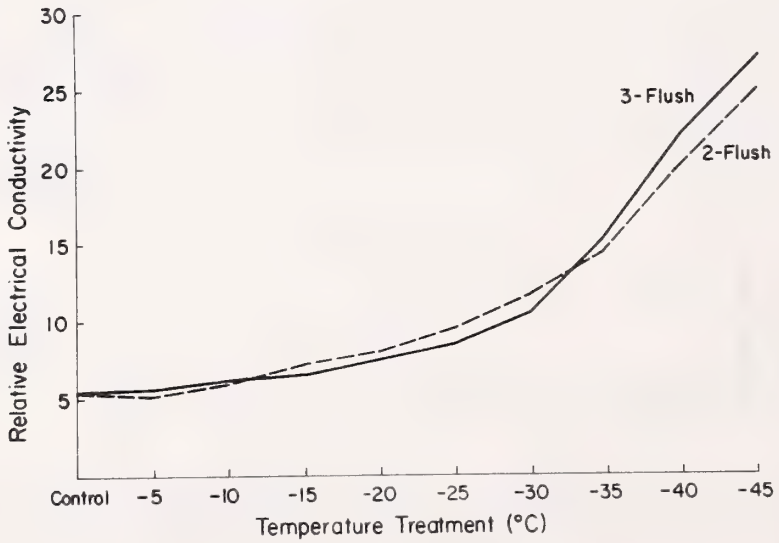

Figure 3.--December 1982 injury (Relative Electrical Conductivity) to needle tissues versus temperature treatment for upper internodes of "two-flush" and "three-flush" branches. Graphs adjusted to a common y-intercept.

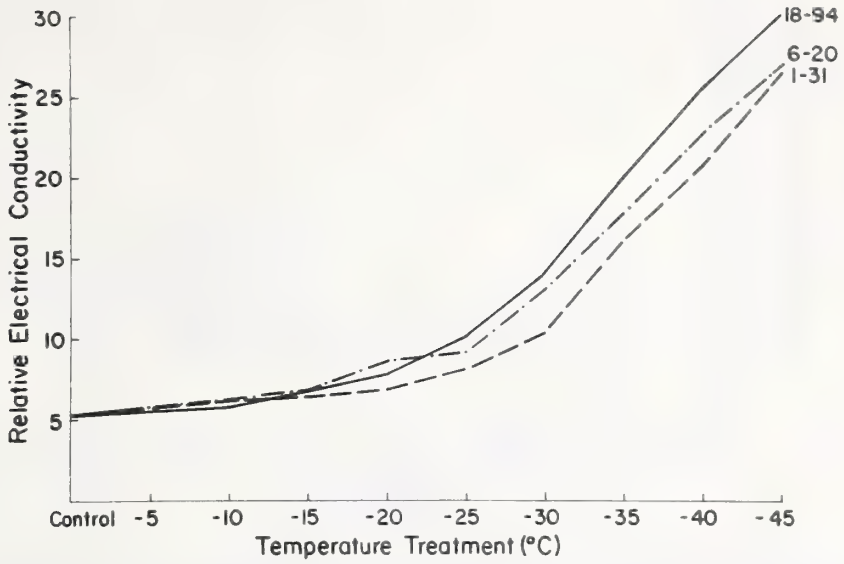

Figure 4.--December 1982 injury (Relative Electrical Conductivity) to needle tissues versus temperature treatment for lower internodes of three open-pollinated families. Graphs adjusted to a common y-intercept.

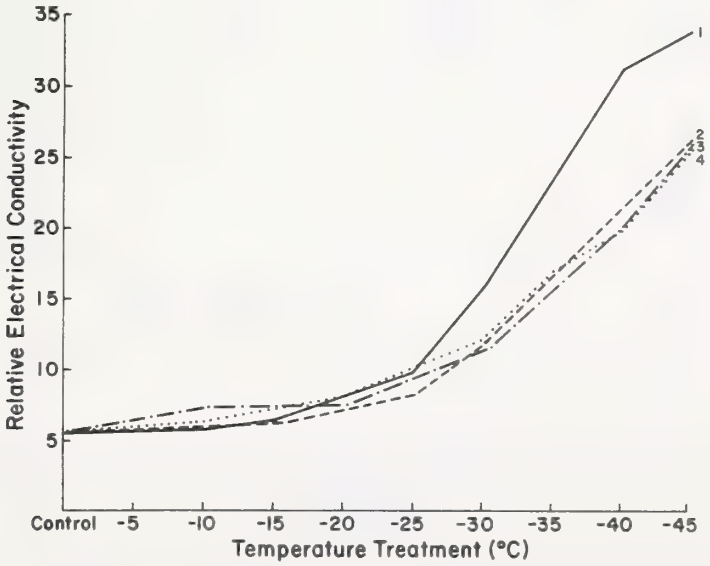

Figure 5.--December 1982 injury (Relative Electrical Conductivity) to needle tissues versus temperature treatment for four field blocks. Graphs adjusted to a common y-intercept.

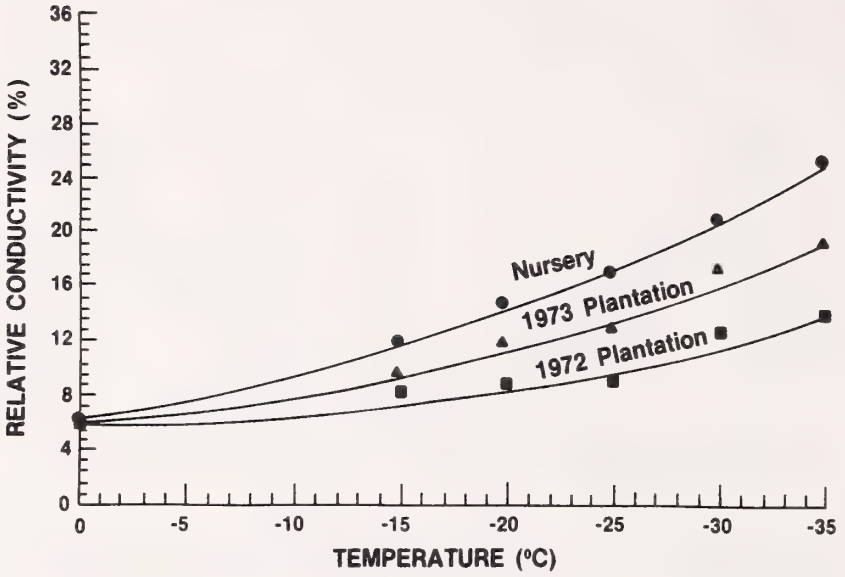

Figure 6.--February 1984 injury (Relative Conductivity) to needle tissues versus temperature treatment for four families in each of three environments. Graphs adjusted to a common y-intercept.

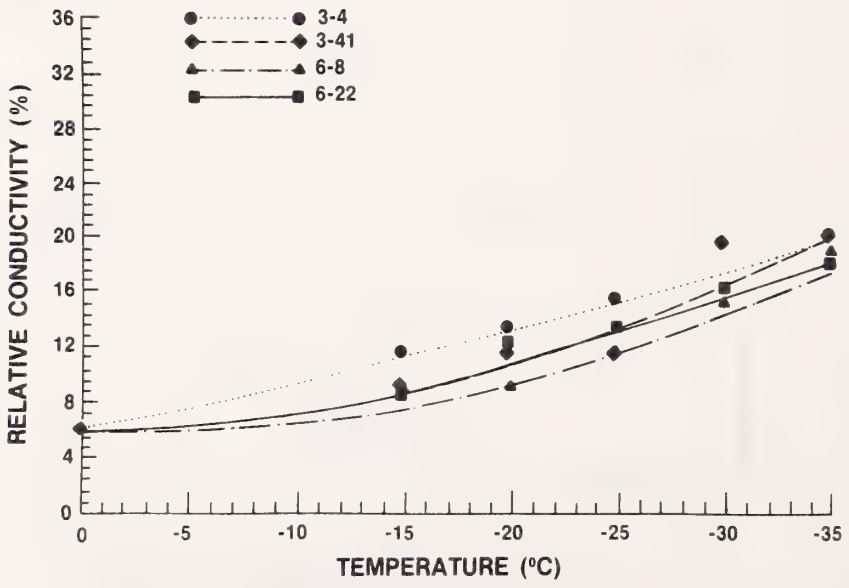

Figure 7.-- February 1984 injury (Relative Conductivity) to needle tissues versus temperature treatment for each of four families growing in the 1973 plantation environment. Graphs adjusted to a common y-intercept.

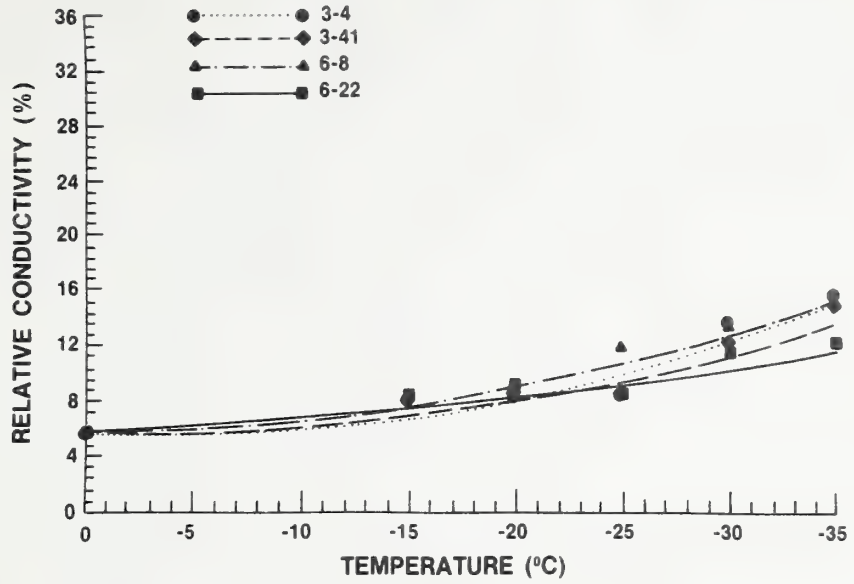

Figure 8.--February 1984 injury (Relative Conductivity) to needle tissues versus temperature treatment for each of four families growing in the 1972 plantation environment. Graphs adjusted to a common y-intercept.

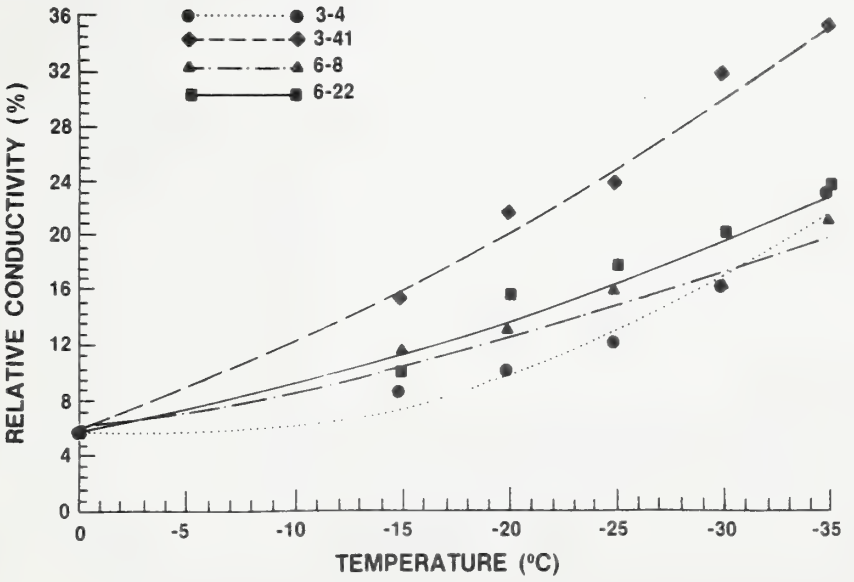

Figure 9.--February 1984 injury (Relative Conductivity) to needle tissues versus temperature treatment for each of four families growing in the nursery environment. Graphs adjusted to a common y-intercept.

POSTERS

ADVENTIVE EMBRYOGENESIS IN YELLOW-POPLAR TISSUE CULTURES: A PRELIMINARY REPORT

S. A. Merkle, H. E. Sommer and C. L. Brown[1]

Among hardwood forest tree species, there have been only a few reports of adventive embryogeny. We initiated cultures of yellow-poplar (Liriodendron tulipifera) from embryos from seeds collected from a single tree at two week intervals between mid-August and late October, 1984. Proembryogenic nodules developed from the explants 5-6 weeks after cultures were transferred to a medium containing 2,4-D and 6BA. Within a month following transfer of proembryogenic nodules to a hormone-free medium supplemented with casein hydrolysate, embryoids differentiated. Prembryogenic cultures have also been grown in suspension culture and produced embryoids. All four cultures that produced embryoids originated from immature embryos from seed collected during the last week of August and the first week of September. Although most embryoids appeared abnormal, those with well-formed cotyledons and radicles were capable of developing into normal plantlets.

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[1]Postdoctoral Associate, Associate Professor and Professor Emeritus, School of Forest Resources, University of Georgia, Athens, GA 30602.

DIFFERENCES IN SEED PROPERTIES AMONG RECIPROCAL CROSSES OF LOBLOLLY PINE
(A preliminary report)

Thomas O. Perry and Theodore H. Shear

ABSTRACT

In order to determine the relative effects of seed and pollen parents on seed properties and initial germination and growth of loblolly pine seedlings, selected reciprocal crosses among 14 loblolly pine clones were made. Measurements of the total seed weights, seed coat and gametophyte weights, and the percent of filled seeds are reported in this display.

Both the seed parent and the pollen parent affected the weight of the seed. Statistical analyses indicated that the seed parent accounted for 66% of the variation in total seed weight and the pollen parent accounted for an additional 15%. Some clones were particularly poor seed parents while they functioned well as pollen parents.

Planned germination studies and progeny tests will determine the differences in progeny performance among reciprocal crosses. In the meanwhile, tree improvement workers should be aware that there are large and significant differences in the quality of seeds between a cross of A x B and its reciprocal B x A.

VARIATION IN FUSIFORM RUST STEM GALLS ON FIVE- AND SIX-YEAR-OLD SLASH PINES

Charles H. Walkinshaw[1]

Infection of slash pines (Pinus elliottii Engelm. var. elliottii) by Cronartium quercuum (Berk.) Miyabe ex Shirai f. sp. fusiforme produces many identifiable external symptoms as well as internal damage. This study classifies symptoms on galled 5- and 6-year-old slash pine stems. Observations were made in progeny tests near Gulfport, Mississippi; Greenwood, Florida; and Savannah, Georgia. Data and photographs of approximately 20 families and 5 replications per location serve as the basis for this poster.

Galls on apical dominant stems are thin, one-sided, small, typical, or fat. The first three of these five types appear inocuous unless they develop into constricted galls. Some galls cause a loss or reduction of apical dominance and stimulate the production of auxiliary stems. These are stem to branch, branch to stem, double stems, or multiple stems (often termed witches brooms). Trees with auxiliary stems may split in windstorms and generally die 2-5 years following infection. By the fifth growing season 23% of rust-affected trees have formed witches brooms and another 22% have grown auxiliary stems while retaining a dominant main stem. Galls on dominant or abnormal stems can kill affected slash pines by girdling the cambium. Constriction and girdling of pine stems have long been associated with wounding by mechanical means, incompatibility of scion and stock, and infections such as fusiform rust. Stem tissues above the constriction increase to form an elongated upward taper. Constrictions may form in thin, typical or fat galls; the cambium is destroyed within the gall. Incidence of constricted stem galls in the progeny tests was 0 to 27% for control-pollinated families.

These preliminary results indicate that infected progeny from different parents exhibit large variations in external symptoms. Families M-601 and C-115 in the Florida test had similiar percentages of infected trees. However, their percentages with branch galls were 80 and 46, respectively. Incidence of witches brooms was 25 and 38%, and mortality was 19 and 54%, respectively. Thus, disease severity was significantly different in these two families. Disease severity is governed by type of stem gall and the age of the tree at time of infection. Thin, one-sided, and small stem galls appear to cause minimal tree distortion unless they become constricted galls. Galls which cause loss of apical dominance ruin the affected trees for forest products.

Knowledge of gall types may have application in selecting for rust resistance in slash pine. A family which forms a high percentage of thin, one-sided or small galls on dominant stems has the greatest chance of survival. Typical and fat stem galls, and especially those showing constriction reduce height growth. Once this occurs, mortality usually follows within one or two growing seasons.

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[1]Principal Plant Pathologist, USDA, Forest Service, Southern Forest Experiment Station, P.O. Box 2008, GMF, Gulfport, MS 39505.

APPENDIX

SOUTHERN FOREST TREE IMPROVEMENT COMMITTEE

Membership List

May 5, 1985

GROUP A

Representatives Appointed for 6-year term

Committee Membership	Term Ends (at end of Annual meeting)	Representing
E. C. Burkhardt Route 12, Box 92 Vicksburg, MS 39180 (601)638-2305	1989	Hardwood Research Council
Marvin Zoerb Union Camp Corp. P. O. Box 216 Rincon, GA 31326 (912)826-5556	1989	Pulp and Paper Industry
Siroos T. Jahromi International Paper Co. Southlands Experiment Forest Rt. 1, Box 571 Bainbridge, GA 31717 (912)246-3642	1986	Pulp and Paper Industry
Dave Canavera Westvaco Summerville, SC 29483 (803)871-5000	1986	Pulp and Paper Industry
Mr. Garner Barnum Management Forester Arkansas Forestry Commission P. O. Box 4523, Asher Station 3821 W. Roosevelt Rd. Little Rock, AR 72214 (501)664-2531	1988	State Forestry Agencies
Mr. C. R. Nichols Asst. State Forester South Carolina Forestry Comm. P. O. Box 21707 Columbus, SC 29221 (803)758-6900	1986	State Forestry Agencies

Committee Membership	Term Ends (at end of Annual meeting)	Representing
James Bright Tree Improvement Forester Mississippi Forestry Comm. 908 Robert E. Lee Bldg. Jackson, MS 39201 (601)359-1386	1988	State Forestry Agencies
Charles G. Tauer 08 AG Hall Oklahoma State University Stillwater, OK 74074 (405)624-5462	1989	Forestry Schools
Bruce Bongarten School of Forest Resources Univ. of Georgia Athens, GA 30602 (404)542-7247	1986	Forestry Schools
Sam B. Land, Jr. Dept. of Forestry Mississippi State Univ. P. O. Drawer FR Mississippi State, MS 39762 (601)325-2946	1988	Forestry Schools
Sam Foster Crown-Zellerbach Corp. Southern Timber Division Box 400 Bogalusa, LA 70427 (504)735-4602	1986	Southern Forest Products Assoc.

GROUP B

Representatives Appointed for Indefinite Terms

John Kraus USDA Forest Service Southeastern For. Exp. Sta. Georgia Forestry Center Route 1, Box 182A Dry Branch, GA 31020 (912)744-0266	U. S. Forest Service

Committee Membership	Term Ends (at end of Annual meeting)	Representing
O. O. Wells Institute of Forest Genetics P. O. Box 2008, GMF Gulfport, MS 39503 (601)864-3972		U. S. Forest Service Southern For. Exp. Sta.
Clark Lantz Nursery/Tree Improvement Specialist U. S. Forest Service Suite 300 1720 Peachtree Road, N.W. Atlanta, GA 30356 (404) 881-3551		U. S. Forest Service State & Private Forestry

GROUP C

Specialists Appointed for 6-year Term

O. O. Wells Institute of Forest Genetics P. O. Box 2008, GMF Gulfport, MS 39503 (601) 864-3972	1984	Racial Variation
Tom Miller, IPM School of Forest Resources University of Florida Gainesville, FL 32611 (904) 392-4826	1987	Pathology
Harry O. Yates Southeastern For. Expt. Sta. Carlton Street Athens, GA 30601 (404) 546-2467	1984	Entomology
Floyd Bridgwater N. C. State University Box 8002 Raleigh, NC 27650-8002 (919) 737-3168	1987	Pollen Management

Committee Membership	Term Ends (at end of annual meeting)	Representing
Warren Nance U.S.D.A. - Forest Service Southern For. Expt. Sta. Forestry Sciences Lab. Box 2008 Gulfport, Mississippi 39503	1990	Stand Dynamics
Ralph Mott Botany Dept. Box 7612 North Carolina State Univ. Raleigh, N.C. 27650-7612	1990	Forest Biotechnology
	GROUP D	
James L. McConnell U. S. Forest Service Suite 828 1720 Peachtree Road, N.W. Atlanta, GA 30309 (404) 881-3846		U.S. Forest Service Regional Office Region 8
J. P. van Buijtenen Forest Genetics Lab College Station, TX 77843 (713) 845-1325		Western Gulf Regional Tree Improvement Cooperative
Tim White School of Forest Resources and Conservation University of Florida Gainesville, FL 32611 (904) 392-1850		University of Florida Tree Improvement Cooperative
Bob Weir Forestry Department Box 8002 Raleigh, NC 27650-8002 (919) 737-3168		North Carolina State Tree Improvement Cooperative

SOUTHERN FOREST TREE IMPROVEMENT CONFERENCE

May 21-23, 1985

Registrants

Abbott, Jerry E.
Kirby Forest Industries, Inc.
P. O. Box 577
Silsbee, TX 77706
(409) 385-5201

Adams, John C.
School of Forestry
Louisiana Tech University
Ruston, LA 71270

Allen, Robert M.
Dept. of Forestry
Clemson University
Clemson, SC 29631
(803) 656 3302

Arnold, Billy G.
Georgia Kraft Co.
Genetics Dev. Sec.
P. O. Box 296
Greensboro, GA 30642
(404) 485-8363

Askew, Dr. George R.
Clemson University
P. O. Box 596
Georgetown, SC 29442
(803) 546-4402

Bailian, Li
Dept. of Forestry
NC State University
709 Latta St.
Raleigh, NC 27607

Barbour, Jill
Owens Illinois, Inc.
P. O. Drawer A
White Springs, FL 32096
(904) 397-2171

Barnum, Garner
Arkansas Forestry Commission
P. O. Box 4523, Asher Station
Little Rock, AR 72214
(501) 664-2531

Barras, Dr. Stanley
USDA-Forest Service
RM T-10210 701 Loyola Ave.
New Orleans, LA 70113
(504) 589-3003

Beineke, Walter
Dept. of Forestry Purdue
Purdue University
West Lafayette, IN 47907
(317) 494-3611

Benson, James D.
Dept. of Forestry
Clemson University
Lehotsky Hall
Clemson, SC 29631
(803) 656-3302

Bartlum, Guy N.
St. Joe Paper Co.
Rt 1 Box 70
Lamont, FL 32336
(904) 997-0526

Blanton, Steve A.
Alabama Forestry Commission
Rt 1 P.O. Box 88-B
Thorsby, AL 35171

Blush, Tom
Westvaco
P. O. Box 1950
Summerville, SC 29484
(803) 871-5000 Ext 547

Boltz, Brett
School of Forest Resources
University of Georgia
Athens, GA 30602
(404) 542-7247

Bongarten, Bruce
School of Forest Resources
University of Georgia
Athens, GA 30602
(404) 542-7247

Bramlett, David L.
U.S. Forest Seravice
Rt 1 Box 182A
Dry Branch, GA 31020
(919)744-0261

Bright, Jim
Mississippi Forestry Commission
908 Robert E. Lee Bldg
Jackson, MS 39201
(601) 359-1386

Bridgewater, Floyd
School of Forest Resources
NC State University
P. O. Box 8002
Raleigh, NC 27695

Brooks, Terrell
Georgia Forestry Commission
P. O. Box 819
Macon, GA 31298
(912) 744-3369

Brouard, John
NC State University
201 B Dixie Trail
Raleigh, NC 27607
(919) 737-3420

Brown, Raymond
Champion International
P. O. Box 97
Tillery, NC 27887
(919)826-4182

Bryan, Harry
Hiwassee Land Company
Chatsworth, LA 30705
(404) 334-2422

Bryant, Richard
International Paper Company
Rt 1 Box 571
Bainbridge, GA 31717
(912) 246-3642

Buckles, Oliver W.
U.S. Forest Service
P. O. Box 757
Moncks Corner, SC 29461
(803) 336-3246

Buford, Marilyn
Virginia Polytechnic Inst.
Department of Forestry
Blacksburg, VA 24061
(703) 961-6603

Byram, Tom
Texas Forest Service
Forest Science Laboratory
College Station, TX 77843
(409) 845-2556

Canavera, David
Westvaco
P. O. Box 1950
Summerville, SC 29484
(803) 871-5000

Chase, Charles D.
St. Joe Paper Company
Rt 1 Box 70
Lamont, FL 32336
(904) 997-0526

Coleman, Stephen
Boise Cascade
P. O. Box 45
Singer, LA 70660
(318) 463-9681

Comer, C. W.
University of Florida
118 N-Z Hall
Gainesville, FL 32611
(904) 392-4851

Cox, Russell A.
Tennessee Div of Forestry
P. O. Box 120
Pinson, TN 38366
(901) 988-5221

Crane, Barbara S.
Georgia Kraft Company
Genetics Dev. Sec.
Greensboro, GA 30642
(404) 485-8363

Cunningham, Michael
NC State University
School of Forest Resources
Box 8002
Raleigh, NC 27695
(919) 737-3420

Davison, R. Marc
Champion International
P. O. Box 97
Tillery, NC 27887
(919) 826-4182

Debarr, Gary L.
USDA-Forest Service
Carlton Street
Athens, GA 30602
(404) 546-2467

Dewald, Laura E.
Virginia Polytechnic Institute
and State University
228 Cheatham Hall
Blacksburg, VA 24061
(703) 961-5300

Dinus, Dr. Ronald J.
International Paper Company
P. O. Box 797
Tuxedo Park, NY 10987
(914) 351-2101

Duba, Dr. Stuart
Auburn University
School of Forestry
Auburn University, AL 36849
(205) 826-4050

Dvorak, William S.
Camcore Cooperative
NC State University
P. O. Box 8007
Raleigh, NC 27195
(919) 737-2738

Einspahr, Dean W.
Institute of Paper Chemistry
P. O. Box 1039
Appleton, WI 54912
(414) 734-9241
Ext 282

Emery, Bruce
NC State University
2829 Mayview Rd
Raleigh, NC 27607
(919) 737-3168

Feret, Dr. Peter P.
Dept of Forestry
Virginia Polytechnic Institute
and State University
Blacksburg, VA 24060
(703) 961-5943

Fins, Dr. Lauren
University of Idaho
Inland Empire Tree
Improvement Coop
Dept of Forest Resources
Moscow, ID 83843

Foster, G. Sam
Crown Zellerbach Corp.
Southern Timber Division
Box 400
Bogalusa, LA 70427
(504) 735-4602

Frampton, L. John
School of Forest REsources
NC State University
P. O. Box 8002
Raleigh, NC 27695-8002
(919) 737-3168

Gates, James
USFS Kisatchie Natl Forest
2500 Shreveport Hwy
Pineville, LA 71360
(318) 473-7123

Gill, John J.
Georgia Kraft Company
P. O. Box 108
Rome, GA 30161

Goddard, Ray
University of Florida
School of Forest Res. & Conserv.
Gainesville, FL 32611
(904) 3921850

Greenwood, Michael S.
College of Forestry
Nutting Hall
University of Maine
Orono, ME 04469
(207) 581-2838

Griggs, Margene M.
U.S. Forest Service
P. O. Box 2008, GMF
Gulfport, MS 39505
(601) 864-3972

Guiness, Bill
Catawba Timber Company
Box 128
Catawba, SC 29704
(803) 329-6653

Hamlin, Jim
U.S. Forest Service
P. O. Box 1008
Roseburg, OR 97470
(503) 672-6601

Handley, Lee
Westvaco
Box 1950
Summerville, SC 29484
(803) 871-5000

Harbin, Michael C.
Chesapeake Corp
Box 311, 19th & Main St
West Point, VA 23181
(804) 843-5652

Hart, Joel David
Mississippi State Univ
P. O. Drawer FR
Mississippi State, MS 39762

Hartwell, Dianna Lynn
NC State University
2834 Mayview Rd
Raleigh, NC 27607
(919) 737-3420

Hatcher, Alice
School of Forest Resources
NC State University
P. O. Box 8002
Raleigh, NC 27695-8002
(919) 737-3168

Hendrickson, John A.
Scott Paper Company
P. O. Box 899
Saraland, AL 36571
(205) 675-2932

Hodge, Gary
NC State University
School of Forest Research
Box 8002
Raleigh, NC 27695
(919) 737-3420

Hodges, James F.
Brunswick Pulp Land Company
Box 860
Brunswick, GA 31521
(912) 265-5780

Hollowell, Alfred
Weyerhaeuser Comapny
P. O. Box 1060
Hot Springs, AR 71902

Hood, Jim
Ontario Ministry of Natural Res
Forest Res. Branch, Queens Park
Toronto, Ontario L4C 1C6 Canada
(416) 963-1334

Hopkins, Sam
Gulf States Paper Corporation
P. O. Box 3199
Tuscaloosa, AL 35404
(205) 553-6200

Huang, F. H.
University of Arkansas
316 Plant Science Bldg
Fayetteville, AR 72701
(501) 575-2603

Hutto, Erika M.
Alabama Forestry Commission
Rt 2 P. O. Box 151
Kinston, AL 36453

Isik, Kani
University of California
Forestry Department
Mulford Hall 145
Berkeley, CA 94720

Jahromi, Siroos T.
International Paper Coampny
Southlands Experimental Forest
Rt 1 Box 571
Bainbridge, GA 31717
(912) 246-3642

Jernigan, Richard
Georgia Forestry Commission
P. O. Box 819
Macon, GA 31298-4599
(912) 744-3369

Jett, J. B.
School of Forest Resources
NC State University
P. O. Box 8002
Raleigh, NC 27695-8002
(919) 737-3168

John, Sally
NC State University
201B Dixie Trail
Raleigh NC 27607
(919) 737-3420

Dr. Jon Johnson
Department of Forestry
University of Florida
118 N-Z Hall
Gainesville, FL 32611
(904) 392-4851

Jurado-Blanco, Juan B.
NC State University
P. O. Box 5716
Raleigh, NC 27650
(919) 737-2739

Kanashiro, Milton
NC State University
School of Forest Resources
Box 8002
Raleigh, NC 27695
(919) 737-3420

Karrfalt, Robert
USDA Forest Service
Rt 1 Box 182 B
Dry Branch, GA 31020
(912) 744-3314

Kitchens, Frank
School of Forestry
Branch, GA 31020
(912) 744-0266

Kitchens, Robert
USFS Timber RO
1720 Peachtree Rd NW
Atlanta, GA 30367
(404) 881-4039

Kok, H. R.
University of Florida
118 Newins-Ziegler Hall
Gainesville, FL 32611
(904) 392-1850

Kolb, Tom
Penn State University
302-D Forest Resources Lab
University Park, PA 16802
(814) 865-2130

Kormanik, Paul P.
SE Forest Exp Station
Carlton Street
Athens, GA 30602
(404) 546-2435

Kossuth, Dr. Susan V.
USDA Forest Service
1143 Fifield Hall
University of Florida
Gainesville, FL 32611
(904) 371-4360

Kraus, John F.
Southeastern Forest Exp. Station
Rt 1 Box 182-A
Dry Branch, GA 31020
(912) 744-0266

Kung, Dr. Fan H.
Department of Forestry
Southern Illinois University
Carbondale, IL 62901
(618) 453-3341

Lafarge, Timothy
USDA Forest Service
1720 Peachtree Rd NW
Atlanta, GA 30367
(404) 881-4046

Lambeth, Clements C.
Weyerhaeuser Company
900 Whittington Ave.
Hot Springs, AR 71902
(501) 624-8516

Land, Samuel B., Jr.
Mississippi State University
P. O. Drawer FR
Mississippi State, MS 39762
(601) 325-2946

Lantz, Clark W.
USDA Forest Service
1720 Peachtree Rd NW
Atlanta, GA 30367
(404) 881-3554

Layton, Patricia
Building 1503
Oak Ridge National Laabs
P. O. Box X
Oak Ridge, TN 37831
(615) 574-7364

Ledig, Thomas
Institute of Forest Genetics
U.S. Forest Service, PSW
Box 245
Berkeley, CA 94701
(415) 486-3458

Leach, Greg
Chamption International Corp.
P. O. Box 87
Cantonment, FL 32533
(904) 968-6614

Lesney, Mark
University of Florida
Dept. of Forestry
118 N-Z Hall
Gainesville, FL 32611
(94) 392-4851

Lewis, Ralph A.
USDA Forest Service
380 Rock Meadow Dr.
Stone Mountain, GA 30367
(404) 881-4664

Libby, Dr. Bill
University of California
Dept. of Forestry & Conserv.
Mulford Hall
Berkeley, CA 94720

Lowe, William J.
Texas Forest Service
Forest SCience Lab
College Station, TX 77843
(409) 845-2523

Lowerts, George A.
Union Camp Corporation
P. O. Box 216
Rincon, GA 31326
(912) 826-5556

Mabry, Will
Catawba Timber Co.
Box 128
Catawba, SC 29704
(803) 329-6653

Manchester, Edwin H.
USDA Forest Service
201 Woodland Dr.
Murphy, NC 28906
(704) 837-5152

Manley, David
International Paper Co.
Rt 3, Box 312-B
Natchez, MS 39120
(601) 442-7421 Ext 583

Massie, William E., Jr.
Mississippi Forestry Commission
P. O. Bos 468
Lumberton, MS 39544
(601) 796-8892

McConnell, James L.
U.S. Forest Service
1720 Peachtreee Rd
Atlanta, GA 30367
(404) 881-4045

McCutchan, Barbara G.
Westvaco
P. O. Box 1950
Summerville, SC 29484
(803) 871-5000

McKeand, Steve
School of Forest Resources
NC State University
P. O. Box 8002
Raleigh, NC 27695-8002
(919) 737-3168

McKinley, Craig R.
Texas Forest Service
Texas A&M University
Caollege Station, TX 77840
(409) 845-5078

Merkle, Scott A.
University of Georgia
School of Forest Resources
Athens, GA 30602
(404) 542-6112

Miller, Dot
Texas Forest Service
Route 1 P. O. Box 160
Hillister, TX 77624

Miller, Larry
Temple-Eastex, Inc.
229 N. Bowie
Jasper, TX 75951
(409) 384-3434

Morrow, Dan
Dept of Natural Resources
Buckingham Forest
Tree Nursery
Harmans, MD 21077
(301) 768-7367

Mott, Ralph L.
NC State University
Dept of Botany
Raleigh, NC
737-3525

Moye, Jim
Alabama Forestry Commission
Route 2
Kinston, AL 36453

Murray, Gordon
Canadian Forestry Service
Petawawa Natl Forestry Inst.
Chalk River, Ontario
Canada KOJ 1JO
(613) 589-2880

Neal, Ronald B.
American Can Company
P. O. Box 315
Butler, AL 36904
(205) 459-3002

Nelson, Neil D.
Biotechnology Program Leader
NC Forest Experiment Station
Forestry Sciences Laboratory
Box 898
Rhinelander, WI 54501
(715) 362-7474

Newton, R. J.
Dept of Forest Science
Texas A&M University
College Station, TX 77843
(409) 845-8279

O'Gwynn, Claude H.
Weyerhaeuser Company
208 S. Moore Road
Hot Springs, AR 71913
(501) 767-7255

Padgett, Bill
Alabama Forestry Commission
513 Madison Avenue
Montgomery, AL 36130
(205) 261-2532

Pait, John
Container Corp. of America
Box 626
Callahan, FL 32011
(904) 879-3051

Park, Yill Sung
Canadian Forestry Service
P. O. Box 4000
Fredericton, NB E3B 1P4 Canada
(506) 452-3585

Platt, Adlai
Champion International Corp
P. O. Box 834
Newberry, SC 29108
(803) 276-5529

Post, Boyd W.
Coop State Research Service
J. S. Morrill Bldg
15th & Independence
Washington, D.C. 20251
(202) 447-2016

Powell, Gregory L.
University of Florida
118 Newins-Ziegler Hall
Gainesville, FL 32611
(904) 392-1850

Ranney, J. W.
Oak Ridge National Lab
Box X
Oak Ridge, TN 37831
(615) 574-7364

Reddy, K. V.
University of Florida
118 N-Z Hall
Department of Forestry
Gainesville, FL 32611
(904) 392-1850

Reighard, Greg
University of Florida
118 Newins-Ziegler Hall
Gainesville, FL 32611
(904) 392-1850

Rhinehardt, George
International Paper Company
Bellamy Seed Orchard
Rt 3 Box 405
Marianna, FL 32446
(904) 594-6001

Rockwood, D. L.
University of Florida
118 Newins-Ziegler Hall
Gainesville, FL 32611
(904) 392-1852

Rounsaville, Marc
USFS
P. O. Box 248
Wiggins, MS 39577
(601) 928-4792

Rousseau, Randall
Westvaco
221 Lansing Dr.
Paducah, KY 42001
(502) 335-3156

Ruzin, Dr. Steven E.
Plant Genetics, Inc.
1930 Fifth St.
Davis, CA 95616
(916) 753-1400

San Fratello, Guy
SC Forestry Commission
P. O. Box 157
Tillman, SC 29943
726-3845

Schlarbaum, Scott E.
Dept of Forestry, Wildlife
and Fisheries
University of Tennessee
Knoxville, TN 37901-1071
(615) 974-7126

Schmidtling, Ron
U. S. Forest Service
P. O. Box 2008, GMF
Gulfport, MS 39505
(601) 864-3972

Schoenihe, Roland E.
Clemson University
Department of Forestry
Clemson, SC 29631
(803) 656-3302

Schultz, Emily B.
Mississippi State University
School of Forest Resources
P. O. Drawer FR
Mississippi State, MS 39762
(601) 325-2946

Sederoff, Ron
Forest Genetics, PSW
P. O. Box 245
Berkeley, CA 94701
(415) 486-3134

Shaw, Douglas U.
International Forest Seed Co.
P. O. Box 290
Odenville, AL 35120
(205) 323-0150

Shear, Ted
5016 Newcastle Road
Raleigh, NC 27606
(919) 851-0374

Shimzu, Jarbas Yukio
Embrapa-Centro Nacional De Presq.
De Florestas
Caixa Postal 3319
8000 Curitiba-Parana, Brazil

Sluder, Earl R.
SE Forest Experiment Station
Rt 1 Box 182A
Dry Branch, GA 31020
(912) 744-0266

Smeltzer, Richard H.
International Paper Company
Route 3, Box 312-B
Natchez, MS 39120
(601) 442-7421 Ext 587

Snow, Glenn A.
U. S. Forest Service
P. O. Box 2008, GMF
Gulfport, MS 39505
(601) 864-8256

Sommer, Harry E.
School of Forest Resources
University of Georgia
Athens, GA 30602
(404) 542-2535

Sprague, Jerry
School of Forest Resources
NC State University
P. O. Box 8002
Raleigh, NC 27695-8002
(919) 737-3168

Squillace, A. E.
School of For. Res. & Conserv.
University of Florida
N-Z Hall, Rm 118
Gainesville, FL 32611
(904) 392-1850

Stauder, Albert
Texas Forest Service
P. O. Box 617
Alto, TX 75925
(409) 858-4202

Steele, James A.
Temple-Eastex Forests
229 N. Bowie
Jasper, TX 75951
(409) 382-3434

Steigerwalt, Mark
KMI Land Resources
P. O. Box 7038
Garden City, GA 31418
(912) 964-1871

Stelzer, Henry
Dept. of Forestry
Perdue University
West Lafayette, IN 47907
(317) 494-3613

Stonecypher, Roy W.
Weyerhaeuser Company
534 N. Tower Street
Centralia, WA 98531
(206) 736-8241

Stubbs, Olin L.
Louisiana Office of Forestry
P. O. Box 1628
Baton Rouge, LA 70821
(504) 925-4507

Studyvin, Charles
U.S. Forest Service
P. O. Box 255
Mt. Ida, AR 7197
(501) 867-2101

Stuhlinger, Chris
LSU
School of Forestry, Wildlife
& Fisheries
Baton Rouge, LA 70803

Talbert, Cheryl B.
Weyerhaeuser Company
WTC-2H2 Technology Center
Tacoma, WA 98477
(206) 924-6991

Tauer, Charles G.
Oklahoma State University
013 S Agriculture Hall
Stillwater, OK 74078
(405) 624-5462

Thielges, Bart A.
University of Kentucky
Department of Forestry
Lexington, KY 40546-0073
(606) 257-7596

Todhunter, Michael N.
International Paper Company
Southlands Experiment Forest
Bainbridge, GA 31717
(912) 246-3642

Toliver, Dr. John
LSU School of Forestry
Baton Rouge, LA 70803
(504) 388-4131

Turner, Gene F.
NC Div of Forest Resources
2411 Garner Rd
Clayton, NC 27529
(919) 553-6178

Tuskan, Gerald A.
North Dakota State Univ.
Dept. Horticulture & Forestry
Fargo, ND 58105
(701) 237-8476

Van Buijtenen, J. P.
Forest Genetics Lab.
Texas Forest Service
College Station, TX 77843
(409) 845-1325

Walkinshaw, Charles H.
USDA Forest Service
Southern Forest Expt Stn
P. O. Box 2008, GMF
Gulfport, MS 39505
(601) 864-8256

Waxler, Michael S.
Weyerhaeuser Company
P. O. Box 1060
Hot Springs, AR 71902
(501) 624-8494

Weir, Robert J.
School of Forest REsources
NC State University
P. O. Box 8002
Raleigh, NC 27695-8002
(919) 737-3168

Wells, Ozzie
Southern Forest Expt Stn
P. O. Box 2008, GMF
Gulfport, MS 39505
(601) 864-3972

White, Tim
University of Florida
Gainesville, FL 32611

Wilder, Jean
Louisiana State University
P. O. Box 22809
Baton Rouge, LA 70893
(504) 388-4131

Williams, Claire G.
North Carolina State University
P. O. Box 8002
Raleigh, NC 27695
(919) 737-3420

Williford, Mike
American Can Company
P. O. Box 315
Butler, AL 36904
(205) 459-3007

Wiselogel, Arthur
Texas A&M University
Forest Science Dept
College Station, TX 77843
(409) 845-5004

Wright, Lynn
Oak Ridge National Lab
Bldg 1503
Oak Ridge, TN 37831
(615) 574-7378

Yates, Harry O. III
Forestry Sciences Laboratory
USDA Forest Service
Carlton Street
Athens, GA 30602
(404) 546-2467

Young, Mike
Georgia Forestry Commission
P. O. Box 819
Macon, GA 31298-4599
(912) 744-3369

Zeringue, Mr. Furcy
LSU School of Forestry
Baton Rouge, LA 70803
(504) 388-4131

Zoerb, Marvin H., Jr.
Union Camp Corp
Box 216
Rincon, GA 31326
(912) 826-5556